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Antibody response to 1.0 and 0.5 mL doses of an inactivated bacterial vaccine against bovine respiratory disease in young Holstein calves: a field trial

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Abstract

Introduction: Early vaccination of cattle with an inactivated commercial bacterial vaccine against bovine respiratory disease has been reported to increase antibody production and can alleviate the disease. However, its dosage has been little investigated in young Holstein calves. This study addresses the need to establish guide values for vaccine dosage in these animals. **Material and Methods:** Healthy calves received an inactivated vaccine for *Histophilus somni*, *Pasteurella multocida* and *Mannheimia haemolytica* intramuscularly at the ages of 1 and 4 weeks. Administered vaccine doses were 1.0 mL for the primary and booster vaccinations (1.0 + 1.0 group), 0.5 mL for the primary and 1.0 mL for the booster vaccination (0.5 + 1.0 group), or 0.5 mL for both vaccinations (0.5 + 0.5 group). **Results:** Differences in the vaccine responses between the 1.0 + 1.0 group and 0.5 + 1.0 group were minor. However, the number of calves with a positive vaccine response to *H. somni* in the 0.5 + 0.5 group was less than half of that in the 1.0 + 1.0 and 0.5 + 1.0 groups. In logistic regression analysis, although the booster vaccination dose was positively correlated with seropositivity for *H. somni*, the primary vaccination dose was not correlated with vaccine response. The number of calves with positive vaccine responses to *M. haemolytica* was low even after booster vaccination regardless of the dose. **Conclusion:** The dose of 0.5 mL can be used for primary vaccinations in newborn Holstein calves, but 1.0 mL may be required for booster vaccinations.

Keywords: bovine respiratory disease, early vaccination, vaccine dose, young calves.

Introduction

Bovine respiratory disease (BRD) is one of the leading causes of death in pre-weaned calves and weaned dairy heifers (15). It has been difficult to develop effective control strategies because the disease is multifactorial, but vaccination can help reduce the risk of this disease (14). Previous studies have reported that booster vaccinations with a commercially available inactivated *Histophilus somni*, *Pasteurella multocida* and *Mannheimia haemolytica* vaccine resulted in increased antibody titres in young calves (5, 9). It has already been reported that calves which received this vaccine had a significantly lower incidence of respiratory disease and higher antibody titres (7). Therefore, increasing antibody production through this vaccine is likely to alleviate BRD in young calves.

When young children are vaccinated, the doses are frequently lower than when adults are vaccinated (3, 11). According to Qadri *et al.* (10), the immunogenicity of a full, a half and a quarter dose of an inactivated enterotoxigenic *Escherichia coli* vaccine in human children was comparable. Furthermore, in newborns and infants aged up to one year, a 0.025 mg dose of intradermal Bacillus Calmette–Guérin induced an immune response comparable with the response to a 0.05 mg dose (13). Therefore, lower doses of the inactivated bacterial BRD vaccine than the 1.0 mL dose

administered in previous studies may be suitable for young Holstein calves (5, 6). The optimal dose of this vaccine in young Holstein calves has not been investigated thoroughly.

Voysey *et al.* (16) reported that using a half dose for the primary COVID-19 vaccination and a standard dose for the booster was as effective as using a standard dose for both primary and booster vaccinations. In general, a higher antigen dose improves antigen availability and stimulates more memory cells during booster vaccinations. Conversely, lower doses are thought to favour memory B cell induction during priming. Based on these findings, we hypothesised that the doses of vaccine required for primary and booster vaccinations might differ.

A previous study (6) reported that a satisfactory body mass index (BMI) was positively correlated with antibody production following administration of the inactivated vaccine in young Holstein calves. It is therefore necessary to ensure that the BMI at the time of vaccination is similar between groups to study the effect of dosage.

In this study we present antibody responses in young Holstein calves matched for BMI receiving a dose of 1.0 mL or of 0.5 mL of the inactivated *H. somni*, *P. multocida* and *M. haemolytica* vaccine in the field. The main objective of this study was to determine whether the dosage of this vaccine could be reduced from 1.0 mL to 0.5 mL for these animals.

Material and Methods

For this study, 66 healthy female Holstein calves on a commercial dairy farm were selected as subjects. Veterinarians from the Rakuno Gakuen University Animal Medical Center's Large Animal Clinical Services team regularly visited this farm and vaccinated the calves. All procedures described in this study were conducted and all necessary animal care was given in accordance with the guidelines of the Rakuno Gakuen University Animal Experiment and Care Committee. Verbal informed consent from the herd's owner was obtained for the field experiment and the Rakuno Gakuen University Animal Experiment and Care Committee approved the acceptability of such a form of consent.

All calves were born on the farm and were separated from their dams within 12 h of birth. Calves received adequate amounts of colostrum replacer (Calfsupport Dash, Zenoaq, Fukushima, Japan) and were reared in isolation in hutches. Three experimental groups each of 14 calves and two control groups each of 12 calves were created. One experimental group was administered 1.0 mL of the vaccine as the primary vaccination and 1.0 mL as the booster. Another experimental group was administered 0.5 mL of the vaccine as the primary vaccination and 1.0 mL as the booster. The final experimental group was administered 0.5 mL of the vaccine as the primary vaccination and 0.5 mL as the booster. A 20 mL vial of vaccine contained inactivated whole bacterial cells of *H. somni* ($0.5–1.0 \times 10^{11}$ colony-forming units – CFU) and *P. multocida* ($1.0–2.0 \times 10^{11}$ CFU) cultured in an artificial medium, 2 mL of centrifugal supernatant of *M. haemolytica* cultured in an artificial medium and 200 mg of aluminium chloride hexahydrate adjuvant (strain # 26-1; Kyoto Biken Laboratories, Inc., Uji, Japan). All three experimental groups of calves were vaccinated intramuscularly at one and four weeks of age. Calves in the primary-vaccination control (PV-Control) group were vaccinated once at the age of four weeks. Calves in the no-vaccination control (NV-Control) group were not vaccinated. Table 1 displays the vaccine dosages and sample sizes.

Table 1. Dosages of the vaccine and sample size

Group	Dose at one week of age	Dose at four weeks of age	Sample size
1.0 + 1.0	1.0 mL	1.0 mL	n = 14
0.5 + 1.0	0.5 mL	1.0 mL	n = 14
0.5 + 0.5	0.5 mL	0.5 mL	n = 14
PV-Control	Not vaccinated	1.0 mL	n = 12
NV-Control	Not vaccinated	Not vaccinated	n = 12

PV-Control – primary-vaccination control; NV-Control – no-vaccination control

The body weights and withers heights of the calves were measured on the day of primary vaccination (experimental group) or at one week of age (control group), and each calf's BMI was calculated by dividing the body weight in kilograms by the square of the height in metres (16).

Blood was drawn immediately before each vaccination and again three weeks later. Sampling was on the same day as when the calves were weighed (at one week of age) in the PV- and NV-Control groups and was additionally performed at four and seven weeks of age in the NV-Control group. To isolate the serum, blood samples were collected into plain vacutainer tubes and centrifuged at 3,500 rpm for 8 min. The serum was stored at −30°C until the analysis, and the antibodies to *H. somni*, *P. multocida* and *M. haemolytica* were detected using an ELISA with the same antigens as described in a previous report (7). The antibody titre for *P. multocida* and *M. haemolytica* was calculated as a 50-fold dilution when detected with a dilution less than 100-fold. Antibody titres greater than 0.604 (*H. somni*), 100 (*P. multocida*) and 200 (*M. haemolytica*) at three weeks after booster vaccination were considered to be positive. Similarly, the titres greater than those values at the time of primary vaccination were considered to be high maternal antibody levels (MAL) (8). Calves were divided into positive and negative groups based

on post-vaccination titres of antibodies against *H. somni*, *P. multocida* and *M. haemolytica*, or into high and low MAL groups based on titres at primary vaccination (5, 6).

The data were expressed as geometric mean ± standard error of the mean. All analyses were carried out using XLSTAT 2021.3.1 (Addinsoft, Paris, France). The Kruskal–Wallis and the Dwass–Steel–Critchlow–Fligner tests were used to compare antibody titres and BMIs between groups. Differences between titres at primary vaccination (or one week of age), booster vaccination (or four weeks) and three weeks after final vaccination (or seven weeks) were examined using the Friedman and Nemenyi tests. Fisher's exact test of independence was used to compare the number of calves with positive and negative vaccine responses or the number of calves with high and low MAL. In experimental groups of calves, multivariable logistic regression was used to examine the relationship between dosage and seropositivity for *H. somni* or *M. haemolytica*. A P-value <0.05 was considered statistically significant.

Results

Table 2 shows the BMIs of the calves at the time of primary vaccination. There were slight differences between groups (P = 0.286). The P-values between groups for the BMI parameter are also shown in Table 2.

Figure 1 depicts antibody responses in young Holstein calves following early vaccination with the inactivated bacterial preparation. Calves in all three experimental groups exhibited significantly increased titres of anti-*H. somni* and -*P. multocida* antibodies after vaccination (at age seven weeks compared to at age one week). The post-vaccination titres of antibodies against *H. somni* in the 1.0 + 1.0, 0.5 + 1.0, 0.5 + 0.5 and PV-Control groups were significantly higher than those in the NV-Control group (P < 0.05) and the antibody responses were better, with respective mean 3.6-, 4.1-, 3.5- and 2.2-fold increases. Additionally, the titres in the 0.5 + 1.0 group were significantly higher than those in the PV-Control group (mean 1.9-fold increase; P < 0.01). The titres of anti-*P. multocida* antibodies were significantly higher in the 1.0 + 1.0, 0.5 + 1.0 and 0.5 + 0.5 groups than in the PV-Control group (P < 0.01, 0.01 and 0.05), with respective mean 2.6, 2.8 and 2.5-fold increases, and likewise were higher than in the NV-Control group (P < 0.01, 0.01 and 0.05), with mean 2.3, 2.4 and 2.1-fold increases. After vaccination, the antibody titre against *M. haemolytica* in the 1.0 + 1.0 and 0.5 + 1.0 groups was significantly higher than in the PV-Control group (P < 0.01 and 0.05) and the antibody responses were also better in this instance, with mean 1.8-fold increases in both groups. In contrast, the titres of anti-*M. haemolytica* antibodies in calves in the 0.5 + 0.5 and PV- and NV-Control groups had significantly decreased at seven weeks of age compared with those at one week (P < 0.01) (Fig. 1).

The numbers of calves with high and low MAL and those with positive and negative vaccine responses are shown in Table 3. Although there was generally no significant difference between groups in the number of calves with high and low MAL, there tended to be a difference in the number of calves with high and low MAL against *M. haemolytica* (P = 0.104). After vaccination, there were significant interactions for antibodies against *H. somni* and *P. multocida* (P < 0.01). Calves in the PV- and NV-Control groups provided significantly fewer samples positive for *H. somni* and *P. multocida* antibodies. Almost all the calves in the experimental groups tested positive for *P. multocida* antibodies. Although there was little difference in the number of *H. somni*-positive calves between the 1.0 + 1.0 and 0.5 + 1.0 groups, the number of positive calves in the 0.5 + 0.5 group was less than half of those of the 1.0 + 1.0 and 0.5 + 1.0 groups. The number of calves with positive vaccine responses to *M. haemolytica* was low in all groups (Table 3).

The standardised regression coefficients from the logistic regression analysis for seropositivity in experimental group calves are shown in Fig. 2. Although the dose of the booster vaccination was positively correlated with seropositivity for *H. somni,* the dose of the primary was not correlated with vaccine response. For *M. haemolytica*, no significant factor for seropositivity was identified in this study (Fig. 2).

Table 2. Body mass index (BMI) values for the experimental and control groups of young Holstein calves and null hypothesis validity of the values

Group	BMI	P-value				
		1.0 + 1.0	0.5 + 1.0	0.5 + 0.5	PV-Control	NV-Control
1.0 + 1.0	64.9 ± 0.8	1	0.898	1.000	0.731	0.715
0.5 + 1.0	64.0 ± 0.9	0.898	1	0.975	0.568	0.534
0.5 + 0.5	64.6 ± 0.8	1.000	0.975	1	0.731	0.584
PV-Control	66.8 ± 1.5	0.731	0.568	0.731	1	0.999
NV-Control	66.8 ± 1.7	0.715	0.534	0.584	0.999	1

PV-Control – primary-vaccination control; NV-Control – no-vaccination control

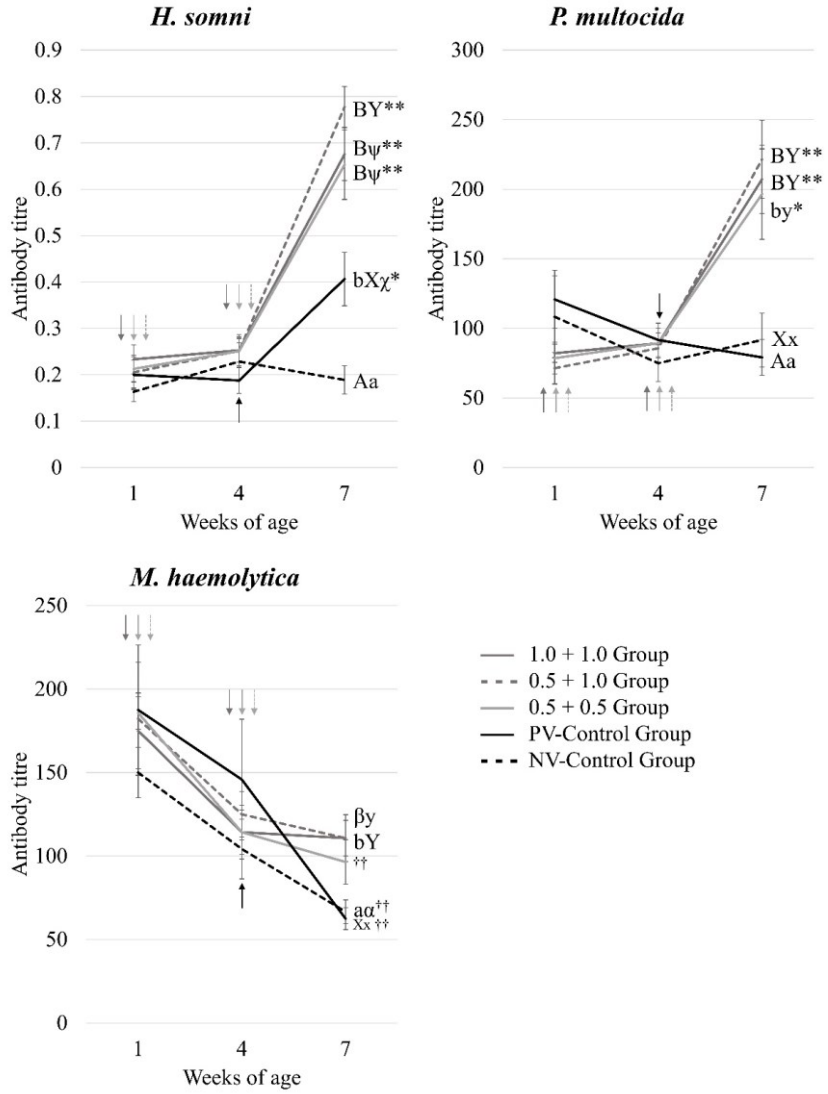

Fig. 1. Antibody responses in young Holstein calves vaccinated with a dose of 1.0 mL or 0.5 mL of an inactivated bacterial vaccine against bovine respiratory disease
PV-Control – primary-vaccination control; NV-Control – no-vaccination control; arrows – vaccination times; **, * – significant increase compared to titre at one week of age ($P < 0.01$, $P < 0.05$); †† – significant decrease compared to titre at one week of age ($P < 0.01$); A-B, X-Y, a-b, x-y, α-β, χ-ψ – differences in final antibody titres ($P < 0.01$, $P < 0.01$, $P < 0.05$, $P < 0.05$, $P < 0.10$, and $P < 0.10$, respectively)

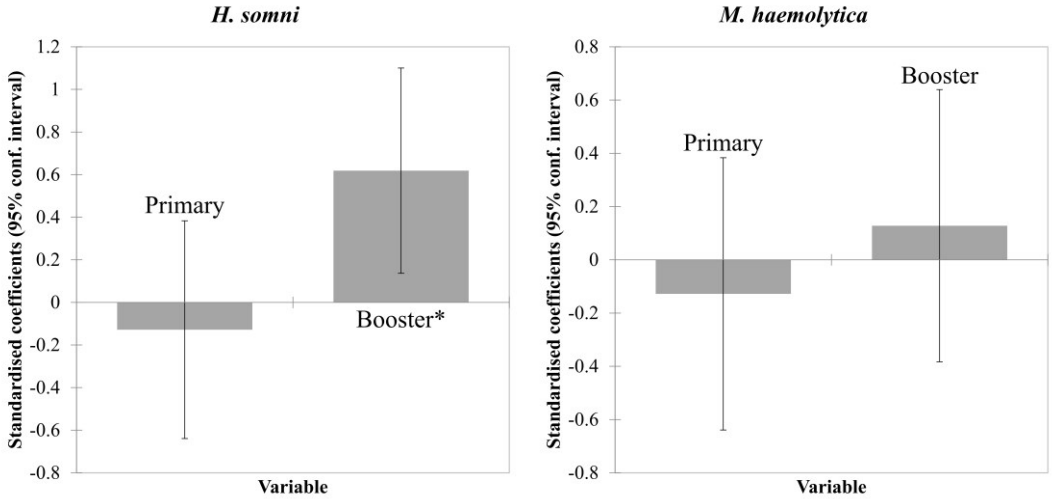

Fig. 2. Standardised regression coefficients from the logistic regression for seropositivity
Primary – primary vaccination; Booster – booster vaccination; * – $P < 0.05$

Table 3. The number of calves with high and low maternal antibody levels and with positive and negative vaccine responses to (a) *H. somni*, (b) *P. multocida* and (c) *M. haemolytica*

(a)	One week of age			Seven weeks of age		
H. somni	High MAL	Low MAL	P-value	Positive	Negative	P-value
1.0 + 1.0	0	14	-	11	3	0.005**
0.5 + 1.0	0	14	-	12	2	0.000**
0.5 + 0.5	0	14	-	5	9	0.556
PV-Control	0	12	-	1	11	0.008**
NV-Control	0	12	-	0	12	0.000**
P-value		1.000			0.000**	

(b)	One week of age			Seven weeks of age		
P. multocida	High MAL	Low MAL	P-value	Positive	Negative	P-value
1.0 + 1.0	9	5	-	14	0	0.015*
0.5 + 1.0	4	10	-	14	0	0.015*
0.5 + 0.5	6	8	-	13	1	0.159
PV-Control	7	5	-	5	7	0.006**
NV-Control	6	6	-	4	8	0.000**
P-value		0.402			0.000**	

(c)	One week of age			Seven weeks of age		
M. haemolytica	High MAL	Low MAL	P-value	Positive	Negative	P-value
1.0 + 1.0	9	5	-	2	12	-
0.5 + 1.0	6	8	-	3	11	-
0.5 + 0.5	12	2	-	2	12	-
PV-Control	5	7	-	0	12	-
NV-Control	6	6	-	0	12	-
P-value		0.104			0.275	

Antibody titres greater than 0.604 (*H. somni*), 100 (*P. multocida*) or 200 (*M. haemolytica*) were determined as positive or high maternal antibody levels. MAL – maternal antibody level, PV-Control – primary-vaccination control, NV-Control – no-vaccination control, ** – $P < 0.01$, * – $P < 0.05$

Discussion

Recent studies have found that young calves receiving an inactivated commercial bacterial vaccine have higher antibody titres and can mount an effective immune defence against BRD (5, 9). The appropriate dosage of this vaccine in young calves, however, had been little studied prior to the present research. In this study, we investigated antibody response in young Holstein calves which received 0.5 mL or 1.0 mL of the vaccine in the field.

This study revealed that calves in all experimental groups showed increased antibodies against *H. somni* and *P. multocida* as well as higher antibody titres than those without vaccination. Therefore, early vaccination with this vaccine is expected to produce a certain level of antibody response in young Holstein calves even at a dose of 0.5 mL. Furthermore, the differences in vaccine responses between the 1.0 + 1.0 and 0.5 + 1.0 groups were very minor, and the titres of antibodies to all three bacteria in the 0.5 + 1.0 group were significantly higher than those in the PV-Control group. The effect of a standard-dose COVID-19 booster vaccination in humans is reported to be maintained even when the primary vaccination dose is halved (15). A lower vaccine antigen volume is already known to favour memory B cell generation in primary immunisation (2). Although this study's findings cannot be assumed to completely rule out the possibility of a difference in elicited response between the 1.0 and 0.5 mL doses of primary vaccination, reducing this from 1.0 to 0.5 mL is probably feasible in newborn Holstein calves.

Conversely, the 0.5 + 0.5 group had a lower number of *H. somni* antibody–positive calves than the group administered the 1.0 mL booster vaccination dose. Given that the majority of calves in the experimental group had a positive vaccine response to *P. multocida*, logistic regression analysis for *P. multocida* was impossible; however, the standardised regression coefficients revealed that booster vaccination dosage was a significant factor for seropositivity for antibodies against *H. somni*. Sieng *et al.* (12) discovered that cattle given a half dose of an inactivated foot-and-mouth

disease vaccine had significantly lower antibody titres following a booster vaccination than cattle given the full dose. Hopkins *et al.* (4) found that healthy adults who received multiple half-dose anthrax vaccines tended to have lower post-vaccination serum toxin-neutralising antibody levels than those who received the full dose on the same schedule. It was demonstrated that for adequate antibody serum titres, relatively high antigen doses were required (2). We estimate that the antigens in 0.5 mL of this vaccine as a booster are insufficient to produce antibodies in an adequate quantity to protect the calves; thus, we conclude that a booster vaccination with this preparation in young Holstein calves will most likely require a 1.0 mL dose.

Antibody titres also increased at some points in the control groups. The antigens in the vaccines were known as bacterial elements of the nasal microbiome (1), and it cannot be ruled out that there was antigenic stimulation from the bacteria present in the control animals' nasal cavities. However, as all calves in this study were born on the same farm and only healthy calves were used, we believe that antigen stimulation by normal intranasal flora was generally equal between groups. A field trial using the same vaccine and methods has been reported (5). It is contended that the increased antibody titres after vaccination were due to both the antigenic stimulation by the bacterial flora and vaccination.

In this study, however, the number of calves with positive vaccine responses to *M. haemolytica* was low even after the booster vaccination. A previous study (6) investigating the same vaccine as used in this research reported that a higher BMI was a positive factor for successful early vaccination and that the calves with positive vaccine responses to *M. haemolytica* had higher BMIs (mean 70) than those responding positively to *H. somni* and *P. multocida.* Although there were slight differences in BMI between the groups, the mean BMI of the calves in this study was similar to that of the *M. haemolytica*-negative calves in the previous report (6). We therefore presume that the inadequate antibody response to *M. haemolytica* in this study is due to the low BMI of the recipient calves. In this field trial, small differences in MAL for *M. haemolytica* may have made it challenging to determine differences in antibody responses. Additional studies may be warranted on the effect of dosage on the antibody response to *M. haemolytica*.

In conclusion, we propose that a dose of 0.5 mL can be administered for primary vaccination with this commercial BRD prophylactic in newborn Holstein calves aged one week, but the dose of 1.0 mL may be required for the booster vaccination. Vaccine response, however, may differ depending on the BMI, the week of age and the breed. Further studies are justified to confirm the comparable immunogenicity and effectiveness of the doses of 1.0 mL and 0.5 mL for primary vaccination in young calves.

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Animal Rights Statement: All animals were regular patients of Farm Animal Clinical Services located at the Rakuno Gakuen University Animal Medical Center. All procedures carried out and animal care provided in the study were in accordance with guidelines approved by the Rakuno Gakuen University Animal Experiment and Care Committee and were not commenced until verbal informed consent was obtained from the animals' owners. The sufficiency of verbal informed consent for a field experiment was approved by the Rakuno Gakuen University Animal Experiment and Care Committee.

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