

# Antibody Response in Calves Following Administration of Attenuated Infectious Bovine Rhinotracheitis (IBR) Vaccines

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## ABSTRACT

The serum antibody response of calves vaccinated against infectious bovine rhinotracheitis by the intramuscular route was compared to calves vaccinated subcutaneously. Immunological response in the calves as determined by serum neutralization tests was highly variable; however, a significantly greater percentage (87.5%) of the calves inoculated subcutaneously responded to vaccination by producing a four-week post-vaccinal serum titer of two or higher as compared to only 47.8% of the calves that were vaccinated intramuscularly. Of those calves that were vaccinated a second time, all maintained or had produced titers of two or higher within four weeks after the second immunization. However, the existing circulating serum antibody titers resulting from the first vaccination of nine of 22 calves were lowered by repeat vaccination.

## RÉSUMÉ

Cette étude visait à comparer la concentration d'anticorps sériques de veaux vaccinés contre la rhino-trachéite infectieuse bovine, par la voie intra-musculaire, à celle d'autres veaux immunisés par la voie sous-cutanée. La réponse immunologique de ces animaux, telle que déterminée par des épreuves de séro-neutralisation, s'avéra très variable; toutefois, un pourcentage sensiblement plus élevé (87.5%) des

veaux vaccinés par la voie sous-cutanée réagissent à la vaccination en développant, en l'espace de quatre semaines, un titre sérique de deux ou plus, comparativement à seulement 47.8% des sujets vaccinés par la voie intramusculaire. Parmi les veaux que l'on vaccina une seconde fois, tous avaient maintenu ou développé un titre de deux ou plus, au bout de quatre semaines après la deuxième injection. Cependant, la teneur du sérum en anticorps circulants, à la suite de la première injection, diminue, après la seconde vaccination, chez neuf d'un groupe de 22 veaux.

## INTRODUCTION

The ubiquity of the infectious bovine rhinotracheitis-infectious pustular vulvovaginitis (IBR-IPV) virus and its inherent ability to produce a diversity of disease syndromes has resulted in widespread use of attenuated IBR vaccines. The efficacy of these vaccines has been the subject of recent investigations and considerable controversy (1-16). The objective of this investigation was to determine the serum antibody response in calves following intramuscular and subcutaneous administration of attenuated infectious bovine rhinotracheitis virus vaccines.

## MATERIALS AND METHODS

### EXPERIMENTAL ANIMALS

Fifty-five Holstein and Holstein-Angus cross calves between three and nine months of age were housed in a conventional barn facility. They were fed a commercial alfalfa pellet ration.

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VACCINATION AND SERUM COLLECTION

Collection of blood by jugular vein puncture was made prior to initial vaccine administration (controls) and at four and eight weeks after the first vaccination. Twenty-nine of the calves were inoculated a second time at the time of the four-week bleeding. Sera were separated from the blood clots by centrifugation after refrigeration overnight at 4°C. All sera were heat inactivated before titration.

EXPERIMENTAL PROCEDURE

This investigation was initially designed to compare the efficacy of subcutaneous (SC) administration versus intramuscular (IM) administration of attenuated IBR vaccines using a currently popular commer-

cial brand of IBR vaccine. However, it was later deemed necessary to use several different brands of vaccine to eliminate potential product differences. Availability of experimental animals greatly restricted the number of animals assigned to each treatment. Table I illustrates the number of animals treated with the various combinations of the different brands of vaccine.

Five different commercial brands of attenuated infectious bovine rhinotracheitis (IBR) virus vaccines which are readily available to stockmen were used in this study. Each experimental animal received 2 ml of vaccine parenterally, administered either by the intramuscular (IM) or subcutaneous (SC) route. Vaccines were deposited with one-inch 16 gauge needles using care to prevent drainback. Intramuscular inoculations were deposited deep in

TABLE I. IBR Antibody Titers in Serums Produced by Vaccination with Attenuated IBR Vaccines

Calf No.	Route <sup>a</sup> of Immun	Vaccine <sup>b</sup> Commercial Brand		Titer <sup>d</sup>		Calf No.	Route <sup>a</sup> of Immun	Vaccine <sup>b</sup> Commercial Brand		Titer <sup>d</sup>	
		(1)	(2) <sup>c</sup>	4 week	8 week <sup>c</sup>			(1)	(2) <sup>c</sup>	4 week	8 week <sup>c</sup>
1	SC	A	A	4	4	29	SC	D	D	2	2
2	SC	A	A	2	8	30	SC	D	D	<2	2
3	SC	A	A	4	4	31	SC	D	D	8	4
4	SC	A	A	4	2	32 <sup>e</sup>	SC	D	—	<2	—
5	SC	A	A	4	2	33	IM	A	—	2	—
6	SC	B	—	8	—	34	IM	A	—	2	—
7	SC	B	—	4	—	35	IM	A	—	<2	—
8	SC	B	—	<2	—	36	IM	A	—	4	—
9	SC	B	—	<2	—	37	IM	A	—	<2	—
10	SC	B	—	4	—	38	IM	A	—	<2	—
11	SC	B	—	16	—	39	IM	A	—	4	—
12	SC	B	—	4	—	40	IM	A	—	<2	—
13	SC	B	—	4	—	41	IM	A	A	<2	16
14	SC	B	—	2	—	42	IM	A	A	<2	4
15	SC	B	B	16	8	43	IM	B	—	<2	—
16	SC	B	B	32	16	44	IM	B	—	<2	—
17	SC	B	B	4	2	45	IM	B	—	<2	—
18	SC	B	B	8	4	46	IM	B	—	8	—
19	SC	B	B	4	2	47	IM	B	—	2	—
20	SC	B	B	2	2	48	IM	B	—	2	—
21	SC	B	B	4	8	49	IM	B	—	4	—
22	SC	B	C	2	32	50	IM	B	—	2	—
23	SC	C	C	8	4	51	IM	B	B	4	16
24	SC	C	C	8	8	52	IM	B	B	4	32
25	SC	C	C	8	8	53	IM	B	C	<2	16
26	SC	C	C	8	2	54	IM	D	E	<2	64
27	SC	C	C	2	4	55	IM	D	E	<2	32
28	SC	D	D	2	4						

<sup>a</sup>Route of Immunization: SC = Subcutaneous; IM = Intramuscular

<sup>b</sup>Vaccine Commercial Brand: (1) = Initial inoculation; (2) = Second inoculation

<sup>c</sup>—Indicates no test

<sup>d</sup>Titer: Prevaccinal serum antibody titers of all calves were zero

<sup>e</sup>Calf No. 32 died five weeks after the first vaccination

**TABLE II. Four Week Antibody Titers to IBR**

A. Group I (Subcutaneous)							
Titer	0	2	4	8	16	32	64
Total No Calves	4	7	11	7	2	1	9
Percentage of Calves	12.5	21.9	34.4	21.9	6.3	3.1	0
B. Group II (Intramuscular)							
Titer	0	2	4	8	16	32	64
Total No Calves	12	5	5	1	0	0	0
Percentage of Calves	52.2	21.7	21.7	4.3	0	0	0

**TABLE III. Eight Week Antibody Titers to IBR**

A. Group I (Subcutaneous)							
Titer	0	2	4	8	16	32	64
Total No Calves	0	8	7	5	1	1	0
Percentage of Calves	0	36.4	31.8	22.7	4.6	4.6	0
B. Group II (Intramuscular)							
Titer	0	2	4	8	16	32	64
Total No Calves	0	0	1	0	3	2	1
Percentage of Calves	0	0	14.3	0	42.9	28.6	14.3

the muscle and SC deposition was performed by lifting the skin prior to insertion of the needle.

The test virus used in the serum neutralization test was 15th passage IBR virus, Cooper isolate (Colorado) grown on bovine endocardial cells (BEC). The virus titer was  $10^{6.5}$  TCID<sub>50</sub>/ml. The tissue cultures were of BEC origin, and were between fifth and 20th passage. The tissue cultures were maintained in minimum essential medium (MEM) consisting of Hank's salt with 1% lactalbumin hydrolysate, 10% sodium pyruvate, and 10% heat inactivated calf serum.

Serum antibody levels were titered using two-fold dilutions by conventional neutralization procedures. The test virus preparation (1 ml) and serum dilution (1 ml) were mixed and allowed to remain at room temperature for 45 minutes. Two-tenths ml of the virus-serum mixture was placed in each of three tubes of BEC tissue culture. The inoculated tubes were again incubated at room temperature for 45 minutes. Then 0.8 ml of MEM was added to each tube and incubation was continued at 37°C for 48 hours. Titers were reported as the reciprocal of the highest serum dilution exhibiting complete neutralization of the cytopathogenic effect of the test virus. The data compiled in this study were tested for statistical significance using the Chi-square test.

## RESULTS

The prevaccinal serum of all calves were devoid of serum antibodies specific for IBR virus. Immunological response of the calves used in this study was highly variable. However, a significantly greater percentage (87.5%) of the calves in Group I inoculated by the SC route responded to vaccination by producing a serum titer of two or higher as compared to 47.8% of the calves in Group II that were vaccinated IM ( $P < .005$ ). A similar immunological presentation was observed at titer of four and higher with 65.5% of Group I calves and 26.1% of the calves in Group II in this category ( $P < .01$ ). Only 4.3% of the calves in Group II had a titer of eight, while in Group I, 21.9% had a titer of eight and 9.4% had a titer of 16 or higher (Table II). The percentage difference between the subcutaneous and intramuscular groups at titer of eight was also statistically significant ( $P < .025$ ).

Seven of the 23 calves in Group II were vaccinated a second time four weeks after the initial inoculation. All seven of these calves demonstrated a titer of four or higher (Table III). Twenty-two of the 32 calves in Group I were revaccinated subcutaneously. All 22 of these animals had a titer of two

or higher four weeks after the second vaccination (Table III). Of the calves in Group I, 36.4% had a titer of two after the second vaccination, an increase of 14.5% from the percentage observed after one vaccination. Inspection of the immunological response of the individual revealed that of the 22 calves vaccinated subcutaneously a second time, the serum titers of six calves were increased, ten were reduced and the titer of six calves remained unchanged (Table I). There was no observable difference in immunogenicity between the five individual commercial brands of vaccine used in this investigation.

## DISCUSSION

Calves vaccinated with attenuated IBR virus vaccines had variable serum titers four weeks after vaccination. The calves that were administered vaccine subcutaneously responded more favorably by producing higher levels of serum antibody than the calves that were vaccinated intramuscularly. Fifty-two and two-tenths percent of the calves vaccinated intramuscularly failed to respond to the vaccine, while 12.5% of the calves vaccinated subcutaneously failed to respond. Also in those animals that did respond to the vaccine, the serum titers were significantly higher in the calves that had been vaccinated subcutaneously than in the calves that had been vaccinated intramuscularly ( $P < .005$ ). These observations indicated that subcutaneous administration was superior to intramuscular administration for production of antibody titers in the calves utilized in this investigation.

All calves in this investigation that were vaccinated twice had titers of two or higher. The second vaccination did not effectively increase the serum titer in some animals; the titers of ten calves were reduced and the titers of six calves remained unchanged while the titers of only six calves increased after the second vaccination. The lowering of existing serum antibody titers could be the result of binding of circulating antibody by the antigen of the second immunization. This could result in transition of a calf from an immune to a susceptible state.

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