

First description of a Shiga toxin-producing *Escherichia coli* O103:H2 strain isolated from sheep in Brazil

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Introduction

Shiga toxin-producing *Escherichia coli* (STEC) comprise an important group of zoonotic pathogens causing a broad spectrum of disorders in humans, including mild to severe diarrhea, hemorrhagic colitis (HC) and the life-threatening condition hemolytic uremic syndrome (HUS). Although most outbreaks and sporadic cases of STEC have been attributed to strains belonging to serotype O157:H7, the frequency of non-O157 STEC infections is increasing in several regions [1]. More than 400 non-O157 STEC serotypes have been described so far and a considerable proportion of them have been linked to human illness [1,2]. In Brazil, human infections due to STEC are mainly associated with non-O157 serotypes, of which O26:H11, O103:H2 and O111:H8/H- (non-motile) accounted for the majority of cases [3-5].

STEC O103:H2 was first described as a causative agent of HUS in 1992 [6]. Since then, serotype O103:H2 has been implicated either in outbreaks or in sporadic cases of gastroenteritis and HUS in Europe [7,8], Japan [9] and the United States [10,11]. In Brazil, STEC O103:H2 was isolated for the first time in 1986, and re-emerged years later as causative agent of infantile diarrhea and hemolytic anemia [4,5]. Despite the fact that multiple STEC strains have already been isolated from farm animals, such as cattle [12] and pigs [13] in Brazil, none of them belonged to the known pathogenic serotype O103:H2 and did not display the virulence repertoire commonly associated

with severe human disease (i.e., *stx* plus *eae* and/or *ehxA* genes). This is the first report of STEC O103:H2 isolated and characterized from animals in Brazil.

The Study

Ten sheep flocks located on southern Brazil were tested between April and September 2010. Fecal samples were collected from 130 healthy animals, streaked onto MacConkey agar and lactose-fermenting colonies were biochemically characterized as *E. coli* [14]. STEC strains were identified by detection of *stx1*, *stx2*, *eae* and *ehxA* virulence genes [15] and serotyped as previously described [16]. Twenty-three different STEC serotypes were detected (data not shown), including O103:H2, carrying *stx1*, *eae* and *ehxA* genes which was isolated from a 4-week-old lamb. Intimin type ϵ was identified in this strain [16]. The *stx1 eae- ϵ ehxA* virulence gene profile is commonly observed among STEC O103:H2 strains [4,5,7,9]. *Stx1* expression was confirmed by cytotoxicity and neutralization assays on Vero cells [17], and enterohemolysin production was evidenced by the appearance of lysis zone on washed sheep blood agar plates [17]. The genotypic and phenotypic characteristics observed were similar to those of O103:H2 strains previously isolated from patients with diarrhea and hemolytic anemia in Brazil (Table 1).

Table 1. Characteristics of ovine STEC O103:H2 compared to human O103:H2 strains isolated in Brazil.

Strain	Serotype	Origin ⁽¹⁾	Genotypic and phenotypic characteristics ⁽²⁾				Reference
			Virulence profile	Intimin type	Stx	Ehly	
JN2b	O103:H2	S	<i>stx1 eae ehxA</i>	ε	+	+	This study
437/01	O103:H2	HA	<i>stx1 eae ehxA</i>	ε	+	+	[4]
651-1	O103:H2	HD	<i>stx1 eae ehxA</i>	ε	+	+	[4]
495-12	O103:H2	HD	<i>stx1 eae ehxA</i>	ε	+	+	[4,5]
91	O103:H2	HD	<i>stx1 stx2 eae ehxA</i>	ε	+	+	[4]

⁽¹⁾S: sheep; HA: hemolytic anemia; HD: human diarrhea

⁽²⁾Stx: cytotoxic activity; Ehly: production of enterohemolysin

A low prevalence of STEC O103:H2 in sheep has been also documented in other studies [18], supporting our results and suggesting that the occurrence of this serotype in ovine seems to be uncommon. However, STEC O103:H2 outbreaks and sporadic cases have been traced to contact with animals [7] as well as to consumption of contaminated meat [11], indicating that transmission of strains belonging to this serotype can occur between animals and humans.

Conclusion

The potential role of animals in the epidemiology of STEC O103:H2 human infection is still poorly understood in Brazil. In the present study, we described for the first time the isolation and characterization of STEC O103:H2 of animal origin in this country. This strain exhibited virulence features similar to those of human clinical strains, suggesting that sheep may be carriers, albeit at low frequency, of potentially human-pathogenic STEC O103:H2. However, more studies are needed to establish sheep and other animal species as source of STEC O103:H2 infection in our settings.

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