Archives of Environmental Sciences and Environmental Toxicology

Augustino SMA et al. Arch Environ Sci Environ Toxicol 5: 136.

www.doi.org/10.29011/2688-948X.100136

www.gavinpublishers.com

Review Article

Enterotoxigenic *E. coli* (ETEC) and Porcine Epidemic Diarrhea Virus (PEDV) Diarrheal Infections and Resistance in Piglets

Serafino MA Augustino[1,2*], Erneo B Ochi[3], Qinglei Xu[5], VML Jackson[4], Ying Yu[2]

[1]School of Natural Resources and Environmental Studies, University of Juba, Juba, South Sudan

[2]College of Animal Science and Technology, China Agricultural University, Beijing, China

[3]School of Veterinary Sciences, University of Juba, Juba, South Sudan

[4]School of Applied and Industrial Sciences, University of Juba, Juba, South Sudan

[5]College of Animal Science and Technology, Nanjing Agricultural University, Nanjing, China

[*]**Corresponding author:** Serafino MA Augustino, College of Natural Resources and Environmental Studies, University of Juba, Juba, South Sudan

Citation: Augustino SMA, Ochi EB, Xu Q, Jackson VML, Yu Y. (2022) Enterotoxigenic E. coli (ETEC) and Porcine Epidemic Diarrhea Virus (PEDV) Diarrheal Infections and Resistance in Piglets. Arch Environ Sci Environ Toxicol 5: 136. DOI: 10.29011/2688-948X.100136

Received Date: 05 April, 2022; **Accepted Date**: 18 April, 2022; **Published Date**: 22 April, 2022

Abstract

Diarrhea is considered one of the most frequent major problems in piggery industries, particularly during newborn, weaned and post weaned periods, accompanied by tremendous economic losses owing to higher morbidity and mortality rate. Enterotoxigenic *Escherichia coli* (ETEC) is considered a major cause of bacterial diarrhea. It accounts for 56.2% of the cases of piglet diarrhea and 24.7% of the death. Virulence factors in ETEC diarrhea are enterotoxins and fimbrial adhesins. Fimbrial adhesins mediate bacterial attachment and colonization on the surface of host epithelium cells whereas enterotoxins, including plasmid-encoded heat-stable (STa and/or STb) and heat-labile enterotoxins (LT), stop intestinal fluid homeostasis causing fluid hyper-secretion that leads to diarrhea. Porcine Epidemic Diarrhea Virus (PEDV) on the other hand, causes Porcine Epidemic Diarrhea (PED), which is characterized by massive dehydration with a high death toll in baby pigs due to severe vomiting and diarrhea. When improving the environment and the use of vaccines and other preventive measures can only reduce the incidence rate of diarrhea. However, if measures that improve the resistance level of piglets to diarrhea from the genetic side are taken and cultivate excellent strains with resistance ability, can radically reduce the incidence of diarrhea in piglets and improve the safety of pork products. The implication of a single genetic locus responsible for disease resistance or pathogenesis has been demonstrated for a few infectious diseases. Well-known examples in livestock are the dominant alleles responsible for receptors providing access to host cells for certain retroviruses in chicken, and enter-toxigenic E. coli in swine. Therefore, equipped with molecular genetic tools, we can, locate the major genes that affect the immune ability of pigs or associate with the disease, find the biological mechanism and functional mutations, and search for effective genetic markers for disease resistance in pigs. This review focused on the diarrheal infections caused by ETEC and PEDV and underlying virulence factors and resistance candidate genes in piglets

Keywords: Piglet's immunity; Diarrhea; ETEC; PEDV; Resistance; Virulent factors

Abbreviations: ETEC: Enterotoxigenic *Escherichia coli*; STa: heat-stable a; STb: heat-stable b; LT: heat-labile; PEDV: Porcine Epidemic Diarrhea Virus; PED: Porcine epidemic diarrhea; MAFF: Ministry of Agriculture, Fisheries and Food; FAWC: Farm Animal Welfare Council; EPEC: Enteropathogenic *Escherichia coli*; EIEC: Enteroinvasive *Escherichia coli*; VTEC: Verotoxigenic *Escherichia coli*; EHEC: Enterohaemorrhagic *Escherichia coli*; EaggEC: Enteroaggregative *Escherichia coli*; PWD: Post-weaning diarrhea; EAST1: Enteroaggregative *Escherichia coli* heat-stable enterotoxin 1; Stx2e: Shiga toxin 2e; ssRNA: Single-strand RNA; pAPN: Porcine aminopeptidase N

Introduction

Under natural conditions, the piglet's weaning process is carried out gradually with piglets getting weaned at ages ranging from 14-17 weeks [1], whereas commercially, according to pig husbandry practised in the UK, piglets are traditionally weaned at ages ranging between 3-4 weeks [2,3]. However, the early weaning of piglets may cause a series of adverse effects on growth and development, resulting in physiological disorders in weaned piglets and the occurrence of diarrhea and edema.

It is well documented in many studies that, diarrhea is believed to be one of the most frequent major leading problems in piggery industries, particularly during both pre-and post-weaning periods [4-7]. This is not unusual due to weak/poor disease resistance, gastrointestinal anomaly and environmental stress thus resulting in huge economic losses. In some situations, it causes severe morbidity as well as mortality [8-11]. This disease alone is responsible for 11.5%-29.5% of deaths in piglets. *Escherichia coli,* the major infectious agent causing diarrhea in swine accounts for 56.2% of the cases of piglet diarrhea and 24.7% of the deaths as a result of diarrhea. On the other hand, the Porcine Epidemic Diarrhea Virus (PEDV), a causative agent of Porcine Epidemic Diarrhea (PED), an acute and highly contagious disease of swine leads to massive dehydration with a high mortality rate in new-born piglets due to severe vomiting and diarrhea. In 2013-2014, swine producers in the US lost $900 million to $1.8 billion as a result of the PED outbreak [12].

In actual production, some measures have been taken to prevent and treat piglet diarrhea, such as feeding antibiotics, probiotics, enzyme preparations, antibacterial peptides, specific yolk antibodies and pharmacological doses of Zinc Oxide [13-15]. These drugs and feed have achieved some effective additives in the prevention and treatment of piglet diarrhea, but some also have brought disadvantages to the pig industry [16]. This has made several countries reduce and/or totally ban the use of antibiotics as growth promoters [17]. In 2003, Korea announced phasing out plans for the use of antibiotics supplements in compound feed, which came into effect in May 2005 and the use of antibiotics was totally discontinued on July 1, 2011 [18]. Similarly, in January 2006, the European Union has joined the move and banned the use of antibiotics as feed additives in food animals [19]. Subsequently, the Chinese Ministry of Agriculture has also issued a series of new regulations prohibiting the use of some antibiotics in edible animals [20]. More recently, in December 2013, the United States phased out the use of non - Medical antibiotics [21]. All these cautionary measures are initiatives to mitigate the disadvantages associated with the use of antibiotics as feed additive in food animals and to ensure safeguard and wholesome livestock products are produced.

In addition, for acute diarrhea, such as diarrhea caused by the porcine epidemic diarrhea virus, there is no commercial vaccine that has been proven effective in pig farms in the United States [22,23]. In the long run, we should carry out disease resistance breeding programs, improve resistance to pathogens by genetic means, and combine immunization to control the disease of livestock, which is essentially a way to solve the root of the problem or mitigate the diseases of livestock and poultry. This review aimed at focusing on piglets' diarrheal infections caused by enterotoxigenic *E. coli* (ETEC) and porcine epidemic diarrhea virus (PEDV) as well as underlying virulence factors and resistance candidate genes.

Types of Piglet diarrhea

Piglet diarrhea is a typical multifactorial disease. There are a wide variety of factors that cause diarrhea to piglets, which can be divided into two categories, non-infectious and infectious factors: **Non-infectious Factors** such as inadequate piglet immune system, dyspepsia, malnutrition, weaning stress, etc. can lead to piglet diarrhea. **Infectious Factors** include viral diarrhea, bacterial diarrhea and parasitic diarrhea [24]. The viral diarrhea is mainly transmissible gastroenteritis in piglets, epidemic diarrhea and rotavirus infection. Bacterial diarrhea is commonly induced yellow scour of piglets, white scour of piglets [25-27], post-weaning diarrhea of piglets [28] and porcine edema disease [29].

In production practice, bacteria and viruses are the main causes of diarrhea in weaning piglets, including pathogenic *Escherichia coli* and porcine epidemic diarrhea virus [30]. They have a high fatality rate and cause a great loss to the pig industry. Enterotoxigenic *Escherichia coli* (ETEC) is the main pathogen causing diarrhea in newborn piglets and weaned piglets [31,32]. *E. coli* strains that caused severe diarrhea are usually F4 (K88), F5 (K99), F6 (987P), F41, and F18. Among these, the pathogenic *Escherichia coli* expressing F4 pilus antigen is the most common, and it can cause diarrhea and death in newborn piglets and early-weaned piglets [33], while ETEC F18 mainly cause diarrhea and edema in weaned piglets [34]. Porcine epidemic diarrhea virus (PEDV), a member of the Alphacoronavirus, causes acute diarrhea, vomiting, dehydration, and high mortality in newborn piglets, resulting in a significant economic loss [35]. In the United States, PEDV has caused a sharp rise in the mortality of piglets and continued to spread throughout the United States. The first case of the disease occurred in April 2013. Only a year later (March 2014), about 50% of the 27 states in the US were found infected with PEDV. This extremely serious negative impact has forced international cooperation among countries to work together combinatorially to mitigate its impact and stop the virus from further spreading [36].

Bacterial diarrhea caused by Enterotoxigenic *Escherichia coli*

Theodore Escherich (1857-1911) a German pediatrician and a bacteriologist, in 1885 discovered a bacterium that was found in the faeces of healthy individuals which he named Bacterium coli commune (Escherich, 1885, cited in [37]. Currently, this microorganism is known as *Escherichia coli* (renamed after him, in 1919). However, some *E. coli* strains have developed specific abilities which turn them into primary pathogens, responsible for

a range of different diseases in both man and animals [38]. The enteric *E. coli* pathogens are further divided into six serotypes on the basis of virulence properties, such as Enterotoxigenic *Escherichia coli* (ETEC), Enteropathogenic *Escherichia coli* (EPEC), Enteroinvasive *Escherichia coli* (EIEC), Verotoxigenic *Escherichia coli* (VTEC), Enterohaemorrhagic *Escherichia coli* (EHEC), Enteroaggregative *Escherichia coli* (EaggEC) [39,40]. Enterotoxigenic *E. coli* (ETEC) is the major infectious agent causing diarrhea in swine, which accounts for most cases of deaths in porcine herds [12].

ETEC Virulence Factors

The most important ETEC virulence factors in diarrhea are fimbrial adhesins and enterotoxins [41]. **Fimbrial Adhesins:** mediate bacterial attachment and initiation of bacterial colonization on the surface of host epithelium cells. The adhesive fimbriae most frequently found in ETEC from pigs with post-weaning diarrhea (PWD) are those belonging to types F4 (heretofore known as K88) and F18 (F107, 2134P, 8813) [42,44]. **Enterotoxins:** play a pivotal role in the inhibition of intestinal fluid homeostasis causing fluid hypersecretion into the gut lumen thus, leading to diarrhea. The K88 (F4) fimbrial ETEC strains often produce both heat-labile (LT) and heat-stable (STb) enterotoxins with or without STa. The F18 fimbrial ETEC strains produce STa and STb heat-stable toxins, occasionally with Stx2e toxin [45]. The most frequently observed enterotoxin combinations are LT and STb, or LT, STa, and STb [46].

The main enterotoxins detected in porcine ETEC are heat-labile toxin (LT), heat-stable toxin a (STa) and heat-stable toxin b (STb). Some strains can produce both enterotoxins and a Shiga toxin, which is usually Stx2e subtype. Some authors classify these strains as ETEC rather than Shiga toxin-producing *E. coli* (STEC) [47]. Despite its relevance, only a few recent studies investigating the occurrence and the distribution of fimbriae and virulence factors among *E. coli* isolates from cases of PWD in Europe are available and in many cases, they do not differentiate between *E. coli* isolates recovered from PWD cases, edema disease and pre-weaning diarrhea [8]. For ED and/or PWD-causing *E. coli*, the most common adhesins are associated with fimbriae F18, F4, F5, F6 and F41, while the predominant toxins are heat-labile enterotoxin (LT), heat-sensitive enterotoxin (ST) and Shiga toxin 2e (Stx2e) [48].

F4 fimbria can exist as antigenic variants, F4ab, F4ac and F4ad, all of which allow adhesion to villous enterocytes throughout the small intestine. There are also other fimbrial structures, such as F5 (K99), F6 (987P), F41, etc., which are associated with porcine ETEC strains but not frequently associated with PWD strains [42]. In addition, F18 (F107) fimbria has been identified on porcine *E. coli* strains connected with edema disease [48] and post-weaning diarrhea [4,34]. The proper characterization of *E. coli* strains isolated either from diarrheal or edema disease cases requires the determination of both fimbrial and toxin properties. Fimbrial antigens of F4, F5, F6, F18 and F41 in the 215 isolates of *E. coli* were detected by Xiang et al. from pigs with post-weaning diarrhea in Eastern China [49]. Luppi et al. identified two different fimbrial subtypes of ETEC in one farm, F4-ETEC (F4, STa, STb, LT) and F18-ETEC (F18, STa, STb) [50]. Reports from Poland, Slovakia and Denmark suggest differences between countries in the prevalence of the main fimbrial types in *E. coli* isolates recovered from pigs with PWD [51].

Infections with ETEC that, express the F4ab or F4ac fimbriae, cause diarrhea and mortality in neonatal and weaned piglets, these pathogens bind with their F4ab/ac fimbriae to F4 specific receptors (F4R) on the brush border of small intestinal enterocytes, resulting in colonization of bacteria to porcine intestinal epithelial cells (Figure 1) [52].

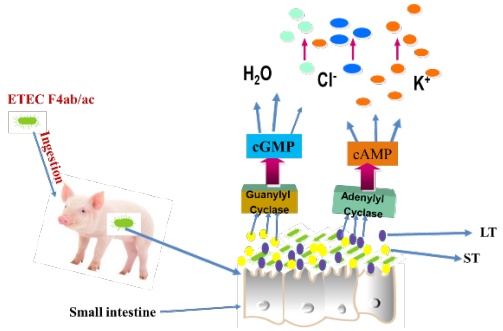

Figure 1: The mechanism of pathogenicity by which ETEC F4 cause diarrhea in piglets (A. Mirhoseini et al., 2018): Briefly, first after ingestion, the ETEC bacteria bind to a specific receptor on the epithelial cells of the small intestine then establish colonization. Subsequently, the colonizing bacteria secret toxins ST & LT, these toxins activate Guanylyl cyclase (GC) and Adenylyl cyclase (AC) respectively. Guanylyl cyclase catalases the cyclization of guanine monophosphate to cyclic guanine monophosphate (cGMP) whereas Adenylyl cyclase enzyme catalases the cyclization of adenine monophosphate to generate cyclic adenine monophosphate (cAMP). Accumulation of cGMP and cAMP causes hypersecretion of fluids into the gut lumen resulting in diarrhea.

Colonizing bacteria then release harmful enterotoxins that lead to more secretion of electrolytes into the gut lumen and stop the process of fluid homeostasis, consequently permitting water to flow into the gut lumen causing diarrhea [34]. However, some pigs are naturally resistant to ETEC, because they don't have receptors on their epithelial cell brush borders to which the fimbriae bind [54,55], the absence of receptors causes resistance to ETEC induced diarrhea [56] and their expression is genetically determined and inherited in a dominant way following Mendelian inheritance [53-58].

Viral diarrhea caused by the epidemic diarrhea virus

Porcine epidemic diarrhea virus is an enveloped single-strand RNA (ssRNA) virus [59] that belongs to the family Coronaviridae,

genus Alpha coronavirus [60-62] causes porcine epidemic diarrhea (PED), an acute and highly contagious disease of pigs, which leads to massive dehydration and high mortality rate in baby pigs due to severe vomiting and diarrhea [63,64]. Thus resulting in huge and significant economic setbacks in pig farms [65].

PEDV was first reported in 1971 in England [60] and now worldwide including the USA, Mexico, Italy, Japan, France, Belgium, Korea, Thailand and Canada [66] poses huge economic losses. Serious PED was reported in 2010 in China [59].

Swine, the only known natural host of porcine epidemic diarrhea virus (PEDV) [67], the infection in swine is facilitated by porcine aminopeptidase N (pAPN), a cellular functional receptor that binds to spike proteins of PEDV coronaviruses [68,69]. The virus then replicates in the differentiated enterocytes of the porcine small intestine thus, causing severe diarrhea [70,71]. Viral pathogens are not the same as bacterial pathogens as far as their structures are concerned but some of their properties are similar in terms of their virulence and pathogenicity. It has a 28 kb genome consisting of 7 ORFs, one 5' UTR and one 3' UTR with a polyadenylated tail. The seven ORFs encode four different structural proteins and three different non-structural proteins. The four structural proteins are spike (S), envelope (E), membrane (M) and nucleocapsid (N) and the three non-structural proteins are replicases 1a, 1b and ORF3 [72,73] (Figure 2).

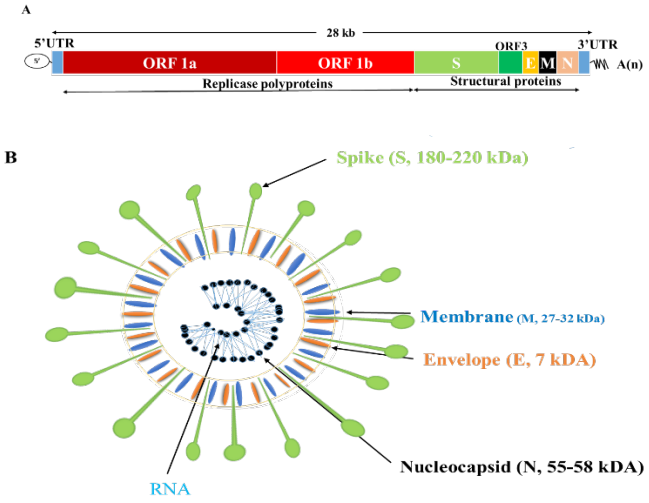

Figure 2: PEDV genome organization and virion structure; Modified from (Lee et al., 2015). A. Showing PEDV genomic region, different color indicates the different region in the genome. B. Showing virion structures with their size in (kDa), different color indicates a different structure.

The S protein holds a specific receptor binding site which is necessary for fusion of cell membrane and entry of virus and is an antigenic target that neutralizes antibodies [74] whereas the M protein, the most abundant protein on the surface, is co-expressed with E protein forming pseudo-particles that results in interfering genic activity [75]. The N protein is highly conserved, binds to virion RNA for providing a structural basis to the helical nucleocapsid, and is being used for early diagnosis [76]. On the other hand, the non-structural proteins, namely; replicases 1a and 1b are both multi-functional and are associated with the replication of the viral genome [77], and the ORF3 protein is thought to have an effect on virulence [78].

PEDV Virulence Factors

Adhesion of viruses to the host cells is mediated by adhesins, however, certain viruses which are enveloped depend on antigenic variation which is not recognizable by the immune system of the host thus avoiding immune defences. In any viral infection, the first step is the viral adhesion to specific receptors on the cells' surface. This process is facilitated by adhesins that are part of the viral capsid or membrane envelope. The viral adhesins and viral-specific cell receptors interaction define the tropism (preferential targeting) of viruses for specific cells or tissues in the body [79]. Porcine epidemic diarrhea virus has a restricted tissue tropism, replicates well efficient in the epithelial cells of the porcine small intestinal villous. Porcine amino-peptidase N (pAPN) is largely expressed on the epithelial cells surface of the small intestine and has been recognized as the cellular receptor for this virus [71,80-82].

Lee et al. in their study on the N-terminal region of the porcine epidemic diarrhea virus's spike protein showed that the N-terminal region of the PEDV spike protein S1 domain is necessary for the recognition of the pAPN receptor. Thus, the entry of PEDV into the host cells commences with the binding to pAPN then followed by the invasion of the virus into target cells through direct membrane fusion, subsequently, the viral genome is released into the cytosol after un-coating thus marking the process of genome replication [83]. However, contrary to the aforementioned evidence for pAPN acting as a receptor for PEDV infection, Li et al. [84] and Shirato et al. [85] suggested that pAPN is not required for PEDV infection and therefore, is not the cellular receptor for PEDV. Interestingly, Shirato et al. [85] further suggested that, albeit pAPN is not the cellular receptor for PEDV, its activity promotes PEDV infection.

Piglet's immunity

Having described the two pathogens (ETEC and PEDV), their virulent factors and pathogenicity, we need to throw some light on piglets' immunity because the disease can never be discussed in isolation from immunity. The newborn piglet lacks protective immunity against diseases, thus, it largely depends on the colostrum intake from sow's milk to obtain passive immunity from its mother, which in turn lasts for 10-14 days after birth [86,87].

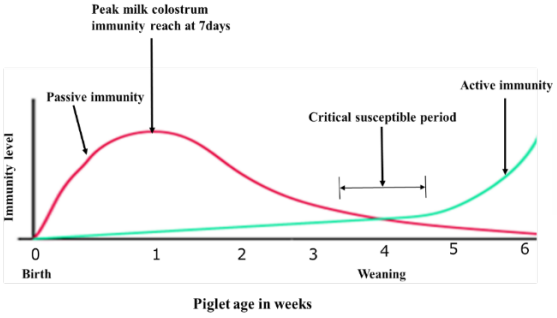

Figure 3: Piglets' immunity curve: passive immunity in piglets rises after birth upon taking of sow's milk colostrum and reaches its maximum on day 7 then declines afterwards and reaches its lowest level in days 21 up to 28, simultaneously active immunity will start to develop. The critical susceptible period range is one week before and after weaning. Figure Modified from two Sources: http://www.circumvent-g2.com/immunology.aspx and https://www.purinamills.com/swine-feed/education/detail/why-do-weaned-pigs-get-sick-easily

Then, once the age of the piglet reaches 21-28 days, its own immune system will start to develop, therefore piglets weaned at the ages of 14-28 days are vulnerable to getting infected because their immunity to fight disease infections is at its lowest level [88]. At this juncture, any contact with causative agents can have very serious consequences [88-90] (Figure 3).

The immune system in the pig is a complex network and is responsible for recognizing as well as repelling pathogens, thereby helping in providing protection against disease infection. The immune system fights against infections in three different ways [91] as follows:

Innate Immunity: The genetic actions of innate immunity stimulate adaptive immunity, once the innate immunity identifies a foreign organism (body), the circulating T-cells will interact with a foreign antigen, they can either kill infected cells directly or they can return to lymph tissue to incite (stimulate) a B cell response which in turn will produce the antibody that can fight the infection. The innate immune system is the first defence barrier to infection in pigs. **Passive Immunity**: Due to the nature of epitheliochorial of the placenta in the porcine, it is necessary for the newborn baby pig to acquire maternal immune-globulins from sow's milk colostrum to obtain passive immune to provide protection against infections until its immune system is fully developed [91,92]. **Adaptive Immunity**: Adaptive immunity is a defence system of pigs, which is built on specific cellular targeting, it takes up to 96 hours after infection to develop its weaponry (antibodies) and is far more effective due to its accuracy (precision). The adaptive immunity system reacts when exposed to pathogenic organisms and vaccine antigens, thereby providing long-term immunity against future infection by similar invading pathogens and it functions throughout life [91].

An overview of the porcine genome and candidate genes associated with diarrhea resistance in piglets

Genome assembly of the pig (Sscrofa10.2) comprises 2.60 Gigabases (Gb) assigned to 18 autosomes and X and Y chromosomes with a further 212 megabases (Mb) in unplaced scaffolds [99] (Table 1).

The use of genomics tools, such as genetic and physical maps, sequencing and gene expression profiles studies provided powerful tools to uncover genetic markers and genes, which can be used to improve disease resistance. Equipped with the knowledge of the genetic basis of resistance to diseases is very important in improving animal well-being by the use of conventional molecular breeding methods [100]. Previous studies revealed that resistance to infection of certain pathogens is heritable and the identification of indicator traits that can be used in breeding programs is of paramount importance in time to come in the pig industry. *Edfors-Lilja et al*. showed that heritability estimates of responses to *E. coli* antigens range from 0.29 to 0.45 [100].

Estimation and application of genetic parameters on the basis of phenotypic records are one of the big challenges in the selection process for resistance to disease in the pig. Fewer genes have been identified to control disease in the pig. A typical example is the presence or absence of the receptor of F4, a cell surface antigen on some *Escherichia coli*, which can cause diarrhea in the pig [34,101].

Assembly	Place	Unplaced	Annotation*
Total length	2,59,66,39,456	21,18,69,922	21,640 Protein-coding genes
Ungapped length	2,32,36,71,356	19,54,90,322	380 Pseudogenes
Scaffolds	5,343	4,562	2,965 ncRNAs†
Contigs	73,524	1,68,358	197,675 gene exons
Scaffold N50	6,37,332	98,022	26,487 gene transcripts
Contig N50	80,720	2,423	

*Numbers refer to the annotation performed by Ensembl (release 67). Results of an independent annotation by the NCBI can be obtained from http://www.ncbi.nlm.nih.gov/mapview/stats/BuildStats.cgi?taxid=9823&build=4&ver=1. †An improved ncRNA annotation with 3,601 ncRNAs and structured elements is available as a separate track in Ensembl version 70 and for download from http://rth,dk/resources/rnannotator/susscr102. N50, 50% of the genome is in fragments of this length or longer.

Table 1: Assembly and annotation statistics.

A further example is piglets' genetic resistance to the causative agent of neonatal diarrhea. A receptor found in the intestinal tract to which a certain strain of *E. coli* adheres is too genetically determined, whereas, a commercially available DR2 gene marker test makes possible routinely identification of genetically resistant individuals and a DR1 gene marker test further identifies those individuals which are genetically susceptible to F18 *E. coli*. Usage of these tests in breeding practices has significantly improved genetic resistance in those populations where it has been applied [100]. Additional examples explaining how genetically resistant individuals are being favored during selection include the usage of a commercial test for the porcine syndrome (PSS) [102].

The involvement of a single genetic locus responsible for disease resistance or pathogenesis has been demonstrated for a few infectious diseases. Some good known examples in livestock are the dominant alleles responsible for receptors providing access to host cells for certain retroviruses in chicken [103], and enter-toxigenic *E. coli* in swine [58].

Rasschaert, et al. found that the probability of an SR/SS genotype for MUCIN 4 increases significantly with increasing F4ab ETEC per 250 mm villi ($P = 0.029$), with the odds ratio for each unit increase of F4ab equal to 1.036 (95% CI [1.004-1.069]) [104]. For F4ac, the probability of an SR/SS genotype for MUC 4 increases significantly with increasing F4ac ETEC per 250 mm villi ($P = 0.030$), with the odds ratio for each unit increase of F4ac equal to 1.018 (95% CI [1.002–1.034]). This variance is due to the fact that some pigs do not have any expression (due to the lack of the gene) and that the expression of the F4 receptor genes varies when the gene is present [57]. Another factor, which could explain the individual variation is the age of the pigs. The villus length decreases with aging [104]. Various potential host receptors for F4 fimbriae have been described, including MUC4, MUC13, MUC20, ITGB5, and TFRC [4,10,109]. Grange and Mouricourt (1996) already demonstrated that F4ab can bind to a receptor of the transferrin family but there should also exist an additional receptor(s) for F4ac. As a consequence, not only MUC4 gene polymorphism but also the expression of these other receptor/s has or have to be included in the screening assay for F4ac/ab receptor-negative pigs [105]. This has led to MUC4 gene polymorphisms in the intron 7 region being used as an important biomarker in the classification of the majority of piglets as resistant or susceptible to F4+ ETEC infections [110,111]. Consistently, Nguyen et al. confirmed that MUC4 polymorphisms and their candidate glycoprotein receptors are highly associated with the MUC4-susceptible genotype [112]. In addition, Peng et al. and Zhang et al. revealed that alleles in MUCIN genes have a strong association with susceptibility to enterotoxigenic *Escherichia coli* F4ab/ac in the pig [106,107]. Similarly, Ren et al. and Zhou et al. in our laboratory, both found that susceptibility/resistance toward ETEC F4ac is conferred by the MUC13 gene in pigs [4,10]. However, Schroyen et al. in their study on MUC13 and MUC20 reported that neither of these genes is related to ETEC F4ac susceptibility in piglets [113]. Goetstouwer et al. however, recently confirmed that MUC4 and MUC13 genotypes are not completely associated with F4ab/ac ETEC susceptibility and there are likely to be other receptors [114]. Further development concerning piglets' susceptibility to ETEC F4 diarrhea, Fu et al. also in our laboratory, identified HEG1 and ITGB5 as two novel candidates and most promising genes that underlie F4ab/F4ac susceptibility to ETEC diarrhea considering their functions and positions in chromosome 13. This finding has provided novel evidence for understanding the genetic mechanism behind the risk of diarrhea in piglets.

Yan et al. demonstrated that susceptibility or resistance to ETEC F4 is correlated with whether or not brush borders of the pig intestinal epithelium bind to the bacteria as can be visualized by phase-contrast microscopy [108]. The SNPs and the candidate genes, which are confirmed to be associated with susceptibility or resistance are summarized in Table 2. Melkebeek et al. reported that porcine aminopeptidase N (APN) serves as a receptor protein for F4ac+ ETEC. APN, can promote intestinal epithelial cell endocytosis in F4Rs piglets and is involved in the induction of mucosal immunity [115].

Xia et al. recently, found that IPEC-J2 cells express APN and that F4 E. coli was able to adhere/ bind to IPEC-J2 cells in an APN dependent manner. Pre-incubation with APN polyclonal antiserum and anti-F4 fimbriae monoclonal antibody both reduced ETEC adherence to IPEC-J2 cells. Results from Y2H and pulldown assays also showed that FaeG binds directly to APN.

Xia et al. did not find an impact on APN-FaeG binding after treating samples with metaperiodate (NaIO4), according to them, this suggests that at least under their in vitro conditions, APN glycosylation does not play a significant role in FaeG binding [6].

However, Xia et al. concluded that the molecular details regarding APN-FaeG interactions and their roles in ETEC adherence await further experimentation [6].

Candidate	**Chr.**	**SNP**	**Study**	**Pig**	**ETEC**	**Author/**
Gene	**No.**		**Method**	**breed**	**strain**	**source**
ZFAT	4	ALGA00 22658	GWAS	White Duroc × Erhualian	ETEC F41 strain	Ji et al. 2016
MUC13	13	ASGA0089965,	GWAS	Large White, Belgian Landrace, Large White x	(0101:K30:F41 ETEC F4ab/ac	Goetstouwers et al. 2014
		ASGA0091537		Belgian Landrace crossbreds, Large White x		
MUC13	13	ALGA0106330	Gene silencing	Pie´train crossbreds, and crossbreds of multiple breeds. IPEC-J2 cells	ETEC F4ac	Zhou et al. 2013
MUC13	13	ASGA0058923	GWAS	White Duroc x Erhualian, Western commercial	ETEC F4ac	Ren et al. 2012
		MARC0096736		population, Sutai		
MUC13	13	c. 576 C>T,	Genotyping	White Duroc X Erhualian	ETEC F4ab/ac	Zhang et al. 2008
ITGB5	13	c. 908 A>G, c. 935A>C	and SNP identification Gene	IPEC-J2 cells	ETEC F4ac	Zhou et al. 2013
			silencing			
ITGB5	13	H3GA0037348 H3GA0037351	GWAS	Landrace, Yorkshire, Songliao Black	ETEC F4ab/Ac	Fu et al. 2012
HEG1	13	MARC0002946 ASGA0058925 ALGA0072075	GWAS	Landrace, Yorkshire, Songliao Black	ETEC F4ab/Ac	Fu et al. 2012

TFRC	13		Microarray and qPCR	Landrace X large white, Pietrain	ETEC F4ab/ac	Schroyen et al 2012
TFRC	13	g.19750 G>T	Genotype	Wild boar, Large White, Landrace, Yorkshire	ETEC F4ab/ac	Jacobsen et al. 2011
TFRC	13	g. 19759 G>T	Haplotype	Duroc Wild Boar x Swedish white	ETEC F4ab/ac	Jacobsen et al. 2009
TFRC	13	c. 291 C>T	mapping Genotyping	White Duroc, Erhualian	ETEC F4ab/ac	Yang et al. 2007
KIAA0226	3	g. 15137 C>T	Genotyping	Wild Boar, Large White, Landrace, Yorkshire	ETEC F4ab/ac	Jacobsen et al. 2011
KIAA026	3	g. 73754 G>A	Haplotype	Duroc Wild Boar x Large White	ETEC F4ab/ac	Jacobsen et al. 2009
MUC20	13	g. 191 C>T, c. 1600 C>T	Mapping Genotyping DTD	White Duroc, Erhualian	ETEC F4ab/ac	Ji et al. 2011
MUC20	13	g. 136484 C>T	Haplotype	Wild Boar x Large White	ETEC F4ab/ac	Jacobsen et al. 2009
ACK1 (TNK2)	13	g. 95821 G>T,g. 95830 G>A, g 95834 G>C, g. 97460 A>G, g. 97740 C>G, g. 93765 G>-, g. 94600 C>T, g. 107371 A>C, g. 108013 T>C, g. 113132A>G, g. 114703 A>G, g. 116847 A>G	mapping mapping Haplotype mapping	Wild Boar x Large White	ETEC F4ab/ac	Jacobsen et al. 2009
MUC4	13		RH mapping	White Duroc • Erhualian	ETEC F4ac	Ren et al 2009
MUC4	13	g. 95755 G>A,	Haplotype	Wild Boar x Large White	ETEC F4ab/ac	Jacobsen et al. 2009
MUC4	13s	g. 8227 G>C	FISH	Wild Boar, Swedish Yorkshire	ETEC F4ab/ac	Jorgensen et al. 2003
MYLK	13	g. 1673 A>G	Genotyping TDT	White Duroc, Erhualian	ETEC F4ab/ac	Huang et al. 2008
SLC12A8	13	g. 159 A>G	Genotyping TDT	White Duroc, Erhualian	ETEC F4ab/ac	Huang et al. 2008
KPNAI	13	g. 306 A>G	Genotyping TDT	White Duroc, Erhualian	ETEC F4ab/ac	Huang et al. 2008

Table 2: SNP and candidate genes associated with the ETEC F4ab/ac.

On the other hand, Interferons (IFNs) as antiviral agents play vital roles in both adaptive and innate immune responses against viral infections [93]. Type III IFNs (IFN-λ) were first identified by Sheppard et al. in 2003 and found to be encoded by 1–3 functional genes in most species of mammals [94]. Among the four interferons (IFNλ1, IFN-λ2, IFN-λ3, and IFN-λ4) found in humans [95,96] only two (IFN-λ1 and IFN-λ3) have been identified in pigs [97]. Shen, et al. [98] showed that porcine interferon Type III (poIFN-λ3) has antiviral properties against PEDV and thus, may serve as an important bio-therapeutic tool for inhibiting PEDV in pigs.

Conclusions

Both pre-weaning and post-weaning diarrhea caused by Enterotoxigenic *E. coli* (ETEC) and porcine epidemic diarrhea virus in piglets remains major hurdles affecting economic growth in the piggery industries. Many studies have been carried out with the aim to have a better comprehension of the pathogenesis of the disease, which may lead to improved methods of prevention, disease prediction and treatment. The fact that the major virulence genes for ETEC are on plasmids means that various combinations of these genes are possible but there is a tendency for certain virulence genes to cluster together, suggesting that there are functional relationships that are not yet understood. ETEC of O-group 149 has been recognized as the dominant type of ETEC worldwide, but the basis for this dominance is not known. F4 and F18, the major fimbrial antigens present on the *E. coli* that cause PWD in pigs, have been identified as good targets for active and passive immunization and utilized for the selection of intestinal receptor-negative pigs.

Although diarrhea is a factor of huge economic loss in pig production, a few solutions or strategies have been put forward in attempts to mitigate the diarrhea problem. None of the attempted solutions or strategies to the problem of diarrhea in piglets has been consistently effective [8]. Newborn baby pigs may be protected by vaccination of their dams with pilus antigens that induce effective passive local immunity [116]. However, after weaning, loss of protective antibody in milk, transportation, mixing of pigs from several sources, fighting, and transition to a new diet all predispose pigs to diarrhea. Orally administered chicken egg-yolk antibodies against F4 and F18 fimbriae have failed to be effective when low doses were administered [117]. The most successful approach on a particular pig farm will probably involve combinations of molecular genetic tools, diet modification and other preventive measures.

References

1. Jensen P, Recén B (1989) When to wean-Observations from free-ranging domestic pigs.□ Applied Animal Behaviour Science. 23: 49-60.
2. FAWC (1993) Report on Priorities For Animal Welfare Research and Development. 1-19.
3. "Ministry of Agriculture, Fisheries and Food. (1983) Codes of recommendations for the welfare of livestock: pigs. MAFF Publications, London... Shi QS, (2003) The review of the receptors of ETEC F4. Pigs and Poultry, 23: 33-35. Shinkai H, Matsumoto T, To."
4. Ren J, yan x, Ai H, Zhang Z, Huang X, et al. (2012) "Susceptibility towards Enterotoxigenic *Escherichia coli* F4ac Diarrhea Is Governed by the MUC13 Gene in Pigs." PLoS One. 7: 1-11.
5. Schroyen M, Stinckens A, Verhelst R, Geens M, Coxet E, et al. (2012) "Susceptibility of piglets to enterotoxigenic *Escherichia coli* is not related to the expression of MUC13 and MUC20." Anim Genet. 43: 324-327.
6. Xia P, Wang Y, Zhu C, Zou Y, Yanget Y, et al. (2016) "Porcine aminopeptidase N binds to F4+enterotoxigenic *Escherichia coli* fimbriae." Vet Res 47: 1-7.
7. Rhouma M, Fairbrother JM, Beaudry F, Letellier A (2017) "Post weaning diarrhea in pigs : risk factors and non - colistin - based control strategies." Acta Vet Scand 59: 1-19.
8. Frydendahl K (2002) "Prevalence of serogroups and virulence genes in *Escherichia coli* associated with postweaning diarrhoea and edema disease in pigs and a comparison of diagnostic approaches." Vet Microbiol. 85: 169-182.
9. Halas D, Heo JM, Hansen CF, Kim JC, Hampson DJ, et al. (2007) "Organic acids, prebiotics and protein level as dietary tools to control the weaning transition and reduce post-weaning diarrhoea in piglets." CAB Rev Perspect Agric Vet Sci Nutr Nat Resour 2.
10. Zhou C, Liu Z , Liu Y, Fu W, Ding X, et al. (2013) "Gene Silencing of Porcine MUC13 and ITGB5: Candidate Genes towards *Escherichia coli* F4ac Adhesion." PLoS One. 8: 1-8.
11. Kim JS, Hosseindoust A, Lee SH, Choi YH, Kim MJ et al. (2017) "Bacteriophage cocktail and multi-strain probiotics in the feed for weanling pigs: Effects on intestine morphology and targeted intestinal coliforms and Clostridium." Animal 11: 45-53.
12. Langel SN, Paim FC, Lager KM, Vlasova AN, Saif LJ (2016) "Lactogenic immunity and vaccines for porcine epidemic diarrhea virus (PEDV): Historical and current concepts." Virus Res 226: 93-107.
13. Cromwell GL (2002) "Why and How Antibiotics Are Used in Swine Production." Anim Biotechnol 13: 7-27.
14. Xu Y, Li X, Jin L, Zhen Y, Lu Y, et al. (2011) "Application of chicken egg yolk immunoglobulins in the control of terrestrial and aquatic animal diseases: A review." Biotechnol Adv 29: 860-868.
15. Poulsen HD (1995) "Zinc oxide for weanling piglets." Acta Agric Scand A Anim Sci 45: 159-167.
16. Schwarz S, Kehrenberg C, Walsh TR (2001) "Use of antimicrobial agents in veterinary medicine and food animal production." Int J Antimicrob Agents 17: 431-437.
17. Stein HH, Kil DY (2006) "Reduced use of antibiotic growth promoters in diets fed to weanling pigs: Dietary tools, part 2." Anim Biotechnol 17: 217-231.
18. SK GAIN (2011) Korea phases out antibiotic usage in compound feed. USDA Foreign Agricultural Service. Report number: KS1128, Seoul, "20."
19. Http://ec.europa.eu/health/ph/others/antimicrob_resist/am_02_en.pdf).

20. (2011) Https://www.washingtonpost.com/news/wonk/wp/2013/12/14/the-fda, "USDA STAFF AND NOT NECESSARILY STATEMENTS OF OFFICIAL U.S Https://www.washingtonpost.com/news/wonk/wp/2013/12/14/the-fda GOVERNMENT China - Peoples Republic of China Re-issues Banned Drugs and Substances in Feed and Animal Production Approved By.176.

21. A (2013) TC for FS and PH www. aasv. org/aas Website/Resources/Diseases/PED. "24."

22. (2013) Porcine Epidemic Diarrhea. The Pig Site. 25. The Pig Site Article 345, "25."

23. (1995) RP, AO of SD, AH (1st edition) AW-B. Cowart, "26 1st edi 51MtJiNkjiL."

24. (1971) Mouwen JMVM, "White Scours in Piglets," 380: 364-380.

25. (2013) DJ, PD (9th ed.) 28. Taylor. "28."

26. The Pig Site: Http://www.thepigsite.com/diseaseinfo/31/e-coli-scour-diarrhoea/, "29." .

27. Montagne L, Cavaney FS, Hampson DJ, Lallès JP, Pluske JR (2004) Effect of diet composition on postweaning colibacillosis in piglets. 82: 2364-2374.

28. IISUPI https://www. amazon. com/Disease. S.-3-fkmr2&keywords =diseases+of+swine+8th+edition. 31. Bertschinger HU,Fairbrother JM (1999) *Escherichia coli* infections. In BE Straw, SD'Allaire, WL Mengeling, & DJ Taylor (Eds.), Diseases of swine (Eighth, p. Chapter 32). Ames "31.".

29. Zhang Q, Hu R, Tang X, Wu C, He Q, et al. (2013) "Occurrence and investigation of enteric viral infections in pigs with diarrhea in China." Arch Virol 158: 1631-1636.

30. Verdonck F, Snoeck V, Goddeeris BM, Cox E (2004) "Binding of a monoclonal antibody positively correlates with bioactivity of the F4 fimbrial adhesin FaeG associated with post-weaning diarrhoea in piglets." J Immunol Methods 294: 81-88.

31. Shepard SM, Danzeisen JL, Isaacson RE, Seemann T, Achtman M, et al. (2012) "Genome sequences and phylogenetic analysis of K88- and F18- positive porcine enterotoxigenic *Escherichia coli*." J Bacteriol 194: 395-405.

32. Rapacz J, Hasler-Rapacz J (1986) "Polymorphism and inheritance of swine small intestinal receptors mediating adhesion of three serological variants of *Escherichia coli* producing K88 pilus antigen." Anim Genet 17: 305-321.

33. Moon HW, Hoffman LJ, Cornick NA, Booher SL, Bosworth BT (1999) "Prevalences of some virulence genes among *Escherichia coli* isolates from swine presented to a diagnostic laboratory in Iowa." J Vet Diagnostic Investig 11: 557-560.

34. Jung K, Saif LJ (2015) "Porcine epidemic diarrhea virus infection: Etiology, epidemiology, pathogenesis and immunoprophylaxis." Vet J 204: 134-143.

35. Stevenson GW, Hoang H, Schwartz KJ, Burrough ER, Sun D, et al. (2013) "Emergence of Porcine epidemic diarrhea virus in the United States: Clinical signs, lesions, and viral genomic sequences." J. Vet. Diagnostic Investig 25: 649-654.

36. Http://www.asm.org "39."

37. Xiande X, Chen M, Wang DQ, Wang Y (2006) "Melting and vitrification of plagioclase under dynamic high pressures." Acta Petrol Sin 22: 503-509.

38. Henton MM, Engelbrecht MM (1997) "*Escherichia coli* serotypes in pigs in South Africa." Onderstepoort J Vet Res 64: 175-187.

39. Gomes TAT, Elias WP, Scaletsky ICA, Guth BEC, Rodrigues JF, et al. (2016) "Diarrheagenic *Escherichia coli*." Braz J Microbiol 47: 3-30.

40. Zhang W, Berberov EM, Freeling J, He D, Moxley RA, et al. (2006) "Significance of heat-stable and heat-labile enterotoxins in porcine colibacillosis in an additive model for pathogenicity studies." Infection and Immunity. 74: 3107-3114.

41. Nagy B, Casey TA, Moon HW (1990) "Phenotype and genotype of *Escherichia coli* isolated from pigs with postweaning diarrhea in Hungary." J Clin Microbiol 28: 651-653.

42. P. A. Co, "杖 組銅 川 中 X," no. 12, 2010.

43. Nguyen UV, Coddens A, Melkebeek V, Devriendt B, Goetstouwers T, et al. (2017) "High susceptibility prevalence for F4+and F18+*Escherichia coli* in Flemish pigs." Vet Microbiol 202: 52-57.

44. Francis DH (2002) "Enterotoxigenic *Escherichia coli* infection in pigs and its diagnosis." Journal of Swine Health and Production. 10: 171-175.

45. Celemín C, Rubio P, Echeverria P, Suárez S (1995) Gene toxin patterns of *Escherichia coli* isolated from diseased and healthy piglets." Vet Microbiol 45: 121-127.

46. (2012) C Rapp-Gabrielson VJO, SR; Pijoan, Haemophilus parasuis. In: Straw BE, Zimmernann JJ, D'allaire S, Taylor DJ. (ed.).

47. Bertschinger HU, Bachmann M, Mettler C, Pospischil A, Schraner EM, et al. (1990) "Adhesive fimbriae produced in vivo by *Escherichia coli* O139:K12 (B):H1 associated with enterotoxaemia in pigs." Vet Microbiol 25: 267-281.

48. Chen X, Gao S, Jiao X, Liu XF (2004) "Prevalence of serogroups and virulence factors of *Escherichia coli* strains isolated from pigs with postweaning diarrhoea in eastern China." Vet Microbiol 103: 13-20.

49. Luppi A, Gibellini M, Gin T, Vangroenweghe F, Vandenbroucke V, et al. (2016) "Prevalence of virulence factors in enterotoxigenic *Escherichia coli* isolated from pigs with post-weaning diarrhoea in Europe." Porc Heal Manag 1-6.

50. Khac HV, Holoda E, Pilipcinec E, Blanco M, Blanco JE, et al. (2006) "Serotypes, virulence genes, and PFGE profiles of *Escherichia coli* isolated from pigs with postweaning diarrhoea in Slovakia." BMC Vet Res 2: 1-8.

51. (2004) Geenen PL, Van der Meulen J, Bouma A, De Jong MCM, "Estimating transmission parameters of F4+ E. coli for F4-receptor-positive and -negative piglets: One-to-one transmission experiment," Epidemiol. Infect 132:1039-1048.

52. Jorgenssen US7785778B2.pdf.".

53. BakerDR, Billey LO, Francis DH (1997) "Distribution of K88 *Escherichia coli*-adhesive and nonadhesive phenotypes among pigs of four breeds." Vet Microbiol 54: 123-132.

54. Rampoldi A, Bertschinger HU, Bürgi E, Dolf G, Sidler X, et al. (2014) Inheritance of porcine receptors for enterotoxigenic *Escherichia coli* with fimbriae F4ad and their relation to other F4 receptors." Animal 8: 859-866.

55. Sellwood R, Gibbons RA, Jones GW, Rutter JM (1975) "Adhesion of enteropathogenic *Escherichia coli* to pig intestinal brush borders: the existence of two pig phenotypes." J Med Microbiol 8: 405-411.

56. Bijlsma IGW, Bouw J (1987) Inheritance of K88-mediated adhesion of *Escherichia coli* to jejunal brush borders in pigs: a genetic analysis. Vet Res Commun 11: 509-518.

57. Gibbons RA, Sellwood R, Burrows M, Hunter PA (1977) "Inheritance of resistance to neonatal E. coli diarrhoea in the pig: examination of the genetic system." Theor Appl Genet 51: 65-70.

58. Klempa B, Koivogui L, Sylla O, Koulemou K, Auste B, et al. (2010) "Serological Evidence of Human Hantavirus Infections in Guinea, West Africa." J Infect Dis 201: 1031-1034.

59. "分子動力学シミュレーションに用いられる計算アルゴリズムの安定性の検討 NII-Electronic Library Service."

60. Pensaert MB, Martelli P (2016) Porcine epidemic diarrhea: A retrospect from Europe and matters of debate. Virus Res 226: 1-6.

61. Gong L, Lin Y, Qin J, Li Q, Xue C, et al. (2018) Neutralizing antibodies against porcine epidemic diarrhea virus block virus attachment and internalization. Virol J 15: 133.

62. Pensaert MB, de Bouck P (1978) A new coronavirus-like particle associated with diarrhea in swine. Arc Virol 58: 243-247.

63. Lee C (2015) Porcine epidemic diarrhea virus: An emerging and re-emerging epizootic swine virus. Virol J 12: 193.

64. Song D, Park B (2012) Porcine epidemic diarrhoea virus: A comprehensive review of molecular epidemiology, diagnosis, and vaccines. Virus Genes 44: 167-175.

65. Nolen S (2015) While new salaries grow, debt remains a drag: AVMA report is most thorough study of veterinary debt and income to date. J Am Vet Med Assoc 246: 1268-1271.

66. Harris DC (2012) Porcine Epidemic Diarrhea. The Merck Manual.

67. Godet M, Grosclaude J, Delmas B, Laude H (1994) Major receptor-binding and neutralization determinants are located within the same domain of the transmissible gastroenteritis virus (coronavirus) spike protein. J Virol 68: 8008-8016.

68. Siebert DN (2007) Análise de formas discretas da equação de Boltzmann para problemas térmicos bi-dimensionais. 77: 8801-8811.

69. Sánchez CM, Gebauer F, Suñé C, Mendez A, Dopazo J, et al. (1992) Genetic evolution and tropism of transmissible gastroenteritis coronaviruses. Virology 190: 92-105.

70. Li BX, Ge JW, Li YJ (2007) Porcine aminopeptidase N is a functional receptor for the PEDV coronavirus. Virology 365: 166-172.

71. Bridgen A, Kocherhans R, Tobler K, Carvajal A, Ackermann M (1998) Further analysis of the genome of porcine epidemic diarrhoea virus. Adv Exp Med Biol 440: 781-786.

72. Kocherhans R, Bridgen A, Ackermann M, Tobler K (2001) Completion of the porcine epidemic diarrhoea coronavirus (PEDV) genome sequence. Virus Genes 23: 137-144.

73. Duarte M, Laude H (1994) Sequence of the spike protein of the porcine epidemic diarrhoea virus. J Gen Virol 75: 1195-1200.

74. Baudoux P, Carrat C, Besnardeau L, Charley B, Laude H (1998) Coronavirus pseudoparticles formed with recombinant M and E proteins induce alpha interferon synthesis by leukocytes. J Virol 72: 8636-8643.

75. Curtis KM, Yount B, Baric RS (2002) Heterologous gene expression from transmissible gastroenteritis virus replicon particles. J Virol 76: 1422-1434.

76. Brian DA, Baric RS (2005) Coronavirus genome structure and replication. Curr Top Microbiol Immunol 287: 1-30.

77. Liu GM, Zhang LH, Dong DJ, Tang XW (2003) Formation factors influencing parameters Tma and m in acoustic formation factor (AFF) formula. Jianghan Shiyou Xueyuan Xuebao/Journal Jianghan Pet. Inst 25: 384-391.

78. https://courses.lumenlearning.com/microbiology/chapter/virulence-factors-of-bacterial-and-viral-pathogens/

79. Nam E, Lee C (2010) Contribution of the porcine aminopeptidase N (CD13) receptor density to porcine epidemic diarrhea virus infection. Vet Microbiol 144: 41-50.

80. Liu C, Tang J, Ma Y, Liang X, Yang Y, et al. (2015) Receptor usage and cell entry of porcine epidemic diarrhea coronavirus. J Virol 89: 6121-6125.

81. Kamau AN, Park JE, Park ES, Yu JE, Rho J, et al. (2017) Porcine amino peptidase N domain VII has critical role in binding and entry of porcine epidemic diarrhea virus. Virus Res 227: 150-157.

82. McKeown TJ (2009) Case Studies and the Statistical Worldview: Review of King, Keohane, and Verba's Designing Social Inquiry: Scientific Inference in Qualitative Research. International Organization 53: 161-190.

83. Li W, Luo R, He Q, van Kuppeveld FJM, Rottier PJM, et al. (2017) Aminopeptidase N is not required for porcine epidemic diarrhea virus cell entry. Virus Res 235: 6-13.

84. Shirato K, Maejima M, Islam MT, Miyazaki A, Kawase M, et al. (2016) Porcine aminopeptidase N is not a cellular receptor of porcine epidemic diarrhea virus, but promotes its infectivity via aminopeptidase activity. J Gen Virol 97: 2528-2539.

85. Tuboly S, Bernáth S, Glávits R, Medveczky I (1988) Intestinal absorption of colostral lymphoid cells in newborn piglets. Vet Immunol Immunopathol 20: 75-85.

86. Heaney JP (2007) History of the Department of environmental engineering sciences, University of Florida. Environ Water Resour Milestones Eng Hist 33: 29-35.

87. Rooke JA, Bland IM (2002) The acquisition of passive immunity in the new-born piglet. Livest Prod Sci 78: 13-23.

88. Bland IM, Rooke JA, Bland VC, Sinclair AG, Edwards SA (2003) Appearance of immunoglobulin G in the plasma of piglets following intake of colostrum, with or without a delay in sucking. Anim Sci 77: 277-286.

89. https://www.thepigsite.com/disease-and-welfare/managing-disease/acquired-specific-immunity

90. http://www.circumvent-g2.com/immunology.aspx

91. Schanbacher FL, Talhouk RS, Murray FA (1997) Biology and origin of bioactive peptides in milk. Livest Prod Sci 50: 105-123.

92. Bauer R (2009) Hemispheric studies. PMLA 124: 234-250.

93. Sheppard P, Kindsvogel W, Xu W, Henderson K, Schlutsmeyer S, et al. (2003) IL-28, IL-29 and their class II cytokine receptor IL-28R. Nat Immunol 4: 63-68.

94. Hermant P, Michiels T (2014) Interferon-λ in the context of viral infections: Production, response and therapeutic implications. J Innate Immun 6: 563-574.

95. Prokunina-Olsson L, Muchmore B, Tang W, Pfeiffer RM, Park H et al. (2013) A variant upstream of IFNL3 (IL28B) creating a new interferon gene IFNL4 is associated with impaired clearance of hepatitis C virus. Nat Genet 45: 164-171.

96. Sang Y, Rowland RRR, Blecha F (2010) Molecular characterization and antiviral analyses of porcine type III interferons. J Interferon Cytokine Res 30: 801-807.

97. Shen H, Zhang C, Guo P, Liu Z, Sun M, et al. (2016) Short communication: antiviral activity of porcine IFN-λ3 against porcine epidemic diarrhea virus *in vitro*. Virus Genes 52: 877-882.

98. Groenen MAM, Archibald AL, Uenishi H, Tuggle CK, Takeuchi Y, et al. (2012) Analyses of pig genomes provide insight into porcine demography and evolution. Nature 491: 393-398.

99. Edfors-Lilja I, Petersson H, Gahne B (1986) Performance of pigs with or without the intestinal receptor for *Escherichia coli* K88. Anim Prod 42: 381-387.

100. Gibbons RA, Jones G, Sellwood R (1975) An attempt to identify the intestinal receptor for the K88 adhesin by means of a haemagglutination inhibition test using glycoproteins and fractions from sow colostrum. J Gen Microbiol 86: 228-240.

101. Jovanovic S, Savic M, Zivkovic D (2009) Genetic variation in disease resistance among farm animals disease. Biotechnology in Animal Husbandry 25: 339-347.

102. Crittenden LB (1985) Identification and Cloning of Genes for Insertion. Poultry Science 65: 1468-1473.

103. Cera KR, Mahan DC, Cross RF, Reinhart GA, Whitmoyer RE (1988) Effect of age, weaning and postweaning diet on small intestinal growth and jejunal morphology in young swine. J Anim Sci 66: 574-584.

104. Grange PA, Mouricout MA (1996) Transferrin associated with the porcine intestinal mucosa is a receptor specific for K88ab fimbriae of *Escherichia coli*. Infect Immun 64: 606-610.

105. Peng QL, Ren J, Yan XM, Huang X, Tang H, et al. (2007) The g.243A>G mutation in intron 17 of MUC4 is significantly associated with susceptibility/resistance to ETEC F4ab/ac infection in pigs. Anim Genet 38: 397-400.

106. Zhang B, Ren J, Yan X, Huang X, Ji H, et al. (2008) Investigation of the porcine MUC13 gene: Isolation, expression, polymorphisms and strong association with susceptibility to enterotoxigenic *Escherichia coli* F4ab/ac. Anim Genet 39: 258-266.

107. Yan X, Huang X, Ren J, Zou Z, Yang S, et al. (2009) Distribution of *Escherichia coli* F4 adhesion phenotypes in pigs of 15 Chinese and Western breeds and a White DurocxErhualian intercross. J Med Microbiol 58: 1112-1117.

108. Rampoldi A, Jacobsen MJ, Bertschinger HU, Joller D, Bürgi E, et al. (2011) The receptor locus for *Escherichia coli* F4ab/F4ac in the pig maps distal to the MUC4-LMLN region. Mamm Genome 22: 122-129.

109. Jacobsen M, Cirera S, Joller D, Esteso G, Kracht SS, et al. (2011) Characterisation of five candidate genes within the ETEC F4ab/ac candidate region in pigs. BMC Res Notes 4: 225.

110. Ouyang J, Zeng W, Ren J, Yan X, Zhang Z, et al. (2012) Association of B3GNT5 polymorphisms with susceptibility to ETEC F4ab/ac in the white Duroc × Erhualian intercross and 15 outbred pig breeds. Biochem Genet 50: 19-33.

111. Nguyen VU, Goetstouwers T, Coddens A, Van Poucke M, Peelman L, et al. (2013) Differentiation of F4 receptor profiles in pigs based on their mucin 4 polymorphism , responsiveness to oral F4 immunization and *in vitro* binding of F4 to villi. Vet Immunol Immunopathol 152: 93-100.

112. Schroyen M, Stinckens A, Verhelst R, Niewold T, Buys N (2012) The search for the gene mutations underlying enterotoxigenic *Escherichia coli* F4ab / ac susceptibility in pigs : a review. Vet Res 43: 70.

113. Goetstouwers T, Poucke MV, Coppieters W, Ut Nguyen V, Melkebeek V et al., (2014) "Refined candidate region for F4ab/ac enterotoxigenic *Escherichia coli* susceptibility situated proximal to MUC13 in pigs." PLoS One. 9: 4-11.

114. Melkebeek V, Rasschaert K, Bellot P, Tilleman K, Favoreel H et al. (2012) "Targeting aminopeptidase N, a newly identified receptor for F4ac fimbriae, enhances the intestinal mucosal immune response." Mucosal Immunol 5: 635-645.

115. Moon HW, Bunn TO (1993) Vaccines for preventing enterotoxigenic *Escherichia coli* infections in farm animals. Vaccine 11: 213-220.

116. Sun Y, Kim SW (2017) Intestinal challenge with enterotoxigenic *Escherichia coli* in pigs, and nutritional intervention to prevent postweaning diarrhea. Anim Nutr 3: 322-330.

117. Mirhoseini A, Amani J, Nazarian S (2018) Review on pathogenicity mechanism of enterotoxigenic *Escherichia coli* and vaccines against it. Microb Pathog 117: 162-169.