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Prevalence and distribution of *Clostridium difficile* PCR ribotypes in cats and dogs from animal shelters in Thuringia, Germany

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ABSTRACT

Clostridium difficile is an important cause of nosocomial diarrhoea in humans. Pet animals and livestock are discussed as potential natural reservoirs and sources of infection. In this study faecal samples from dogs and cats were collected at 10 animal shelters in Thuringia, Germany. *C. difficile* was isolated from 9 out of 165 (5.5%) canine and 5 out of 135 (3.7%) feline samples. Five PCR ribotypes (010, 014/020, 039, 045, SLO 066) were identified. PCR ribotypes 010 and 014/020 were detected in more than one shelter and PCR ribotypes 014/020 and 045 were isolated from dogs and cats. MLVA profiles of strains of a PCR ribotype from one shelter were identical or closely related, while strains of the same PCR ribotype from different shelters showed significant differences. This study shows that dogs and cats kept in animal shelters are a reservoir of *C. difficile* PCR ribotypes which can infect also humans.

1. Introduction

Clostridium difficile is the most common cause of antibiotic-associated diarrhoea in humans [1]. The main virulence factors are two high-molecular-weight toxins, toxin A (enterotoxin; TcdA) and toxin B (cytotoxin; TcdB) [1]. Some strains additionally produce the binary toxin CDT [1]. During the last decade, clinical presentation and epidemiology of *C. difficile* infection (CDI) changed towards an increased morbidity and mortality [1]. Health-care-associated *C. difficile* infection incidence rates from 0 to 36.3 (mean: 4.1) per 10.000 patient-days have been reported for European hospitals [3]. Although CDI is still a nosocomial infection in general it is increasingly recognized as cause of community acquired diarrhoea and there is some evidence that animals might be reservoirs of virulent *C. difficile* and a possible source of infection for community acquired cases [2]. Numerous wild animals (e.g. primates, ostriches and prairie dogs), companion animals (horses, rodents) and livestock (especially pigs) can be affected by CDI [4]. Human pathogenic PCR ribotypes were found in several mammals including cattle, horses and pigs [5–7]. Furthermore, several studies demonstrated a significant contamination of food (meat and salads) with pathogenic *C. difficile* [2]. However, the zoonotic potential of the pathogen is still discussed controversially [2,6].

The relevance of *C. difficile* as a cause of disease in dogs and cats is not well understood yet. Intestinal colonization with *C. difficile* has been described to range from 1 to 57% in dogs and from 2 to 38% in cats (Table 1). Prevalences investigated in diarrhoeic dogs vary from 2 to 25% and were 7% and 16% in cats respectively (Table 1). Some studies showed a correlation between colonization and diarrhoea in dogs and cats [8–11]. However, the attempt to provoke CDI in healthy adult dogs by administering *C. difficile* with and without antibiotics failed [13]. Strains isolated from canines, felines and humans are often of the same PCR ribotypes [5,7,14]. Most studies concerning the prevalence of *C. difficile* in dogs and cats so far focus on veterinary hospitals (Table 1). Weese et al. [14] survey the prevalence of *C. difficile* in dogs and cats in the household environment in Canada. They isolated *C. difficile* from 14/139 dogs (10%) and from 3/14 cats (21%). McKenzie et al. [12] found 61/135 racing sled dogs (45%) to be positive for *C. difficile*. The situation in animal shelters has been rarely considered. Struble et al. [15] could not detect *C. difficile* in faecal specimens of 42 dogs collected in an unspecified number of shelters in the USA. Perrin et al. [16] showed that 1/74 dogs (1%) entering a kennel in Switzerland harboured *C. difficile*. Al Saif et al. [17] collected stool samples from 2 veterinary

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Table 1
Reported prevalences of *Clostridium difficile* in dogs and cats.

Country	Sample origin	Total individuals	Culture positive	Diarrhoeic	Culture positive	Non-diarrhoeic	Culture positive	Used methods for typing and characterization	Reference
Canine									
Netherlands	Diagnostic samples	116	29 (25%)	116	29 (25%)	0	0	PCR ribotyping; MLVA; PCR toxin genes A, B, CDT	[7], 2011
USA	Racing sled dogs	135	61 (45%)	35	n.a.	94	n.a.	ELISA toxins A, B	[12], 2010
Canada	Household environment	139	14 (10%)	0	0	139	14 (10%)	PCR ribotyping; PCR toxin genes A, B, CDT; Toxinotyping	[14], 2009
Canada	Vet. hospital, ICU	360	70 (19%)	n.a.		n.a.		PCR ribotyping; PCR toxin genes A, B	[26], 2008
USA	Vet. hospital	143	33 (23%)	100	20 (20%)	43	13 (30%)	PCR toxin genes A, B; ELISA toxin A, B; Cytotoxicity	[27], 2006
Canada	Hospital visiting dogs	102	58 (57%)	n.a.		n.a.		PCR ribotyping; PCR toxin genes A, B, CDT	[28], 2006
USA	Vet. hospital	334	52 (16%)	260	47 (18%)	74	5 (7%)	ELISA toxin A	[29],[a] 2002
USA	Vet. hospital	132	17 (13%)	32	5 (16%)	100	12 (12%)	ELISA toxin A; PCR toxin genes A, B	[10], 2002
Canada	Vet. hospital	142	2 (1%)	87	2 (2%)	55	0	ELISA toxin A, B	[8], 2001
UK	Vet. hospital, shelter	100	10 (10%)	n.a.		n.a.		EIA toxin A	[17], 1996
USA	Vet. hospital, shelter	194	28 (14%)	42	7 (17%)	110	21 (19%)	PCR toxin genes A, B	[15], 1994
Switzerland	Litters, shelter	158	73 (46%)	11	1 (9%)	147	72 (49%)	GLC; Enzymatic activities; Cytotoxicity; ELISA toxin A	[16], 1993
Australia	Vet. hospital	60	24 (40%)	n.a.		n.a.		Cytotoxicity	[30], 1991
Germany	Vet. practices	150	9 (6%)	75	2 (3%)	75	7 (9%)	Cytotoxicity	[31], 1989
UK	Vet. hospital	52	11 (21%)	n.a.		n.a.		Cytotoxicity, Hamster bioassay	[32], 1983
Feline									
Netherlands	Diagnostic samples	115	18 (16)	115	18 (16%)	0	0	PCR ribotyping; MLVA; PCR toxin genes A, B, CDT	[7], 2011
Canada	Household environment	14	3 (21%)	0	0	14	3 (21%)	PCR ribotyping; PCR toxin genes A, B, CDT; Toxinotyping	[14], 2009
Canada	Vet. hospital, ICU	42	3 (7%)	n.a.		n.a.		PCR ribotyping; PCR toxin genes A, B	[26], 2008
USA	Vet. hospital	294	23 (8%)	n.a.	10	80	0	PCR toxin genes A, B; AP-PCR (Genotyping)	[33], 1999
UK	Vet. hospital, shelter	100	2 (2%)	n.a.		n.a.		EIA toxin A	[17], 1996
Australia	Vet. hospital	21	8 (38%)	n.a.		n.a.		Cytotoxicity	[30], 1991
Germany	Vet. practices	175	14 (8%)	75	5 (7%)	100	9 (9%)	Cytotoxicity	[31], 1989
UK	Vet. hospital	20	6 (30%)	n.a.		n.a.		Cytotoxicity, Hamster bioassay	[32], 1983

[a] Data shown in the abstract differ from the presented results.

clinics and an animal shelter. They isolated *C. difficile* from 10/100 dog samples and from 2/100 cat samples. However, they do not distinguish between samples from the clinics and the shelter. All these studies considering animal shelters are from the nineties and were using EIA/ELISA of toxin A and cytotoxicity assays or toxin gene PCR for detecting *C. difficile*. Information on the PCR ribotype diversity in shelter animals is so far not available [Table 1].

2. Materials and methods

2.1. Sampling

Twenty five shelters of the state Thuringia, Germany, were invited to attend this investigation. The shelters differed in size and in their proportional composition of animal species. A total of 10 shelters volunteered to participate. Reasons for non-participation were not requested. Faecal specimens of dogs and cats were collected between January and March 2010. In all shelters dogs were kept individually or in groups of 2. Cats were mostly kept in groups up to 60 animals (Shelter A: approx. 60 cats in 1 group; B: approx. 60 cats in 6 groups; C: 50 cats in 7 groups; D: no cats; E: 2 cats kept individually; F: 31 cats in 13 groups; G: 16 cats in 7 groups; H: 8 cats in 4 groups; I: 25 cats in 10 groups; J: 48 cats in 2 groups). Canine faecal samples were taken from the floor of the kennels. Each sample could be assigned to an individual animal. Feline faecal specimens were usually taken from litter trays (up to 4/litter tray, mostly 1–2) which made an assignment of samples to individuals impossible. We assumed that all feline faecal samples originated from different cats. The samples were transported on ice and processed within 2–4 h.

2.2. Isolation and identification

Isolation of *C. difficile* was performed using direct plating and enrichment culture in parallel. Depending on its consistency 1–4 inoculation loops of each sample (approximately 0.5 g) were re-suspended in 10 ml *C. difficile* moxalactam/norfloxacin broth (CDMN, Oxoid, SR173) containing 0.1% sodium-taurocholate (Sigma–Aldrich, 86339). 100 μl of this mixture was immediately plated onto CDMN agar. Plates were incubated for 1–3 days, the enrichment culture for 14–21 days at 37 °C under anaerobic conditions.

In order to select spores, 900 μl of each enrichment culture was mixed with the same volume of 99% ethanol and left at room