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Genes Encoding Shiga Toxin and the Intimin Receptor Detected in Faecal Samples Collected from Wild Canids

Camila Imperico Riboldi[1], Cassiane Elisabete Lopes[1], Patrícia Bernardes Rodrigues Witt[2], Victor Hugo Valiati[2], João Fábio Soares[1] & Franciele Maboni Siqueira[1]

ABSTRACT

***Background*:** Enteropathogenic *Escherichia coli* (EPEC) and Shiga toxin-producing *E. coli* (STEC) are diarrheagenic *E. coli* that can cause disease in humans. The pathotype EPEC leads to the attaching and effacing lesion, causing damage to the microvilli following to diarrhea. STEC pathotypes produces cytotoxins, which in humans are responsible for hemorrhagic colitis or hemolytic uremic syndrome. Animals are the reservoirs of these pathotypes, especially ruminants. However, other animal's species can be associated as carriers of EPEC and STEC strains. The aim of this study was to analyze wild canid crab-eating fox (*Cerdocyon thous*) as potential natural carriers of STEC and EPEC *E. coli*.

***Materials, Methods & Results*:** Seven fecal samples were analyzed from the crab-eating fox of free-living, captured in a peri-urban area. Samples were collected from the rectal ampulla, and the animals were clinic evaluated, being considered healthy at the captured moment. The feces were inoculated on medium MacConkey agar, and then the plates were incubated at 37°C for 24 h. All colony forming units (CFU) were collected by plate washing with ultrapure water (2 mL) and posterior freezing at -20°C. The total bacterial DNA from the CFU collected was extracted, followed by PCR assay to search for three genes: *stx1*, *stx2* (responsible for the synthesis of the Shiga toxin) and *tir*, which encodes the translocated intimin receptor, related to the A/E lesion formation. Three samples were detected as positive, being one animal detected as carrier of the *stx2* gene (STEC strain), while two animals were identified as carrier of the *tir* gene (EPEC strains).The *stx1* gene was not identified on the samples. Also, in the samples, only the presence of one gene studied at a time was observed. Therefore, we have found out that the crab-eating fox can act as reservoirs of STEC and EPEC strains.

***Discussion*:** The carrier's animals of STEC and EPEC strains do not have receptors for the Shiga toxin, serving as asymptomatic vehicle. The wild canids collected and positives to STEC and EPEC *E. coli* strains analyzed here, did not shown any clinical signs, wherefore they have the potential of being a source of pathogens to other animals and even humans. The observation of this additional wild species, the crab-eating fox, as carriers of STEC and EPEC reinforces the importance of monitoring wild species and the need for caution because of the potential zoonosis. Nevertheless, there are other species of wild animals that were described as rarely-carriers of these pathogens, like deer, wild ruminants and birds. These *E. coli* pathotypes, potentially foodborne zoonotic pathogens, are acquired by humans from food and water contaminated, by oral-fecal route, or by direct contact with carries animals and their feces. The human contact with wild animals is getting ordinary; because of that, wild animals, like wild canids, may constitute a considerable risk to animal and humans health, mostly by the transmission of these bacteria strains to the environment. In conclusion, the identification of genes encoding Shiga toxin and the intimin receptor in wild canids feces highlight that STEC and EPEC pathogens could be spread by these wild animals. We reinforce that STEC and EPEC pathogens could be naturally carried by wild animals, such as the crab-eating fox, and therefore, is needed further research for better understanding their potential effect, and also the interaction between the pathogen and the host.

Keywords: *Escherichia coli*, EPEC, STEC, crab-eating fox, PCR.

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[1]Veterinary College, Federal University of Rio Grande do Sul (UFRGS), Porto Alegre, RS, Brazil. [2]University of Vale do Rio dos Sinos (UNISINOS), São Leopoldo, RS. CORRESPONDENCE: F.M. Siqueira [franciele.siqueira@ufrgs.com - Tel.: +55 (51) 3308 6115]. Veterinary Bacteriology Laboratory (LaBacVet), Department of Clinical Pathology (DPCV).Veterinary College, Federal University of Rio Grande do Sul (UFRGS). Av. Bento Gonçalves n. 9090. Bairro Agronomia. CEP 91540-000 Porto Alegre, RS, Brazil.

INTRODUCTION

The two most important diarrheagenic *Escherichia coli* that can cause disease in humans are the enteropathogenic *E. coli* (EPEC) and the Shiga toxin-producing *E. coli* (STEC) [4], being these pathotypes considered foodborne zoonotic pathogens [6]. EPEC causes the attaching and effacing (A/E) lesion, which is responsible for diarrhea, and effacement of surface-absorptive microvilli [11]. The bacteria bind to the host cells by the secretion and insertion of a translocated intimin receptor, which is encoded by the *tir* gene. This receptor is recognized by the intimin, an adhesion protein located on the surface of *E. coli*.

The STEC produces at least one type of a potent cytotoxin, named Shiga toxin or verotoxin [6]. Shiga toxins in humans are responsible by potentially life-threatening diarrhea and hemorrhagic colitis (HC) or hemolytic uremic syndrome (HUS) associated [7], being the HUS characterized by acute renal failure [14]. Also, some strains of STEC can develop A/E lesions [8], being considered as enterohemorrhagic *E. coli* (EHEC) pathotype [3].

Ruminants are the most important natural reservoir for EPEC and STEC strains [1,6]. However, there are other animals that can be rarely carriers of these pathogens, such as wild animals, like deer, wild ruminants [7], wild boars [1] and birds [1,4,5]. Humans usually acquire EHEC/STEC or EPEC from food and water contaminated or by direct contact with carries animals [9].

The aim of the present study was to evaluate feces from wild canid crab-eating fox (*Cerdocyon thous*) to screening marker genes to STEC and EPEC strains.

MATERIALS AND METHODS

We analyzed seven samples of feces from crab-eating fox of free living. These wild canids were randomly captured in a peri-urban area, in South Brazil. All samples were collected from the rectal ampulla, using sterile materials for collection. The animals were clinic evaluated by the responsible veterinarian, being considered healthy at the captured moment, without clinical signs of a systemic disease, and all the feces are non-diarrheic. After sample collection, the animals were again released into the wild. The feces were collected and stored in sterile containers and later transported at ice, arriving in less than 24 h in our laboratory.

The feces samples were inoculated on medium MacConkey agar[1], and then the plates were incubated at 37°C for 24 h in the aerobic atmosphere. Following the growth period, all colony forming units (CFU) were collected by the washing of the plate with ultrapure water (2 mL) and freezing at -20°C. Total bacterial DNA, from all the recovered CFU of each animal, was extracted with the kit QIAamp DNA Mini Kit[2], according to the manufacturer instructions. We have executed the PCR assay, as already previously described searching for three genes: *stx1*, *stx2* and *tir* [2] with primer-individual reactions. As a positive control, we have used the *E. coli* O157:H7 Sasaki strain. Amplicons were analyzed in electrophoresis by agarose gel stained with GelRed™[3] and UV light. Additionally, the PCR fragments were sequenced by Sanger technology to confirm the fragment identity.

RESULTS

During the capture period, seven animals were cached. The Table 1 shows the PCR results of the studied gene. Among the animals, three were detected as positive, to at least one of the screened genes, *stx1*, *stx2* or *tir*, by the PCR reactions. One animal was detected as carrier of the *stx2* gene, while two other animals were identified as carrier of the tir gene. The presence of *stx2* gene mean that the animal carrier STEC strains, while the presence of tir gene determined that the animals were carriers of EPEC strains. The *stx1* gene was not identified on the samples. Besides that, each sample has the detection of only one studied gene.

Table 1. PCR assay analysis of EPEC and STEC gene identification from wild canids feces samples.

Animal	Genes		
	stx1	*stx2*	*tir*
G04	N	N	P
G05	N	N	N
G22	N	N	N
G23	N	P	N
G26	N	N	N
G27	N	N	P
G29	N	N	N

N: no identification; P: positive identification.

DISCUSSION

In this study, we have found out that the crab-eating fox, a wildlife native canid of South America, can be reservoirs of STEC and EPEC pathotypes. By our knowledge, this is the first report of wild canids as carriers of these zoonotic pathogens.

We have performed the detection of the genes encoding Shiga toxin and the intimin receptor from faecal samples from wild canids. The genes *stx1* and *stx2* are responsible for the synthesis of the Shiga toxin; while the tir gene encodes the translocated intimin receptor, which is related to the A/E lesion formation. These animals did not show any clinical signs, wherefore they have the potential of being source of pathogens to other animals, even humans since the collection areas are close to human living. It is import to mention that wild animals can share habitat with livestock animals, performing a cycle of transmission among wild animals, domestic animals, and humans.

Ruminants are identified as the most important reservoir for EPEC and STEC [1,6], and frequently their feces contaminated food and water, included the meet-produced, being these pathotypes considered foodborne zoonotic pathogens. Sources of contaminated food include raw milk, undercook beef, cheese, unpasteurized juice and vegetables [6].

There are few studies that provide information about some wild animals as carriers of STEC and EPEC [1,4,5,7], therefore, the present observation of this additional wild species (crab-eating fox) that could act as carriers of STEC and EPEC highlights the importance of a surveillance system and the need for preventive measures to awareness of a potential zoonosis. Also, we have found only the *stx2* gene on the feces samples, which is described in other studies as predominant over the *stx1* gene [1].

Recently was reported 3.3% of wild boars, 1.3% of wild birds, 7.6% of deer, and 100% of muflon with STEC serotypes, while 1.3% of wild birds, 3.3% of wild boars, and 7.6% of deer analyzed were detected as carrier of EPEC serotypes [1].

Wild animals as a carrier of the serotype O157:H7 was described in Spain. In that study, 3.41% and 1.25% of wild boars and Iberian ibexes fecal samples tested for O157:H7 *E. coli* were positive, respectively [10]. Similarly, other studies concluded that deer can be a source of STEC [7,13].

Otherwise, some studies suggest that wild animals are not a significant source of O157 EHEC, however, the authors emphasize that other STEC-serotypes were identified, being potentially pathogenic to humans [5]. Altogether, all these studies reinforce that wild animals can be a source of STEC and EPEC pathotypes, demonstrating the requirement of knowledge in news species that can act spreading these pathogens.

The STEC/EHEC and EPEC pathotypes are zoonotic bacteria, which could be carried by a range of animals, both wild and domestic. Some carriers animals, like bovines, do not have receptors for the Shiga toxin, serving only as asymptomatic vehicle [12]. When humans get infect whit these pathogens, after contact whit contaminated food and water, or direct contact with carries animals and their feces, they can develop from diarrhea to a potentially life-threatening disease such as HC or HUS. Therefore wild animals may constitute a considerable hazard to human and animal health by transmission of bacteria strains to the environment.

CONCLUSION

In summary, the identification of genes encoding Shiga toxin and the intimin receptor in wild canids feces highlight that STEC and EPEC pathogens could be spread by these wild animals. Therefore, further researches are necessary to better understand the interaction between pathogen and hosts and their potential effects.

MANUFACTURERS

[1]HiMedia Laboratories Private Limited. Mumbai, Maharashtra, India.

[2]Qiagen GmbH. Hilden, Germany.

[3]Biotium Inc. Fremont, CA, USA.

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***Ethical approval*.** Wild canids capture were performed according to permissions from Brazilian biodiversity authorities (license number: SISBIO 51252-2), and the Institutional Ethics Animal Research Committee of University from Vale do Rio dos Sinos (license number 01/2017).

***Declaration of interest*.** None of the authors of this paper has a financial or personal interest that could inappropriately influence or impair the paper.

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