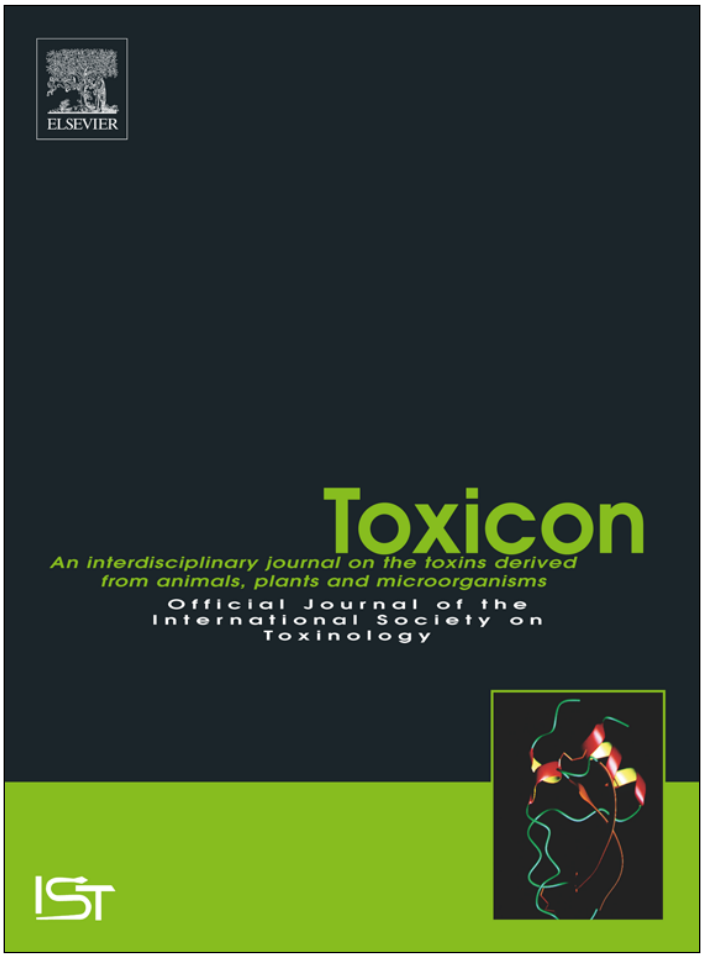

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Review

Overview of the role of Shiga toxins in porcine edema disease pathogenesis

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ABSTRACT

Shiga toxin-producing *Escherichia coli* (STEC) have been implicated as the cause of enterotoxemias, such as hemolytic uremic syndrome in humans and edema disease (ED) of pigs. Stx1 and Stx2 are the most common types found in association with illness, but only Stx2e is associated with disease in the animal host. Porcine edema disease is a serious affection which can lead to dead causing great losses of weaned piglets. Stx2e is the most frequent Stx variant found in porcine feces and is considered the key virulence factor involved in the pathogenesis of porcine edema disease. Stx2e binds with higher affinity to Gb4 receptor than to Gb3 which could be due to amino acid changes in B subunit. Moreover, this subtype also binds to Forssman glycosphingolipids conferring upon Stx2e a unique promiscuous recognition feature. Manifestations of edema disease are caused by systemic effects of Stx2e with no significant morphologic changes in enterocytes. Endothelial cell necrosis in the brain is an early event in the pathogenesis of ED caused by Stx2e-producing STEC strains. Further studies are needed to generate techniques and tools which allow to understand the circulation and ecology of STEC strains in pigs even in resistant animals for diagnostic and epidemiological purposes.

Contents

1. Introduction

Shiga toxins (Stx) of *Escherichia coli* were first described in the late 1970s when it was noted that filtrates from certain *E. coli* strains were toxic to Vero cells (derived from the kidney of African

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green monkeys) *in vitro* and they were originally designated as verocytotoxins (Konowalchuk et al., 1977). Verocytotoxins were subsequently recognized for their structural and functional similarity to Shiga toxin of *Shigella dysenteriae* type I and were designated Stx1 and Stx2 (O'Brien et al., 1992). Therefore, *E. coli* strains producing toxins are designated Shiga toxin-producing *E. coli* (STEC) and have been implicated as the cause of enterotoxemias, particularly edema disease (ED) of pigs and hemolytic uremic syndrome in humans. Toxin subtypes are recognized by sequencing and by differences in biological activity and/or immunoreactivity (Scheutz et al., 2012). Regarding Stx1a, only two variants have been identified: Stx1c and Stx1d (Ohmura-Hoshino et al., 2003; Zhang et al., 2002). Respect to Stx2a subtypes, it has been described that Stx2c and Stx2d are more closely related to Stx2a than Stx2b and Stx2e, Stx2f and Stx2g (Scheutz et al., 2009).

Shiga toxins are encoded by the late genes of lambdoid prophages integrated in the bacterial chromosome. These toxins have an AB5 structure non-covalently linked, with an enzymatically active A subunit that inhibits protein biosynthesis at ribosomal level and the pentameric B subunit which binds to glycosphingolipid receptor (Beddoe et al., 2010). Stx1a recognizes globotriaosylceramide (Gb3Cer) and also globotetraosylceramide (Gb4Cer) to a considerable extent, whereas Stx2a preferentially binds to Gb3Cer and to a lesser extent to Gb4Cer. Advances in glycolipid biology indicate that Gb3 is localized to lipid rafts rich in cholesterol (Falguières et al., 2001; Müthing et al., 2009) and that not all Gb3 in the plasma membrane is bioavailable (Johannes and Römer, 2010; Lingwood et al., 2010; Mahfoud et al., 2010).

Stx1 and Stx2 have the same mode of action (Strockbine et al., 1986). After binding of Stx to lipid raft-associated Gb3, Stx is delivered to the cytoplasm where A subunit is cleaved by furin into an active A1 subunit and an A2 fragment associated with the B pentamer (Garred et al., 1995). A1 binds to the large ribosomal subunit and inhibits protein synthesis by cleaving off a single adenine residue from the 28S rRNA (Endo et al., 1988). Although inhibition of protein synthesis itself does not kill the host cell, it triggers a ribotoxic stress response which eventually leads to apoptosis. Retrograde Stx transport to the endoplasmic reticulum and nucleus has been linked to cytotoxicity as toxin trafficking is different in Stx resistant cells. In macrophages and dendritic cells, Gb3 is not associated with lipid rafts and Stx is transported along the endosomal/lysosomal pathway and ultimately degraded in lysosomes (Falguiéres et al., 2001).

2. Shiga toxin 2e and swine edema disease

Porcine edema disease, also called gut edema or bowel edema, is a serious affection causing great losses of weaned piglets. Usually, ED is considered a common cause of illness and death in swine and it is mostly a weaned pig disease with signs occurring within two weeks after weaning. However, the condition may develop in older animals as in case of feeding medicated feed mixtures to piglets immediately after weaning; isolated cases have been reported (Alexa et al., 2004). It manifests as abruptly sporadic cases with lethality rate from 50% to 90% last between 4 and 14 days. Recurrence of ED in the herd is frequent.

ED is clinically characterized by swelling of the eyelids, paralysis, unusual squeal or snoring sound, neurologic signs such as ataxia, incoordination and recumbency and/or subcutaneous and submucosal edema in various tissues (Matise et al., 2000). Vascular damage with resultant infarction and malacia in the brain stem is the main cause of death in affected pigs. In addition, edema may course with postweaning diarrhea because some *E. coli* strains can produce both diseases (Fairbrother and Gyles, 2012). Susceptibility of piglets depends on several variables, especially on genetic resistance, diet, environmental factors (stress, transportation, mixing animals) and immunity (Bertschinger et al., 1993; Smith and Linggood, 1971).

Stx is considered the key virulence factor involved in the pathogenesis of porcine ED. The typical Stx produced by *E. coli* isolated from pigs with ED is Stx2e (Marques et al., 1987) whose gene has been found located in the chromosome since no Stx converting phages could be isolated opportunely (Muniesa et al., 2000). Stx2e and Stx2a show a high nucleotide and amino acid sequence homology of their A- and B-subunits of 94 and 84%, respectively (Gyles et al., 1988; Piérard et al., 1998; Weinstein et al., 1988) and less homology to Shiga toxin genes of *S. dysenteriae* type I and Stx1 from *E. coli* (55–60%) (Weinstein et al., 1988). Additionally, in contrast to Stx1/2, Stx2e binds with higher affinity to Gb4 receptor than to Gb3 which could be due to amino acid changes in B subunit (DeGrandis et al., 1989; Karve and Weiss, 2014). Moreover, this subtype also binds to Forssman glycosphingolipids that constitutes a GalNAcα3-elongated Gb4Cer structure conferring upon Stx2e a unique promiscuous recognition feature (Müthing et al., 2012).

Stx2e is the most frequent Stx variant found in porcine feces (Fratamico et al., 2008) and it was the second most common Stx2 variant in environmental STEC isolates (Vernozy-Rozand et al., 2004). Although Stx2e, Stx2f and Stx2g are associated with animal STEC infection (Fratamico et al., 2008; Schmidt et al., 2000; Leung et al., 2003), only Stx2e is associated with disease in the animal host. Stx2e-producing STEC has been also isolated from food (Beutin and Fach, 2014) and has been only occasionally isolated from humans with uncomplicated diarrhea (Piérard et al., 1998; Friedrich et al., 2003; Beutin and Fach, 2014). In pigs with ED, most of the identified *E. coli* strains are assigned to O138, O139, O141 and O147 serogroups with the fimbrial variants F18ab and F18ac (DebRoy et al., 2009) while in humans with symptomatic disease, STEC strains belonging to serotypes O157:H7, O145:H28, O111:H8, O103:H2, and O26:H11 have been identified in different countries worldwide but do not usually carry Stx2e genes. Whereas Stx2e-producing *E. coli* isolates from humans and pigs were found to differ in their virulence abilities and therefore allow them to adapt to the hosts and cause several forms of disease (Sonntag et al., 2005), the information about the interaction of Stx2e with target cells is scanty. It is assumed that colonization in pigs develops between 3 and 6 days; furthermore, fimbrial antigens seem to be essential for adherence to swine epithelial cells and to play a role in specificity and adaptation to the host. By the way, differential expression of adhesin receptors on the intestinal epithelium between adults and newborn animals could explain association to postweaning diseases and age (Etcheverría and Padola, 2013). The susceptibility to disease is determined by the presence of these receptors on small intestinal epithelial cells and is inherited as a dominant trait; resistance is present in a low percentage of pigs. Clinical signs and lesions are largely the result of Stx2e activity and the absence of circulating neutralizing antibodies against Stx in serum is considered to be the decisive factor in disease pathogenesis (Gannon and Gyles, 1990).

Experimental induction of ED is very difficult. Several studies have so far been performed in which pig tissues were analyzed for their expression of Stx2e receptors. Thin-layer chromatography overlay assays of glycosphingolipid extracts from a collection of pig tissues indicated the presence of Gb3Cer and Gb4Cer mainly in red blood cells, colon, spleen, stomach, lung and heart, but only small quantities in the kidney (Boyd et al., 1993). In a more detailed study, Stx2e was found to bind to Gb3Cer and Gb4Cer from microvillus membranes of pig jejunum and ileum but not to crude mucus (Waddell et al., 1996). The presence of Gb3Cer and Gb4Cer was also reported for a porcine aortic endothelial cell line, indicating relationship between cell susceptibility and content of Gb4Cer

(Valdivieso-Garcia et al., 1996). Considering that Gb4 expression is age-dependent, it might affect susceptibility to F18 + *E. coli* disease, as was suggested earlier for another toxin receptor (Grange et al., 2006).

2.1. Intestinal effects

The rabbit small intestine has been extensively used as a model to study the pathogenesis of STEC-associated intestinal and extra-intestinal disease. The effects of Stxs in the small intestines of rabbits of different ages have been well characterized (Fuchs et al., 1986; Kandel et al., 1989; Keenan et al., 1986; Li et al., 1993; Mobassaleh et al., 1989). In contrast to other Stxs, Stx2e alone does not cause fluid accumulation in rabbits (MacLeod et al., 1991) or local damage to the intestinal mucosa in ligated loops of the pig small intestine (Waddell et al., 1996). The reason for the lack of sensitivity of the pig intestine to luminal Stx2e is not clear since the toxin is stable in pig intestinal washings (MacLeod et al., 1991), and Gb3 and Gb4 receptors are present in pig intestinal mucosal scrapings (Samuel et al., 1990). Differences in the nature of interaction of Stx2e with the intestines of the pig and the rabbit may account for the propensity of Stx2e-producing *E. coli* to cause systemic but not enteric disease in pigs. Stx2e was shown to bind to intestinal microvillus membrane from the jejunum and ileum of weaned pigs (Waddell et al., 1996) and to the small intestinal microvillus membrane of 6-week-old rabbits which are also responsive to the enterotoxic effects of Stxs and Stx2e (MacLeod and Gyles, 1990). In contrast, the intestinal microvillus membrane obtained from 1-week-old rabbits, which are not responsive to the enterotoxic effects of Stxs (Keusch et al., 1991), did not bind Stx2e (Waddell et al., 1996). The binding of Stx2e to intestinal microvillus membrane from pigs and rabbits was correlated with the presence of Stxs receptor glycolipids. In the pig, Stx2e binds to the enterocytes at the base of the villi and not to the apical absorptive enterocytes, as it does in the rabbit (Kandel et al., 1989). Although Stx receptor glycolipids are present in the intestine of the pig, these glycolipids apparently cannot mediate uptake of Stx2e to the systemic vasculature since intraintestinal administration of Stx2e does not result in toxemia (Waddell et al., 1996). Intestinal colonization of pigs with ED strains of *E. coli* has been shown to decrease from the tip to the base of the villi (Bertschinger and Pohlenz, 1983). Significant morphologic changes to enterocytes are not reported to occur (Methiyapun et al., 1984) and it is probably that much of the toxin that enters in cells is degraded in the endocytic compartment. However, systemic Stx2e absorption occurs soon after colonization and vascular lesions characteristic of Stx2e are evident by two days post-inoculation (Methiyapun et al., 1984). In experimental models of infection, local production of Stx2e within the intestinal lumen occurs as early as 1 day post-inoculation and Stx2e fecal titers peak at 4–6 days post-inoculation (Cornick et al., 2000). Therefore, a local environment at the villus tip lacking specific Stx2e receptors may allow toxin to traverse the gut barrier by a nonspecific mechanism. It should be considered that ED strains that elaborate one or more enterotoxins, excluding Stx2e, may cause diarrhea prior to clinical edema signs. In experimental ED, absorption of Stx2e occurred without visible damage to the intestinal epithelium in pigs whose intestines had been colonized with a Stx2e pathogenic strain of *E. coli* (Bertschinger and Pohlenz, 1983; Methiyapun et al., 1984). Also, the intraintestinal inoculation of pigs with a relatively high dose of Stx2e alone was not able to reproduce ED (Waddell et al., 1996). Normally, the intestine of the postweaning pigs prevents the transmucosal passage of macromolecular proteins (Weström et al., 1984), but the intestinal absorption of toxin can be increased experimentally with sodium deoxycholate, reproducing signs of ED. Sodium deoxycholate is an unconjugated bile salt that causes increased permeability of the intestinal mucosa to macromolecules (Waddell and Gyles, 1995). It is likely that higher toxin would be necessary or that particular conditions are required for the transport of toxin from the gut to the systemic vasculature, including increased intestinal permeability due to changes in the intestinal microflora or diet. Indeed, postweaning pigs present modified intestinal cells related with malabsorption and high protein feed contribute to a rapid proliferation of *E. coli.* The possible mechanisms of absorption of Stx2e from the intestine of the pig include specific receptor-mediated transcytosis, nonspecific transcytosis through enterocytes, paracellular transcytosis and antigenic sampling through M cells. Strains of STEC may pass from intestine to the mesenteric lymph nodes and produce toxin there, providing another mechanism for absorption of Stx2e into the blood (Fairbrother and Gyles, 2012).

2.2. Systemic effects

Following intestinal absorption, Stx2e is presumed to travel in the systemic circulation bound to the surface of erythrocytes, which express high levels of its preferred ligand, Gb4. Because of the interaction of Stx2e and erythrocytes, the distribution of Stx2e *in vivo* was affected by tissue blood flow, unlike Stx1 or the GT3 mutant, which did not bind significantly to erythrocytes (Fuller et al., 2011).

Clinical manifestations of ED are caused by systemic effects of Stx2e and despite the fact that they depend of the presence or absence of Stx2e receptor, there are additional factors conditioning if toxin leads to intoxication (Paton and Paton, 1998). The disease is clinically characterized by neurologic signs such as ataxia, incoordination and recumbency and/or subcutaneous and submucosal edema in various tissues (Matise et al., 2000); usually no diarrhea or fever is presented. A spectrum of clinical signs is reported although sudden death without apparent clinical signs could result from severe infections. In this presentation a subclinical edema may occur and pigs did not show characteristic neurologic signs. However, animals decreased growth rate and develop vascular lesions with no subcutaneous edema. Chronic disease occurs from acute episodes of edema or diarrhea caused by strains that also produce Stx2e.

The classical lesion is vascular necrosis of arterioles, commonly in the brain (brainstem, meninges, and cerebrum) and intestinal tract (submucosa of stomach, intestine and mesentery). Rarely, vascular lesions can be identified in other organs including pancreas, liver, and kidney (Kausche et al., 1992). Vascular damage with resultant infarction and malacia in the brain stem is the main cause of death in affected pigs. An increase of blood pressure was observed after injection of a partial purified Stx2e preparation as a result of edema and vessels damage could be exacerbated by resultant hypertension (Clugston et al., 1974).

A post mortem examination determined pathological changes in various parts of the body including subcutaneous edema, which is typically most pronounced on the eyelids, in the submandibular, thoracic and abdominal areas including the mesocolon (Imberechts et al., 1992). Histological examinations revealed vascular edema in the brain. In piglets, a determined dose of intravascular Stx2e induced characteristic lesions of clinical ED such as necrosis of arterioles and brain hemorrhage, and higher doses of Stx2e resulted in surface epithelial necrosis of colon and lesions of kidney (Gannon and Gyles, 1990). Endothelial cell necrosis in the brain is an early event in the pathogenesis of ED caused by Stx2e-producing STEC strains suggesting weakening and leakage of the endothelial blood–brain barrier, which might explain severe brain tissue lesions during the disease (Matise et al., 2000). Stx2e exhibited strong cytotoxic effects on the endothelial monolayer and a rapid

collapse of the blood–brain barrier in an *in vitro* model (Meisen et al., 2013). It has been hypothetized that the rapid decline of the transepithelial electrical resistance of the blood–brain barrier model is the initiation process that may end up in severe endothelial damage as a result of necrotic processes as shown by the *in vivo* observation of Matise et al. (2000). These data suggest the involvement of Stx2e in cerebral vascular damage with resultant neurological disturbance, characteristic of ED. The comparative effects of Stx2e with those of other Stx-like toxins in pigs and in other experimental animals show some differences. For all Stx-like toxins, the underlying mechanism is damage to the vascular endothelium. There is, however, a variation in the organs affected. In hemolytic uremic syndrome associated with Stx producing *E. coli*, the kidneys are affected. In contrast, the kidneys are not affected in experimentally reproduced ED in pigs.

3. STEC prevalence in swine population and production chain in Argentina

The prevalence of STEC in swine has been reported in numerous studies in multiple regions of the world indicating that strains are widely distributed and their frequency can vary from one region to another and over time (Tseng et al., 2014). The presence of STEC in swine populations has also been documented in Argentine. Cicuta et al. (1999) studied diarrheic or asymptomatic piglets from intensive farms in several regions of the country (Corrientes, Chaco, Santa Fe, Buenos Aires) and detected 2.1% Stx2e positive samples. In 2000, Parma et al., described the existence of porcine toxigenic *E. coli* in farms from central and northeast region of the country in 5% of the piggeries analyzed. Samples were derived from animals with or without diarrhea. Although no toxin gene (Stx1, Stx2a, Stx2e) was detected in healthy pigs they were found in animals showing delayed growth and, as expected, in those with diarrhea. Another group from Santa Fe suggested that healthy pigs are a reservoir of STEC due to they found a high percentage of STEC and the presence of human infection-associated serogroups (Notario et al., 2000). In contrast, isolates from nondiarrheic animals from central region of Argentina (Buenos Aires) had not genes related with human illness such as intimin (eae) or STEC autoagglutinating adhesin (saa), among others. Most of them had combination of genes indicators of pathogenicity for pigs (aidA, fedA and astA). STEC serogroups/stx2e detected were not those commonly associated with post-weaning diarrhea or edema (O138, O139, O141, and O149) diseases (Moredo et al., 2012). In the same year, another group updated information about presence of *E. coli* among newborn piglets in an intensive farm from Córdoba (central region of the country) concluding that in spite of the application of strict sanitary measures, virulence factors of *E. coli* are still detected (Alustiza et al., 2012).

In relation to Stx2e in pig retail, data is not consistent; differences are derived from multiple factors including types of product and technical protocols (sampling, isolation, diagnostic test). Regarding to STEC – Stx2e, it had been isolated from pork products and detected from human hosts with diarrhea, hemolytic uremic syndrome or uncomplicated diarrhea (Sonntag et al., 2005; Kaufmann et al., 2006; Trotz-Williams et al., 2012). Although outbreaks associated with pigs have been rarely reported, these animals cannot be excluded as source of STEC (Baranzoni et al., 2016). Numerous STEC genotypes coexist in swine population without clinical manifestation of neonatal or postweaning diarrhea or ED in different production stages (Moredo et al., 2015). Recently, Colello reported the prevalence and characterization of STEC throughout the pork production chain in Argentina (2016). They suggested the vertical transmission from farm to market even when the prevalence of Stx2e decreased from animals to products. However, it is unknown if the contamination occurs during the processing or by cross contamination (Tseng et al., 2014). Considering that ED causes economic losses in pork production (Kaufmann et al., 2006) this work emphasizes the need to improve a STEC control system in production.

4. Control and perspective

Edema disease management is arduous; actually, few commercial vaccine or effective treatments are available. Several experimental approaches have been shown to be effective, but none are economical. Modifications of feed (high fiber and low protein diet), limiting the amount of feed or *ad libitum* feeding of fiber modulate fimbrial receptors (Kelly et al., 1994) which may be involved in a reduced colonization by *E. coli* after weaning (Bertschinger et al., 1993) and might decreased production of toxic protein metabolites (Halas et al., 2007). Furthermore, it seems that these strategies might prevent new cases during outbreaks of disease. Inclusion of organic and inorganic acids in the feed did not alter lethality rate due to ED (Johansen et al., 2000). Some promising results related with minor incidence of clinical signs were obtained using pre and probiotic supplementation (Konstantinov et al., 2008; Tsukahara et al., 2007). Supplementation of piglets diet with ZnO might reduces postweaning diseases through reducing *E. coli* adhesion and intestinal permeability, and modulation of cytokine gene expression instead of growth modulation (Roselli et al., 2003; Kim et al., 2012). However, this practice is not fully accepted due to environmental accumulation of minerals.

Control of bacterial proliferation in ED is difficult due to the toxin has already been absorbed into the circulation and bound to receptors when clinical is clear. Antimicrobial therapy, often used to protect unaffected animals, may increase antibiotic-resistant isolates of healthy pigs or with clinical signs non-compatible with diarrhea (Moredo et al., 2007). Indeed, isolates from *E. coli* edema disease show the highest rate of resistance within porcine *E. coli* (Fairbrother and Gyles, 2012). Moreover, due to the rapid course of the illness, treatment comes too late for piglets with clinical signs.

Immunoprophylaxis has been also employed in ED management including vaccinating pregnant sows and piglets with purified/detoxified wild and recombinant toxin or Stx2e preparations (Johansen et al., 1997; MacLeod and Gyles, 1990; Oanh et al., 2012) with inactivated *E. coli* strains (Cicuta et al., 1999), purified F18 fimbriae (Verdonck et al., 2007) or passive systemic immunization with antitoxin (Johansen et al., 2000) or specific antibodies (Imberechts et al., 1997; Owusu-Asiedu et al., 2002). The best results in edema prevention and reduction of mortality were achieved with vaccination with toxin preparations.

Further studies are needed to generate techniques and tools which allow to understand the circulation and ecology of STEC strains in pigs even in resistant animals for diagnostic and epidemiological purposes.

Conflicts of interest

The authors declare no conflict of interest.

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References

Alexa, P., Hamrik, J., Stouracova, K., Konstantinova, L., Salajka, E., 2004. Passive immunoprophylaxis of edema disease in weaned piglets. Vet. Med. – Czech 49, 447–452.

Alustiza, F.E., Picco, N.Y., Bellingeri, R.V., Terzolo, H.R., Vivas, A.B., 2012. Frequency of virulence genes of *Escherichia coli* among newborn piglets from an intensive pig farm in Argentina. Rev. Argent. Microbiol. 44, 250–254.

Baranzoni, G.M., Fratamico, P.M., Gangiredla, J., Patel, I., Bagi, L.K., Delannoy, S., Fach, P., Boccia, F., Anastasio, A., Pepe, T., 2016. Characterization of shiga toxin subtypes and virulence genes in porcine shiga toxin-producing Escherichia coli. Front. Microbiol. 7, 1–10. https://doi.org/10.3389/fmicb.2016.00574.

Beddoe, T., Paton, A.W., Le Nours, J., Rossjohn, J., Paton, J.C., 2010. Structure, biological functions and applications of the AB5 toxins. Trends biochem. Sci. 35, 411–418. https://doi.org/10.1016/j.tibs.2010.02.003.

Bertschinger, H.U., Pohlenz, J., 1983. Bacterial colonization and morphology of the intestine in porcine *Escherichia Coli* enterotoxemia (edema disease). Vet. Pathol. 20, 99–110. https://doi.org/10.1177/030098588302000111.

Bertschinger, H.U., Stamm, M., Vogeli, P., 1993. Inheritance of resistance to oedema disease in the pig: experiments with an Escherichia coli strain expressing fimbriae 107. Vet. Microbiol. 35, 79–89. https://doi.org/10.1016/0378-1135(93)90117-P.

Beutin, L., Fach, P., 2014. Detection of shiga toxin-producing Escherichia coli from nonhuman sources and strain typing. Microbiol. Spectr. 2, 1–23. https://doi.org/10.1128/microbiolspec.EHEC-0001-2013.Correspondence.

Boyd, B., Tyrrell, G., Maloney, M., Gyles, C., Brunton, J., Lingwood, C., 1993. Alteration of the glycolipid binding specificity of the pig edema toxin from globotetraosyl to globotriaosyl ceramide alters in vivo tissue targetting and results in a verotoxin 1-like disease in pigs. J. Exp. Med. 177, 1745–1753.

Cicuta, M., Parma, A., Viñas, M., Sanz, M., Boehringer, S., Roibón, W., Benitez, M., Barceló, M., Vena, M., 1999. Factores de virulencia de Escherichia coli aisladas de porcinos en Argentina. Rev. Vet. 10/11, 11–13.

Clugston, R.E., Nielsen, N.O., Smith, D.L., 1974. Experimental edema disease of swine (E. coli enterotoxemia). 3. Pathology and pathogenesis. Can. J. Comp. Med. 38, 34–43.

Colello, R., Caceres, M.E., Ruiz, M.J., Sanz, M., Etcheverria, A.I., Padola, N.L., 2016. From farm to table: follow-up of Shiga toxin-producing Escherichia coli throughout the pork production chain in Argentina. Front. Microbiol. 7, 1–7. https://doi.org/10.3389/fmicb.2016.00093.

Cornick, N.A., Matise, I., Samuel, J.E., Bosworth, B.T., Moon, H.W., 2000. Shiga toxin–producing *Escherichia coli* infection: temporal and quantitative relationships among colonization, toxin production, and systemic disease. J. Infect. Dis. 181, 242–251. https://doi.org/10.1086/315172.

DebRoy, C., Roberts, E., Scheuchenzuber, W., Kariyawasam, S., Jayarao, B.M., 2009. Comparison of genotypes of Escherichia coli strains carrying F18ab and F18ac fimbriae from pigs. J. Vet. Diagn. Invest. 21, 359–364. https://doi.org/10.1177/104063870902100310.

DeGrandis, S., Law, H., Brunton, J., Gyles, C., Lingwood, C.A., 1989. Globotetraosylceramide is recognized by the pig edema disease toxin. J. Biol. Chem. 264, 12520–12525.

Endo, Y., Tsurugi, K., Yutsudo, T., Takeda, Y., Ogasawara, T., Igarashi, K., 1988. Site of action of a Vero toxin (VT2) from Escherichia coli O157:H7 and of Shiga toxin on eukaryotic ribosomes. RNA N-glycosidase activity of the toxins. Eur. J. Biochem. 171, 45–50.

Etcheverría, A.I., Padola, N.L., 2013. Shiga toxin-producing Escherichia coli: factors involved in virulence and cattle colonization. Virulence 4, 366–372. https://doi.org/10.4161/viru.24642.

Fairbrother, J.M., Gyles, C.L., 2012. Colibacillosis. In: Diseases of Swine, tenth ed. Wiley-Blackwell. https://doi.org/10.1017/CBO9781107415324.004.

Falguières, T., Mallard, F., Baron, C., Hanau, D., Lingwood, C., Goud, B., Salamero, J., Johannes, L., 2001. Targeting of Shiga toxin B-subunit to retrograde transport route in association with detergent-resistant membranes. Mol. Biol. Cell 12, 2453–2468.

Fratamico, P.M., Bhagwat, A.A., Injaian, L., Fedorka-Cray, P.J., 2008. Characterization of Shiga toxin-producing Escherichia coli strains isolated from swine feces. Foodborne Pathog. Dis. 5, 827–838. https://doi.org/10.1089/fpd.2008.0147.

Friedrich, A.W., Borell, J., Bielaszewska, M., Fruth, A., Tschäpe, H., Karch, H., 2003. Shiga toxin 1c-producing Escherichia coli strains: phenotypic and genetic characterization and association with human disease. J. Clin. Microbiol. 41, 2448–2453.

Fuchs, G., Mobassaleh, M., Donohue-Rolfe, A., Montgomery, R.K., Grand, R.J., Keusch, G.T., 1986. Pathogenesis of Shigella diarrhea: rabbit intestinal cell microvillus membrane binding site for Shigella toxin. Infect. Immun. 53, 372–377.

Fuller, C.A., Pellino, C.A., Flagler, M.J., Strasser, J.E., Weiss, A.A., 2011. Shiga toxin subtypes display dramatic differences in potency. Infect. Immun. 79, 1329–1337. https://doi.org/10.1128/IAI.01182-10.

Gannon, V.P.J., Gyles, C.L., 1990. Characteristics of the Shiga-like toxin produced by Escherichia coli associated with porcine edema disease. Vet. Microbiol. 24, 89–100. https://doi.org/10.1016/0378-1135(90)90054-Y.

Garred, O., van Deurs, B., Sandvig, K., 1995. Furin-induced cleavage and activation of Shiga toxin. J. Biol. Chem. 270, 10817–10821.

Grange, P.A., Parrish, L.A., Erickson, A.K., 2006. Expression of putative Escherichia coli heat-labile enterotoxin (LT) receptors on intestinal brush borders from pigs of different ages. Vet. Res. Commun. 30, 57–71. https://doi.org/10.1007/s11259-005-3225-9.

Gyles, C.L., De Grandis, S.A., MacKenzie, C., Brunton, J.L., 1988. Cloning and nucleotide sequence analysis of the genes determining verocytotoxin production in a porcine edema disease isolate of Escherichia coli. Microb. Pathog. 5, 419–426.

Halas, D., Heo, J.M., Hansen, C.F., Kim, J.C., Hampson, D.J., Mullan, B.P., Pluske, J.R., 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, 13. https://doi.org/10.1079/PAVSNNR20072079.

Imberechts, H., De Greve, H., Lintermans, P., 1992. The pathogenesis of edema disease in pigs. A review. Vet. Microbiol. 31, 221–233.

Imberechts, H., Deprez, P., Van Driessche, E., Pohl, P., 1997. Chicken egg yolk antibodies against F18ab fimbriae of Escherichia coli inhibit shedding of F18 positive E. coli by experimentally infected pigs. Vet. Microbiol. 54, 329–341.

Johannes, L., Römer, W., 2010. Shiga toxins–from cell biology to biomedical applications. Nat. Rev. Microbiol. 8, 105–116. https://doi.org/10.1038/nrmicro2279.

Johansen, M., Andresen, L.O., Jorsal, S.E., Thomsen, L.K., Waddell, T.E., Gyles, C.L., 1997. Prevention of edema disease in pigs by vaccination with verotoxin 2e toxoid. Can. J. Vet. Res. 61, 280–285.

Johansen, M., Andresen, L.O., Thomsen, L.K., Busch, M.E., Wachmann, H., Jorsal, S.E., Gyles, C.L., 2000. Prevention of edema disease in pigs by passive immunization. Can. J. Vet. Res. 64, 9–14.

Kandel, G., Donohue-Rolfe, A., Donowitz, M., Keusch, G.T., 1989. Pathogenesis of Shigella diarrhea. XVI. Selective targetting of Shiga toxin to villus cells of rabbit jejunum explains the effect of the toxin on intestinal electrolyte transport. J. Clin. Invest. 84, 1509–1517. https://doi.org/10.1172/JCI114327.

Karve, S.S., Weiss, A.A., 2014. Glycolipid binding preferences of shiga toxin variants. PLoS ONE 9 (7), e101173. https://doi.org/10.1371/journal.pone.0101173.

Kaufmann, M., Zweifel, C., Blanco, M., Blanco, J.E., Blanco, J., Beutin, L., Stephan, R., 2006. Escherichia coli O157 and non-O157 Shiga toxin-producing Escherichia coli in fecal samples of finished pigs at slaughter in Switzerland. J. Food Prot. 69, 260–266.

Kausche, F.M., Dean, E.A., Arp, L.H., Samuel, J.E., Moon, H.W., 1992. An experimental model for subclinical edema disease (Escherichia coli enterotoxemia) manifest as vascular necrosis in pigs. Am. J. Vet. Res. 53, 281–287.

Keenan, K.P., Sharpnack, D.D., Collins, H., Formal, S.B., O'Brien, A.D., 1986. Morphologic evaluation of the effects of Shiga toxin and E coli Shiga-like toxin on the rabbit intestine. Am. J. Pathol. 125, 69–80.

Kelly, D., Begbie, R., King, T.P., 1994. Nutritional influences on interactions between bacteria and the small intestinal mucosa. Nutr. Res. Rev. 7, 233–257. https://doi.org/10.1079/NRR19940013.

Keusch, G.T., Jacewicz, M., Mobassaleh, M., Donohue-Rolfe, A., 1991. Shiga toxin: intestinal cell receptors and pathophysiology of enterotoxic effects. Rev. Infect. Dis. 13 (Suppl. 4), S304–S310.

Kim, J.C., Hansen, C.F., Mullan, B.P., Pluske, J.R., 2012. Nutrition and pathology of weaner pigs: nutritional strategies to support barrier function in the gastrointestinal tract. Anim. Feed Sci. Technol. 173, 3–16. https://doi.org/10.1016/j.anifeedsci.2011.12.022.

Konstantinov, S.R., Smidt, H., Akkermans, A.D.L., Casini, L., Trevisi, P., Mazzoni, M., De Filippi, S., Bosi, P., de Vos, W.M., 2008. Feeding of Lactobacillus sobrius reduces Escherichia coli F4 levels in the gut and promotes growth of infected piglets. FEMS Microbiol. Ecol. 66, 599–607. https://doi.org/10.1111/j.1574-6941.2008.00517.x.

Konowalchuk, J., Speirs, J.I., Stavric, S., 1977 Dec. Vero response to a cytotoxin of Escherichia coli. Infect. Immun. 18 (3), 775–779.

Leung, P.H., Peiris, J.S., Ng, W.W., Robins-Browne, R.M., Bettelheim, K.A., Yam, W.C., 2003. A newly discovered verotoxin variant, VT2g, produced by bovine verocytotoxigenic Escherichia coli. Appl. Environ. Microbiol. 69 (12), 7549–7553.

Li, Z., Bell, C., Buret, A., Robins-Browne, R., Stiel, D., O'Loughlin, E., 1993. The effect of enterohemorrhagic Escherichia coli O157:H7 on intestinal structure and solute transport in rabbits. Gastroenterology 104, 467–474.

Lingwood, C.A., Binnington, B., Manis, A., Branch, D.R., 2010. Globotriaosyl ceramide receptor function - where membrane structure and pathology intersect. FEBS Lett. 584, 1879–1886. https://doi.org/10.1016/j.febslet.2009.11.089.

MacLeod, D.L., Gyles, C.L., 1990. Purification and characterization of an Escherichia coli Shiga-like toxin II variant. Infect. Immun. 58, 1232–1239.

MacLeod, D.L., Gyles, C.L., Valdivieso-Garcia, A., Clarke, R.C., 1991. Physicochemical and biological properties of purified Escherichia coli Shiga-like toxin II variant. Infect. Immun. 59, 1300–1306.

Mahfoud, R., Manis, A., Binnington, B., Ackerley, C., Lingwood, C.A., 2010. A major fraction of glycosphingolipids in model and cellular cholesterol-containing membranes is undetectable by their binding proteins. J. Biol. Chem. 285, 36049–36059. https://doi.org/10.1074/jbc.M110.110189.

Marques, L.R.M., Peiris, J.S.M., Cryz, S.J., O'Brien, A.D., 1987. *Escherichia coli* strains isolated from pigs with edema disease produce a variant of Shiga-like toxin II. FEMS Microbiol. Lett. 44, 33–38. https://doi.org/10.1111/j.1574-6968.1987.tb02237.x.

Matise, I., Sirinarumitr, T., Bosworth, B.T., Moon, H.W., 2000. Vascular ultrastructure and DNA fragmentation in swine infected with Shiga toxin-producing Escherichia coli. Vet. Pathol. 37, 318–327. https://doi.org/10.1354/vp.37-4-318.

Meisen, I., Rosenbrück, R., Galla, H.-J., Hüwel, S., Kouzel, I.U., Mormann, M., Karch, H., Müthing, J., 2013. Expression of Shiga toxin 2e glycosphingolipid

receptors of primary porcine brain endothelial cells and toxin-mediated breakdown of the blood-brain barrier. Glycobiology 23, 745–759. https://doi.org/10.1093/glycob/cwt013.

Methiyapun, S., Pohlenz, J.F.L., Bertschinger, H.U., 1984. Ultrastructure of the intestinal mucosa in pigs experimentally inoculated with an edema disease-producing strain of *Escherichia coli* (0139:K12:H1). Vet. Pathol. 21, 516–520. https://doi.org/10.1177/030098588402100511.

Mobassaleh, M., Gross, S.K., McCluer, R.H., Donohue-Rolfe, A., Keusch, G.T., 1989. Quantitation of the rabbit intestinal glycolipid receptor for Shiga toxin. Further evidence for the developmental regulation of globotriaosylceramide in microvillus membranes. Gastroenterology 97, 384–391.

Moredo, F.A., Piñeyro, P.E., Márquez, G.C., Sanz, M., Colello, R., Etcheverría, A., Padola, N.L., Quiroga, M.A., Perfumo, C.J., Galli, L., Leotta, G.A., 2015. Enterotoxigenic Escherichia coli subclinical infection in pigs: bacteriological and genotypic characterization and antimicrobial resistance profiles. Foodborne Pathog. Dis. 12, 704–711. https://doi.org/10.1089/fpd.2015.1959.

Moredo, F.A., Vigo, G.B., Cappuccio, J.A., Piñeyro, P., Perfumo, C.J., Giacoboni, G.I., 2007. Antimicrobial resistance of Escherichia coli isolated from pigs in Argentina. Rev. Argent. Microbiol. 39, 227–229.

Moredo, F. a, Cappuccio, J. a, Insarralde, L., Perfumo, C.J., Quiroga, M. a, Leotta, G. a, 2012. Genotypic characterization of toxigenic Escherichia coli isolated from pigs with postweaning diarrhea (PWD) and edema disease (ED). Rev. Argent. Microbiol. 44, 85–88.

Muniesa, M., Recktenwald, J., Bielaszewska, M., Karch, H., Schmidt, H., 2000. Characterization of a Shiga Toxin 2e-Converting Bacteriophage from an Escherichia coli Strain of Human Origin. Infect. Immun. 68 (9), 4850–4855.

Müthing, J., Meisen, I., Zhang, W., Bielaszewska, M., Mormann, M., Bauerfeind, R., Schmidt, M.A., Friedrich, A.W., Karch, H., 2012. Promiscuous Shiga toxin 2e and its intimate relationship to Forssman. Glycobiology 22, 849–862. https://doi.org/10.1093/glycob/cws009.

Müthing, J., Schweppe, C.H., Karch, H., Friedrich, A.W., 2009. Shiga toxins, glycosphingolipid diversity, and endothelial cell injury. Thromb. Haemost. 101, 252–264.

Notario, R., Fain, J.C., Prado, V., Ríos, M., Borda, N., Gambandé, T., 2000. Prevalence of enterohemorrhagic Escherichia coli in a cattle area of Argentina. Genotypic characterization of the strains of animal origin. Rev. Med. Chil. 128, 1335–1341.

Oanh, T.K.N., Nguyen, V.K., De Greve, H., Goddeeris, B.M., 2012. Protection of piglets against edema disease by maternal immunization with Stx2e toxoid. Infect. Immun. 80, 469–473. https://doi.org/10.1128/IAI.05539-11.

O'Brien, A.D., Tesh, V.L., Donohue-Rolfe, A., Jackson, M.P., Olsnes, S., Sandvig, K., Lindberg, A.A., Keusch, G.T., 1992. Shiga toxin: biochemistry, genetics, mode of action, and role in pathogenesis. Curr. Top. Microbiol. Immunol. 180, 65–94.

Ohmura-Hoshino, M., Ho, S.-T., Kurazono, H., Igarashi, K., Yamasaki, S., Takeda, Y., 2003. Genetic and immunological analysis of a novel variant of Shiga toxin 1 from bovine Escherichia coli strains and development of bead-ELISA to detect the variant toxin. Microbiol. Immunol. 47, 717–725.

Owusu-Asiedu, A., Baidoot, S.K., Nyachoti, C.M., Marquardt, R.R., 2002. Response of early-weaned pigs to spray-dried porcine or animal plasma-based diets supplemented with egg-yolk antibodies against enterotoxigenic Escherichia coli. J. Anim. Sci. 80, 2895–2903.

Parma, A., Sanz, M., Viñas, M., Cicuta, M., Blanco, J., Boehringer, S., Vena, M., Roibon, W., Benitez, M., Blanco, J., Blanco, M., 2000. Toxigenic Escherichia coli isolated from pigs in Argentina. Vet. Microbiol. 72, 269–276. https://doi.org/10.1016/S0378-1135(99)00167-4.

Paton, J.C., Paton, A.W., 1998. Pathogenesis and diagnosis of Shiga toxin-producing Escherichia coli infections. Clin. Microbiol. Rev. 11, 450–479.

Piérard, D., Muyldermans, G., Moriau, L., Stevens, D., Lauwers, S., 1998. Identification of new verocytotoxin type 2 variant B-subunit genes in human and animal Escherichia coli isolates. J. Clin. Microbiol. 36, 3317–3322.

Roselli, M., Finamore, A., Garaguso, I., Britti, M.S., Mengheri, E., 2003. Zinc oxide protects cultured enterocytes from the damage induced by Escherichia coli. J. Nutr. 133, 4077–4082.

Samuel, J.E., Perera, L.P., Ward, S., O'Brien, A.D., Ginsburg, V., Krivan, H.C., 1990. Comparison of the glycolipid receptor specificities of Shiga-like toxin type II and Shiga-like toxin type II variants. Infect. Immun. 58, 611–618.

Scheutz, F., Beutin, L., Piérard, D., Karch, H., Tozzoli, R., Caprioli, A., O' Brien, A., Melton-Celsa, A., Teel, L., Strockine, N., 2009. Nomenclature of verotoxins: a review, a proposal, and a protocol for typing vtx genes. In: 4th Annu. Workshop Commun. Ref. Lab. E. coli VTEC. ISS, Rome, Italy.

Scheutz, F., Teel, L.D., Beutin, L., Pierard, D., Buvens, G., Karch, H., Mellmann, A., Caprioli, A., Tozzoli, R., Morabito, S., Strockbine, N.A., Melton-Celsa, A.R., Sanchez, M., Persson, S., O'Brien, A.D., 2012. Multicenter evaluation of a sequence-based protocol for subtyping shiga toxins and standardizing stx nomenclature. J. Clin. Microbiol. 50, 2951–2963. https://doi.org/10.1128/JCM.00860-12.

Schmidt, H., Scheef, J., Morabito, S., Caprioli, A., Wieler, L., Karch, H., 2000. A new shiga toxin 2 variant (Stx2f) from Escherichia coli isolated from pigeons. Appl. Environ. Microbiol. 66 (3), 1205–1208.

Smith, H.W., Linggood, M.A., 1971. The effect of antisera in protecting pigs against experimental Escherichia coli diarrhoea and oedema disease. J. Med. Microbiol. 4, 487–493. https://doi.org/10.1099/00222615-4-4-487.

Sonntag, A.-K., Bielaszewska, M., Mellmann, A., Dierksen, N., Schierack, P., Wieler, L.H., Schmidt, M.A., Karch, H., 2005. Shiga toxin 2e-producing Escherichia coli isolates from humans and pigs differ in their virulence profiles and interactions with intestinal epithelial cells. Appl. Environ. Microbiol. 71, 8855–8863. https://doi.org/10.1128/AEM.71.12.8855-8863.2005.

Strockbine, N.A., Marques, L.R., Newland, J.W., Smith, H.W., Holmes, R.K., O'Brien, A.D., 1986. Two toxin-converting phages from Escherichia coli O157:H7 strain 933 encode antigenically distinct toxins with similar biologic activities. Infect. Immun. 53, 135–140.

Trotz-Williams, L.A., Mercer, N.J., Walters, J.M., Maki, A.M., Johnson, R.P., 2012. Pork implicated in a Shiga toxin-producing Escherichia coli O157:H7 outbreak in Ontario, Canada. Can. J. Public Health 103, e322–e326.

Tseng, M., Fratamico, P.M., Manning, S.D., Funk, J.A., 2014. Shiga toxin-producing Escherichia coli in swine: the public health perspective. Anim. Heal. Res. Rev. 15, 63–75. https://doi.org/10.1017/S1466252313000170.

Tsukahara, T., Inoue, R., Nakanishi, N., Nakayama, K., Matsubara, N., Ushida, K., 2007. Evaluation of the low dose level of a heat-killed and dried cell preparation of Enterococcus faecalis to prevent porcine edema disease using experimental infection model with enterotoxemic Escherichia coli in weaning pigs. J. Vet. Med. Sci. 69, 103–109. https://doi.org/10.1292/jvms.69.103.

Valdivieso-Garcia, A., MacLeod, D.L., Clarke, R.C., Gyles, C.L., Lingwood, C., Boyd, B., Durette, A., 1996. Comparative cytotoxicity of purified Shiga-like toxin-IIe on porcine and bovine aortic endothelial and human colonic adenocarcinoma cells. J. Med. Microbiol. 45, 331–337. https://doi.org/10.1099/00222615-45-5-331.

Verdonck, F., Tiels, P., van Gog, K., Goddeeris, B.M., Lycke, N., Clements, J., Cox, E., 2007. Mucosal immunization of piglets with purified F18 fimbriae does not protect against F18+ Escherichia coli infection. Vet. Immunol. Immunopathol. 120, 69–79. https://doi.org/10.1016/j.vetimm.2007.06.018.

Vernozy-Rozand, C., Montet, M.P., Bertin, Y., Trably, F., Girardeau, J.P., Martin, C., Livrelli, V., Beutin, L., 2004. Serotyping, stx2 subtyping, and characterization of the locus of enterocyte effacement island of shiga toxin-producing Escherichia coli and E. coli O157:H7 strains isolated from the environment in France. Appl. Environ. Microbiol. 70, 2556–2559.

Waddell, T., Gyles, C., 1995. Sodium Deoxycholate Facilitates Systemic Absorption of Verotoxin 2e from Pig Intestine. Infect. Immun. 63 (12), 4953–4956.

Waddell, T.E., Lingwood, C.A., Gyles, C.L., 1996. Interaction of verotoxin 2e with pig intestine. Infect. Immun. 64, 1714–1719.

Weinstein, D.L., Jackson, M.P., Samuel, J.E., Holmes, R.K., O'Brien, A.D., 1988. Cloning and sequencing of a Shiga-like toxin type II variant from Escherichia coli strain responsible for edema disease of swine. J. Bacteriol. 170, 4223–4230.

Weström, B.R., Svendsen, J., Ohlsson, B.G., Tagesson, C., Karlsson, B.W., 1984. Intestinal transmission of macromolecules (BSA and FITC-labelled dextrans) in the neonatal pig. Influence of age of piglet and molecular weight of markers. Biol. Neonate 46, 20–26.

Zhang, W., Bielaszewska, M., Kuczius, T., Karch, H., 2002. Identification, characterization, and distribution of a Shiga toxin 1 gene variant (stx(1c)) in Escherichia coli strains isolated from humans. J. Clin. Microbiol. 40, 1441–1446.