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Experimental Neonatal Colibacillosis in Cows: Serological Studies

R. A. WILSON[1]* AND J. W. JUTILA

Department of Microbiology, Montana State University, Bozeman, Montana 59715

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Serological studies of passive immunity in experimentally induced bovine colibacillosis was studied in a 41-cow university herd. Pregnant dams were antigenized prepartum with two injections administered by the subcutaneous and intrammamary routes with one of four vaccine preparations (killed bacteria, live bacteria, culture supernatant, or heart infusion broth [control]). The data indicate that 77% of the neonates born to vaccinated dams were strongly protected against oral challenge with *Escherichia coli* strain B-44. Bacterial agglutinin and passive hemagglutination titers of colostral whey directly reflected the efficacy of the vaccines. A notable decrease in the whey titers to somatic and capsular antigens occurred after heat treatment at 56 C for 30 min. Complicity of heat-labile immune factor(s) in protection from scouring was suggested. The nature of the protective antigen is not clearly defined by these studies but there is some evidence that the K antigen may play a vital role in this regard.

Neonatal calf diarrhea (calf scours) is an acute, often fatal infection of the young bovine presenting a myriad of signs including diarrhea, dehydration, malaise, prostration, and finally death. There is evidence that *Escherichia coli* can be the etiological agent of calf scours and that different strains may be involved (13; G. Wramby, Abstr. Vet. Bull. 19:261-262, 1949). Since *E. coli* are common to the intestinal tract of calves (23), it is often difficult to show clear etiological relationships.

Certain cellular constituents of *E. coli* have been examined for their role in promoting intestinal disorders including the O somatic antigen, K capsular antigens, and the enterotoxin(s) produced by some strains of enteropathogenic *E. coli*. Although the O somatic antigenic material may not be involved per se with the pathogenicity and virulence of *E. coli*, the endotoxin portion of the O antigen may act in concert with the virulence factors associated with enteropathogenic coliforms (13).

There is evidence that would implicate K antigens in the virulence of *E. coli* (14). These studies indicated that K antigen reduces the complement sensitivity of *E. coli* by covering the antigenic determinants of the O antigen. It has also been shown that certain K antigens contribute to the tissue adherent qualities of some strains of *E. coli*. Studies by Smith and Linggood (24) indicate that the common K antigen (KcO) produced by certain calf and lamb enteropathogenic *E. coli* may function in a manner similar to K-88 antigens in the pig (16), that is, by facilitating adherence to the intestinal epithelium.

Agglutinin activities in sera or whey from antigenized dams has been used in the past to assess the efficacy of *E. coli* vaccines (8, 12). These studies generally evaluate the effect of route and adjuvants upon the immune response. In a recent clinical trial, designed to evaluate the effectivenss of various *E. coli* vaccines to stimulate immunity in dams and passively transfer to suckling calves, a marked protection of calves from experimentally induced scours was observed (19).The challenge organisms, *E. coli* B-44, caused severe diarrhea only in calves born to control dams, whereas calves born to specifically immunized dams were strongly protected. This report describes the serological parameters of the cow/calf unit described in the earlier communication (19).

MATERIALS AND METHODS

Test animals. A 41-cow herd of pregnant Hereford dams was randomly distributed into three experimental groups of nine cows each and one control group of 14 cows as described earlier (19). Each group was maintained in a separate outdoor pen and provided hay once daily and water ad libitum.

Test organism. The strain of *E. coli* selected for this experiment was strain B-44 (serotype O9:K:NM) furnished by H. W. Smith of Great Britain.

[1] Present address: Department of Veterinary Microbiology, Syntex Research, Palo Alto, Calif. 94043.

E. coli B-44 is nonhemolytic, prototrophic, colicin positive, lactose positive, and demonstrates both smooth mucoid and rough nonmucoid morphology. This strain gives positive ligated gut loops in both the calf and rabbit. The smooth-mucoid colony type was used in the production of culture supernatant and live and killed vaccine, as well as for challenge of the neonatal calves.

Vaccines. The vaccines were described in a previous paper (19). Briefly, B-44 was grown in brain heart infusion broth at 37 C with constant shaking. The live vaccine consisted of a 24-h culture of the test organism which contained about 3×10^{10} viable cells/ml. The killed vaccine contained washed, formalinized (0.2% vol/vol) B-44 organisms at a concentration of approximately 6×10^{10} cells/ml. The culture supernatant preparation was obtained by centrifugation and filtration of brain heart infusion broth culture. The concentrate of this material was dialyzed against water at 4 C before formalin (0.20% vol/vol) was added.

Vaccines were emulsified in an equal volume of adjuvant (96% mineral oil and 4% emulsifier) for administration. The control vaccine consisted of brain heart infusion broth mixed with adjuvant.

Vaccination protocol. Each cow was given 5 ml of antigen plus adjuvant subcutaneously and 4 ml of vaccine by the intramammary route (1 ml per quarter) twice in a 2-week interval. Antigenization was initiated 3 weeks before expected calving for the herd and the second dose was administered 2 weeks later.

Neonatal challenge. The herd was under maximum surveillance to ensure that newborn calves would not have access to colostrum prior to the acquisition of the first postpartum blood specimen. At parturition, the calf was moved immediately to the clinic and the dam was put into a chute to facilitate collection of specimens. Calves were bled from the jugular vein and each calf was cultured by rectal swab. The cows were bled (20 ml) from the jugular vein and a separate sample of colostrum was taken from each quarter. Thereafter, the cow/calf unit was maintained in a small pen to be certain the calf could obtain colostrum prior to challenge. Challenge organisms were administered before the calves were 10 h old. Calves were challenged by gavage with 50 ml of an 18-h culture of *E. coli* B-44. After challenge, the cow/calf unit was moved to a common pen separate from the pregnant dams. The cow/calf units had access to shelter and dry bedding.

Time of onset, duration, and severity of diarrhea (scored as 0, 1+, 2+, 3+, 4+) were determined for each calf (Table 1). A zero score means no diarrhea was observed. Diarrhea of 1+ was assigned if the calf was generally healthy and had normal rectal temperatures (approximately 38.9 C), diarrhea, and no signs of dehydration. Diarrhea was scored as 2+ if the calf had normal temperature, diarrhea, concavity of flanks, some dehydration indicated as partial recession of the eyes, and slight apathy. The criteria for the 3+ score was temperature higher than 37.8 C, diarrhea, moderate dehydration indicated by progressive recession of the eyes, and weakness (but calf retained ability to stand). The disease was scored 4+ when the rectal temperature was less than 37.8 C (often 34 to 36 C), and when the calf had diarrhea, severe dehydration, skin pliability, marked recession of the eyes, and was unable to stand or on occasion was prostrate.

TABLE 1. *Clinical evaluation of calves after challenge*

Vaccine group of dam	Cow-calf no.	Signs of neonatal calf diarrhea: Onset of diarrhea after challenge (h)	Signs of neonatal calf diarrhea: Duration of diarrhea (h)	Score[a]
Control	440-202	21	36	1+
	422-209	7	40	4+
	419-210	5	31	4+
	443-212	7	48	4+
	408-215	7	36	4+
	405-221	6	27	4+
	430-222	6	29	4+
	427-225	11	43	4+
	441-227	8	40	4+
	620-229	5	32	4+
	413-230	4	15	4+
	446-234	4	20	4+
	628-236	8	48	3+
	411-240	7	33	4+
CS	442-201	11	8	1+
	417-204	Negative	0	0
	622-207	12	48	1+
	928-216	6	12	1+
	630-228	9	33	1+
	421-233	4	13	4+
	433-235	4	72	4+
	961-237	6	27	4+
	410-238	17	29	1+
LB	650-206	11	33	4+
	426-208	13	48	4+
	403-211	7	24	1+
	450-214	Negative	0	0
	429-217	6	36	1+
	649-218	5	15	1+
	407-223	Negative	0	0
	937-224	Negative	0	0
	922-239	4	25	1+
KB	432-203	0	0	0
	618-205	7	48	1+
	948-213	Born dead (cause unknown)		
	943-219	Negative	0	0
	921-220	13	12	1+
	444-226	Negative	0	0
	404-231	Born dead (cause unknown)		
	439-232	Negative	0	0
	434-241	Negative	0	0

[a] See text.

Agglutination studies. Bacterial agglutination

studies were done in accordance with methods described in Campbell et al. (5). Somatic antigens described as O antigens were prepared from 18-h broth culture. Cells were washed two times with sterile saline (0.85%) before boiling for 1 h. Boiled cells were then washed with sterile saline and suspended at a final concentration of 10^9 organisms/ml (nephelometric measurement). Titrations performed using B-44 cells, boiled for 1 h, were subsequently shown to include anti-K antibodies indicating that 1 h of boiling does not entirely remove all of the capsular material from this strain of *E. coli*.

External or capsular antigens, designated as K antigens, were prepared from 18-h cultures of sedimented cells washed in the same manner as described above. Packed cells were suspended in formalin (0.20% vol/vol) at a final concentration of approximately 10^9 organisms/ml.

Passive hemagglutination experiments were performed by the method outlined in Campbell et al. (5). Endotoxin (lipopolysaccharide) was extracted from *E. coli* B-44 using the phenol water method of Westphal and Jann (25). Sheep erythrocytes were sensitized to B-44 LPS according to the technique of Neter et al. (20).

Blood samples. On day 1 of the experiment, just before the administration of antigen, a blood sample was taken from each cow by the jugular vein. Subsequent blood samples were obtained at calving and again 3 months postpartum. Blood samples from calves were obtained at the time of birth and at 4 days of age. Sera were stored at −20 C until tested and heated at 56 C for 30 min immediately before titrations were performed.

Whey samples. Colostrum and milk samples were treated with renin (stock solution diluted 1:300) and calcium chloride in a concentration sufficient to create a firm clot at 42 C. Anti-O and anti-K titers were performed on unheated whey samples and on whey samples that had been heated for 30 min at 56 C. Passive hemagglutination titers were determined for heated whey only, since earlier studies in this laboratory indicated an insignificant difference in passive hemagglutination titers before and after such heat treatment.

RESULTS

Passive immunization in experimental colibacillosis: a clinical evaluation. (i) Characteristics of strain B-44. In the preliminary study of strain B-44, viable cells, filtrates, and concentrated toxin preparations caused distention of ligated intestinal segments in rabbits and calves. Strain B-44 had both rough and smooth colony types, and both forms caused distention of ligated intestinal segments. Only the smooth form of strain B-44 was used in these experiments. The viable cell count of strain B-44 grown in heart infusion broth at 37 C for 24 h with vigorous aeration was approximately 3×10^{10} cells/ml.

(ii) Response of calves to oral challenge with live E. coli. A calf scour index was derived by determining the mean diarrhea score for each experimental group from the data presented in Table 1. The control calves had a scour index of 3.7, which is markedly higher than the indexes calculated for the calves in the vaccine groups.

The calf scour index indicates that the calves in the killed vaccine (KB) group had the lowest index, 0.3, followed by 1.3 and 1.9 for the calves in the live bacteria (LB) and culture supernatant (CS) groups, respectively (the index represents an average of the degree of scours based on the clinical evaluation of calves in each group). The onset of diarrhea (Table 1) varied between 4 and 21 h after the calves were challenged and was essentially the same for both the control and vaccine groups. The duration varied from 12 to 72 h, at which time the calves either had expired or were removed from the experiment.

Clinically, all calves gaining a score of 4+ diarrhea had profuse watery diarrhea which appeared several hours before the initial onset of the accompanying signs of dehydration (sunken eyes). As dehydration progressed apathy and anorexia were noted, and then the calf was unable to stand, body temperature decreased, and if untreated, the calf became prostrate and died. Bacteremia was not detected until the calves were moribund. At necropsy, gross pathological changes were not seen.

(iii) Bacteriological evaluation of fecal specimens. Fecal swabs collected from 19 of 39 calves at time of challenge did not produce growth of *E. coli* on Tergitol-7 agar with triphenyltetrazolium chloride. In fecal swabs collected from the remaining calves at challenge a few coliforms of the rough form were isolated. Only two calves were excreting significant numbers of coliform organisms exhibiting smooth colony at the time of challenge.

After calves were challenged with strain B-44, the population of *E. coli* increased, with the majority being smooth and mucoid and having morphological properties identical to those of strain B-44. Smooth organisms resembling strain B-44 were initially isolated from fecal swabs of every calf between 5 and 24 h after challenge. The appearance of the various colony types remained constant for 4 days of fecal swab collections. Usually, rough colony types emerged and became approximately equal in number to the smooth, mucoid form by day 4 of collection.

Serological studies. (i) O agglutinins of the dam. The titration of the preimmune sera from all dams yielded undetectable or negative O agglutinin titers. The titration of cow serum 2 weeks after a single antigenization revealed

TABLE 2. *Cow serum titers 2 weeks after a single antigenization and at parturition*

Experimental group[a]	Anti-O[b]		Anti-K[b]		PHA[b]	
	2 weeks post-injection	At parturition[c]	2 weeks post-injection	At parturition[c]	2 weeks postinjection	At parturition[c]
C	<10[d]	<10[d]	<10[d]	<10[d]	930[d]	1,274[d]
CS	<10	21	<10	<10	2,416	5,440
LB	20	63	10	14	1,719	6,948
KB	80	110	10	14	2,321	6,656

[a] C, Unvaccinated dams.
[b] Anti-O, Reciprocal of the mean titer of agglutinins to *E. coli* B-44 boiled cells; Anti-K, formalinized *E. coli* B-44 cells; PHA (passive hemagglutination), *E. coli* B-44 LPS-sensitized sheep erythrocytes.
[c] Eight to 66 days from the time of the second antigenization.
[d] Serum heated at 56 C for 30 min.

TABLE 3. *Summary of statistical analysis[a] of results*

Data analyzed	Significance between groups[b]			
Anti-O (2 weeks pi)	C	CS	LB	KB
Anti-O (at parturition)	C	CS	LB	KB
Anti-K (2 weeks pi)	C	CS	LB	KB
Anti-K (at parturition)	C	CS	LB	KB
PHA (at 2 weeks)	C	CS	LB	KB
PHA (at parturition)	C	CS	LB	KB

[a] Test of comparison among means by least square difference. PHA, passive hemagglutination; pi, postinfection.
[b] Means not underlined by the same line are different from each other ($P < 0.05$).

TABLE 4. *Agglutinin titers of heated and unheated colostral whey from immune and nonimmune dams*

Experimental group[a]	Anti-O[b]		Anti-K[b]		PHA[b] heated
	Unheated	Heated[d]	Unheated	Heated	
C	19	10	10	10	2,720
CS	77	27	14	10	12,581
LB	134	38	39	16	21,809
KB	276	190	69	54	37,050

[a,b,d] See Table 2.

TABLE 5. *Summary of statistical analysis[a] of results in Table 4*

Data analyzed	Significance between groups[b]			
Anti-O (unheated)	C	CS	LB	KB
Anti-O (heated)[c]	C	CS	LB	KB
Anti-K (unheated)	C	CS	LB	KB
Anti-K (heated)	C	CS	LB	KB
PHA[d] (heated)	C	CS	LB	KB

[a,b] See Table 3.
[c] Heated at 56 C for 30 min.
[d] See Table 2, footnote *b*.

that neither dams in the control group nor in the culture supernatant group had detectable O agglutinins in their serum (Table 2). Dams vaccinated with LB had a mean titer of 20 compared to a mean titer of 80 in the sera of the dams antigenized with KB. These values are not significantly ($P > 0.05$) different (Table 3). The mean titer in serum from the KB group, however, is significantly higher than the control group.

Assays of sera obtained at the time of parturition detected antibody activity in the sera of the CS-treated dams but the titers (21) were very low. A slight increase in titer was seen in the sera of dams in the KB group (110), whereas a threefold increase (63) was observed in the sera of dams antigenized twice with LB.

Investigations conducted a year after these titrations were performed indicated that the procedure used to prepare the O somatic antigens was not rigorous enough to eliminate all of the capsular material from the bacterial cells. Therefore, the O agglutination titers reported may have included a minor contribution by anti-K antibodies present in the sera and fail to express the exact titer. Additionally, K antigen present on the cells may have obscured O determinants.

Colostral whey from immune and nonimmune dams was titrated before and after heating at 56 C for 30 min. A very low titer of anti-O activity was detected in the unheated whey from the control dams, whereas mean titers of 276, 134, and 77 were measured in unheated whey from dams antigenized twice with KB, LB, and CS, respectively (Tables 4 and 5). After heating, the titers dropped 32% in the KB group, 52% in the LB group, and 65% in the CS group. All of the titers, however, were still significantly higher than the mean whey titers of the nonimmunized dams. Agglutinating ac-

TABLE 6. *Calf serum titers at birth and at 4 days old*

Experimental group[a]	Anti-O[b]		Anti-K[b]		PHA[b]	
	At birth	4 days old	At birth	4 days old	At birth	4 days old
C	<10[c]	<10[c]	<10[c]	<10[c]	<40[c]	24[c]
CS	<10	56	12	60	40	214
LB	<10	90	<10	240	<10	1,920
KB	<10	97	<10	121	<10	1,496

[a,b,c] See Table 2.

tivity was not detectable in whey from milk obtained 3 months postpartum in dams given CS or bacterins.

(ii) O agglutinins of the calf. The O agglutinin titers were estimated on serum harvested from calves before colostral feeding and 4 days post-parturition. The results of these titrations are found in Table 6. The data show that few of the calves had antibody activity (mean = 10). Less than three calves in each of the antigenized groups demonstrated titers at birth and none of the titers exceeded 40.

The data show a significant (Tables 6 and 7) increase in the antibody titers in the sera of 4-day-old calves born to KB-, LB-, and CS-vaccinated dams as contrasted to the precolostral serum. None of the control calves acquired detectable serum antibody against the test organism by 4 days of age.

(iii) K agglutinins of the dam. Cow serum obtained before antigenization and again 2 weeks later failed to agglutinate a formalinized preparation of *E. coli* B-44. At parturition, no antibody activity was detected in the serum of dams in the control nor CS groups. A very weak humoral response, to the capsular antigens, was detected only in the sera of dams that had been immunized twice with KB or LB.

Titrations were performed on whey before and after heating at 56 C for 30 min. Both heated and unheated whey from control dams were devoid of antibody activity against the capsular antigens. An insignificant titer (mean = 14) was measured in the unheated whey from dams in the CS group. The antibody contributing to this activity was inactivated by heat. The mean titers of the KB and the LB groups of 69 and 39, respectively, are significantly ($P < 0.01$) different (Table 3) and are also significantly higher than the control titers.

Heating the wheys from the KB group decreased the antibody activity by 22% while decreasing the activity in the LB groups by 51%. None of the milk specimens tested had detectable agglutinating antibody 90 to 120 days after calving.

(iv) K agglutinins of the calf. Except for the low titer of 12 in the CS vaccine group, serum specimens obtained from precolostral calves lacked agglutinating activity to the K antigens of *E. coli* B-44 (Table 6). However, considerable antibody activity was apparently acquired from colostrum by the calves in the CS, LB, and KB groups. Thus, calves in the KB group had a mean titer of 240 compared to titers of 12 and 60 in sera of calves in the LB and CS groups, respectively. The difference observed among groups was not statistically significant ($P > 0.05$) (Table 7).

TABLE 7. *Summary of statistical analysis[a] of results in Table 5*

Data analyzed	Significance between groups[b] ($P < 0.05$)			
Anti-O (at birth)	C	CS	LB	KB
Anti-O (4 days old)	C	CS	LB	KB
Anti-K (at birth)	C	CS	LB	KB
Anti-K (4 days old)	C	CS	LB	KB
PHA (at birth)	C	CS	LB	KB
PHA[c] (4 days old)	C	CS	LB	KB

[a,b] See Table 3.
[c] See Table 2, footnote *b*.

(v) Passive hemagglutination activity of the dam. Antibody activity against the LPS moiety of B-44 was not measured in cow serum prior to antigenization. Two weeks after a single antigenization and again at parturition the cows were tested for serum passive hemagglutination activity. The results are given in Table 4.

After a single antigenization, the serum passive hemagglutination activities detected in the vaccine groups were not significantly different (Table 5). However, the titers were significantly different at parturition. The serum titers showed a threefold increase in all of the vaccine groups, whereas titers in the control group did not show an appreciable change.

The highest colostral whey titer detected in the KB group had a mean titer of 37,000 as comapred to a titer of 12,500 in the CS group and 21,000 in the LB group (Table 4). The differ-

ence among these groups is not significant at the 0.05 level (Table 5). However, the results are significantly different between the vaccine groups and the controls. Contrasting the mean whey titers to the serum titers in the vaccine groups indicated that two to six times more anti-LPS activity was present in the whey of immune cows.

The relative difference between the vaccine groups can be seen in the result of the O antibody titers measured in unheated whey (Table 4).

DISCUSSION

These studies, designated to evaluate the efficiency of three different vaccine preparations, indicated that subcutaneous and intramammary antigenization of preparturient dams afforded significant protection to the neonatal calves. Seventy-seven percent of the calves born to vaccinated dams were protected against an oral challenge with *E. coli* B-44. On the contrary, only two of 14 calves born to dams in the control group failed to reach a 4+ scour condition after oral challenge with the test organism.

A semiquantitative index, derived from the mean diarrhea score for each group of calves, showed that the KB vaccine was superior to the other three preparations. Next in the order of efficiency were the LB and CS vaccine preparation. Interestingly, the CS preparation apparently contained enough somatic and capsular material to elicit a response sufficient to react with the challenge organisms and protect the neonates.

It was noted early in the experiments that challenging with strain B-44 after 20 h of age was clearly less detrimental to the calf than an oral challenge administered within the first 10 h of life. The only calf in the control group that had less than 3+ diarrhea had been challenged beyond 12 h of age; hence, a relationship between age and susceptibility was suggested.

It is known that newborn calves absorb proteins more efficiently during the first 18 to 20 h of life (10), and further, that strains of enteric organisms begin to establish in the intestinal tract at 18 to 24 h of age (23). It would follow then that transfer of bacterial enterotoxins as well as immunoglobulins would be influenced by the time related physiological mechanisms in the neonatal intestine. In addition, it is possible that endotoxin and enterotoxin substances are being released from dead and metabolically active bacteria, respectively, during the time period when the small intestine is exquisitely sensitive and vulnerable to molecular cytotoxic reactions. These factors quite likely contribute to field cases of neonatal diarrhea (scours) as well.

The time of onset and duration of the clinical syndrome caused by strain B-44 (profuse diarrhea, severe dehydration, apathy, decrease in body temperature and morbidity, death) and the absence of bacteremia before the moribund state was reached are typical of field cases of the disease. Bywater (4) reported that physiological changes caused by strain B-44 enterotoxin included a net increase in secretion of fluid, sodium, bicarbonate, and chloride from the blood into the small intestine of the calf. Similar physiological changes in calves have been associated with naturally occurring neonatal diarrhea (7, 11, 18).

Results of the serological studies indicate that, 2 weeks after a single antigenization, a significant agglutinin response to O somatic and K antigen was not detectable in serum of dams. At parturition (8 to 66 days after the second antigenization), only the anti-O serum titers were significantly different from titers of the control dams. Anti-K activity still was not detected in the sera of the controls or the vaccine groups at parturition. These data would agree with the findings of others, that is, the O-agglutinating titers in sera of vaccinated dams are generally higher than are the titers detected against capsular antigens of the vaccine strain (12).

The present studies would indicate that the KB, LB, and CS vaccines, in that order, were effective in eliciting responses to the O-somatic antigens. No such ordering of vaccine efficacy was possible using anti-K titers. Therefore, since both anti-O and anti-K antibody activity are highly likely to be involved in passive immunity, an ordering of the effectiveness of KB, LB, and CS vaccines from the results of serum activity of the dam is questionable.

Since the importance of colostrum for the survival of the calf has been established (1, 9, 15), an evaluation of the three vaccines for production of protective factors in colostrum would be an appropriate indication of vaccine efficiency. Since colostrum-derived antibody had been shown by many investigators to be a critical factor in passive immunity in neonatal calves (2, 3, 26), it would follow that an effective vaccine should elicit protective antibodies that will predominate in the dam's colostrum.

The somatic antigens remaining after heat treatment (boiling for 1 h) were reacted against both heated (56 C, 30 min) and unheated whey obtained from individual quarters of the udder of each cow. In all groups, the titers detected in the unheated whey were distinctly higher than corresponding titers in the heated specimens.

The greatest response, measured as agglutinating activity, was noted in whey from dams antigenized with KB. The agglutinating titer in the LB group was one-half the titer detected in the KB group. Surprisingly, the mean agglutinin response made by dams in the CS group was, again, one-half the titer detected in the LB group. The low agglutinin titers of the control dams were most likely due to low avidity, cross-reactive antibodies directed against the common antigen carried by all *Enterobacteriaceae*.

One of the most interesting findings in the agglutination studies centered about the effect of heat upon the whey titers. For example, LB and KB anti-O titers were not significantly different ($P > 0.05$) in unheated whey but differ significantly in heated specimens. CS titers were not different ($P > 0.05$) from control titers after the whey samples were heated (whey samples were heated prior to titration).

The effect of heat upon whey agglutinins (anti-O) was seen in the results of the anti-K titrations as well. The anti-K titers in colostral whey were analyzed by the least square difference statistical test. Titers of heated whey from dams antigenized with CS were not significantly higher than the titers in the control group. In addition, the mean anti-K titer in the LB group was significantly ($P < 0.05$) lower than the mean titer in the KB group. However, both the KB and LB groups had mean titers significantly higher ($P < 0.05$) than the mean titers in control whey. These data indicate that the CS was not as effective in stimulating anti-K agglutinins as KB or LB. Moreover, the efficacy of the KB and LB vaccine preparations, reflected by anti-O and anti-K titers, was appropriately reflected in the scouring index as well.

The effect of the factor(s) upon the agglutinating activity in whey is apparent; the effect in vivo is unknown. Whether or not the heat-labile factor(s) represents an antibody to common enterobacterial antigen was not determined.

The strong relationship between agglutination titers of unheated whey and vaccine effectiveness suggests the complicity of a heat-labile immune factor(s) in protection from scouring.

The passive hemagglutination titers in serum and whey of vaccinated cows, 2 weeks post-antigenization and at parturition, were significantly higher than titers observed in the sera and whey of control cows. None of the mean titers in any of the vaccine groups, however, were significantly different. The relative difference seen, however, is similar to differences noted in the data for anti-O titers of both heated and unheated whey. Furthermore, the relatedness of mean passive hemagglutination and anti-O titers in whey specimens correlate well with the clinical findings.

Since endotoxin has not been shown to be a critical effector in *E. coli* enterotoxemias, it is doubtful that the passive hemagglutination antibody detected in passive hemagglutination assays functions in the protection against *E. coli*. However, immunological or physiological activities contributing to the death of enteric organisms coupled with a breakdown of the integrity of the intestinal mucus membrane (produced by enterotoxin) may require the interaction of endotoxin neutralizing antibodies. It is apparent from these studies that anti-LPS agglutinating antibody is present in significant amounts in the serum and whey of the vaccinated dams, and like the anti-O agglutinins, deserve consideration as significant factors in scouring.

Since absorption of colostral antibodies is limited to the first 24 to 36 h of life (9), it was not surprising that at birth these calves did not exhibit agglutinating activity against either the O or K antigens. Of particular interest was the passive hemagglutination activity detected in precolostral calf serum in each of the groups, including the control calves. In utero response to an LPS moiety could account for the antibody activity since immunocompetence has been demonstrated in another ungulate, the ovine (22, 21), and more recently in the fetal calf (6).

In contrast to the control calves, the calves in the vaccine groups showed an increase in serum agglutinin activity by day 4. The mean O titers were not significantly different between immune groups, whereas the O titers in colostrum were significantly different. Perhaps the most significant observation in these studies related to the marked increase in the anti-K activity in sera of calves born to all vaccinated dams. The response was most pronounced in the KB and LB groups and stands in contrast to the lack of anti-K activity in the dams' sera. These results strongly suggest then that an effective response to O somatic and K antigens of *E. coli* was produced in dams and that passive transfer of immunity to calves via colostrum was manifest as a marked diminution of the symptoms of and death from scouring. The nature of the protective antigen is not clearly defined by these studies but there is some evidence that the K antigen may play a vital role in this regard.

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LITERATURE CITED

1. Aschaffenburg, R., S. Bartlett, S. K. Kon, P. Terry, S. Y. Thompson, D. M. Walker, C. Briggs, E. Cotchin, and R. Lovell. 1949. The nutritive value of colostrum for the calf. I. The effect of different fractions of colostrum. Br. J. Nutr. 3:187–196.
2. Briggs, C. 1951. The nutritive value of colostrum for the calf. The K antigens of *E. coli*. Br. J. Nutr. 5:349–355.
3. Briggs, C., R. Covell, R. Aschaffenburg, S. Bartlett, S. K. Kon, J. A. B. Roy, S. Y. Thompson, and D. M. Walker. 1951. Nutritive value of colostrum for the calf. VII. Observations on the nature of the protective properties of colostrum. Br. J. Nutr. 5:356–362.
4. Bywater, R. J. 1970. "Some effects of *Escherichia coli* enterotoxin on net fluid, glucose and electrolyte transfer in calf small intestine. J. Comp. Pathol. 80:565–573.
5. Campbell, D. H., J. S. Garvey, N. E. Cremer, and D. H. Sussdorf (ed.). 1964. Methods in immunology, p. 166–167. W. A. Benjamin Co., New York.
6. Conner, G. H., M. Richardson, and G. R. Carter. 1973. Prenatal immunization and protection of the newborn: ovine and bovine fetuses vaccinated with *Escherichia coli* antigen by the oral route and exposed to challenge inoculum at birth. Am. J. Vet. Res. 34:737–741.
7. Dalton, R. G., E. W. Fisher, and W. I. M. McIntyre. 1965. Changes in blood chemistry, body weight and hematocrit of calves affected with neonatal diarrhea. Br. Vet. J. 121:34–41.
8. Dam, A. 1968. Studies on the gammaglobulin levels in sera of calves from herds with colisepticaemia as a problem, and some investigations on the content of specific antibodies in colostrum. Nord. Veterinaermed. 20:449–457.
9. Fey, H. 1971. Immunology of the newborn calf: its relationship to colisepticemia. Ann. N. Y. Acad. Sci. 176:49–00.
10. Fey, H., 1972. Colibacillosis in calves. Hans Huber Publishers, Vienna.
11. Fisher, E.W. 1965. Death in neonatal calf diarrhea. Br. Vet. J. 121:132–138.
12. Gay, C. C. 1965. *Escherichia coli* and neonatal disease of calves. Bacteriol. Rev. 29:75–101.
13. Gay, C. C., K. A. McKay, and D. A. Barnum. 1964. Studies on colibacillosis of calves. 1. The antibody acquired by calves as the result of vaccination of the dam. Can. Vet. J. 5:no. 10, 248–261.
14. Glynn, A. A., and C. J. Howard. 1970. The sensitivity to complement of strains of *Escherichia coli* related to their K antigens. Immunology 18:331–346.
15. Ingram, P. C., R. Lovell, P. C. Wood, R. Aschaffenburg, S. Bartlett, S. K. Kon, J. H. B. Roy, and H. J. Sears. 1953. Further observations of the significance of colostrum for the calf. II. The immunological importance of the colostrum. Proc. Int. Dairy Congr. Den Haag 3:1369–1371.
16. Jones, G. W., and J. M. Rutter. 1972. Role of K-88 antigen in the pathogenesis of neonatal diarrhea caused by *Escherichia coli* in piglets. Infect. Immun. 6:918–927.
17. Kohler, E. M. 1971. Enterotoxic activity of whole cell lysates of *Escherichia coli* in young pigs. Am. J. Vet. Res. 32:731–737.
18. McSherry, B. J., and I. Gringer. 1954. Disturbances in acid-base balance and electrolyte in calf diarrhea and their treatment. A report of eighteen cases. Am. J. Vet. Res. 15:535–541.
19. Myers, L. L., F. S. Newman, R. A. Wilson, and J. E. Catlin. 1973. Passive immunization of calves against experimentally induced enteric colibacillosis by vaccination of dams. Am. J. Vet. Res. 34:29–33.
20. Neter, E., O. Westphal, O. Luderwitz, E. A. Gorzynski, and E. Eichenberger. 1956. Studies on enterobacterial lipopolysaccharides. Effect of heat and chemicals on erythrocyte modifying, antigenic, toxic and pyrogenic properties. J. Immunol. 76:377.
21. Richardson, M., and G. H. Conner. 1972. Prenatal immunization by the oral route: stimulation of brucella antibody in fetal lambs. Infect. Immun. 5:454–460.
22. Silverstein, A. W., D. W. Uhr, K. L. Kraner, and R. J. Lukes. 1963. Fetal response to antigenic stimulus: antibody production by the fetal lamb. J. Exp. Med. 117:799–812.
23. Smith, H. W. 1965. The development of the flora of the alimentary tract in young animals. J. Pathol. Bacteriol. 90:495–513.
24. Smith, H. W., and M. A. Linggood. 1972. Further observations on *Escherichia coli* enterotoxins with particular regard to those produced by atypical piglet strains and by calf and lamb strains: the transmissible nature of these enterotoxins and of a K antigen possessed by calf and lamb strains. J. Med. Microbiol. 5:243–249.
25. Westphal, O., and K. Jann. 1965. Bacterial lipopolysaccharides. Extraction with phenol-water and further applications of procedure, p. 83–91. *In* R. Whistler (ed.) Methods in carbohydrate chemistry, vol. 5. Academic Press Inc., New York.
26. Wood, P. C. 1955. The epidemiology of white scours among calves kept under experimental conditions. J. Pathol. Bacteriol. 70:179–193.