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Some Infectious Causes of Diarrhea in Young Farm Animals

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INTRODUCTION 346
General Considerations 346
Public health aspects of EHEC, rotavirus, and *C. parvum* 346
Bacterial agents 346
Viral agents 346
Parasitic agents 347
NEONATAL IMMUNITY 347
Immunoglobulin Classes 347
Lacteal Immunoglobulins 347
Immune System of the Mammary Gland 347
Immunoglobulin Absorption 348
Failure of Passive Transfer of Immunoglobulins 348
Colostral Antibodies within the Gastrointestinal Tract 348
Circulating Passive Antibodies 349
Active Immunization 349
E. COLI 349
DIARRHEAL DISEASE CAUSED BY ETEC 349
Virulence Factors of ETEC 349
Fimbriae 349
Type 1 fimbriae (F1) 350
K88 (F4) antigen 350
K99 (F5) antigen 351
F41 antigen 351
987P (F6) antigen 351
CFAs 351
Detection of Fimbrial Antigens 352
Enterotoxins 352
LT 352
STs 352
Detection of Enterotoxins 352
Serologic Types of ETEC 353
Pathogenesis 353
Clinical Presentation 354
DIARRHEAL DISEASE CAUSED BY NON-ETEC 354
Virulence Factors of EPEC and EHEC 355
EPEC 355
EHEC 355
Fimbriae 355
Cytotoxins 355
Serologic Types 355
Pathogenesis 356
Clinical Presentation 356
CNF-Positive *E. coli* 356
ROTAVIRUSES 358
Taxonomy of Rotaviruses 358
Serologic Classification 358
Groups (serogroups) 358
Serotypes 358
Subgroups 358
VP4 359
VP6 359
VP7 359
Genome segments 10 and 11 359
Viral particles 359
Rotaviruses in Pigs 359
Group A rotaviruses 359

Non-group A rotaviruses 359
Rotaviruses in Calves and Lambs 360
Group A rotaviruses 360
Group B rotaviruses 360
Detection of Rotaviruses 360
Pathogenesis 360
Clinical Presentation 361
CRYPTOSPORIDIUM SPP. 362
Biology of *Cryptosporidium* spp. 362
Taxonomy 362
Life cycle 362
Animal Cryptosporidiosis 363
Detection of *C. parvum* Infection 363
Pathogenesis 363
Clinical Presentation 364
CONCLUSIONS 365
ACKNOWLEDGMENTS 365
LITERATURE CITED 365

INTRODUCTION

An array of infectious agents including bacteria, viruses, and parasites cause diarrhea in food animal species. Many of these enteropathogens cause severe intestinal lesions, alterations in enzyme activity, alterations in nutrient transport mechanisms, or a combination of these effects. While most of these enteric agents cause diarrhea, the clinical presentation varies. Some of the diarrheas are self-limiting, some are associated with high morbidity, and others are associated with high mortality.

Infectious diarrhea of neonatal animals is one of the most common and economically devastating conditions encountered in the animal agriculture industry (165). Acute infectious diarrhea encountered in a herd is often difficult to manage because of the large number of potential enteropathogens involved, differences in individual animal immunity within the herd, population dynamics, environmental stresses, nutritional status, and difficulty in establishing an etiologic diagnosis (3, 232, 236, 240, 300, 387). The etiologic diagnosis is not determined for a large percentage of cases of neonatal diarrheas.

General Considerations

Public health aspects of EHEC, rotavirus, and *C. parvum*. Increasingly, food animals and their products are being identified as important sources of infectious pathogens for humans. Numerous infectious pathogens capable of causing diarrhea among food animals have been associated with foodborne diseases and zoonoses of humans. Many of these infectious agents have been recently reviewed elsewhere (12).

Contamination of food products with enterohemorrhagic *Escherichia coli* (EHEC) O157:H7 has been associated with hemolytic-uremic syndrome and hemorrhagic colitis in humans (90, 389). Diarrheic and asymptomatic beef and diary cattle have been considered reservoirs for this organism (225, 226, 269). Transmission of EHEC O157:H7 to humans is thought to occur by the consumption of undercooked meat and raw milk (90, 389).

Although rotaviruses of several serogroups can infect multiple mammalian species, antibodies or the antigen of serogroup B rotaviruses have been detected in human (44), rat (100), and pig (44) sera. This finding, along with the severe epidemics of diarrhea among Chinese children and adults, has led to speculation that group B rotaviruses may be a zoonosis (99, 100).

Cryptosporidium parvum infection is an important cause of diarrhea and nutrient malabsorption in humans (11, 53, 59, 76) and animals (75, 115, 155, 238, 370). In humans, enteric cryptosporidiosis occurs as an acute, self-limiting diarrhea in immunocompetent patients or as a chronic, severe, intractable diarrhea and malabsorption condition in patients with acquired immunodeficiency syndrome (11, 59). Furthermore, enteric cryptosporidiosis is common among travelers (75, 255) and children in day care centers (75) and appears to be increasing in importance among the elderly (19). Fecal-oral transmission occurs among humans and animals, and ingestion of contaminated groundwater has been identified as a source of infective oocysts (78).

Bacterial agents. Bacteria can mediate diarrhea by (i) producing enterotoxins that influence the crypt cells to hypersecrete (13, 229, 266), (ii) invading the intestinal mucosa and eliciting an inflammatory response that mediates hypersecretion through prostaglandins and other products of inflammation (13, 266), and (iii) destroying villous absorptive epithelial cells and thus causing a malabsorptive diarrhea (13, 266). Among the bacterial causes of diarrhea in neonatal food animals, *E. coli* and *Salmonella* spp. are the most common and economically important (23, 165), but *Clostridium perfringens* (23, 70), *Bacteriodes fragilis* (34, 251), *Campylobacter* spp. (117, 250, 297), and *Yersinia enterocolitica* (250) have also been identified as causes of enteric disease and diarrhea.

Viral agents. Rotaviruses and coronaviruses are by far the most commonly identified viral causes of diarrhea of neonatal food animals (368). These viruses have also been associated with diarrhea in adult food animals, but their disease incidence in adults is low compared with that in neonates. However, adults infected both clinically and subclinically shed the viruses and provide sources of infection for the young (46, 69).

Early investigations on rotavirus provided the impetus for defining, developing, and implementing certain viral diagnostic procedures. These procedures were employed in the search for nonbacterial agents that may contribute to diarrheas. Subsequently, other viral agents were identified, some of which have been implicated in infectious diarrheas. In 1980, Bridger (36) and Saif et al. (310) described characteristics of viruses that were morphologically identical to conventional rotaviruses but expressed a different genome

profile and lacked the group-specific antigen. These atypical rotaviruses were isolated from diarrheic pigs (36, 310) and subsequently from calves (65, 286, 287, 342), lambs (64, 342), and humans (342). The viruses were assigned the names pararotavirus or group C rotaviruses (32, 312), atypical rotavirus (38, 287), antigenically distinct rotaviruses (313), rotaviruslike viruses or group B rotaviruses (287), and novel rotaviruses (37). Other viruses that have been implicated in diarrhea of young farm animals include togavirus (bovine viral diarrhea virus) (390), parvovirus (353, 354, 411), calicivirus (36, 39, 310, 397), adenoviruses (72, 93, 268), bredaviruses (402, 403), and astroviruses (36, 141, 341, 397).

Parasitic agents. Although parasitic infections occur in neonatal food animals, they are rarely associated with acute gastroenteritis or diarrhea in the neonate (132). The clinical importance of most parasitic infections is manifest in weanling to yearling animals when infections are associated with poor performance, anemias, and, in some animals, diarrhea (132).

NEONATAL IMMUNITY

Early immunity in neonatal farm animals depends almost entirely on their obtaining antibodies via colostrum (161, 316). The transfer of colostral immunoglobulins from the dam is the single most important form of immunologic protection to the neonatal calf, lamb, and pig. Although its immune systems is competent at birth and the neonate is capable of responding to antigenic challenge, such responses are delayed and often ineffective in combating infectious disease (366).

Failure of passive transfer of immunoglobulins predisposes the young animal to gastrointestinal and systemic infections. In farm animals, the exchange of immunoglobulins occurs by the consumption of maternal colostrum. In contrast to the hemochorial placentation of humans, which allows for the transport of maternal immunoglobulins to the fetus, the syndesmochorial placentation of cows and ewes and the epitheliochorial placentation of sows do not transport immunoglobulins (366). Therefore, colostrum serves to supply the newborn with its first line of defense, nutrients, and growth factors (257, 259).

As calf, lamb, and pig fetuses develop within the uterus, they become capable of producing specific antibodies and immunoglobulins in response to antigenic stimulation. Lymphocytes appear in the fetal calf and lamb thymuses at 42 and 43 days of gestation (275, 320), respectively, and in the fetal pig thymus at 38 days of gestation (299). Immunoglobulin M (IgM) is apparent at 59 days of gestation in the fetal calf (320), 70 days in the fetal lamb (108), and 38 days in the fetal pig (298, 299). As fetal development continues and their immune systems mature, the fetuses are capable of responding to an increasing number of antigens. At birth, the neonate can respond to a large number of antigens but lacks the specific immune functions that are required to combat many infections (18).

The sera of newborn unfed calves, lambs, and pigs contain small concentrations of immunoglobulins (IgG1 and IgM) (35, 211, 212). At birth, these neonates have about one-third the adult population of circulating B lymphocytes (18). Shortly after birth, immunoglobulin synthesis increases and the development of local intestinal immunity accelerates. Local immunity begins in the gastrointestinal tract of calves and pigs during the first week of life (49, 293, 294, 384). During this time, IgM predominates, and after about the third week of life, IgA becomes prominent and remains the major immunoglobulin class within the intestinal tract through adulthood. The appearance of local immunoglobulins is influenced by antigenic stimulation, and because newborns are immunocompetent, they can make a primary response to antigens. Further exposure to the antigen results in an anamnestic response.

Immunoglobulin Classes

As in humans, the major immunoglobulin classes in domestic farm animals include IgG, IgA, and IgM (35, 49, 54). IgE is found in very low concentrations in farm animals, and IgD has been found in pigs (366). Differences in the distribution of immunoglobulins in the serum and mammary gland secretions exist among species. The highest concentrations in the serum of most domestic farm animals are of IgG, followed by IgM and IgA. IgA is found in low concentrations except in pigs, in which serum IgM concentration is the lowest (294).

Lacteal Immunoglobulins

Near the end of gestation, cows, ewes, and sows begin the process of transferring serum immunoglobulins to colostrum. Cows and ewes have the unique ability to selectively transport and concentrate large quantities of an IgG subclass immunoglobulin, IgG1, in colostrum (50, 52). The transfer of IgG1 from serum to colostrum causes an increase of colostral IgG1 concentration and a concurrent decrease in concentrations in maternal serum (50, 257). In the transfer of IgG1 from serum to colostrum, IgG1 first diffuses across the vascular epithelium and then is selectively transported by mammary alveolar epithelial cells (50). IgG1 specifically binds via Fc-specific receptors located on the basal membrane of the secretory epithelium (50). IgG1 molecules become engulfed by the basal membrane, forming vesicles that are subsequently released into the colostrum.

In the transition of cow and ewe colostrum to milk, all the immunoglobulins decrease in concentration (50, 330). Relative to the other immunoglobulins, IgG1 is found in higher concentrations throughout lactation. In cows and ewes, IgG1 is the major secretory immunoglobulin (267), and it plays a decisive role in combating enteric infections among calves and lambs (52, 311, 344). IgA is present only in low concentrations in the mammary secretions of the cow and ewe (49, 50, 267).

Similarly, the colostrum of sows is rich in IgG (35, 294, 295). As lactation proceeds, IgG concentrations rapidly decrease and IgA becomes the dominant immunoglobulin class in sow milk. This consistently high concentration of milk IgA provides local protection within the gut. Furthermore, IgA in combination with IgM adsorbs to the crypt epithelium, thereby providing protection against potential enteropathogens (294).

Immune System of the Mammary Gland

In cows and ewes, serum is the main contributor of colostral and milk immunoglobulins. Unless stimulated, the immune system of the mammary gland is fairly inactive during lactation and the dry (nonlactating) period (257, 267). After antigenic stimulation, synthesis of immunoglobulins is enhanced (267) and increased local production of immunoglobulins, particularly IgA and IgG2, occurs. These immunoglobulins are thought to inhibit bacterial attachment, serve as bactericidal agents by lysing bacteria, and act as opsonins

(51). The local synthesis of IgG1, IgG2, IgA, and IgM in the mammary gland protects the gland and provides a source of milk immunoglobulins. Although the immunoglobulin concentration in milk is less than that in colostrum, milk immunoglobulins do provide some local gut protection to unweaned young animals (257, 294, 295).

Evidence suggests that the mammary gland of sows is the main source of milk immunoglobulins in pigs (35). Bourne (35) has suggested that 90% of IgA and IgM and 70% of IgG is produced within mammary tissue. This high concentration of IgA provides a constant source of antibodies within the gastrointestinal tract.

Immunoglobulin Absorption

The successful transfer of immunoglobulins from the dam to the newborn depends on the quality and quantity of immunoglobulins in the colostrum and on the amount consumed and absorbed by the neonate (325). The jejunum is the most active site of immunoglobulin absorption (348, 392). The absorptive epithelial cells within the jejunum are believed to possess receptors which mediate the binding and cellular transport of immunoglobulins (50, 348). Colostral immunoglobulins are selectively absorbed across the jejunal absorptive epithelium, transferred intracellularly, and released into the lymphatic vessels (348). Absorbed immunoglobulin molecules enter the circulation with intestinal lymph by way of the thoracic duct.

The normal neonate suckles colostrum shortly after birth, and serum immunoglobulins are detected by 4 h in the calf (291) and by 3 h in the pig (294). In lambs, intact immunoglobulins have been detected in the thoracic duct lymph from 6 to 120 min after duodenal administration (54). Absorptive epithelial cells can absorb colostral immunoglobulins for up to 24 h after birth in calves (291, 325) and for up to 48 h in lambs and pigs (35, 153, 291). However, efficiency of absorption at these times is reduced. It has been shown that calves and pigs absorb immunoglobulins most efficiently during the first 4 h after birth (291, 294). Lambs appear to require multiple colostrum feedings within 6 h to achieve adequate serum immunoglobulin concentrations (153). As the neonate ages, the absorptive epithelium becomes less permeable through a gradual and progressive process known as closure. In all species, the capacity of the small intestine to absorb immunoglobulins is at an end by 24 to 48 h after birth, a time at which the absorption of large molecules is complete. The mechanism of closure has been described by Stott et al. (355). With successful acquisition and absorption of colostral immunoglobulins, the serum immunoglobulin concentrations of the neonate should be, within 24 h of birth, comparable to those of the adult. Thereafter, serum immunoglobulin concentrations are effective in preventing most systemic infections.

Failure of Passive Transfer of Immunoglobulins

Among neonatal farm animals, the most common disease associated with failure of passive transfer of immunoglobulins is septicemic colibacillosis, a highly fatal disease caused by opportunistic strains of *E. coli* that tend to belong to serogroups O1, O2, O8, O20, O117, O55, O26, and O78 (134, 147, 248, 271). Serotypes O2:K1 and O78:K80 are reported to be the most common among strains isolated from septicemic animals, followed in frequency by O26:K60 and O86:K61 (271, 278). Some colisepticemic strains are considered invasive (278). The mechanism by which the organisms become translocated from the gastrointestinal tract into systemic circulation and internal organs is not completely understood but is believed to be primarily associated with certain colicin V plasmids (278). In addition, the colisepticemic strains often resist the bactericidal effect of serum that is associated with the expression of colicin V plasmids or virulence (Vir) plasmids or both (109, 196, 333). These strains often express fimbrial antigens and produce hemolysins and other cytotoxins (240, 271, 278, 335, 383). Similar properties are observed in strains isolated from extraintestinal infections in humans (83, 106, 142, 151, 186, 224, 247).

No specific clinical signs suggest failure of immunoglobulin transfer. The most accurate indication is serum IgG concentrations of <1,600 mg/dl in calves and lambs (54, 161). Animals with such low IgG concentrations should receive intravenous plasma as soon as possible to prevent sepsis. Affected animals show a variety of nonspecific clinical signs such as lethargy, anorexia, central nervous system depression, and subnormal to elevated rectal temperatures. Omphalophlebitis, hypopyon, meningitis, peritonitis, pneumonia, septic joints, and diarrhea are common in advanced stages of septicemic colibacillosis.

Colostral Antibodies within the Gastrointestinal Tract

Farm animals are born into environments with many potential enteropathogens. They are initially exposed to resident microflora in the vagina of the dam and subsequently to microbes harbored by herdmates. Some microorganisms are potentially harmful, while others are necessary for normal development and function of the gastrointestinal tract. However, once exposed, the intestinal tract is susceptible to infection with potential enteropathogens, and in the absence of protective antibodies, various enteropathogens can become established and cause enteric disease. Within the gastrointestinal tract, colostral immunoglobulins provide protection against potential infectious agents. The local effects of immunoglobulins are best documented in the prevention of adherence by enterotoxigenic *E. coli* (ETEC) (1). The common ETEC strains that express fimbrial antigens and produce enterotoxins colonize the small intestinal epithelium of neonatal calves, lambs, and pigs. Fimbrial antigens attach to intestinal epithelial cell receptors, and after attachment, the bacteria secrete the enterotoxins that cause the secretory diarrhea. Within hours of exposure to ETEC, the brush border epithelium is heavily colonized with bacteria. Attachment of the fimbrial antigens to receptors can be prevented by the presence of anti-fimbrial-antigen antibodies contained in colostrum (1, 3, 174, 244, 254). Antibodies within the intestinal tract coat the binding sites on fimbriae, thereby inhibiting attachment to intestinal epithelial cell receptors.

Vaccinating dams in late gestation with fimbrial-antigen-containing vaccines is an effective means of limiting enteric infections with ETEC. Parenterally administered vaccines induce protective concentrations of antibodies in the colostrum of the dam. Oral administration of monoclonal antibody to neonatal calves shortly after birth is an effective means of preventing attachment of K99-positive ETEC (328).

Neutralization of viral particles is yet another important local effect of colostral antibodies (60, 340, 344). Because of the prevalence of rotavirus in farm environments, most adult farm animals have circulating antibodies to rotavirus and some degree of virus-neutralizing antibodies in their colostrum (400). In the immediate postpartum period, colostral antirotavirus antibodies provide protection from enteric in-

fection for about 3 to 4 days in calves and pigs (1, 4, 294) and for up to about 7 days in lambs (343). After this time, the young animals become susceptible to rotavirus infections because of decreasing virus-neutralizing antibodies in the milk. Bovine colostrum or milk virus-neutralizing antibody titers of >1,024 are protective against rotavirus challenge (60). Milk antibody titers of <640 but >32 are incompletely protective (60, 400), and titers of <32 are not protective (60). Decreasing IgG1 milk titers are highly associated with increased prevalence of rotavirus diarrhea in the 4-day- to 2-week-old calf (1, 4).

In pigs, rotavirus diarrhea can occur in any age group but is most prevalent in pigs less than 5 days old and in the post-weaning-age groups (3 to 6 weeks) (327). In pigs less than 5 days old, rotavirus diarrhea has been attributed to insufficient ingestion of colostrum early after birth and to the lack of specific antibody to rotavirus in the colostrum of the dam (327). Terminating the milk diet, which contains IgA, has been associated with the prevalence of rotavirus diarrhea in the postweaning groups (327).

Serum antibody titers to *C. parvum* are widely distributed in the farm animal population, which indicates widespread exposure (115). However, colostral immunoglobulins are not protective against *C. parvum* infection (15, 155). Diarrhea and oocyst shedding readily occur in calves fed adequate amounts of colostrum (155, 230, 377).

Circulating Passive Antibodies

Even when neonates consume sufficient colostrum and absorb adequate quantities of immunoglobulins (passive antibodies), some will become infected with enteropathogens and develop diarrhea. Infections occur in young animals born to vaccinated as well as unvaccinated dams and frequently are caused by multiple agents. Passively derived serum antibodies have little effect in reducing or eliminating diarrhea caused by ETEC (1) and *C. parvum* (15, 239). In calves, it has recently been shown that circulating passive rotavirus antibodies (IgG1) are transferred to the lumen of the gastrointestinal tract and are protective against rotavirus challenge (24, 25).

Active Immunization

Active immunization of the dam is frequently employed to enhance the production of specific colostral and milk antibodies. The quantity of colostrum consumed, specific-neutralizing-antibody titers of colostrum, and the duration of colostrum feeding are associated with reduction or elimination of infections with potential enteropathogens (1, 4, 225, 344). Modified live vaccines and monoclonal antibodies are also administered orally to the neonate to prevent viral and ETEC (K99) infections, respectively. Active immunization of the neonate does not appear to be as effective in reducing the incidence of enteric disease as immunizing the dam in late gestation and providing the neonate with her colostrum.

E. COLI

E. coli is a gram-negative, motile or nonmotile, facultatively anaerobic, nonsporeforming member of the *Enterobacteriaceae* family. Most strains are commensal inhabitants of the gastrointestinal tract, but some strains express virulence factors that enhance organism ability to cause a variety of intestinal infections and diarrheal syndromes among neonatal farm animals and humans. Identification of certain virulence factors has assisted in defining mechanisms by which various strains cause the different diarrheal syndromes. Levine (188) has described human diarrheagenic *E. coli* and assigned strains to four major categories and a less-well-defined fifth category: ETEC, enteroinvasive, enteropathogenic (EPEC), EHEC, and enteroadherent, which was later described as enteroadherent-aggregative (386). ETEC, EPEC, and EHEC are the diarrheagenic types described as occurring in young farm animals. The importance of ETEC in the etiology of diarrhea among calves, lambs, and pigs is well recognized, and these organisms should not be confused with the rare EPEC and EHEC types that cause the less-common diarrheal syndromes.

DIARRHEAL DISEASE CAUSED BY ETEC

Virulence Factors of ETEC

Investigations into *E. coli* strains associated with individual cases or outbreaks of diarrhea among neonatal calves, lambs, pigs, and humans have helped determine specific virulence factors that can be used to distinguish between pathogenic and commensal strains (91, 109, 128, 147, 169, 264, 334, 337, 393, 408). Two of the more prominent virulence factors thus far identified for ETEC are (i) expression of fimbrial antigens that enable the bacterium to attach to mammalian cells and (ii) elaboration of one or more enterotoxins that influence intestinal secretion of fluids through increased cellular concentrations of cyclic AMP (cAMP) or cGMP. Although the association with serotypes or serogroups does not confer virulence, results of several studies have shown that ETEC strains are limited to a few serotypes or serogroups (128, 241, 346, 347, 393).

Fimbriae

Attachment of bacteria to susceptible host tissue is the initial step in bacterium-induced disease (20). Attachment is by filamentous, proteinaceous appendages called pili (96), fimbriae (95, 96), surface adhesins (265), or colonization factors (104) that are located on the surface of the bacteria (95, 265, 272). In this discussion, the term fimbriae (Fig. 1) is used to describe the surface adhesins that function in bacterial attachment to the intestinal epithelium.

E. coli is capable of synthesizing various types of fimbriae that enable the organism to colonize the intestinal tracts of different animal species. It is generally accepted that diarrheagenic strains express only one fimbrial antigen, which is responsible for colonization of the intestinal tract. However, a single *E. coli* strain or single *E. coli* cell may under natural conditions express multiple distinct fimbriae. A single strain of ETEC has been shown to simultaneously express the K88 and 987P antigens (318). Either of these antigens alone enables *E. coli* to colonize the intestinal tract of susceptible young pigs. The combination of K99 and F41 fimbriae on *E. coli* strains belonging to serogroups O9 and O101 is common (242). Both of these antigens may occur singly or in combination on *E. coli* strains that cause diarrhea in calves, lambs, and pigs.

In the synthesis of fimbriae, some *E. coli* strains may exhibit phase variation, a process of alternating phases of fimbrial expression and repression (42, 358). Although this characteristic has been most often described for type 1 fimbriae, other fimbriae exhibit phase variation. Phase variation can be affected by environmental influences of temperature, oxygen tension, medium, and growth conditions (358).

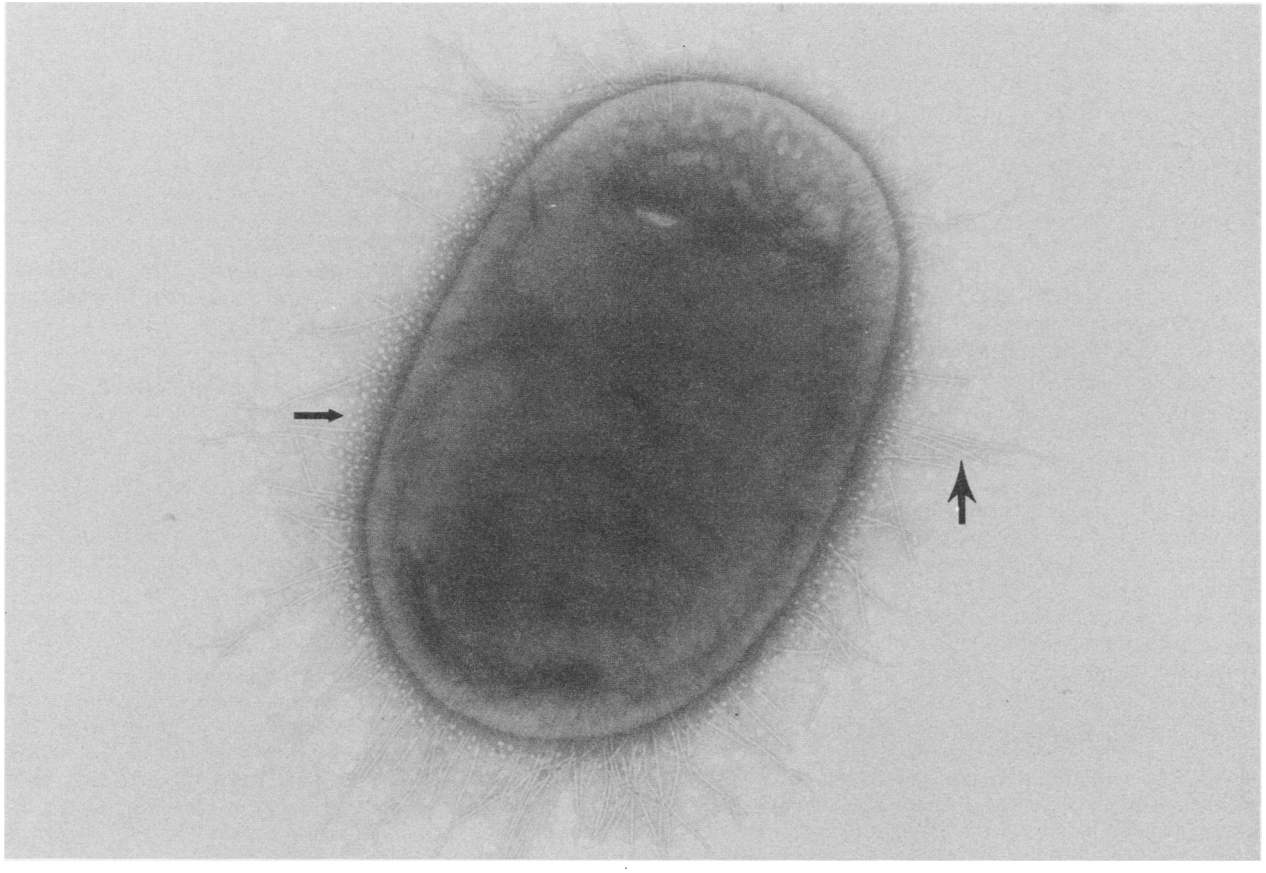

FIG. 1. Electron micrograph of an F41-positive *E. coli* cell negatively stained with 2% sodium phosphotungstate. Numerous fimbriae (large arrow) and outer membrane lipopolysaccharide vesicles (small arrows) are present. Magnification, ×42,500.

Type 1 fimbriae (E1). Type 1 fimbriae, found on both pathogenic and commensal *E. coli* serotypes, are the most common fimbrial antigens. Currently, the relation of type 1 fimbriae to enteric disease is unclear, but they are expressed on strains associated with diarrhea (110, 174, 191). Type 1 fimbriae are encoded by chromosomal DNA (42), mediate bacterial binding to receptors containing D-mannosides on mammalian cell membranes (95, 265, 272), and hemagglutinate the erythrocytes of a variety of animal species but not bovine erythrocytes (272). The hemagglutinating activity can be inhibited in the presence of D-mannose or its analogs, and fimbriae exhibiting this trait are mannose-sensitive hemagglutinins. By contrast, fimbrial antigens resistant to D-mannose are mannose-resistant hemagglutinins (265, 272). Functionally, the majority of *E. coli* strains that cause diarrhea in young farm animals express fimbriae that exhibit mannose-resistant hemagglutination, a characteristic that differentiates them from fimbriae expressed by other *E. coli* serotypes (281).

At first fimbriae were thought to be capsular (K) antigens (273, 274) and were designated K88 or K99 (273, 274, 337). Nomenclature proposed by Ørskov et al. (272) employs the F system to eliminate confusion between capsular and fimbrial antigens. Certain types of fimbriae have been associated with *E. coli* strains that cause secretory diarrhea in young farm animals. The more prominent types include K88 (F4), K99 (F5), 987P (F6), and F41 (128, 281). Recently, putative fimbrial antigens F17 (194), F165 (109–111), CS1541 (43), and CS31A (71, 134) have been isolated from neonatal farm animals with diarrhea. A relationship between CS31A and K88 fimbrial antigens has been proposed (134), and in in vivo studies, bacteria expressing the 987P fimbrial antigens reacted with CS1541 antisera (43), indicating some antigenic relationship.

K88 (F4) antigen. K88 antigen was the first fimbrial antigen detected on ETEC that caused diarrhea in a young animal (274). K88 antigen is a heat-labile protein containing all the common amino acids except cysteine-cystine (281). Seven genes located on a large plasmid code for its production (277).

K88 antigens are found on *E. coli* strains that cause diarrhea in pigs. This antigen mediates the attachment of *E. coli* to specific intestinal epithelial-cell receptors within the brush border epithelium of the small intestine. Sellwood et al. (324), using prepared brush border membranes of intestinal epithelial cells, demonstrated that two brush border phenotypes occur: (i) K88 adhesion positive and (ii) K88 adhesion negative. Pigs that have adhesion-positive brush borders are susceptible to infection with K88-positive *E. coli* strains, and the adhesion-negative pigs are genetically resistant to infection because of the lack of receptors (305, 323, 324). The presence or absence of receptors for the K88 fimbrial antigen is inherited in a simple Mendelian manner (133, 324). The two alleles are present at a single locus, and the allele coding for the receptor is dominant (182, 323, 324).

Further studies by Bijlsma et al. (26) with three K88 fimbrial-antigen variants showed that at least five pig phenotypes can be detected by brush border assays. Organisms

with four of the phenotypes were adhesion positive, and organisms with one were adhesion negative. The phenotypes were designated A through E, depending on susceptibility of the organism to adhesion in the brush border assay. Brush borders of pigs of phenotype A were susceptible to adhesion by all three K88 variants, phenotype B and C pigs were susceptible to two of the three variants, and phenotype D pigs were susceptible to one variant. Phenotype E pigs were resistant to adhesion. Pigs born to phenotype E parents should, under natural conditions, resist intestinal colonization by any of the K88-positive *E. coli*. Such genetic protection could provide a means of reducing neonatal pig morbidity and mortality due to K88-positive ETEC.

Each of the three K88 antigen variants, K88ab, K88ac, and K88ad, and the one subvariant, K88ad(e) (149, 228), are serologically distinct because of the amino acid sequence differences in the fimbrial-antigen proteins. The common a factor of each variant is conserved and binds with a variable antigenic factor designated b, c, or d. The a factor is believed to be responsible for antigen binding to susceptible epithelial-cell receptors (228).

K88 antigens exhibit consistent mannose-resistant hemagglutination of guinea pig erythrocytes (27, 149, 281). In addition to guinea pig erythrocytes, K88ab exhibits mannose-resistant hemagglutination of porcine and chicken erythrocytes, and K88ad exhibits mannose-resistant hemagglutination of porcine erythrocytes. Inconsistent mannose-resistant hemagglutination has been observed with rabbit, sheep, cow, goat, and human erythrocytes (281).

K99 (F5) antigen. *E. coli* strains isolated from newborn calves and lambs with diarrhea express what was believed to be a common K antigen, designated Kco (337). Ørskov et al. (273) named this Kco antigen K99. Subsequently, the K99 antigen was found in association with *E. coli* strains isolated from pigs with diarrhea (233). On the basis of the structure, shape, and surface location of the antigen subunit, Isaacson (168) characterized and described the antigen as a fimbrial antigen.

Six plasmid-encoded genes, designated *fanC* through *fanH*, are responsible for the production of the K99 fimbrial antigen (277). Deletion or mutational inactivation of *fanC*, *fanD*, or *fanE* results in decreased antigen production and adhesive capacity (277). Like other fimbriae on ETEC grown in in vitro systems, expression of the K99 antigen can be inhibited by media that contain the amino acid alanine or glucose (125). Furthermore, growth at 18°C inhibits fimbrial-antigen expression, while growth at 37°C with aeration stimulates production of K99 fimbriae (128). Reduced expression of K99 fimbriae is observed in vivo when intestinal fluid pH is <6.5 (123).

K99 fimbriae show mannose-resistant hemagglutination of sheep and horse erythrocytes (48) and adhere to calf, lamb, and pig jejunal and ileal brush border membranes (281). Calves and lambs are most susceptible to infection with K99-positive ETEC within the first 2 days after birth. After this time they become increasingly resistant to experimental infections (150). The resistance is associated with changes in the small-intestine epithelial-cell surface structures and does not appear to be antibody mediated (303). Runnels et al. (303) showed that intestinal epithelial cells from 1-day-old pigs had greater numbers of adherent K99-positive *E. coli* than did cells from 6-week-old pigs. Similarly, K99-positive *E. coli* adhered to epithelial cells from 12-h-old calves in greater numbers than to epithelial cells from 2-week-old calves. The inherited pattern of resistance to K88-positive *E. coli* exhibited by some pigs does not occur with K99 fimbriae (146).

Although the small-intestine epithelia of older calves, lambs, and pigs resist infection by K99-positive *E. coli*, under natural conditions this resistance is compromised by other enteropathogens. For reasons that are not well understood, intestinal infections with enteric viruses and *C. parvum* extend the period of susceptibility to infection. Hence, K99-positive ETEC is commonly identified in calves older than 2 days with concurrent intestinal infections with other enteropathogens.

F41 antigen. Fimbriae isolated from *E. coli* B41 have been shown by electrophoresis to be composed of two proteins that have different molecular weights and ionic charges (241, 242). The cationic and lower-molecular-weight protein is K99 fimbria, and the anionic, high-molecular-weight protein was eventually designated F41 fimbrial antigen. K99 and F41 antigens are composed of different amino acids and are serologically distinct.

F41 antigen has been shown to have adhesive properties had has been identified on *E. coli* strains that cause diarrhea among calves (241, 242), lambs (241, 242), and pigs (243). F41-positive strains characteristically show mannose-resistant hemagglutination of guinea pig and human type A erythrocytes (281). To (367) reported that F41 antigen agglutinated chicken, goat, and pig erythrocytes. Like other fimbriae, its synthesis is suppressed by low temperatures (18°C) and alanine. F41 antigen production appears to be encoded by chromosomal DNA (245).

987P (F6) antigen. An *E. coli* strain (strain 987) isolated from the intestinal tract of a neonatal pig with diarrhea lacked K88 and K99 fimbriae (253). However, a fimbrial antigen was determined to mediate attachment to pig jejunal and ileal intestinal epithelial cells. Isaacson and Richter (170) purified the antigen, confirmed its fimbrial nature, and designated it 987P.

987P fimbrial-antigen genes are located on a plasmid of at least 35 megadaltons (MDa) (317). The fimbriae are morphologically similar to type 1 fimbriae, but their hemagglutination characteristics are different from those of type 1 fimbriae and the other fimbrial antigens common to ETEC strains. They do not display mannose-resistant hemagglutination of guinea pig erythrocytes and they only weakly agglutinate chicken erythrocytes (281). 987P fimbriae characteristically occur on ETEC strains that cause diarrhea in neonatal pigs.

CFAs. The fimbrial antigens of ETEC that cause diarrhea in humans are antigenically distinct from those that cause diarrhea in animals (107). Fimbriae that mediate attachment of ETEC strains to human small-intestine epithelial cells are referred to as colonization factor antigens (CFAs). Currently there are at least three well-described, serologically distinct CFAs: CFA/I, CFA/II, and PCF8775, which has been designated CFA/IV (210, 357). Evans et al. (102, 104) initially described and characterized CFA/I and CFA/II as the ETEC attachment factors. ETEC strains bearing the CFA/I antigens showed mannose-resistant hemagglutination of human, bovine, and chicken erythrocytes (103, 107), while CFA/II showed mannose-resistant hemagglutination of bovine and chicken erythrocytes (102, 105, 107). Further studies have shown that *E. coli* strains expressing CFA/II may possess two or three immunologically distinct coli surface-associated antigens, CS1, CS2, and CS3 (190, 210, 359). CS1 and CS2 hemagglutinate bovine erythrocytes, and the CS3 antigen is nonhemagglutinating. These antigens appear to be expressed by different *E. coli* strains. CFA/IV (PCF8775) expresses

three coli surface antigens (CS4, CS5, and CS6) that appear on *E. coli* of specific serogroups and that seem to have individually distinct hemagglutination characteristics (209, 210). Coli surface antigens CS4 and CS5 cause mannose-resistant hemagglutination of human and bovine erythrocytes (357), while CS6 is nonfimbriate and nonhemagglutinating (210, 357).

Two less-well-defined CFAs, CFA/III and PCF159 (159, 210, 359), have been described elsewhere. CFA/III was identified on a strain (strain 260-1) that elaborated a heat-labile enterotoxin (LT) and produced nonhemagglutinating fimbriae with heat-stable hydrophobicity (159). *E. coli* expressing the putative PCF159 fimbrial antigen produced both heat-stable enterotoxins (STs) and LT. The fimbrial antigen is nonhemagglutinating (210, 359).

Detection of Fimbrial Antigens

Verification that ETEC is the causative agent of diarrheal disease requires demonstration of the *E. coli* strain and its virulence factors. The most practical yet least reliable procedure is demonstration of the fimbrial antigens on strains isolated from feces or intestinal contents. Identification of the fimbrial antigen alone offers presumptive evidence that the *E. coli* strain is the causative agent.

After primary isolation of the organism, suspect colonies are grown on selective media (125, 150) that promote expression of the various fimbrial antigens: for K88, blood agar and E medium; for K99 and F41, E medium and Minca IsoVitaleX medium; for 987P, Minca IsoVitaleX medium. Fimbriae are then identified by routine slide agglutination procedures (122, 210) with monoclonal antibodies, monospecific fimbrial antisera, or polyvalent antisera (385).

Enzyme immunoassays (EIA) have been developed to detect fimbrial-antigen-expressing bacteria in feces (156, 222, 223). For clinical purposes, an EIA for detecting K99 antigen is commercially available (Coli-Tect 99 kit; Schering-Plough Corp., Union, N.J.). Procedures using fluorescent-antibody techniques are performed on impression smears of intestinal tissue (122). These procedures are more reliable than demonstration of the fimbrial antigens on strains isolated from feces, for they detect the antigen that is attached to the intestinal epithelial cells.

Hybridization assays that simultaneously detect fimbrial antigen and enterotoxin genes in bacterial colonies or fecal material are used by some diagnostic and reference laboratories (199, 200, 227, 234). These assays greatly enhance the reliability of detecting ETEC and eliminate the tedious bioassays that are often required to definitively diagnose ETEC infections.

In herd or flock outbreaks of diarrheal disease in which ETEC is suspected, histopathologic examination of intestinal tissue in combination with bacterial cultures provides a practical and extremely useful means of diagnosing ETEC infections. Histopathologic examination will reveal diffuse or focal areas of colonization of the intestinal epithelium in the absence of extensive damage to the epithelium (Fig. 2 and 3). This histopathologic observation along with the concurrent isolation of fimbria-positive *E. coli* is a reasonable indication that an *E. coli* strain is the causative agent. Hemagglutination studies are not routinely used for diagnostic purposes.

Enterotoxins

After colonization, ETEC produces one or more enterotoxins that cause a hypersecretory diarrhea by receptor activation of the intracellular second-messenger system of cAMP or cGMP (266). Two biologic classes of enterotoxins are produced: heat labile and heat stable (128, 147, 158, 321). Production of both classes of enterotoxins is controlled by transmissible plasmids (331).

LT. LT is a high-molecular-weight toxin that is functionally and structurally related to *Vibrio cholerae* toxin (58, 158). It is inactivated by being heated at 60°C for 30 min. The toxin consists of an A/B subunit with five immunologically active B subunits that bind to ganglioside receptors on the cell surface. Following binding, the biologically active A subunit is enzymatically activated, adenylate cyclase activity is stimulated, and increased intracellular concentrations of cAMP results. The cAMP activates specific protein kinases that release calcium from the intracellular stores, leading to a net secretory response (58, 158).

LT is consistently produced by K88-positive ETEC (184), and none of the other common ETEC strains produce LT. *E. coli* and *V. cholerae* LTs belong to two serogroups designated LTI and LTII (127). Those *E. coli* LTs that are neutralized by antisera prepared against cholera toxin are LTI (serogroup I). Pig *E. coli* LTs in this toxin serogroup have been described (127). LTs that are not neutralized by antisera against cholera toxin are LTII, and *E. coli* strains producing them have been isolated from cattle in Sri Lanka and Thailand (326).

STs. STs are low-molecular-weight toxins that are resistant to temperatures of 100°C for 30 min (335). In neonatal farm animals, STs are produced by all the common ETEC strains (184). However, STs produced by the various ETEC strains represent a heterogeneous class of enterotoxins. On the basis of methanol solubility, biologic activity in the gastrointestinal tracts of infant mice, neonatal pigs, and weanling-age pigs, ability to increase mucosal guanylate cyclase activity, and amino acid composition, STs can be divided into two types, STa and STb (128, 158, 183). STa is active within the gastrointestinal tracts of neonatal pigs and mice (280). STb induces intestinal secretion in newborn pigs and weanling-age pigs (391) but has no apparent activity in mice. STb is produced by porcine (K88-positive) ETEC (202), and the mechanism by which it stimulates the intestinal mucosa to secrete is unknown (187, 391). STa is produced by K99, F41, and 987P ETEC strains. Because of its low molecular weight, STa is nonimmunogenic, although it has been shown to behave as a hapten (307) when coupled to a carrier protein.

STa influences intestinal ion and fluid secretion by activating guanylate cyclase, which leads to increased intracellular cGMP. Increased cGMP inhibits the Na^+-Cl^- cotransport system (92, 266). Two distinct genes coding for STa production have been identified, *staH* and *staP* (199, 200, 202, 234). The *staH* gene is present in ETEC strains that cause diarrhea in humans but is absent from strains that infect animals. The *staP* gene is present in ETEC strains that infect pigs, cattle, and humans (200, 202, 234).

Detection of Enterotoxins

The impediment to studying *E. coli*-induced diarrhea has been in routinely determining enterotoxin activity. Although many procedures have been developed, some are not readily available for routine diagnostic use. Common and practical methods used to detect enterotoxins include biologic and immunologic assays. LT activity is most often detected in cell culture with Y-1 mouse adrenal cells (89, 308), Chinese hamster ovarian cells (CHO) (148), and Vero monkey kidney

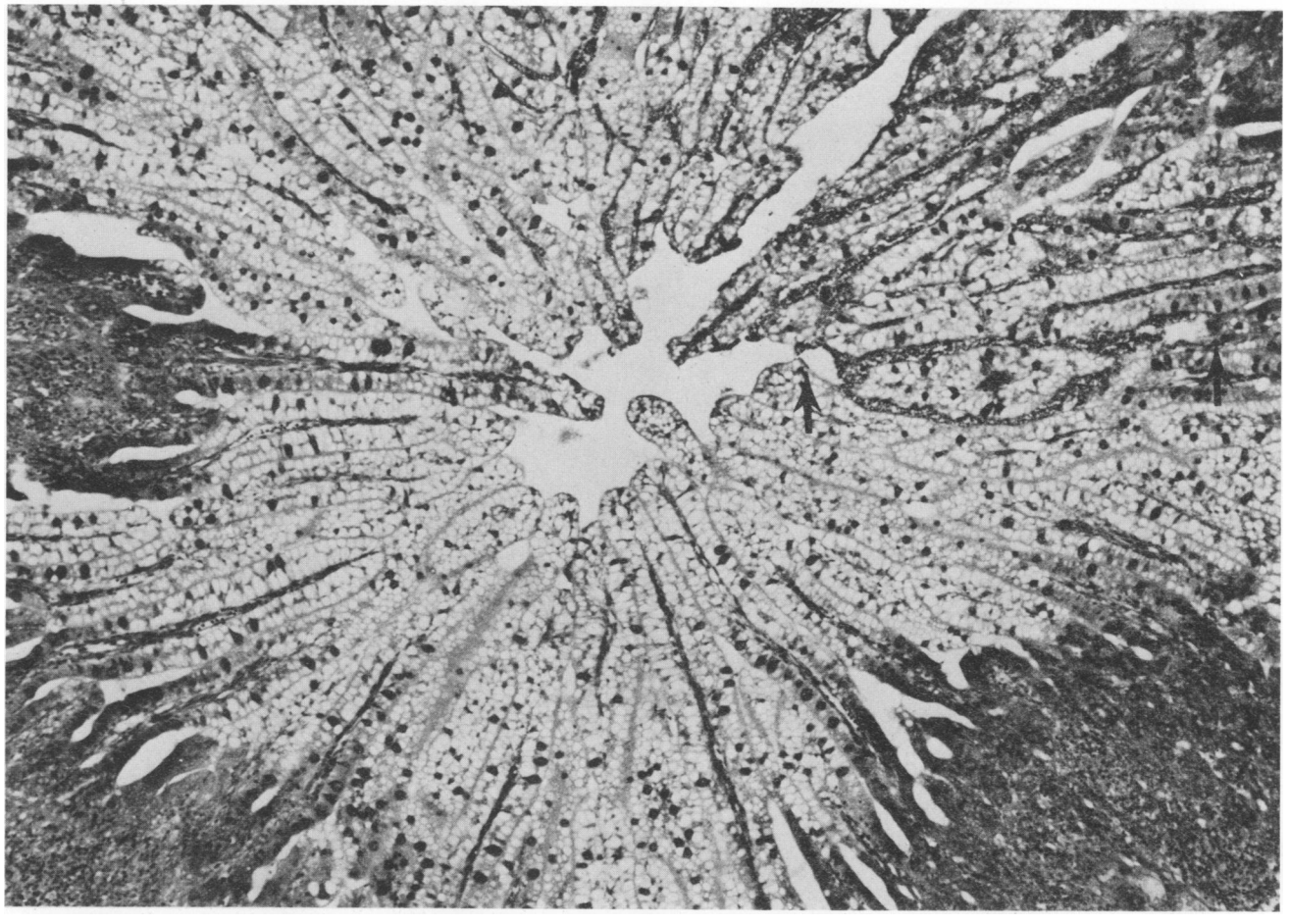

FIG. 2. Photomicrograph of a section of ileum from a gnotobiotic pig infected with 987P-positive ETEC. The bacteria are attached to the villi from the crypts to the tips (arrows). No other microscopic lesions are observed. Giemsa stain. Magnification, ×120.

cells (185). The immunogenic nature of LT has allowed for the development of immunologic tests to detect its presence (160). In contrast, STs are poorly antigenic and their activities are detected by fluid accumulation in calf or pig intestinal loops or in infant mice (81, 308, 321). Recently, DNA probes, DNA colony hybridization techniques, and EIA have been introduced for the detection of LT, STa, and STb (199, 200, 202, 234, 292, 309, 322).

Serologic Types of ETEC

Serologic typing of ETEC is based on the antigenic characteristics of the three major surface antigens (270, 272). Currently, 171 O antigens, 80 K antigens, and 56 H antigens are known. O-antigen determinations provide the basis for serogroup and serotype designations. These antigens are the polysaccharide component of the outer membrane liposaccharide complex. The K antigens are the liposaccharide capsular antigens, and the H antigens are the flagellum antigens. The O antigen is used to designate serogroup, and the O and H antigens constitute the O:H system. O:H and O:K systems are frequently used to designate serotypes.

Before fimbrial antigens and enterotoxin activity were detected, diarrheagenic *E. coli* strains were identified by serogroup or serotype. O-antigen determinations have shown that ETEC strains belong to a limited number of serogroups (128, 189, 346, 393), in contrast to normal commensal flora, which may belong to a wide variety of serogroups. Although serotyping and serogrouping have provided a means of assessing enteropathogenicity, unless the virulence factors that cause diarrhea (e.g., enterotoxins) have been detected, the serologic evidence is only subjective.

Serogroups O8, O9, and O101 are the most common ETEC (K99 and STa) serogroups that cause diarrhea in calves and lambs. However, K99-positive ETEC strains obtained from piglets belong to serogroups O64 and O101, with serogroup O101 occurring most often (346, 347, 393). ETEC strains with the F41 fimbrial antigen isolated from calves and pigs belong primarily to serogroups O9 and O101 (128). Fimbrial antigen K88 has been detected on ETEC strains belonging to serogroups O157, O149, O8, O45, O138, O141, and O147, whereas 987P ETEC strains belong to serogroups O141, O20, and O9 (128, 281, 393).

Pathogenesis

In the calf and pig, facultative anaerobic and strictly anaerobic bacteria become fully established within 24 h after birth (94). However, the small intestine of the neonate contains few bacteria. Strong gut peristalsis, villous pumping, flowing ingesta, and mucus serve to propel bacteria through the small intestine and thereby regulate the establishment of bacteria (126, 231). Experimentally, inocula

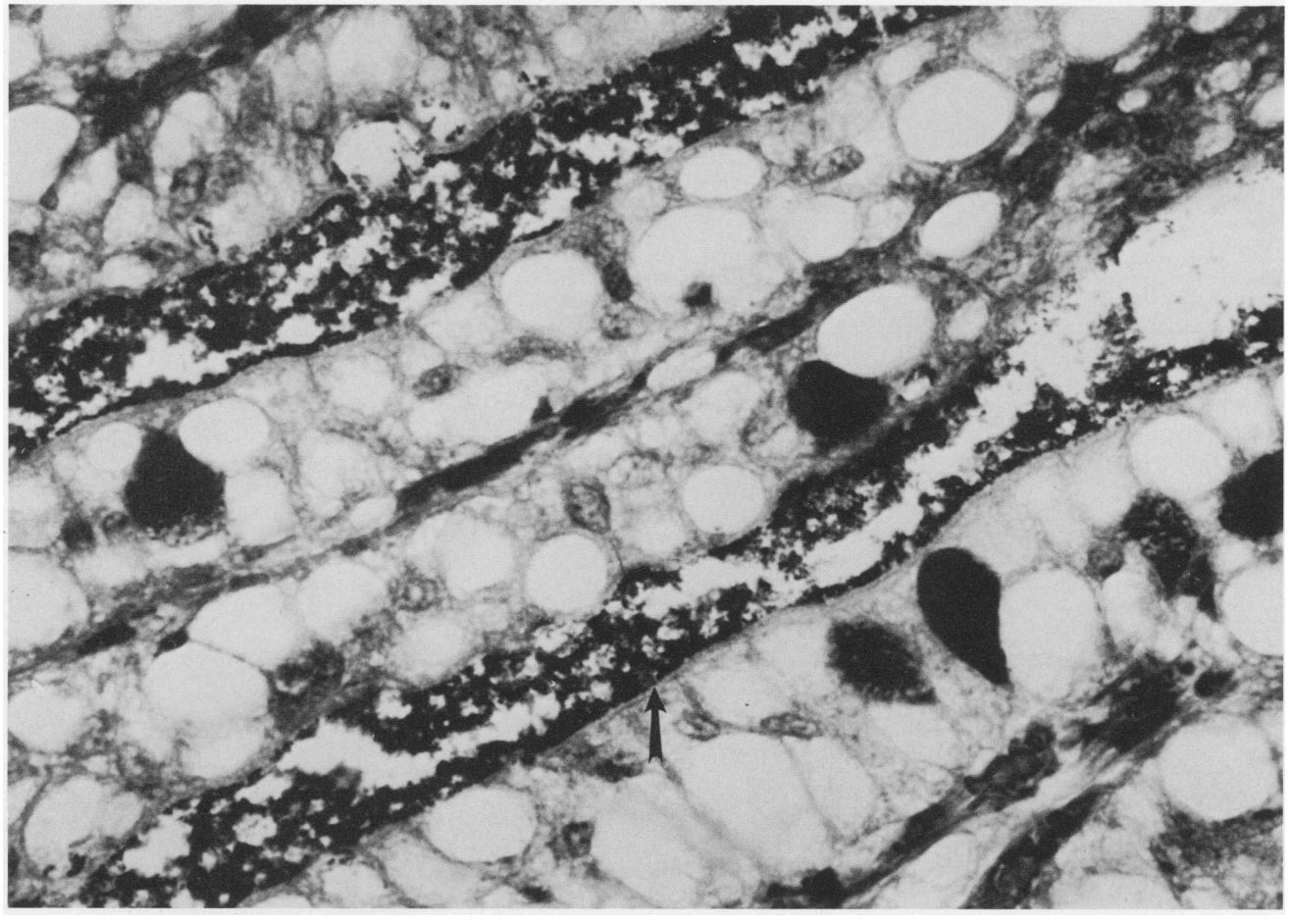

FIG. 3. Photomicrograph of a section of ileum from a gnotobiotic pig infected with 987P-positive ETEC. Layers of bacteria are attached to the epithelium of the villi (arrow). Giemsa stain. Magnification, ×480.

containing 10^9 to 10^{10} ETEC are required in order to induce diarrhea (1). Under natural conditions, it is improbable that a neonatal animal will consume such large numbers of ETEC; therefore, in addition to overcoming the physiological defenses within the gastrointestinal tract, an incubation period is required for ETEC to proliferate to sufficient numbers to cause diarrhea.

Fimbriae enable ETEC to overcome physiological defenses and attach to the membrane receptors of a susceptible host (66, 231). The enterotoxigenic strains become attached and rapidly proliferate within the small intestine. After attachment, the ETEC strains elaborate enterotoxins that cause the secretory diarrhea.

Minimal gross or histopathologic lesions are observed in the intestinal mucosa of animals infected with ETEC. The characteristic histopathologic finding is a uniform layer of adherent bacteria lining the brush border epithelium of the distal jejunum and ileum (Fig. 2 and 3).

Clinical Presentation

The period of susceptibility for enterotoxigenic colibacillosis varies with the species at risk. In neonatal calves, clinical signs may be apparent within 24 h of birth, and pure enterotoxigenic colibacillosis is seldom observed in calves >3 days old. However, the presence of other enteropathogens (rotavirus and *C. parvum*) may extend the period of susceptibility. In pigs the period of susceptibility is not limited. Pigs as young as 12 h to several weeks of age are susceptible to ETEC infections. The disease is characterized by a profuse, effortless, watery diarrhea. In the acute stage of colibacillosis, affected neonates rapidly become dehydrated, losing up to 10 to 12% of their body weight in <6 h. As a result, the affected animal exhibits central nervous system depression, weakness, normal to subnormal body temperature, and tachycardia or bradycardia; if the animal is left untreated, death results from hypovolemic shock and heart failure.

Clinical pathologic evaluation of affected neonates reveals hyperkalemia, uncompensated metabolic acidosis, and prerenal azotemia. Hyperkalemia results from a shift of H^+ into the cells and a reciprocal shift of K^+ out of the cells in response to lactic acidosis. Although the cause of death in these neonates is due to the cardiotoxic effects of a high serum K^+ concentration, a total body potassium deficit is present.

DIARRHEAL DISEASE CAUSED BY NON-ETEC

With increasing awareness of the virulence factors displayed by various strains of *E. coli*, more diarrheagenic *E. coli* strains are being characterized and diarrheal syndromes in neonatal farm animals similar to those described in humans (188) are more frequently reported. EPEC, EHEC,

and cytotoxic necrotizing factor (CNF)-positive *E. coli* will be discussed in this section. Evidence that enteroinvasive strains exhibiting *Shigella*-like invasiveness occur in young farm animals is lacking.

Virulence Factors of EPEC and EHEC

After the experimental inoculation of colostrum-deprived neonatal pigs with *E. coli* O55B5H7, Staley et al. (349–352) observed that the pigs had a mild enteritis and colitis. Microscopic lesions in the ileum and colon included microvillous degeneration, villous atrophy, and colitis. The pigs displaying these lesions also had reduced protein absorption (351). Although virulence factors of strain O55B5H7 were not discussed in the reports, the microscopic lesions induced after experimental inoculations were not typical of those caused by ETEC but were consistent with the lesions associated with *E. coli* strains that attach to and efface the microvilli of intestinal epithelial cells (235, 246).

Attaching and effacing lesions are increasingly being described and detected in association with *E. coli* strains isolated from a variety of animal species (55, 63, 124, 152, 172, 173, 235, 246, 288, 289, 296, 319, 329, 375, 379, 394, 407). Such *E. coli* strains have been categorized as EPEC and EHEC. Unlike ETEC, the precise role of EPEC and EHEC as causative agents of intestinal disease and diarrhea among calves, lambs, and pigs is not completely understood. However, some strains isolated from rabbits, calves, and humans have been characterized.

EPEC. In veterinary medicine, the term EPEC has historically been used to refer to *E. coli* strains that cause intestinal (324, 334, 337) and extraintestinal (281) infections. However, EPEC has also been defined to refer to specific serogroups or serotypes identified as pathogens involved in diarrheal disease of infants and young children (98). The mechanism by which these strains cause diarrheal disease does not involve the production of LT or ST, and they lack *Shigella*-like invasiveness (98).

In animals, under both natural and experimental conditions, EPEC attaches to intestinal epithelial cells and causes effacement of the microvilli. Mechanisms by which EPEC causes the attaching and effacing lesions are not fully defined, since expression of an attachment factor (55, 394) and production of cytotoxins (152, 246) are important but not essential for producing the attaching and effacing lesion.

EHEC. The clinical, epidemiologic, and pathogenetic features of O157:H7 and O26:H11, the EHEC prototypes, provide the standards by which other potential EHEC isolates are judged (188, 375). These EHEC serotypes have been recovered from humans and calves with diarrhea (204). Experimental inoculations of gnotobiotic pigs with these serotypes caused various degrees of attaching and effacing lesions and clinical illness (124, 375, 376). Additionally, serotypes O157:H7 (30, 137), O26:H11 (329, 407), O26:NM (173), O111:NM (173, 319, 329), and O5:NM (63, 219) have been recovered from calves with diarrhea or dysenterylike syndrome. Although EHEC strains are rare in neonatal food animals, they cause diarrhea, and infected calves may be potential reservoirs for EHEC serotypes.

Like EPEC, EHEC strains do not synthesize LT or STs and they are not enteroinvasive. In gnotobiotic pigs, some EHEC strains penetrate the mucosa to reside within the lamina propria (375). Expression of the 60-MDa plasmid that encodes for production of fimbriae and Shiga-like toxins is not essential for enteric disease production in gnotobiotic pigs (376). EHEC strains colonize the intestinal mucosa by intimate attachment to and effacement of microvilli from the epithelial cells (235, 246, 375, 379). They also differ from EPEC strains in that they more consistently synthesize potent cytotoxins (Shiga-like toxins) that cause severe hemorrhagic colitis (180, 188, 258).

Fimbriae

EPEC and EHEC strains express surface structures that mediate their attachment to intestinal epithelial cells. EPEC strains isolated from humans synthesize a plasmid-mediated adherence factor that displays two patterns of adherence (180): (i) localized adherence to HEp-2 cells and (ii) diffuse adherence or no adherence to HEp-2 cells. Strains isolated from animals display similar plasmid-mediated fimbrialike adhesive factors (EAF 44) (410), AF/R1 pilus adhesion (rabbit strains) (55, 394), and mannose-resistant hemagglutinins (264, 410). EHEC strains possess a 60-MDa plasmid that is required for expression of fimbriae that attach to cultured Henle 406 intestinal cells (179).

Cytotoxins

Some EPEC strains and most of the currently described EHEC strains synthesize potent cytotoxins that damage epithelial cells and lead to cell death. *E. coli* cytotoxic activity was first observed by Smith and Lingwood (336). However, Konowalchuk et al. (185) found an *E. coli* cytotoxin that differed from the LT and STs common to *E. coli* strains. The cytotoxin-producing strains were isolated from human infants with diarrhea. Culture filtrates of the isolates were toxic to Vero cells and therefore were called verotoxins. Subsequently, two types of *E. coli* verotoxins were described in strains isolated from human cases of diarrhea (258) and in neonatal farm animals with diarrhea (55, 63, 125, 137, 225, 226, 319, 329). *E. coli* verotoxin type 1 is immunologically and structurally related to the toxin synthesized by *Shigella dysenteriae* type 1. Unlike verotoxin type 1, *E. coli* verotoxin type 2 is not neutralized by anti-Shiga toxin antibodies but is immunologically related to verotoxin type 1. Shiga-like toxins I and II are synonymous with verotoxin types 1 and 2, respectively (180, 258).

Serologic Types

For neonatal farm animals, epidemiologic information concerning the serotypes or serogroups of EPEC and EHEC strains that may cause diarrheal disease is lacking. Listed in Tables 1 and 2 are serotypes and serogroups of Shiga-like-toxin-producing (verotoxin-positive) *E. coli* strains that have been associated with diarrhea in calves and pigs. The term Shiga-like-toxin-positive *E. coli* rather than the terms EPEC and EHEC is used, since EPEC strains inconsistently produce cytotoxins.

Among calf isolates, many Shiga-like-toxin-positive *E. coli* isolates are untypeable (30, 137). The typeable strains are most often (two or more isolates) associated with serogroups O2, O5, O6, O8, O26, O103, O111, O128, and O157. *E. coli* serotypes O5:NM, O8:H49, O26:H11, O86:H26, O111:NM, and O157:H7 were isolated from two or more animals. Among pigs, serogroups O138, O139, and O141 appear to predominate in cases of diarrhea and edema disease. Serotypes O138:NM, O139:K82, and O141:K85 were often observed in association with diarrhea. *E. coli* Shiga-like toxins can be detected by gene probes (201) and by cell culture procedures. Shiga-like-toxin-positive *E. coli*

TABLE 1. Distribution of bovine *E. coli* serotypes and serogroups that produce Shiga-like toxins and CNF

Shiga-like toxins (verotoxins) produced by[a]:				CNF produced by serotype[b]:
Serotype			Serogroup	
O2:H7	O8,O75:H49	O104:K?	O125	O8:K87
O2:H11	O20:K?	O111:NM	O4	O15:K14
O2:K?	O26:NM	O116:NM	O19	O15:K$^-$
O3:K$^-$	O26:H11	O117:K$^+$:H7	O168	O54:K?
O5:K?:H32	O29:H34	O127:NM		O76:K$^-$
O5:K?:H7	O49:K?:H12	O128:H31		O78:K80
O5:K4:NM	O55:H17	O128:K?		O88:K?
O5:NM	O76:H48	O137:H41		O123:K$^-$
O6:H27	O84:K?	O153:H12		O139:K$^-$
OX6:H2	O86:H26	O153:K$^-$		O153:K$^-$
O7:H40	O103:H21	O157:K$^-$:H7		O2:K53,93:H1
O8:49	O103:K$^-$			O8:K87:H$^-$
				O8:K87:H$^-$
				O8:K87:H$^-$
				O11:H$^-$

[a] Compiled from references 30, 85, 137, 225, 246, 319, 329, 332, and 410.
[b] Compiled from references 30, 85, and 137.

of animal origin belong to many serotypes common to human strains (see references 180 and 258 for detailed discussions of Shiga and Shiga-like toxins).

Pathogenesis

Under both experimental and natural conditions, EPEC and EHEC strains cause enteric disease and diarrhea among neonatal farm animals. Experimental inoculation of gnotobiotic pigs with EPEC and EHEC strains isolated from humans, pigs, and rabbits causes diarrhea and the characteristic attaching and effacing lesions in pigs (235, 375, 376, 379). Strains isolated from calves cause similar clinical signs and microscopic lesions in other calves (56, 246, 407), lambs (219), and pigs (137).

While expression of an attachment factor and production of cytotoxins influence the virulence of EPEC and EHEC strains in calves and pigs, they are not essential for establishing the attaching and effacing lesions and diarrhea (55, 152, 376, 394). Their presence, however, influences the distribution and severity of the lesions. Lesions may be mild and scattered throughout the small and large intestines (152, 235), or they may be severe and restricted to the large intestine (319). The cecum and colon are most often affected, but the distal jejunum and ileum may also be involved.

TABLE 2. Distribution of porcine *E. coli* serotypes and serogroups that produce Shiga-like toxins and CNF

Shiga-like toxins produced by[a]:		CNF produced by[b]:	
Serotype	Serogroup	Serotype	Serogroup
O65:H9	O2	O75:K$^-$	O4
O138:NM[c]	O107	O75:K95	O6
O138:H14	O120	O2:K2	O8
O138:K81	O121		O88
O139:K82	O130		
O141:H4[c]			
O139:K82			
O141:K85$_{ab}$			
O141:K85$_{ac}$			
O157:K"V117"			
O149:K91			

[a] Compiled from references 129, 136, and 332.
[b] Compiled from references 136 and 213.
[c] Serotypes associated with edema disease of pigs.

Within the small and large intestines, large numbers of bacteria attach to the epithelium. Often, the bacteria are observed to accumulate in several thick layers. Intimate apposition of the bacteria to the intestinal epithelial cells causes effacement of microvilli. Associated microscopic lesions include erosions and ulcers at the site of bacterial attachment to absorptive cells (152, 235, 246). Epithelial cells in areas of bacterial attachment are cuboidal to low columnar, and the microvilli are atrophic and display various degrees of swelling and elongation (352).

Similar microscopic lesions are observed in the epithelium of the colon (Fig. 4). Bacterial attachment appears to be multifocal and random, for there is no apparent predilection for any particular cells. At the sites of bacterial attachment, the microvilli display various degrees of exfoliation. Cellular degeneration is thought to occur as a result of impaired circulation and edema of the lamina propria and submucosa (152, 296).

Fluid accumulation within the lumen of the intestinal tract and edema of the large intestine are frequently observed upon gross necropsy. Those strains that synthesize Shiga-like toxins appear to cause more-extensive damage to the large-intestine mucosa and epithelium. Extensive edema and congestion, mucosal erosions, ulcers, and blood or hemorrhage are observed in the intestinal lumen. Hemorrhage is believed to occur secondary to the extensive mucosal erosions and ulcerations (152, 296).

Clinical Presentation

Diarrhea and dysenterylike diarrhea are often observed as clinical signs in calves and pigs naturally infected with EPEC. Limited information suggests that EPEC strains can cause diarrhea in calves from 2 days to 4 months of age (172, 173). Other enteropathogens such as rotaviruses and *C. parvum* are often present. The diarrhea resulting from infection with EPEC has been postulated to occur secondary to maldigestion and malabsorption.

Clinically, I have observed and attempted therapy in three calves that had attaching and effacing lesions in the colon (Fig. 4). The calves presented with anemia, they were weak and emaciated, and they passed clots of blood that contained intestinal casts. Hemorrhagic colitis was tentatively diagnosed. Therapy included plasma and whole-blood transfusions, an antiinflammatory agent, intravenous balanced electrolytes, and broad-spectrum antibiotics but was ineffective. The bacteria isolated from these calves did not produce Shiga-like toxins.

True EPEC and EHEC infections appear to be of low prevalence among calves, lambs, and pigs. In the live animal, presumptive diagnosis of EPEC infection is based on clinical findings. Observing the characteristic histopathologic lesions and identifying potential virulence properties of the *E. coli* isolates will assist in defining EPEC infections. Epidemiologic information is not yet available as to what factors (e.g., environmental, host, or nutritional) may contribute to the occurrence of these bacteria in young farm animals. EHEC (O157:H7 Shiga-like toxin positive) are of considerable public health importance because they have been implicated in foodborne illness.

CNF-Positive *E. coli*

In addition to LT, STs, and Shiga-like toxins, some *E. coli* strains have been determined to synthesize CNF. CNF was

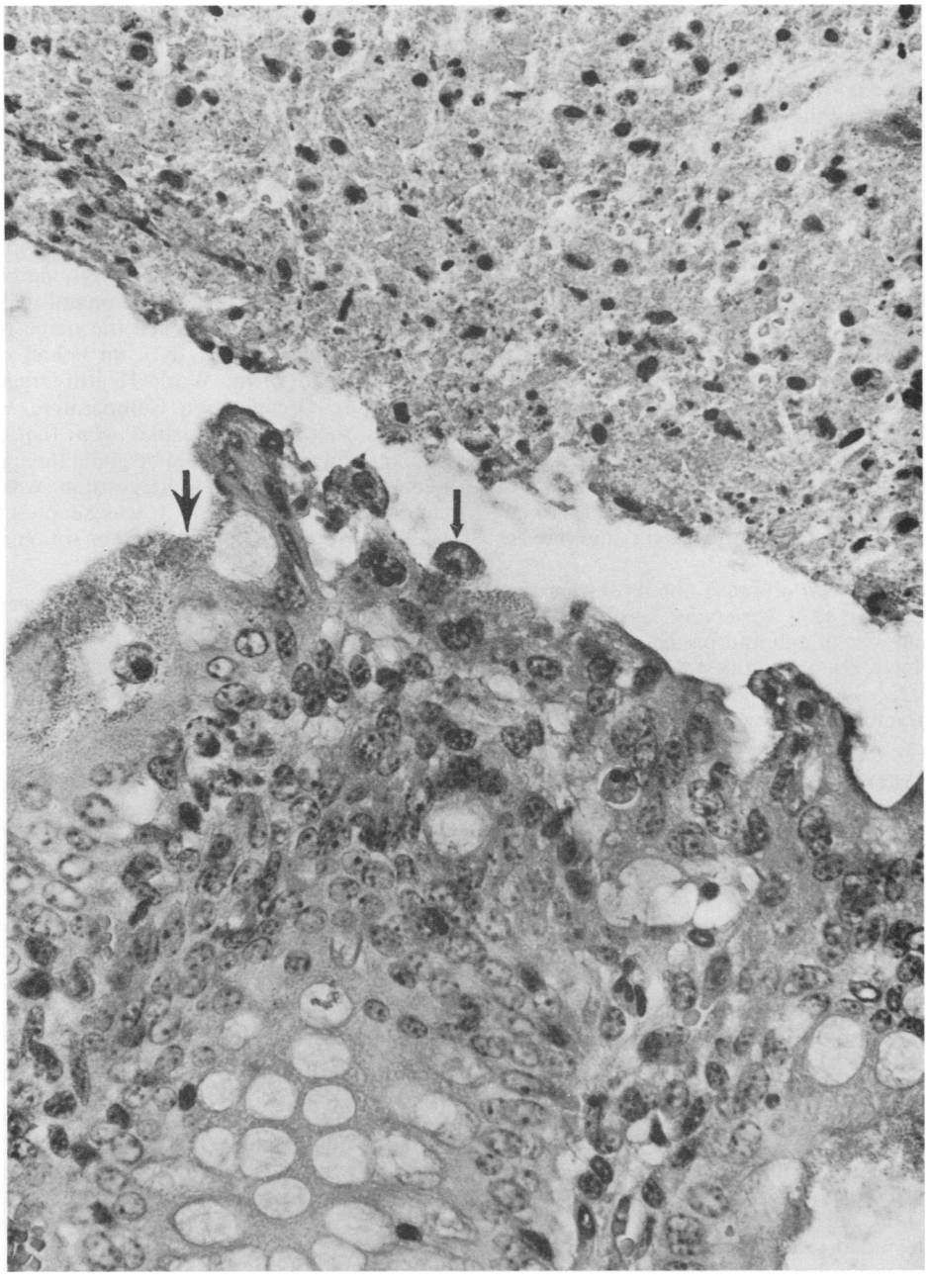

FIG. 4. Photomicrograph of a section of colon from a calf naturally infected with bacteria that caused microscopic lesions consistent with the presence of EPEC. The surface epithelium is irregular and eroded. Bacteria are closely attached to the lumenal surface of the epithelial cells (large arrow), and in these colonized areas, the brush border is no longer distinct. An extruding cell is ready to slough into the intestinal lumen (small arrow). Cellular debris is present in the intestinal lumen. Giemsa stain. Magnification, ×400.

first detected by Caprioli et al. (56) as a cell-associated toxic factor of *E. coli* isolated from young children with diarrhea. Subsequently, CNF-positive *E. coli* were detected in association with diarrhea in calves (30, 85, 136, 137, 213, 225, 226, 332) and pigs (136) and with diarrhea and urinary tract infections in humans (5, 29).

CNF has been shown to be most toxic to HeLa cells but causes the formation of large multinucleated cells when incubated on cultured HeLa, CHO, or Vero cells (57, 84). In in vivo studies, CNF causes local necrosis when injected into rabbit skin (56, 87), and subcutaneous administration in the abdomen of guinea pigs caused widespread systemic hemorrhage (56). CNF was demonstrated in the rabbit ileal-loop assay and suckling mouse assay to be different from *E. coli* LT and STs (56), respectively. It is a heat-labile toxin, for it is inactivated by being heated at 75°C for 15 min or at 60°C for 1 h (85). It is highly lethal to mice (86, 87) and is a protein of 115 kDa (87). Although most *E. coli* strains producing CNF also produce hemolysins, the toxic effects of CNF are independent of hemolytic activity (57).

On the basis of in vivo, in vitro, and seroneutralization studies, two types of CNF have been characterized in animal and human *E. coli* isolates (57, 84, 276). In a study by De Rycke et al. (85), 14% of diarrheic calves <21 days of age

harbored *E. coli* strains that displayed two types of cytopathic effects on HeLa cells. When cells were exposed to sonic extracts of the bacteria, the characteristic effects of enlargement, rounding, and multinucleation typical of CNF was seen and was designated the type 1 response. Moreover, in addition to the type 1 response, some of the *E. coli* sonic extracts produced elongation of HeLa cells and were lethal for chickens. These were designated the type 2 response. Results of further studies (276) have shown that the type 1 and type 2 responses are caused by two partially related cytotoxins and that type 2 cytotoxicity is coded for by a transmissible virulence plasmid (Vir plasmid). The Vir plasmid was initially discovered in *E. coli* strains (333) that produced septicemia or bacteremia in young animals. It is required for synthesis of a toxin and fimbriae (196).

In addition to the different morphologic effects displayed by the two types of CNF, they differ in their responses in the rabbit skin test and mouse footpad test (84, 86). CNF type 1 causes moderate necrosis in rabbit skin and lacks activity in the footpad test, whereas CNF type 2 is associated with an intense necrotic response in the rabbit skin and mouse footpad tests.

Most CNF-positive *E. coli* express mannose-resistant hemagglutinins and belong to a variety of serotypes (29). Thus far, a limited number of calf and pig *E. coli* serotypes that produce CNF have been identified (Tables 1 and 2). Some CNF-positive *E. coli* serotypes recovered from humans have been characterized elsewhere (29).

ROTAVIRUSES

Rotaviruses affect a variety of animal species, including laboratory rodents, wildlife, and humans (67, 74, 120). They are a common cause of diarrhea in lambs, calves, and pigs (120, 165, 368), in which infection may be asymptomatic (73, 74), varying in severity (41, 380), or fatal (41, 399, 404). In infants and young children, rotaviruses are the most commonly identified cause of viral enteritis and diarrhea (74). Results of seroepidemiologic and molecular epidemiologic investigations suggest that rotaviruses have a worldwide distribution in both human and animal populations (44, 45, 99, 121, 131, 164, 205, 207, 290, 302, 306, 364, 382). Human and animal rotaviruses share certain antigenic properties (40, 44, 99, 120, 131, 163, 164, 177, 178, 313, 412), indicating the possibility of cross-species infections (120, 177, 404). Human rotaviruses can infect and cause disease in calves (82, 219, 399), lambs (79, 82, 342), and pigs (79, 82, 399), yet limited evidence has been provided to establish animal rotaviruses as a cause of diarrhea in humans (47, 99, 100).

The importance of viruses as causes of neonatal calf diarrhea was first demonstrated by Mebus et al. (218) in 1969. They collected bulk fecal samples from young calves on a ranch that had yearly epizootics of calf diarrheas and inoculated neonatal calves with bacterium-free fecal filtrates by intraduodenal or oral administration or by spraying the filtrate as a mist over the heads of the calves. The calves inoculated orally and intraduodenally became ill and shed viral particles in their feces. The particles were determined by electron microscopy to have a diameter of approximately 65 nm. Subsequently, viruses that were antigenically and morphologically similar to those identified by Mebus et al. (218) were observed in the feces of calves in other countries (120, 396, 398) and were reported to cause diarrhea in other species (28, 118–120, 396). Further investigations, including virus isolations from different animal hosts, adaptation of the virus to cell culture (216, 218, 388), experimental inoculations (218, 362), and seroepidemiologic investigations (118, 177), aided in determining the worldwide distribution of the virus, its infectivity for potential hosts, and its enteropathogenic nature.

In initial studies, the viruses were referred to as reovirus-like agent or particles (118, 119, 217, 388, 398), neonatal calf diarrhea virus (216), Nebraska calf diarrhea virus (82), neonatal calf diarrhea reovirùslike agent (397), orbivirus (28, 220), and infantile gastroenteritis virus (79, 219). After conducting serologic and morphologic studies of virus particles obtained from the feces of young children with acute gastroenteritis and of the neonatal calf diarrhea virus, Flewett et al. (118) proposed the name rotavirus. In 1978, Derbyshire and Woode (82), on behalf of The Reoviridae Working Team of the World Health Organization/Food and Agriculture Organization Comparative Virology Program, reported that the reoviruslike agent that causes diarrhea in humans and animals would be given the name rotavirus. The neonatal calf diarrhea virus strain was selected as the candidate reference virus. It was adapted to cell culture and was used to characterize all other rotavirus isolates.

Taxonomy of Rotaviruses

Rotaviruses are taxonomically grouped in the *Reoviridae* family (82, 177). This family also includes the orthoreoviruses and orbiviruses, other genera known to infect animals and human beings (82, 177, 397). Rotaviruses are uniquely characterized by a double-stranded RNA genome that consists of 11 gene segments enclosed in a double-shelled protein capsid (177). The 11 RNA gene segments encode viral proteins and have assigned functions (206). The structures and functions of rotavirus proteins have been covered extensively by Estes and Cohen (101). The antigenic characteristics and importance in electropherotyping of gene segments 4, 6, 7, 8, 9, 10, and 11 are briefly discussed here.

Serologic Classification

Groups (serogroups). Initially, the serogroup designation was proposed by Pedley et al. (286, 287) and was based on the presence of distinct group antigens, RNA gene segment migration profiles, and unique terminal fingerprint patterns of the genome segments. Rotaviruses are divided into six distinct groups designated A through F, with evidence accumulating to support the possible inclusion of one more group (37, 47, 287). Group A rotaviruses are the rotaviruses most often associated with diarrhea among young farm animals and humans. Recently, group B and C rotaviruses have been found in animals and humans with diarrhea. Thus far, groups D, E, and F rotaviruses have been found only in animals.

Serotypes. On the basis of the reactivity of viral protein (VP) 7, group A rotaviruses are further classified into nine serotypes (21, 68, 74, 208, 221, 382, 409). Six serotypes of human rotaviruses are recognized: serotypes 1 through 4, 8, and 9 (21, 68, 74, 164, 208, 221). Serotypes 3, 4, 5, 6, and 7 are animal rotaviruses, and serotype 7 includes avian species (164). Serotypes 3 and 4, then, include both animal and human rotaviruses (164, 205, 252).

Subgroups. Nonneutralizing epitopes located on VP6 are used to characterize rotaviruses as to their subgroup specificities. At least three rotavirus subgroups have been defined. They are designated subgroup I, subgroup II, and non-subgroup I or II (143, 162, 176, 178, 205, 382). Most human rotaviruses show subgroup II specificity, while most

animal rotaviruses belong to subgroup I (144, 178). Some animal (Gottfried porcine strain subgroup II and equine F1-14 strain subgroups I and II) and human rotavirus strains have antigenic properties common to both subgroups (33, 162, 252). Non-subgroup I or II contains both mammalian and avian rotavirus strains (162).

VP4. VP4 is an outer capsid protein that is encoded by gene segment 4. It is an 82-kDa protein with hemagglutinating properties (144), is responsible for inhibiting the growth of human rotavirus in cell culture, and is associated with protease-enhanced plaque formation (175). Of the two outer capsid proteins, VP4 has been considered the less potent in eliciting neutralizing antibodies (74, 176). Both VP4 and VP7 are believed to be necessary for infection of target intestinal epithelial cells (195, 261, 262).

VP6. Group A rotaviruses share a common group-specific antigen located on VP6 that is encoded by gene segment 6 (162, 176). The 42-kDa VP6 is the major inner capsid protein and carries distinct epitopes for subgroup specificity (45, 143, 144, 162, 176, 178).

VP7. VP7 is a 32-kDa glycoprotein that is encoded by gene segment 9 of simian SA11 virus, gene segment 8 of the United Kingdom bovine virus, and gene segment 7 of rhesus rotavirus (97, 145, 175, 195). It is a potent immunogen and induces neutralizing antibodies (163). VP7 is the major determinant of serotype specificity (143, 144, 176).

Genome segments 10 and 11. Each of the 11 segments of the viral genome can be separated by electrophoresis in polyacrylamide gels to produce characteristic migration patterns (74, 177). Two electrophoretic mobility patterns, based on the migration of segments 10 and 11, are described in the literature (74, 302, 341). Slow migration of gene segments 10 and 11 yields the so-called short pattern, while more-rapid migration creates a long pattern (74).

Viral particles. Complete rotavirus particles from different serogroups are morphologically identical by electron microscopy, but particles of two sizes are typically observed in feces (74, 120, 218, 313). The large particles have a density of 1.36 g/ml as determined by cesium chloride density gradient centrifugation and are characterized by a 70- to 75-nm diameter and a smooth, double-shelled capsid (74, 120). The smaller particles have a density of 1.38 g/ml, are 60 to 65 nm in diameter, and contain a rough-surface, single-shell capsid (74, 120). Only complete, double-shelled particles are infective (74).

Rotaviruses in Pigs

Rotavirus-associated diarrhea is common in 1- to 4-week-old pigs and in recently weaned pigs (31). In 1975, viruses that were morphologically similar to the neonatal calf diarrhea reoviruslike agent were first seen in the feces of diarrheic pigs in three separate herds in the United Kingdom (396). Subsequently, rotaviruses were determined to have worldwide distribution in the swine population (120, 131).

Group A rotaviruses. Studies by researchers from around the world have identified porcine rotaviruses classified in groups A, B, C, and E (16, 22, 32, 36–38, 40, 44, 65, 205, 252, 282, 310, 313, 341, 360, 365). Group A rotaviruses are most prevalent in swine populations (40, 286), and two serotypes, represented by the OSU and Gottfried strains, have been well characterized (33, 143, 144, 164, 286, 287, 362). Another serotype (serotype 3) has been proposed (252), and the isolation of two new serotypes has been reported (282).

The OSU strain is antigenically similar to the equine rotavirus H-1 strain but is distinct from other mammalian and avian strains (33, 162, 164). The OSU strain is also similar to the equine H-1 strain by plaque reduction neutralization and hemagglutination inhibition tests, and both strains are classified as rotavirus serotype 5 (subgroup 1) (164, 252). Serologic evidence suggests that approximately 95% of swine herds in the United States demonstrate antibodies to the OSU strain (33). The Gottfried strain is antigenically related to the human St. Thomas rotavirus strain and therefore is the first animal rotavirus known to belong to rotavirus serotype 4 (subgroup 2) (164, 252). The Gottfried strain is also antigenically related to canine, simian, and, to a lesser extent, human strains that belong to rotavirus serotype 3 (164, 252).

A third and less-well-defined porcine rotavirus serotype has been proposed by Nagesha and Holmes (252). By immunofluorescence, fluorescent-focus neutralization tests, and electropherotyping, they have identified porcine rotavirus strains that are antigenically related to group 3 rotaviruses (subgroup 1) and to the simian SA11 prototype strain for serotype 3.

Non-group A rotaviruses. In 1980, independent reports published by Saif et al. (310) in Ohio and Bridger (36) in the United Kingdom described the occurrence of "rotavirus-like particles" in intestinal contents of a suckling pig and a "rotavirus-like agent" from pigs experiencing postweaning diarrhea, respectively. The rotaviruses were identified by electron microscopy and were found in combination with other viruses that included caliciviruses, 23-nm viruslike particles, and astroviruses. The rotaviruslike particles originally identified by Saif et al. (310) were separated from the mixed viral population by passage in gnotobiotic pigs. Viral identity and purity were verified by electron microscopy, immunoelectron microscopy, and several serologic procedures (32). On the basis of its size, morphology, and tropism for villous enterocytes, the virus was referred to as porcine pararotavirus and was assigned to rotavirus group C (32, 287). To date, group C rotaviruses have been detected in pigs and humans (37, 38, 40, 45, 205, 207, 290, 361). Although group C rotaviruses were observed in the feces of diarrheic pigs (36, 310), they occurred less than half as often as group A rotaviruses. Seroepidemiologic studies also support the less-frequent occurrence of group C rotavirus in the swine population (38). Group C rotaviruses had been infrequently detected and reported in humans until their widespread occurrence was reported in humans in Australia, Brazil, and the United Kingdom (40). Atypical rotaviruses found in fecal samples from patients in these countries were shown by antigenic and nucleic acid analyses to be related to porcine group C rotaviruses (40). Furthermore, a recent outbreak of gastroenteritis among school children and their teachers in Japan was attributed to group C rotavirus (207). A survey of fecal samples obtained from patients in Thailand, Nepal, England (290), and South India (45) suggests the global occurrence of group C rotavirus in humans. The strains isolated from pigs and humans are antigenically related and have similar double-stranded RNA genome electropherotypes. Porcine strains of group C rotavirus have been successfully adapted and propagated in continuous cell lines (312, 360, 361).

The rotaviruslike particles identified by Bridger (36) were antigenically distinct from group A rotavirus and genetically distinct from the porcine pararotaviruses (36, 286, 287). The virus was referred to as rotaviruslike; under the Pedley classification scheme, it was designated as a group B virus and became the prototype (NIRD/1) to which all other potential group B viruses were compared (287).

Although group B rotaviruses have been identified rarely in swine, seroepidemiologic evidence suggests that exposure to this group is widespread (38, 44). In addition to their activity in swine (36, 365), group B rotaviruses are now known to cause diarrhea in humans (44, 77, 99, 100), calves (341), lambs (341), and rats (100). A strain of group B rotavirus called "adult diarrhea rotavirus" has been determined to be the cause of severe epidemics of diarrheal disease among humans in China over the past 7 years (77, 166). Although the diarrheal disease was especially prevalent among adults (166), group B rotaviruses have been associated with diarrhea among infants (77). Group B rotaviruses were identified as etiologic agents of infectious diarrhea in infant rats and of gastroenteritis in children and adults in Baltimore, Md. (100). Through the use of nucleic acid hybridization and enzymatic and immunofluorescence assays, Eiden et al. (100) have shown that the rat and human group B rotaviruses are closely related to bovine and porcine group B rotaviruses. The relationship of animal group B rotaviruses to human disease has not been clearly defined (412). Further seroepidemiologic investigations, cross-species infectivity studies, and protection studies are required to determine the zoonotic importance of group B rotaviruses.

Serologic evidence suggests that asymptomatic group B rotavirus infection is widespread in the human population, as it is in pigs (37). However, results of a seroepidemiologic survey conducted by Brown et al. (44) showed that group B antibodies are much more prevalent in domestic animals (pigs, 97%; cattle, 71%; sheep, 91%; and goats, 91%) than in humans in general (10%) or in veterinarians (4%), in whom a higher frequency would be expected.

Electron microscopic observations of group B rotavirus from infected pigs indicate that the predominant morphologic form is a corelike particle 52 nm in diameter (313, 365). Experimental inoculation of 5- to 6-day-old gnotobiotically derived pigs induced diarrhea between 15 and 24 h after inoculation (365). The diarrhea was characterized by profuse, watery, yellow feces. The virus infected the villous enterocytes throughout the small intestine and was restricted to the enterocytes on or near the villous tips. In experimental pig infection, histopathologic lesions induced by group B rotavirus were less severe than in group A rotavirus infection (365).

Rotaviruses in Calves and Lambs

Group A rotaviruses. Currently, group A and B rotaviruses are known to infect calves and lambs (64, 65, 116, 167, 338, 341, 364), but group A rotaviruses are most prevalent and clinically important. Four prominent group A calf diarrhea rotaviruses have been characterized: the neonatal calf diarrhea virus, the United Kingdom calf rotavirus, the B641 calf rotavirus, and the B223 calf rotavirus. Three of these strains (neonatal calf diarrhea virus, the United Kingdom strain, and B641 strain) have been assigned to rotavirus serotype 6 (164, 401, 405, 413). The B641 virus is also referred to as the US bovine rotavirus serotype 1 (401, 413). The B223 bovine strain has not been assigned to a rotavirus serotype (413) but is referred to as bovine rotavirus serotype 2 (401, 405).

Two serotypes of group A rotaviruses appear to be prevalent in calves. Rotaviruses belonging to serotype 6 are most often identified in isolates from calf diarrheas. Strains that are distinct from serotype 6 and have serologic and electrophoretic properties similar to those of strain B223 (unassigned serotype) occur less frequently (249, 263, 343, 405).

Group A rotaviruses from calves exhibit extensive genomic diversity, although one electropherotype usually predominates during an outbreak (116). Longitudinal studies within herds have shown that over time, calves may excrete rotaviruses of different electropherotypes, with different antigenic properties, and with different degrees of virulence (41, 116, 140). All bovine group A rotaviruses characterized thus far have the subgroup 1 antigen, and most exhibit a long RNA electropherotype pattern (143, 144). Two recent studies have shown the occurrence of bovine group A rotaviruses with the subgroup 1 antigen and short electropherotype patterns (283, 363). Short RNA electropherotype patterns had not been detected previously in bovine isolates but have been observed frequently in human rotavirus strains. The ID strain described by Theil and McCloskey (363) and the VMRI strain described by Paul et al. (283) had short and super-short electrophoretic migration patterns, respectively. Both bovine strains possess subgroup 1 antigen, and the VMRI strain was assigned to rotavirus serotype 6. This assignment was based on fluorescent-focus neutralization tests with antisera to serotype 6 rotaviruses (B641 and neonatal calf disease virus).

Human rotavirus strains that exhibit the short electrophoretic pattern belong to serotype 2. An exception is the 69M human rotavirus strain, which has a super-short RNA electrophoretic pattern for gene segments 10 and 11 (208). This strain has been assigned to rotavirus serotype 8 (221).

Group B rotaviruses. Although group B rotaviruses are infrequently detected in the feces of diarrheic calves, results of serologic or electron microscopic studies suggest that infection or at least exposure to group B rotavirus is common among young calves and lambs (65, 341, 364). On the basis of electrophoretic patterns and failure of some isolates to react positively in EIAs capable of detecting group A rotavirus, Chasey and Banks (64) have reported that group B rotaviruses are the most common cause of rotavirus diarrhea in neonatal lambs in England and Wales.

Detection of Rotaviruses

Rotaviruses and coronaviruses are the most frequently identified viruses in diarrheas of neonatal food animal. Both viruses are often identified simultaneously in diarrhea specimens from young calves (Fig. 5). Group A rotaviruses are readily detected because of the availability of rapid diagnostic EIAs (Rotazyme I and Rotazyme II EIAs, Abbott Laboratories, Chicago, Ill.; Pathfinder EIA, Kallestad Laboratories, Austin, Tex.). The use of conventional EIA precludes the detection of the atypical rotaviruses, however. Immunofluorescence and electron microscopic procedures are easily employed to diagnose rotavirus infections as well as coronavirus infections. Analysis of electrophoretic migration patterns is often used in epidemiologic studies, but electropherotyping does not predict serologic relationships among the various rotavirus strains (413).

Pathogenesis

Rotaviruses cause disease that varies in severity. The viruses can be carried by an asymptomatic animal, can cause mild, self-limiting diarrhea, or can cause severe diarrhea with excessive fluid loss and severe electrolyte imbalances. Rotaviruses are transmitted by the fecal-oral route and selectively infect the mature villous absorptive epithelial cells (362, 399, 404). Replication takes place within the cytoplasm of the mature enterocyte; crypt cells are spared

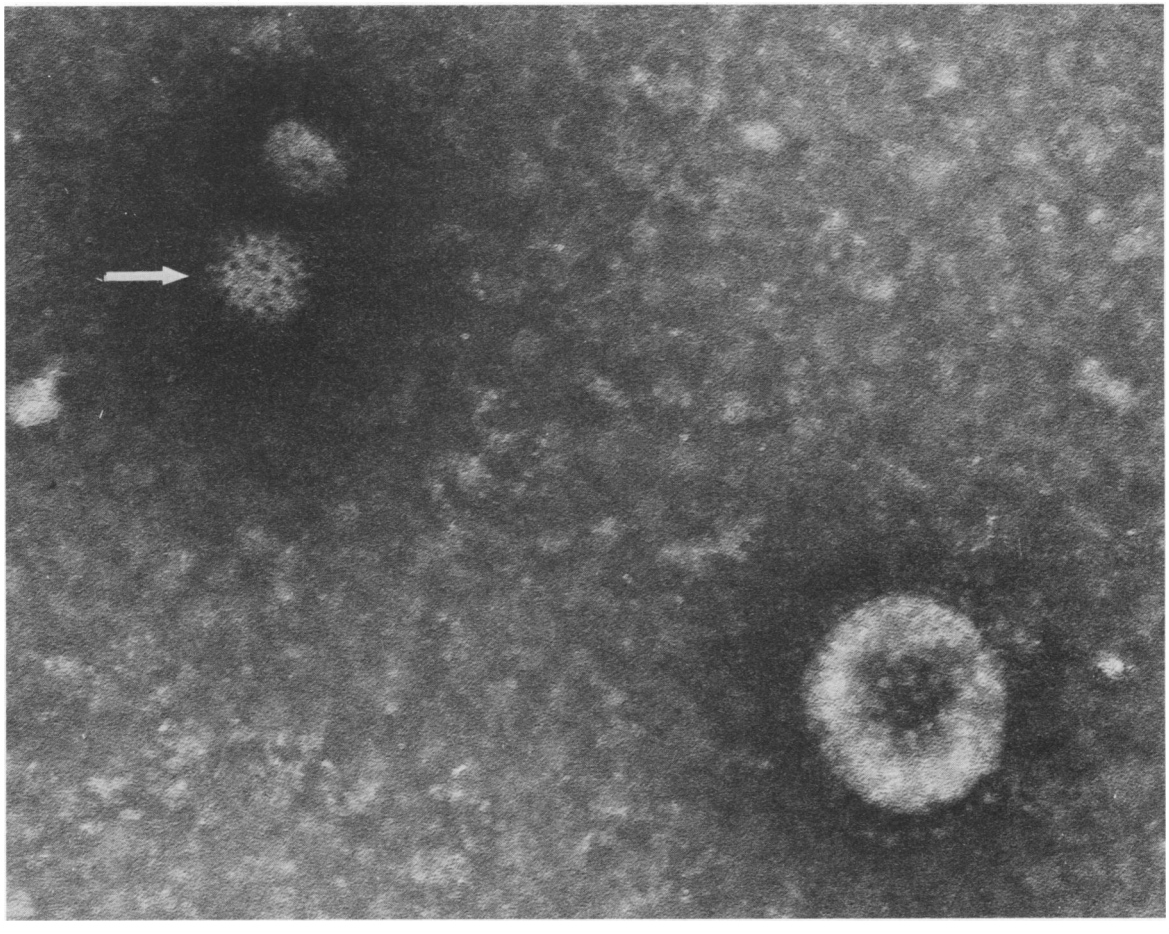

FIG. 5. Calf rotavirus particles (arrow) and a single coronavirus particle as observed by negative-staining electron microscopy. The calf was infected with both viruses simultaneously. Magnification, ×100,000.

infection (368). Characteristically, lesions induced by rotaviruses are limited to the small intestine. Some groups or strains cause more severe lesions than others (69, 365). Variability in virulence among rotaviruses and among individual host responses to infection may account for some of the observed differences in clinical signs (41). Infection results in the loss of mature epithelial cells, which are replaced by immature undifferentiated cells deficient in disaccharidase and sodium-potassium ATPase activities (13, 229). Absorptive capacity is much reduced, as indicated by D-xylose absorption studies, and morphologic changes are characterized by desquamation of infected cells followed by villous atrophy (399, 404). The atrophic villi become covered with immature squamous to cuboidal epithelial cells that have migrated from the crypts. The loss of digestive and absorptive functions leads to nutrient maldigestion and malabsorption. Lactose, the primary carbohydrate of milk and the primary nutrient of young animals, passes through the small intestine undigested and unabsorbed. In the large intestine, lactose is fermented by colonic bacteria to produce short-chain organic acids and gases. The short-chain organic acids lower the colonic pH, causing a shift in the colonic bacterial flora. Bacteria that produce lactic acid and survive at the lower pH become established. Concurrent with the increase in lumenal lactic acid, there is an increase in the intralumenal osmotic pressure, leading to further increased secretion. These two effects contribute to ongoing metabolic acidosis and systemic dehydration.

Clinical Presentation

Rotavirus infection occurs in pigs of all ages, but diarrhea is common in pigs <1 week and 3 to 6 weeks old (327). Rotavirus diarrhea must be differentiated from transmissible gastroenteritis virus (coronavirus) infection, which is characterized by a more-explosive onset of clinical signs, including diarrhea, rapid dehydration, high morbidity and mortality, and rapid spread through the herd (14).

In the susceptible host, porcine rotaviruses have a short incubation period and usually cause an acute infection characterized by sudden onset of diarrhea, anorexia, central nervous system depression, and occasionally vomiting. Recently weaned pigs infected with rotavirus have more severe diarrhea and higher mortality than younger animals. Decreased lactogenic immunity, increased stress due to nutritional changes, weaning, housing, and mixed infections with other microorganisms contribute to the higher mortality in this age group.

Since the initial description by Mebus et al. (218) of the clinical signs of rotavirus-induced diarrhea in calves, much has been learned about the epidemiology of rotavirus infection. Rotaviruses can be found in fecal samples from normal

healthy calves or in calves exhibiting clinical disease of varied severity (80, 380). Variations in clinical disease observed in calves depend on a number of factors, including difference in virulence among rotavirus strains, age of the host, host immune status, dose of the inoculum, occurrence of mixed infections, environmental stresses (weather conditions, housing, overcrowding), and nutrition (2, 215, 219, 300, 369). These factors, along with systemic consequences of electrolyte imbalances, fluid loss, and metabolic acidemia, influence mortality. Fortunately, most rotavirus infections are mild and self-limiting, although there is usually high morbidity.

Among 35 herds, the prevalence of rotavirus diarrhea in young calves has been reported to be approximately 23% (4). In general, the clinical signs attributable to rotavirus infection are variable and mimic those observed with other causes of diarrhea. The classic rotavirus clinical picture describes an epizootic among calves usually less than 2 weeks of age (4). A variable percentage of calves (30 to 40% in a highly susceptible herd) may exhibit clinical signs of disease, including anorexia, central nervous system depression, profuse watery diarrhea, and various degrees of systemic dehydration. In severe cases, death occurs as a result of electrolyte imbalances, dehydration, and cardiac arrest. In the absence of mixed infections with other enteropathogens, clinical signs of disease abate in 2 to 4 days, but fecal shedding of virus particles may last for 5 to 7 days.

Calves approximately 1 week old often have simultaneous infections with *C. parvum*, ETEC, or coronavirus. In the case of mixed infections with *C. parvum*, the disease is characterized by chronic, moderate to severe diarrhea and nutrient malabsorption. The calves often appear cachectic and emaciated, and despite aggressive medical therapy, they succumb to the infection. In calves <5 days old from herds that are experiencing high morbidity and mortality from acute rotavirus infections, ETEC strains are often present concurrently.

CRYPTOSPORIDIUM SPP.

Cryptosporidium spp. are coccidian protozoal parasites first described as a cause of diarrhea in a calf in 1971 (279). This report was followed by the description of cryptosporidiosis in a 3-year-old child (256). Before 1980, cryptosporidiosis was considered rare and relatively innocuous (75). Since that time numerous reports that describe *Cryptosporidium* infection in association with gastroenteritis, diarrhea, and malabsorptive disease in a variety of animal species as well as humans have appeared (9, 13, 59, 75, 76, 115, 314, 370, 395). Among food animals, *C. parvum* is commonly associated with diarrhea in calves, lambs, and, less frequently, pigs (6, 7, 284, 315, 373, 378). In these species, enteric cryptosporidiosis is characterized by diarrhea and high morbidity (237); when the disease is characterized by high mortality, concurrent bacterial and viral infections are common (240, 284, 293, 339).

Infection occurs in immunocompetent as well as immunocompromised animals and humans, with diarrhea being the characteristic clinical sign (11, 19, 112, 115, 157, 198, 230, 345, 377, 395). Diarrhea is usually self-limiting in immunocompetent individuals (157, 345). In contrast, infection in immunocomprised humans is associated with choleralike diarrhea and chronic, severe, malabsorptive disease (75, 76, 345). In many of these patients, infection may disseminate to involve multiple organ systems (255, 345). Mammalian cryptosporidia lack host specificity and, therefore, are zoonotic agents (115, 238).

Biology of *Cryptosporidium* spp.

Taxonomy. To date, the exact number of *Cryptosporidium* spp. has not been clearly defined. However, *Cryptosporidium* spp. have been named according to the host species from which they were isolated (192) or which they were capable of infecting (381). Fayer and Ungar (115) compiled a list of 20 named species of *Cryptosporidium* from various publications. Several of these species have been determined to be other coccidian protozoa or not true *Cryptosporidium* spp. (75, 115). The results of early cross-transmission experiments conducted with cryptosporidia isolated from a diarrheic calf showed a lack of host specificity (371). On the basis of these results, Tzipori et al. (371) proposed *Cryptosporidium* as a single-species genus. More recent reports have established two species that can infect birds (*C. meleagridis* and *C. baileyi*) (193, 238, 356) and at least two species that can infect mammals (*C. muris* and *C. parvum*) (238, 381).

The genus *Cryptosporidium* is the only known genus in the Cryptosporidiidae family (115, 255). The Cryptosporidiidae family along with the Sarcocystidae and Eimeriidae families constitutes the suborder Eimeriina. Genera of the Sarcocystidae (*Sarcocystis* and *Toxoplasma*) and Eimeriidae (*Eimeria* and *Isospora*) families are well-known pathogens for different animal species. Four characteristics distinguish mammalian *Cryptosporidium* spp. from other coccidian protozoa: (i) a lack of host specificity (75, 381), (ii) differentiation after attachment within a parasitophorus membrane in an intracellular extracytoplasmic location (197, 203), (iii) an oocyst containing four naked sporozoites (115), and (iv) oocysts that are fully sporulated before passage in the feces (115, 406).

Life cycle. The life cycle of *Cryptosporidium* spp. is monoxenous and closely resembles that of other coccidian protozoa (115, 255). The sporulated *Cryptosporidium* oocysts are readily infective when ingested by a susceptible host. Unlike those of other true coccidia, the oocysts of *Cryptosporidium* spp. require minimal exposure to digestive enzymes and bile salts in order to release infective sporozoites (113, 301). Results of in vitro experiments have shown that sporozoites excyst from oocysts suspended in water, saline, or salt solutions in the absence of reducing conditions or digestive enzymes. However, excystation was more complete after incubation in the presence of trypsin and bile salts in Ringer solution at 37°C, but it also occurred at 20°C. Pretreatment of oocysts with dilute aqueous sodium hypochlorite also enhanced excystation (301).

After they are ingested, sporulated oocysts release sporozoites that infect the microvillous brush border. The sporozoites invaginate the microvillous membranes, which contain thin cytoplasmic extensions. The sporozoites become incorporated in the microvillous membrane and are internalized in a membrane sac of the host cell. Sporozoites and all subsequent developmental stages occur within this membrane sac or parasitophorus vacuole located at the cell surface. This intracellular extracytoplasmic site of development is unique to *C. parvum* (197, 203). Within the parasitophorous vacuole, the sporozoite differentiates into trophozoites. The nucleus of each mature trophozoite undergoes asexual division (merogony or schizogony), resulting in the development of type I and type II meronts. Type I meronts (first-generation meronts) mature and divide to contain six to eight merozoites. The mature merozoites are released and invade previously uninfected host cells, in which they differentiate into other type I or type II meronts. The mature

type II meront (second-generation meront) contains four merozoites. The sexual phase (gamogony) is initiated when the type II meronts are released and invade susceptible host cells. The type II meronts differentiate into either microgametocytes (male) or macrogametocytes (female). The microgametocytes contain microgametes, which fertilize the macrogametes to produce the zygotes. The zygotes develop into thick-walled or thin-walled oocysts containing four naked sporozoites. The thick-walled oocysts are passed in the feces and are readily infective to susceptible hosts. The thin-walled oocysts rupture in the host and release sporozoites that can invade uninfected epithelial cells. Sporozoites released from thin-walled oocysts permit crytosporidia to maintain persistent infections in the immunocompromised host.

Animal Cryptosporidiosis

Cryptosporidia are recognized worldwide as primary pathogens associated with naturally occurring outbreaks of diarrhea among neonatal farm animals (6, 7, 154, 372, 374, 377, 381). *C. parvum* is a major cause of infectious diarrhea among young farm animals and also humans (75, 192, 238, 381). *C. parvum* can be differentiated from *C. muris* and the avian *Cryptosporidium* spp. by its size (4 to 5 μm) (238, 381). *C. muris* infects the intestinal tract of mice (238, 381) and has been associated with abomasal cryptosporidiosis of adult cattle (8). *C. muris* oocysts are approximately 7 to 8 μm (381).

Transmission is by the fecal-oral route, but infection can result from consumption of contaminated groundwater or contaminated feed material or by contact with fomites and other animals shedding infective oocysts. On most farms, environmental contamination is widespread, and many potential sources of infection exist. After ingestion, cryptosporidia infect the brush border epithelium and undergo a complete life cycle, primarily in the distal small intestine (6, 9, 154, 293, 377). Infections frequently occur in the large intestine, and other organs or systems may be infected less often (230). In calves, lambs, and pigs, enteric cryptosporidiosis is characterized by high morbidity (230, 240) and high mortality and is associated with concurrent bacterial and viral infections (240, 339).

Coccidian protozoa are usually host and tissue specific (132). Cross-transmission experiments using cryptosporidia of mammalian origin have shown that these parasites are not restricted to a single host, although *C. muris* exhibits more host specificity than *C. parvum* (381). Under experimental and natural conditions, *C. parvum* caused enteric disease in calves, lambs, pigs, humans, and laboratory rodents (230, 372, 380). Similarly, *C. parvum* obtained from humans can cause diarrhea in a variety of animal species, and human-to-human transmission is suspected (370, 372).

Avian cryptosporidia (*C. meleagridis* and *C. baileyi*) exhibit characteristics that are unique and that differentiate them from mammalian cryptosporidia. The organisms have a predilection for infecting the epithelium of both the respiratory and the digestive tracts of avian species (88, 135, 138, 139, 171). Although cross-species studies are incomplete, *C. meleagridis* and *C. baileyi* appear to be host specific for avian species (193). Organisms isolated from the bursa of Fabricius of naturally infected broiler chickens failed to induce disease and cause lesions in laboratory rodents and pigs. Furthermore, avian species appear to be relatively resistant to infection with mammalian cryptosporidia (260, 371). *C. parvum* isolated from calves and humans did not cause diarrhea in avian species; however, an isolate from calves caused villous atrophy in chicks (371).

Despite numerous evaluations, no compound effective against cryptosporidial infection has been found (10, 52, 61, 62, 237). Furthermore, no suitable prophylaxis that reduces susceptibility to infection has been developed. In animals, a number of antimicrobial agents including coccidiostats, broad-spectrum antibiotics, and anthelminthics have been found ineffective. In acquired immunodeficiency syndrome patients with symptomatic cryptosporidiosis, some resolution of clinical illness has been observed after therapy with spiramycin (62), octreotide (181), and dialyzable leukocyte extract (214).

In animals, passive immunization by consumption of maternal colostrum is not entirely protective. Calves on farms where they are persistently exposed develop enteric cryptosporidiosis even after consuming sufficient maternal immunoglobulins. Results of a recent study provided evidence that hyperimmune colostrum obtained from a cow immunized with *C. parvum* oocysts reduced both duration of diarrhea and oocyst shedding in challenged calves (112, 114). Although clinical illness was less severe, the calves developed cryptosporidiosis despite the hyperimmune colostrum. Neither circulating nor local immunoglobulins are entirely protective for young calves exposed shortly after birth.

Detection of *C. parvum* Infection

C. parvum infection is diagnosed microscopically by demonstration of oocysts in feces or by examination of stained intestinal tissue (Fig. 6). Fecal examination is noninvasive and can be performed easily on fecal samples collected from the same animal on a daily basis. When dealing with individual animals in which enteric cryptosporidiosis is suspected, multiple fecal examinations may be required because some animals shed oocysts intermittently. However, oocysts are usually shed throughout the course of clinical illness. To the untrained observer, oocysts may be difficult to detect because of their smallness and because they are often confused with yeasts.

Various techniques used in the recovery and identification of oocysts have been evaluated (130, 198). A three-step stool examination procedure which consists of staining wet mounts with iodine, staining them with Kinyoun acid-fast stain, and then concentrating oocysts by Sheather sugar cover slip flotation has been recommended as a simple diagnostic method (198). In another study, the modified Ziehl-Neelsen carbol fuchsin staining method was recommended over 14 other methods evaluated (130). Oocysts can also be detected in feces and intestinal tissue by immunofluorescence procedures (304). Identification of *C. parvum* infection is most reliable when developmental stages are observed in intestinal epithelium (Fig. 6).

Pathogenesis

Enteric cryptosporidiosis has been studied in specific-pathogen-free, colostrum-deprived, and conventionally raised calves, lambs, and pigs. Because cryptosporidium oocysts contain naked sporozoites and are sporulated when passed in the feces, the organisms can cause disease in young animals shortly after ingestion. The onset of clinical illness may follow inoculation in 2 to 7 days. The prepatent period may be influenced by host susceptibility. Cryptosporidia do not appear to cause diarrhea in adult cattle, although developmental stages of *C. muris* have been found within the abomasal mucosa of adult cattle (8).

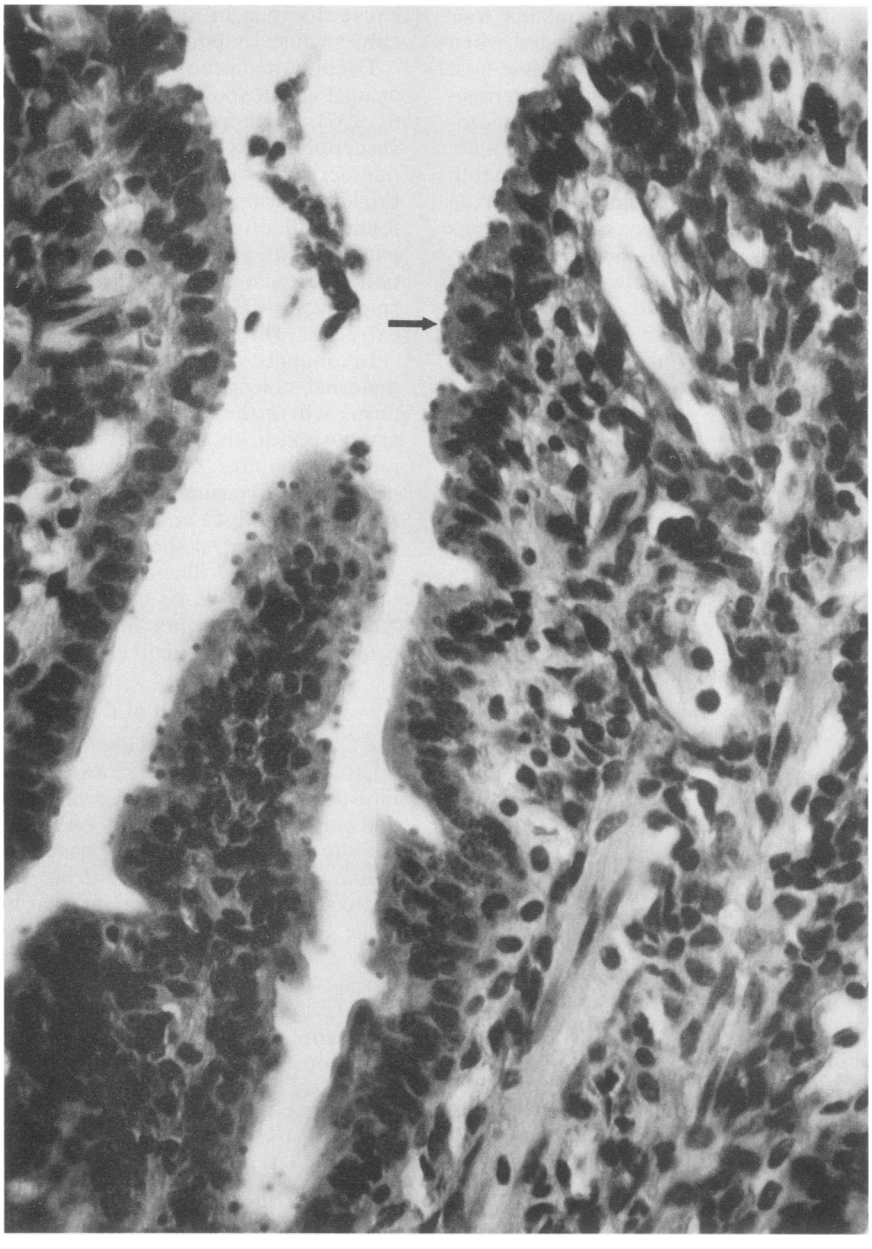

FIG. 6. Photomicrograph of a section of ileum from a calf infected with *C. parvum*. The organisms are visible along the brush border surface of the small intestinal villi (arrow). Hematoxylin and eosin. Magnification, ×400.

C. parvum infects the distal small intestine and colon, where it proliferates asexually and sexually. The parasite does not invade beyond the apical surfaces of the epithelial cells but adheres to the intestinal epithelium, where it can be observed microscopically on the lumenal surface (Fig. 6). Histologic lesions include cryptitis, decreased mucosal height, villous atrophy, and villous fusion (154, 230, 284, 285, 377, 378). Biochemically, mucosal enzymatic activities are decreased (372, 377). The loss of microvilli, decreased mucosal enzymatic activities, decreased villous function, and reduced villous absorptive surface area result in compromised intestinal digestion and absorption of dietary nutrients. Impaired digestion and nutrient malabsorption occur in young calves infected with *Cryptosporidium* spp. (155). The accumulation and subsequent fermentation of osmotically active nutrients within the large intestine contribute to an osmotic catharsis, which is at least a partial cause of *Cryptosporidium* species-induced diarrhea.

Clinical Presentation

There are no specific clinical signs to differentiate *C. parvum* infection from other kinds of diarrhea. Diarrhea is the most consistent clinical sign and is accompanied by increased frequency of defecation, tenesmus, anorexia, emaciation, central nervous system depression, dehydration, and, in protracted cases, malnutrition. During the early stages of infection, blood-tinged feces and intestinal casts may be observed. In contrast to immunocompromised humans, in whom diarrhea and nutrient malabsorption may

persist for weeks (19, 59, 76, 314), young farm animals usually do not show long-term shedding of oocysts (155).

Neonatal animals are most susceptible to infection, because resistance to infection develops with increasing age. In contrast, previously unexposed humans are susceptible at all ages (19, 53, 314). Additionally, clinical illness is more severe in younger animals. Age-associated resistance and less-severe clinical illness have been shown experimentally following inoculation of young farm animals and mice (230, 372, 373). Experimental enteric cryptosporidiosis was most severe in 5-day-old lambs (7, 372, 373). Clinical illness was characterized by protracted diarrhea, wasting, and death. Lambs infected at 30 days of age became infected but did not exhibit severe signs of clinical disease (373). Gnotobiotic pigs inoculated with calf isolates developed severe enterocolitis when inoculated at 1 day of age, moderate diarrhea when inoculated at 7 days of age, and subclinical infection when inoculated at 15 days of age (230, 378). In pigs inoculated at <3 days of age, the mucosae of the small intestines were severely damaged, with lesions of the small intestines being similar to those in calves (378). Young animals recovering from enteric cryptosporidiosis develop immunity and resist further infections.

Supportive therapy in the form of oral or intravenous fluid administration of balanced electrolyte solutions and provision of adequate nutrition is often sufficient in rehabilitating young calves. Those that are malnourished, severely debilitated, and unresponsive to conventional therapy respond well to total parenteral nutrition (17). Severe clinical illness and poor response to therapy are observed when mixed infections with other enteric pathogens are present, when milk and other nutrients have been needlessly withheld, and when the weather is extremely cold. Mixed infections of *C. parvum* with ETEC, rotavirus, coronavirus, and other potential enteropathogens are common and in many instances exaggerate the clinical course of the disease. *C. parvum* infections appear to potentiate the ability of ETEC to infect and cause disease in older calves.

Young animals have high nutritional requirements (a 45-kg calf requires 2,900 kcal [1 cal = 4.184 J] of metabolizable energy per day) (17). However, because of the nature of the enteric lesions caused by *C. parvum* infection, milk is commonly withheld for extended periods. Although calves respond to reduced milk intake by a corresponding reduction in fecal output, in the face of ongoing nutrient malabsorption, milk deprivation greatly contributes to animal mortality.

CONCLUSIONS

ETEC, rotaviruses, and *C. parvum* are common causes of diarrhea among neonatal farm animals. These enteropathogens cause specific enteric infections that are associated with nonspecific signs of clinical illness. Common clinical signs include frequent voiding of watery to semiformed feces, generalized weakness, anorexia, and dehydration. Although clinical signs tend to be nonspecific, age of the animal at onset of diarrhea, characteristics of the feces (e.g., color, volume, and odor), duration and severity of diarrhea, and response to therapy can be used to establish a tentative diagnosis. In treating the viable individual animal, definitive diagnosis is based on identifying the causative agents by various diagnostic procedures and employing aggressive therapeutic measures.

An epizootic of diarrheal disease among neonatal animals in a flock or herd is a complex, multifactorial problem that is complicated by the interplay of multiple causative agents, host immunity, environmental factors, nutritional factors, and management conditions. When dealing with epizootics, fecal samples should be collected from healthy and sick animals and evaluated for causative agents. In addition, the presence of associated microscopic lesions should be demonstrated in the intestinal tract. Even though one causative agent may predominate, infections with multiple enteropathogens should always be considered when diarrheal diseases are evaluated. Moreover, the environment and management practices should be evaluated as well.

To what extent young food animals serve as reservoirs for potential zoonotic agents is not known. However, *C. parvum* has zoonotic potential, and EHEC O157:H7 (Shiga-like toxin positive) consumed in undercooked hamburger and raw milk has been incriminated as the causative agent of hemorrhagic colitis and hemolytic uremic syndrome among humans. The occurrence of *C. parvum* and Shiga-like-toxin-positive *E. coli* in young food animals makes them candidate reservoirs of zoonotic agents.

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