Enteric disease continues to be a substantial problem in the swine industry, contributing to poor growth performance, increased morbidity and mortality rates, compromised welfare, and economic losses. Advances in the understanding of swine management, vaccine technology, and prophylactic antimicrobial regimens have substantially reduced the impact of certain diarrheal diseases of swine, but several pathogens continue to pose major challenges to the swine industry. Intensive management practices and changes in genetics have likely led to increased susceptibility of pigs to common enteric pathogens and the emergence of new pathogens that were once considered commensal. Several of these pathogens have not been fully characterized, or their pathophysiologic features are not well understood. Since a review of the mechanisms of diarrhea by Moon1 in 1978, the basic understanding of pathophysiologic mechanisms of diarrheal disease has increased considerably. Elucidation of the molecular basics of intestinal ion transport and how these molecular events become dysregulated by enteric pathogens have not only helped us better understand the disease process, but have also provided us with important information aiding in the development of diagnostic, management, and therapeutic strategies to combat these disorders. The objective of this report was to review the current understanding of the basic mechanisms of diarrheal diseases in swine, with particular emphasis on the ability of specific enteric pathogens to alter intestinal ion transport and fluid movement across intestinal epithelium. Although this review is focused on enteric diseases in pigs, basic mechanisms discussed apply to all veterinary species.

**Abbreviations**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Name</th>
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<tr>
<td>ATP</td>
<td>Adenosine triphosphate</td>
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<td>ENS</td>
<td>Enteric nervous system</td>
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<td>VIP</td>
<td>Vasoactive intestinal peptide</td>
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<td>5HT</td>
<td>Serotonin</td>
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<tr>
<td>NKCC1</td>
<td>Na⁺-K⁺-2Cl⁻ cotransporter</td>
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<td>CFTR</td>
<td>Cystic fibrosis transmembrane regulator</td>
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<tr>
<td>PKA</td>
<td>Protein kinase A</td>
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<td>cAMP</td>
<td>Cyclic adenosine monophosphate</td>
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<td>AA</td>
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<td>NHE1</td>
<td>Na⁺/H⁺ exchanger</td>
</tr>
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<td>Sodium-glucose–linked transporter-1</td>
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<td>NHE3</td>
<td>Na⁺/H⁺ exchanger isoform 3</td>
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<td>TGE</td>
<td>Transmissible gastroenteritis</td>
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<td>ETEC</td>
<td>Enterotoxigenic <em>Escherichia coli</em></td>
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<tr>
<td>NSP4</td>
<td>Nonstructural protein 4</td>
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<td>CaCC</td>
<td>Ca²⁺-activated Cl⁻ channel</td>
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**Figure 1**—Illustration of certain anatomic features of the small intestinal mucosa.
tion of electrolytes. For example, the Na\(^+\)-K\(^+\) ATPase transporter is localized to the basolateral portion of the membrane of enterocytes and generates an electrical potential across the cell that provides the energy to move other ions either into or out of the cell. The Na\(^+\)-K\(^+\)-ATPase maintains Na\(^+\) at relatively low intracellular concentrations (15 mEq of Na\(^+\) intracellular vs 150 mEq of Na\(^+\) in plasma), which preferentially allows Na\(^+\) to enter the cell via a number of apical transporters on the apical membrane. Thus, these apical transporters use the electrochemical gradient setup by Na\(^+\)-K\(^+\) ATPase.

A further anatomic consideration is the organization of the mucosa into glandular crypts and villi (in the small intestine) or intercrypt surface epithelium (in the colon). The crypt-villus axis divides the epithelium into secretory epithelium and absorptive epithelium. The epithelium that performs these opposing functions is derived from stem cells located within the crypts. Newly formed epithelial cells migrate along the crypt-villus axis, maturing and acquiring differing functions until they are ultimately sloughed from the tip of the villus approximately 5 days after their creation. Paneth cells remain at the base of the crypt and therefore do not take part in the migration of cells toward the surface. However, there is evidence that suggests this population of cells does not exist in pigs. Overall, immature epithelium within the crypts performs predominantly secretory functions, whereas the more mature epithelium on the surface is predominantly involved in absorption. This becomes critically important in a number of diarrheal diseases that preferentially injure the surface of infected epithelial cells, or inflammatory stimuli.

The functional structure of the colon is similar, including the location of stem cells and immature epithelial cells within crypts and the migration of immature cells progressively out of the crypts. However, there are no villi in the colon. Instead, there is simply intercrypt surface epithelium, which performs similar absorptive functions as the small intestine. In addition, the array of transporters in the colon differs. Because the colon is the principal site of fluid and electrolyte absorption in most species, this arrangement would seem to put the colon at a disadvantage because of the lack of surface area imparted by villi; however, the colon is capable of compensating for fluid loss in the crypts, suggesting that its ability to absorb fluid is not adversely affected by the lack of villi. This may be in part because the greatest contribution to increased absorptive capacity is the presence of epithelial microvilli, which are present throughout the gastrointestinal tract. In addition, the colonic epithelium is less “leaky” than small intestinal epithelium as a result of close apposition of tight junctions, suggesting there is less back flow of fluid into the lumen in the colon following absorption.

Supepithelial components, such as the ENS and immune cells, are intimately involved in the regulation of secretion and absorption under normal and pathophysiologic processes in the gastrointestinal tract. The ENS consists of an extensive network of nerve cells that play a critical role in intestinal ion transport. The ENS is composed of 2 major plexuses (between the 2 muscle layers) and the submucosal plexus, which interconnect and regulate motor and sensory neural input, respectively. The ENS can receive central input from the CNS via the parasympathetic and sympathetic branches of the autonomic network while also operating independently from the CNS. Chemical mediators of the ENS consist of a multitude of neurotransmitters; however, acetylcholine and VIP are the major neurotransmitters released by enteric nerves that stimulate epithelial secretion. Norepinephrine is the predominant neurotransmitter released by nerves that have proabsorptive effects by activating α-2 receptors on enterocytes and nerves. The neural effect of this mediator is principally inhibitory. The ENS is activated by numerous toxic, endocrine, and inflammatory mediators, resulting in intestinal secretion, and thus plays an important role in many diarrheal diseases. One important mechanism by which activation of the ENS stimulates secretory processes is via localized neural reflex arcs. The reflex arc consists of sensory nerves and interneurons that transmit to motor nerves that are mainly VIP and cholinergic. Sensory nerves in the intestinal mucosa are stimulated by bacterial toxins, products of infected epithelial cells, or inflammatory stimuli to regulate secretion by afferent-interneuron-secretomotor reflex arcs (Figure 2).

![Figure 2](image-url)
Another vital component of the intestinal mucosa is immune cells capable of mounting a response to invading microorganisms or their toxins. In terms of innate immunity, the principal cell population comprises neutrophils, which are normally resident within the lamina propria and can also be rapidly recruited from the circulation. Once the epithelium is activated by injury or microorganisms, neutrophils migrate toward the epithelium and, ultimately, across the epithelial cell monolayer between tight junctions. This is beneficial to the host in that the phagocytosis of organisms or toxins can occur rapidly, but may be detrimental because neutrophils may harm gastrointestinal tissues either by release of mediators such as reactive oxygen metabolites or by physically injuring epithelial cells as they migrate across the mucosa. Mast cells also reside in subepithelial and lamina propria tissues and serve as a critical first line of defense at the epithelial barrier. Mast cells are located in close proximity to enteric neurons, blood vessels, and epithelial cells and, when activated, release a variety of secretory and proinflammatory mediators, including histamine, prostaglandins, 5HT, and proteases, that are central to several diarrheal diseases.

The adaptive immune response is carried out by the regulated presentation of antigens to lymphocyte populations that are also present within the lamina propria. Cells involved in the process are M cells located within the epithelium, which process and present antigen, and clustered populations of lymphocytes, particular in Peyer's patches located principally in the submucosa at the antimesenteric border of the ileum. This process results in the subacute response to specific antigens, typically from microorganisms or their toxins, and supersedes the innate immune response because subacute responses are far more targeted.

**Ion Transport That Results in Intestinal Secretion**

Fluid secretion in the intestine is a normal physiologic process that serves to flush mucus into the lumen from the crypts and provides an aqueous luminal environment required for digestive process and gastrointestinal transit. Secretion is also an important defense mechanism that aids in ridding the host of enteric pathogens. However, uncontrolled secretion such as occurs in toxin-induced diarrhea can induce fluid loss sufficient to have profound and potentially fatal consequences. The principal secreted ion that results in fluid movement into the lumen is Cl⁻, and it may be secreted via a number of apical Cl⁻ channels. Secretion of Cl⁻ results in transmucosal movement of both Na⁺ and water, which are drawn across the paracellular space and into the lumen in response to the electrical and osmotic gradient (Figure 3). However, before Cl⁻ can be secreted by the epithelial cell, it requires a method of entering the cell. This is achieved via the NKCC1 cotransporter on the basolateral membrane, which allows 2 Cl⁻ ions, 1 Na⁺ ion, and 1 K⁺ ion to enter the cell. This process is driven by the electrochemical gradient of Na⁺ setup by Na⁺-K⁺-ATPase. One of the characteristics of the NKCC1 cotransporter is that it is inhibited by loop diuretics such as furosemide and bumetanide (in the kidney, the NKCC1 transporter is on the apical membrane of the cell and is involved in Cl⁻ uptake from the tubular lumen). In intestinal epithelium, Cl⁻ that enters the cell via NKCC1 transporters escapes via an apical Cl⁻ channel. Although the process of Cl⁻ secretion would seem to be somewhat passive on the basis of this description, it is driven by the intracellular environment, which is relatively electronegative as a result of continued pumping of 2 Na⁺ ions out of the cell for every K⁺ ion that enters the cell via Na⁺-K⁺-ATPase. Furthermore, there are K⁺ channels on the basolateral membrane that allow K⁺ to leak back out of the cell, further increasing the electronegative intracellular environment. Alternatively, K⁺ channels on the apical membrane provide a method for excreting excess K⁺. This is thought to occur predominantly in the colon and appears to be partially under the control of aldosterone, which likely alters the conductance of apical K⁺ channels.

The principal Cl⁻ channel is the CFTR. The CFTR is intimately involved in cystic fibrosis, a genetic disease involving abnormal CFTR expression in children. Without adequate function of this Cl⁻ channel, ion and fluid secretion is insufficient to keep the luminal environment in the lung and intestine moist, resulting in mucous plugging. There are a number of other Cl⁻ channels that play a relatively minor role in fluid secretion, including ClC-2, which is predominantly expressed adjacent to apical tight junctions, and the outwardly rectifying Cl⁻ channel. These channels cannot compensate for absent or dysfunctional CFTR channels. Recently, it has been determined that HCO₃⁻ also uses the CFTR and that secretion of this ion is particularly important in the proximal portion of the small intestine to buffer
The principal ion involved in absorptive processes is Na⁺, which takes advantage of the electrochemical gradient of Na⁺ setup by the basolaterally located Na⁺-K⁺-ATPase to enter the cell (Figure 4). Although Na⁺ can be passively absorbed, most of this ion is absorbed via transporters located on the apical membrane that are linked to another ion or solute transporters. For example, there are Na⁺-linked glucose, AA, and B-vitamin transporters and there are NHEs.

The classic example of an Na⁺-linked solute transporter is the SGLT-1, which transports 2 molecules of Na⁺ with each molecule of glucose into small intestinal enterocytes. This provides a means for the intestinal epithelium to absorb a much needed nutrient along with absorption of Na⁺. The importance of this transporter is highlighted by the underlying principal for most orally administered rehydration solutions, which stimulate Na⁺ and water absorption by supplying glucose to the epithelium.¹⁸ The increase in Na⁺ uptake with provision of glucose is up to 4-fold, depending upon the concentration of glucose used. Thus, glucose-based polyionic solutions are far more efficient at reversing extracellular fluid loss in diarrheal disease than simple saline (0.9% NaCl) solutions. Once inside the cell, Na⁺ is pumped out of the cell into the extracellular environment by use of Na⁺-K⁺-ATPase, and the diffusion of glucose out of the cell is facilitated by use of glucose transporter 2 on the basolateral membrane. Although debated, it is also thought that this Na⁺-glucose transport enhances absorption of Na⁺ and water by use of a mechanism called solute drag, in which fluid and solutes are drawn from the lumen into the paracellular space in response to the osmotic gradient generated by Na⁺ and glucose as they exit the basolateral membrane. Alternatively, there is evidence to support SGLT-1-induced alteration of cytoskeletal tone as a result of activation of myosin light-chain kinase, which has the effect of opening tight junctions (tight junctions are intimately associated with the actinomysosin cytoskeleton).¹⁹ Amino acids are also transported via Na⁺-linked transporters, although these transporters typically transport a family of AAs and usually transport Na⁺ and AAs in a 1:1 ratio. For example, the B AA system transports glutamine, leucine, and methionine. However, these transporters are not as well characterized as glucose transporters and likely represent a group of proteins with overlapping AA transport functions. Probably the most important mechanism for transport of Na⁺ into the cell is the NHE family. These transporters (primarily NHE3 and NHE2) are expressed on the apical membrane in the small intestinal mucosa and the mucosa of the proximal portion of the colon.¹⁶ They are electroneutral in that they exchange Na⁺ for H⁺ (moving out of the cell) with no net change in charge. As such, they are driven by the internal pH of the cell and by the volume of the cell.¹⁷ Thus,
when the pH decreases inside the cell, typically because of metabolism, NHE2 and NHE3 will begin to expel protons in exchange for Na+. This process is thought to be linked, perhaps somewhat loosely, to Cl−-HCO3− exchange. Therefore, in theory, if electroneutral and normal acid-base status of the cell is maintained, it is possible to absorb NaCl in exchange for H+ and HCO3−. The NHEs contribute to almost all of the intestinal Na+ absorption that occurs between meals, and NHE3 appears to be the predominant isofrom. To highlight the importance of the NHE3 isoform, NHE3 knockout mice have chronic diarrhea because of loss of this exchanger. Like the CFTR, NHEs are regulated by cAMP and PKA. However, unlike CFTR activity, which is activated when CFTR is phosphorylated by PKA, NHE function is inhibited by PKA phosphorylation. Therefore, the net effect of activated PKA is inhibition of Na+ absorption and stimulation of Cl− secretion. This is the reason that bacterial toxins and inflammatory mechanisms that increase cAMP are such potent stimuli of diarrhea. The NHEs are also expressed in epithelial cells on the villus compartment of the crypt-villus axis, at a site that may be spared from villus destruction that is associated with viral diseases such as TGE and caused by rotavirus. This is important because some nutrients, particularly glutamine, are capable of driving NHE even in the presence of villus atrophy associated with TGE. Other mediators that regulate neutral NaCl uptake include catecholamines, which stimulate NaCl absorption. Thus, the renin-angiotensin-aldosterone system, which initially responds to reduced extracellular fluid volume (eg, during diarrhea) at the level of the kidney by releasing renin, causes production of angiotensin II in the lung. Angiotensin II stimulates sympathetic nerves in the ENS to release noradrenaline, which stimulates neutral NaCl absorption. Aldosterone may also be involved in stimulation of NaCl absorption. Furthermore, aldosterone stimulates electronegenic Na+ absorption via Na+ channels in the distal portion of the colon of pigs, horses, and sheep.

Pathophysiologic Mechanisms of Diarrheal Disease in Pigs

Historically, diarrheal diseases in pigs have been characterized into 4 major types: hypersecretion, malabsorption, hypermotility, and increased permeability. However, diarrheal disease in pigs is generally multifactorial in nature, involving alterations in secretory and absorptive functions. In addition, disruption in intestinal barrier function (increased intestinal permeability) is a common pathophysiologic event occurring in secretory and malabsorptive diarrheal diseases, which can result in activation of inflammatory cascades that may further increase secretion and exacerbate diarrhea. Finally, a relative increase in luminal ions as a result of secretion and reduced absorption may exacerbate diarrhea as a result of the increased osmotic load within the lumen. In the present report, disease mechanisms of specific swine enteric pathogens will be discussed under 3 general categories: diseases characterized by toxin-induced secretion (so-called secretory diarrhea), diseases characterized by intestinal damage and villus atrophy, and diseases associated with mucosal inflammation.

Diarrheal diseases associated with hypersecretion—The classic example of secretory diarrhea in veterinary species is that caused by ETEC (Table 1). Enterotoxigenic E coli attaches to enterocyte microvilli via surface antigens called fimbria (eg, K88, K99, 987P, and F41) and elaborate enterotoxins that stimulate secretion and severe dehydrating diarrhea. K88 strains tend to be more virulent because of their ability to extensively colonize the entire small intestine, compared with K99, 987P, and F41 strains, which predominantly colonize the ileum. The enterotoxins produced by ETEC are of 3 major categories: large molecular-weight heat-labile toxins (LT), small molecular-weight heat-stable toxins (StA and StB), and enteroaggregative E coli heat-stable enterotoxin-1 (EASt1). Although individual E coli enterotoxins stimulate secretion, they do so by activation of different signaling pathways (Figure 5). The LT toxin of ETEC consists of a single A domain and 5B subunits that bind predominantly to GM1 ganglioside receptors on cell surfaces. Once bound, the A1 fragments translocate into the cell, where they activate adenylate cyclase, leading to unrelenting production of cAMP and activation of PKA, which phosphorylates the CFTR and apical NHE isoforms. The net result of these phosphorylation steps is opening of the CFTR, which

<table>
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<tr>
<th>Pathogen</th>
<th>Hypersecretion</th>
<th>Malabsorption</th>
<th>Inflammation</th>
<th>Increased intestinal permeability</th>
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<td>X</td>
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<tr>
<td>Isospira suis</td>
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*Group A rotaviruses; 1Brachyspira hyodesenteriae and Brachyspira pilisicoli.*
greatly enhances Cl− secretion and inhibition of Na+ absorption via NHE. This process is also the secretory mechanism of human cholera toxin. Heat-stable toxin STa and EAST-1 enterotoxins stimulate hypersecretion by binding to glycoproteins associated with luminal membrane guanylate cyclase receptor, thus activating guanylate cyclase. This, in turn, leads to intracellular accumulation of cGMP, fluid secretion, and inhibition of Na+ absorption. The action of STb is less well-known, but STb is thought to stimulate secretion by activating enteric nerves.25

Although the intracellular signaling pathways of enterotoxin-mediated secretion are well-defined, the importance of these pathways to overall fluid secretion has been questioned. For example, it is not known whether enterotoxins penetrate deep into the crypts to cause secretion in vivo, which has prompted investigations into other mechanisms of secretion by enterotoxins.2 There is convincing evidence for major involvement of the ENS in enterotoxin-induced fluid secretion. For example, in rodent studies, a major portion (> 60%) of E coli enterotoxin-mediated fluid secretion can be inhibited by enteric neural toxins, suggesting that activation of the ENS plays an important role in enterotoxin-mediated diarrhea.25,26 These effects appear to be mediated by the binding of STb enterotoxin, probably to enteroendocrine cells, thereby stimulating release of 5HT and PGE2 into the subepithelial tissues.27 This, in turn, activates a neural secretory reflex arc via release of VIP, thus stimulating secretory processes in the crypts.2,27 Overall, it is clear that direct toxic effects on epithelial cells and neural mechanisms play a critical role in ETEC-induced diarrheal disease.

The acute stages of ETEC infection in pigs are independent of any mucosal epithelial histologic changes. However, if fluid loss and dehydration become severe, ischemic necrosis, epithelial cell sloughing, and villus atrophy can occur, which have major consequences. Ischemic lesions facilitate bacterial translocation across the intestinal epithelium, facilitating the onset of septicemia.28 Additionally, increased permeability can incite mucosal inflammatory processes, which can further exacerbate diarrheal disease.

Diarrheal diseases associated with villus atrophy and malabsorption—Villus atrophy and malabsorption are general characteristics of viral diarrhea in pigs. Because rotavirus is an important cause of human infant deaths in underdeveloped countries, extensive research has been conducted on

Figure 5—Illustrations of the ETEC model of secretory diarrhea (A), the Clostridium difficile model of inflammatory diarrhea (B), and the rotavirus model of malabsorptive diarrhea (C).
the mechanism by which this organism causes diarrhea. Rotavirus is also of major importance in neonatal large animal species, including pigs, foals, lambs, and calves. Rotavirus attaches to mature enterocytes on the upper third of the villus. Once bound to the receptor on the enterocyte (thought to involve sialic acid-dependent and independent mechanisms), rotavirus enters the enteroctye via Ca²⁺-dependent endocytosis, where it replicates within the cytoplasm (Figure 5). Rotavirus infection results in marked changes in histologic features of the intestine, including stunted villi and a change from columnar to cuboidal epithelium within 24 hours of infection. These effects are most pronounced in the proximal portion of the small intestine. Rotavirus impairs glucose absorption via 2 mechanisms. First, rotavirus impairs Na⁺-glucose cotransport by inhibiting SGLT-1. Second, intestinal disaccharidases (sucrase, maltase, and lactase) responsible for cleaving monosaccharides for Na⁺-glucose cotransport located on the brush-border membrane of enterocytes are markedly attenuated in rotavirus enteritis. Despite substantial impairment of Na⁺-glucose cotransport, glucose-based oral rehydration solutions are effective in replacing fluid losses during rotaviral diarrhea, which suggests that some SGLT-1 transport mechanisms remain intact. The principal mechanisms associated with rotaviral diarrhea have long been thought to be malabsorption and osmosis. However, there are several lines of evidence that place the importance of these mechanisms in question. For instance, rotavirus-induced diarrhea develops prior to extensive histologic evidence of damage to the absorptive intestinal epithelium. Infection of neonatal pigs with porcine rotavirus results in watery diarrhea 8 hours after infection, whereas histologic damage to the jejunum is not evident until 48 hours after infection. Furthermore, in a study of the effect of nutritional status on rotavirus-induced diarrhea, 9 days after infection, intestinal structural damage was similar between nourished and malnourished piglets, but the duration of the diarrheal period was significantly longer in malnourished piglets. With regard to the latter finding, greater indices of intestinal inflammation were observed in rotavirus-infected pigs and were prolonged in malnourished piglets, which suggests an important role of nutritional status and inflammatory responses in rotaviral diarrhea. These findings can be explained by emerging evidence of a novel secretory mechanism associated with rotavirus infection. Rotavirus secretes an enterotoxin called NSP4, which induces enterocyte secretion by inducing increases in intracellular Ca²⁺. On the basis of known ion transport mechanisms, these increases in Ca²⁺ would be expected to induce secretion via effects on basolateral K⁺ channels, with resultant increases in Cl⁻ secretion via the CFTR. However, NSP4 induces secretion of Cl⁻ to a similar extent in cystic fibrosis tissues in which the CFTR is essentially nonfunctional. These findings suggest a novel CaCC on the apical membrane. In addition, there is evidence to indicate that NSP4 interferes with NaCl and solute absorption. For example, NSP4 directly damages or interferes with the function of the apical Na⁺-glucose transporter SGLT, further exacerbating diarrhea. The NSP4 also inhibits Na⁺/K⁺ ATPase function, thus impairing both NaCl and Na⁺-linked nutrient transport. Interestingly, NSP4 toxin has little effect in adult animals, possibly because of age-dependent decreases in CaCC expression. It should be noted that the mechanistic studies on NSP4 have been performed almost exclusively in laboratory rodents and rabbits. However, a specific NSP4 antibody response has been detected in gnotobiotic pigs infected with human rotavirus. To date, NSP4 has been found only in group A rotaviruses, but pigs can be infected with multiple groups including groups A, B, C, and E. Interestingly, group A rotavirus was more commonly detected in suckling pigs with diarrhea, compared with groups B to E.

Rotavirus-induced fluid secretory responses are greatly reduced (by > 60%) in the presence of enteric neural blockers such as tetrodotoxin and lidocaine, suggesting that activation of the ENS is a major component of rotaviral diarrhea. However, the exact mechanisms by which rotavirus or NSP4 activates the ENS remain unclear, although recently, 5HT and VIP have been determined to be the major neurotransmitters involved in rotaviral secretory responses, suggesting activation of neural secretory reflex arcs. Villus ischemia and enhanced tight junction permeability have also been detected in rotavirus-infected animals and may also contribute to diarrhea.

Another important viral enteric disease of pigs is TGE. Transmissible gastroenteritis is caused by a coronavirus that induces severe villus atrophy and loss of mature absorptive epithelium, leaving a stunted villus lined by a relatively immature epithelium. Transmissible gastroenteritis-infect ed porcine intestine has markedly reduced brush-border enzymatic activities and impaired Na⁺ and Cl⁻ absorption. The intestinal crypts are spared during TGE and become hyperplastic so that secretion continues in the presence of severely impaired absorption, resulting in diarrhea. An osmotic gradient caused by undigested carbohydrates in the intestine is thought to draw water into the lumen, thus contributing to diarrhea. Transmissible gastroenteritis affects pigs of all ages; however, clinical disease is generally less severe in older pigs, which is attributable in large part to the compensatory fluid absorption that occurs in the large intestine of older pigs, compared with younger pigs. For instance, reduced clinical severity of TGE-associated diarrhea in 3-week-old pigs, compared with 3-day-old pigs, is correlated with increased short-chain fatty acids and net water absorption across the colon of older pigs. It is thought that development of microbial digestion in older pigs enables undigested carbohydrates such as lactose to be converted to short-chain fatty acids, which are absorbed in the colon, thus driving water absorption and reversing fluid losses that occur in the proximal portion of the intestine. Like rotavirus, TGE-affected pigs are also responsive to oral rehydration solution, suggesting intact absorptive mechanisms despite substantial damage to the mature absorptive epithelium. Oral rehydration solutions containing AAs such as i-alanine, in addition to glucose, are more effective in stimulating Na⁺ absorption in TGE-affected jejenum of neonatal pigs, compared with either AAs or glucose alone. Increased permeability of the epithelium has also been detected in TGE-affected...
tissues, which may contribute to TGE-induced diarrhea by facilitating access of luminal antigens to the lamina propria and activating inflammatory and enteric neural pathways.

There are 2 major spirochetal organisms known to cause predominantly malabsorptive diarrhea and decreased weight gain in grower and finisher pigs. *Brachyspira* (Serpulina) *hyodysenteriae* causes swine dysentery, and *Brachyspira* (Serpulina) pilosicoli causes porcine intestinal spirochosis. *Brachyspira hyodysenteriae* causes severe catarrhal and hemorrhagic colitis thought to be predominantly malabsorptive in nature. Severely impaired Na⁺ and water-absorptive capabilities have been detected in *B hyodysenteriae*-infected colonic epithelium. *Brachyspira hyodysenteriae* infection is not associated with increases in the second-messenger cAMP or cGMP or with enhanced responsiveness to the cAMP secretagogue, theophylline, suggesting that diarrhea is predominantly attributable to malabsorption rather than activation of intestinal secretory processes. Large intestinal diarrhea predominates in *B hyodysenteriae* infection, whereas the small intestine is largely unaffected. In fact, glucose-dependant stimulation of solute and water absorption is unaltered in infected jejunum, suggesting that oral rehydration solution would be beneficial in restoring extracellular fluid losses associated with this disease. The role of the 2 toxins of *B hyodysenteriae*, hemolysin and lipopolysaccharide, in the pathogenesis of diarrhea is unclear. Injection of hemolysin toxin into ligated ileal and colonic loops of gnotobiotic pigs results in marked epithelial sloughing at the villus tips and subsequent villus contraction; however, no fluid accumulation, hemorrhage, or inflammation is evident. Whipp et al infused colonic loops of commercial-bred pigs with known pathogenic isolates of *B hyodysenteriae* and observed histopathologic lesions in addition to fluid accumulation, hemorrhage, and inflammation. Results of these studies may suggest that although hemolysin may be important in epithelial destruction, other mechanisms, toxins, or both are likely required for development of diarrhea in pigs. Mechanisms of *B piliscoli*-induced diarrhea are similar to *B hyodysenteriae* (malabsorption), although *B piliscoli* spp induce less severe histopathologic lesions and milder diarrhea; nevertheless, reductions in growth performance can be substantial.

*Isospora suis* (neonatal pig coccidiosis) is a major cause of diarrhea in pigs from 7 to 21 days of age. Penetration of *I suis* sporozoites into enterocytes results in sloughing of small intestinal villus epithelium, villus atrophy, and necrotic enteritis. Sloughed and necrotic epithelial cells are rapidly replaced by flattened, immature epithelium, which causes predominantly malabsorptive diarrhea. To the authors' knowledge, no other diarrheal mechanisms (eg, hypersecretion or inflammation) for neonatal pig coccidiosis have been identified.

**Inflammatory diarrheal disease—Salmonella**

Typhimurium is the cause of one of the best-studied inflammatory diarrheal diseases that affects pigs. *Salmonella* spp attach to enterocytes and M cells of the ileum and proximal portion of the large intestine and subsequently invade the host cells and trigger activation of inflammatory signaling cascades (Figure 5). *Salmonella* spp have evolved sophisticated virulence mechanisms for attachment and invasion into host cells. A critical event associated with virulence of salmonellae is the formation of a needle-like structure that docks with host cell membranes. Through this so-called type III secretory system, a variety of bacterial effector proteins are injected into the host cell and trigger inflammatory signaling cascades. Insertion of the bacterial protein flagellin into the host cell is responsible for inducing an inflammatory response. Flagellin traverses the cell, reaching the basolateral membrane, after which it interacts with toll-like receptor 5. This ultimately results in production of the potent chemokine interleukin 8 by the basolateral membrane via nuclear κ-B pathways, which attracts neutrophils to the basolateral surface of the epithelium. Other organism-induced proteins induce transepithelial migration of neutrophils. Once in the vicinity of the epithelium, neutrophils may induce enterocyte secretion by a number of mechanisms, including oxidant- and interleukin-1β-induced upregulation of prostaglandin production via cyclooxygenase-2 within subepithelial fibroblasts. Cyclooxygenase-2-derived PGE₂ can directly activate enterocyte Ω secretion, whereas PGI₁ is capable of activating enteric nerves. Intraluminal 5HT concentrations are also markedly increased in response to *Salmonella* spp infection and the 5HT4 receptor antagonist attenuates secretory responses in the porcine intestine. However, it is not known whether 5HT-mediated secretion is a result of 5HTs direct action on epithelium or via activation of a neural reflex arc as described for other agents. In rats, *S Typhimurium*-induced secretion in the jejunum and ileum is mediated by enteric nerves, and prostanadlin inhibition blocks this response.

In calf models of *Salmonella* spp infection, inflammatory processes result in denudation of the epithelium; necrosis of the intestinal mucosa; and, in severe cases, mucosal collapse. Histologic changes coincide with accumulation of fluid, protein, and neutrophils in the intestinal lumen, suggesting that exudative processes in the intestine contribute to diarrhea.

Another disease characterized by a marked inflammatory response is that caused by *Clostridium difficile*. *Clostridium difficile* releases toxin A and toxin B, which bind to epithelium and trigger the opening of epithelial tight junctions via glucosylation of several small guanine triphosphatases that are involved in cytoskeletal organization at the level of the tight junction. This may, in part, cause excessive loss of fluid into the intestinal lumen as a result of increased permeability, but of far more importance is toxin A-induced release of cytokines via activation of nuclear factor κ-B in epithelial cell lines. Interleukin-8 is increased in response to toxin A in human colon, whereas tumor necrosis factor-α, leukotriene B₄, and PGE₂, are released in rabbit and rat intestine. The net effect of the release of an array of proinflammatory mediators is a robust inflammatory response. However, the mechanisms whereby the inflammatory response is induced are complex. It appears that the initial effect of cytokines released in response to toxin A is stimulation of enteric sensory nerves. These nerves release substance P and calcitonin gene-related peptide, which act on mast cells, and...
these cells appear to be responsible for recruiting neutrophils. Substance P can also activate interneurons with subsequent activation of secretomotor nerves, resulting in cholinergic stimulation of epithelial secretion. Thus, inhibiting enteric sensory nerves or reducing mast cell activity markedly reduces inflammation and secretory diarrhea.

**Diarrheal disease for which the pathophysiological mechanisms remain unclear**—Diarrheal diseases in this category include proliferative enteropathies associated with *Lawsonia intracellularis*, edema disease associated with certain strains of *E. coli*, and diseases associated with *Clostridium perfringens*.

*Lawsonia intracellularis* is the etiologic agent of proliferative enteropathy or ileitis. *Lawsonia intracellularis* invades proliferative, immature crypt cells via a vacuole and replicates in the cytoplasm. Entry of *L. intracellularis* is thought to be mediated through expression of the surface antigen Lsa, which mediates attachment and invasion into epithelial cells. Once infected, crypt cells continue to undergo mitosis but fail to develop into mature absorptive enterocytes. Therefore, the absorptive capability of the epithelium is reduced, and this is thought to be a mechanism of diarrhea. However, it is not known whether *L. intracellularis* disrupts secretory processes in these enterocytes. The form of proliferative enteropathy has an important association with clinical signs. Porcine intestinal adenomatosis is characterized by intestinal hypertrophy, and heavy infections induce diarrhea and inflammatory lesions. Associated lesions may cause colic, obstructions, and reduced feed intake and performance. Acute hemorrhagic forms of proliferative enteropathy such as porcine hemorrhagic enteropathy are associated with more severe clinical signs of diarrhea in which inflammation is thought to play a larger role. Porcine hemorrhagic enteropathy is characterized by severe denudation of the epithelial cell lining and bleeding into the lumen as a result of leakage from the capillaries. Well-developed lesions of proliferative enteropathy consist of predominantly mononuclear leukocyte infiltrates in the lamina propria, including CD8 cells. However, macrophages are thought to be the key immunocytes involved in the pathogenesis in porcine hemorrhagic enteropathy and may influence vascular permeability and hemorrhage.

Verotoxigenic *E. coli* causes edema disease that is associated with the postweaning period. The *E. coli* involved in this disease, F18, adheres to the intestinal epithelium and releases a potent vasotoxin, verotoxin 2e. Verotoxin 2e (also known as Shiga-like toxin IV) migrates across the intestinal epithelium and enters the circulation, resulting in vascular necrosis, neurologic signs, and edema in several tissues without causing any visible architectural damage to the intestinal mucosa. Experimental inoculation of large doses of verotoxin 2e into the intestine of pigs does not induce edema disease. However, when pigs are inoculated with verotoxin 2e in the presence of sodium deoxycholate, a bile salt that disrupts the intestinal barrier and thus increases the intestinal permeability, edema disease develops, suggesting that an impaired intestinal barrier is a prerequisite for development of disease. Intestinal acidosis may be important regarding the pathogenesis of edema disease, but the definitive role of intestinal acidosi remains unclear. Rapid changes in microflora and nutrient composition of the diet have also been implicated in the pathogeneity of verotoxigenic *E. coli*. The pathophysiological features of diarrhea related to edema disease are associated with the causative strains ability to produce enterotoxins (Sta, STb, or both).

*Clostridium perfringens* is the cause of the most common clostridial disease in pigs. *Clostridium perfringens* proliferates rapidly and becomes the predominant bacterium in the gastrointestinal microflora during clostridial disease. The finding that neonates have less developed and less diverse intestinal microflora likely explains why neonates are more susceptible to *C. perfringens* infection than are older pigs. There are 2 major types of *C. perfringens* that cause disease in neonatal pigs: type A and type C. *Clostridium perfringens* type C produces α and β toxins and generally causes more severe disease than type A. These toxins cause massive destruction of the intestinal mucosa and necrotic enteritis. Type C organisms attach to villus tips of the jejunum and cause sloughing of the epithelium and proliferation of the organism along the basement membrane. Necrosis of lamina propria and villus epithelial cells extending into the crypts, muscularis mucosa, submucosa, and muscle layers is observed. Therefore, exudation is the major mechanism of diarrhea caused by *C. perfringens* type C, loss of the epithelium causes loss of extracellular fluid, proteins, and blood into the intestinal lumen. Infections with *C. perfringens* type A are more common and the histologic and clinical disease is less severe, compared with type C infections. Unlike type C, *C. perfringens* type A organisms do not attach to villus epithelium and diarrhea associated with this organism is thought to be secretory in nature, with α and β playing a predominant role. The difference in severity between type A and type C infections is likely because β toxin is produced by type C, but not by type A. The β toxin is considered to play an important role in tissue destruction because the use of β toxoid alone prevents mucosal damage. However, inoculation of β toxin experimentally in intestinal loops does not cause epithelial necrosis, suggesting that other factors other than the presence of the toxin are required to initiate damage. Inflammatory cells, including neutrophils, lymphocytes, plasma cells, and macrophages, are thought to play a role in the actions of β toxin. Conversely, α toxin has a secretory role. Purified α toxin stimulates electrogenic secretory processes while inhibiting Na+-glucose cotransport in the small intestine of laying hens. Other toxins such as β2 and *C. perfringens* enterotoxin are produced by all *C. perfringens* and may also play a role in disease. More than 90% of strains isolated from pigs with neonatal enteritis produce β2 toxin, but its exact role in pathogenesis remains elusive. *Clostridium perfringens* enterotoxin toxin causes villus necrosis and fluid secretion in ileal loops. The role of toxins and their mechanism of action are not clear, and more work is needed to understand their importance.

**Factors affecting onset and severity of diarrhea in pigs**—The presence of the pathogenic agent alone is often insufficient to cause diarrhea in pigs, suggesting that other factors are important components of the disease process. Use of various challenge models has
revealed that social and physical stress can increase susceptibility to enteric disease. For example, intestinal colonization with ETEC alone is insufficient to cause clinical disease in neonatal pigs. However, clinical disease results when experimental ETEC challenge is combined with stressors such as weaning and mixing pigs with nonlittermates, reduced environmental temperature, and coinfecion with other organisms. 86, 87 Piglets subjected to cold stress followed by inoculation with TGE virus have severe disease with 100% morbidity rate, compared with nonstressed pigs inoculated with TGE virus, which do not develop clinical disease. 87 Although it appears that stress plays a critical role in the development of enteric disease in pigs, the mechanisms that link stress to intestinal disease are unclear; recent studies have begun to elucidate these mechanisms. Weaning stress in 19-day-old pigs results in marked disruption of the intestinal barrier function (increased intestinal permeability) and enhanced intestinal secretory tone. 88 These mucosal disturbances are a result of activation of stress signaling pathways, including activation of intestinal corticotropin-releasing factor and its receptors and subsequent degranulation of mast cells residing within the intestinal mucosa. Activation of enteric nerves also appears to be an integral component of this intestinal stress response. Stress-induced intestinal dysfunction in weaned pigs can be attenuated by increasing weaning age (from 19 to 28 days of age). 89 This indicates that knowledge of stress pathways in pigs and management factors that influence them will be helpful in the prevention of stress-induced enteric disease.

The age of the pig can also be associated with susceptibility to enteric disease. Organisms such as rotavirus have a predilection for infecting young animals, which may relate to age-specific expression of CaCC. Another example is diarrhea caused by E.coli K88. Intestinal receptors for E.coli K88 are abundant in neonates and decrease with age, which is the reason disease caused by this organism mainly affects young pigs. 90 Similarly, intestinal receptors for E.coli F18 increase with age, which is the reason infection with that organism occurs only in older weaned pigs. 91 As mentioned, severity of TGE is less in older animals because of their disease resistance regulator mouse models. J Biol Chem 2004:279:22276–22283.

17. Berberov EM, Zhou Y, Francis DH, et al. Relative importance of heat-labile enterotoxin in the causation of severe diarrheal disease in the gnotobiotic piglet model by a strain of enterotoxi-


