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REVIEW VIRULENCE NATURE OF *Escherichia coli* IN NEONATAL SWINE

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ABSTRACT: Piglet disease due to Enterotoxigenic *Escherichia coli* (ETEC) are classical and associated typically with severe watery diarrhea within the first two weeks of life and occasionally some days after weaning in pigs. *E.coli* is a well-known and diverse organism though normally harmless commensal, but when it acquires mobile genetic elements becomes a highly pathogenic organism capable of causing a range of diseases. ETEC adhere to the small intestinal microvilli without inducing morphological lesions and produce enterotoxins acting locally on enterocytes. This leads to hyper-secretions and reduced absorption of electrolytes. The virulence attributes of ETEC are adhesions and toxins and the successful management of the disease is dependent on good understanding of these virulence factors. In pigs ETEC, the commonest adhesions are the fimbriae on the surface K88, K99, 987p, F18ab and F18ac. The enterotoxine of pigs ETEC are further classified into heat-labile (LT) and heat-stable (ST). Other subdivisions of enterotoxin *E. coli* are LT, STb, STa, Stx2e. The adhesive fimbriae and enterotoxins of piglet ETEC can be evaluated using plasmids. Polymerase chain reaction (PCR) is a specific test and had been used for virulence gene detection of ETEC. In this reviews, we focus on current opinions and knowledge of the various pathogenic pathways that *E.coli* uses to cause disease in piglet.

Keywords: ETEC, Fimbria, Toxins, Piglets, Virulence

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INTRODUCTION

One of the important disease of swine herds around the world is neonatal Colibacillosis (Blickwede and Schwarz 2004; Hart et al., 2004). The ability of adhesion of Enterotoxigenic *Escherichia coli* to intestinal epithelial cell is mainly due to the production of thin (3-7nm) proteinaceous surface appendages (fimbriae or pili), which can be morphologically, biologically and antigenically different on various strains. Some of them morphologically resemble the common fimbriae (type 1 fimbriae) of *E.coli* (Duguid et al., 1955). The ability of the fimbriae to agglutinate red blood cells of different species was recognized early (Elsinghorst and Weitz, 1994) and has been used. Neonatal and post-weaning diarrhea has been associated with different types of *Esherichria coli* pathotypes among which are enterotoxigenic *Escherichia coli* (ETEC) and attaching or enteropathogenic *E. coli* (AEEC). The disease called edema is caused by a Shiga toxin – producing *E. coli* (STEC). There is a high mortality rates in piglets as a result of these disease (Choi et al., 2002, Hariharan et al., 2004). The enterotoxigenic *E. coli* makes use of the fimbriae (F4, F5, F6, F18 and F41) to adhere to the small intestine and produce enterotoxins (STa, STb, and LT) that interact with enterocytes causing water and electrolyte hypersecretion and impairing nutrient absorption (Bertschinger and Fainbrother, 1999; Amezcua et al., 2002). A very important factor or marker of ETEC virulence from swine is hemolysins (Docic and Bilkei, 2003). The contamination of piglets occurs via contact with environment or from the sow and this has resulted in disease producing a very high mortality rate in the first 24 hours of life. The piglet susceptibility is associated with several factors such as presence of intestinal *E. coli* fimbrial receptors, stress, very poor hygiene and low quantity of ingested colostrums (Bertschinger and Fairbrother, 1999). In Nigeria the case of neonatal piglet diarrhea is alarming and this inform the essence of this review. The study takes a look at important characteristics features that occurs in piglet due to ETEC and these include: fimbriae adhesions, enterotoxins, virulence spread determinants, age-related ETEC strain susceptibility as well as its lineage to ETEC, common receptor in piglet enterocytes and diagnostic methods.

Fimbriae Adhesins

The piglet ETEC isolates are known to produce about five different adhesins, all of which are fimbriae also called pili among which are K88, (F4), K99 (F5), 987P(F6), (F7) and F18 (Wilson and Francis, 1986; Casey et al.,1992). Fimbrial ahesins K88 and F18 occur in several antigenic forms. The K88 fimbrial variants are K88ab, K88ac and K88ad. In a country like United States of America, the only form of K88 found is K88ac (Westerman et

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al., 1988). The variants of F18 include F18ab and F18ac. The strains that express F18ab maybe more commonly associated with edema disease than strains expressing F18ac. The F41 type of adhesion was commonly and often found in association with K99, but has significance in naturally occurring disease. The Fimbrial adhesions are known to be involve in target of specific receptors on piglet intestinal brush border epithelial cells (enterocytes), enabling the bacteria to colonize the cell surface and also excrete toxins whose action includes production of watery stool in the piglet. The exposure of these receptors to the luminal surface is responsible for the susceptibility of piglets to ETEC. The exposure of piglets to clinical infection of ETEC is limited to animal genetics and age (Francis et al., 1998; Imberechts et al., 1997).

Enterotoxins

The ETEC strain that causes diarrhea piglets produces toxins. The toxins are of five different pathotypes namely; heat labile enterotoxin (LT), heat stable enterotoxin type A (STa); heat stable enterotoxin type B (STb); Shiga toxin type 2e (Stx2e), and enteroaggregative *E.coli* heat stable enterotoxin 1 (EASTI). Choi et al. (2001) enterotoxin Stx2e, also known as edema disease factor is responsible for lesions that are associated with edema disease in piglets. When these toxins are absorbed into the blood stream, they cause destruction of endothelial cells resulting in hemorrhage, blood dot, necrosis and edema in important organs among which is the brain (Clugston et al., 1974). The importance of entero-aggregative *E. coli* heat stable enterotoxin 1 (EAST 1) in piglet diarrheal is yet to be known.

Table 1 - Features of enterotoxigenic *Escherichia Coli* (ETEC) strains in relative with diseased pigs of different ages.

	ETEC	Features		Age group affected	
Serogroup	Fimbrial	Toxins	Hemolysins	Weaned	Neonatal
08	K99	STa	-	No	Yes
09	K99, 987p	STa	-	No	Yes
0101	K99	STa	-	No	Yes
0141	987P	STa	-	No	Yes
0149	K88	LT, STb ± STa	+	Yes	Yes
0157	K88	LT, STb ± STa	+	Yes	Yes
0138	F18ab, F18ac	STa, STb ± St x 2e	+	Yes	No
0139	F18ab	STa, STb ± St x 2e	+	Yes	No
0141	FI8ac	STa, STb ± St x 2e	+	Yes	No
0157	F18ac	STa, STb ± Stx 2e	+	Yes	No

The neonate were affected by enterotoxigenic *E coli*, (08, 09, 0141 and 0149) while the weaned were affected by enterotoxigenic *E.coli* (014, 0139, 0141 and0157); Source: Wilson, R.A and Francis, D.H. (1986); + = Presence, - = absence of hemolysin.

Determinants of Virulence pattern/spread

Virulence determinants are not randomly distributed among the virulent strains, rather they occur in patterns associated with specific serogroups and fumbrial. Table 1 above demonstrates clustering of virulence determinants around serogroup and the ages of pigs that are commonly infected with ETEC of each virulence type. All the strains of ETEC isolated from weaned pigs with diarrhea contain the gene in post-weaning diarrhea and this strongly suggests that ST_b plays an important role in pathogenesis that is not duplicated by other enterotoxins. Furthermore, very few ETEC Strains possess the features of only ST_b genes. Postweaning isolates genes for ST_a- LT or ST_a-LT.

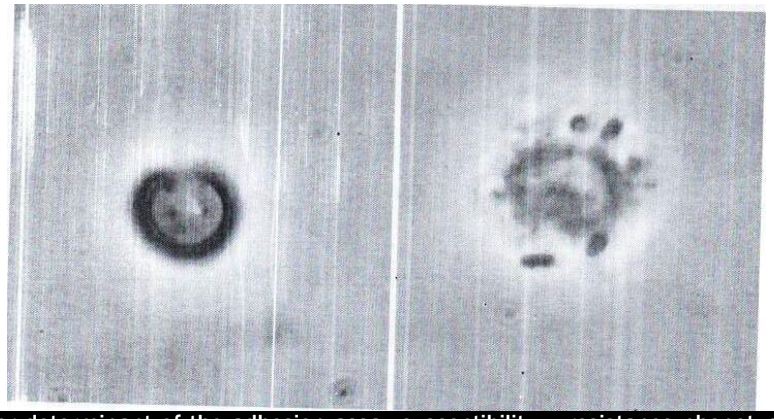

Figure 1 - The brush border determinant of the adhesion assay susceptibility – resistance phenotype of piglet k88+ and f18+ enterotoxigenic *Escherichia coli.* (Bacteria) with F18 (Fimbrial) adhere to brush border residue of pigs of the susceptible phenotype right (x1200) and non for left. Brush borde determinant of the adhesion assay susceptibility –resistant phenotype of pigletK88+ and f18+ enteritoxigenic *Echerichia coli*. (Bacteria) with F18 (Fimbrial) do not adhere to the brush border determinant of the adhesion assay susceptibility-resistant phenotype left (x1200).

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Age associated spread susceptibility to various ETEC strains

It has been shown that a large majority of piglets clinically exposed to ETEC strains revealed 987P and K99 are neonates, even though most pigs may be susceptible to these strains for about one week post birth. As piglets grow into grower stage, the receptors of K99 fimbriae decrease in concentration which is an indication that ETEC susceptibility is a function of expression of receptors (Runnels et al., 1980). The receptors for K88 and F18 also appear to be present in large quantity in adult pigs which is an indication that stop in receptor expression is inconsequential with respect to age related resistance of adult pigs to ETEC expressing these fimbrial related resistance of adult pigs to ETEC expressing these fimbrial (Erickson et al., 1992). There exists a little correlation between the type of enterotoxin produced by ETEC strain and the age at which pigs become resistant. Furthermore, even adult humans are susceptible to traveler's diarrhea caused by ETEC.

This suggests that some adult pigs are not resistant to all types of *E-coli* enterotoxins. The explanation for this occurrence could be that changes in the intestinal brush border glycocalyx associated with aging render the fimbrial receptors less available for bacterial attachments (Erickson *et al.,* 1992). Since receptors for the different fimbrial adhesions are different in size and probably different in presentation from each other, on the surface of the enterocyte, diet and age associated changes in the glycocalyx may mask fimbrial of different receptors at varying animal ages (Grange et al*.,* 1999).

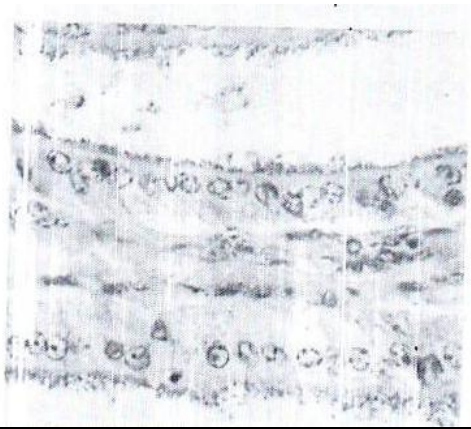

Figure 2 - Hematoxylin-eosin stained histologic section of small intestine from a pig infected with enterotoxigenic *Escherichia coli.* Bacteria form a confluent layer on the epithelial surface. (x400)

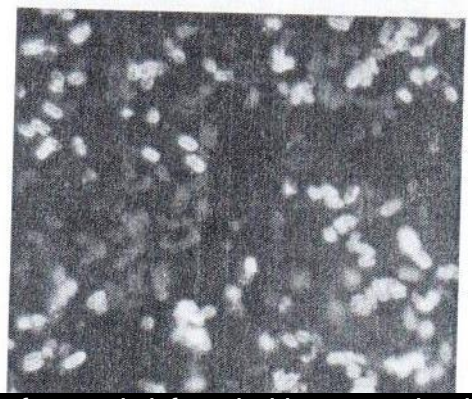

Figure 3 - Intestinal impression smear from a pig infected with enterotoxigenic *Escherichia coli* stained by indirect immunofluorescence with anti-fimbria antibodies and fluorescein-isothiocyanate-conjugated anti-immunoglobulin. Many fluorescing bacteria are present (x1200).

Besides age related resistance of piglets to $K88^+$ and $F18^+$ ETEC strains, there may be lineage-related resistance to infection. In each of the cases, susceptibility is an autosomal dominant trait and the inability of the pigs to express the required receptors on enterocytes is due to resistance. An experiment mounted to demonstrate expression of K88 receptors from four different breeds of pigs showed that there was substantial variation in the prevalence of susceptible pigs among the breeds (Bakar et al., 1997). The prevalence of pigs of the F18 susceptibility phenotype has not been determined, though casual observation suggests that it is considerably higher than that 88 ETEC susceptibility K88 and F18 receptors present on swine erythrocytes. K88ab and K 88ac in swine erythrocyte possess a receptor which has been identified as a mucin-type sialoglycoprotein (MTSG). This molecule often gets attached to the border of the brush surface of erythrocytes (Erickon et al., 1992). Those pigs that show (MTSG) are susceptible to $K88ab^+$ and $K88ac^+$ ETEC and the piglet acceptance to K88 ETEC correlates with the

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expression of (mucin-type sialoglycoprotein (Francis et al., 1998). The brush boarder adherence assay is a test useful for $K88^+$ ETEC susceptibility-resistance phenotypes of pigs as reflected (Figure 1). There is no genetic marker of $K88^+$ ETEC resistance-susceptibility identified. However, the determinant for resistance susceptibility rest on the 13^{th} chromosome (Peelman, 1999). The receptor for F18ab and F18ac is yet to be known, but a genetic marker for loss of expression of receptor had been shown to be closely linked to the marker for swine blood group inhibitor (s) and swine stress syndrome. The marker was mapped to an alpha (1,2) fuicosyltransferase (FUT) gene on the 6^{th} chromosome (Meijerink et al., 1997). Difference exists between resistant and susceptible fuicosyltransferase gene and open reading frames (ORFS) were polymorphism at lease pairs 307 and 857 resulted in substitution of amino acid at position 103 and 286 for (threonine substituted for alanine) and glutamine substituted for arginine). The animals resistant to F18 ETEC are in hologous for threonine at amino acid 103 of the fuicosyltransferase enzyme. The level of FUT enzyme are significantly lower in F15 ETEC resistant animals (Meijerink et al., 2000).

Methods of Diagnosis & Enterotoxigenic *E. coli* (ETEC) Characterization

At necropsy, no obvious sign may be seen in pigs infected with ETEC beside dehydration. Histological evaluation may show bacilli adherent diffusely on the villous surface of the small intestine (figure 2). Large number of Gram negative bacilli is seen in the ileum of clinically infected pigs on making a smear (Francis, 1983) with few organisms present. Enterotoxigenic *E. coli* cultured from infected neonatal pigs may be either hemolytic or non-hemolytic but only hemolytic ETEC colonize weaned pigs (Imberechts et al., 1997) confirmation that E coli isolates are pathogenic strains may be by phenotypic or genotypic analysis. However, for the purpose of this study, genotypic assessment will be considered. Genotypic analysis by multiplier polymerase chain reaction (PCR) identifies genes for virulence factors, among which are fimbriae K88, K99, 987P, F18 and F41 (Figure 4). It also identifies genes for toxins LT, ST_a, ST_b and Stx2e (Casey and Bosworth, 1997). PCR testing eliminates difficulties associated with gene expression under laboratory conditions. However, PCR have its demerits because results obtained are subject to interpretation and superfluous bands may mix with genes of virulence factors. The presence of genes or its fragment evidenced by a band on the agarose gel does not necessarily show that gene is expressed as a functional virulence factor.

Genes contained within bacterial plasmids may easily be spread via a bacterial populations. Many virulence factor genes of ETEC are plasmid borne and the presence of a typical combination of genes in ETEC strain is expected. The fact that extra genes add little if at all any advantage is on the premise of low isolation incidence of each typical combination. Phenotypic analysis is best performed by assessment for production of adhesive fimbriae, either by Enzyme-Linked Immunosorbent Assay (ELISA) or by Indirect Immunofluorescence Assay (IFA) as described (Mullaney et al., 1991). Monoclonal antibodies for each type of fimbria are available for these tests. The ELISA rest requires culture of the organisms from infected tissues. The IFA may be performed either on impression smears or frozen sections of infected intestine or on smears prepared from cultures of the bacteria (Francis, 1983) as described (Figure 3). A potential pitfall in using organisms cultured from infected tissue is that cultured organism may not always express fimbrae. In vitro expression is parta problem with 987P and F18ab fimbriae (Imberechts et al., 1997; Mullaney et al., 1991)

CONCLUSION

Esherichia coli particurlaly enterotoxigenic *E. coli* is responsible for causing severe diarrhea in neonate swine. The virulence nature of *E.coli* is embedded in the fimbriae which produces the toxin and it also posses adhesive property. The virulence form is associated with certain age type of pigs. The toxins STa affects only the neonatal pigs and not the weaned. Only non-haemolytic *E. coli* colonize the neonate pig.

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