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Use of a Fluorometric Immunoassay to Determine Antibody Response to *Pasteurella haemolytica* in Vaccinated and Nonvaccinated Feedlot Cattle†

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A retrospective study of the antibody response to *Pasteurella haemolytica* was conducted by using sera from 368 feedlot cattle divided among five experiments. In three experiments, live vaccines or a bacterin were administered to some of the cattle and others were left as nonvaccinated controls. In two experiments, cattle were not vaccinated. Clinical signs of disease with subsequent recovery developed in 48.0% of the cattle, and 10.3% of the cattle died. Vaccination had no apparent effect on morbidity or mortality. At the time of purchase, 78% of the cattle had low antibody titers (<25) as measured by a quantitative fluorometric immunoassay. In most groups of cattle (both vaccinated and nonvaccinated), there was a significant rise in mean antibody titers between the time of purchase and days 28 to 32 in the feedlot. The antibody titers at the time of shipment and days 28 to 32 and the ratio of the two antibody titers were compared with the health status of cattle. The antibody ratios were significantly greater for cattle that became sick and then recovered compared with those of cattle that remained healthy. Although significance could not be established, antibody titers at the time of shipment were higher for cattle that remained healthy compared with cattle that became sick and then recovered.

Bovine pneumonic pasteurellosis (shipping fever) is a severe fibrinous pneumonia of feedlot cattle (8). *Pasteurella haemolytica* biotype A serotype 1 has been demonstrated to be an essential component in the etiology of shipping fever (3, 4).

Studies of mechanisms of resistance to *P. haemolytica* in cattle have included serum antibody responses in cattle exposed to the organism under natural and experimental conditions (5, 6, 9–13, 15). A positive correlation has been demonstrated between naturally occurring serum antibody titers to *P. haemolytica* at entry to feedlots and resistance to pneumonic pasteurellosis (12; C. Reggiardo, abstr. Rumin. Immun. Syst. 1981, p. 818). In one study, serum antibody titers to *P. haemolytica* increased in cattle after their arrival in a feedlot (12). It has been demonstrated recently that feedlot cattle dying of fibrinous pneumonia have lower serum antibody titers than those dying of other causes (10). Experimental inoculation with bacterins or live bacteria have demonstrated rises in serum antibody titers to *P. haemolytica* (5, 6, 13, 14). Correlative studies, however, between antibody titers to *P. haemolytica* after vaccination and health status have been limited to small groups of experimentally challenged cattle (6, 14).

The data reported here are from a retrospective study of the antibody response to *P. haemolytica* as detected by a quantitative fluorometric immunoassay, using sera obtained from feedlot cattle that had been used in experimental vaccine or nutritional studies. The objective of this investigation was to study the serum antibody response to *P. haemolytica* in vaccinated and nonvaccinated feedlot cattle and to correlate this response with health status.

MATERIALS AND METHODS

Animals. A total of 368 cattle, aged 6 to 10 months, were used in five experiments. In experiments 1, 2, 3, and 5, steers (weighing ca. 136 to 250 kg each) were purchased through an order buyer in Newport, Tenn. (samples provided by Texas A & M Research and

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Experiment Station, Amarillo). Cattle were held at those facilities from 1 to 5 days before shipment by truck to an experimental feedlot in Bushland, Tex. While in the feedlot, cattle were fed a 70% concentrate diet, and individual daily feed intake was determined by automatic feed monitoring devices (Pinpointers; VIS, Inc., Cookeville, Tenn.).

Cattle in experiment 4 were crossbred heifers (ca. 136 kg each) purchased through an order buyer in Warren, Ark. Cattle were held at this facility for 10 days before shipment by truck to a commercial feedlot in Follett, Tex. On arrival, calves were given a single injection of antibiotics; vaccinated parenterally for infectious bovine rhinotracheitis, *Leptospira interrogans* subvar. *pomoma*, and *Clostridium chauvoei*; placed in a standard pen; and fed a 65% concentrate diet from standard feeders.

Vaccines. The vaccine used in experiment 3 (vaccine 1) was a lyophilized preparation of *P. haemolytica* serotype 1 that was reconstituted in distilled water and administered intradermally as a single 0.5-ml dose (A. H. Robbins, Co., Richmond, Va.). Each dose contained ca. $10^{6.3}$ CFU. For experiment 4, *P. haemolytica* serotype 1 was grown on a supplemented brain heart infusion agar for 20 h as previously described (7). The cultures were removed from the incubator, placed in insulated chests, and taken to the experiment site (transit time, ca. 2 h). Organisms were harvested and suspended in sterile phosphate-buffered saline (0.01 M, pH 7.4) at a concentration of ca. 10^9 CFU/ml. Calves were vaccinated by aerosol (vaccine 2) or subcutaneous (vaccine 3) routes. Each calf receiving vaccine 2 was exposed for 15 min to an aerosol of 40 to 60 ml of suspension produced by an ultrasonic nebulizer (model 65; DeVilbiss Co., Somerset, Pa.) as previously described (7). Subcutaneous inoculation was performed by using 5 ml of bacterial suspension. In experiment 5, steers were injected intramuscularly with a single 5-ml dose of an autogenous bacterin (vaccine 4) produced from *P. haemolytica* serotypes 1 and 4 isolated previously at the order buyer facilities.

Serology. Serum antibody titers to *P. haemolytica* somatic antigens were determined as previously described by a rapid quantitative fluorometric immunoassay (FIAX; International Diagnostic Technology, Inc., Santa Clara, Calif.) that has been shown to be comparable to the indirect bacterial agglutination test (5). The antigen used was Formalin-killed *P. haemolytica* serotype 1 obtained from a 22-h culture. Titers of <25 were considered to be low, whereas titers of >100 were considered to be high and indicative of substantial exposure to *P. haemolytica* (5).

Experimental design. The basic design of each experiment is outlined in Table 1. Cattle were randomly allotted to treatment or control groups. In each experiment, a necropsy was performed on each animal that died, and bacterial culture of the lungs was performed. In experiments 1, 2, 3, and 5, cattle were observed daily for 56 days after their arrival in the feedlot. Health status was determined by the assignment of points to clinical signs of disease as follows: 2 points, absolute anorexia; 2 points, rectal temperature of 40°C or greater; 1 point, nasal discharge; 1 point, ocular discharge; 1 point, severe depression. An animal with four or more of seven possible points on a given day was considered sick and treated with antibiotics for 4 days. In experiment 4, cattle were observed for 32 days, and similar criteria were used to evaluate health status, except that absolute anorexia could not be definitively evaluated because animals were in a commercial feedlot.

Serum samples were obtained in all experiments on the days outlined in Table 1. In experiment 3, 93 steers were identified for purchase. Of these, 47 were randomly selected and inoculated with vaccine 1 over a 3-day period while on their respective farms of origin. The remaining 46 steers were left as nonvaccinated controls. Sera were obtained from all calves on the day of vaccination (days −15 to −13, designated hereafter as day −14). Calves remained on their respective farms for 13 to 15 days until being shipped to the order buyer facility, where they were comingled for an additional 3 days before shipment to the feedlot.

In experiment 4, 58 calves were purchased and comingled in a pen at the order buyer facility. On day −10, 20 calves received vaccine 2, 19 calves received vaccine 3, and 19 calves received phosphate-buffered saline via the aerosol route (10 calves) or the subcutaneous route (9 calves). After 7 days (day −3), the vaccinations were repeated.

In experiment 5, 100 steers were purchased at the order buyer barn. One half of the cattle were given vaccine 4 immediately before shipment (day 0).

Data analysis. Health status, vaccination status, antibody titers at day 0 (AB1) and day 28 to 32 (AB2), and the ratio of AB2 to AB1 were analyzed by analysis

TABLE 1. Experimental designs

Expt	*P. haemolytica* vaccine	No. of cattle	State of origin	Texas destination	Serum samples obtained (day)	Vaccination (day)
1	None	52	Tenn.	Bushland	0[a], 32[b]	ND[c]
2	None	65	Tenn.	Bushland	0, 28	ND
3	None	46	Tenn.	Bushland	−14, 0, 31	ND
	Live intradermal (vaccine 1)	47			−14, 0, 31	−14
4	Phosphate-buffered saline	19	Ark.	Follett	−10, 0, 30	−10, −3
	Live aerosol (vaccine 2)	20			−10, 0, 30	−10, −3
	Live subcutaneous (vaccine 3)	19			−10, 0, 30	−10, −3
5	None	50	Tenn.	Bushland	0, 28	ND
	Killed intramuscular (vaccine 4)	50			0, 28	0

[a] Day 0, Day sampled before shipment (AB1).
[b] Day 28 to 32, AB2.
[c] ND, Not done.

TABLE 2. Health status of cattle

Expt	Vaccine	Total no. of cattle	No. of cattle (% total)		
			Healthy	Sick	Dead
1	None	52	22 (42.3)[a]	19 (36.5)	11 (21.2)
2	None	65	6 (9.2)	59 (90.8)	0
3	None	46	32 (69.6)	13 (28.3)	1 (2.2)
	Vaccine 1	47	36 (76.6)	10 (21.3)	1 (2.1)
4	Phosphate-buffered saline	19	13 (68.4)	6 (31.6)	0
	Vaccine 2	20	16 (80.0)	4 (20.0)	0
	Vaccine 3	19	9 (47.4)	9 (47.4)	1 (5.3)
5	None	50	9 (18.0)	26 (52.0)	15 (30.0)
	Vaccine 4	50	10 (20.0)	31 (62.0)	9 (18.0)

of variance for the 329 cattle from which both AB1 and AB2 values were known (1). Geometric mean antibody titers at different samplings were analyzed by Student's *t* test and health status data was analyzed by the chi-square test (2).

RESULTS

Morbidity and mortality. More than one half of the cattle in the five experiments either became sick or died (Table 2). Cattle that died had severe fibrinous pleuropneumonia, and *P. haemolytica* was isolated in pure culture from the lungs of >90% of them. In experiments 3, 4, and 5, there were no significant differences ($P > 0.05$) between vaccinated and nonvaccinated groups with respect to the number of cattle that remained healthy, became sick, or died. Vaccinated calves in experiment 3 developed small swellings at the sites of injection that lasted for 2 to 3 weeks. Vaccines 2, 3, and 4 did not cause any observable reactions.

Serology at the time of purchase. Antibody titers were determined for 362 calves at the initial serum collections. Titers ranged from 0 to 224 (geometric mean, 8.5 ± 4.1). The frequency distribution of antibody titers indicated that 283 (78.2%) of 362 cattle had low titers of <25, and 22 (6.1%) cattle had high titers of >100 (Table 3).

Serology for nonvaccinated cattle. In each experiment, except experiment 3, there was a significant increase ($P < 0.05$) in mean antibody titers for nonvaccinated cattle between the time that they were initially sampled and day 28 to 32 in the feedlot (Table 4). Antibody was not detected, however, in sera from 34 nonvaccinated cattle at the final sampling. Of these cattle, 29 were in experiment 3. AB2 values of >100 were detected in 85 (37%) of 230 nonvaccinated cattle. Only 3 (6.8%) of 44 control animals in experiment 3 had AB2 values of >100. In experiment 4, nonvaccinated cattle had a significant rise in mean titer during the 10 days that they were comingled before shipment. During that time, signs of respiratory disease were seen in these cattle, but health status was not recorded for individual animals. Subsequently, in this experiment, mean antibody titer for the nonvaccinated group did not increase during the 30 days in the feedlot.

Serology for vaccinated cattle. Groups of vaccinated cattle had significant increase ($P < 0.01$) in mean antibody titers between the time of vaccination and the second serum sampling (Table 4). In experiments 3 and 4, antibody to *P. haemolytica* was not detected in 14 (30.4%) and 1 (2.7%) calves, respectively, 10 to 14 days after vaccination. At the final sampling, antibody to *P. haemolytica* was not detected in sera from nine (19.6%) vaccinated cattle in experiment 3 and one (2%) vaccinated steer in experiment 5. At that time, 63 (70.8%) of 89 vaccinated cattle in experiments 4 and 5 had AB2 values of >100. Only 12 (26.0%) of 46 vaccinated cattle in experiment 3 had AB2 values of >100.

Serological response compared with health status. AB1, AB2, and the ratio of AB2 to AB1 were compared with health status (Table 5). There was a tendency (although statistically

TABLE 3. Frequency distribution of antibody titers to *P. haemolytica* at the time of purchase

Titer range	Frequency	% (Cumulative %)
<25	283	78.2 (78.2)
26–50	34	9.4 (87.6)
51–75	18	4.9 (92.5)
76–100	5	1.4 (93.9)
101–125	6	1.7 (95.6)
126–150	5	1.4 (97.0)
>151	11[a]	3.0 (100.0)

[a] $N = 362$.

TABLE 4. Means and ranges of antibody titers to *P. haemolytica*

Expt	Geometric mean titer ± SEM (range)		
	Day −10 or −14[a]	Day 0 (AB1)	Day 28 to 32 (AB2)
1	ND[b]	4.0 ± 0.7 (0–224)	51.6 ± 0.7 (0–263)[c]
2	ND	4.0 ± 0.5 (0–148)	79.4 ± 0.4 (2–401)[c]
3			
Control	4.6 ± 0.8 (0–216)	4.9 ± 0.8 (0–202)	3.0 ± 0.9 (0–267)
Vaccine 1	5.0 ± 0.8 (0–216)	44.7 ± 0.5 (0–231)[c]	28.2 ± 1.1 (0–405)[d]
4			
Control	7.1 ± 0.9 (0–73)	50.1 ± 0.6 (0–250)[e]	51.4 ± 1.1 (0–263)
Vaccine 2	35.5 ± 0.8 (0–176)	97.5 ± 1.1 (0–407)[c]	153.3 ± 0.5 (50–457)[e]
Vaccine 3	18.6 ± 0.9 (0–151)	90.1 ± 0.7 (17–389)[c]	127.0 ± 0.5 (43–389)[e]
5			
Control	ND	9.1 ± 0.6 (0–139)	120.2 ± 0.4 (0–361)[c]
Vaccine 4	ND	11.8 ± 0.7 (0–197)	112.2 ± 0.5 (0–504)[c]

[a] Date of purchase in experiments 3 and 4.
[b] ND, Not done.
[c] $P < 0.01$ compared with previous sampling.
[d] $P > 0.05$ compared with previous sampling.
[e] $P < 0.05$ compared with previous sampling.

insignificant) for AB1 to be higher (1.9 times) and AB2 to be lower in cattle that remained healthy compared with values obtained from cattle that became sick and then recovered. The lower AB2/AB1 ratios for healthy cattle compared with those for sick cattle were significant when all cattle or nonvaccinated cattle alone were considered ($P < 0.05$). Cattle that died had AB1 values similar to those of cattle that became sick and then recovered. For vaccinated cattle, the mean AB1 value for healthy cattle was 1.8 times higher than that for sick cattle.

AB2 values and AB2/AB1 ratios were available for only four cattle that died because most of the cattle died before day 28. Those four cattle had individual AB2 values of 0, 42, 182, and 245. Antibody titers were also determined on sera obtained from cattle in experiment 1 on day 14. All cattle were alive at that time and mean titers were 7.9 ± 1.4, 5.1 ± 1.2, and 7.8 ± 1.6 for cattle that remained healthy, became sick and then recovered, and died, respectively. These differences were not significant ($P > 0.05$).

TABLE 5. Comparison of antibody titers on days 0 (AB1) and 28 to 32 (AB2), the ratio AB2 to AB1, and health status

Health status	Geometric mean antibody titers ± SEM (no. of available sera)		Mean AB2/AB1 ± SEM
	AB1	AB2	
All cattle			
Healthy	15.6 ± 1.2 (151)	28.7 ± 1.2 (151)	1.9 ± 1.2
Sick	8.2 ± 1.1 (174)	67.5 ± 1.1 (174)	9.0 ± 1.2
Dead	8.3 ± 1.3 (37)	37.2 ± 3.5 (4)	2.4 ± 5.1
Nonvaccinated			
Healthy	8.0 ± 6.1 (81)	16.1 ± 1.3 (78)	2.1 ± 1.3
Sick	5.0 ± 1.2 (121)	62.0 ± 1.2 (119)	13.7 ± 0.7
Dead	7.0 ± 1.3 (27)	37.2 ± 3.5 (4)	2.4 ± 5.1
Vaccinated			
Healthy	46.1 ± 1.2 (70)	66.0 ± 1.2 (66)	1.8 ± 1.3
Sick	25.2 ± 1.3 (53)	71.8 ± 1.3 (52)	3.5 ± 1.3
Dead	12.8 ± 1.8 (10)	—[a]	—

[a] No sera available.

DISCUSSION

In these five experiments, most cattle appeared healthy at the time of purchase and had low antibody titers (<25) to *P. haemolytica*, as determined by a quantitative fluorometric immunoassay. Similarly, we recently demonstrated that 105 (64%) of 164 beef calves of the same age range purchased from a closed herd in Oklahoma had low serum antibody titers at the time of purchase (A. W. Confer, R. E. Corstvet, R. J. Panciera, and J. A. Rummage, Vet. Microbiol., in press). A low prevalence rate and low titers of antibody to *P. haemolytica* are probably typical for pastured calves and indicate low exposure rates.

Vaccinations with live or killed *P. haemolytica* in these experiments had no apparent effect on the prevalence of sickness or death. All groups of vaccinated cattle, however, had a statistically significant rise in mean antibody titers after vaccination. Not all cattle in experiments 3 and 4, however, developed detectable antibody by day 14 after vaccination, indicating that not all cattle respond immunologically even to live *P. haemolytica* vaccines. Clinical signs of disease occurred in cattle in experiment 4 before shipment, and part of the immune response in vaccinated animals may have been due to natural infection. Mean titers were higher in both vaccinated groups than in control cattle at the time of shipment, implying that the vaccines had a positive effect on the serum antibody response. In experiment 5, vaccination with the bacterin at the time of shipment did not appear to enhance the serological response to *P. haemolytica*.

It was shown recently that cattle that died of fibrinous pneumonia had lower antibody titers to *P. haemolytica* somatic antigens at the time of death than those cattle that died of other diseases (10). In the present study, we were unable to corroborate these findings. From the data in Table 5, it might appear that vaccinated cattle that died had substantially lower mean AB1 values than those of cattle that became sick and then recovered. However, of the 10 vaccinated cattle that died, 9 were in experiment 5. Because the vaccine in this experiment was administered at the time of shipment, AB1 values for these cattle could not be compared with those from experiments 3 and 4 in which cattle were vaccinated before shipment. We were also unable to establish any relationship between later antibody titers to *P. haemolytica* and death due to fibrinous pleuropneumonia.

In the present study, mean AB2/AB1 ratios were significantly greater in cattle that became sick and then survived compared with those that remained healthy. Therefore, a marked increase in antibody titer to *P. haemolytica* probably indicated that these animals became actively infected with *P. haemolytica*, responded to treatment, and recovered. This is not to imply that *P. haemolytica* was the only pathogenic agent involved in the observed illnesses in these experiments. In fact, in experiments 3 and 4 there were no changes in mean antibody titers for nonvaccinated cattle, implying that agents other than *P. haemolytica* may have been involved in the production of disease in these experiments.

In conclusion, the fluorometric immunoassay detected differences in serum antibody titers to *P. haemolytica* in cattle before and after vaccination and in many nonvaccinated cattle after 28 to 32 days in a feedlot. A rise in antibody titer to somatic antigens of *P. haemolytica* correlated with clinical signs of disease, suggesting that a natural challenge with the organism occurred in many cases. There is a need for controlled experimental studies to further determine whether there is a protective role for antibody to *P. haemolytica* in respiratory diseases of cattle.

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