175 Holstein bulls and heifers were randomly assigned at birth to groups of equal size. The groups consisted of treatment calves that received a microbial culture product and control calves that received a placebo or no treatment. The calves were weighed at assignment and at trials terminating 28 days later. Grain intake was determined daily. Evaluation and treatment of diarrhea and respiratory disease were reported and recorded daily. The response variables were recorded as geometric means and were analyzed for significance by analysis of variance using SAS-GLM.

There were no significant differences at the 0.05 level between the treatment or control groups for any of the parameters examined in either trial. The results favored the treatment groups in total feed intake and weight gain, though these trends are not significantly different at the 0.05 level. When feed intake was examined by repeated measure analysis, it showed a marginal significance at the 0.1 level favoring the treatment group.

These calves were fed whole milk throughout this study, which may have reduced the anticipated benefit of feeding microbial culture and fermentation products. Perhaps under less optimal management systems, the positive trends observed in these trials would be enhanced.

Comparisons of Colostral and Serum Antibody Titres in Cows Vaccinated with *E. coli* K99 Antigens

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A field trial was conducted on a large dairy farm to evaluate the serum and colostral antibody responses to four commercially available enterotoxigenic *E. coli* (ETEC) diarrhea vaccines. Multiparous cows (n=192) and 114 first calf heifers were randomly assigned to six treatment groups (Table 1). Groups A-D were vaccinated twice subcutaneously; the first dose 50-90 days prior to the expected calving date (at drying off); the second dose three weeks prior to calving. Group E was vaccinated only once at three weeks prior to calving and Group F served as the non-vaccinated controls.

All groups were bled via evacuated blood collection tubes at assignment to the trial, and the serum was separated, labeled and stored at -4° C. At calving, 30cc of colostrum and a second blood sample were collected, the serum was separated, and both were labeled and frozen. At the end of the trial, all samples were shipped to the testing laboratory[c] to be analyzed. Samples were coded so that treatment group identity could not be determined by the testing laboratory.

E. coli antibody titres were determined by microtitre plate agglutination. The antigen was a whole cell preparation of strains B41 (0101:K28:K99) and 3509 (09:K$^-$:K99).

The former strain is the standard used by most researchers and the strain 3509 was used in this trial because the K99 antigen was the only antigen common to the four vaccine antigens, except for the 09 fraction of the Group B vaccine. (Table 2)

[c] *Beecham Laboratories, Bristol, Tennessee 37620*

Results

Serum 1 antibody titres were less than 5 in all treatment groups. Table 3 gives the serum 2 and colostral antibody titres using the B41 antigen. Groups B and C showed the greatest serological response and were followed by the other three vaccinated groups. The control animals' antibody level remained the same as the pre-vaccination levels.

The colostral titres followed the serum titres with the exception of Group B which did not respond as favourably in the colostrum as it did in the serum. Again, the control group shows virtually no antibodies to the B41 antigens. The titre generated by Treatment C was significantly greater than all other groups.

TABLE 1. Treatment Groups[1]

A.	Coli-Bovis 2x (Beecham)—2 ml
B.	Colligen (Ft. Dodge)—5 ml
C.	Vicogen (Pitman-Moore)—5 ml
D.	Scour Guard 3 (Norden)[2]—2 ml
E.	Coli-Bovis 1x (Beecham)—2 ml
F.	Controls

[1] Approximately 50 animals per group.
[2] Viral component of this vaccine was not administered.

TABLE 2. **E. coli** Strains used in the four vaccines and the Antigen for agglutination.

Vaccine	Strain	Antigens
A. Coli-Bovis (Beecham)	1474	K12: K99
B. Colligen (Ft. Dodge)	B41	08:K85: K99 09:K35: K99 0101:K28: K99 0101:K30: K99
C. Vicogen (Pitman-Moore)	B41	0101:K28: K99
D. Scour Guard 3 (Norden)	?	?
Agglutinating antigen	3509	09:K—: K99

A similar response was seen when the more K99 specific *E. coli* strain 3509 antigen was used. (Table 4) In this case, all treatment groups were significantly different ($P<0.05$) from the control group.

When colostral antibody was compared to parity of the dam, heifers were found to respond better than multiparous cows. (Table 5)

Discussion

Many researchers have found that colonization of the small intestine with ETEC is dependent on the presence of pili that allow adherence to the villus epithelium.[1,2] In cattle, the pilus antigen has been designated as the K99 antigen and lacteal immunity depends on the presence of anti-K99 antibodies in the lumen of the gut.[3,4]

Vaccines incorporating ETEC have been proven by others to be effective in lessening the severity of diarrhea caused by ETEC. Several different strains of ETEC are used in the four vaccines used in this trial (Table 2). Coli-bovis[a] is a genetically engineered variant of strain K12 and has the potential to produce large amounts of K99 pilus antigen. Pitman-Moore's product, Vicogen, is a B41 strain that also has a high concentration of K99 antigen. Coligen[b] is a multivalnt bacterin composed of four field strains of ETEC.

The antigen strains of ETEC in Norden's Scour Guard 3 were not made available to the author. Antibody response in colostrum as determined by either B41 or 3509 strains of antigen was greatest when Vicogen was used although this was not a significant difference. A single vaccination using Coli-bovis appears to produce an adequate titre for protection of the calf. Other methods of determining antibody to K99 are available and more accurate, but those used were relatively inexpensive and felt to be accurate enough to carry out the objectives of the trial.

[a] *Beecham Laboratories*

[b] *Fort Dodge Laboratories, Fort Dodge, Iowa 50501*

TABLE 3. Geometric Mean Serum and Colostrum Anti-K99 Titres using **E. coli** Strain B41 Antigen.

Treatment	No.	Serum 2	Range Serum 2	Colostrum	Range Colostrum
A	49	31.6[b]	4— 512	>275.4[b]	8— >4096
B	50	53.7[a]	4—2048	239.9[b]	4 — 2048
C	50	79.4[a]	16—1024	>549.5[a]	32— >4096
D	51	34.7[b]	8—1024	>263[b]	16— >4096
E	51	27.5[b]	4—2048	128.8[c]	8 — 1024
F	49	4.8[c]	0—16	10.7[d]	2 — 256

A = Coli-Bovis 2X (Beecham)
B = Colligen (Ft. Dodge)
C = Vicogen (Pitman-Moore)
D = Scour Guard 3 (Norden)
E = Coli-Bovis 1X (Beecham)
F = Control

[a,b,c,d] Means with common superscripts are not significantly different at the 0.05 level of significance.

TABLE 4. Geometric Mean Colostrum Anti-K99 Titres Using **E. coli** Strain 3509 Antigen.

Treatment	Titre	Range
A	232[a]	32-4096
B	180[a]	4-2048
C	289[a]	64-1024
D	197[a]	32-4096
E	161[a]	16-4096
F	12.5[b]	4-128

A = Coli-Bovis 2X
B = Colligen
C = Vicogen
D = Scour Guard 3
E = Coli-Bovis 1X
F = Control

[a,b] Means with common superscript are not significantly different at the 0.05 level of significance.

TABLE 5. Geometric Mean Serum and Colostrum Anti-K99 Titres Versus Parity of the Cow.

Parity	Serum 2	Colostrum
1	40.7	>204.2[a]
2	30.2	>166.0[b]
3	19.5	>109.6[b]

[a,b] Means with common superscripts are not significantly different at the 0.05 level of significance.

The higher antibody levels found in heifers' colostrum as compared to multiparous cows may be due to dilution of K99 antibodies by larger volumes of colostrum produced by older cows.

The importance of early feeding of high quality colostrum cannot be overstressed but one must also appreciate the fact that protection of neonatal calves from ETEC diarrhea is a local phenomena and absorbed anti-K99 antibodies will not protect the calf from this type of diarrhea.[5,6] Colostrum feeding for the first 3-5 days of life is essential for adequate "gut immunity". The calf does seem to produce its own immunity after the third day of life[7] and whole milk or milk replacer feeding can be started at that time.

Summary

It was found that all of the commercially available vaccines tested in this field trial increased colostral and serum antibodies against ETEC. Calf performance or challenge studies were not done because of the sheer numbers of animals and that was beyond the scope of this trial.

It also appears that a single vaccination using Coli-bovis at 2-3 weeks pre-partum will produce protective antibody levels in colostrum.

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Infectious Bovine Keratoconjunctivitis: Comparison of Immunological Response and Disease Reproduction in Vaccinated and Non-Vaccinated Calves

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Introduction

Moraxella bovis is considered to be the main causative agent of infectious bovine keratoconjunctivitis (IBK), commonly known as pinkeye[1 2]. IBK has been reproduced with *M. bovis* organisms alone[3] or in combination with other enhancing factors[4 8].

Numerous attempts have been made to produce a *M. bovis* vaccine utilizing viable and nonviable organisms in both experimental and natural environmental conditions[9 13]. In most cases these vaccines consisted of a heat-killed, formalin-killed, or viable autogenous *M. bovis* bacteria injected at weekly intervals intramuscularly or into the third eyelid. While in many cases *M. bovis* antibodies were produced, fewer positive cultures were obtained, and the severity of lesions were frequently reduced, vaccinations did not produce practical protection against the disease[12 13]. Other factors such as age, vaccination schedule, and the use of homologous stains of *M. bovis* have been studied[14 16].

M. bovis may exist in either a smooth or rough colony form, with rough colonies exhibiting pili extending from the cell walls[17]. These pili are delicate elongated unbranched filaments which contain no central pore and have a peritricous distribution. Pili appear to provide additional extracellular antigens[18], which may be of importance in development of resistance to the organism. Studies using a *M. bovis* pilus vaccine indicated the stimulation of immune response to *M. bovis* which may provide a more protective immunity than previous vaccines[19 21].

Local resistance to bacterial infection of corneal and conjunctival surfaces is a complex system involving several antibacterial substances, including secretory IgA, lysozyme, beta-lysin, and lactoferrin[22 23]. *M. bovis* antibodies have

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