




Isolation and genotyping of *Clostridium perfringens* from goats in Minas Gerais, Brazil

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ABSTRACT: Despite the known importance of *Clostridium perfringens* as an enteropathogen in small ruminants, little is known about the role of its additional virulence factors or the frequency of the various *C. perfringens* genotypes in healthy goats; this complicates the laboratory diagnosis of the infections caused by this microorganism. In light of this, the aim of the present study was to isolate and genotype *C. perfringens* from stool samples from healthy goats in Brazil. Stool samples from 250 apparently healthy adult goats from 17 different herds in Minas Gerais, Brazil were collected, and isolation and genotyping of *C. perfringens* was performed. *C. perfringens* type A was isolated from 189 (75.6%) goats, whereas *C. perfringens* types C and D were each detected in one goat (0.4%). All isolates were negative for enterotoxin-, NetB-, NetE-, and NetF-encoding genes. These results confirmed *C. perfringens* type A as part of the microbiota in these animals, and they suggested that *C. perfringens* type C and D are rarely isolated from healthy goats.

Key words: *Clostridium perfringens* type D, microbiota, epsilon, beta.

Isolamento e genotipagem de *Clostridium perfringens* isolados de cabras em Minas Gerais, Brasil

RESUMO: Apesar da reconhecida importância de *Clostridium perfringens* como enteropatógeno de pequenos ruminantes, pouco se sabe sobre a frequência dos genótipos ou do papel de fatores de virulência adicionais de *C. perfringens* em cabras saudáveis, dificultando o diagnóstico laboratorial da infecção causada por esse micro-organismo. Dessa forma, o presente estudo teve como objetivo caracterizar *C. perfringens* de amostras de fezes de cabras adultas saudáveis. Amostras de fezes de 250 cabras saudáveis de 17 rebanhos diferentes em Minas Gerais, Brasil, foram submetidas ao isolamento e genotipagem de *C. perfringens*. *C. perfringens* tipo A foi isolado de 189 (75,6%) cabras, enquanto *C. perfringens* tipos C e D foram detectados em um animal (0,4%) cada. Todos os isolados foram negativos para os genes codificadores das toxinas NetB, NetE, NetF e enterotoxina. Os resultados apresentados confirmam *C. perfringens* tipo A como parte da microbiota de cabras saudáveis e sugere que *C. perfringens* tipos C e D são raramente encontrados em caprinos saudáveis.

Palavras-chave: *Clostridium perfringens* tipo D, microbiota, épsilon, beta.

Clostridium perfringens is classified into five types (A-E) based on the capacity to produce one or more of the four major toxins (alpha, beta, epsilon, and iota). *C. perfringens* type A is commonly isolated as part of the microbiota in animals and humans, whereas the other types are rarely isolated from healthy individuals (UZAL et al., 2014). In addition to the four major toxins, *C. perfringens* can produce several additional virulence factors that are associated with disease in humans and some domestic animals (LINDSTRÖM et al., 2011; SILVA & LOBATO, 2015). In the last few years, three pore-forming toxins (NetB, NetE, and NetF) have been described in *C. perfringens* strains from broiler chicken, horses, and dogs with enteritis (KEYBURN et al., 2008;

GOHARI et al., 2015). However, there is no study evaluating the presence of the genes for these toxins in *C. perfringens* isolates from goats.

Enterotoxaemia caused by *C. perfringens* type D is a disease of great economic and sanitary importance for ruminants farming worldwide, including in Brazil (BALDASSI et al., 1995; COLODEL et al., 2003; OLIVEIRA et al., 2010; SUMITHRA et al., 2013). The detection of the epsilon toxin in intestinal contents or stool samples using the serum neutralization test in mice or enzyme-linked immunosorbent assay (ELISA) is considered the “gold standard” method to diagnose enterotoxaemia; however, these techniques are available in very few laboratories. In addition, the epsilon toxin is

extremely unstable, which commonly leads to false-negative results (SUMITHRA et al., 2013). Therefore, a diagnosis of enterotoxaemia is commonly based on the combination of history, clinical signs, and isolation of *C. perfringens* type D in the intestinal contents of the affected animals (SUMITHRA et al., 2013; UZAL et al., 2014). Despite the importance of *C. perfringens* type D as an enteropathogen in goats, there have been few studies on *C. perfringens* in healthy individuals, and none has been conducted in Brazil. The majority of studies included only samples from diseased animals (UZAL et al., 1996; MISEREZ et al., 1998; UZAL et al., 2008). Thus, the frequency of *C. perfringens* type D in healthy animals, which can influence the laboratory diagnosis of enterotoxaemia in these animals, is still not known.

C. perfringens type A has also been described as a cause of enteric disease in goats (DRAY, 2004; MIYAKAWA et al., 2007). Once it was commonly recovered from healthy animals; several studies have been conducted to detect the additional virulence factors that could be used as markers in the diagnosis of *C. perfringens*-associated disease in domestic animals (KEYBURN et al., 2008; GOHARI et al., 2015). However, no study has evaluated the frequency of these recent described virulence factors in healthy goats.

Thus, the absence of information regarding the types of *C. perfringens* in healthy goats and the absence of studies on the role of additional virulence factors complicate the laboratory diagnosis of the infections caused by this microorganism. In light of this, the aim of the present study was to isolate and genotype *C. perfringens* from stool samples from healthy goats in Brazil.

Stool samples from 250 apparently healthy adult goats from 17 different herds in Minas Gerais, Brazil, were collected. To perform the isolation of *C. perfringens*, 0.08–0.12g of feces were serially diluted by factors of 10, ranging from 10^{-1} to 10^{-3} . Aliquots of 10 μ l of each dilution were plated on sulfite-polymyxin-sulfadiazine agar (SPS, Difco Laboratories, USA) and anaerobically incubated at 37°C for 24 hours (SILVA et al., 2013). After the incubation, at least three sulfite-reducing colonies from each dilution were tested using a previously described PCR protocol (VIEIRA et al., 2008) for the detection of genes encoding the major *C. perfringens* toxins (alpha, beta, epsilon, and iota), beta-2 toxin (*cpb2*), and enterotoxin (*cpe*). For the detection of the NetB-, NetE-, NetF-, and NetG-encoding genes (*netB*, *netE*, *netF*, and *netG*, respectively), the PCR protocols described by KEYBURN et al. (2008)

and GOHARI et al. (2015) were applied. The following strains were used as positive controls: *C. perfringens* ATCC 3629, *C. perfringens* ATCC 3626, *C. perfringens* D7 (*netE*⁺, *netF*⁺, *netG*⁺, *cpe*⁺), and *C. perfringens* CP149 (*tpeL*⁺, *netB*⁺).

C. perfringens was isolated from 191 (76.4%) of the goats sampled (Table 1). In the present study, even though three colonies were tested per animal, none of the goats had more than one genotype. Thus, only one isolate per goat was further analyzed. *C. perfringens* type A (positive only for alpha-toxin-encoding gene) was the most common genotype, corresponding to 98.9% (189/191) of isolates. This isolation rate is similar to the ones previously described in other ruminant and non-ruminant domestic animals (SIPOS et al., 2003; FARZAN et al., 2013; SILVA et al., 2013; SILVA & LOBATO, 2015; SILVA et al., 2015; DINIZ et al., 2017), and it confirmed that *C. perfringens* type A is common as part of the microbiota in goats.

C. perfringens type E, previously reported as a possible enteropathogen in goats and calves (SONGER & MISKIMMINS, 2004; KIM et al., 2013), was also not detected in the present study. *C. perfringens* type C (strains positive for alpha- and beta-encoding genes) and D (strains positive for alpha- and epsilon-encoding genes) were each isolated from one goat (0.4%). Previous studies in small ruminants with clinical signs of enterotoxaemia have reported a high isolation rate of this microorganism (MISEREZ et al., 1998; UZAL et al., 2008). Together these previous reports and the present research suggested that, in absence of techniques to detect the epsilon toxin, the isolation of *C. perfringens* followed by the detection of the epsilon-encoding gene (*etx*) by PCR is useful in the presumptive diagnosis of enterotoxaemia in goats.

Table 1 - Isolation and genotyping of *Clostridium perfringens* strains from apparently health goats (n=250) in Minas Gerais, Brazil.

	Stool samples from apparently health goats (%)
<i>Clostridium perfringens</i> ¹	191/250 (76.4)
Type A	189/250 (75.6)
Type C	1/250 (0.4)
Type D	1/250 (0.4)
Negative	59/250 (23.6)

¹All isolates were negative for *net B*, *net E*, *net F*, *net G* and *cpe* genes.

Although, some reports suggested that *C. perfringens* type A is cause of diarrhea in small ruminants (DRAY et al., 2004; MIYAKAWA et al., 2007), this hypothesis cannot be confirmed because there are no additional virulence factors that can be used as markers in the diagnosis of *C. perfringens* type A-associated disease in goats and sheep. In contrast, three pore-forming toxins (NetB, NetE, and NetF) have been recently described to have a strong association with the enteritis caused by *C. perfringens* type A in dogs, foals, and broiler chickens (GOHARI et al., 2015). In the present work, all isolates were negative for NetB-, NetE-, and NetF-encoding genes. In addition, enterotoxin-encoding-gene (*cpe*), which is responsible for diarrhea in humans (LINDSTRÖM et al., 2011) and has previously been detected in a goat with necrotizing enterocolitis (MIYAKAWA et al., 2007), was also not detected. These results suggested that these additional virulence factors are not frequent in healthy goats. Further studies in goats with intestinal disorders can clarify if these recently described toxins play a key role in the intestinal disorders due to *C. perfringens* type A.

This is the first study on the genotypes of *C. perfringens* in goats in Brazil, and it had the highest number of samples compared to all other studies in the literature. The results of the present study have confirmed that *C. perfringens* type A is part of the microbiota in these animals, and they suggest that *C. perfringens* types C and D are rarely isolated from healthy goats.

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BIOETHICS AND BIOSSECURITY COMMITTEE APPROVAL

All animal procedures were approved by the Comitê de Ética em Experimentação Animal (Ethical Committee for the Use of Experimental Animals) (CEUA/UFV), protocol 012/2013.

DECLARATION OF CONFLICTING OF INTERESTS

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

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