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A Review of Enterotoxigenic *Escherichia coli* Infection in Piglets: Public Health Importance

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ABSTRACT

Diarrhea in piglets can cause health problems and even death. The cause is often infection by enterotoxigenic Escherichia coli (ETEC). This condition can have an impact on the growth of pigs and the economy of farmers, the cause can also be transmitted to humans which has an impact on public health. Diarrhea is one of the main health problems of piglets, because it attacks the digestive tract, especially the intestines. The main virulence factors are adhesin (fimbriae) and enterotoxins, with the most frequently found being ETEC F4 (K88) and F18. Fimbriae F4 (K88) ETEC causes diarrhea in neonatal pigs, while fimbriae F18 causes diarrhea in post-weaning pigs (PWD). Meanwhile, enterotoxin is divided into two types, namely heat labile enterotoxin (LT) and heat resistant enterotoxin (ST). After attaching it to the intestinal mucosa, E. coli will colonize and produce enterotoxins. Neonatal diarrhea is usually observed in piglets 1-4 days of age, while post-weaning diarrhea occurs in piglets 2-3 weeks after weaning with a peak diarrhea occurring 6-8 weeks post weaning, and even at 12 weeks. The large amount of water and electrolyte secretions causes dehydration, metabolic acidosis, osmotic diarrhea and a high probability of death before 2 weeks. Currently, there are many incidents of antibiotic resistance, so an alternative use of antibiotics is needed in pig farms to prevent ETEC infection. Alternative antibiotics that can be used to prevent infection with ETEC infection in piglets are immunoprophylaxis, antimicrobial minerals (such as zinci oxide and cupri sulfate), acidifiers, blood plasma, egg yolk antibodies, probiotics, nucleotides, bacteriophages and so on. These kinds of alternatives and feed additives can improve intestinal health and prevent diarrhea in piglets. This review contains the latest research from various journals discussing how ETEC can infect piglets and the management against the disease.

Keywords: enterotoxigenic Escherichia coli (ETEC), diarrhea, piglets, public health

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INTRODUCTION

The digestive tract (GIT) is an important channel in the metabolism and defense system of pigs. One of the important parts of this channel is the intestine. The intestine is responsible for digesting food, absorption of nutrients, and for protecting the body from toxins or pathogens. So that knowledge is needed in maintaining the health of piglets. The most common cases of piglets are neonatal diarrhea, post-weaning diarrhea (PWD) and edema. This is caused by enterotoxigenic Escherichia coli (ETEC) infection. ETEC can cause health problems and even death in piglets around the world (1,2). So that the pathogenesis of this infectious agent is important to be discussed in this review. ETEC attaches to the epithelium of the small intestine of pigs, then there is an increase in the secretion of water and electrolytes in the intestinal lumen. This is due to the production of enterotoxins and subsequent changes in enterocyte function. The large amount of water and electrolyte secretions causes dehydration, metabolic acidosis, osmotic diarrhea and a high probability of death before 2 weeks (3).

Diarrhea in pigs caused by E. coli is classified into six pathogenic strains based on virulence factors and pathogenic characteristics. Namely enterotoxigenic E. coli (ETEC), enterohemoragic E. coli (EHEC) or shiga-toxicigenic E. coli (STEC), enteroagregative E. coli (EAEC), enteropathogenic E. coli (EPEC), enteroinvasive E. coli (EIEC), and diffusely attached E. coli. The most common and pathogenic strain in piglets is the ETEC strain (4,5).

The incidence of diarrhea in piglets is usually associated with the virulence factors of ETEC. Another very influential factor besides enterotoxin is fimbriae. The main fimbriae in pigs are F18 fimbrae in post-weaning piglets and F4 (K88) fimbrae in newborn pigs (neonates) (6-8). The large number of E. coli bacteria that are resistant to certain antibiotics has prompted research on preventive and control measures, one of which is finding a substitute for antibiotics. Abraham et al. (2014) stated that in their research, E. coli in pigs in Australia was resistant to several antibiotics including third generation cephalosporins and fluroquinolones (9). Another Australian study found E. coli resistance to colistin antibiotics, streptomycin spectinomycin, ampicillin and trimethoprim-sulfamethoxazole (10). Another study, ETEC infection in swine in Switzerland, occurred resistance to antibiotics ampicillin, gentamicin, kanamycin, sulfonamide, streptomycin, tetracycline, and trimethoprim (11). In Kupang, Indonesia, the dominant resistance of E. coli isolates from pigs to erythromycin and cephalotin antibiotics (12). Even the research of Rosager et al. (2017) suggested that resistance was greater in E. coli ETEC strains than in non-ETEC (13). This indicates that it is difficult to control E. coli in piglets which causes diarrhea. With the incidence of diarrhea in piglets caused by ETEC,

knowledge of preventive and control measures against this problem is required. Furthermore, this review focuses on alternative strategies for preventing ETEC infection in piglets.

Description of E. coli causing colisepticemia and pathogenesis

Enterotoxigenic E. coli disease (ETEC) in piglets causes huge economic losses with high morbidity, high mortality, growth retardation and high medical costs (13). Neonatal diarrhea is usually observed in piglets ages 1-4, while post-weaning diarrhea occurs in piglets 2-3 weeks after weaning with a peak diarrhea occurring 6–8 weeks post weaning, and even at 12 weeks. The period of ETEC infection to cause diarrhea is around 1 to 5 days depending on many factors, especially the success of bacterial colonization of the small intestinal mucosa (14).

Diseases caused by E. coli can cause diarrhea and septicemia in piglets. In addition, it can also cause diarrhea, edema during weaning, mastitis and cystitis in broodstock (15). Diarrhea in piglets often occurs as a result of a single infection or various types of E. coli, including ETEC, toxin-producing E. coli vero or shiga, necrotoxigenic E. coli, enteropathogenic E. coli, enterohemorrhagic E. coli, enteroaggregative E. coli, and enteroinvasive E. coli. Among these E. coli strains, ETEC is a common cause of severe and watery diarrhea in piglets (16). There are two important parts of ETEC infection in causing diarrhea, namely intestinal epithelial cells and the production of toxins from bacteria. The most well-known enterotoxin genes are the heat-labile enterotoxin LT and the heat-stable enterotoxins STa, STb. These toxins have the ability to cause diarrhea due to various changes in electrolyte balance (2,17).

Virulence Factors in Escherichia coli

The main pathotype of E. coli causing diarrhea in piglets is ETEC. These bacteria live and multiply in the intestinal epithelium. Diarrhea can cause health problems, death and economic decline for pig farmers. Enterotoxins produced by bacteria can also cause damage to enterocyte cell function so that they can interfere with fluid homeostasis (increasing fluidity and reducing water absorption) in the small intestine (6,7). Apart from predisposing environmental conditions and host factors, as well as high bacterial proliferation in the gut, virulence factors are very important in playing a role in the process of diarrhea. Virulence factor refers to the molecules produced by bacteria when interacting with the host (18). The main virulence factors of ETEC are adhesives with attachments such as fimbriae and enterotoxins. These fimbriae will help the bacteria attach to specific receptors. It is important for the pathoogenesis process are adhesins and enterotoxins. These specific receptors make the ETEC strain host specific (10).

The first step in the pathogenic process is an interaction between adhesins and ligands on the microvilli of the small intestine, this allows bacteria to attach to the microvilli (19). The relationship between ETEC and intestinal epithelium is caused by adhesive fimbriae such as F4 (faeG) and F18 (fedA), as well as F5 (fanC), F6 (fasA), and F41 (fim41A). ETEC classification in pigs at least in E. coli isolates found one enterotoxin gene (elt, sta, stb), along with one gene encoding fimbriae, including F4, F5, F6, F18, and F41 (2). The main fimbrae in pigs are F18 fimbrae in post-weaning pigs and F4 (K88) fimbrae in newborn pigs (7).

Morphologically, fimbriae can be classified into 2 categories, namely pili and fibrillae (20). Fimbriae are extracellular appendages 0.5–10 μm long and 2–8 nm wide. Fimbriae are involved in adhesion and many other functions, including interactions with macrophages, biofilm formation, gut persistence, and bacterial aggregation (21). There are two antigenic variants that have been identified, namely F18ab (previously known as F107) and F18ac (previously known as 2134P and 8813), the word a means an antigen factor and the word b, c is a specific factor (22, 23). The majority of the ETEC F18 strains were capable of producing heat-resistant enterotoxins including STa and STb, whereas the ability to produce Shigatoxin was more related to F18ab (23). F18 is coded by the fed gene of five units, namely, fed A (major subunit), fed B (usher), fed C (chaperone), fed E (minor protein) and fed F (adhesin) (24).

Fimbriae F18 are filaments 1 to 2 mm long based on the main structural protein, called fed A (15.1 kDa) (16). The main structural subunit of F18 fimbriae is fed A, but the small adhesive fed F subunit also plays a central role in binding to the host receptor and is highly conserved among the F18 strains (25,26). F18ab is usually associated with strains that produce Stx2e (STEC) or shigatoxin E. coli, and F18ac is associated with ETEC causing diarrhea in post-weaning piglets (PWD) (27). The ETEC F18 + strain frequently produces heat-resistant enterotoxins including STa and STb, whereas heat labile (LT) enterotoxins are rarely produced in these strains (28, 29). ETEC colonization in the small intestine is initiated by the interaction of the fimbriae receptor, namely F18 fimbriae which binds to glycoproteins on the microvilli or brush border of the small intestine (24, 30). Genetically, some piglets do not have the F18 fimbriae receptor and thus these pigs may be resistant to F18 + ETEC colonization (31,32).

Enterotoxigenic E. coli (ETEC) K88 expresses F4 fimbriae, which are proteinaceous filamentous adhesins consisting of repeated copies of the major fimbrial subunit fae G and several additional minor subunits (33). The ETEC strain expressing F4 fimbriae is associated with neonatal diarrhea and post-weaning diarrhea in piglets. Three variations of the F4 fimbriae antigen are F4ab, F4ac, and F4ad, with the most popular being F4ac. These antigen variants differ in amino acid composition from the main subunit, fae G, and each has a related but different receptor binding profile (34, 35).

Amino acids along the 125 - 163 subunits of FaeG are effectively essential to the binding of fimbrial F4. Amino acid residues 140–145 and 151–156 were identified as functional sites for F4ab fimbriae. Meanwhile, other amino acids, namely 148–150 and 156–158, were reported to inhibit the attachment of F4ab to the host cell. F4ac has an amino acid length of 147 to 160, this being the defining epitope that controls the fimbrial binding capacity (36). In contrast to the ab and ac variants, the F4ad FaeG subunit interacts with the minimal galactose-binding epitope via the D'-D"-α1-α2 binding domain, resulting in different structural and attachment properties (34). Recent studies have shown that oral inoculation of ETEC / VTEC / EPEC F4 ab or ac in freshly weaned piglets causes enteritis and a severe systemic inflammatory response (37). In another study, in vitro enterotoxigenic binding of F4 + E. coli (ETEC) to piglet enterocytes was partially blocked by dose-specific neoglycans (38). FaeG is the main fimbriae subunit and adhesion for F4 fimbriae compared to F18 fimbriae. All adhesins F4 bind to carbohydrate glycoproteins in intestinal epithelial cells and intestinal mucus. F4ad adheres more to glycolipids, whereas adhesins F4ab and F4ac tend to bind glycoproteins more (36).

Other associated fimbriae that have a lower prevalence are F5 (K99), F6 (987P), F17 (Fy / Att25) and F41, with the number of active receptors in intestinal epithelial cells decreasing as the pigs and cows age. This fimbrae is usually found in post weaning piglet diarrhea (PWD) together with F18 and / or F4, or individually (6). The results of the most recent study regarding colonization factors identified among the ETECs isolated from swine neonatal diarrhea were F4, F5 + F41, and F6 (40.5, 16.7, and 11.9%, respectively), whereas in post-piglet diarrhea, weaning (PWD) there were no positive isolates for F5, F6, and F41 (8).

Enterotoxin Characteristics

Enterotoxins are extracellular proteins or peptides secreted by bacteria, one of which is ETEC. ETEC can cause disease by colonizing the small intestine or colonization factors (CFs), the production of heat-resistant or labile toxin (LT) and / or heat-resistant or stable toxin (ST) toxins. The proportion of ETEC strains producing single LT, single ST, or both toxins (LT + ST) varies depending on geographic location and seasonality. However, overall, the majority of ETEC produced ST and LT (17,39).

LT is an 84-kDa polymer protein consisting of enzymatically active A subunits (28 kDa) which are noncovalently linked to pentameric B subunits (11.5 kDa each). Subunit A consists of two components, A1 and A2. The A1 subunit (21 kDa), the enzymatically active portion of the toxin, is noncovalently linked to pentamer B via peptide A2 (7 kDa) (40,41). STp or stabile toxin produced by pigs is a short peptide, consisting of 18 or 19 amino acids, originally produced as 72-structure amino acids by bacteria (17). The STa gene encodes 72 precursors of pre-pro-peptide amino acids. Just like STa, STb is synthesized as 71 amino acid prepeptides, consisting of a signal peptide and an adult STb enterotoxin namely 48 amino acids (5.2 kDa) (42).

Labile enterotoxin (LT) is a member of the AB5 toxin family, which is similar to the cholera toxin secreted by Vibrio cholerae. These toxins have structural similarities and mechanisms of action. The LT structure consists of one catalytic subunit A and a pentameric ring of subunit B as a receptor binding. The subunit is encoded by genes from plasmids eltA and eltB, and then transcribed as operons (41,42,43). Subunit A contains 240 amino acids, while subunit B contains 103 amino acids (16).

Subunit A is enzymatically active, divided into A1 and A2. Domain A1 is enzymatically active, domain A2 is shorter active and five B subunits mediate binding of glycolipid and glycoprotein receptors to host cells. Pentamer GM1-binding B subunit (LTB) (11.5 kDa) interacts with A subunit (28 kDa) via noncovalent binding of the A2 domain to form the holotoxin AB5 structure. The B subunit binds primarily to the monocialotetrahexosylganglioside (GM1) receptor on the cell surface. Domain A1 is responsible for ADP-ribosylating stimulation of G protein. A1 will translocate to the endoplasmic reticulum and activate the cyclic adenylate system to increase cellular cyclic adenosine monophosphate then the intracellular cAMP concentration is uncontrolled. As a result, the ions open, then the chloride anion and then the water molecules are released. This is a characteristic feature of diarrhea caused by ETEC infection, namely increased secretion of fluids and electrolytes followed by decreased water absorption (44,45). In addition to its enterotoxicity function, LT also acts as an adhesin, which binds bacteria to GM1 in the plasma membrane of small intestinal epithelial cells. Elimination of the LT gene can reduce the severity of diarrhea and ETEC colonization in the small intestine of pigs (46,47).

Heat resistant enterotoxin (ST) produced by ETEC is a peptide which is divided into two types, namely STa and STb. STb is more deadly in animals, especially in post-weaning piglets (PWD). Then the STa enterotoxin is more relevant for diarrhea in humans, newborn piglets and calves. These peptides are encoded by two genes, estA (STI) and estB (STII), which are located in plasmids, and can be differentiated from one another by their solubility in methanol and their protease sensitivity (42).

STa has poor resistance due to its small size (19 amino acids with 3 disulfide bonds), but STa is a very strong enterotoxin (48). Enterotoxin STa is soluble in methanol and resistant to proteolytic enzymes. According to the host species, STa is further classified into two subtypes, known as STp and STh, which were initially isolated from the ETEC strains of pigs and humans. While STb is a peptide consisting of 48 amino acids with 4 cysteine residues included in 2 disulfide bonds (42).

Serogroups and serotypes in diarrhea caused by ETEC

ETEC has many variations of the toxin coding genes, so that scientists classify them based on specific serogroups and specific serotypes. ETEC specific serogroups, namely O and H serogroups, were determined based on lipopolysaccharide and flagella. The O antigen consists of repeating subunits that extend over the surface of the bacteria. Research in China, the most O antigen found in pigs is O8, O64, O141 and O149 (49-51). This is in line with research in Vietnam, where the O8 antigen found the most (52). Other serogroups that cause diarrhea are O2, O6, O9, O, 11, O20, O26, O40, O45, O48, O54, O86, O87, O115, O101, O138, O139, O147, O152, O157 and O161. Meanwhile, serogroup O causes edema, namely O138, O139 and O141 (49,51). Specific serogroup H are determined by the flagella antigen, which also serves as an antigenic marker. This antigenic will be useful as a component of the ETEC vaccine. When compared with serogroup O, serogroup H has less association, namely O141: H4, O2: HNM, O139: H1, O26: H11 and O2: H40 antigens (8). In addition, fimbrial adhesins which also play an important role in diarrhea caused by ETEC in pigs are F4 (K88), F5, F6, F18 and F41 (53).

Pathogenesis of Enterotoxigenic E. coli (ETEC).

Adhesins and enterotoxins in the pathogenesis of ETEC play an important role as virulence factors. Adhesin in ETEC is useful for facilitating binding to specific receptors of small intestinal epithelial cells. Adhesin also plays an important role in the colonization, proliferation and development of ETEC infection. Enterotoxins are also considered to be direct virulence factors that cause disease and may also play a role in the colonization process. (54).

There are four main fimbriae in ETEC as fimbrial adhesins, namely F4 (K88), F5 (K99), F6 (987P), and F41. One particular ETEC strain usually only expresses one fimbrial type, so that each strain has a different fimbrial type. ETEC possessing fimbrial adhesins F4 which release enterotoxins is a characteristic pathogen causing diarrhea in piglets (55). The ETEC strain expresses two main types of enterotoxins as previously described, namely heat-labile (LT) enterotoxins and heat-stable (ST) enterotoxins. Enterotoxin ST is subdivided into STa and STb according to differences in protein structure and pathogenesis. Since each section targets a specific site, it is necessary to develop vaccinations against ETEC targeting fimbriae and enterotoxins.

How much bacteria colonize can determine the severity of the disease. Fimbriae attach to specific receptors for small intestinal epithelial cell membranes and non-specific on the epithelial mucous membrane of the small intestine, in this case the jejunum (56). The molecular epidemiology of diarrhea in pigs showed that the ETEC F4 receptor was expressed in the small intestine of newborn to adult pigs, whereas the ETEC F18 receptor was expressed increased in 3 weeks old pigs (57). Therefore, the F18 receptor does not cause diarrhea in newborn pigs.

Immunoglobulin G (IgG) antibodies or immunoglobulin A (IgA) piglets were obtained from the colostrum of sows that developed E. coli in their intestines. However, this only applies to weaning (54,58). As previously explained, piglets with ETEC usually show watery diarrhea in the first week after infection with ETEC. Some piglets died suddenly without symptoms of diarrhea, when necropsy was performed, intestinal edema occurred. The diarrhea causes significant dehydration due to impaired intestinal absorption. The pathogenetic mechanism of LT and ST enterotoxins, namely damaging the function of small intestinal epithelial cells which results in increased secretion of water and electrolytes (Na + and Cl-), decreased fluid absorption and dehydration, and even acidosis.

The mechanism of action of LT is divided into 2 subunits, namely, the A subunit is enzymatically active and five B subunits which mediate the binding of glycolipids and glycoprotein receptors to host cells. Pentamer GM1-binding B subunit (LTB) interacts with the subunit via noncovalent binding of the A2 domain. The B subunit binds to the monocialotetrahexosylganglioside (GM1) receptor on the cell surface. Domain A1 is responsible for ADP-ribosylating stimulation of G protein. Then A1 will translocate to the endoplasmic reticulum and activate the cyclic adenylate system to increase cellular cyclic adenosine monophosphate then intracellular cAMP concentration is not controlled. Furthermore, protein kinase A is stimulated by cAMP which phosphorylates the cystic fibrosis transmembrane conductance regulator (CFTR). As a result, the ions open, then the chloride anion and then the water molecules are released. This is a characteristic feature of diarrhea caused by ETEC infection, namely increased secretion of fluids and electrolytes (Cl-) followed by decreased water absorption (44,45).

STa binds and activates guanylyl cyclase C (GC-C) in the brush border of the intestinal epithelium. It then induces intracellular cGMP accumulation and activates several interrelated signal transduction routes resulting in an uncontrolled osmotic flow of water to the intestinal lumen (Na + and Cl-) (4,59). The STb transduction pathway does not involve cyclic nucleotides as secondary messengers. Research shows that STb binds to the sulfatide receptor (3-O-sulfogalactosylceramide) and is internalized into small intestinal epithelial cells. Subsequently, the protein G cascade was activated, and the intracellular calcium concentration increased. This makes a number of enzymes active. The first phase is calmodulin dependent PKII, which opens a specific chloride pathway and activates PKC. The result is phosphorylation of CFTR and inhibits absorption of Na +. Next is the activation of phospholipase A2 and C enzymes.The function of this enzyme is to catalyze the release of arachidonic acid from the phospholipid membrane and the formation of prostaglandins E2 and serotonin, which are known as secretory agents from enterochromaffin cells (60).

Incidence of diarrhea caused by ETEC in piglets.

There were cases of antibiotic resistance to ETEC strains in Switzerland with certain characterizations (11). In Brazil, ETEC strains were also found with the types LT, STa and STB, with the most cases being the type STb (61). In addition, there were cases of diarrhea in neonatal pigs in Spain, one of which is due to ETEC infection. The ETEC patotypes that often occur are STa and STb, with fimbriae F4 (62). In Spain, out of 122 pigs to be studied for antibiotic resistance, 94 were stricken with ETEC diarrhea (63).

Furthermore, in South Africa, the dominant STb type ETEC case appeared with high frequency in newborn and post-weaning piglets. A total of 74.4% of ETEC cases presented with diarrhea symptoms and 69.2% without diarrhea (14). A recent study in Korea from 2008 to 2016, found cases of diarrhea in piglets with a percentage of 61.3% ETEC, with various types of enterotoxins (27). Until now, there are still many cases that occur in piglets caused by ETEC. In addition, ETEC has a specific coding gene with a specific target which can be used as a reference for research related to vaccination of this disease. This encourages research to conduct research on how to prevent and control diseases caused by ETEC infection (11).

Preventive measures against diarrhea caused by ETEC

The diagnosis of diarrhea in piglets must take into account clinical signs and lesions, as well as epidemiological patterns and detection of infectious agents (64). However, it would be difficult to diagnose diarrhea caused by ETEC on the basis of clinical signs and necropsy lesions alone, which are considered non-specific. Isolation and identification of E. coli still does not prove the relationship of disease, because E. coli is a normal bacterial flora in the intestines of animals. Thus, a phenotypic or genotypic description of the isolated strains is needed to recognize bacterial fimbriae. Furthermore, it can be carried out characterization of genes encoding fimbrial proteins and enterotoxins (65).

Virulence factors such as adhesins and enterotoxins can be detected by ELISA (Enzyme-linked Immunosorbent Assay) (66, 67). More recently, DNA-based molecular detection methods for determining serotypes, such as enterotoxins and fimbrial genes, can use PCR with samples from the faecal or internal organs of the intestine. Until recently detection using PCR was more effective and simplest for diagnosis in pigs (68, 69). Another study using PCR multiplex has proven useful for the rapid, sensitive and specific detection of enteric pig pathogens (70).

Neonatal pigs can form immune reactions such as tolerance or mucosal defense against certain antigens (71). Vaccination of weaned piglets can be used to control diarrhea due to ETEC infection. Continuous supply of SIgA to piglets via milk is required for passive immunity. Piglets must have active immunity to ETEC after weaning because passive immunity cannot continuously protect against ETEC infection (72). So that targeted vaccination is needed to protect the intestinal mucosa. The vaccine must activate the immune system in the intestinal mucosa as well as immunoglobulin specific to the antigen, namely IgA and IgM.

There are several methods of applying the ETEC vaccine to pigs. The first is via intramuscular injection. Economically, injection vaccines tend to be expensive. This vaccine stimulates a systemic immune response rather than the mucosal barrier needed to prevent ETEC infection in the small intestine (73). The injection site is usually done in

the neck. After the first vaccine injection, a booster is carried out 2 weeks later (54).

Next is the oral vaccine with live attenuated vaccines. It is also possible with the live wild strain E. coli vaccine, which is non-enterotoxigenic, which carries the fimbrial adhesin. Attenuated live vaccines can protect pigs from the ETEC K88 + LT + STb + strains (74). Oral vaccination of piglets with recombinantly produced FaeG can induce specific mucosal and systemic immune responses (73). Several studies have shown that oral vaccination with purified F4 fimbriae is an antigen that can induce mucosal immunity in pigs (72). Oral vaccination of fimbriae F18 encapsulated into weaned pigs may not produce a significant serum antibody response, or in other words no decrease coli colonization. However, in Canada, the live vaccine F4 + ETEC has been commercialized, and has been shown to protect pigs from ETEC infection (58,73).

Other vaccines include the inactivated, multi-adhesins, intact cell ETEC bacterin mixture, or the purified ETEC fimbrial subunit vaccine (54). This purification of fimbriae F4ac is a vaccine that has strong immunogenicity (58). This purification involves many processes, one of which is chemical conjugation which is long and complicated, especially when the toxoid STa or STa is to be purified from wild strains. Basically, the latest research has been able to clone wild-strain STa, making the purification process easier (74, 75). The orally purified fimbriae are introduced into the small intestine in a pellet form with an enteric coating. Although the interaction of the purified fimbriae with the coating polymer reduces the biological activity of the purified fimbriae, this is not a problem. However, this requires further research to find an effective way for purified fimbriae to target the small intestine without losing its biological activity (76).

Recent studies suggest that the addition of zinc oxide (ZnO) at a level of 3100 mg / kg in feed resulted in a decrease in the number of ETEC isolated from pig faeces. This addition was associated with small intestinal morphology, increased the number of goblet cell villi and resulted in favorable changes in the ratio of lactic acid bacteria to coliforms (77,78). Another study, namely the addition of zinc oxide (ZnO) to post-weaning pig feed as much as 2,000 to 3,000 ppm can reduce diarrhea and increase intestinal morphological growth and performance (79). So that the presence of free zinc ions continuously in the luminal compartment is very important for intestinal protection, decreasing the inflammatory response and reducing the small intestinal morphological damage to ETEC K88 infection (80,81). Zinc (Zn) is a mineral that is important as a micronutrient for pigs. Zinc deficiency can lead to growth retardation and reduced overall enzyme activity in tissues (82).

Another mineral that has a pharmaceutical effect to inhibit bacterial development is cupric sulfate (CuSO4). Cupric sulfate is actually a mineral to stimulate growth, but also has antimicrobial properties. A concentration of 175 mg / kg in pig feed is required. It is also based on the Cu inclusion rates currently allowed in EU countries. This dose can also affect the level of bacterial development in the digestive tract, in this case the fecal coliform bacteria and E. coli (83,84). E. coli is damaged and even killed due to oxidation of the membrane, which causes loss of membrane integrity. This bactericidal effect occurs due to the fusion of cupri alloy with cupric sulfate (CuSO4) (85).

Several feed additives that can function as antimicrobials, one of which is an acidifier. Acidifier-based feed when given to pigs with diarrhea, has a significant effect. In recent studies, the use of fatty acids (medium chain fatty acids) in pigs induced enterotoxigenic β-hemolytic E. coli (ETEC), serotype O149: K91: K88, has antibacterial effects (86). It has also been recognized in recent studies that volatile fatty acids (VFAs), such as acetic, propionic, butyric, valeric and lactic acids, have antibacterial activity. Propionic and butyric acid are very important metabolites because they have a specific inhibitory effect against enteric bacteria, such as E. coli in pigs (87). Hydrochloric acid secreted from the stomach can also act as an antibacterial. After weaning piglets, solid feed can increase the pH of the digestive tract. So that feeding with organic acid supplementation can be an effective way to control the pH of the gastrointestinal tract, in order to control the growth of bacteria in the stomach and intestines.

Diets containing blood plasma have been shown to inhibit the development of ETEC in pigs. Recent studies have shown that natural IgG purified directly from pig plasma and given in the form of a feed additive can be used to increase pig production. This is because IgG has a positive effect on reducing the colonization of ETEC bacteria and can be an alternative use of antibiotics (88). This product can be used given 5 days before weaning and 10 days after weaning.

Recently, chicken egg yolk antibody called immunoglobulin Y (IgY) from chickens that were immunized against a specific pathogen was effective in preventing and controlling disease. Specific IgY acts to effectively inhibit various intestinal pathogens including Salmonella, coronavirus, rotavirus, viral gastroenteritis and ETEC (89). Oral administration of specific IgY has great potential to control diarrheal diseases and increase pig growth. This can be an alternative to antibiotics (90,91). There is research that egg yolk antibodies may be ineffective in pigs 3 to 4 weeks of age. This is because gastric pH and digestive enzymes can break down the antibodies in GIT thereby reducing the amount of egg antibodies available to protect pigs from E. coli infection (92). Antibodies must also remain in the intestines continuously. A total of 1.5 g per day per piglet is sufficient to prevent diarrhea caused by infection with 1010 ETEC. The addition of 0.2% egg yolk antibody to feed can prevent diarrhea in commercial pig farms (91). However, research on how many doses are needed for the effectiveness of egg yolk antibodies is still being done to date.

Probiotics or Direct fed microbials (DFM) are live microorganisms which, when given in sufficient quantities, provide health benefits to the host. These microbes are categorized into 3 main groups, including lactic acid-producing bacteria, yeast, and Bacillus (93). Probiotics can work against various pathogenesis processes. The main thing is to inhibit the invasion of pathogens to target cells. Blockade of this process is important, because it is the first step in the pathogenesis process of ETEC. Furthermore, it prevents the colonization of bacteria in the intestinal mucosa. It has also been proven related to the inhibition of enterotoxin production from ETEC. Moreover, it reduces the inflammatory process in the intestine due to ETEC infection (94). However, these results can be effective at the end of the probiotic administration. This may be due to decreased appetite, dehydration and a lack of cytokine response in the pig's body (95).

A recent study, when pigs were induced by ETEC F18, then treated with DFM1, it seemed that no change had occurred. But with DFM2 therapy can provide effectiveness by decreasing colonization of ETEC in the intestine. In addition to colonization, ETEC induction also results in impaired intestinal barrier integrity, disruption of OCLN

and ZO-1 junction proteins, decreased intestinal mucosal sIgA, and activation of the immune response, namely increased local and systemic IL-8 production. DFM1 contains 3 strains of Bacillus amyloliquefaciens, while DFM2 contains 2 strains of Bacillus amyloliquefaciens (96).

Other probiotics such as Lactobacillus also provide antimicrobial benefits. One of them is L. plantarum ZLP001. This probiotic can prevent the growth of ETEC by producing certain antimicrobial substances which are then combined to produce a relatively acidic environment. L. plantarum ZLP001 adheres to IPEC-J2 cells and inhibits ETEC adhesion primarily through exclusion and induces HDP expression and secretion in intestinal epithelial cells (97,98). The Bacillus subtilis DSM25841 strain also has an antimicrobial effect against ETEC F4ac infection in weaned pigs. B. subtilis can improve intestinal health of pigs by reducing the abundance of Enterobacteriaceae. It also increases the regulation of genes related to immunity, increases metabolism and utilization of amino acids (99). Overall, given the good potential in the use of probiotics to treat diarrheal disease in pigs, it can be said that probiotics are an alternative as a substitute for antibiotics and can avoid bacterial resistance to antibiotics.

Plant extracts, also known as phytobiotics, have been widely researched and have many benefits as a nutritional enhancer for animal feed, as an antimicrobial, anti-inflammatory, antioxidant, and anti-parasitic. Some studies consider that plant extracts at minimum inhibitory concentrations (MICs) of 100 - 1000 µg / ml in in vitro bacterial susceptibility tests have antibacterial activity. Until now, there are several kinds of phytobiotics, such as phenolics / polyphenols, terpenoids / essential oils, alkaloids and lectins / polypeptides. There are many variations in the composition of phytobiotics based on several influencing factors, namely biological factors (type of plant, location of growth and harvest conditions), manufacturing (extraction, distillation and stabilization), and storage conditions (light, temperature, oxygen tension, and time) . These phytobiotics have different antimicrobial activities (100,101). Previous research, tannic acid can inhibit the growth of bacteria in the intestine, one of which is E. coli. Alkaloids can inhibit DNA synthesis by killing the topoisomerase enzyme. In addition, saponins can be antimicrobial by forming sterol complexes and damaging the bacterial membrane. Usually these phytobiotics become feed additives (102).

Phytobiotics have now become a trend as a substitute for the growth promoter antibiotic (AGP), whose use has been banned. Like the Labiatae family plants, namely thyme, oregano and sage can reduce the number of E. coli bacteria in the small intestine of animals (103). Macleaya cordata extract can increase pig palatability, body weight and amino acid concentration. In addition, it has the effect of increasing immunity by regulating phagocytes, haptoglobin and amyloid A. Another effect is that it is an important barrier to breast milk with the action of the ZO-1 and claudin-1 proteins (104). The effectiveness of phytobiotics and any plants that can be antimicrobial needs to be studied further, because until now there are so many plants that can be used as antimicrobials.

Nucleotide feed supplements have a role as bioactive molecules in the functions of metabolism and the immune system. Recent studies suggest that giving pig feed supplements containing nucleotides has shown positive effects. Namely the increase in growth, by increasing the effectiveness of intestinal absorption, controlling the concentration of bacteria in the intestine, reducing the severity of diarrhea, increasing the performance of enzymes in the ileum and immune stimulation (105,106). Feeding supplements to pigs containing 0.2 to 1 gram / kilogram of feed, can have a positive effect in pigs (107). There are still many studies related to how nucleotides can be used as feed additives for the prevention of diarrhea in piglets.

Bacteriophage is a kind of virus that has the effect of inhibiting growth and even killing bacteria. Virulent bacteriophages can be isolated from sources such as pig manure, wastewater and soil indicating that these phages are widely distributed in the environmental areas of pigs. Bacteriophage therapy has good potential for control of infections in pigs, such as diarrhea caused by ETEC (108). Pork feed containing bacteriophages can inhibit the development of ETEC F4 + infection. It is characterized by decreased bacterial adhesion in the ileum and cecum. It can also improve villi morphology (109). There is still much that needs to be deepened regarding how bacteriophages can become feed supplements and antimicrobial substitutes.

Preventive methods were used to avoid improper use of antibiotics. In the field of veterinary medicine, there have problems related with antimicrobial resistance such as in livestock (110-117), pets (118- 122), poultry (123-128) and in fisheries (129-132). The concept of antimicrobial replacement treatment is needed, not only to overcome the problem of diarrhea in piglets caused by ETEC, but also to prevent transmission to humans. So that public health is maintained properly.

CONCLUSION

The intestines are responsible for digestion, absorption of nutrients and protection of the body from toxins or pathogens. The incidence of diarrhea in piglets that has been rife recently has resulted in health problems and even death. The diarrhea is caused by E. coli enterotoxigenic infection (ETEC). An understanding of the disease features, virulence factors, ETEC characteristics and pathogenesis of the disease is required. Antibiotics are expected to be an effective way to prevent and manage infections. However, the more recent continuous use of antibiotics has resulted in E. coli resistance to some antibiotics. Preventive action and control strategies are needed against this disease, one of which is the use of alternative antibiotic substitutes. Alternative antibiotics that can be used to prevent infection with ETEC infection in piglets are immunoprophylaxis (vaccines), antimicrobial minerals (such as zinci oxide and cupri sulfate), acidifiers, blood plasma, egg yolk antibodies, probiotics, nucleotides, bacteriophages and so on. These kinds of alternatives and feed additives can improve intestinal health and prevent diarrhea in piglets. This review contains the latest research from various journals discussing the pathogenesis of ETEC in infecting piglets and the management of disease, which also benefits to public health.

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