The Role of Electron Microscopy in the Study of Calf Scours

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The pathophysiology of neonatal calf diarrhea (calf scours) is complex. This type of diarrhea results from a serious infection of the small intestine which causes a decrease in absorption or a complete breakdown of the epithelial cells. During the last few years researchers have identified numerous microbial agents believed to be associated with the disease. The list of microbes includes bacteria, virus and protozoa (1). A pathogenic variety of the bacterium *E. coli* is the one organism that is most frequently associated with neonatal calf diarrhea. Other microbes commonly associated with the disease are coronavirus and rotavirus. There is also evidence suggesting that both environmental factors and management practices may play an important role in the occurrence of calf scours.

Generally, if the organisms are present in sufficient number to result in a serious infection of the intestinal lining they can also be expected to be found in fecal samples from the same animals. Virus are much too small to be observed and studied with the light microscope. They are, however, of sufficient size to be detected and identified with the aid of a transmission electron microscope.

Virus and virus-like particles are routinely found in fecal samples from calves having diarrhea. Unfortunately, the mere presence of virus or bacteria in the fecal samples is not necessarily evidence that they are responsible for the problem. It has been demonstrated, for example, that the rotavirus and coronavirus believed to be causative agents can be isolated from the fecal samples of apparently healthy animals (2,3). A great deal of additional research is needed to determine the roles and interrelationships between the various types of microbes isolated from fecal samples of calves having diarrhea.

Electron microscopy has become a useful tool in the study of calf scours because, unlike many other techniques, it can provide a rapid assessment of the microbes present. Electron microscopy permits the identification of many different types of organisms in a single sample preparation. Information can be obtained using this technique without the need of tissue samples or of sacrificing the animal.

Virus present in fecal samples can generally be identified to family or group, which is frequently all the information requested by the attending veterinarian. Other types of laboratory tests may be more sensitive if the virus are present only in very low concentrations. The relatively new technique of immunoelectron microscopy has, however, increased the sensitivity of transmission electron microscopy (4) and has allowed for the utilization of more than just morphological characteristics.

The standard electron microscopic technique for the identification of virus and virus-like particles associated with neonatal diarrhea includes the separation of these small structures from a fecal sample. The sample is centrifuged at two speeds, one for clarification or removal of heavy components and a second centrifugation to concentrate the virus into a small pellet. The pellet is then resuspended and a portion placed on a small coated grid and stained with phosphotungstic acid. A more rapid technique used by some researchers eliminates the centrifugation and simply involves spraying a small amount of stained fecal material directly on the grid.

The microscope used in this laboratory is a JEOL JEM 100CX (Fig. 1). This transmission electron microscope was recently purchased with the cooperation of the College of Science and Math, the Agricultural Experiment Station, and the North Dakota Beef Commission. This instrument has the capability of magnifications up to 250,000 times and is able to distinguish objects as small as three angstroms (1

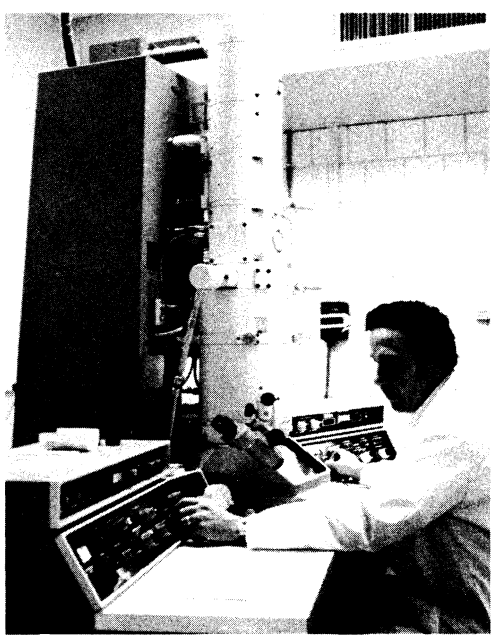

Fig. 1. Dr. Freeman working with the transmission electron microscope.

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angstrom = 1/254,000,000 inch). With this degree of magnification and resolution the major limiting factor becomes the number of virus present in the sample.

In addition to rotavirus (Fig. 2) and coronavirus (Fig. 3) commonly associated with neonatal diarrhea, fecal samples frequently contain other virus and virus-like structures (Fig. 4), as well as membrane particles (Fig. 5, 6, 7). Without specific laboratory tests in addition to electron microscopy it is not possible to positively identify many of these structures. Many of the non-viral structures are probably of bacterial membrane origin.

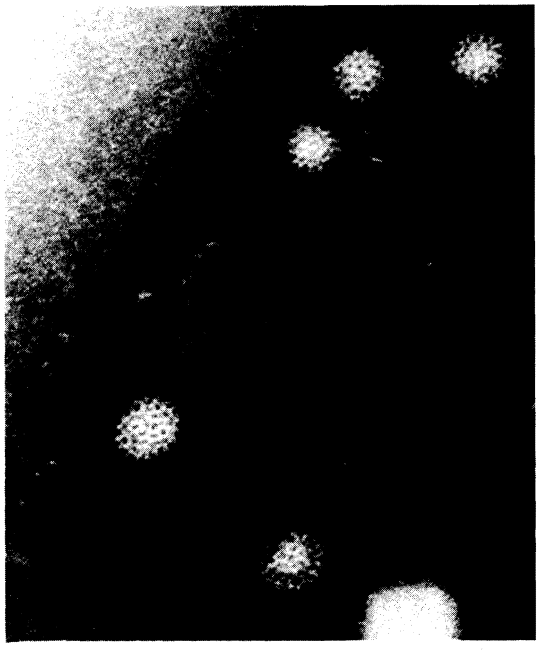

Fig. 2. Rotavirus isolated from fecal sample of scouring calf. Magnification 140,000 X.

Fig. 3. Coronavirus isolated from fecal sample of scouring calf. Magnification 140,000 X.

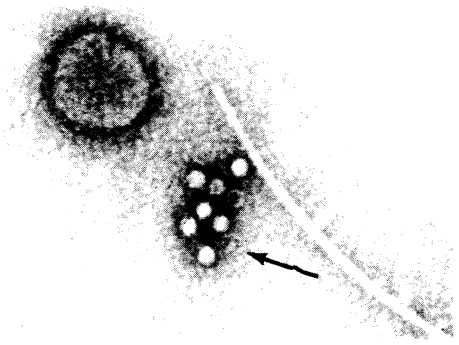

Fig. 4. Example of other virus (arrow) and virus-like particles found in fecal samples. Magnification 100,000 X.

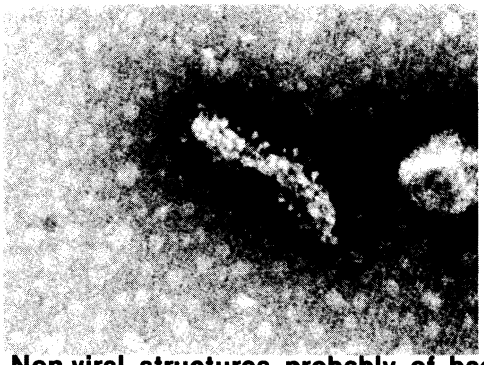

Fig. 5. Non-viral structures probably of bacterial membrane origin. 140,000 X.

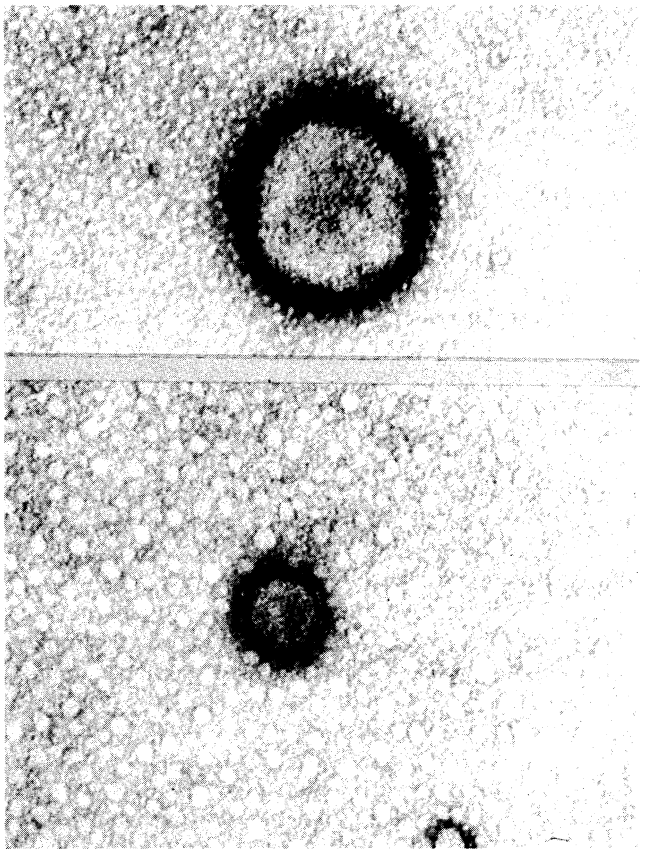

Fig. 6, 7. Biological membranes commonly confused with corona virus. 100,000 X.

The problem of positive identification is further complicated by the normal variation of the virus (5) as well as by morphological modifications which result from incomplete viral synthesis and preparatory procedures. As an example, very small corona-like particles are frequently observed (Fig. 8).

The bacteria present in the original fecal samples are generally not found on the same grids as the virus following centrifugation. Bacteria have sufficient weight to be removed during the first centrifugation. If desirable, bacteria can also be separated and examined with the transmission electron microscope or with the scanning electron microscope. Bacteriophage (virus which attack bacteria) (Fig. 9) and bacterial flagella (Fig. 10) are often present in large numbers in fecal preparations.

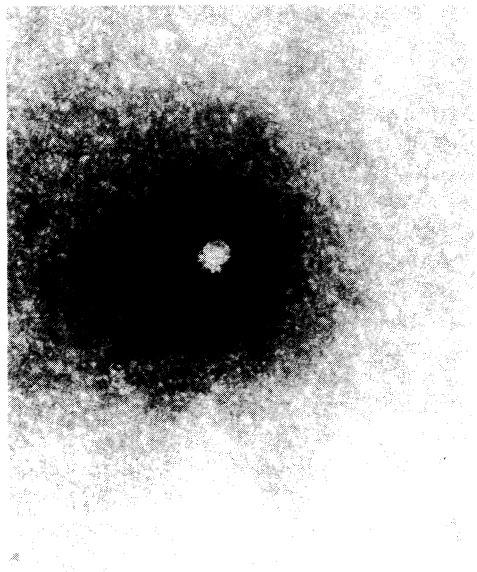

Fig. 8. Small corona virus. 100,000 X.

Fig. 9. An example of one type of bacteriophage seen in fecal samples of scouring calves. Magnification 100,000 X.

The use of electron microscopy in the study of calf scours does not need to be restricted simply to the detection of virus in fecal samples. A great deal more needs to be known regarding the interaction of virus and cell surfaces which may result in diarrhea. This type of information can be obtained only by the examination of tissue samples at the ultrastructural level. The procedures used in preparing tissue samples for electron microscopy require considerable time and effort. However, there is no other method of documenting changes in the cell fine structure.

Electron microscopy has proven to be a valuable and reliable tool for the rapid detection of virus in fecal samples. Electron microscopy is also a basic tool in determining the effects of disease at the cellular level.

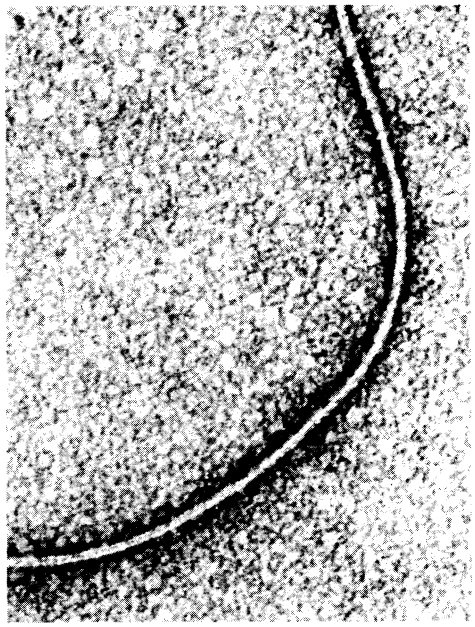

Fig. 10. Bacterial flagella. 100,000 X.

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