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Gastrointestinal carriage of *Clostridium difficile* in cats and dogs attending veterinary clinics

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SUMMARY

Cats and dogs being treated at two veterinary clinics were investigated for gastrointestinal carriage of *Clostridium difficile* using selective solid and enrichment media. Thirty-two (39·5%) of 81 stool samples yielded *C. difficile*. There were significant differences in isolation rates between clinics, 61·0% of animals being positive at one clinic compared to 17·5% at the other (Chi-square, $P < 0{\cdot}005$). Of 29 animals receiving antibiotics, 15 (52·0%) harboured *C. difficile* while 11 (23·9%) of 46 animals not receiving antibiotics were positive (Chi-square, $P < 0{\cdot}01$). There was no difference in carriage rate between cats (38·1%) and dogs (40·0%). The environment at both veterinary clinics was surveyed for the presence of *C. difficile*. Fifteen of 20 sites at one clinic were positive compared to 6 of 14 sites at the other clinic. Both cytotoxigenic and noncytotoxigenic isolates of *C. difficile* were recovered from animals and environmental sites. These findings suggest that household pets may be a potentially significant reservoir of infection with *C. difficile*.

INTRODUCTION

Although many sources of *Clostridium difficile* have been reported it is still not known whether the usual niche for *C. difficile* infecting humans is endogenous or exogenous, or if both sources are of epidemiological significance [1]. Exogenous sources of *C. difficile* may be common but only a few studies have examined the distribution of *C. difficile* in the environment, concentrating primarily on soil, peat and marine sediments [2]. *C. difficile* has been isolated infrequently from the gastrointestinal tract of healthy animals. It has been recovered from the stools of cattle, camels, horses and donkeys, hamsters and a snake, and from the contents of the large intestine of a Weddel seal [see references 2, 3]. Faecal carriage of *C. difficile* by household pets (mainly cats and dogs) was reported to be common (23%) and it was suggested that infection from a household pet was possible [3].

Recently, using restriction endonuclease analysis of chromosomal DNA, we showed that the majority of relapses in patients with *C. difficile*-associated diarrhoea were actually reinfections with a strain different from the original infecting organism [4]. In addition, *C. difficile* may be a common cause of community-acquired diarrhoea [5–7]. These results pointed strongly to acquisition

of the infecting strain from the environment and we therefore investigated further the possibility that household pets could act as a reservoir of *C. difficile*.

MATERIALS AND METHODS

Specimens

Animals were investigated at two veterinary clinics within the metropolitan area of Perth, Western Australia. The Karrinyup Small Animal Hospital (KSAH) was situated approximately 10 miles north, and the Bassendean Veterinary Hospital (BVH) approximately 10 miles east, of the city centre. Faecal samples from cats and dogs, admitted to the clinics for a variety of reasons, were collected into 50 ml sterile screw-cap plastic containers. They were transported to the laboratory at 4 °C and processed on the day of collection or stored overnight at 4 °C and processed the following day. We have shown previously that *C. difficile* remained viable for up to 4 days in stool samples provided that an adequate quantity of specimen was collected [8].

Isolation of C. difficile *and cytotoxin detection*

The methods employed for the isolation of *C. difficile* from stools have been described previously [9, 10] and included using a selective enrichment broth containing gentamicin 5 mg/L, cycloserine 250 mg/L and cefoxitin 8 mg/L (GCC broth) [11]. Screening of selective enrichment broths and final identification of *C. difficile* was performed using a commercially available latex particle agglutination test (Mercia Diagnostics Ltd., Guildford, Surrey) [12]. Cross-reacting species were identified according to the criteria and methods of Brazier [13]. Isolates of *C. difficile* were tested for cytotoxin production after subculture into prereduced supplemented brain heart infusion broth (BHIB-S) and anaerobic incubation for 72 h at 37 °C [11]. Sterile filtrates of the BHIB-S cultures were examined for cytotoxin as described previously [11].

Environmental sampling

Contact plates with a 55 mm diameter (Sterilin, Disposable Products Pty. Ltd., South Australia) were prepared containing modified CCFA [9], modified CCFA with 0·25 g/L sodium cholate (Sigma Chemical Co., Cat. No. C-1254) and modified CCFA with ciprofloxacin 4 mg/L replacing the cefoxitin. Sodium cholate 0·25 g/L was also added to this medium (CCCA) to enhance spore germination. These selective media were used to isolate *C. difficile* by pressing the contact plate gently onto various surfaces at the veterinary clinics under investigation.

RESULTS

A total of 81 stool samples from 21 cats (aged 5 months to 13 years) and 60 dogs (aged 6 months to 14 years) was examined, 32 (39·5 %) of which yielded *C. difficile* by either direct or enrichment culture. Using direct plating onto CCFA, *C. difficile* was isolated from 25 specimens, while 28 samples contained *C. difficile* by enrichment culture. Enrichment culture detected *C. difficile* in an additional seven samples not positive by direct culture, while direct culture on CCFA yielded four isolates which were not recovered by enrichment broth.

Table 1. *Comparison of isolation sites, media and cytotoxin production for environmental isolates of* C. difficile *at KSAH*

Site	Growth on			Cytotoxin production by isolate from		
	CCFA	CCFA(c)	CCCA	CCFA	CCFA(c)	CCCA
Holding room floor 1	+	+	−	−	−	NA
Holding room table	−	−	−	NA	NA	NA
Holding room bench	−	+	−	NA	−	NA
Holding room floor 2	+	+	−	−	−	NA
Holding room cage	+	+	−	−	+	NA
Isolation room floor 1	+	+	+	−	−	−
Isolation room floor 2	+	−	−	+	NA	NA
Isolation room cage	+	−	−	−	NA	NA
Operating room floor	−	+	−	NA	+	NA
Operating room sink	−	−	−	NA	NA	NA
Operating room table	−	−	−	NA	NA	NA
Operating room bench	+	−	−	−	NA	NA
Treatment room floor 1	+	+	+	+	−	−
Treatment room floor 2	−	+	+	NA	+	−
Treatment room X-ray table	+	−	−	−	NA	NA
Treatment room table	+	−	+	−	NA	−
Treatment room bench	−	−	−	NA	NA	NA
Treatment room cage	+	+	−	−	−	NA
Examination room table	−	−	−	NA	NA	NA
Examination room floor	+	+	+	+	+	−

NA, not applicable.
CCFA(c), CCFA plus 0·25% sodium cholate.

Of the 81 stool specimens examined, 41 were obtained from the KSAH while the remainder were supplied by the BVH. It was noted that the proportion of animals carrying *C. difficile* was not evenly distributed between the two locations. Of the 41 samples from the KSAH, 25 harboured *C. difficile* either by direct or enrichment procedures, a carriage rate of 61·0% in these animals. The carriage rate of *C. difficile* by animals from the BVH was much lower, with only 7 of 40 pets being colonized by the organism (17·5%). This difference was statistically significant (Chi-square, $P < 0{\cdot}005$). Of the 25 strains of *C. difficile* isolated at the KSAH, 22 were tested for cytotoxin production *in vitro* and 12 were positive. Only 1 of the 4 isolates of *C. difficile* from the BVH available for testing produced cytotoxin *in vitro*.

Details concerning antibiotic therapy of 6 pets from the KSAH were unknown. Twenty-two of the remaining 35 pets had received antibiotics, most commonly a combination of penicillin and streptomycin. Of these animals 13 (50%) yielded *C. difficile* while 6 of the animals not receiving antibiotics were positive (46%). Only 7 of the pets from the BVH had received antibiotics prior to sampling and 2 of these were carrying *C. difficile*. The proportion of animals from the BVH harbouring *C. difficile* was higher in the animals receiving antibiotics (28·6%) compared to those not receiving antibiotic therapy (15·1%). Of the 29 animals receiving antibiotics, regardless of location, 15 harboured *C. difficile* (52·0%) while 11 (23·9%) of the 46 animals not receiving antibiotics were positive for *C. difficile*.

Table 2. *Comparison of sites of isolation, media and cytotoxin production for environmental isolates of* C. difficile *at BVH*

Site	Growth on			Cytotoxin production by isolate from		
	CCFA	CCFA(c)	CCCA	CCFA	CCFA(c)	CCCA
Operating room floor	+	+	−	−	−	NA
Operating room sink	−	−	−	NA	NA	NA
Operating room table	−	−	−	NA	NA	NA
Operating room bench	−	−	−	NA	NA	NA
Treatment room floor	−	−	−	NA	NA	NA
Treatment room table	−	−	−	NA	NA	NA
Treatment room bench	−	−	+	NA	NA	+
Holding room (large) floor	−	+	−	NA	+	NA
Holding room (large) bench	−	−	−	NA	NA	NA
Holding room (large) table	−	−	−	NA	NA	NA
Holding room (large) drain	+	+	−	−	−	NA
Holding room (large) cage	−	−	−	NA	NA	NA
Holding room (small) floor	−	+	−	NA	+	NA
Holding room (small) table	−	+	−	NA	−	NA

NA, not applicable.
CCFA(c), CCFA plus 0·25% sodium cholate.

This was a statistically significant difference in isolation rate (Chi-square, $P < 0{\cdot}01$). There was no difference between the carriage rates in dogs (40·0%) and cats (38·1%).

The results of environmental sampling are shown in Tables 1 and 2. Fifteen (75%) of 20 sites at the KSAH were positive (Table 1), as were 6 (42·8%) of 14 sites at the BVH (Table 2). Also shown in Tables 1 and 2 is the utility of the various media used for environmental sampling. Of the 34 sites investigated, 14 were positive on CCFA, 15 were positive on CCFA containing 0·25 g/L sodium cholate and only 6 were positive on CCCA. Preliminary experiments had shown that recovery of pure cultures of spores of *C. difficile* on CCCA was equivalent to recovery on blood agar (data not shown). It was noted, however, that many CCCA plates were overgrown with other spore-forming clostridia, such as *C. perfringens*, obscuring any growth of *C. difficile*. It is also possible that other bacteria inhibited *C. difficile* growth *in vitro*.

One colony of *C. difficile* from all positive culture plates was tested for its ability to produce cytotoxin *in vitro*. These results are also shown in Tables 1 and 2 and indicate, in keeping with findings from animal isolates, that both cytotoxigenic (28·6%) and noncytotoxigenic (71·4%) strains were present at the same site.

DISCUSSION

A variety of reservoirs of *C. difficile* are recognized. These include endogenous carriage, environmental contamination and zoonoses [1], but the relative importance of these sources with respect to the epidemiology of *C. difficile*-associated disease is unknown.

Animals constitute a potentially important reservoir of *C. difficile* although there have been few reported isolations of *C. difficile* from animals. There has been

only one published report focusing on the incidence of *C. difficile* in domestic pets [3]. This work showed that *C. difficile* was commonly found in domestic pets with isolation rates of 21·0% and 30·0% for dogs and cats, respectively. In the present study the isolation rates for *C. difficile* were even higher, 40·0% for dogs and 38·1% for cats. These differences in isolation rates may have been due solely to variations in cultural techniques. Enrichment culture, in addition to culture on solid media, was used in the present study, while the earlier work relied on the use of solid media (CCFA with 0·1% sodium taurocholate) and an alcohol shock procedure [3]. In a previous study [14] comparing enrichment culture and the alcohol shock procedure, enrichment was significantly better than alcohol shock. Clearly though, there was also a difference in isolation rate between the two veterinary clinics we investigated and the significance of clinic-to-clinic variation needs to be considered.

Fifty per cent of animal isolates and 71·4% of environmental isolates were noncytotoxigenic. These findings are similar to those of Borriello and colleagues [3] who isolated mostly noncytotoxigenic strains.

In contrast to the work of Borriello and colleagues [3] a statistically significant difference in isolation rates was seen between animals which had received antibiotic therapy and those which had not. This was only apparent, however, for the combined clinic results and not for the results from each clinic. The isolation rate of *C. difficile* from animals receiving antibiotics (most commonly a combination of penicillin and streptomycin) was double that in animals not receiving antibiotics. Thus the situation in animals does appear to be analogous to the situation in humans where antibiotic therapy is the major factor predisposing to *C. difficile* colonisation or infection.

Significant differences in isolation rates were seen between stool specimens supplied by the two veterinary clinics. The isolation rate of *C. difficile* from pet samples supplied by the KSAH was over three times that from pets treated at the BVH. The reasons for this disparity are unknown, although, clearly, the more extensive use of antibiotics at the KSAH may have played some role. It should be noted, however, that the isolation rate in animals not receiving antibiotics was still much higher in pets from the KSAH compared with pets from the BVH. It had been reported by the staff at the KSAH that clindamycin had been used extensively over the previous several years, although this practice had declined prior to the commencement of our study. In humans clindamycin remains one of the major antibiotics predisposing to *C. difficile*-associated diarrhoea [15]. We hypothesized that, if clindamycin had been used in the past, it was possible that animals treated at that time acquired *C. difficile* and subsequently contaminated the environment at the KSAH. This environmental contamination could be responsible for the high incidence of *C. difficile* among pets from the KSAH, both in the group of animals given antibiotics and those who were not.

To test our hypothesis we carried out an extensive survey of the environment at both veterinary clinics. The high number of positive sites (75%) at the KSAH would seem to confirm that the environment was a potentially significant source of *C. difficile* at that clinic. We were surprised that 43% of sites were positive at the BVH considering the lower isolation rate recorded for animals of 17%. A comparison of the results of our environmental sampling with those of Borriello

and colleagues [3] is also interesting. Both investigations looked at over 30 sites although in the present investigation these were at two different clinics. However, 21 of our 34 sites yielded *C. difficile* compared with 4 of 35 sites reported by Borriello and colleagues [3], and one of these was the sole of an investigator's shoe.

We also took the opportunity, while conducting the environmental investigation, to assess two modifications of CCFA for their ability to recover spores of *C. difficile*. Previous investigators have shown that the recovery of *C. difficile* spores was enhanced by the addition of 0·1% sodium taurocholate [16]. Subsequently, however, Wilson [17] indicated that the source and purity of the taurocholate used was important. Because of the expense of sufficiently pure sodium taurocholate we evaluated media containing 0·25% sodium cholate. In laboratory trials our results looked encouraging and recovery of *C. difficile* spores on both media was almost equivalent to that on blood agar plates. In the field, however, the medium containing ciprofloxacin was often overgrown with other spore-forming clostridia and was deemed unsuitable. Overall CCFA containing 0·25% sodium cholate performed slightly better than conventional CCFA and, in view of the saving achieved by using sodium cholate instead of sodium taurocholate, may warrant further investigation.

Borriello and colleagues [3] speculated that household pets may act as a source of infection for people in the community. In view of our recent findings that *C. difficile* was commonly associated with community-acquired diarrhoea [6] and that many relapses of *C. difficile*-associated diarrhoea were reinfections with a different strain of *C. difficile* apparently from the environment [4], we would reiterate the suggestion that domestic animals may be a significant source of infection. We cannot conclude, however, that the high carriage rates we found in animals at veterinary clinics represents the normal state, as this may just be a reflection of transient colonization due to the high level of environmental contamination. To some extent we showed this with the differences in results between the two veterinary clinics investigated. Nonetheless, until longitudinal studies are carried out domestic pets should be viewed as a potential risk. Indeed, during the course of the investigations reported here we learnt of two staff members at one of the veterinary clinics who had complained of recurrent diarrhoeal disease for some time. Perhaps *C. difficile*-associated diarrhoea may be yet another occupational hazard for veterinary workers.

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