Prevalence of enterotoxigenic and Shiga toxin-producing Escherichia coli in pigs slaughtered in Mato Grosso, Brazil

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Brief Original Article

Prevalence of enterotoxigenic and Shiga toxin-producing *Escherichia coli* in pigs slaughtered in Mato Grosso, Brazil

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Abstract

Introduction: This study aimed to estimate the prevalence of enterotoxigenic *Escherichia coli* (ETEC) and Shiga toxin-producing *Escherichia coli* (STEC) strains in pigs slaughtered in abattoirs located in the state of Mato Grosso, Brazil.
Methodology: Intestinal samples from 74 animals were aseptically dissected and lumen content was plated on MacConkey agar. Confluent colonies from each plate were screened for the presence of ETEC and STEC strains by PCR assays.
Results: It was verified that the prevalence of STEC and ETEC carriers was 1.35% and 9.46% respectively. One (1.35%) of the 74 samples tested was positive for the stx2 gene, and seven (9.46%) for st1, of which two (2.70%) were also positive for lt1.
Conclusion: The results provided represent a benchmark for future research on pathogenic *E. coli* of porcine origin in Mato Grosso.

Key words: STEC; ETEC; PCR; slaughterhouse; Mato Grosso

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Introduction

Escherichia coli is one of the main inhabitants of the intestinal tract of mammalian species [1]. Until the 1950s this microorganism was regarded as a normal non-pathogenic cohabitant of the enteric tract [2]. However, during the last decades, a remarkable amount of research has established *E. coli* among the important etiological agents of enteritis and several extraintestinal diseases [3].

Enterotoxigenic *E. coli* (ETEC) is considered a major cause of infantile diarrhoea in developing countries and is frequently associated with traveller's diarrhoea [4]. In pigs, ETEC is a major cause of postweaning diarrhoea [5]. This *E. coli* pathotype causes diarrhoea by adhering to intestinal mucosa through its unique colonization factors and producing either heat-labile enterotoxins (LT-I and LT-II), heat-stable enterotoxins (STa and STb), or both [4]. Human diarrhoea due to ETEC, like other diarrhoeal illnesses, may be the result of ingestion of contaminated food or water [3]. However, due to the species-specific binding of the fimbrial adhesins, ETEC is not regarded as a zoonotic agent [2].

Another group of pathogenic *E. coli*, defined as Shiga toxin-producing *E. coli* (STEC), is a naturally occurring organism in the gut microbiota of cattle [6] and has been isolated from the intestine of other animals such as sheep [7] and goats [8]. According to Wasteson [2], STEC has emerged as an important zoonotic food-borne pathogen. Infection in humans is mainly associated with the ingestion of foods contaminated with these zoonotic bacteria, and clinical signs include watery diarrhoea, haemorrhagic colitis (HC), and/or haemolytic uraemic syndrome (HUS) [3]. The ability of STEC strains to cause severe disease is related to the production of one or more types of Shiga toxin (Stx1, Stx2 or variants) [6]. Pigs are not considered a major source of STEC [9] since its prevalence is usually very low in this species [1]. However, Shiga toxin-positive bacteria have been isolated from pork and some pork products involved in human infections [10]. It has been reported that, besides acting as sources of contamination of STEC-free animals via faeces [6], STEC carriers can introduce this pathogen into slaughterhouses [11].

Table 1. Sequences, amplicon sizes and annealing temperatures of oligonucleotide primers used

Gene	Sequence (5' – 3')	AT[a] (ºC)	Amplicon size (bp)	Reference
stx1	AGGTTGCAGCTCTCTTTGAATA TGCAAACAAATTATCCCCTGAG	53	364	[16]
stx2	GGGCAGTTATTTTGCTGTGGA GTATCTGCCTGAAGCGTAA	53	386	[16]
lt1	TATCCTCTCTATATGCACAG CTGTAGTGGAAGCTGTTATA	48	480	[16]
st1	TCCGTGAAACAACATGACGG ATAACATCCAGCACAGGCAG	60	244	[16]

[a] Annealing temperature

In Brazil, few epidemiologic studies on the occurrence of STEC have been conducted [12] and very few laboratories are able to perform STEC screening [13]. Furthermore, studies concerning ETEC from swine have focused on weaned or newborn pigs [14] and data regarding finishing pigs are not available. Given these considerations, this study aimed to estimate the prevalence of ETEC and STEC strains in the intestines of pigs slaughtered in abattoirs located in the state of Mato Grosso, Brazil, by PCR assays.

Methodology

Sample collection

Fragments of intestine (n = 74) were collected from pigs slaughtered in abattoirs located in the state of Mato Grosso, Brazil. Each fragment of approximately 5 cm was sampled from a different animal immediately after evisceration. Samples were placed in sterile plastic bags and kept under refrigeration until required for laboratory procedures (less than eight hours). To estimate the prevalence of ETEC and STEC carriers, a statistically adequate sample size was calculated on the basis of the number of 800,000 pigs slaughtered per year, an expected prevalence of 5%, a 95% confidence level and an accuracy of 5% using Win Episcope 2.0 (Universidad de Zaragoza, Zaragoza, Spain).

Enrichment and plating procedures

Each intestine fragment was aseptically dissected and a sterile swab was used to collect content from the lumen. All swabs were enriched in 10 ml of buffered peptone water (Oxoid, Basingstoke, Hampshire, United KingdomUnited Kingdom) and incubated in aerobiosis at 37º C for 24 hours. Afterward, about 10 µl of the culture was streaked on MacConkey agar (Oxoid, United Kingdom) plates and incubated in aerobiosis at 37ºC for 24 hours.

Screening for STEC and ETEC carriers by PCR assays

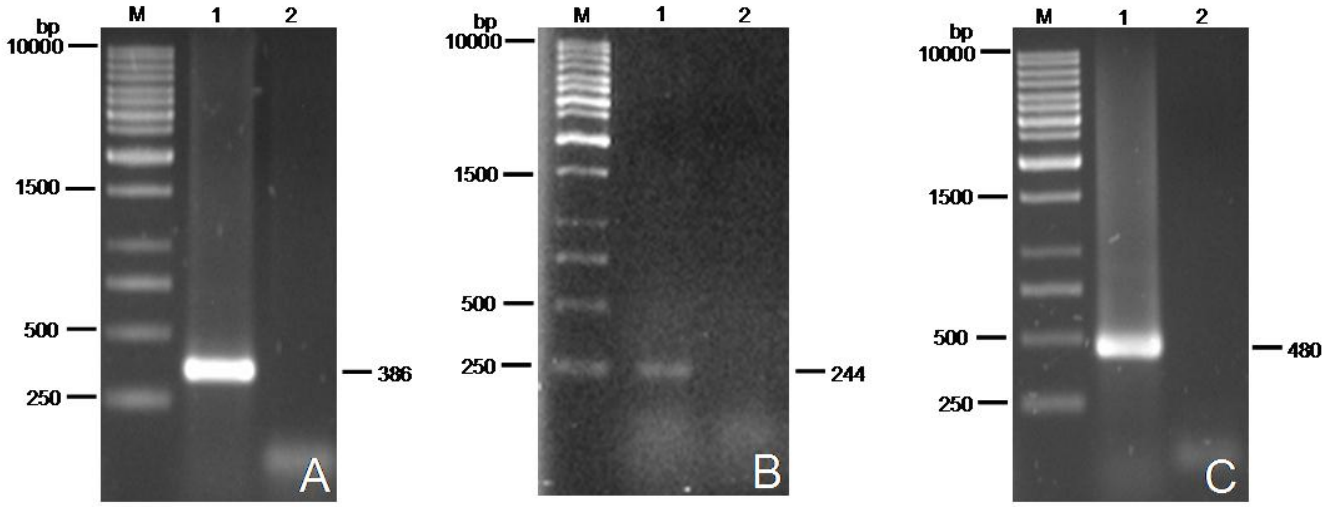
To detect STEC and ETEC strains, a loopful of confluent colonies from each MacConkey plate was tested by PCR assays for the presence of genes coding for Shiga toxin (*stx1* and *stx2*), heat-labile (*lt1*) and heat-stable (*st1*) enterotoxins. For DNA extractions, colonies collected were suspended in 200 µl of Ultra High Quality (UHQ)-water, boiled for 10 minutes and subsequently centrifuged at 10,000 x g for 2 minutes. The supernatant was used as template for PCR. PCR assays were conducted as described by Siqueira *et al*. [15]. Sequences, predicted sizes of the products, and specific annealing temperatures of the primers employed are described in Table 1. *E. coli* K12C600 was used as a negative control for the reactions. The STEC strains H30 (O26:H11) and J2 (O157:H-) were used as positive controls for *stx1* and *stx2* respectively. For the *lt1* and *st1* genes, the ETEC strain H100407 was employed as a positive control.

Pigs were considered STEC or ETEC carriers as follows: animals harbouring strains positive for *stx1* and/or *stx2* were considered STEC carriers; those harbouring isolates positive for *lt1* and/or *st1* were considered ETEC carriers.

Results and discussion

The observed prevalence of STEC and ETEC carriers was 1.35% and 9.46% respectively. One (1.35%) of the 74 samples was positive for *stx2*, and seven (9.46%) were positive for *st1*, of which two

Figure 1. Amplification of *stx2* (A), *st1* (B) and *lt1* (C) genes by PCR. Lanes M are DNA size markers (GeneRuler™ 1 kb DNA Ladder, Fermentas, USA). Lanes 1 and 2 are representative positive and negative strains respectively. bp – base pairs.



(2.70%) were also positive for the *lt1* gene (Figure 1). None of the samples tested positive for the *stx1* gene.

The low prevalence of STEC carriers asserted in this study is in agreement with preceding records. Leung *et al*. [9] reported the presence of STEC in 2.1% of pigs slaughtered in Hong Kong, and Oporto *et al*. [7] observed the absence of STEC in faecal samples from 17 Spanish swine herds. Bonardi *et al*. [17] and Heuvelink *et al*. [18] detected the occurrence of *E. coli* O157:H7, a highly human-virulent STEC strain, in 0.7% of pigs slaughtered in both Italian and Dutch abattoirs.

Results regarding ETEC revealed a higher prevalence when compared to STEC. It is well established that ETEC from animals normally do not infect humans, since these strains harbour colonization factors with remarkable host specificity [2]. However, infections by this *E. coli* pathotype have an important impact on porcine health and negative implications in the efficiency of porcine production systems [19]. In pigs, ETEC is often implicated in cases of postweaning diarrhoea [4]. Nevertheless, this pathogen is observed less frequently in the faeces of healthy animals [20] and rarely causes clinical infections in adult pigs [21]. Interestingly, almost 10% of the animals analysed in this study were ETEC carriers, and this result could indicate that adult carriers are sources of ETEC in piggeries from Mato Grosso. Faecal contamination is a contributing factor to the persistence of ETEC in the environment, and it also contributes to the transmission of this pathogen to animals through the contamination of food crops and water sources or from direct contact [19]. Therefore, it could be inferred that control of finishing pigs may help in the reduction of infections by ETEC at the herd level.

The low prevalence of STEC carriers asserted in this study suggests that slaughter pigs do not represent an important source of STEC in Mato Grosso. However, further research is necessary to better understand the role of carriers in the epidemiology of ETEC infections in pigs from this region.

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