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Short Communication

Impacts of *Mycoplasma gallisepticum* Vaccine on Newcastle Disease Vaccination and Protection in Parent Stock Flocks

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The present study reports on the impact of *Mycoplasma gallisepticum* (MG) vaccination on vaccine respond and subsequent protection against Newcastle disease (ND) in parent stock flocks of Department of Livestock Services (DLS) using MG killed vaccine and conventional ND vaccines. Birds were grouped into four groups, each consisted of 50 birds from the same flock. Group A birds were vaccinated with ND, group B with ND and MG, group C with MG, and group D birds were kept as unvaccinated control. The parameters studied included detection of ND antibody, MG seroprevalance, mortality (%), and cause of death. The sera of groups B and C were sero-positive after administration of MG vaccine. The haemagglutination-inhibition (HI) titres of group A were higher than group B from week 4 after administration of MG vaccine to the birds of group B. The mortality was very low; one bird of group C died at week 5 due to traumatic injury and another bird of group D died at week 2 due to infectious bronchitis virus (IBV). After challenge, birds of groups A and B showed no clinical signs and normal post mortem findings were found. Birds of groups C and D showed clinical signs from day 3 and different pathological lesions were found in post mortem. The MG vaccination did not improve other parameters. Therefore, inoculation of MG INAC vaccine is not justified and is too expensive at farm levels.

Keywords: *Mycoplasma gallisepticum* (MG) vaccine, Newcastle disease (ND) vaccination, Protection, Mortality

Mycoplasmosis is one of the important poultry diseases causing significant economic losses in many countries[1]. Most of these losses are related directly or indirectly to *Mycoplasma gallicepticum* (MG) infection, with or without complicating factors. *M. gallisepticum* is the most economically significant mycoplasma pathogen of poultry, and has a worldwide distribution[1]. Economic losses from downgrading of carcasses, reduced feed and egg production efficiency, and increased medication costs are additional factors that make this one of the costliest disease problems confronting the poultry production. Prevention and control programmes, which may include vaccination, account for additional costs[2]. There is some evidence that *M. gallicepticum* is also present in small backyard poultry flocks[3]. In Bangladesh, overall seroprevalance of avian mycoplasmosis is about 57% of which 30% in breeding farms, 67% in government poultry farms, and 50% in commercial poultry farms[4]. It is evident that *M. gallisepticum* infection has been prevailing in Bangladesh in improved breeds of chickens[5]. Therefore, the present study aims to identify the impacts of MG vaccine on Newcastle disease (ND) vaccination and their subsequent protection.

The present study was carried out in the Bangladesh Agricultural University (BAU), Mymensingh, Bangladesh. Vaccines used in this experiment were MG killed vaccine (MG INAC from Intervet International BV Boxmeer, Holland) and conventional ND vaccines. Four groups of birds were used in this experiment and each group consisted 50 birds from same flock (Central Poultry Farm, Mirpur, Dhaka). Birds of group A were vaccinated against Newcastle disease (ND); birds of group B were vaccinated against Newcastle disease and *M. gallisepticum*; birds of group C were vaccinated against *M. gallisepticum*; and birds of group D were kept as unvaccinated control. Table 1 shows the vaccination schedule in four groups of poultry birds. In addition, all birds (Group A-D) were vaccinated against Marek's, IBD and pox with the exception that IBD was not repeated on day 21. Serum samples were collected from 10 randomly selected birds from each group at different intervals for haemagglutination-inhibition (HI) titres against ND and antibody against MG[6-7]. Rapid serum plate agglutination test (RSPAT) was performed by placing a drop of serum on a white porcelain plate and mixed with equal amount of stained MG antigen. It was then mixed to make a spot of about 2 cm in diameter, rotating the plate gently, and the test was read within 2 min[1]. To perform the haemagglutination-inhibition (HI) test[8], 0.025 ml of PBS was dispensed into each well of a plastic V-bottomed microtitre plate and 0.025 ml of

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serum was placed into first well of plate. Two-fold dilutions of 0.025 ml volume of the serum were made across the plate. To each well 4 HAU virus/antigen (0.025 ml) was added and left for a minimum 30 min at room temperature (*i.e.*, about 20°C). After reaction, 0.025 ml of 1% chicken RBC was added to each well and allowed to settle RBC for 40 min at room temperature. The HI titre was defined as the highest dilution of the serum causing complete inhibition of 4 HAU of antigen. During the study period the weekly mortality was recorded in all 4 groups. The record was taken from day-old to 12 weeks of age. Before challenge, embryo lethal dose 50 (ELD_{50}) of antigen (Ag) was determined. Ten birds from each group were challenged by 0.5 ml of field strain of ND virus of known virulence at 10 weeks of age and administered through intra-muscular route. Following challenge, the birds were observed for 2 weeks. All birds were subjected post mortem examination at the end of the experiment, including parasitological examination.

Table 1. *Vaccination schedule in four groups of poultry birds*

Age of birds (Day)	Vaccine	Group (n = 50)			
		A	B	C	D
Day-old	Marek's disease vaccine	+	+	+	+
3	ND live: BCRDV (F strain)	+	+	-	-
14	Gumboro live (D78)	+	+	+	+
21	MG killed: Nobilis MG INAC	-	+	+	-
28	ND live: BCRDV (F Strain)	+	+	-	-
35	Fowl pox live (LRI, DLS)	+	+	+	+
60	ND live: RDV (M strain)	+	+	-	-

ND = Newcastle disease; BCRDV = Baby chicks Ranikhet disease vaccine; MG = *Mycoplasma gallisepticum*; LRI = Livestock Research Institute; DLS = Department of Livestock Services; RDV = Ranikhet disease vaccine

Sero-conversion was observed in the sera of group B group C birds after administration of MG INAC vaccine (Table 2). The HI titres of group A were considerable different from group B from week 4 to week 12 (Table 3). This indicates that MG INAC vaccine produced effects on Newcastle disease (ND) vaccine, lowered the HI titres and thus had a negative impact. The mortality (%) of the birds was very low. Only bird of group C died at week 5 due to traumatic injury associated with abscess in the neck as evident from the post mortem investigation. Another bird of group D died at week 2 due to IBD. The post mortem findings showed swollen BF.

Before challenge, ELD_{50} of Ag was determined. After challenge, birds of groups A and B showed no clinical signs and the post mortem findings were normal in these cases. Birds of groups C and D showed clinical signs from day 3 and different pathological lesions like haemorrhages in the proventriculus, hemorrhagic plaques on cecal tonsil, haemorrhages in the trachea etc. were seen in post mortem.

Table 2. *Seroprevalence of Mycoplasma gallisepticum (MG) in the birds of different groups after administration of MG INAC vaccine*

Age of birds	Seroprevalence of MG (No. positive/No. negative)			
	Group A	Group B	Group C	Group D
Day-old	0/10	0/10	0/10	0/10
2-week	0/10	0/10	0/10	0/10
4-week	0/10	10/0	10/0	0/10
6-week	0/10	10/0	10/0	0/10
8-week	0/10	10/0	10/0	0/10
10-week	0/10	10/0	10/0	0/10
12-week	0/10	10/0	10/0	0/10

Table 3. *Haemagglutination-inhibition (HI) titres against Newcastle disease (ND) of the birds of different groups at various time intervals*

Age of birds	Haemagglutination-inhibition (HI) titre			
	Group A	Group B	Group C	Group D
Day-old	$2^{4.0 \pm 0.47}$	$2^{4.0 \pm 0.47}$	$2^{4.0 \pm 0.47}$	$2^{4.0 \pm 0.66}$
2-week	$2^{5.3 \pm 0.82}$	$2^{5.3 \pm 0.82}$	$2^{1.5 \pm 0.52}$	$2^{1.5 \pm 0.5}$
4-week	$2^{7.6 \pm 0.51}$	$2^{5.5 \pm 1.26}$	$2^{0.5 \pm 0.52}$	$2^{0.3 \pm 0.48}$
6-week	$2^{8.0 \pm 0.66}$	$2^{6.0 \pm 0.81}$	2^{0}	2^{0}
8-week	$2^{6.7 \pm 0.48}$	$2^{4.6 \pm 0.51}$	2^{0}	2^{0}
10-week	$2^{8.4 \pm 1.17}$	$2^{6.2 \pm 0.78}$	2^{0}	2^{0}
12-week	$2^{8.0 \pm 0.66}$	$2^{6.6 \pm 0.69}$	2^{0}	2^{0}

In addition to a negative effect of MG vaccine on ND vaccination, the administration of MG vaccine also showed negative impact, to some extent, on protection after challenge with a virulent ND virus. The HI titres of group A were remarkably different from group B from week 4 to week 12 (Table 3). At week 4, it was $>2^7$ in group A and $<2^7$ in group B. At week 6, HI titre was $>2^8$ in group A, and $<2^8$ in group B. At week 8, it was $>2^6$ in group A and $<2^6$ in group B, and at weeks 10 and 12, they were $>2^8$ in group A and $<2^8$ in group B. Thus MG INAC vaccine showed negative impact on Newcastle disease vaccines. The birds of group C were vaccinated against MG and one bird persisted challenge up to day 11 and showed clinical signs at day 7. All birds of group D (unvaccinated control group) showed clinical signs at day 3 and died within the day 7. The MG vaccination seems not promising. Interest in MG vaccines originated in the late 1970s as it became apparent that MG infection was enzootic in some multiple-age, egg-laying complexes[2]. MG bacterins with oil-emulsion adjuvant were reported to protect young chickens from intrasinus challenge with virulent MG, and commercial egg layers from MG-induced drop in egg production[9]. Some investigators found that such bacterins could protect broilers from air-sacculitis[10-11] or layers from reduction in egg production[12], while other did not detect much efficacy in commercial egg layers with enzootic MG infection[13]. Vaccination with bacterins has been shown to reduce, but usually not eliminate, colonization by MG following challenge[11-12]. Therefore, inoculation of MG INAC vaccine is not justified and is too expensive at farm levels.

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