Evaluation of 793/B-like and Mass-like vaccine strain kinetics in experimental and field conditions by real-time RT-PCR quantification

C. M. Tucciarone,[*,1] G. Franzo,[*] G. Berto,[†] M. Drigo,[*] G. Ramon,[‡] K. C. Koutoulis,[§] E. Catelli,[#] and M. Cecchinato[*]

[*]*Department of Animal Medicine, Production and Health (MAPS), University of Padua, Viale dell'Università 16, 35020 Legnaro (PD), Italy;* [†]*Ceva Salute Animale, Viale Colleoni 15, 20864 Agrate Brianza (MB), Italy;* [‡]*Ceva Santé Animale, 10 Avenue de la Ballastiere, 33500 Libourne, France;* [§]*Department of Poultry Diseases, Faculty of Veterinary Science, University of Thessaly, Trikalon 224, 43100 Karditsa, Greece; and* [#]*Department of Veterinary Medical Sciences, University of Bologna, Via Tolara di Sopra, 50 - 40064 Ozzano dell'Emilia (BO), Italy*

ABSTRACT Infectious bronchitis virus (IBV) is a great economic burden both for productive losses and costs of the control strategies. Many different vaccination protocols are applied in the same region and even in consecutive cycles on the same farm in order to find the perfect balance between costs and benefits. In Northern Italy, the usual second vaccination is more and more often moved up to the chick's first d of life. The second strain administration together with the common Mass priming by spray at the hatchery allows saving money and time and reducing animal stress. The present work compared the different vaccine strains (Mass-like or B48, and 1/96) kinetics both in field conditions and in a 21-day-long experimental trial in broilers, monitoring the viral replication by upper respiratory tract swabbing and vaccine specific real time reverse transcription PCR (RT-PCR) quantification. In both field and experimental conditions, titers for all the vaccines showed an increasing trend in the first 2 wk and then a decrease, though still remaining detectable during the whole monitored period. IBV field strain and avian *Metapneumovirus* (aMPV) presence also was also investigated by RT-PCR and sequencing, and by multiplex real-time RT-PCR, respectively, revealing a consistency in the pathogen introduction timing at around 30 d, in correspondence with the vaccine titer's main decrease. These findings suggest the need for an accurate knowledge of live vaccine kinetics, whose replication can compete with the other pathogen one, providing additional protection to be added to what is conferred by the adaptive immune response.

Key words: infectious bronchitis virus, vaccination, protectotype, quantification

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INTRODUCTION

Infectious bronchitis virus (**IBV**) is an avian *Gammacoronavirus* that causes a highly contagious and effectively spreading disease, responsible for substantial economic losses. IBV can affect young birds as a respiratory or renal infection, with high morbidity (100%) and mortality rates, even over 50% when secondary infections or virulent strains occur (Jackwood, 2012). It also has been associated with reproductive disorders, mainly production drop and poor egg quality, as the outcome of a reproductive tract infection (Cook et al., 2012). IBV presents a global distribution and an extreme variability, with more than 50 variants classified on antigenic and genetic bases (Valastro et al., 2016), sharing around 75 to 80% of identity in the S1 gene (Cavanagh, 2007). The vivid necessity of controlling the disease has guided the attention towards the optimization of vaccination strategies. One of the pivotal points was represented by the establishment of the protectotype concept, consisting on the association of unrelated IBV vaccine strains in an attempt to widen the conferred protection (Cook et al., 1999). Indeed, many studies have been performed to assess vaccine kinetics and cross-protection level promoted by different vaccines, through several experimental trials, administration routes and timing, settings, and evaluation methods, such as vaccine quantification from tracheal swabs (Jackwood et al., 2009), ciliostasis observation, or challenge virus detection (De Wit and Cook, 2014; Jackwood et al., 2015).

An important adjustment has been introduced in the broiler vaccination program by moving up the second administration to the first d of age. Previously, the vaccination was usually performed at different times of the production cycle, starting with priming on day-old

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[1]Corresponding author: claudiamaria.tucciarone@phd.unipd.it

birds (typically with Mass-based vaccines) and boosting at 14 d (De Wit and Cook, 2014). The rationale behind this approach was that a better tracheal epithelium recovery between administrations, a milder vaccination impact on animals with fewer adverse reactions (De Wit and Cook, 2014), and a stronger immune response (van Ginkel et al., 2015) were proven. Nevertheless, the earlier second vaccine supply could balance other negative management aspects, such as irregular animal vaccination, often causing rolling-reactions, and prevent vaccine administration in the field, when poor conditions or secondary pathogens are already present (De Wit and Cook, 2014).

Above all, the extreme IBV strain variability and heterogeneity over the different parts of the world imply the need of an accurate evaluation of the vaccine protection against every new variant and the choice of the vaccination protocol on local epidemiologic bases (Bande et al., 2015). Up to 2011, the Italian scenario appeared to be dominated by the 793/B genotype, together with other less frequent strains such as QX, Massachusetts, It02, 624/I, B1648, and D274 (Taddei et al., 2011), when the strong emergence of the QX genotype as the most commonly detected one and the attribution of the 793/B persistence to homologous vaccination (Franzo et al., 2014) changed the epidemiologic considerations. Updates in the epidemiological knowledge have occasionally prompted the tuning of the vaccination approach to face these changes, most remarkably through the introduction of a QX homologous vaccine instead of the 793/B genotype to be associated with the Mass one, even though the Mass-793/B association is often reported to confer a wide protection (Cook et al., 1999) against a broader strain range and an effective protection especially towards QX strain (Terregino et al., 2008; Sarueng et al., 2014; Awad et al., 2016). Anyway, vaccination strategies including the 793/B genotype are still the most commonly applied, and they are unceasingly evolving. In fact, over time, several 793/B-based vaccines have been developed, originating from different progenitors and with different attenuation processes. So, it is expected that these strains would display different biological features that could reflect on their efficacy, virulence, and interaction with other strains.

Among the several issues acting on poultry productivity, avian *Metapneumovirus* (**aMPV**) is emerging as a growing health problem also in broiler farming (Catelli et al., 2004), causing clinical signs as a secondary agent or even as the only detected pathogen (Cecchinato et al., 2013a). In Italy, no vaccination is currently employed in the control of the disease in this sector, whereas it is more widely adopted among layers and breeders to achieve adequate protection (Cecchinato et al., 2012), with mostly live or inactivated subtype B-based vaccines according to the subtype B being the most prevalent field strain circulating (Catelli et al., 2004; Cecchinato et al., 2012; Cecchinato et al., 2013a). aMPV infection has been identified mainly in late production stages (Cavanagh et al., 1999; Cecchinato et al., 2013a), and many explanations for its delayed appearance have been proposed, such as a peculiar slower replication of aMPV (Cook et al., 2001) and the shared target of the upper respiratory tract epithelium, leading to an interference between aMPV and IBV vaccine/field strain replication (Cavanagh et al., 1999). Unfortunately, comprehensive data about aMPV epidemiology, different IBV vaccine and pathogen interactions, and the relevance of application timing are often lacking or anecdotal.

Keeping this in mind, we performed an experimental trial aimed to evaluate the kinetics and safety of a recently commercialized 793/B based vaccine (i.e., 1/96 strain) as well as its interactions with a Mass-based vaccine (i.e., B48 strain), when co-administered at one d of age. Considering that experimental settings can be poorly representative of the actual field conditions, we performed a parallel large-scale epidemiological study following several farms where Mass-like and 1/96-based vaccines were administered at one d of age. The vaccine titer trends were monitored along the productive cycles, and their effect on IBV field strain or aMPV entry was evaluated.

MATERIALS AND METHODS

Real-Time RT-PCR

Two different real time reverse transcription PCR (**RT-PCR**) were validated in order to specifically detect and quantify the vaccine strains used in this study. One assay aimed to detect the Mass-like strains (i.e., Mass H120 strain and B48 strain CEVAC® MASS L, Ceva Santé Animale, Libourne, France), and the other was specific for the 1/96 (CEVAC® IBird, Ceva Santé Animale, Libourne, France) one. Primers and probes were designed on the variable regions of aligned reference sequences (Table 1). The assays were performed using SuperScript® III Platinum® One-Step qRT-PCR Kit (Invitrogen™, Carlsbad, CA, USA) on LightCycler® Nano Instrument (Roche, Basel, Switzerland). The 2 assays were validated on titrated vaccines (5.4 $\log_{10}$ EID_{50}/mL for the Mass H120 strain, 4.1 $\log_{10}$ EID_{50}/mL for the B48 strain, and 5.5 $\log_{10}$ EID_{50}/mL for the 1/96 strain), accounting for the real-time RT-PCR detecting both viable and non-viable viral particles in vaccines, as well as in experimental and field samples. The real-time RT-PCR limit of detection (**LoD**) was evaluated using serial 10-fold viral RNA dilutions, performed in quadruplicate, ranging from the undiluted vaccine to a final 10^{-7} dilution. LoD was defined as the lowest viral amount detectable in at least 50% of the replicates. LoD was $10^{-0.49}$ EID_{50}/mL for IBV 1/96, $10^{-0.49}$ EID_{50}/mL for the B48 strain, and $10^{-0.1}$ EID_{50}/mL for the Mass H120 strain. The specificity of each assay was assessed by testing vaccine strains different from the targeted ones. All assays showed an efficiency between 92 and

Table 1. Primer and probe sequences designed for the study.

Primer or Probe	Sequences (5′ to 3′)	Direction
1/96 F	CCTGGTTCAGGTTGGCATTT	Sense
1/96 R	ATGCACTGCCTGCATTGTTG	Antisense
1/96 Probe	FAM-TCTACTGCATAAGCACCCCCATG-BHQ1	Antisense
Mass F	AGCAGACGCAGGTTTGGCTA	Sense
Mass R	TGGTTGACATCTTCGCAAGG	Antisense
Mass Probe	FAM-CATCTGGTTCCATAGACATCTTTGTCG-BHQ1	Antisense

103% and a coefficient of determination higher than 0.97.

Experimental Trial

Experimental Design. 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. Four groups (1 to 4) of 20 one-day-old Ross 308 chicks were chosen from the same hatchery and breeders. Animals were individually marked and housed in 4 separated rooms, under high biosecurity measures. Twenty other animals from the same batch were blood sampled to gather information about maternally derived antibody (**MDA**) levels. At d one of the trial, each group of day-old birds was vaccinated by spray vaccination, following the recommended procedures, with different vaccine strains: group 1 was vaccinated with H120 strain, group 2 with B48 strain, group 3 with both B48 and 1/96 strains, and group 4 with 1/96 strain only. Vaccine suspensions were titrated by the specific real-time RT-PCR used for the study, revealing titers of $10^{4.99}$ EID_{50}/mL for H120 strain, $10^{3.90}$ EID_{50}/mL for B48, $10^{3.97}$ EID_{50}/mL for 1/96 strain, and $10^{3.98} + 10^{3.86}$ EID_{50}/mL for B48+1/96, respectively. Ten animals out of each group were randomly selected and marked on the first d, and sampling was carried out by performing oro-pharyngeal swabs (choanal cleft and pharynx). The other 10 animals were not sampled but maintained to simulate within-flock vaccine virus circulation. Swabs were collected at different time points: 12 h and 2, 4, 7, 10, 14, 18, and 21 days. Swabs were divided into 2 pools of 5 swabs each, always including the same animals, air-dried for 10 min to avoid bacterial and mold growth, and then stored at -80°C until processing. Swabs were eluted in 2 mL of PBS, and RNA extraction was performed using the High Pure RNA Isolation Kit (Roche, Basel, Switzerland), following the manufacturer's instructions. Samples underwent both the Mass-like and 1/96 specific real-time RT-PCR, as previously described, for the detection of the applied vaccine and the exclusion of a possible contamination by the other strain.

Serology. Blood samples were obtained from 20 one-day-old Ross 308 chicks of the same origin to evaluate MDA levels. Infectious Bronchitis Virus Antibody Test Kit (IDEXX, Westbrook, Maine, USA) was used following the manufacturer's instructions. Samples with S/P absorbance ratios of less than or equal to 0,2 were considered negative (corresponding to an antibody titer of $10^{2.60}$).

Field Trial

Animals and Vaccine Strains. The screening activity focused on 70 broiler flocks distributed in Northern Italy and sampled over different production cycles. All sampled animals were vaccinated on the first d of life with 2 different IBV vaccine strains (i.e., 1/96 and Mass-based vaccines, excluding B48 strain), following the protectotype concept.

Samples. Samples consisted of swabs taken from the upper respiratory tract, and sampling was arranged in at least 2 time points of the bird life span per productive cycle, at the beginning and at the end, for commercial reasons, in order to monitor the presence of the vaccines and the bird health. Ten swabs for each flock or house were pooled, air-dried, stored at −80°C until extraction, and processed as previously described.

Diagnostic Assays Towards Vaccines and Field Pathogens. All samples were tested, and IBV vaccine amount was quantified with the 2 previously described real-time RT-PCR. Additionally, diagnostics for IBV field strain detection, based on a RT-PCR able to detect all IBV genotypes currently circulating in Italy, was performed on at least one sample per cycle from the latest period (>30 d). Furthermore, whenever clinical signs were reported (i.e., about 16% of the total amount of the tested samples), related samples were tested independently from animal age. All positive samples were sequenced to evaluate the presence of vaccine strains and their genetic stability or the entry of a field strain. Briefly, the generic IBV RT-PCR described by Cavanagh et al. (1999)(Cavanagh et al., 1999) was performed with minor modifications, using SuperScript® III One-Step RT-PCR System with Platinum® Taq DNA Polymerase kit (Invitrogen™, Carlsbad, CA, USA) on Applied Biosystems 2720 Thermal Cycler (Applied Biosystems, Foster City, CA, USA), and the amplicons were then sequenced using the same primers. The sequences, encompassing the hypervariable region of the S1 gene, were used for a phylogenetic-based classification by comparison with reference strains (Valastro et al., 2016). Starting from the fourth wk of the production cycle, when most of the aMPV detections are expected (Cecchinato et al., 2013a), samples were systematically analyzed also for aMPV detection

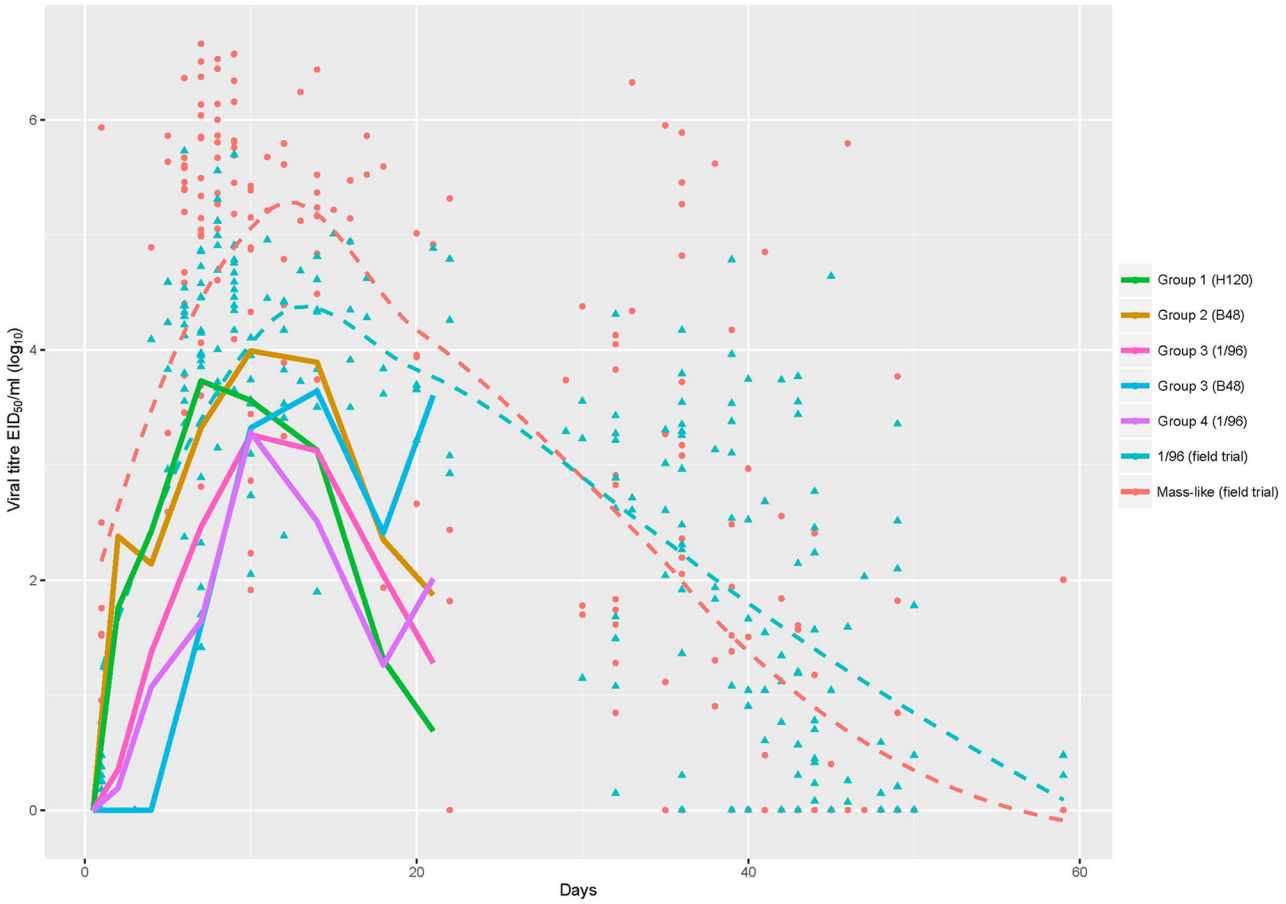

Figure 1. Vaccine titer trends relative to animal age in experimental and field conditions: titers from the experimental trial are represented by continuous lines color-coded for each group (1 to 4); titers from the field trial are marked by red dots for Mass-like strains and by blue triangles for 1/96 strain, and the overall trend is depicted by red and blue lines, respectively.

using a multiplex real-time RT-PCR (Cecchinato et al., 2013b).

Database Setting. Samples were organized in a comprehensive database of available information about flocks, sampling time, chicken age, clinical signs, and lab results, such as vaccine detection and titers, sequencing results, and aMPV positivity.

Statistical Analysis. The effect of sampling age and genotype on vaccine viral titers was evaluated using the robust analysis of covariance (**ANCOVA**) (Wilcox, 2012). This method compares the trimmed means of the 2 group (i.e., vaccine genotype) viral titers at different time points along the covariate (i.e., sampling age). The trimming level was set to 0.2. Two thousand bootstrap replicates were performed to estimate the confidence intervals. The effect of Mass-like and 1/96 vaccine viral titers in conditioning the likelihood of IBV field strain detection was assessed using a logistic regression. A similar approach was used to evaluate the protective effect of IBV vaccines against aMPV infection. The presence of a non-random association between IBV field strains and aMPV was investigated by a Chi-square test. All analyses were performed using the R statistical software and the significance level was set to P-value <0.05.

RESULTS

Experimental Trial

Serology. MDA levels appeared to be definitely homogeneous among the sampled birds, ranging from $10^{2,95}$ to $10^{3,88}$ with a mean of $10^{3,57}$.

Vaccine Kinetics. No cross-contamination was detected among the groups, and similar viral titers between the 2 pools from the same sampling moment were highlighted for all groups. Mean viral titers were calculated between the 2 pooled samples collected at each time point (Figure 1). All groups were negative for the RNA of the respective applied vaccine at 12 h post vaccination. At every other sampling time, all groups were positive, except group 3, which appeared negative only for B48 RNA until 7 d p.v. In group 1, H120 titers showed an increase until d 7 ($10^{3.73}$ EID_{50}/mL) and then a decrease. In group 2, B48 titers displayed a peak at d 10 ($10^{3.99}$ EID_{50}/mL), reaching higher titers than the other Mass-like strain in group 1 and presenting also a higher titer persistence. B48 strain presented a different behavior when administered with 1/96 strain, being undetectable until 7 d post vaccination with a subsequent peak at 14 d ($10^{3.64}$ EID_{50}/mL), a slight decrease at

d 18 ($10^{2.41}$ EID_{50}/mL), and a second peak at 21 d ($10^{3.60}$ EID_{50}/ml). In group 4, the maximum 1/96 RNA viral titer has been detected at 10 d ($10^{3.28}$ EID_{50}/mL), similar to 1/96 viral titer peak in group 3 ($10^{3.26}$ EID_{50}/mL at d 10), while the 2 kinetics trends revealed the strain tendency to decrease when in association with Mass-like strain and to increase again towards d 21 when alone instead.

Field Trial

Samples. Two-hundred-forty-seven samples were analyzed by the 2 specific real-time RT-PCR: 87.86% of the samples were positive for 1/96 strain, while 70.05% for Mass-like strains.

Vaccine Kinetics in Field Conditions. The vaccine titer distribution over time revealed equally an effect of time and genotype on viral titers. Both genotypes displayed a positively skewed curve, with viral titers rapidly rising during approximately the first 2 wk of life and then progressively decreasing (Figure 1). In fact, the few negative values for both Mass and 1/96 strains were concentrated within the first 3 d and after the 36th day. However, a genotype-specific significant difference between titers was detectable, with Mass titers being higher in the first half of the cycle but more quickly decreasing than 1/96 titers towards the cycle end. The detection of vaccine strain sequences was well spanned all along the productive cycle, and the presence of identical vaccine sequences showed the genetic stability of the used vaccines, opposite to their prolonged persistence and circulation.

IBV Field Strain Detection. IBV generic RT-PCR and sequencing were performed on 99 (40.08%) samples. The resulting sequences belonged to one of the administered vaccines in 56 (56.57%) samples, to the QX genotype in 22 (22.23%), to 793/B vaccine-like strains different from 1/96 in 15 (15.16%). Five (5.06%) samples were classified as Q1 and one (1.02%) was defined as a recombinant strain derived from a combination of QX and 793/B (different from 1/96 strain) parental strains. Field strains were detected from the 35th d in correspondence with the second half of the productive cycle and in particular with the 1/96 vaccine titer decline, but occasionally with high Mass-like vaccine titers instead (Figure 2). When the relationship between vaccine titers and field strain detection was investigated, significantly lower 1/96 vaccine strain titers were demonstrated in field strain positive samples compared to the negative ones. No significant difference was demonstrated for the Mass-type vaccine (Figure 3).

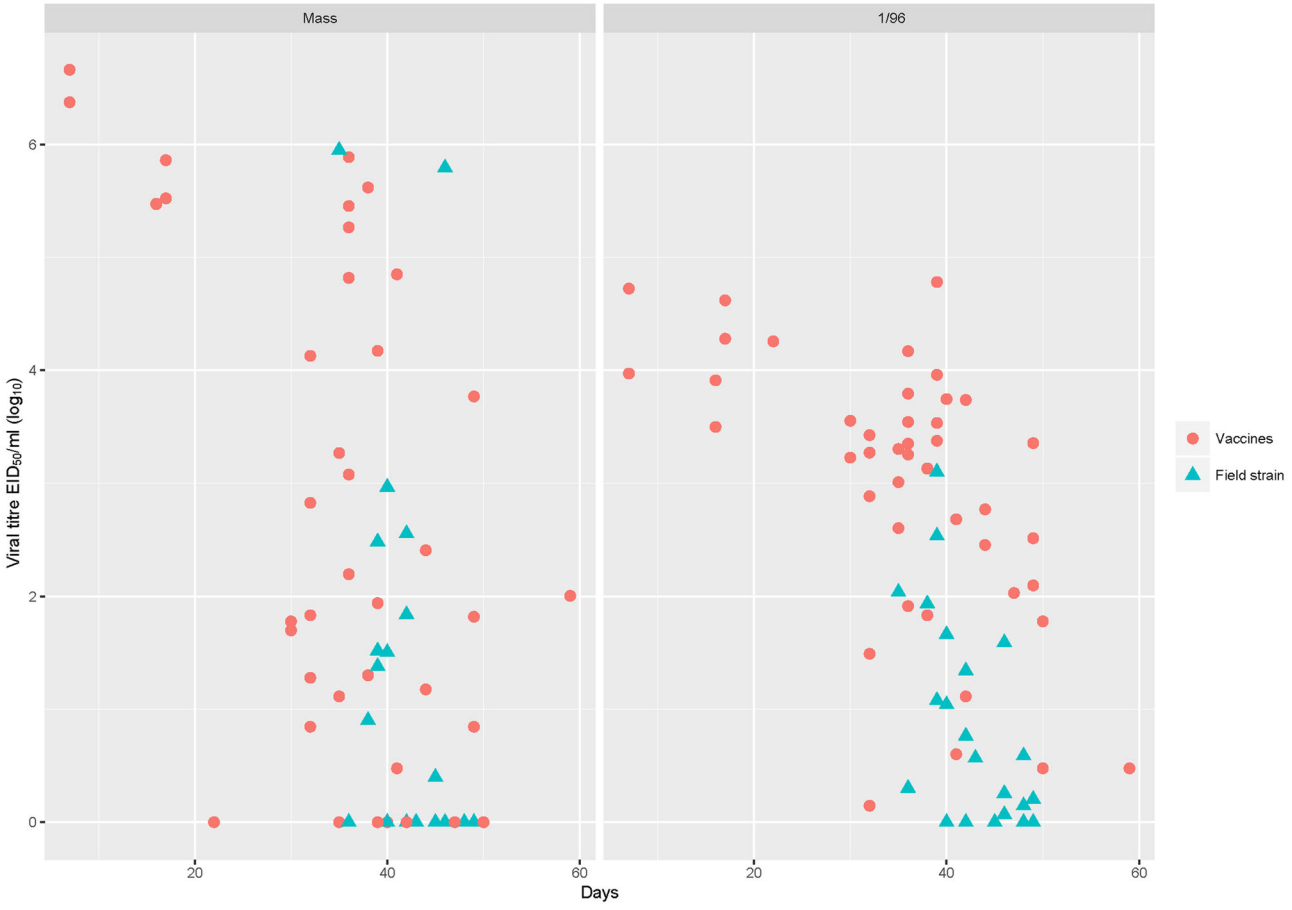

Figure 2. IBV vaccine titers in samples in which vaccine and field strain sequences were detected. Red dots represent Mass or 1/96 vaccine sequences, while blue triangles represent field strain sequences, placed along the axes in correspondence with vaccine titers and d of age.

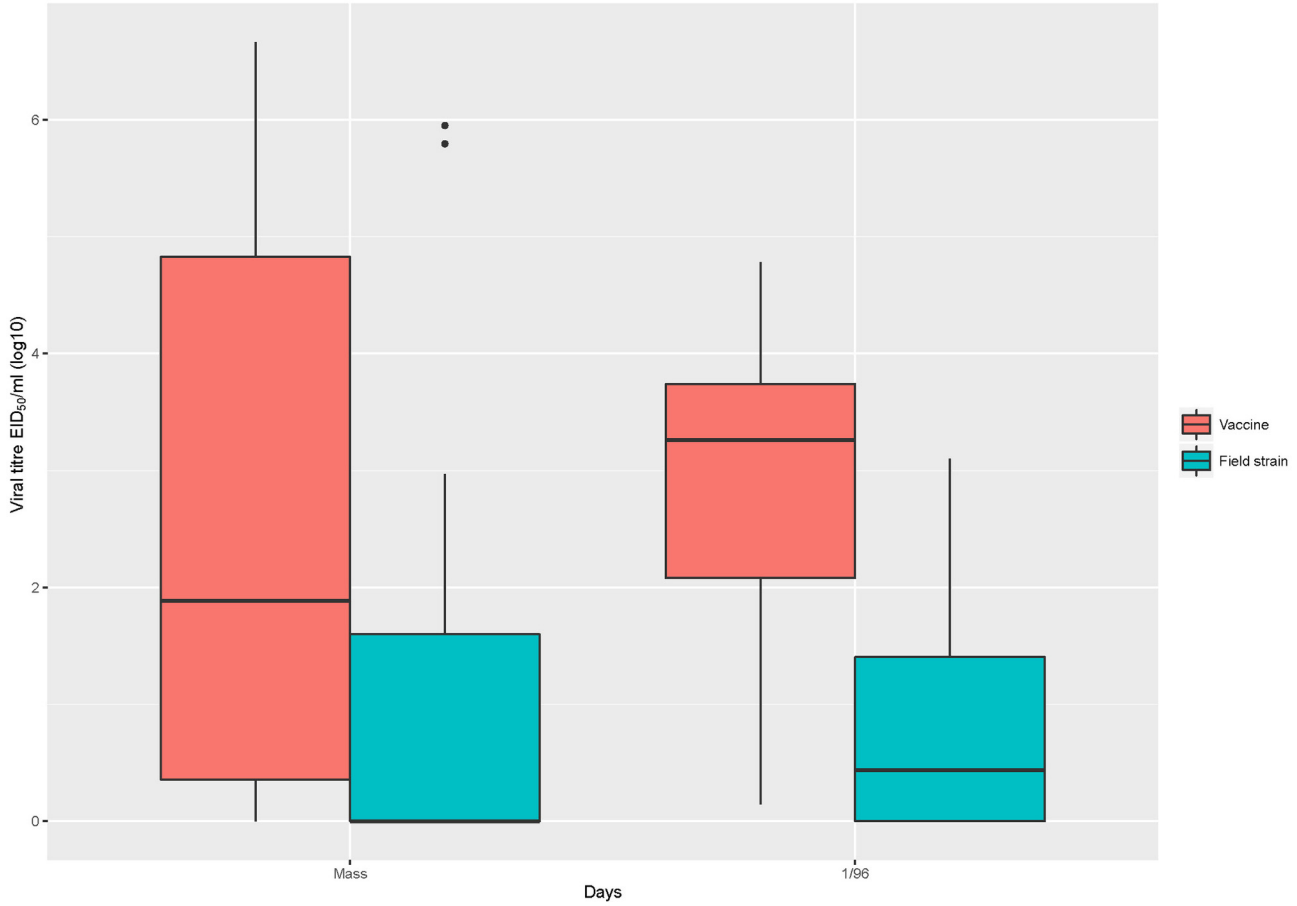

Figure 3. IBV vaccine titer comparison among samples in which IBV field strain sequences and vaccine ones were detected.

aMPV Detection. Eighty-two samples (33.2%) were tested for aMPV, and 33 (40.25%) of them were aMPV subtype B positive. From the 35th d of the cycle, aMPV subtype B was found frequently (Figure 4), and its presence was recognized also together with IBV field strains (12 samples), among which 6 samples belonged to QX genotype, even though a statistically significant association between aMPV and IBV infection could not be proven. Interestingly, a tendency to display lower IBV vaccine titers emerged in aMPV positive samples (Figure 5), although not significant from a statistical point of view.

DISCUSSION

The huge IBV diffusion and its extreme genetic variability produce daily challenges in the matter of control strategies, which are mainly relying on 2 aspects: the vaccine immunological properties and managerial aspects. The first ones have prompted the research and development of vaccine combinations, providing an effective cross-protection against different strains, with a particular focus on the locally circulating ones. Unfortunately, practical constraints related to economical and organizational choices in farm management led to a decreased vaccine efficacy compared to experimental conditions. Thus, the need for providing vaccines able to efficiently spread in field conditions and to induce good protection, even in the presence of other vaccines and co-infections, has led to commercialization of new vaccine strains and to the implementation of new administration plans, while minimizing at the same time the number of interventions. In the current study, the replication dynamics of different IBV vaccines co-administered at one d of life were investigated both in experimental and field conditions. As a matter of fact, a strict and extended monitoring of vaccine titers could allow verifying the vaccine administration efficacy from a managerial point of view and also from a biological one. In experimental conditions, viral titers displayed a common trend characterized by a rapid increase, reaching the highest values around 10 d, then decreasing until the conclusion of the trial. The only exception was represented by the behavior of B48 strain, when administered in combination with 1/96 strain, which did not appear before d 7, and it showed a titer rise in correspondence with the 1/96 expected decrease, thus testifying its persistence and replication during the early d, even if at extremely low levels, probably due to the prevailing 1/96 replication. The effects of this peculiar behavior of B48–1/96 co-administration on the vaccine-induced protection are not clear and will need further investigations. On the contrary, 1/96 strain did not present any major titer alteration, either by standing

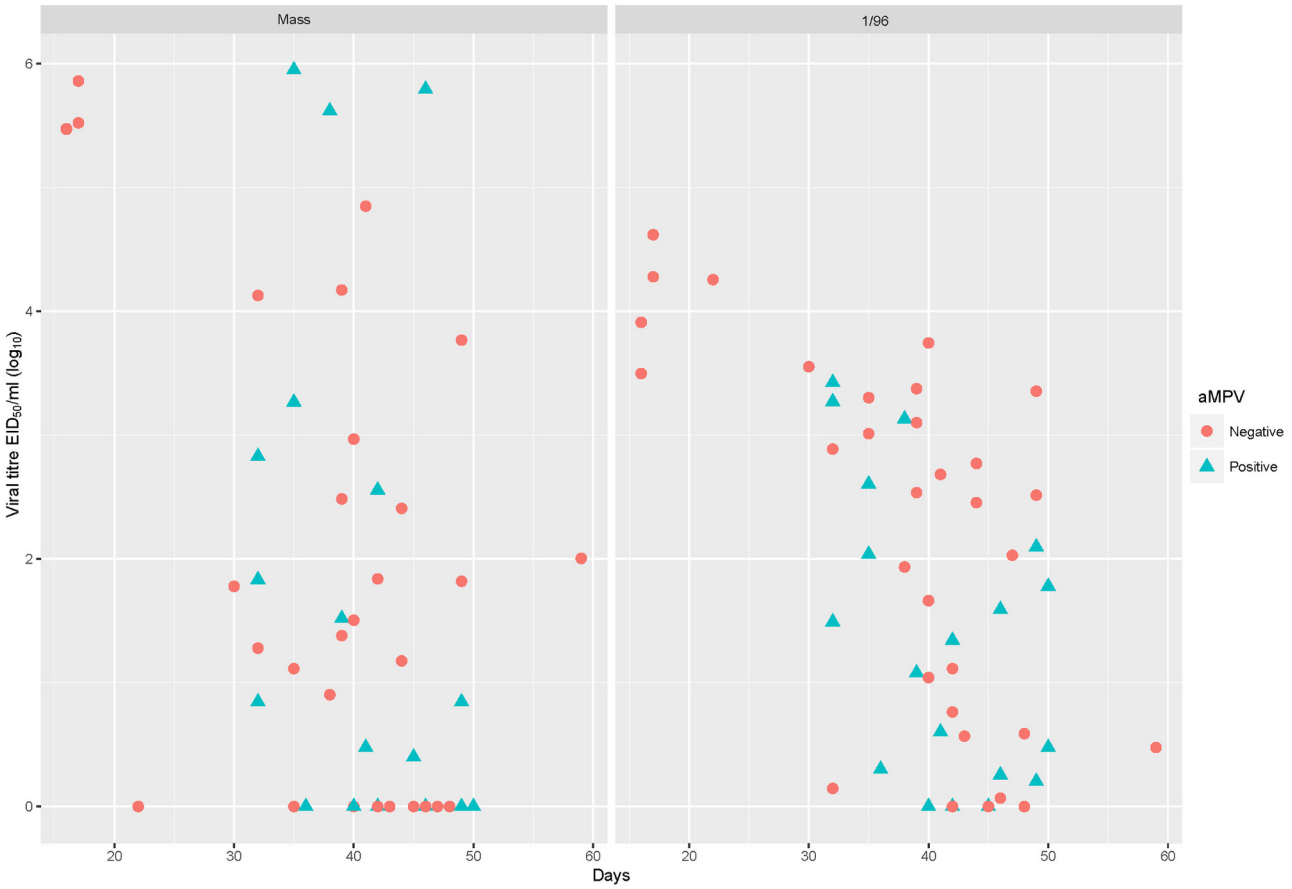

Figure 4. IBV vaccine titers in samples in which aMPV was detected.

alone or in combination with B48 strain administration, thus supporting the inferences about the strong replication and co-replication ability of this vaccine strain (Figure 1).

When an analogous scenario was examined in the field, significantly overlapping results were obtained. Similar to the experimental conditions (Figure 1), an effect of both time and genotype was revealed on vaccine viral titers. Both genotypes displayed a positively skewed curve, with viral titer rapidly rising during approximately the first 2 wk of age and then progressively decreasing (Figure 1). Particularly, the few negative results were mostly located at the very beginning of the cycle, when the viral replication could not be homogeneous among animals and the viral spreading could not have reached all the birds yet. In the last phase of the cycle, negative samples were more frequent, as a consequence of the generalized titer decline. Interestingly, the differences in the 2 strain kinetics highlighted a certain higher replication capacity for the Mass-like strains in the upper respiratory tract at first, opposite to the longer persistence of the 1/96 strain. This was partially mimicked by the dynamics in experimental conditions in which H120 and B48, when administered alone, displayed a higher titer compared with 1/96 until d 18 and 21, respectively. The end of the trial did not allow gathering further conclusions about the following B48 titer trend; nevertheless, another study (Tatár-Kis et al., 2016) explored the second half of the productive cycle of similarly vaccinated animals: B48 appeared always lower than 1/96, which displayed a notable persistence. Therefore, the observed pattern could be attributed to actual viral biological properties and not to the particular scenario.

A separate discussion must be reserved to the interaction between the 2 vaccines. Even if presenting some dissimilarities, both vaccine trends were quite overlapping, and high viral titers were frequently encountered, hence suggesting the absence of a competition between the 2 genotypes, and a parallel coexistence also when administered on the same day. This clearly conflicts with the experimental evidences, in which an obvious interference was observed. Two main hypotheses can be introduced to explain this discrepancy; at first a Mass vaccine different from B48 is currently used in Italy, so the different biological properties of this virus could explain its lower susceptibility to 1/96 competition. Additionally, in field conditions, the Mass vaccine is often provided at the hatchery, while the 1/96 is provided upon arrival at the farm. Considering that a higher affinity for the tracheal receptor has been proposed as a source of the increased replication of 793/B genotypes (Cook et al., 1999), this short lag phase between the 2 administrations could enhance the Mass vaccine cell adhesion and replication.

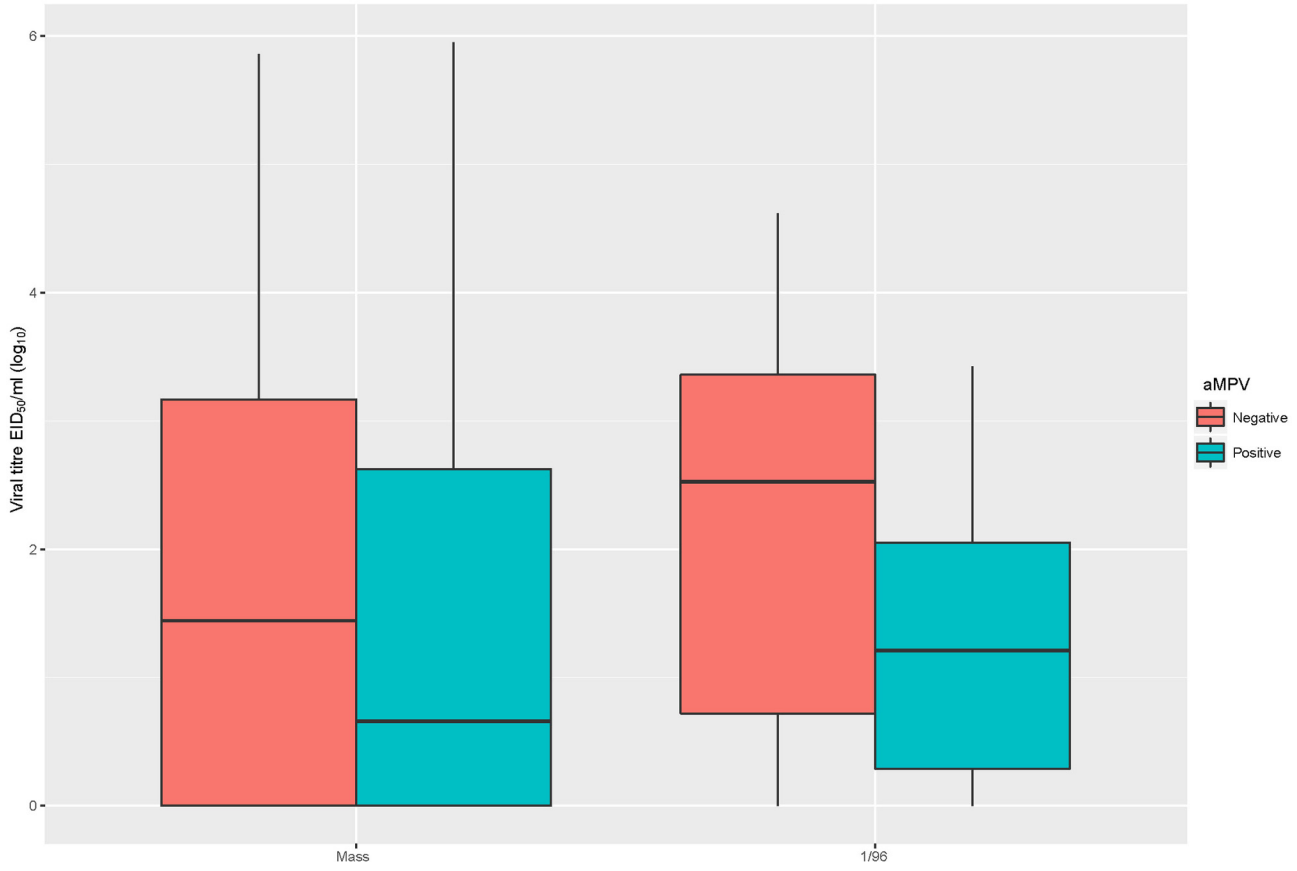

Figure 5. IBV vaccine titer comparison between aMPV negative and positive samples.

It must be stressed also that experimental trials are often poorly representative of the sub-optimal conditions found in the field, and thus they should be interpreted with caution.

In fact, comparing the prolonged vaccine persistence in the field with the experimental trial trend, the experimental conditions could have influenced the viral titers because of the small number of involved animals, leading to a lower viral circulation. On the other hand, a controlled environment could have minimized the risk of other infections or the presence of stressing and immunosuppressive conditions, placing the premises for unbiased results by other pathogens' competitive actions. The abovementioned field scenario raises a question about vaccine viral attenuation and the efficacy of the immune response, which appears to be insufficient for the vaccine clearance within production cycles, allowing a continuous re-infection of the animals because of a high environmental infectious burden.

However, it must be clearly stated that the prolonged vaccine circulation could be advantageous by increasing the "vaccination coverage" and granting a protective effect ascribable also to the interference against the field strains. In fact, higher 1/96 strain titer seem to confer better protection against field strains. Literature reports how this particular genotype, in association with Mass vaccine administration, could ensure strong protection against QX strain (Terregino et al., 2008; Sarueng et al., 2014; Awad et al., 2016), which is the most prevalent in Italy and the most detected one in this study as well. Moreover, the frequency of Italian QX outbreaks has been correlated to the 793/B vaccine application, with its peaks quite explained by the 793/B administration suspension (Franzo et al., 2016). Afterward, a clear effect of the viral competition emerges from this study, because of the IBV field strain occurrence mainly in the second part of the productive cycle, when the vaccine titers are decreasing, but the immunity should still be effective because of the continuous stimulus imposed by vaccine circulation. Even if the sampling nature of this study has created a lack of information from the 22nd to the 30th d of the cycle, due to the primary aim of assessing the vaccination adequacy, the vaccine persistence, and possible field strain infection in the case of clinical symptoms, the viral titer trend suggested a similar decreasing behavior in that gap. So a slight underestimation of IBV field strains or aMPV entrance time also could have occurred, since their first detections from the 35th d of the cycle on, but this finding seems to be in line with the expected timing, especially for aMPV (Cavanagh et al., 1999; Cecchinato et al., 2013a). As a matter of fact, another proposed competing effect is the one between IBV and aMPV (Cavanagh et al., 1999; Cook et al., 2001; Cecchinato et al., 2013a), which appeared to be lightened by the sporadic finding of

aMPV and IBV field strain co-infection in the last period of the productive cycle, whereas the paucity of aMPV positivity in the first part of the cycle would enforce the competitive role sustained by high IBV vaccine titers. The tendency to detect aMPV in presence of lower IBV vaccine titer (Figure 5) seems to support a certain interference between the 2 viruses, potentially due to the reduction of the susceptible cell number or to the pre-activation of the innate immune response, which could hamper aMPV replication. aMPV field relevance appears more urgent nowadays, even though the clinical and productive impact of this pathogen is still partially neglected. Being this study focussed on IBV, no epidemiological conclusions can be drawn on aMPV; still, its wide presence in broilers should promote more accurate surveys.

Moreover, further studies will be necessary to understand also if the apparent inverse relationship between IBV vaccine titer and aMPV infection is actually due to competition, to the vaccine kinetics itself, or to other unconsidered factors.

CONCLUSIONS

The importance of a simple vaccine kinetics monitoring through real-time RT-PCR appears evident when the titer kinetics and vaccine persistence suggest different evaluations about the exerted protection. The extended vaccine presence until the cycle end would more likely propose a competitive role of the vaccines against the field strain entry rather than a more classical immunity, which should assure a quicker clearance of the vaccine strain itself. Additionally, the implications and features of aMPV and IBV coexistence, either of vaccine or field nature, could be interesting and even likely resourceful, if consciously managed. Anyway, these considerations will benefit from further studies, accounting for serology, immunity, and protection evaluations.

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CONFLICT OF INTERESTS

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