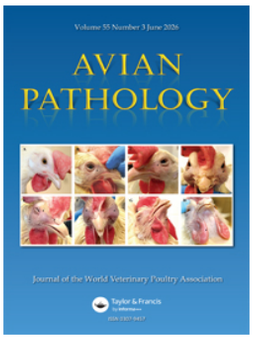

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Broad IBV protection induced by a live IBV-NDV vaccine containing a Mass and Q1 strain

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ORIGINAL ARTICLE

Broad IBV protection induced by a live IBV-NDV vaccine containing a Mass and Q1 strain

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ABSTRACT

Five vaccination-challenge trials were performed with a live attenuated vaccine which includes an infectious bronchitis virus (IBV) Massachusetts strain (H120, GI-1), an IBV Q1 strain (BNF 28/86, GI-16), and a Newcastle disease virus (NDV) cloned strain. The efficacy of the vaccine was tested in maternally-derived antibody-positive (MDA+) commercial layers against challenge with IBV M41, 793B, D388(QX) or Q1 challenge virus. The efficacy of the vaccine was also tested in SPF layers against challenge with IBV M41, D388(QX) or Variant 2 challenge virus. The vaccine was applied at day of hatch, and all challenges were performed at 3 weeks of age. At 5 days post-challenge, the ciliary activity of tracheal explants was examined to determine the level of protection. The level of protection induced by the vaccine in commercial layers against IBV M41, 793B, D388(QX) and Q1 challenge was 91%, 94%, 73% and 84%, respectively. The level of protection in the SPF layers against IBV M41, D388(QX) and Var2 challenge was 89%, 69%, and 96%, respectively. In summary, vaccination with a live attenuated vaccine which includes an IBV Massachusetts strain (H120, GI-1) and an IBV Q1 strain (BNF 28/86, GI-16) provides a substantial level of protection against the homologous and most heterologous challenge viruses, which are representatives of the IBV strains circulating worldwide.

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Introduction

Avian infectious bronchitis virus (IBV) can cause infections in chickens of all ages and types. The virus mainly causes respiratory disease and nephritis in young chickens, but it can also result in reduced or abnormal egg production and quality (Cavanagh & Gelb, 2008). IBV is notable for its growing number of variants, known as serotypes or genotypes (Valastro *et al.*, 2016), which arise due to its high mutation rate and the occurrence of recombinations (Jackwood & de Wit, 2020). Some exotic strains (e.g. D1466) might be the result of an occasional introduction of gammacoronavirus from wild birds to domestic chickens (De Wit & Cook, 2019). As a result, chickens in most regions of the world face challenges from multiple IBV variants, requiring broader protection than a single vaccine strain can offer (Cook *et al.*, 1999; De Wit *et al.*, 2013). This provides a continuous challenge to existing vaccination programmes.

It is of great value if a vaccine combination can protect birds against infections of both the homologous strains covered by the vaccine combination and other heterologous strains that may be prevalent. The combination of a live-attenuated IBV Massachusetts (GI-1) and 793B-4/91 (GI-13) vaccine was one of the first combinations shown to increase the level of protection of young chicks for multiple heterologous IBV variants (Cook *et al.*, 1999). Now, the approach of combining vaccine strains of different genotypes or serotypes to induce broad heterologous protection has become common practice in a major part of the world. Many papers have been published that show a high level of heterologous protection induced by a vaccine combination of genotype lineage GI-1 (Mass) with a second IBV strain of for example GI-11 (BR1), GI-12 (D274), GI-13 (793B), GI-19 (QX) or GI-23 (Variant 2) (Terregino *et al.*, 2008; De Wit *et al.*, 2011; Awad *et al.*, 2015; Chhabra *et al.*, 2015; De Wit *et al.*, 2015; Awad *et al.*, 2016; Bru *et al.*, 2017; De Wit *et al.*, 2017; Kutle *et al.*, 2020; Houta *et al.*, 2024; Bataille *et al.*, 2025). The level of protection that can be induced by the combination of genotype lineage GI-1 (Mass) and GI-16 (Q1) has not been described before.

In this paper, the results of five vaccination-challenge trials are reported using Vaxxon® CHB, a live attenuated IBV and NDV combo vaccine that includes an IBV Massachusetts strain (H120, GI-1) and an IBV Q1 like strain (BNF 28/86, GI-16) plus a Newcastle disease virus (NDV) cloned LaSota strain. The efficacy of the vaccine against challenge with homologous and heterologous IBVs M41, 793B, D388(QX), Q1 or Variant 2 (hereafter Var2) was evaluated in both SPF and

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commercial laying chickens, by assessing the ciliary activity of the tracheal epithelium post challenge.

Materials and methods

Chickens and experimental design

The experimental design of the trials is summarized in Table 1. The design of each trial was based on the IB vaccine (live) monograph of the European Pharmacopoeia (8th and 10th editions (European Directorate for the Quality of Medicines & HealthCare (EDQM), 2013, 2020)). For trial A and B 1-day-old specified pathogen free (SPF) layer type chickens were obtained from the SPF facility of Royal GD in Deventer, the Netherlands. For trials C, D and E fertilized eggs from vaccinated commercial layer type chickens were obtained from a commercial hatchery and hatched at Royal GD in Deventer, the Netherlands.

The chickens were housed per group in isolators with filtered air under negative pressure. Feed and tap water were supplied *ad libitum*. Each trial included at least one vaccinated challenged group and one non-vaccinated challenged group (positive control group). The vaccine was applied at day of hatch. At 21 days post-vaccination (dpv) the chickens were challenged with an IB challenge virus. At five days post-challenge (dpc) the chickens were euthanized by electrical stunning and exsanguination, and the tracheas were collected for the ciliary activity test. The chickens were observed daily for clinical signs.

Live-attenuated IBV vaccine

Vaxxon® CHB (Vaxxinova International B.V., Nijmegen, the Netherlands) was used in all five trials. This live attenuated IBV vaccine includes an IBV Massachusetts strain (H120, GI-1), an IBV Q1 strain (BNF 28/86, GI-16) and a Newcastle disease virus (NDV) clone strain. One dose of vaccine was administered to each chicken via the oculo-nasal route, 0.05 ml in one eye and 0.05 ml in one nare (0.1 ml per bird). One dose of vaccine contained at least $10^{3.0}$ EID_{50} (embryo infective dose 50%) of each IBV strain, according to the manufacturer's recommended dose.

IBV challenge strains

Challenge strains IBV M41, 793B, D388(QX), Q1 and Var2 were obtained from Royal GD in Deventer, the Netherlands (De Wit *et al.*, 2011, 2017, 2019, 2022). The challenge viruses were administered in a dose of $10^{4.0}$ EID_{50} per 0.1 ml for the IBV M41, D388(QX) and Var2 challenge viruses, in a dose of $10^{4.5}$ EID_{50} per 0.1 ml for the Q1 challenge virus and in a dose of $10^{5.0}$ EID_{50} per 0.1 ml for the 793B challenge virus. These challenge doses are able to induce high levels of ciliostasis in the non-vaccinated challenged birds based on results of previous studies (data not shown). The challenge viruses were administered via the ocular route, 0.05 ml in each eye (0.1 ml per bird).

Ciliary activity test

The tracheas were collected immediately after euthanasia and placed in Hank's Minimum Essential Medium (HMEM, Gibco, ThermoFisher Scientific, Eindhoven, the Netherlands) with 1% antibiotic-antimycotic solution (Gibco). The ciliary activity test was used to determine the level of protection following challenge with IBV, by estimating the percentage of the surface of the tracheal epithelium that shows ciliary movement post challenge.

The ciliary activity of the tracheal epithelium was examined at 5 dpc. The optimal day for performance of the ciliary activity test was determined in previously performed studies (data not shown).

In the first trial (A) five tracheal rings from each trachea were cut using a scalpel, equally divided over the length of the trachea. In the other trials ten tracheal rings per trachea were cut using a scalpel: three from the upper part, four from the middle part and three from the lower part of the trachea. The tracheal rings were placed into 24-well plates containing HMEM with 1% antibiotic-antimycotic solution, one ring per well, and examined using a light microscope within 2 h after collection. The activity (beating) of the cilia in each tracheal ring was scored as follows: 4 (< 25% beating of cilia), 3 (25–50% beating), 2 (51–75% beating), 1 (76–99% beating) or 0 (all beating) (De Wit *et al.*, 2013).

The percentage of protected chickens in a group was calculated using three different methods:

(1) The EP method: a chicken was considered protected if at least nine out of 10 tracheal rings showed normal ciliary activity. A tracheal ring was considered "normal" when at least 50% of the ring showed vigorous ciliary activity (score 0, 1 or 2) (European Directorate for the Quality of Medicines & HealthCare (EDQM), 2013, 2020).
(2) The BPS (binomial protection score) method: a chicken was considered protected if all tracheal rings showed normal ciliary activity. A tracheal ring was considered "normal" when at least 50% of the ring showed vigorous ciliary activity (score 0, 1 or 2) (De Wit *et al.*, 2013).
(3) The CPS (ciliostasis protection score) method: the cumulative ciliary activity scores (sum of five or 10 rings) were calculated for each bird and the CPS per group was calculated as follows: (1-(mean cumulative ciliary activity score of a group/maximum achievable cumulative ciliary activity score of a bird in that group (20 or 40))) × 100 (Cook *et al.*, 1999).

Table 1. Study designs of the vaccination-challenge trials using SPF or commercial (MDA+) chickens vaccinated with Vaxxon® CHB or non-vaccinated and challenged at 21 dpv.

		D0	D21		
Trial	Chicken type	Vaccination at day of hatch	IBV challenge strain	Challenge dose	Challenged chickens per group (*n*)
A	SPF	Vaxxon® CHB	M41	$10^{4.0}$ EID_{50}	10
		Not vaccinated		$10^{4.0}$ EID_{50}	6
		Vaxxon® CHB	D388(QX)	$10^{4.0}$ EID_{50}	10
		Not vaccinated		$10^{4.0}$ EID_{50}	6
B		Vaxxon® CHB	Var2	$10^{4.0}$ EID_{50}	10
		Not vaccinated		$10^{4.0}$ EID_{50}	10
C	MDA+	Vaxxon® CHB	M41	$10^{4.0}$ EID_{50}	20
		Not vaccinated		$10^{4.0}$ EID_{50}	5
D		Vaxxon® CHB	Q1	$10^{4.5}$ EID_{50}	20
		Not vaccinated		$10^{4.5}$ EID_{50}	5
		Vaxxon® CHB	D388(QX)	$10^{4.0}$ EID_{50}	20
		Not vaccinated		$10^{4.0}$ EID_{50}	5
E		Vaxxon® CHB	793B	$10^{5.0}$ EID_{50}	20
		None		$10^{5.0}$ EID_{50}	10

Statistical analysis

The sum of the ciliary activity scores (five or 10 rings per chicken) was compared between vaccinated and non-vaccinated chickens that were challenged with the same IBV challenge strain. Since the residuals in the linear regression model were not normally distributed, a non-parametric Wilcoxon rank sum test was used to compare the vaccinated and non-vaccinated groups. The significance level was adjusted for multiple testing by Bonferroni correction ($P < 0.004$). Pearson's correlation test was used to check the correlation between the calculation methods. All analyses were performed in Stata Statistical Software: Release 19 (2025), StataCorp LLC.

Ethical statement

All five trials were performed in accordance with the Dutch Animal Procedure Act and approved by the Animal Welfare Body of Royal GD.

Results

Clinical observations

In only 2% (2/92) of the vaccinated MDA+ chickens mild transient respiratory signs, such as dyspnoea and coughing, were observed at 5 and 6 dpv. In the vaccinated SPF chickens transient respiratory signs were observed, in 37% (18/49) of the chickens, from 5 up until 21 dpv. After challenge no clinical signs were observed in any of the chickens.

Ciliary activity of tracheal explants

The percentage of protected birds per group is shown in Table 2 (according to different calculation methods) and in Figure 1 (CPS only). The level of protection induced by the live IBV Mass + Q1 vaccine in MDA+ chickens against IBV M41, 793B, D388(QX) and Q1 challenge was 91%, 94%, 73% and 84%, respectively (CPS calculation method). The level of protection in the SPF chickens against IBV M41, D388(QX) and Var2 challenge was 89%, 69% and 96%, respectively (CPS calculation method). The correlation between the percentage protected birds calculated using the EP, BPS and CPS calculation method was high ($r \geq 0.9824$); therefore, the EP and BPS scores as listed in Table 2 are not discussed separately here. The ciliary activity scores of the individual birds in each group are shown in Figure 2. The sum of the ciliary activity scores was significantly lower in vaccinated chickens compared to non-vaccinated chickens that were challenged with the same IBV challenge strain. In every trial, all non-vaccinated challenged chickens showed severe damage to the tracheal epithelium, except for two out of 10 chickens in the IBV 793B challenged group.

Discussion

To protect chickens against multiple IBV variants, a frequently used approach is to combine vaccine strains of different genotypes or serotypes to induce broad heterologous protection (protectotype vaccination strategy). The level of protection that can be induced by vaccinating with the combination of genotype lineage GI-1 (Mass) and GI-16 (Q1) has not been described before. In addition to obtaining general knowledge about the ability of combinations of IBV vaccines to induce cross-protection, the information is of paramount importance for areas where IBV Q1 occurs. Q1 is a dominant genotype of IBV in large parts of South America (Marandino *et al.*, 2015). It is also detected in parts of Asia, such as China and Vietnam (Le *et al.*, 2019), the Middle East (Ababneh *et al.*, 2012), and Southern Europe (De Wit *et al.*, 2021).

Five trials were performed to assess the level of protection induced by the live IBV Mass + Q1 vaccine

Table 2. The level of protection determined per group at 5 days post-challenge using the ciliary activity test.

		D0	D21		D26		
					Level of protection (%) using the ciliary activity test		
Trial	Chicken type	Vaccination at day of hatch with Vaxxon® CHB (GI-1 and GI-16)	Challenge with IBV strain (GI)	Challenge was homologous or heterologous	EP[a]	BPS[c]	CPS[d,e]
A	SPF	Vaccinated	M41 (GI-1)	Homologous	[b]	100	89[A]
		Not vaccinated			[b]	0	0[B]
		Vaccinated	D388(QX) (GI19)	Heterologous	[b]	70	69[A]
		Not vaccinated			[b]	0	0[B]
B		Vaccinated	Var2 (GI-23)	Heterologous	100	100	96[A]
		Not vaccinated			0	0	5[B]
C	MDA+	Vaccinated	M41 (GI-1)	Homologous	95	90	91[A]
		Not vaccinated			0	0	5[B]
D		Vaccinated	Q1 (GI-16)	Homologous	80	80	84[A]
		Not vaccinated			0	0	4[B]
		Vaccinated	D388(QX) (GI-19)	Heterologous	70	65	73[A]
		Not vaccinated			0	0	3[B]
E		Vaccinated	793B (GI-13)	Heterologous	95	90	94[A]
		Not vaccinated			10	10	38[B]

[a]EP (European Pharmacopoeia 8th and 10th edition) = percentage of protected chickens (a chicken was considered protected if at least nine out of 10 rings showed normal ciliary activity (normal is score 0, 1 or 2)).
[b]EP criteria could not be applied to trial A since five rings per bird were examined for ciliary activity, whereas for the calculation using the EP criteria 10 rings are required.
[c]BPS (binomial protection score) = percentage of chickens with more than 50% cilia beating (only ciliostasis scores 0, 1 or 2).
[d]CPS (ciliostasis protection score) = 1 - (mean cumulative ciliostasis score of a group/maximum cumulative ciliostasis score (20 or 40)) x 100.
[e]The sum of the ciliary activity scores was compared between a vaccinated and non-vaccinated group challenged with the same strain in the same study; significant differences are indicated with [A] or [B] in the CPS column.

Vaxxon® CHB against five different IBV challenge strains. The level of protection over all trials varied from 69-96% (CPS method), with an average of 85%. The level of protection against the two homologous strains was on average 88% (range 84-91%, CPS method). The level of cross-protection against the three heterologous strains was on average 83% (range 69–96%, CPS method), which is higher than the 74% that was reported in a meta-analysis performed by De Wit *et al.* (2013). Although the level of protection against some heterologous strains was higher than the level of protection against the homologous strains, those differences were small and not significant. As was described in previous studies, not all non-vaccinated 793B challenged chickens showed severe damage to the tracheal epithelium (Bru *et al.*, 2017; Bataille *et al.*, 2025). The 793B challenge viruses in general showed more variation in the level of ciliostasis in the control birds than the other challenge viruses.

Three methods were used to calculate the level of protection. The calculation method that is described in the European Pharmacopeia (EP) is considered the gold standard to assess the efficacy of an IB vaccine. However, it relies on the results of 10 trachea rings for the calculation. The EP calculation method could not be used to analyse the results of trial A, because five rings per trachea were examined. The BPS calculation method considers a chicken protected if all tracheal rings show normal ciliary activity (scores 0, 1, 2). This method is independent of the number of rings that are examined. The CPS calculation method described by Cook *et al.* (1999) is another calculation method that is frequently used in trials assessing the efficacy of IB vaccines (Sultan *et al.*, 2019; Keep *et al.*, 2020). This method includes the mean

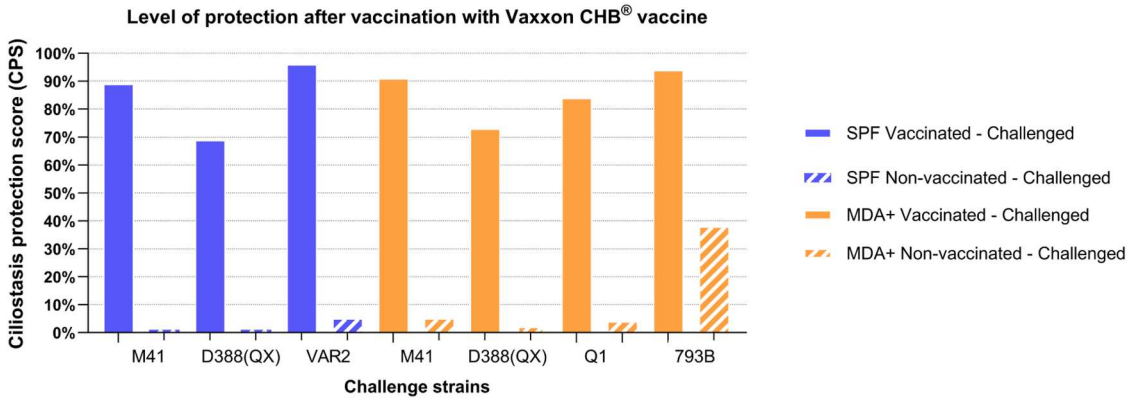

Figure 1. Ciliostasis protection scores (CPS) determined for each group at 5 days post-challenge with different IBV challenge strains.

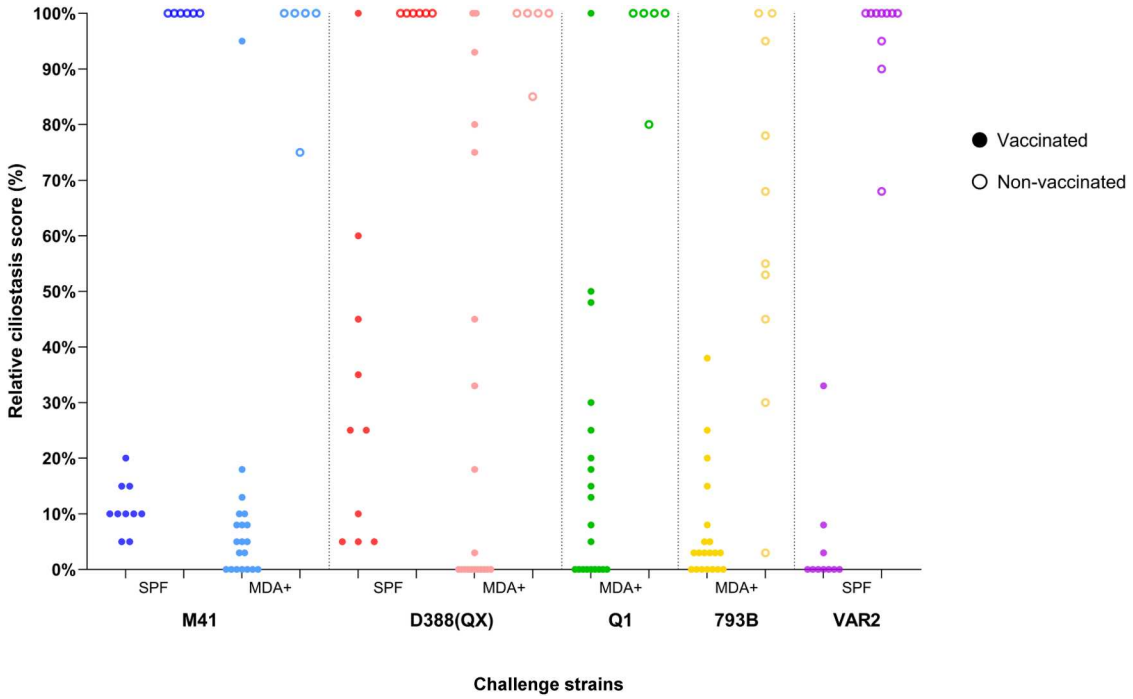

Figure 2. Relative ciliostasis scores of each individual bird in a group at 5 days post-challenge with different IBV challenge strains, calculated as the cumulative ciliary activity score (sum of five or 10 rings) divided by the maximum achievable cumulative ciliary activity score of a bird in that group (20 or 40).

cumulative ciliary activity score of a group and compares it with the maximum score that could have been obtained, i.e. score 20 when five rings are examined, score 40 when 10 rings are examined. The three methods EP, BPS and CPS showed a high level of correlation in this study ($r \geq 0.9824$).

Vaccination with Vaxxon® CHB provided a high level of protection against all tested challenge viruses, showing compliance with the protective efficacy threshold mentioned in EP (at least 80% protection) for all tested challenge viruses except for IBV D388(QX), showing cross-protection level just below the threshold. The levels of protection of the vaccinated SPF and MDA+ chickens were comparable after challenge with Mass or D388(QX). This implies that the presence of (maternal) antibodies did not affect the level of protection induced by vaccination. However, the presence of MDA decreased the vaccination reaction as reviewed in Jackwood and de Wit (2020).

The results of these trials show that the combination of IBV H120 and Q1 can be added to the list of successful combinations of IBV strains that are able to induce high levels of cross-protection against a number of heterologous challenge viruses (Cook *et al.*, 1999; Terregino *et al.*, 2008; De Wit *et al.*, 2011; Awad *et al.*, 2015; Chhabra *et al.*, 2015; De Wit *et al.*, 2015; Awad *et al.*, 2016; Bru *et al.*, 2017; De Wit *et al.*, 2017; Bataille *et al.*, 2025). This shows once more that various combinations of IBV strains can raise broad protection and that this is not a unique trait for one certain combination. The fact that both IBV strains are combined in one vaccine with a NDV strain is highly advantageous, since it reduces the number of vaccinations that a chicken should receive to be protected.

Based on these trials it can be concluded that, under experimental conditions, Vaxxon®CHB was able to induce high levels of protection against challenge with IBV strains that are representatives of the IBV variants currently circulating in a major part of the world.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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