Immune Responses of Fasted Calves to Infectious Bovine Rhinotracheitis Virus

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Introduction

Acute viral respiratory tract disease is commonly observed in nutritionally stressed calves. Short-term food deprivation or fasting is a stressor often associated with weaning and with transportation of calves to feedlots. It may result from both lack of feed and from inappetence brought on by changes in diet, fatigue and anxiety. Because respiratory disease is often associated temporally with nutritional stresses, many consider nutrition one of the important factors in the pathogenesis of bovine respiratory tract disease (1-4). Although malnutrition decreases the resistance of animals to most bacterial and parasitic infections, undernutrition may increase resistance to certain viral infections (5-7). The purpose of this study, therefore, was to investigate the effects of fasting on the resistance of calves to infection with infectious bovine rhinotracheitis (IBR) virus, a virus frequently isolated from naturally occurring cases of bovine respiratory disease (8), and frequently used (in attenuated forms) to vaccinate cattle at weaning and on arrival at feedlots.

In a previous report, we described the effects of a 3-day fast on IBR virus excretion and interferon production in calves (9). We reported that overall mean IBR virus excretion in fasted calves did not differ from, or was lower than that of control fed calves, but their overall interferon production (as measured in nasal secretions) was higher than in the controls. Fasting appeared to enhance interferon production. Here we describe the effects of the 3-day fast on the immune response of these calves. Both humoral and cell mediated immunity are considered important in recovery from herpesvirus infections (10) and malnutrition can affect both (11). We also describe clinical signs, and changes in hematocrit, leukocyte counts, serum protein and cortisol concentrations associated with fasting and infection.

Materials and Methods

Virus

The IBR virus was propagated in bovine fetal kidney cells

TABLE I Neutralizing antibody titers in sera and NS of calves inoculated with IBR virus on day 0.

		Experimental days				
Group	Calf No.	0	14	,	28	
1 (inoculated 24 hours i	nto fast)			_		
	3	8	16	(4)	8	
	5	0	8	(0)	4	
	8	0	8	(0)	8	
	14	0	8	(0)		
	15	0	8	(0)	4	
	16	0	16	(4)	4	
2 (inoculated at end of f	ast)					
,	['] 6	0	4	(0)	4	
	11	0	32		32	
	13	0	4	(0)	16	
	17	0	4	(0)	4	
	18	4	16	(4)	8	
	19	0		(0)	4	
3 (nonfasted control)				• •		
,	1	0	4	(0)	4	
	7	4		(4)	16	
	20	0	4	(8)	4	
	21	0	8	(4)	4	
	22	Ō	8	(0)	4	

Antibodies were not detected in any NS on days 5 and 7 after inoc-

Titers are the reciprocals of dilutions which protected at least 3 of 4 wells; 0 = < 4.

from a strain of virus developed for intranasal vaccination (12). Virus stocks were stored at -80°C and had a titer of 10^7 median tissue culture infectious doses (TCID₅₀)/ml. For the lymphocyte blastogenesis tests, virus was inactivated at 56° C for 30 min.

Experimental Design

Calves (Angus, Hereford, and Angus-Hereford crosses) were purchased locally, and weighed between 120 and 200 kg. They were pastured (fescue grass) upon arrival for about 3 weeks and supplemented as a group with approximately 1.5 kg of a grain concentrate per head daily. Three calves with low antibody titers to IBR virus were included in the study (Table 1); all others were seronegative. Virus was not isolated from nasal swabs taken at the time of arrival and just before

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inoculation.

Two lots of calves were purchased. The first (8 calves) was divided into 3 treatment groups (groups 1, 2 and 3 with 3, 3 and 2 calves respectively) and used in a first study. The second (9 calves) was also divided into 3 treatment groups, 3 calves per group, and used to repeat the first study (replicate study). Treatment groups were selected at random and housed in separate buildings 3 days before inoculation. Calves in groups 1 and 2 were fasted for 3 days, while those in the third group (controls) were fed throughout the experiment. All were inoculated intranasally with 2 ml of IBR virus (106 TCID₅₀/ml), 1 ml per nostril, with a gas powered atomizer on day 0. At this time, group 1 calves (inoculated during fasting) had been fasted for 1 day, and group 2 calves (inoculated at the end of fasting) for 3 days.

Except for the fasting period, confined calves were fed a grain concentrate (average 1.5 kg/head daily) in addition to grass hay provided *ad libitum*. Feed and bedding were removed from the pens during the fast. Water was available at all times. The calves were observed on a daily basis and rectal temperatures recorded whenever samples were taken. Samples were collected at approximately the same time each morning.

Neutralization tests

Neutralizing antibody titers against IBR in both sera and nasal secretions (NS) were determined in bovine fetal kidney cell cultures by a microtitration technique using microtransfer plates (13). Serums were obtained upon arrival, and on days 0, 14 and 28. Nasal secretions were collected by nasal tampons on days 0, 5, 7 and 14. All samples were inactivated (56°C for 30 min) before being tested. Titers were recorded as the reciprocal of the highest final dilution of sample which completely protected at least 3 of 4 wells against a challenge virus dose of 100 TCID₅₀. When NS collected on days 5 and 7 were tested for secretory antibodies, monolayers were washed with Hank's BSS after adsorption of the virus-antibody mixture for 1 hour in order to remove any interferon.

Blast transformation of lymphocytes to IBR virus

The cell mediated immune (CMI) response of calves to IBR virus was determined by measuring the incorporation of tritiated thymidine by peripheral lymphocytes using blood cultures. Bloods were collected on preservative-free heparin on days -3, 0, 3, 5, 7, 11 and 14 and diluted 1:5 in RPMI 1640 medium to which glutamine and antibiotics had been added. After dilution, 100 ul of each sample were added to triplicate microtiter wells containing control medium and three dilutions (1:10, 1:50 and 1:100) of the heat-inactivated IBR virus. Flat-bottomed 96-well tissue culture plates were used. After incubation for 4 days at 37° C, the cultures were pulsed with 25 uCi of (methyl-3H) thymidine, incubated for an additional 24 h and terminated by freezing at -80° C. Leukocytes were collected on glass fiber filters using a multichannel cell harvester. The uptake of tritiated thymidine was determined in a liquid scintillation counter. The measure of lymphocyte transformation was expressed as

a stimulation index (SI). The SI was determined by dividing the mean cpm of sample wells by the mean cpm of nonstimulated control wells.

Leukocyte count, hematocrit, serum protein and cortisol

Blood samples were collected in vacutainer tubes on days -3, 0, 1, 2, 3, 4, 5, and 7. Samples for leukocyte count and hematocrit were collected in tubes containing disodium edetate. Total blood leukocytes were counted with a hemacytometer and packed cell volumes were determined by the microhematocrit method.

Total serum protein was determined with a refractometer (American Optical Co., Buffalo, NY), and sera from calves in both studies were further subjected to electrophoresis to separate the albumin and globulin fractions. Correction for dehydration was achieved for each group by using the mean hematocrit value on day -3 as a baseline for that group. Mean protein values on any subsequent day were then converted to a "corrected value" by multiplying that value to a ratio obtained by dividing the mean baseline hematocrit value by the mean hematocrit value on that day.

Serum cortisol was determined in serum samples collected on days -3, 0, 1, 2, 3 and 4, and was quantitated by a solid phase radioimmunoassay (Radioassay System Laboratories, Inc., Carson, CA).

Statistical analysis

All variables with repeated daily observations were analysed as a 2-factor experiment (day, treatment) using a split-plot in time analysis of variance (14). The effect of replication (repeat study) was also included in the analysis. The least significant difference (LSD) was used to effect separation of means.

Results

Clinical observations

A 3-day fast in calves receiving water ad libitum did not, by itself, result in any clinically apparent ill effects. After inoculation with IBR virus, all calves showed an increase in serous nasal discharge which tended to become mucoid and in some cases purulent after a few days; no difference was noticed between fasted and nonfasted control calves. Transient cough and diarrhea were observed in a few calves early during infection. The diarrhea usually lasted 2 days and was present in a few animals only, mainly in fasted calves upon refeeding. Dullness and lethargy were particularly noticeable in control calves on day 4. Ten of 17 (58%) calves developed fever of 40°C or greater, usually of 1 or 2 days duration, by the 4th or 5th postinoculation day. These calves were distributed approximately equally amongst groups.

Immune response to IBR virus

All 14 seronegative calves seroconverted by day 14. Two of the 3 calves with preexisting antibody titers developed a significant increase in serum neutralizing antibodies. Neu-

TABLE II Blastogenic response of whole blood cultures to inactivated IBR virus.

	Colf		Experimental days								
Gr	Calf - oup No.	0*	3	5	7	11	14	21			
1	(inoculated 24 hour	s into fast)									
	3	160(0)	0(0)	310(0)	13980(23)	530(0)	1410(5)	2602(6)			
	5	0(0)	0(0)	0(0)	200(0)	210(0)	0(0)	0(0)			
	8	1410(0)	0(0)	120(0)	0(0)	0(0)	0(0)	0(0)			
2	(inoculated at end o	f fast)			. ,	, ,					
	6	0(0)	0(0)	0(0)	770(2)	0(0)	0(0)	0(0)			
	11	440(0)	350(0)	460(0)	240(0)	340(0)	380(0)	0(0)			
	13	0(0)	0(0)	570(0)	140(0)	0(0)	0(0)	0(0)			
3	(nonfasted control)	• •			, ,						
	1 1	0(0)	0(0)	1100(3)	0(0)	0(0)	0(0)	0(0)			
	7	290(0)	0(0)	0(0)	480(0)	0(0)	300(0)	0(0)			

^{* =} all calves inoculated with IBR virus.

Data expressed as counts per minute (cpm); 0 = <100.

Values for stimulation index (SI) shown in parentheses: 0 = <2.

tralizing antibodies against IBR virus were not detected in the NS of calves on days 5 and 7, but were present in 7 out of 17 on day 14. Responses were similar between groups (Table I).

A significant CMI response, occurring on day 7, was noted in only 1 of the 8 calves tested. This calf was seropositive at the time of inoculation, presumably due to an earlier active immune response. Two other calves responded with low stimulation indexes (SI = 2 or 3), with responses in each noted only once, on day 5 and 7 after inoculation (Table II). Since differences in response between treatment groups were not evident, and since 8 of 9 calves used in the second (replicate) study were seronegative, these tests were not repeated.

Hematology

Mean leukocyte counts in all groups were within normal range on all days and neither fasting nor infection had any obvious deleterious effects (Table III). Mean hematocrit values during fasting, increased significantly (P<0.01) above baseline values before fasting (Table IV). In both fasted groups, mean values recorded 3 days after onset of the fast were also significantly higher than corresponding values for nonfasted calves. A mild degree of dehydration was thus associated with fasting. Infection itself did not appear to

affect hematocrit concentration.

Serum protein concentration

Mean total serum protein levels also increased significantly (P<0.01) during fasting. In both fasted groups, mean serum protein concentrations by the end of the 3-day fast were significantly higher than the respective mean concentrations obtained before fasting, with a significant increase already evident by 24 hours after the onset of the fast in one group (Table V). Mean A:G ratios decreased markedly during fasting as a result of an absolute decrease in albumin concentration (data not shown).

Since changes observed in both serum protein and hematocrit during fasting were very similar, we were curious to know how much of the changes in serum protein could be attributed to dehydration. After correcting for dehydration, the marked increases in serum protein during fasting were no longer evident (Table V). Significant changes in mean serum protein levels that could be attributed to viral infection were not observed.

Cortisol

A significant (P<0.01) increase in mean serum cortisol levels over prefast values was recorded in group 2 calves at the end of the fast (Table VI). Mean values in the other

TABLE III Mean total leukocyte counts per ml of whole blood.

	-	Experimental days									
Gro	up <u>—3</u>	0*	1	2	3	4	5	7			
1	(inoculated 24 hou 15600	rs into fast) 11500	12000	12600	14600	13800	13500	11200			
2	(inoculated at end		12000	12000	14000	13600	13300	11200			
	` 10600 (nonfasted control)	10200	12800	10600	11100	12000	11900	9700			
Ū	11000	11800	11100	10900	11000	12500	12800	11300			

^{* =} time of inoculation with IBR virus.

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TABLE IV Mean hematocrit values (Hct%).

	Experimental days									
Gro	up <u>—3</u>	0*	1	2	3	4	5	7		
1	(inoculated 24 hou	irs into fast)								
	32.0⁰	<u>36.0</u> ª	36.0^{a}	35.5ab	33.8abc	33.3bc	31.5⁰	31.30		
2	(inoculated at end									
	31.8⁰	36.7a	34.5ab	32.5bc	30.5◦	31.3¢	30.3⁰	30.8¢		
3	(nonfasted control)									
	32.6	33.8	33.9	32.5	32.0	32.8	32.5	32.3		

Values in same row with different lettered superscripts differ (P < 0.01).

Means obtained during fasting are underlined.

fasted group was highest at 24 hours after onset of the fast, but decreased thereafter. In both groups, a marked drop in mean values was noted within the first 24 hours after refeeding.

During infection, mean cortisol levels tended to decrease in all 3 groups. Even in the group inoculated during the fast, where the cortisol levels were expected to increase with fasting, a significant drop occurred between day 0 and 2.

Discussion

In this study, fasted calves were infected with a strain of IBR virus developed for intranasal vaccination (12). We utilized this strain, as opposed to a more virulent field strain, because attenuated strains are commonly used to vaccinate nutritionally stressed calves, either at the time of weaning or on arrival at feedlots. We measured neutralizing antibodies in serum and NS, and the blastogenic response of lymphocytes to inactivated IBR virus antigen. Serum protein levels, cortisol concentration, hematocrit values and leukocyte numbers were also measured during fasting. A 3-day fast was chosen since during this time a high percentage of newly received feeder or stocker beef calves do not eat (15).

Although clinical signs of disease were mild in all the three groups, the signs observed in our calves appeared to be more

TABLE V Mean total serum protein concentration (g/dl).

		Experimental days							
Gr	oup <u>—3</u>	0*	1	2	3	4	5	7	
1	(inoculated	24 hour	s into f	ast)					
	6.2c	6.9ab	7.0a	7.1a	6.6 ^b	6.2c	6.1¢	6.1€	
	(6.2)	(6.1)	(6.2)	(6.4)	(6.2)	(6.0)	(6.2)	(6.2)	
2	(inoculated	at end o	f fast)	•	• •	` ,	` ,	, ,	
	5.9cd	6.8a	6.46	6.2bc	5.8d	6.0cd	5.9cd	6.0cd	
	(5.9)	(5.8)	(5.9)	(6.1)	(6.1)	(6.1)	(6.2)	(6.2)	
3	(nonfasted o	control)	, ,	` ,	. ,	, ,	, ,	` ′	
	6.1	6.2	6.2	6.2	6.0	6.2	6.1	6.1	
	(6.1)	(6.0)	(6.0)	(6.2)	(6.1)	(6.2)	(6.2)	(6.1)	

Values in same row with different lettered superscripts differ (P < 0.01).

Means obtained during fasting are underlined.

Values in parentheses are values corrected for dehydration.

severe than those reported by others in a study using the same strain of virus (12). This may have been due, in part, to the different methods used to inoculate the virus, namely intranasal inoculation by aerosol vs instillation.

Seroconversion after vaccination with IBR virus is a functional measure of humoral immunity and one of the criteria used to determine whether vaccines meet licensing requirements. Malnutrition can affect specific antibody responses and both serum and secretory immunoglobulin levels; however, its effect on humoral immunity is complex and can vary depending on the severity and duration of malnutrition and on the type and dose of the antigen (11). In our study, neutralizing antibody response after virus inoculation was not different between groups. Sixteen of 17 calves developed significant increases (seroconversion or at least 4-fold increase in titer) in IBR serum antibodies, and the presence of serum neutralizing antibodies in 3 calves at the time of inoculation did not prevent them from excreting substantial amounts of virus for at least 5 days after virus inoculation (data not given). In NS, antibodies were first detected on day 14. This is in agreement with findings by some investigators (12, 16), while others have reported low levels of NS antibodies in some calves earlier (7 days) after intranasal vaccination with IBR virus (17) or none at all (18-20).

Cell-mediated immune response and interferon production, which occur earlier after infection, are also thought to play important roles in recovery from IBR virus infection (10). Although the primary purpose of this study was to investigate the effects of fasting on virus replication and interferon production (9), a specific parameter of CMI response, in vitro lymphocyte blast transformation using inactivated IBR virus as antigen, was measured at various times during infection. Epidemiological and clinical evidence suggests that CMI is affected by malnutrition (11). In the present study, lymphocytes collected from fasted and nonfasted calves had similar (essentially negative) blast transformation responses, with the exception of one fasted seropositive calf (sensitized animal) that responded with high SI. This is in agreement with observations that significant systemic lymphocyte responses were detected only after IBR virus challenge and not after primary infection with an IBR vaccine (16). Although we expected only sensitized calves to

^{* =} time of inoculation with IBR virus.

^{* =} time of inoculation with IBR virus.

TABLE VI Mean serum cortisol concentration (ug/dl).

			Experime	ental days		-
Gro	up <u>—3</u>	0*	1	2	3	4
1	(inoculated 24	hours into	fast)			
	3.23abc	4.26a	3.53ab	2.43bcd	1.50d	1.90 ^{cd}
2	(inoculated at	end of fas	st)			
	1.56 ^b	4.25a	2.40b	1.87b	1.45b	1.30b
3	(nonfasted co	ntrol)				
	2.66	2.72	1.63	1.63	1.61	1.31

Values in same row with different lettered superscripts differ (P < 0.01).

Means obtained during fasting are underlined.

respond positively, we did not know how fasting would affect the response of calves not previously exposed to IBR virus. Protein deficiency in mice has been reported to enhance rather than depress cell-mediated immunity (7).

Although water was provided ad libitum during the fast, some dehydration was observed in fasted calves as indicated by significant increases in hematocrit. This was not unexpected, since cattle deprived of food also limit their water intake, and similar degrees of hemoconcentration are observed whether or not water is provided (21). Significant increases in protein concentration, which we attributed to dehydration, have also been reported in steers after a 5-day fast (22).

Since lack of food is a form of stress, any changes observed or masked in our calves might have been mediated purely through stress, as evidenced by increased cortisol levels. Although serum cortisol levels were expected to increase during fasting, only in one group was the rise significant. Mean values in this group peaked at the end of fasting at the time of inoculation. After virus inoculation, mean values tended to decline in all groups with no significant differences noted between fasted and fed groups. The higher values at time of inoculation did not result in higher overall virus excretion, nor were they related to differences in interferon production (9).

Although the 3-day fast significantly affected some of the serum and blood components measured, it did not adversely affect the immune response of calves as measured in this study. Systemic and local neutralizing antibody responses were not compromized, and one fasted calf actually developed a significant CMI response to inactivated IBR virus during infection. These results are consistent with recent observations in humans that acute starvation or fasting are not always deleterious on immune functions (23). Although the effects of fasting on other mediators of host resistance (i.e. alveolar macrophages, complement, neutrophils, natural killer cells, interleukins) were not measured in this study, others have reported enhanced monocyte bactericidal activity and natural killer cell cytolytic activity during a 14-day fast in humans (23). In view of these observations, and of the fact that enhanced nasal interferon

levels were reported in these fasted calves (9), we conclude that acute nutritional deprivation may not necessarily increase the susceptibility of calves to viral infections, nor adversely affect their immune response after vaccination.

Acknowledgements

Supported by USDA-ARS Cooperative Agreement 12-14-3001-522 and by Agricultural Experiment Station Project No. MO-00254. Contributions from the Missouri Agricultural Experiment Station, Journal Series No. 10134.

This report represents a portion of a dissertation submitted by the senior author to the graduate faculty of the University of Missouri in partial fulfillment of the requirements for the PhD degree.

The authors thank Gary Krause and Mark Ellersieck for aid in statistical analysis and Gerald Buening for help in lymphocyte transformation tests.

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^{* =} time of inoculation with IBR virus.

infectious bovine rhinotracheitis virus after infection by viral aerosol or intramuscular inoculation. Am J Vet Res 38:1497-1502, 1977. 20. Jericho KWF and Babiuk LA: The effect of dose, route and virulence of bovine herpesvirus 1 vaccine on experimental respiratory disease in cattle. Can J Comp Med 47:133-139, 1983. 21. Rumsey TS and Bond J: Cardiorespiratory patterns, rectal temperature, serum electrolytes and packed cell

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Abstracts

Inactive ovaries in high-yielding dairy cows before service: Aetiology and effect on conception

O. Markusfeld

Veterinary Record (1987) 121, 149-153

The overall rate of inactive ovaries diagnosed by two consecutive rectal examinations was 8.5 per cent for 7751 lactations. Inactive ovaries had an inverse association with increasing parity and were directly associated with twinning, retained placenta, primary metritis and high milk yield after calving in heifers. No independent associations were established with stillbirth, a low milk yield in the last 120 days before calving, a long dry period, ketonuria or a high serum glutamate oxaloacetate transaminase activity in the first week after calving. The presence of inactive ovaries in the previous lactation was a significant predictor of the trait. Cows with either metritis or inactive ovaries were at a greater independent risk of not conceiving within 150 days after calving and the combined risk associated with both factors was higher than their sum. It is concluded that while damage to the uterus repeatability and an energy shortage after calving are responsible for ovarian inactivity, this trait was not associated with fatty liver. Such an association could be due to an indirect causal relationship, the outcome of post parturient uterine diseases which are associated independently with overfeeding before calving and inactive

Effect of vaccination of the dam on rotavirus infection in young calves

M. S. McNulty, E. F. Logan

Veterinary Record (1987) 120, 250-252

Vaccination of cows with a combined, inactivated, adjuvanted rotavirus and $Escherichia\ coli$ vaccine resulted in increased neutralising antibody titres to rotavirus in serum and colostral whey. Evidence was obtained that vaccination resulted in a decreased incidence of rotavirus shedding and of abnormal faeces or diarrhoea in young calves fed colostrum and milk from the vaccinated dams. The $E\ coli$ component of the vaccine was not evaluated because no natural challenge was evident.

Oxfendazole pulse release intraruminal devices and bovine parasitic bronchitis: Comparison of two control strategies in a field experiment

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Veterinary Record (1987) 121, 221-224

Lungworm-infected seeder calves were used on two 1.41 ha paddocks to ensure that groups of 11 susceptible trial calves would be exposed to heavy early season challenge with Dictyocaulus viviparus. This produced conditions for an artificially severe test of two control strategies. The first employed a front-loaded oxfendazole pulse release bolus, ie, an intraruminal device which released one therapeutic anthelmintic dose immediately and five subsequent pulses at approximately three-weekly intervals. These front-loaded boluses were given to five of 11 calves on one paddock as soon as parasitic bronchitis had become clinically obvious (34 days after turnout) while the remaining six calves were kept as untreated controls. Clinical signs quickly subsided in the treated animals and no further respiratory problems occurred despite continued exposure to reinfection. The other control strategy involved the administration at turnout of an oxfendazole pulse release device which released the first of five anthelmintic doses approximately three weeks after administration, to all 11 calves on the other paddock. This strategy was almost completely successful in preventing patent infections from establishing and reduced the infectivity of the pasture in August and September by 94-1 per cent as shown by tracer calf studies. The calves treated at turnout performed better than the calves treated with the front-loaded boluses for most of the season and had an average weight-gain advantage of 20.4 kg at housing (P<0.01). Prepatent disease did, however, occur when the susceptible calves were exposed to heavy challenge after the first anthelmintic pulse had been released from the bolus three weeks after turnout. Two calves required emergency treatment but otherwise the disease episode was terminated by the second pulse of oxfendazole.