

Virulence factors of *Escherichia coli* in relation to the importance of vaccination in pigs

Fatores de virulência da *Escherichia coli* associados à importância da vacinação nos suínos

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— REVIEW —

ABSTRACT

Enterotoxigenic Escherichia coli (ETEC) is the major cause of diarrhea in newborn and weaned pigs. Bacteria adhesion to the host cell is considered a specific phenomenon among fimbrial and non-fimbrial adhesins with their respective receptors on enterocytes. Enteric disorders are related with the fimbriae F4 (K88), F5 (K99), F6 (987P), F41, and F18. In addition to ETEC, another category of *E. coli*, porcine pathogenic *E. coli* (PEPEC), can cause diarrhea in pigs; it produces the porcine attaching and effacing-associated (Paa) adhesin in, which is capable to cause a typical lesion known as an attaching and effacing (A/E) lesion. Immunization of sows with adhesin is important to stimulate the production of antibodies and their subsequent transfer to piglets through colostrum. The aim of this paper is to illustrate the main impacts of enteric diseases caused by *E. coli* in swine production and to highlight the importance of continuing research on this bacterium to improve disease prevention through vaccination.

Key words: colibacillosis, diarrhea, vaccines, swine, colostrum.

RESUMO

Escherichia coli (*E. coli*) enterotoxigênica (ETEC) é considerada importante causa de diarreia em suínos neonatos e desmamados. A adesão da bactéria à célula do hospedeiro é considerada um fenômeno específico entre as adesinas fimbriais e não fimbriais com seus respectivos receptores nos enterócitos. Os distúrbios entéricos estão relacionados com as fímbrias F4 (K88), F5 (K99), F6 (987P), F41 e F18. Além da ETEC, outra categoria de *E. coli* pode causar diarreia nos suínos, denominada de PEPEC (porcine pathogenic *E. coli*), a qual produz a adesina Paa (Porcine attaching adherence), capaz de provocar uma lesão típica denominada A/E (attaching and effacing). A imunização das

matrizes com adesinas é importante para estimular a produção de anticorpos e a consequente transferência através do colostro aos leitões. O objetivo deste trabalho foi mostrar os principais impactos das doenças entéricas causadas por *Escherichia coli* na produção de suínos, e mostrar a importância de atualizar o estudo dessa bactéria para prevenir a doença através da vacinação.

Palavras-chave: colibacilose, diarreia, vacinas, suínos, colostro.

INTRODUCTION

Enteric diseases of bacterial etiology, particularly those caused by *Escherichia coli*, are the major concern in pig farming and have a heavy impact on pork industry (MENIN et al., 2008). Therefore, to avoid losses in pig farming, care during the initial disease phase is essential through a quick and accurate enteric diagnosis associated with a full history, clinical signs, and pathology. Recent advances in diagnostic methods have enabled a better understanding of diseases and have helped to reduce the effects in swine (WILLS, 2000).

The main cause of economic losses in young pigs is related to enteritis because it causes diarrhea, decreased weight loss in food conversion, culling, and sudden death. The diarrheal syndrome is responsible for 6% of piglets mortality on farms (CARAMORI JÚNIOR et al., 2010). Among the agents that cause

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diarrhea in suckling piglets are *Isospora suis*, coronavirus, rotavirus, and *Clostridium perfringens* type C, although enterotoxigenic *E. coli* (ETEC) can be considered the most important agent capable of causing illness and death in newborn and weaned piglets (ALMEIDA et al., 2007). Colibacillosis may have a mortality rate of approximately 25% if measures such as prevention and treatment are not adopted (MORÉS & MORENO, 2007).

Etiology

E. coli is a facultative anaerobic bacterium belonging to the family *Enterobacteriaceae*; it is a Gram-negative rod, non-sporulating, and either non-mobile or moved by peritrichous flagella (HIRSH et al., 2003). These bacteria have the ability to reduce nitrate to nitrite and to ferment glucose. They also are oxidase-negative and metabolize a wide variety of substances such as carbohydrates, proteins, amino acids, lipids, and organic acids. They produce catalase and glucose and use ammonia as the only source of carbon and nitrogen (TRABULSI & ALTERTHUM, 2005).

Although *E. coli* bacteria are part of the normal flora of human and other animal species intestinal tracts, a few strains are pathogenic due to specific features. These can be classified into six groups: enteropathogenic *E. coli* (EPEC), enterohemorrhagic *E. coli* (EHEC), enterotoxigenic *E. coli* (ETEC), entero aggregative *E. coli* (EAEC), entero invasive *E. coli* (EIEC), and diffusely adherent *E. coli* (DAEC) (TRABULSI et al., 2002). Among these, the bacteria belonging to the ETEC group are primarily responsible for colibacillosis in both newborn and weaned pigs (MELKEBEEK et al., 2013).

Serological classification of *E. coli* is based on the four surface antigens: O (somatic antigen), K (capsular antigen), H (flagellar antigen), and F (fimbrial antigen) (ORSKOV et al., 1977). ETEC strains that cause diarrhea in pigs have two fundamental factors of virulence, the adhesins and enterotoxins (MOON & BUNN, 1993). These may be expressed by several genes located on different segments of chromosomes and/or plasmids, and they are differentiated by their hemagglutinating, serological, and morphological properties. The majority of the protein subunits of the fimbriae are attached to the erythrocytes' integrated sugars, causing hemagglutination in the presence of D-mannose (BLANCO et al., 1993).

The ETEC found in pigs have shown several adhesin in types, defined as fimbriae F4, F5, F6, F18, and F41 (VIDOTTO et al., 2009; LIU et al., 2014). The expression of these fimbriae is stimulated

in vitro by growth conditions such as pH, rearing temperature, aeration, and osmolarity of the medium, with a specific medium for each fimbria (GAASTRA & GRAAF, 1982).

Pathogenesis associated with the bacterial virulence factors

Colibacillosis begins when there is binding of the bacteria to specific receptors of enterocytes through the fimbrial adhesins. Toxins are then released to activate the production of the enzymes adenylate cyclase and guanylyl cyclase, which increases the intracellular concentration of cyclic adenosine monophosphate (cAMP), generating a greater transfer of sodium bicarbonate and water from the gut lumen cells (MORÉS & MORENO, 2007). Colibacillosis, when caused by ETEC toxins, causes dehydration leading to animal death by secretory diarrhea, with fluid excretion higher than the absorption capacity generating a dysfunction of the physiological processes (ZLOTOWSKI et al., 2008).

The enterotoxins are classified into thermo stable (STa and STb) and thermo labile (LT-1 and LT-2); these are responsible for causing imbalances in the absorption and secretion of electrolytes and water by the enterocytes, leading to diarrhea, dehydration, and death of animals (MORÉS & MORENO, 2007). ETEC is able to produce several protein toxins that differ in weight, molecular structure, and biological and immunological activity (GYLES et al., 1992).

The K88 fimbria, also currently known as F4, was the first studied after it was discovered in 1961 by serological testing from pigs with edema disease and enteritis (ORSKOV et al., 1961); its antigenic variants were differentiated into K88ab, K88ac, and K88ad (FAIRBROTHER et al., 2005), with the K88ac variant being most commonly reported in piglets with diarrhea. This fimbria is characterized by a diameter of 2.1nm and molecular weight of 27.5 kilodalton (kDa). The specific culture medium for expression of F4 is agar glucose, and this fimbria enables *E. coli* to hemagglutinate guinea pig erythrocytes in the presence of mannose (MOON, 1983).

RUTTER & JONES (1973) tested the first F4 vaccine in sows to protect the newborn piglets with passive immunity via colostrum. An F4 vaccine was able to protect piglets that received antibodies through colostrum during weaning (NAGY & FEKETE, 1999). A study conducted with samples from a diarrhea outbreak in the Paraná state of Brazil found a 52% prevalence of F4 in the analyzed samples (SOUZA et al., 2007).

The K99 fimbria, currently known as F5, has a molecular weight of 18.5kDa and a diameter of 4.8nm. F5 can be found in ETEC samples in calves and swine (MOON et al., 1977). Selective medium Minca is used to induce expression of the fimbriae.

Purification and characterization of F41 was reported by De GRAAF & ROODA (1982); this fimbria has a molecular weight of 29.5kDa and a diameter of 3.2nm. Its production can be compared with the production of F5. Use of F41 as an antigen in vaccines is less well defined when compared with the fimbriae F4, F5, and F6 (MOON, 1990). This fimbria is usually reported in bovines and swine (GAASTRA & GRAAF, 1982). F5 and F41 can hemagglutinate human, guinea pig, and sheep erythrocytes in the presence of mannose (MOSELEY et al., 1986).

The strains most commonly found in newborn piglets have F5 (K99) and F6 (987P) fimbriae. During the first week of life, the receptors for F5 are found in enterocytes of piglets and then decrease significantly (ALFIERI et al., 2010). The 987P fimbria was analyzed by electron microscopy, showing a diameter of 7nm, molecular weight of 18.9kDa, and pH of 3.7 (MORGAN et al., 1978). It is usually found in newborn piglets (GAASTRA & GRAAF, 1982); however, the receptors in pigs for this fimbria are also present in young and adult animals (ALFIERI et al., 2010). Blood agar culture medium is more selective for inducing this fimbria (FRANCIS et al., 1982). Fimbria 987P does not have hemagglutinating properties (ISAACSON & RICHTER, 1981), but attaches to glycoprotein receptors on swine enterocytes.

The F18 fimbria was expressed only in special laboratory culture conditions (WITTING et al., 1994). This fimbria has two variants, F18ab and F18ac, and their identification was analyzed by serology and polymerase chain reaction (PCR) (IMBERECHTS et al., 1994). According to CODDENS et al. (2007), F18 has the ability to colonize the intestinal epithelium, adhere to specific receptors, and cause edema disease or diarrhea after weaning.

In pig farming, fimbriae F4, F5, F6, and F41 are linked to enteric disorders, and the F18 fimbria is linked with edema disease (FAIRBROTHER et al., 1986). Edema disease is an illness that affects weaned piglets, and it is related to *E. coli* strains linked to different virulence factors such as fimbriae and toxins (GYLES, 1992). MOON et al. (1980) reported that the impact of the fimbriae in the diagnosis and vaccination depends on the number and prevalence of antibody types, favoring intestinal colonization of occurring species.

In addition to ETEC, another class of *E. coli* that causes diarrhea in newborn pigs is porcine pathogenic *E. coli* (PEPEC), which is similar to the *E. coli* that causes enteric infections in humans and animals through the lesion type A/E (attaching and effacing) (AN et al., 1999). These lesions are characterized by close adherence to the membranes of the enterocytes and activation of a variety of chromosomal gene products that interact with components of the host cells, leading to the destruction of enterocytes and cytoskeletons edges (LEVINE, 1987).

Phenotypic and genotypic characterizations of PEPEC showed that they lack the virulence factors of ETEC but present porcine attaching and effacing-associated (*paa*) nucleotide sequences, associated with the ability to produce injury type A/E in piglets and pig ileum sections (LECLERC et al., 2006). The *E. coli* A/E lesion is caused by an outer membrane protein, known as intimin, which is encoded by the *eae* gene and facilitates bacterial adhesion (MALIK et al., 2006).

Histopathological changes caused by A/E injuries are indistinguishable from those produced in humans by EPEC. A/E injuries are also caused by other bacteria (including EHEC), by agents of hemorrhagic colitis, and by uremic syndrome in humans (KAPER & O'BRIEN, 1998); EPEC also causes diarrhea specifically in rabbits (MOON et al., 1983). All genes involved in A/E injuries are located on the locus of enterocyte effacement (LEE) chromosome.

The gene identified in *paa* PEPEC was cloned, and it showed strong correlation with the *eae* gene of EHEC O157:H7 and with isolates of dogs, rabbits, and pigs (AN et al., 2000). Characterization of the *paa* gene suggests its contribution to the early stages of PEPEC virulence (BATHISSON et al., 2003) and its rPaa protein weights (PEREIRA et al., 2015b). In Paraná, 300 stool samples of 100 pigs from different farms were analyzed using PCR for the *paa* gene and its link to the enterotoxins Sta, Stb, and LT. Results were 22% positive for the *paa* gene, which is associated with the presence of genes for other adhesins and toxins (F4, F18, F41, Paa, Sta, Stb, and LT). This demonstrates the importance of the *paa* gene as a virulence factor (VIDOTTO et al., 2013). The characteristics described above are listed in table 1.

Diagnosis

It is difficult to diagnose diarrhea caused by ETEC using clinical signs and necropsy lesions, which are considered non-specific. Because *E. coli* are commonly present in the intestinal flora of animals, their discovery is not a conclusive diagnosis.

Table 1 - Summary of *E. coli* groups and their relation to different species.

Group of <i>E. coli</i>	Adhesin	Molecular weight	Affected species	Disease
ETEC	F4 (K88)	27.5kDa	Newborn, post-weaning, and finishing pigs	Colibacillosis
ETEC	F5 (K99)	18.5kDa	Calves, lambs, and newborn pigs	Colibacillosis
ETEC	F41	29.5kDa	Cattle and piglets	Colibacillosis
ETEC	F6 (987)	18.9kDa	Young and adult pigs	Colibacillosis
ETEC	F18	36kDa	Post-weaning pigs	Edema disease
EPEC	Paa	28kDa	Pigs	Colibacillosis
EHEC	Intimin	94kDa	Humans	Hemorrhagic Colitis and Hemolytic and Uremic Syndrome

A phenotypic or genotypic description of the isolated strain is required to recognize bacterial fimbriae and characterize the genes encoding the fimbrial proteins and enterotoxins (ALFIERI et al., 2010).

Control measures

A number of factors influence the presence of diarrhea within pig production. One of the main problems is improper disinfection of the stall and poor sanitation among the sows. A lack of adequate temperature control for the piglets and sows (the ideal temperature is 33°C), and poor air circulation also play a role. Another factor is low attention by the handler to hygiene aspects, such as the use of dirty boots, which serve as a source of infection for the piglets. Additionally, diets low in digestibility can cause the substrate to remain longer in the small intestine of piglets, favoring the multiplication and prevalence of *E. coli*. Finally, environmental conditions and the immune state of sows also influence the appearance of diarrhea (MORÉS & MORENO, 2007).

There are several ways to minimize the damage caused by ETEC, some of which include sow vaccination before labor, autogenous vaccines in piglets, use of prophylactic antibiotics, and selection of animals lacking genes encoding receptors for fimbriae. Other methods to reduce losses due to ETEC

are active immunization (intramuscular immunization with fimbriae or oral immunization with attenuated or non-virulent strains which contain fimbriae), passive immunization (oral administration of yolk immunoglobulin from eggs (IgY) against fimbriae), use of powdered plasma proteins (FAIRBROTHER et al., 2005), and intranasal vaccines in piglets to prevent diarrhea (LIN et al., 2013). Measures of control and prevention are listed in table 2.

Vaccination schedules proposed by companies that produce vaccines against colibacillosis recommend vaccination of swine matrices during pregnancy in two ways: at four and two weeks before labor in gilts, and a revaccination two weeks before labor in sows. The first contact of the mother with the vaccine should be preconized in two doses to stimulate a higher immune response. As weaned piglets are often affected by ETEC, they must be able to produce immunity through antibodies received in colostrum (ANAMI, 2008). The colostrum can be absorbed by the enterocytes in piglets up to the first 24 hours after birth, as this passive immunization is sufficient for protection by the immunoglobulin (IgG) to prevent diarrhea (JENSEN et al., 2001). The vaccination programs are listed in table 3. PEREIRA et al. (2015a) evaluated the humoral immune response of immunized sows with recombinant proteins of ETEC (F4, F5, F6,

Table 2 - Measures of control and prevention in piglets, gilts, and sows.

Age of animals	Measures of control and prevention
Piglets	Autogenous vaccines, oral vaccine, oral immunization with immunoglobulin (IgY), plasma protein powder, prophylactic antibiotics, or selection of animals that lack genes encoding the fimbrial receptors
Gilts	Adequate temperature control, active and passive immunization, or vaccination with bacterins or recombinant protein (2 doses) four and two weeks before delivery
Sows	Adequate temperature control, active and passive immunization, vaccination with bacterins or recombinant protein (1 dose) two weeks before delivery

Table 3 - Vaccination programs according to each manufacturer.

Vaccines	Dosage	Age	Manufacturer
Porcilis® coli (LT toxoid, F4ab, F4ac, F5, F6)	2ml	Unvaccinated gilts and sows 6-8 weeks before delivery – 1 st dose 4 weeks after the first dose Revaccination: a single dose in the second half of pregnancy	MSD Animal Health
Porcilis® 2*4*3 (K88, K99, 987P, F41)	2ml	Unvaccinated gilts and sows 6-8 weeks before labor; 1 st dose 4 weeks after the first dose Revaccination: a single dose in the second half of pregnancy	MSD Animal Health
Suiseng (F4ab, F4ac, F5, F6, LT)	2ml	Gilts 70 days of gestation: 1 st dose 90 days of gestation: 2 nd dose Sow 90–100 days of gestation: one dose Gilts 5 weeks before delivery: 1 st dose 2 weeks before delivery: 2 nd dose	HIPRA
PILI SHIELD® PORCINO (K88, K99, 987P, F41)	2ml	Sows 2 weeks before delivery: 1 st dose Gilts 5 weeks before delivery: 1 st dose 2 weeks before delivery: 2 nd dose	Novartis
SERKEL GASTRO RV® (K88, 987P, K99, F41)	2ml	Sows 2 weeks before delivery: 1 st dose	Vencofarma

F18, and F41), and the response was compared with a group that received a commercial vaccine containing ETEC bacterins and a control group. There was a statistically significant increase in the levels of fimbriae F4, F5, F6, and F18 antibodies in sows vaccinated with recombinant proteins compared to the control group. Furthermore, all piglets showed significantly elevated antibody levels to all fimbriae, demonstrating the transfer of antibodies via colostrum.

Studies show that the swine matrices immunized with colibacillosis vaccines are able to better protect their piglets as compared to unvaccinated ones (HUR & LEE, 2012). The vaccine is capable of inducing the production of specific antibodies, which decrease bacterial adhesion receptors on the intestinal cells and neutralize enterotoxins. The commercial vaccines contain inactivated bacterins or purified vaccine fimbrial subunits, and some already contain enterotoxin LT; they are given via a parenteral route (ALFIERI et al., 2010). The rPaa protein could be

included in the development of a vaccine against colibacillosis (PEREIRA et al., 2015b).

Commercial vaccines are not completely efficient, owing the dependence on maternal antibodies, however, the commercial vaccines administered parenterally to pregnant sows are effective against newborn diarrhea if the vaccine has all fimbrial antigens that occur on the farm, if there is sufficient intake of colostrum per animal, and if there is a low pressure of infection (HAESEBROUCK et al., 2004).

CONCLUSION

Immunization of sows with adhesins is essential for stimulating the production of antibodies and conferring these to piglets through colostrum. Vaccination may be considered an excellent strategy and quite effective when used properly, and it is considered one of the best ways to prevent diseases

within a production system where the pressure of infection is high. At the same time, risk factors also need to be avoided through control measures; this is fundamental to ensure a healthy environment free of disease. Although, the risk of death in swine after a single infection with ETEC is not high, complex infections with ETEC, rotavirus, and coccidia are often found in piglets at both newborn and post-weaning stages, leading to high economic losses for pig producers.

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