A field study on the evaluation of day-of-hatch and in grow-out application of live infectious bursal disease virus vaccine in broiler chickens

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ABSTRACT Infectious bursal disease (**IBD**) is an acute, highly contagious, economically important disease of young chickens caused by *Avibirnavirus*, the infectious bursal disease virus (**IBDV**). The causative virus is highly resilient in poultry environments and vaccination is the most effective measure for IBDV control. However, the susceptibility of highly attenuated IBDV vaccine strains to neutralization by maternally derived antibodies (**MDA**) and overwhelming virulence of partly attenuated strains have limited the application of conventional live IBDV vaccines in pre- and posthatch chicks. Nevertheless, preliminary data have raised questions about the validity of this prevailing dogma. India is an IBD endemic country and the disease causes sizeable economic losses in the sector. To evaluate the feasibility of application of live IBDV vaccine strain, the IBDV MB-1, to the maternally immunized day-of-hatch chicks in Indian production environment, 4 large-scale field trials have been conducted. The 4 trials have measured the relative safety, IBDV immunization parameters, and production performances of MB-1 vs. the established live and immune complex IBDV vaccines in a variety of commercial broiler systems. The overall health and production performances in all 4 trials have been better in the MB-1 groups. The results challenge the prevailing notion that live IBDV strains may be neutralized or break through maternal immunity and induce permanent damage to the young broiler chick's immune response. A delayed replication phenomenon following parenteral administration of the live IBDV vaccine strain has been observed, while the delayed replication mechanism remains to be elucidated. This study warrants further research on the molecular mechanism of live IBDV vaccine strain, MB-1, and its interaction with the chicken immune system.

Key words: infectious bursal disease, live conventional IBDV vaccine strain, IBDV MB-1, maternally derived antibodies, delayed replication

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INTRODUCTION

Infectious bursal disease (**IBD**) is an acute, highly contagious, viral disease that affects young chickens worldwide bringing forth sizeable economic losses in the poultry industry (Berg, 2000). The etiologic agent of IBD is infectious bursal disease virus (**IBDV**), an *Avibirnavirus* in the family Birnaviridae, where bursa of Fabricius (**BF**) is the main location of virus replication that targets actively dividing immunoglobulin M-expressing (IgM^+) B cells (Hirai et al., 1981; Nakai and Hirai, 1981; Eterradossi and Saif, 2013). IBDV causes depletion of lymphoid cells resulting in long-lasting immunosuppression in young chickens (upto 3 wk of age) or severe symptomatic disease in older birds. The immunosuppression associated with IBDV has the most-significant economic implications due to vaccination failure, susceptibility to opportunistic pathogens, and overall loss of performance (Berg, 2000; Sharma et al., 2000; Van den Berg et al., 2000).

As with other infections, the immune response to IBDV challenge comprises the nonspecific innate immune reaction followed by the induction of the adaptive immune response. The importance of the humoral immunity to IBDV protection has been well documented (Winterfield, 1969) and the additive protective effect of cell-mediated immunity (CMI) has been demonstrated (Rautenschlein et al., 2001). Maternal antibodies can protect chicks from early IBDV infection and prevent the immunosuppressive effect of the virus. However, maternal immunity may interfere with the stimulation of active immunization following vaccination (Skeeles et al., 1979).

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The transmission of IBDV is horizontal; high resistance to many disinfectants make it cumbersome to remove from contaminated poultry premises (Benton et al., 1967). Besides biosecurity, vaccination is the only viable option against IBD (Berg, 2000). Breeder vaccination to induce a high level of maternal immunity followed by active immunization of the progeny appears beneficial; maternally derived antibodies (**MDA**) will protect young chicks when their B-lymphocytes are most vulnerable to IBDV infection while live-attenuated vaccine strains can induce active immunity in young chicks. The optimum application timing of live-attenuated vaccines to induce active immunity is challenging due to the interference of MDA with attenuated strains replication and the overwhelming virulence of partly attenuated strains. Most commercially available, conventional live IBDV vaccines are based on attenuated classical strains. Those classified as ''mild'' vaccines exhibit only poor efficacy in the presence of MDA, even at low levels. Alternatively, ''intermediate,'' ''intermediate plus, or ''hot'' vaccines have a much better efficacy and may break through higher levels of MDA; however, they can induce moderate to severe bursal lesions (Mazariegos et al., 1990; Tsukamoto et al., 1995; Kumar et al., 2000; Rautenschlein et al., 2003; Rautenschlein and Haase, 2005). Apparently, the timing of vaccination and safety of live IBDV vaccines remains a major concern.

To solve this predicament, genetically engineered viral vectors to express the VP2 surface protein of IBDV were developed. These commercially available vector vaccines can be administered in ovo or on day-of-hatch (**DOH**) because their replication and expression of the VP2 protein are not hindered by MDA (Perozo et al., 2009). However, delayed onset of immunity, partial protection from IBDV field strains challenge, and potential risk of immunosuppression in young chicks associated with vectored vaccines render them unsuitable, especially in IBD-endemic areas (Gelb et al., 2016; Kurukulasuriya et al., 2017).

Another type of vaccine that has been developed for day-of-hatch parenteral applications (subcutaneous route, s.c.) in IBDV maternally immunized chickens is the immune complex vaccine, Icx (Whitfill et al., 1995). This complex contains a combination of IBDV-specific antibodies and live-attenuated IBDV. The Icx vaccines were found to induce active immunity and protection against IBDV challenge in the face of variable levels of MDA (Haddad et al., 1997). However, it appeared that IBDV complexing with specific antibodies caused a delay in the vaccine virus replication in the BF of approximately 5 to 7 d. This delay may lead to a window of susceptibility between the waning of maternal-derived antibodies and the development of vaccine-induced antibodies (Jeurissen et al., 1998; Ivan et al., 2005).

MB-1, a derivative of IBDV M.B. live vaccine strain (Lazarus et al., 2008) has been recently developed for in ovo and DOH parenteral applications. Field studies in Latin America, Africa, and Israel have demonstrated that MB-1 induces active immunity in broiler chickens having varying maternal immunity with no adverse effects on the growth performance indices (Ashash et al., 2019).

In India, the IBD was first reported by Mohanty et al. (1971) and it remained in its classical form until 1990s. The emergence of very virulent IBDV strains caused 10 to 75% mortality in layers and 10 to 40% in broilers in 1992-94 while during early 2000s, variant IBDV strains emerged with heavy mortality and economic losses in young chicken flocks (Sah et al., 1995; Mor et al., 2010).

With this background, the present field study was conducted in different production conditions in India to assess the feasibility of MB-1 and Icx vaccine parenteral application (s.c.) in day-of-hatch (DOH) broiler chickens vis-à-vis to the current IBD vaccination protocols.

MATERIALS AND METHODS

Ethical Approval

Experiments were carried out in accordance with the guidelines laid down by the Institute of Animal Ethics Committee for the use of poultry birds.

Experimental Design and General Bird Husbandry

The study was composed of a series of 4 independent, consecutive commercial broiler field trials during the year 2019. The trials were conducted on commercial production farms in Coimbatore, India and designated 1-4 in accordance to the chronologic trial order.

Trial 1 Hatch date: August 13th, 2019, Coimbatore, India. A total of 7,300 as-hatched Vencobb commercial broiler chicks (initial mean body weight 42.0 ± 0.5 g) were selected for this trial which lasted for 40 d. The 7,300 day-old chicks were randomly divided immediately post hatch into 2 groups of 3,650 chicks each and treated in accordance with the company's protocol. The first group was vaccinated in the hatchery with a commercial IBDV Icx subcutaneously while the second group was vaccinated in the hatchery with the MB-1 vaccine subcutaneously. The hatchery vaccination facility and equipment were thoroughly cleaned and disinfected between the first and second group vaccination to avoid cross-contamination of the 2 live IBDV vaccine products between the 2 trial groups. All trial groups were under the same management and vaccination programs (Table 1).

Trial 2 Hatch date: August 16th, 2019, Coimbatore, India. A total of 7,000 as-hatched Vencobb commercial broiler chicks (initial mean body weight 42.0 ± 0.5 g) were selected for this trial which lasted for 40 d. The 7,000 day-old chicks were randomly divided immediately posthatch into 2 groups of 3,500 chicks each and treated in accordance with the company's protocol. The first group of 3,500 chicks was vaccinated in the hatchery with the MB-1 vaccine subcutaneously while the second

Table 1. Trial treatments and vaccination programs.

	Trial 1		Trial 2		Trial 3		Trial 4	
Hatch date	August 13, 2019		August 16, 2019		November 11, 2019		November 11, 2019	
Trial treatment	MB-1, 0 DOA SC	Icx, 0 DOA SC	MB-1, 0 DOA SC	MB, 12 DOA DW	MB-1, 0 DOA SC	Icx, 0 DOA SC	MB-1, 0 DOA SC	228E, 14 DOA DW
Number of birds	3650	3650	3500	3500	4600	4600	2500	2500
Hatchery vaccination	N/A		N/A		Volvac ND Inactivated (SC)		N/A	
Farm vaccination	ND master clone, 5 DOA (ED) and 18 DOA (DW)		ND VH, 5 DOA (ED) and 18 DOA (DW)		ND clone30, 5 DOA (ED) and 18 DOA (DW)		ND clone30, 5 DOA (ED) and 18 DOA (DW); ND VH, 28 DOA (DW)	

Abbreviations: DOA, day of age; DW, drinking water; ED, eye drop; Icx, Immune complex vaccine; MB-1, MB-1 vaccine; N/A, not applicable/not done; ND, Newcastle disease; ND master clone/NDVH/ND clone30, live attenuated ND vaccine; SC, subcutaneous; 228E, live attenuated IBD vaccine.

group of 3,500 chicks was vaccinated on the farm at 12 d of age (**DOA**) with the IBD M.B. vaccine *via* drinking water (**DW**). Both trial groups were allocated into 2 separate broiler houses and were under the same management and vaccination programs (Table 1).

Trial 3 Hatch date: November 11th, 2019, Coimbatore, India. A total of 9,200 as-hatched Vencobb commercial broiler chicks (initial mean body weight 42.5 ± 0.5 g) were selected for this trial which lasted for 40 d. The 9,200 day-old chicks were randomly divided immediately post hatch into 2 groups of 4,600 chicks each and treated in accordance with the company's protocol. The first group was vaccinated in the hatchery with a commercial IBDV Icx subcutaneously while the second group was vaccinated in the hatchery with the MB-1 vaccine subcutaneously. The hatchery vaccination facility and equipment were thoroughly cleaned and disinfected between the first and second group vaccination to avoid cross-contamination of the 2 live IBDV vaccine products between the 2 trial groups. All trial groups were under the same management and vaccination programs (Table 1).

Trial 4 Hatch date: November 11th, 2019, Coimbatore, India. A total of 5,000 as-hatched Vencobb commercial broiler chicks (initial mean body weight 42.5 ± 0.5 g) were selected for this trial which lasted for 40 d. The 5,000 day-old chicks originated from the same broiler breeder flock (as in trial 3) and were randomly divided after hatch into 2 groups of 2,500 chicks. The first group of 2,500 chicks was vaccinated in the hatchery with the MB-1 vaccine subcutaneously while the second group of 2,500 chicks was vaccinated on the farm at 14 DOA with a live attenuated IBD vaccine via drinking water (DW). Both trial groups were allocated into 2 separate broiler houses and were under the same management and vaccination programs (Table 1).

The birds were raised in open-sided poultry sheds on litter composed of paddy straw and space was allocated according to the industry standard of about 0.14 m^2 per bird. Incandescent lighting was used throughout the trial period and lighting schedule involved 24 h light during the first week and 20 h of light up to the end of trial period. The test farm facilities and birds were observed twice daily for general flock condition, lighting, water, feed, ventilation and unanticipated events and records were maintained from the beginning whenever any bird was found dead, culled or sacrificed due to any reason. All the mortalities were subjected to necropsy to determine the probable cause of death.

IBDV Vaccines and Application

The MB-1 vaccine strain is a derivative of the IBDV M.B. strain previously described by Lazarus et al. (2008). The MB-1 live vaccine was titrated to approximately 103 EID50/mL and administered to post hatched broiler chicks in a volume of 0.2 mL by subcutaneous injection. The live attenuated IBD vaccine is a commercially licensed intermediate plus live vaccine

containing 228E strain, approximately 102 EID50 IBDV/mL. The IBD M.B. is a commercially licensed live vaccine containing the IBDV M.B. strain and was used in field trials 2 in accordance with the manufacturer's instructions. The 228E was used in the field trial 4 in accordance with the manufacturer's instructions. The IBDV Icx vaccines used in trial 1 and 3 were licensed commercial live vaccines originated from the WF2512 IBDV strain and were used in accordance with the manufacturer's instructions.

Serology

In trial 3 and 4, twenty blood samples were collected and sera were extracted from each trial group at 4 to 7 d interval from the DOH and through the duration of the trial. IBDV ELISA antibodies were analyzed in all the collected sera samples with the IDEXX Ab Test (IDEXX Laboratories, Inc., Westbrook, ME) in accordance with the manufacturer's instructions.

Bursa to Bodyweight Ratio (BBWR)

In trial 3 and 4, six birds from each trial group were euthanatized and sampled for BBWR at 4 to 8 d intervals from 17 DOA and throughout the duration of the trial in accordance to method described by Cazaban et al., (2015). The birds were weighed, necropsied, and the bursas (BFs) were removed. Each bursa (BF) was weighed before processing and the BBWR was calculated in order to determine the degree of mass increase or decrease of the BF. The ratio was established as follows: weight of BF(g) × 100/body weight(g).

Histopathologic Bursal Analysis

In trial 1 and 2, six birds from each trial group were euthanatized and sampled for BF histopathology at 3 to 4-d intervals from 14th d and through the duration of the trial. Bursal tissues were fixed in 10% neutral buffered formalin and paraffin embedding procedures, and the sections were stained with hematoxylin and eosin (H&E). The bursas of the 4 trials were evaluated microscopically by the same certified veterinary pathologist and scored in the range of 0 for normal bursa to 5 for severely affected bursa in accordance with the European Pharmacopeia bursal lesion score scale (Ashash et al., 2019).

IBDV RNA Extraction, PCR Investigations and Sequence Analysis of PCR Products

In trial 3 and 4, six birds from each trial group were euthanatized and sampled for IBDV PCR at 4 to 8 d intervals from 17 to 40 DOA. Specimens were produced by direct bursal smear on FTA cards, one bursal smear per each card circle. FTA cards were sent to Clinic for Poultry and Fish Medicine, Department for Farm Animals and Public Health in Veterinary Medicine (Vetmeduni Vienna) for PCR amplification and amplicon sequence analysis of pooled samples. RNA extraction of all samples was performed using QIAamp cador Pathogen Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions.

Initially, all RNA samples were investigated by the real-time RT-PCR for the presence of IBDV RNA, using primers and probe for the VP3 gene in the A segment of the IBDV genome (Escaffre et al., 2010; Ashash et al., 2019). Real time PCR was performed in 20 μL reaction mixture on Agilent Mx3000P using TaqMan chemistry (Brilliant III Ultra-Fast qRT-PCR Master Mix (Agilent Technologies, Santa Clara, CA) with 30 nM ROX as reference dye, 0.2 μM primer, and 0.25 μM TaqMan probe. Thermal profile of reactions was as follows: 50°C for 10 minutes, 95°C for 3 minutes, followed by 40 cycles of 15 seconds at 95°C and 20 seconds at 60°C. Fluorescence was detected and reported at each cycle during the 60°C step.

Samples positive for the IBDV were further subjected to conventional in-house RT-PCR to amplify 750 bp region of the *VP2* gene. Amplification products were electrophoresed in a 1% Tris acetate EDTA agarose gel, stained with GelRed (Biotium, Fremont, CA) stain and visualized under UV light (Biorad Universal Hood II, Bio-Rad Laboratories, Hercules, CA). Fragment sizes were determined with reference to 100bp DNA ladder (Invitrogen, Life Technologies, Vienna, Austria). Standard precautions were applied to avoid PCR contamination. PCR reagents were aliquoted; aerosol barrier tips, dedicated pipette sets, laminar flow hoods and separate laboratory areas were used for each step of the procedure. PCR products of the expected sizes were excised from the gel and purified using the QIAquick Gel Extraction Kit (Qiagen, Vienna, Austria) according to the manufacturer's instructions. Direct fluorescence-based sequencing was performed by LGC Genomics GmbH (Berlin, Germany) using the PCR primers. Assembly and analyses of sequences, as well as nucleotide and deduced amino acid sequence alignments were performed with Accelrys Gene, version 2.5 (Accelrys, San Diego, CA). Primer binding sites were excluded from sequences used in the analysis.

Growth Performance Indices

In trial 3 and 4, the body weights (BW) of birds were recorded at weekly interval, and average daily body weight gain (ADG) was calculated during 1 to 14 DOA, 15 to 35 DOA, and 1 to 35 DOA. Body weight (BW) was assessed as the average of a random selection of 25 birds per group during 1st, 2nd, 3rd, and 4th wk of the trial, while the final BW at 35 DOA was assessed by dividing the total weight per trial group by the number of birds alive before slaughtering. Feed consumption of birds of each trial group was recorded at weekly intervals on flock basis and average feed consumption per bird per week was calculated which was used to derive data on average daily feed intake (**ADFI**) during 1 to 14 DOA,

15 to 25 DOA, and 1 to 35 DOA. Feed conversion ratio (**FCR**) was calculated as a ratio between feed intake over body weight during corresponding growth periods as detailed above. Mortality, if any, was recorded as it occurred, and the data were used to adjust subsequent measurements. European performance efficiency factor (**EPEF**) and European broiler index (**EBI**) were calculated using following formula (Wang et al., 2013; Selvam et al., 2018):

$$\text{EPEF} = \text{BW(kg)} \times \%\ \text{liveability} \times 100/\text{FCR} \times \text{trial duration(d)}$$

$$\text{EBI} = \text{ADG(g/bird/d)} \times \%\text{liveability} \times 0.1/\text{FCR}$$

Statistical Analysis

Antibody titers were expressed as the geometric mean titers. Mean BBWR and mean bursal scores were calculated for each group. Statistical differences were determined using the Student t test, two-sample, assuming unequal variance. All statistical differences among the groups were measured at $P < 0.05$, two-tailed.

RESULTS

The controlled field trials measured the relative safety, IBD immunization parameters, and production performance of MB-1 in comparison to the Icx vaccine and the current IBDV live vaccines in commercial broiler production systems. The humoral immune response for IBDV, BF integrity, IBDV live vaccines replication in the BF, and broiler live production indices were evaluated.

Serology

The results of IBDV ELISA of trial 3 and 4 are summarized in Table 2. The maternal antibody titers of the treatment groups at DOH were identical in trial 3 and 4; probably due to same parental origin of day-old chicks. In trial 3, the antibody level rapidly dropped in the Icx group relative to the MB-1 group (58 vs. 121) at 21 DOA, despite having identical levels at 14 DOA. In trial 4, the antibody decay rates in both the groups (MB-1 vs. 228E) were similar and reached low and identical levels (158 vs. 121, respectively) at 21 DOA. The initial titer elevations were observed at 29 DOA in trial 3 and 4, where highest titer ($P \leq 0.05$) was produced in the MB-1 groups at 29 and 40 DOA in trial 3. In trial 4, highest antibody titer ($P \leq 0.05$) was produced in the MB-1 and 228E group at 29 and 40 DOA, respectively.

Bursa to Bodyweight Ratio (BBWR)

The BBWR results of trial 3 and 4 are summarized in Table 3. In trial 3, the BBWR of the compared groups (MB-1 vs. Icx) showed similar patterns, with peak ratios of 0.22-0.26 during 17 and 24 DOA, followed by declining ratios (0.08 vs. Icx) at 28 DOA and reaching upto 0.05 in both the groups at 40 DOA. The differences in BBWR across both the groups (MB-1 vs. Icx) were statistically insignificant ($P > 0.05$) throughout the trial period. In trial 4, the BBWR of the 228E vaccinated group had numerically higher ($P > 0.05$) values relative to the MB-1 group during 17 to 28 DOA and declined drastically to identical levels (0.04 vs. 0.05) during 32-40 DOA.

Histopathologic Bursal Analysis

The bursal lesion score results (Mean ± SEM) of the trial 1 and 2 are summarized in Table 4. In trial 1, the bursal lesion scores in the MB-1 vaccinated group were numerically lower ($P > 0.05$) than the Icx group at 21, 28, 36, and 40 DOA. In trial 2, the bursal lesions scores were numerically lower ($P > 0.05$) in the MB-1 group vis-à-vis MB group throughout the trial period, except at 21 DOA, and the differences were statistically significant ($P \leq 0.05$) at 29 and 32 DOA.

Table 3. Bursa to bodyweight ratio (BBWR) average by treatment.

	Trial 3		Trial 4	
Age (days)	MB-1, 0 DOA	Icx, 0 DOA	MB-1, 0 DOA	228E, 14 DOA
17 d	0.26	0.23	0.23	0.28
21 d	0.22	0.24	0.15	0.17
24 d	0.23	0.26	0.15	0.20
28 d	0.08	0.06	0.12	0.15
32 d	0.05	0.06	0.04	0.05
40 d	0.05	0.05	0.04	0.04

Abbreviations: Icx, Immune complex vaccine; MB-1, MB-1 vaccine; 228E, live attenuated IBD vaccine.

Table 2. IBDV ELISA titers average by treatment.

	Trial 3				Trial 4			
Age (days)	MB-1, 0 DOA	CV%	Icx, 0 DOA	CV%	MB-1, 0 DOA	CV%	228E, 14 DOA	CV%
4 d	3505	77.5	3505	77.5	3505	77.5	3505	77.5
14 d	269	60.4	287	131.2	474	75	548	93
21 d	120	255	58	194	151	120	128	91
29 d	**10438a**	55.9	**2839b**	89.1	**3496a**	79.4	**1613b**	128
40 d	**11622a**	46.5	**8572b**	35.6	**8160a**	47.9	**11100b**	44.2

Abbreviations: CV%, coefficient of variation; Icx, immune complex vaccine; MB-1, MB-1 vaccine; 228E, live attenuated IBD vaccine.
Bold letters, t-test, indicates statistically significant difference; $P \leq 0.05$.

Table 4. Histopathologic BF lesion score (Mean ± SEM) by treatment.

	Trial 1		Trial 2	
Age (days)	MB-1, 0 DOA	Icx, 0 DOA	MB-1, 0 DOA	MB, 12 DOA
14 d	0.333 ± 0.21	0.333 ± 0.21	0.167 ± 0.17	0.333 ± 0.21
17 d	0.5 ± 0.22	0.333 ± 0.21	0.333 ± 0.21	0.833 ± 0.17
21 d	0.667 ± 0.21	0.833 ± 0.31	0.667 ± 0.21	0.333 ± 0.21
24 d	0.333 ± 0.21	0.167 ± 0.17	0.667 ± 0.21	0.833 ± 0.31
28 d	0.833 ± 0.17	1.000 ± 0.26	**0.167 ± 0.17a**	**1.333 ± 0.33b**
32 d	1.333 ± 0.21	1.000 ± 0.37	**0.500 ± 0.22a**	**2.5 ± 0.34b**
36 d	2.000 ± 0.26	2.500 ± 0.22	1.833 ± 0.4	2.667 ± 0.21
40 d	1.333 ± 0.21	1.500 ± 0.22	2.667 ± 0.21	3.000 ± 0.52

Abbreviations: Icx, Immune complex vaccine; MB, live attenuated IBD vaccine containing MB strain; MB-1, MB-1 vaccine.

Bold letters, t-test, indicates statistically significant difference; $P \leq 0.05$.

IBDV RNA Extraction, PCR Investigations and Sequence Analysis of PCR Products

The BF IBDV PCR results and the vaccine strain identification analysis of pooled samples in trial 3 and 4, respectively are summarized in Table 5. In trial 3, MB-1 vs Icx vaccines, the infection of the BF with respective vaccines was detected at 21 DOA and onward in both trials. In trial 4, MB-1 vs 228E vaccines, initial infection of the BF with 228E was detected at 17 DOA only, disappeared at 21 DOA and replaced by MB-1 at 24 DOA and onward while the MB-1 was detected at 21 DOA and onward.

Growth Performance Indices

Data (Mean ± SEM) on BW, ADG, FCR, EPEF, and EBI of the birds in trial 3 and 4 are presented in Tables 6 and 7, respectively. There were significant ($P \leq 0.05$) variations between the vaccinated flocks with regard to BW, ADG, and FCR of the birds at different periods of grow out in both the trials where the MB-1 vaccine did not affect BW, ADG, and FCR as observed in the Icx and the 228E vaccinated birds. However, the differences in BW and ADG between the vaccinated flocks (MB-1 vs. 228E vaccines) were not significant at 14 DOA and during 22 to 35 DOA, respectively, in trial 4. Additionally, the difference in FCR between the vaccinated flocks (MB-1 vs. 228E vaccines) was not significant during 22 to 35 DOA in trial 4.

In both the trials, mortality was same and of nonspecific nature in the vaccinated flocks (data not shown). Hence, liveability was not affected by different vaccines.

DISCUSSION

The application timing of live-attenuated IBDV vaccines to induce active immunity in maternally immunized DOH chicks is challenging and well documented. It stems from the fact that the MDA interferes with the replication of attenuated strains and overwhelming virulence of less-attenuated strains in posthatch chicks (Helmboldt and Garner, 1964; Winterfield et al., 1980). It plausibly hindered the DOH application of conventional live IBDV vaccines at hatchery. Although a few studies reported the efficacy and safety of DOH application of live IBDV vaccines (Gagic et al., 1999; Giambrone et al., 2001; Rauntenschlein and Haase, 2005), other report showed limited protection and immunosuppressive effects of live IBDV vaccines in young chicks, casting doubts on the hatchery usage of live IBDV vaccines (Corley and Giambrone, 2002).

Researchers had worked over past 2 decades on possible alternatives for mass-application of vaccines at hatchery in order to pacify the efficacy and safety concerns associated with the live IBDV vaccines. It had resulted in the development of IBDV vector recombinant (rHVT-IBD) and Icx vaccines which are not neutralized by MDA and relatively safe to B lymphoblasts, marking biggest shift in the hatchery vaccination technologies (Haddad et al., 1997; Perozo et al., 2009). However, rHVT-IBD vaccines may induce only moderate levels of protection from an overwhelming IBDV challenge, and both rHVT-IBD and Icx vaccines appear to have delayed onsets of active immunity (Jeurissen et al., 1998; Ivan et al., 2005; Gelb et al., 2016; Kurukulasuriya et al., 2017).

In an effort to improve the protective characteristics of IBDV hatchery vaccination, we have evaluated the prevailing dogma concerning conventional live IBDV vaccination of in-ovo and posthatch maternally immunized chicks with the MB-1 live vaccine strain. Our previous field studies on MB-1 in Latin America, Africa, and Israel have been shown to be safe and effective in commercial broiler chickens having varying MDA (Ashash et al., 2019). Here in, we have proposed to evaluate the feasibility of DOH application of MB-1 to commercial broiler chickens in India.

The 4 unrelated field trials incorporated in this study compared the broiler performances of posthatch MB-1 vaccination with the Icx vaccine and established IBDV vaccination protocols in broiler integration operation. The results of each trial can be evaluated and

Table 5. Bursa of Fabricius (BF) IBDV PCR and strain differentiation analysis of pooled samples by treatment.

	Trial 3		Trial 4	
Age (days)	MB-1, 0 DOA	Icx, 0 DOA	MB-1, 0 DOA	228E, 14 DOA
17 d	Neg	Neg	Neg	Pos (228E)
21 d	Pos (MB)	Pos (WF2512)	Pos (MB)	Neg
24 d	Pos (MB)	Pos (WF2512)	Pos (MB)	Pos (MB)
29 d	Pos (MB)	Pos (WF2512)	Pos (MB)	Pos (MB)
32 d	Pos (MB)	Pos (WF2512)	Pos (MB)	Pos (MB)
40 d	Pos (MB)	Pos (WF2512)	Pos (MB)	Pos (MB)

Abbreviations: MB-1, MB-1 vaccine; Neg, negative; Pos, positive; WF2512, Winterfield 2512 vaccine strain; 228E, live attenuated vaccine strain.

Table 6. Broiler performance indices in trial 3.

Treatments/Parameters	MB-1	Icx	SEM	*P*-value
BW				
14 d	505.2[a]	452.3[b]	4.94	<0.001
21 d	989.6[a]	960.5[b]	4.37	<0.001
28 d	1573.8[a]	1529.2[b]	5.41	<0.001
35 d	2030.0[a]	1970.4[b]	5.99	<0.001
ADG				
1−21 d	45.1[a]	43.7[b]	0.21	<0.001
22−35 d	80.0[a]	77.7[b]	0.46	0.009
1−35 d	58.5[a]	56.7[b]	0.18	<0.001
FCR				
1−21 d	1.263[a]	1.287[b]	0.006	0.03
22−35 d	1.641[a]	1.756[b]	0.012	<0.001
1−35 d	1.461[a]	1.532[b]	0.006	<0.001
EPEF	397.2	366.6		
EBI	400.2	370.1		

Abbreviations: ADG, average daily body weight gain; BW, body weight; EBI, European broiler index; EPEF, European performance efficiency factor; FCR, feed conversion ratio; Icx, immune complex vaccine; MB-1, MB-1 vaccine; 228E, live attenuated vaccine strain.

[a,b]Means bearing different superscripts within a row differ significantly.

interpreted independently due to different production environment prevailing across the field trials. In trial 3, the IBDV molecular detection data in BF indicated similar timing of viral replication in the MB-1 groups vis-à-vis the Icx group. It appeared that the identification of MB-1 and the Icx vaccines in BF at 21 DOA were in tandem with the declining levels of MDA. However, the IBD ELISA titer during 29 to 40 DOA was significantly higher ($P \leq 0.05$) in the MB-1 group as compared with the Icx vaccinated birds, suggesting delayed onset of active immunity in the later. Our findings are in agreement with previous reports where delayed replication of the Icx vaccines led to a delayed onset of active humoral immunity (Ivan et al., 2005; Ashash et al., 2019). Such a delay may create a window of susceptibility between the waning of maternally derived antibodies and the development of vaccine-induced antibodies in Icx vaccinated birds. Although the MB-1 vaccine was applied as free viral particles, its replication in the BF was not detected prior to 21 DOA, which could allow adequate differentiation and maturation of bursal-derived lymphocytes. In trial 4, early virus replication was detected in 228E group at 17 DOA, followed by unexpected disappearance at 21 DOA, given the fact that IBDV can be detected d 14 to 28 postvaccination in BF (Ivan et al., 2005; Thomrongsuwannakij et al., 2021). It is speculated that inability to invade bursa follicles compounded by improper uptake of 228E vaccine by the experimental flock resulted in the disappearance. Interestingly, the MB-1 vaccine virus appeared in the 228E-vaccinated house at 24 DOA, plausibly due to aided transmission across houses, replacing the later completely. The effect of MB-1 vaccine virus transmission in the 228E-vaccinated shed was reflected in the active humoral immunity where significantly higher IBDV ELISA titer was detected in the 228E group vis-à-vis the MB-1 vaccinated birds at 40 DOA, despite higher IBDV ELISA titer in later at 29 DOA. The molecular evaluation of IBDV nucleic acid in the BF could not demonstrate IBDV field strain infection in trials 3 and 4. It could be concluded that results of both the trials were not affected by an IBDV field strain challenge.

Table 7. Broiler performance indices in trial 4.

Treatments/Parameters	MB-1	228E	SEM	*P*-value
BW				
14 d	399.5	393.0	2.48	0.92
21 d	865.2[a]	814.4[b]	5.56	<0.001
28 d	1412.2[a]	1375.68[b]	4.02	<0.001
35 d	2099.8[a]	2039.84[b]	5.38	<0.001
ADG				
1−21 d	39.17[a]	36.76[b]	0.26	<0.001
22−35 d	88.19	87.53	0.40	0.42
1−35 d	60.44[a]	58.75[b]	0.16	<0.001
FCR				
1−21 d	1.215[a]	1.290[b]	0.009	<0.001
22−35 d	1.629	1.650	0.008	0.173
1−35 d	1.422[a]	1.466[b]	0.004	<0.001
EPEF	405.1	377.7		
EBI	425	400.7		

Abbreviations: ADG, average daily body weight gain; BW, body weight; EBI, European broiler index; EPEF, European performance efficiency factor; FCR, feed conversion ratio; MB-1, MB-1 vaccine; 228E, live attenuated vaccine strain.

[a,b]Means bearing different superscripts within a row differ significantly.

Previous reports showed that the induction of humoral immunity clearly correlated with the induction of bursal lesions and IBDV replication while conventional live IBDV vaccine inflicts transient bursal damage increasing the bursal lesion scores in broiler chickens (Rautenschlein et al., 2003; Geerligs et al., 2015). In present study, the bursal lesion scores evaluated in trial 1 and 2 showed numerically lower scores in the MB-1 groups vis-à-vis the Icx vaccine and the conventional live vaccine, M.B. group, suggesting lesser stress on birds and quicker recovery. The present findings are in contrary to our previous report where the MB-1 vaccinated birds had early higher bursal lesions scores in comparison to the Icx and the M.B. vaccinated birds and the same after 35 d of age (Ashash et al., 2019). As the production indices, BBWR, serology, and BF integrity could not be studied in trial 1 and 2, further correlation with bursal lesion score results was not possible.

The overall health and production performances in trial 3 and 4 were significantly better in the MB-1 groups. It could be plausibly due to early elicitation of active immunity, lesser BF damage, and quick recovery post viral replication in the MB-1 vaccinated birds as compared with the other IBDV vaccine groups.

The present study was first of its kind in India and were conducted in typical commercial environmental settings, in virulent IBDV challenge regions, and provided real-life demonstration of the feasible administration of a live conventional IBDV vaccine to DOH broiler chickens at hatchery. Our findings may provide valuable information to poultry veterinarians and producers in devising robust strategy for prevention of Gumboro disease in India.

Furthermore, the study highlights the interaction between the vaccine virus and the maternally immunized chicken host. In contrast to the existing constraints of neutralization by MDA and failure to mount adequate active immunity, following the application of a

live IBDV vaccine to maternally immunized chicks (Muller et al., 2012), we have shown that DOH parenteral applications consequently delayed replication and elicited strong active immunity. Rauntenschlein and Haase (2005) reported delayed replication of live IBDV vaccines in 14-day-old orally vaccinated broilers. They found that the onset of bursal lesions and recovery of IBDV-vaccinated broilers was delayed but not fully neutralized in the presence of maternal antibodies. However, they could not demonstrate a similar delay in 12-day-old orally vaccinated broilers with higher levels of MDA. The present study indicated delayed replication of the MB-1 vaccine in DOH chicks having varying MDA.

The delayed replication observation raises queries about the mechanism of this phenomenon: what are the implications of the vaccination route (parenteral vs. peros); where does the virus reside for 3 to 4 wk; what causes the onset of replication and immunization; and does the vaccine virus replication synchronize with the decay of MDA. We hypothesize that MDA and macrophages have a pivotal role in the delayed replication, safety, and immunization mechanisms of live IBDV vaccination of DOH chicks. It was reported that IBDV may cause alteration to macrophage functions and remain viable in these cells (Lam, 1998). Jeurissen et al. (1998) proposed that the working mechanism of the IBDV Icx vaccine may be related to its specific cellular interaction with follicular dendritic cells in the spleen and BF. They were able to demonstrate a delayed onset of replication; however, the delayed replication mechanism and the localization of the Icx vaccine strain during the period of delay could not be explained. According to our study on the mode of action of MB-1, we have found that MDA mediates the timing of replication and it is related to the MDA titre of the individual bird (Rosenzweig et al., 2019).

CONCLUSIONS

Herein, we first report the DOH application of a live, conventional IBDV vaccine to commercial broiler chickens in Indian production environments. The data demonstrate the lack of adverse effects and adequate performances associated with the vaccinated broiler chickens. The study's findings challenge the prevailing dogma that live IBDV vaccine strains may be neutralized or break through MDA and induce permanent damage to the young broiler chick's immune response. We have observed a delayed replication phenomenon following parenteral administration of a live IBDV strain, which is in alignment with our previous findings. This study warrants further research on the molecular mechanism of live IBDV vaccine strain, MB-1, and its interaction with the chicken immune system.

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DISCLOSURES

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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