The Microbial Environment of the Calf With Diarrhea

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Introduction

The bacterium *Eschrichia coli* was first associated with diarrhea in calves before 1900 when veterinary workers found that white scours could be produced in healthy calves by feeding the animals *E. coli* isolated from diseased calves. Since that time researchers have learned more about the nature of the virulence mechanisms that enable disease-causing strains to produce diarrhea in young animals. Vaccines against these pathogenic strains designed to increase specific antibody levels in immune colostrum have been developed to protect newborn calves. The use of antibiotics with various supportive care methods has proven to be effective in treating calves that develop diarrhea. Despite these advances, neonatal calf diarrhea continues to be the most important cause of calf mortality.

The coliform bacteria are among those that inhabit the intestine of man and animals. They can be differentiated into two groups, the coliforms and the fecal coliforms, on the basis of their ability to grow at elevated temperatures. Both groups grow at 37C (98.6F) but only the fecal coliforms are able to grow at 44.5C (112F). The most important member of the fecal coliforms from the point of view of this study is *E. coli.*

Smith and Crabb (1956) in a study employing bacteriophage typing of *E. coli* strains isolated from the feces of cows and their calves, concluded that the dam did not appear to be a frequent source of the strains responsible for disease and that the pen in which the calves were kept was a more probable source. In a study of the husbandry factors influencing the occurance of colibacillosis in calves, Wray and Thomlinson (1975) concluded that the use of calf-houses free of fecal contamination could break the cycle of infection.

In light of these findings, workers at the NDSU veterinary science and bacteriology departments began a two-year study of the microbial environment of the calf with diarrhea as a part of the NDSU Calf Diarrhea Research Program in the fall of 1979. In this study the occurence, number and pathogenic properties of the *E. coli* types found in soils from calving barns, calf pens, feedlots and pastures were investigated.

Experimental Methods

Soil samples from four cooperating cattle-raising operations designated as Herds A, C, D and E were taken in the fall of 1979 prior to the 1980 calving season. These samples were tested for the presence of coliform bacteria, fecal coliform bacteria, fecal streptococci, and total heterotrophic bacteria. Total heterotrophic bacteria were enumerated by standard plate count method (1), coliform bacteria numbers were determined by the multiple tube fermentation method (1) and fecal coliform bacteria were counted using the multiple tube fermentation-elevated temperature method (1). Fecal streptococci were counted by standard plate count method (1) using m-Enterococcus medium (Baltimore Biological Laboratories).

Soil samples were taken at various sites at each operation, including clean control sites where no cattle had been kept. Fecal specimens were obtained from these four cattle-raising operations during the 1980 and 1981 calving seasons. Specimens were collected from healthy calves as well as from those with diarrhea. A second set of soil samples were taken at the beginning of the 1981 calving season at a fifth cattle-raising operation designated as Herd M, and the samples were tested as before. Bedding straw samples were also taken at this time. A third group of soil samples were taken in early September at Herd M feed lots.

The *E. coli* strains isolated from soil samples and from fecal specimens were serotyped using O and K typing sera. The O typing sera used were obtained from Difco Laboratories, Detroit, Michigan. The K typing sera used were purchased from the Department of Veterinary Science, Pennsylvania State University, University Park, PA. Bloods were obtained from the North Dakota State University Department of Veterinary Science.

The erythrocytes were separated by centrifugation from blood in Alsever's solution, washed three times in 0.85% (w/v) NaCl solution (saline) and made up to a 3% (v/v) suspension in fresh phosphate buffered saline. They were used immediately or after only a few days' storage at 3-5 degrees C.

Results and Discussion

The results of the testing of soil samples from Herds A, C, D and E are shown in Figures 1-4. As may be seen in these figures, no fecal coliforms or fecal streptococci were ever isolated from these control soil samples. In contrast, soil samples taken from barns, feedlots and pastures contained both fecal coliforms and fecal streptococci.

It should be noted that *E. coli* was not found in clean control soil samples where no cattle had been kept. However, this organism was consistently isolated from soil samples taken from barns, calf lots and pastures. These findings suggest that the cattle themselves are the source of *E. coli* strains associated with calf diarrhea, and clean native soil free of fecal contamination is not a source of these organisms.

If contaminated soils are a source of the *E. coli* strains causing calf diarrhea as these findings suggest, one way to demonstrate such a relationship would be to recover the same strain of *E. coli* from barnyard soils and from the feces of calves with diarrhea.

Evidence to support this relationship was obtained at two cooperating cattle-raising operations. *E. coli* strains isolated from soil samples at Herds A and C were tested for the presence of 10 O-type and two K-type antigens. Similarly, *E. coli* strains isolated from the diarrheic stools of calves born in the 1980 spring calving season from the same cattle operations were also tested for the presence of these same antigens.

The O type antigens of *E. coli* represent certain polysaccharide (sugar) molecules associated with the cell wall of this bacterium. If the antigen knwn as O26 is injected into a healthy laboratory animal — a rabbit for instance — the rabbit will produce an antibody that will clump or agglutinate a strain of *E. coli* that has this particular antigen on its cell wall. An *E. coli* so agglutinated is designated as O type O26. The reaction between an antigen and its corresponding antibody is highly specific. An animal serum containing specific antibody (a typing serum) can be used to accurately identify *E. coli* strains. Table 1 shows the agglutination patterns of serologically identical strains of *E. coli* found in barnyard soils taken in the fall of 1979 and in the feces of

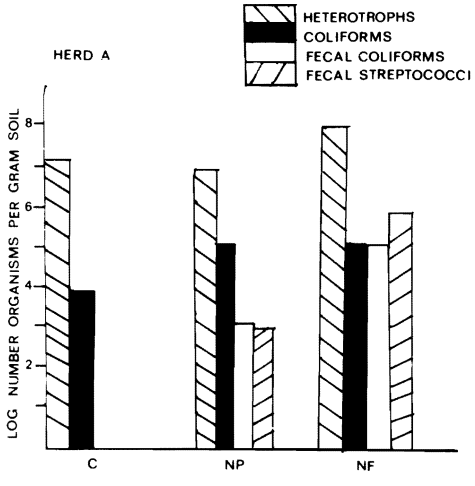

FIGURE 1. BACTERIA ISOLATED FROM SOIL ASSOCIATED WITH HERD A.
C = Control; NP = North Pole Barn; NF = North Feed Lot

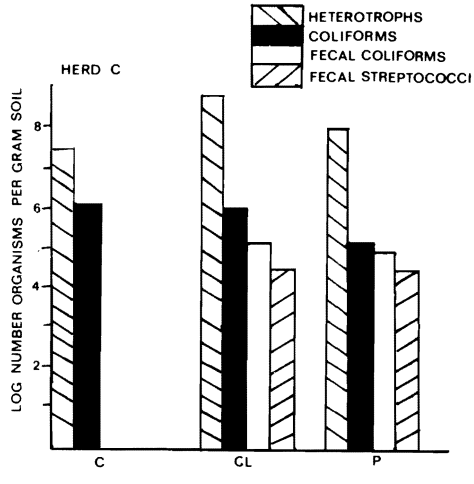

FIGURE 2. BACTERIA ISOLATED FROM SOIL ASSOCIATED WITH HERD C.
C = Control; CL = Calf Lot; P = Pasture

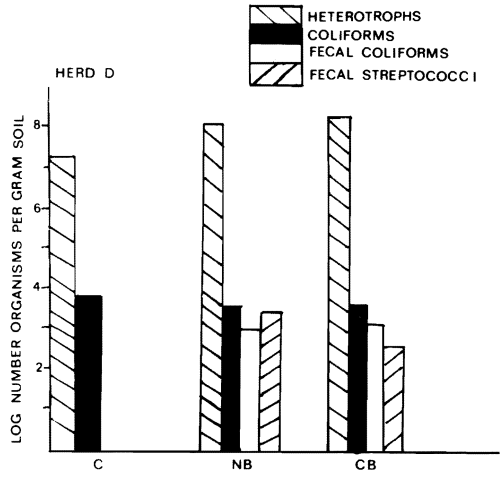

FIGURE 3. BACTERIA ISOLATED FROM SOIL ASSOCIATED WITH HERD D.
C = Control; NB = North Barn; CB = Calving Barn

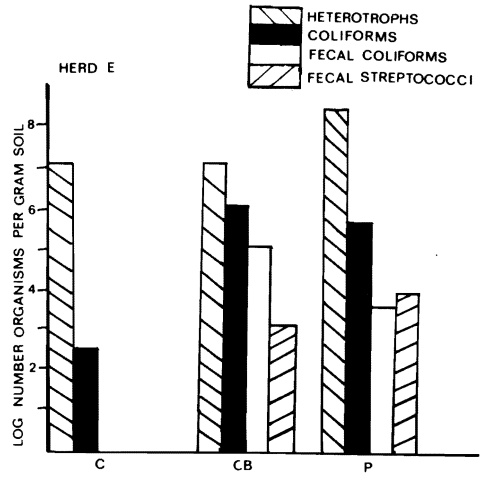

FIGURE 4. BACTERIA ISOLATED FROM SOIL ASSOCIATED WITH HERD E.
C = Control; CB = Calf Barn; P = Pasture

calves with diarrhea born the following spring at the same two cattle-raising operations. These results suggest that calves may acquire pathogenic strains of *E. coli* from soil contaminated with feces.

These studies made it apparent that calves may acquire diarrhea-producing strains of *E. coli* from a reservoir of pathogenic strains residing in contaminated soils. The further knowledge of the occurence of pathogenic *E. coli* strains in barnyard soils during the springtime calving season would be useful in determining which barnyard locations would have the lowest numbers of these potential pathogens and would be the most desirable sites for calving and rearing calves through the period of their greatest susceptability to diarrhea.

Soil samples were taken at cattle-raising operation M at the beginning of the 1981 calving season. The data obtained are shown in Figures 5 and 6. Of the six sites examined, the soil taken from a pasture that had not been used for grazing cattle for one full year had the lowest number of fecal coliforms. Furthermore, the fecal coliforms isolated from this soil were not *E. coli* but rather *Klebsiella* and *Enterobacter* species that have rarely been implicated in calf diarrhea. The most desirable location for calving at this operation was the pasture.

These findings are applicable to ranchers who have available pasture land to make practical use of such findings. However, it offers little solice to the rancher with an intensified operation or at operations where calving in a pasture location is not practical. What can be done to clean up heavily contaminated soils found in calving lots and barns where contaminated soil cannot be avoided?

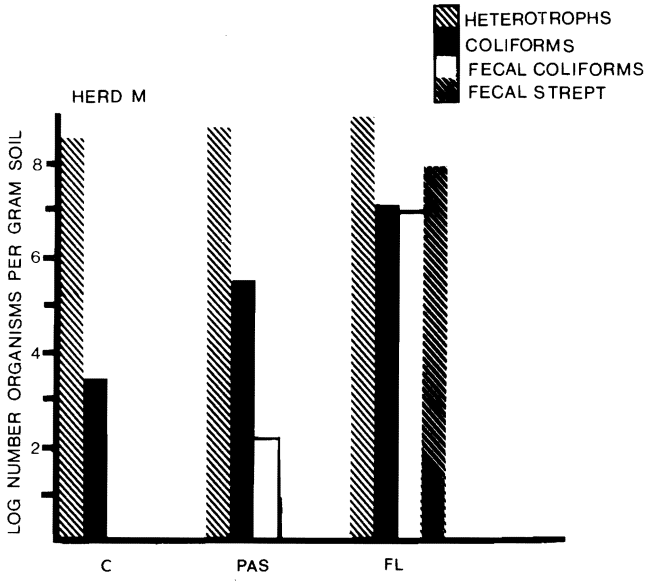

FIGURE 5. BACTERIA ISOLATED FROM SOIL ASSOCIATED WITH HERD M.
C = Control; PAS = Pasture; FL = Feed Lot

Table 1. O and K antigens present on *E. coli* strains isolated from soil samples and the feces of calves with diarrhea.

	O AND K ANTIGENS											
	POLY A SERUM GROUPS				POLY B SERUM GROUPS							
	026	055	0111	0127	086A	0119	0124	0125	0126	0128	K88	K99
HERD A												
SOIL ISOLATE	+	+	+	+	+	+	+	+	—	+	—	+
FECAL ISOLATE	+	+	+	+	+	+	+	+	—	+	—	+
HERD C												
SOIL ISOLATE	+	+	+	—	+	+	—	+	—	+	—	+
SOIL ISOLATE	+	+	+	—	+	+	—	+	—	+	—	+
FECAL ISOLATE	+	+	+	—	+	+	—	+	—	+	—	+

\+ Agglutination With Specific Serum
— No Agglutination With Specific Serum

	O AND K ANTIGENS TESTED FOR											
	026	055	0111	0127	086A	0119	0124	0125	0126	0128	K88	K99
HERD A												
SOIL ISOLATE	+	+	+	+	+	+	+	+	—	+	—	+
FECAL ISOLATE	+	+	+	+	+	+	+	+	—	+	—	+
HERD C												
SOIL ISOLATE	+	+	+	—	+	+	—	+	—	+	—	+
SOIL ISOLATE	+	+	+	—	+	+	—	+	—	+	—	+
FECAL ISOLATE	+	+	+	—	+	+	—	+	—	+	—	+

\+ Agglutination With Specific Serum
— No Agglutination With Specific Serum

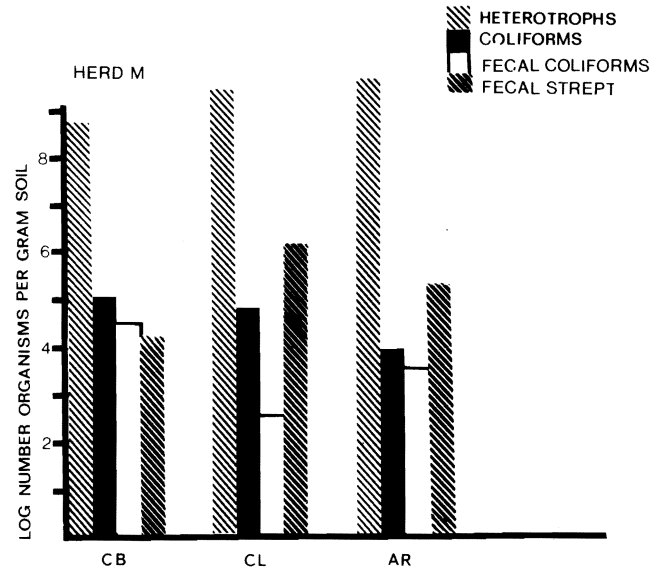

FIGURE 6. BACTERIA ISOLATED FROM SOIL ASSOCIATED WITH HERD M.

CB = Calving Barn; CL = Calf Lot; AR = Access Road.

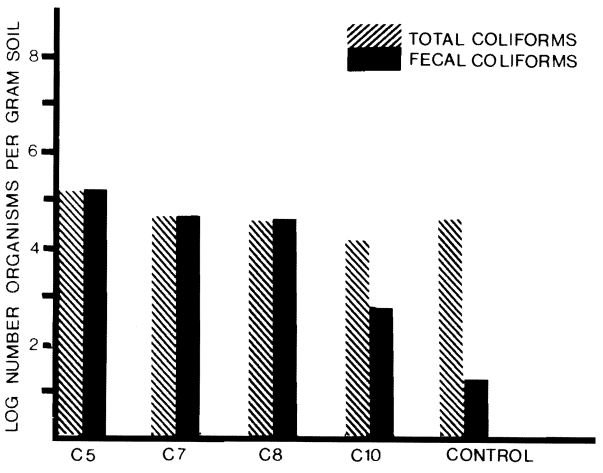

FIGURE 7. TOTAL COLIFORM AND FECAL COLIFORM NUMBERS IN FEED LOT SOILS AT HERD M.

To assess the effect of two common practices used to reduce the level of soil contamination (limeing and the natural drying of soil), soil samples were taken at heavily contaminated feedlots. These sites were treated with ground limestone. Samples taken one week after application indicated that ground limestone did not have a disinfectant effect. No significant decline in coliform, fecal coliform or fecal streptococcus numbers were detected. A repeat of the procedure using calcium oxide as a soil disinfectant is planned.

To determine the effect of natural drying of soils during the summer months on coliform numbers, soil samples were taken at the same set of feedlots. Four sites were selected for sampling. Three of the lots tested had not held cattle since mid-May. Samples were taken in early September. These lots had a history of heavy use and had held calves with diarrhea in previous seasons. The fourth lot sampled had a history of lighter use and had been used throughout the summer to hold only a few head of cattle. This lot appeared to have the best drainage of the four lots. The soil was dry and friable. Conditions in the other three lots ranged from muddy with standing water to dry with large clumps of hardened soil. The coliform numbers found in these lots are shown in Figure 7. The soils from the lots with the history of the heaviest use still had up to 1,000,000 fecal coliforms per gram although the lots had stood empty in the summer sun for nearly four months. The lot with the fewest cattle (C-10) had the lowest fecal coliform numbers. These results emphasize the lethal affect of drying on fecal coliforms as well as the influence of the past use of the site. A control sample was also taken at the same time from a clean control site where no cattle had been kept. Coliforms were detected, but as was the case in the pasture soil at this operation, no *E. coli* were isolated. The coliforms found were *Klebsiella* and *Enterobacter* species that have rarely been associated with calf diarrhea.

In this investigation of the microbial environment of the calf with diarrhea, bedding straw samples were taken from unbroken bales and from the floor of unused calving barns dressed with clean bedding straw. These were tested for the presence of fecal coliforms and fecal streptococci. No fecal streptococci were detected in the three samples tested. Coliforms were detected in one of these samples. When the isolated coliforms were identified, no *E. coli* were found, but an *Enterobacter* species was identified. This finding indicates the use of bedding straw in calving lots and barns with heavily contaminated soil to be desirable.

A final aspect of this study was to investigate the pathogenic properties of the *E. coli* strains isolated from barnyard soils and from the feces of calves with diarrhea. One such pathogenic property of disease causing strains is the presence of tiny thread-like structures on the bacterial cell surface known as pili or as fimbriae (3). These structures extend from the surface of the *E. coli* cell and aid in the attachment of the bacterium to the epithelial cells that line the intestine of the newborn calf (2). Once so attached to the cells lining the animal gut, disease-causing *E. coli* produce a toxin that causes the loss of electrolytes (salts) and water by the epithelial cells of the intestine, producing diarrhea in the infected animal (4).

The pili that allow toxin-producing *E. coli* to attach to intestinal cells are known as K88 (5,7) and K99 antigens (2). These antigens can be detected, as can the O-type antigens, using a typing serum containing specific antibody to these antigens. As shown inTable 1, the K99 antigen was detected in the *E. coli* strains isolated from soils and from the feces of calves with diarrhea.

K88 and K99 pili can be visualized using electron microscopy. Figures 8-12 are electron micrographs of *E. coli* isolated from calves with diarrhea. The small thread-like projections extending from the cell surface are the pili.

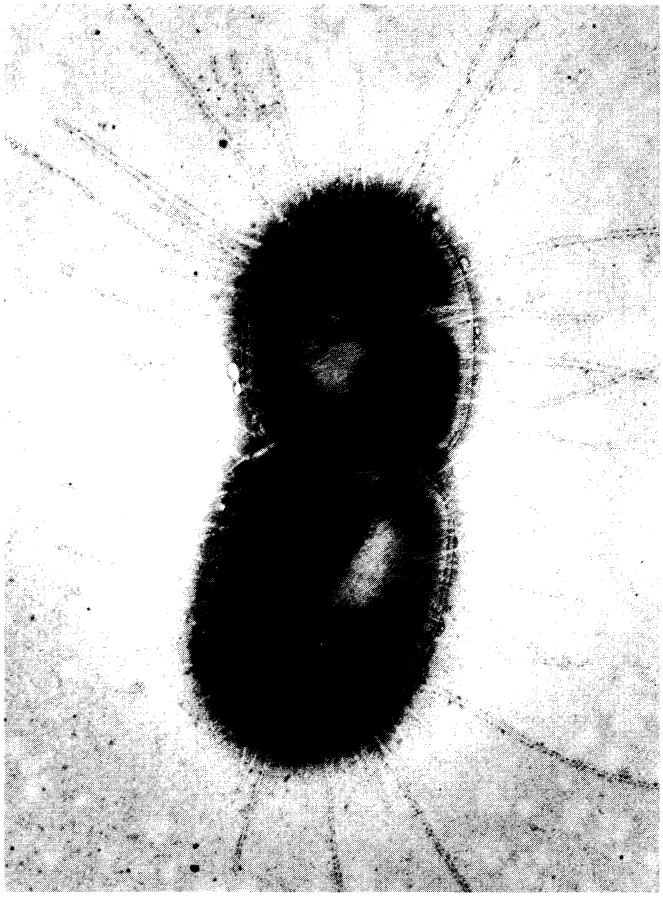

FIGURE 8. A K88 Positive *E. Coli* Strain 2N6 92,800X

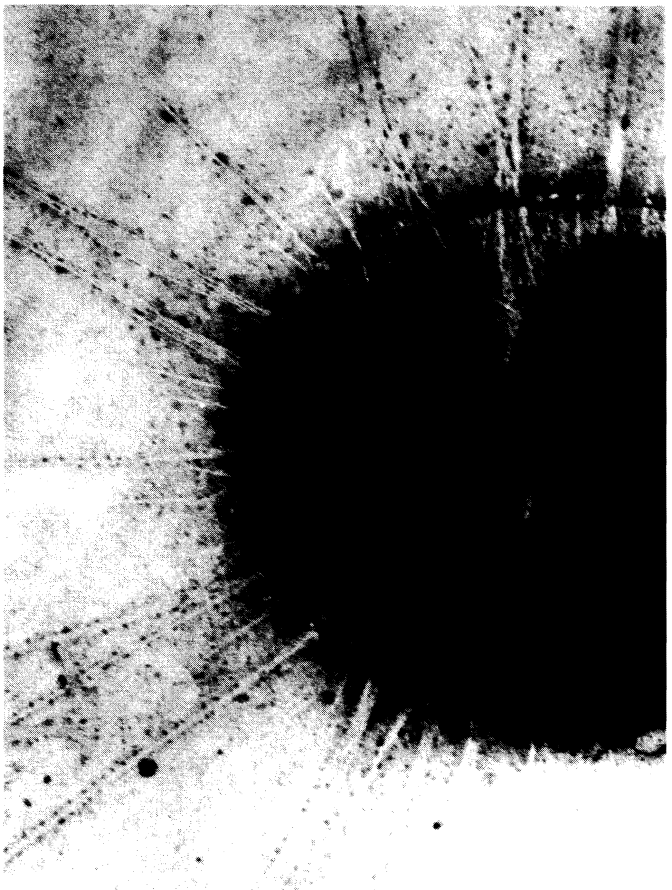

FIGURE 9. K88$^+$ *E. Coli* 2N6 Magnification 290,000X

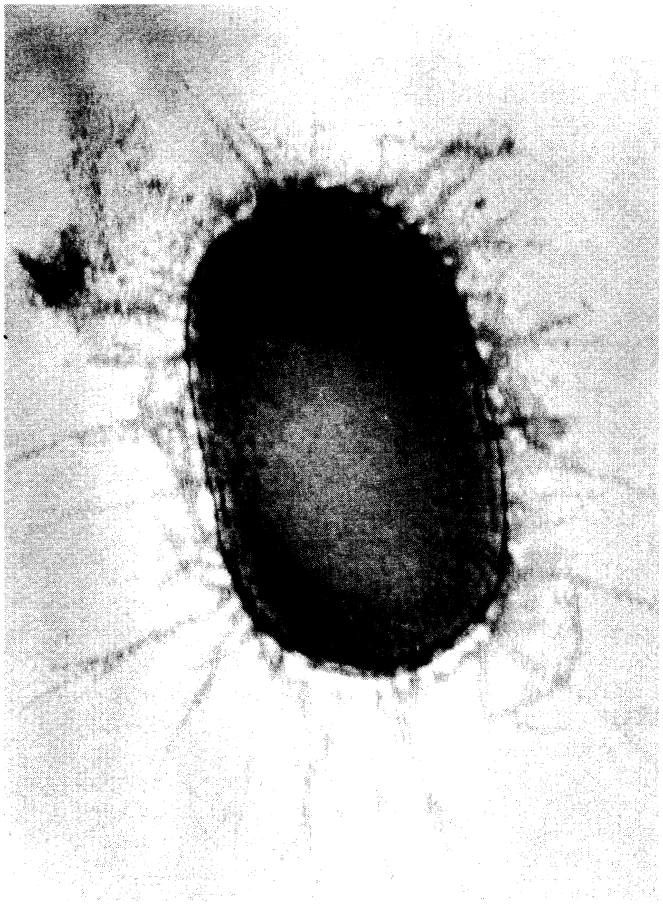

FIGURE 10. K88$^+$K99$^+$ *E. Coli* 3N3. 122,000X

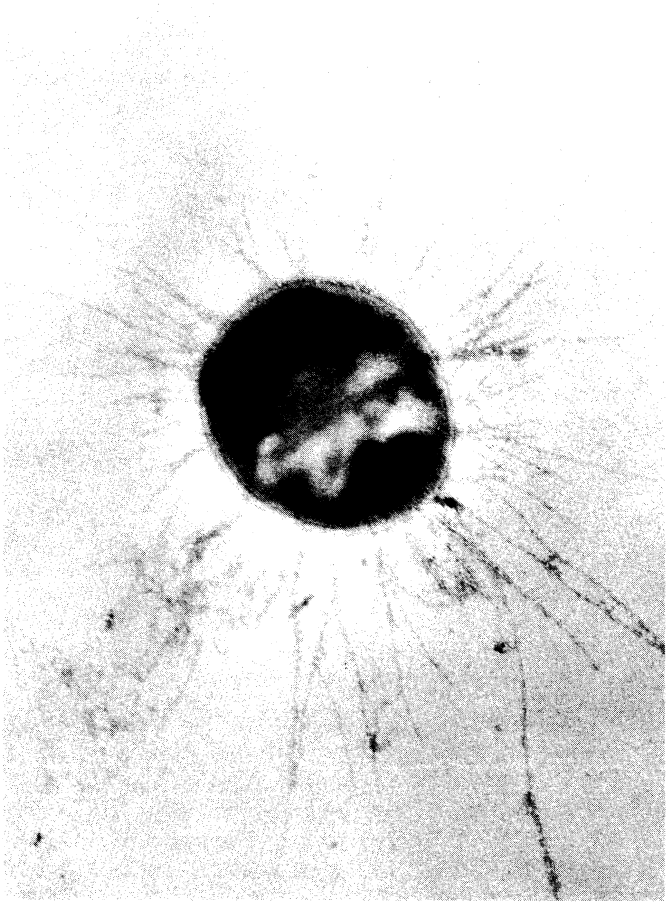

FIGURE 11. K88$^+$K99$^+$ *E. Coli* 3N3 98,000X

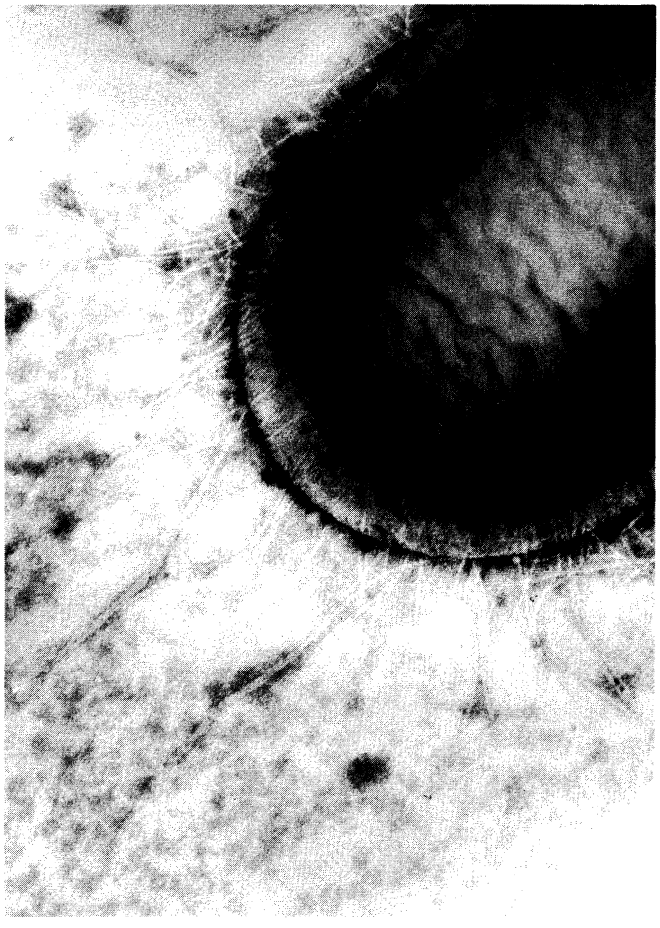

FIGURE 12. K88$^+$K99$^+$ *E. Coli* 3N3 290,000X

The ability of pathogenic *E. coli* strains to produce K88 or K99 pili is determined by a gene carried by a genetic element known as a plasmid that exists separately from the main chromosome of the bacterial cell (9, 12). Such plasmids can be transferred from a donor bacterial cell to another cell in a form of sexual reproduction in bacteria known as conjugation. A K99 negative *E. coli* that does not bear K99 pili could become K99 positive should it receive the plasmid borne K99 gene during conjugation with a K99 positive *E. coli* donor strain. In this way plasmids carrying either K88 or K99 genes can be spread between *E. coli* strains.

At this writing all published data for the occurence of K88 and K99 antigens on *E. coli* strains indicated that only one of these two K antigens would be present on a given *E. coli* cell (6, 10). In this study *E. coli* strains were isolated from calves that bore both these pilar antigens. Evidence for the presence of both pilar antigens on *E. coli* strains isolated is summarized in Table 2. The strains were tested for the presence of these antigens using the slide agglutination test.

The presence of K88 and K99 pili could also be demonstrated by the ability of these strains to agglutinate the erythrocytes (red blood cells) of various animals. K88 positive strains cause the agglutination of guinea pig erythrocytes in the presence of the sugar mannose (8). This phenomenon is known as mannose resistant eluting (MRE) hemagglutination. Similarly, K99 positive strains are able to agglutinate sheep erythrocytes in the presence of mannose (2). The demonstration of the hemagglutinin properties of the strains giving positive slide agglutination with typing sera confirms the presence of both K88 and K99 pili on these isolates. This finding of both K88 and K99 antigens on an *E. coli* strain is not surprising given the fact that both these antigens are coded for by genes located on transmissible plasmids.

Table 2. Evidence For The Presence Of K88 and K99 Antigens On *E. Coli* Strains Isolated From Calves With Diarrhea.

	SLIDE AGGLUTINATION		MRE HEMAGGLUTINATION	
STRAIN	ANTI-K88	ANTI-K99	GUINEA PIG RBC	SHEEP RBC
K99$^+$ CONTROL*	—	+	—	+
K88$^+$ CONTROL*	+	—	+	—
NO K$^+$	—	—	—	—
2N6*	+	—	+	—
3N3*	+	+	+	+
32N2*	+	+	+	+

* API Identification — *E. Coli* Excellent Identification
\+ API Identification — *E. Coli* Very Good Identification

E. Coli	SLIDE AGGLUTINATION		MRE HEMAGGLUTINATION	
STRAINS	ANTI-K88	ANTI-K99	GUINEA PIG RBC	SHEEP RBC
K99$^+$ CONTROL*	—	+	—	+
K88$^+$ CONTROL*	+	—	+	—
NO K$^+$	—	—	—	—
2N6*	+	—	+	—
3N3*	+	+	+	+
32N2*	+	+	+	+

\+ Indicates A Positive Slide Agglutination Or Hemagluttination Reaction
— Indicates No Slide Agglutination Or Hemagglutination Reaction

In summary, the results of this study of the microbial environment of the calf with diarrhea indicate a reservoir of potentially pathogenic *E. coli* strains in barnyard soils contaminated with bovine feces. Investigation of the pathogenic properties of these strains demonstrated the presence of K99 pili in such strains. Furthermore, strains were isolated from diseased calves that bore both K88 and K99 pilar antigens that are associated with the ability of *E. coli* to cause disease in newborn calves.

Acknowledgments

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grams and are redirecting some of our resources to speak more directly to some of the short range problems. Promotion of nutrition misinformation is presently on the upswing. The department is presently determining the nutrient composition of meat from different species of animals, both in the raw and in the cooked form. The means to combat deceptive nutrition information which capitalizes on people's fears (of feed or food additives, pollution) and hopes (of freedom from disease, increased longevity) is a difficult task.

The basis for all of animal agriculture is grass. Producers often take for granted production of the optimum kinds and amounts of forage necessary for red meat and wool production for the livestock industry. North Dakota has approximately 13 million acres classified as native range. An increased effort is underway which will assist North Dakota livestock producers in the management of this resource. The complex relationships which exist between forages, soils, animals and weather mandate that an interdisciplinary approach is necessary to answer the appropriate research questions. Production and management research relating to each of the species, beef cattle, dairy cattle, sheep, swine and poultry, will be continued as in the past but with some redirection of effort.

I would like to close by mentioning some observations about research which were discussed by Dr. Roy Arnold of the Nebraska State Agricultural Experiment Station.

A. Research seeks answers to questions or problems.
B. Questions or problems which guide research come from a wide variety of resources.
C. The nature of the answers sought ranges from fundamental knowledge to practical information.
D. Answers cannot be predicted accurately in advance (if the answer is known the research is not needed).
E. Sometimes a research project does not provide an answer or a result.
F. Sometimes surprises occur which may lead research into new, exciting and productive directions.
G. Research takes time, requiring both ideas and effort.
H. Research costs money.
I. Research can't be turned on and off at will.
J. Like other facets of human enterprise, time spent planning research pays big dividends.
K. Research is of no value to society if no one knows about it.
L. In biological systems, variation is a fact of life.
M. It follows that replication is necessary for a single observation does not establish a fact.
N. Variables which influence agriculture and other biological systems are numerous and complex inter-relationships exist among these variables.
O. Biological systems adapt and change; new problems emerge over time.
P. Increasingly, research projects require team effort and broad integrated approaches.
Q. The nature of urgent problems cannot be predicted in advance or some flexibility in research programming is needed.
R. It is important not to lose sight of long range goals and not divert effort totally to shorter range problems.
S. Scientists are a lot like people.
