

Improved Procedure for Challenging Neonatal Dairy Calves with Enteropathogenic *Escherichia Coli*

S. E. Gilliland, L. J. Bush
and R. D. Contreras

A procedure for challenging animals with the intestinal pathogen of interest is necessary to evaluate the effectiveness of various preventative measures in controlling intestinal infections in newborn dairy calves. Enteropathogenic *Escherichia coli* is generally accepted as the pathogen most often involved in causing diarrhea in neonatal dairy calves. A common method for preparing an *E. coli* culture to be used as a challenge dose is to grow the organism in 10 ml volumes of a broth medium such as Trypticase Soy Broth. The entire 10 ml of culture is then fed to the test animal in an attempt to produce the infection. This represents a rather massive dose of the organisms since it would contain approximately 10 billion cells of *E. coli*. Such a challenge dose may merely overload the intestinal system of the animal with *E. coli* since it is not likely that the calf would be exposed to this large a dose of enteropathogenic *E. coli* in its natural environment. Furthermore, attempts to reproduce results which have been reported using such challenge procedures are often disappointing. We did not obtain satisfactory results in challenge studies using *E. coli* prepared in this manner and given to the animal at the indicated dosage level. A transient diarrhea was produced, but the calves remained alert and recovered quickly without treatment.

Enteropathogenic *E. coli* that contain the K-99 antigen have been identified as the strains being responsible for causing intestinal infections resulting in diarrhea in calves. Apparently the K-99 antigen is needed for the organism to attach to the intestinal wall enabling it to become established and grow to cause the infection. This antigen is often difficult to detect if the bacteria have been grown on a "nutrient-rich" medium, apparently because it is masked by excessive capsular material on the cells. The antigen appears to be more readily exposed on cells grown on a medium containing minimal nutrients due to a reduced amount of capsular material. Thus, growth of enteropathogenic *E. coli* on a minimal nutrient medium might provide a culture which would be more infective in calves and more useful in conducting successful challenge studies.

For our experiments the enteropathogenic *E. coli* cultures were grown on the surface of Minca Iso VitaleX agar as described by Guinee et al (1977). Twenty ml of the sterile medium was placed aseptically in sterile dilution bottles which were then laid on their sides to permit the medium to solidify. One-half ml of a 1:10 dilution of a Trypticase Soy Broth culture of the *E. coli* was spread over the surface of the solidified medium. The inoculated bottles were incubated 18 hours at 37° C. After incubation the cells were washed from the surface of the medium with 20 ml volumes of sterile physiological saline solution. The resulting cell suspensions were placed in an ice bath until needed. The challenge dose for each animal was prepared by diluting the cell suspension of *E. coli* 1:10 then adding 1 ml to milk to be fed to the animal. This resulted

in a challenge dose containing approximately 1×10^8 cells. The challenge doses were used the same day they were prepared.

Newborn Holstein or Ayrshire bull calves were used as test animals for evaluating the challenge procedure. After the challenge dose was administered, the animals were observed periodically during the next 24-hour period for the development of diarrhea. Two colostrum-deprived calves which were challenged at the first feeding after birth with a mixture of five strains of *E. coli* (1×10^8 cells of each) developed diarrhea within 12 hours. In additional experiments enteropathogenic *E. coli* B-44, which is the strain most often utilized in working with neonatal dairy calves, was used alone as a challenge dose. Challenge of two colostrum-deprived calves with this single strain (1×10^8 cells) also produced diarrhea within a 12-hour period. Three calves permitted to obtain colostrum from their dams soon after birth did not develop diarrhea after being given either the B-44 culture or a mixture of six strains of *E. coli* in milk at 12 or 24 hours after birth.

Challenge of colostrum-deprived neonatal dairy calves with enteropathogenic *E. coli* prepared as described above can provide a model system for studying some factors that may prevent infection by this organism. The doses used in this study represent a level of inoculation of the animal 100 times less than would be achieved by challenging the animals with 10 ml of a broth culture of the *E. coli*. Additional evaluation will be needed to determine whether smaller doses of the organism will produce desirable results.

An example of a way in which this challenge system might be utilized would be to determine the effectiveness of preparations containing *Lactobacillus acidophilus* in preventing infections caused by enteropathogenic *E. coli* in neonatal dairy calves. It would also provide a useful model for evaluating the relative merits of various procedures for processing colostrum to be used in feeding newborn calves. Additional work is needed to develop a challenge system effective for colostrum-fed calves because it is well known that infectious diarrhea does occur in such calves. Moreover, discovery of means for complementing the natural immunity of calves acquired by consumption of colostrum would have immediate application in raising dairy herd replacements.

Literature Cited

- Guinee, P. A. M., J. Veldkamp and W. H. Jansen. 1977. Infection and Immunity. 15:676.
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