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Duration of carriage and transmission of *Yersinia enterocolitica* biotype 4, serotype 0:3 in dogs

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SUMMARY

Human infections with pathogenic strains of *Yersinia enterocolitica* have been linked to contact with dogs excreting these microorganisms. This study examines the carriage and transmission of *Y. enterocolitica* biotype 4, serotype 03 in dogs. Fourteen 6-month-old cross-bred dogs were separated into 5 groups, 2 containing 4 dogs (I and II) and the others 2 dogs (III–V). Each of the 4 dogs in Group I and 2 of the dogs in Group II were inoculated orally with the test strain. Bacteriological examination of faecal samples showed that dogs can be readily infected and can carry the organism for up to 23 days. The two in-contact dogs in Group II started to shed the test organism after 5 days. Subsequent transfer of these dogs to Group III and those in Group III to Group IV showed that *Y. enterocolitica* biotype 4, serotype 03 can be readily transmitted between dogs. At no time did any of the dogs show clinical signs of infection. Group V served as a negative control for the trial. These findings suggest that dogs can carry *Y. enterocolitica* biotype 4, serotype 03 asymptomatically and hence might act as a potential source of infection for people.

INTRODUCTION

During the last three decades *Yersinia enterocolitica* has become recognized worldwide as an important human pathogen causing a number of clinical syndromes, of which acute and chronic enteritis are the most commonly recorded [1]. It is considered to be principally a foodborne pathogen and studies have been undertaken to identify potential sources of human infection [2]. Although results from these studies have identified pigs as the major reservoir of human pathogenic strains of *Y. enterocolitica* [3] there is evidence to suggest that household pets, in particular dogs, can also carry such strains [4] and may in some instances be associated with human disease [5].

In 1973, circumstantial evidence was published that implicated sick dogs as the source of infection in a large inter-familial outbreak of *Y. enterocolitica* enteritis which involved the death of two people. Unfortunately, surviving dogs were destroyed without culture of their faeces [6]. In a second case, involving one child, faecal specimens were examined from the three surviving puppies in a litter in which eight animals had died of a wasting illness. *Y. enterocolitica* biotype 1b, serotype 0:20, was isolated from the animals, a dried stool specimen and the sick child [5].

Surveys carried out in Denmark [7, 8] and Japan [4, 9] showed that dogs may carry strains of *Y. enterocolitica* potentially pathogenic for man (e.g. serotypes 0:3, 0:5, 27 and 0:9) however, the prevalence in dogs was far lower than that reported in pigs [10, 11].

Although dogs appear mainly to be asymptomatic carriers of yersiniae, sporadic cases of enteritis have been recorded in Norway [12], USA [13] and Italy [14] associated with *Y. enterocolitica* biotype 4, serotype 0:3, the most commonly isolated strain from human infections worldwide. In addition biotype 1b, 0:8 and 0:20 have been associated with disease in dogs in the USA [5, 6]. During the last year serotype 0:3 strains were isolated from two dogs presented to the Small Animal Clinic of the Faculty of Veterinary Science, Massey University (Fenwick, unpublished). One dog had enteritis and the other acute pharyngitis.

These cases prompted us to initiate a study to answer a number of questions regarding *Y. enterocolitica* infections in dogs, such as the persistence and site of infection, transmission and the clinical significance of the organism, so that the potential role of dogs as a reservoir of yersiniae for humans could be assessed.

MATERIALS AND METHODS

Dogs

Fourteen 6-month-old Huntaway-cross dogs from 2 litters were divided into 5 separately housed groups. Solid walls separated each group and measures to avoid cross-contamination included the use of clean boots and disinfectant footbaths (Virkon S, Antec International, Sudbury, England) at the entrance to each pen, provision of individual thermometers stored in disinfectant (Virkon S) for each group and the use of disposable plastic gloves for handling and taking samples from the dogs. Pens were cleaned thoroughly each day. The dogs were fed a commercial dried dog feed once a day and water was provided *ad libitum*. The duration of the trial was limited to 30 days by the availability of the experimental dogs.

Experimental design

Group I. This group contained four dogs (1–4), all of which were inoculated experimentally at the beginning of the trial.

Group II. Four dogs, of which two (5 and 6) were inoculated orally with *Y. enterocolitica*. Dogs 7 and 8 were left in contact with them for 1 week before being moved to Group III.

Group III. This group initially contained two uninfected dogs (9 and 10). One week after the trial started, the two dogs (7 and 8) from Group II were moved in with them for a further week.

Group IV. This group initially comprised two uninfected dogs (11 and 12). Two weeks after the trial started, the original two dogs (9 and 10) from Group III were moved in with them for the last 2 weeks of the trial.

Group V. This group contained two dogs (13 and 14) which acted as uninfected controls for the duration of the trial.

Inoculation of the dogs

All dogs in Group I and dogs 5 and 6 in Group II were inoculated orally with 5 ml of an overnight broth culture (Nutrient broth, Difco, Detroit, USA)

containing 10^9 cells per ml of *Y. enterocolitica* biotype 4, serotype 0:3, originally isolated from a human enteric infection. Virulence tests for yersiniae, including calcium dependency and auto-agglutination, were performed on the strain used, both before and after inoculation, as putative evidence of the presence of a virulence plasmid [15].

Sampling protocol

Swabs of rectal faeces and the pharynx were taken from the dogs during the 5 weeks of the trial, as outlined in the scheme below. Temperatures were recorded for all dogs daily and the animals were monitored for evidence of illness such as diarrhoea or inappetance.

Sampling scheme

Pre-trial. Rectal swabs were collected from all 14 dogs on three occasions during the week before the trial.

Groups I and II. Pharyngeal and rectal swabs were taken at 1–3 day intervals for 30 days after inoculation.

Groups III and IV. Pharyngeal and rectal samples were taken weekly from the start of the trial and at 1–3 day intervals following contact with infected or potentially infected dogs.

Group V. Pharyngeal and rectal swabs were taken at weekly intervals throughout the trial.

All swabs were cultured to detect the presence of yersiniae.

Isolation of Yersinia enterocolitica

The methods used for the isolation and identification of *Y. enterocolitica* were as described by Mair and Fox, 1986 [15]. Rectal swabs were placed in transport medium immediately after collection and used to inoculate *Yersinia* selective agar (CIN – Difco, Detroit, USA) within 2 h. The swabs were then agitated vigorously in 10 ml phosphate buffered peptone water (PBPW). The CIN agar plates were incubated for 24 h at 28 °C and examined for typical bullseye colonies. These were identified, biotyped [16] and the serotype determined by slide-agglutination using commercial antisera (Eco-Bio, Woudstraat, Belgium). The PBPW bottles were incubated at 4 °C for 3 weeks before subculture onto CIN agar.

Pharyngeal swabs were inoculated into PBPW. This was subcultured on CIN agar after 3 weeks incubation at 4 °C and isolates identified as above.

Enumeration of Y. enterocolitica *in faeces*

On three occasions during a period in which the dogs were actively excreting *Y. enterocolitica* biotype 4 (days 1–10), three faecal samples were collected from the floor of the pen housing animals in Group I. Approximately 1 g of faeces was added to 9 ml of distilled water (DW) and vortexed vigorously for 1 min. Three decimal dilutions of the suspension were made in DW and three CIN agar plates inoculated with 0·1 ml of each. The plates were incubated at 28 °C for 24 h and the number of colony-forming units (cfu) per gram calculated.

RESULTS

A number of *Yersinia* spp. were isolated from the dogs during the week prior to the start of the trial (Table 1). These included *Y. pseudotuberculosis* serotype III.

Table 1. *Isolation of* Yersinia *spp. from swabs of rectal faeces taken on three occasions during the week prior to commencement of the trial*

Dog no.	Sampling occasion* 1	2	3
1	YE3	YE3	YE3
2	YE1/YI	YE3	YE3
3	—	YE3	—
4	YP/YE1	—	YE1
5	—	—	—
6	YP/YE1	—	YE1
7	—	—	YE1
8	—	YE3	YE1
9	—	—	YE2
10	YE1	—	YE2
11	YE3/Y1	—	YE1
12	—	—	YE1
13	YI	YE3	YE1
14	YE1/Y1	—	YE1

* YP, *Y. pseudotuberculosis*; YI, *Y. intermedia*; YE1, *Y. enterocolitica* biotype 1a; YE2, *Y. enterocolitica* biotype 2; YE3, *Y. enterocolitica* biotype 3.

Y. intermedia, *Y. enterocolitica* biotype 1a, *Y. enterocolitica* biotype 2, serotype 0:9 and *Y. enterocolitica* biotype 3, serotype 0:5, 27. No strains of *Y. enterocolitica* biotype 4, serotype 0:3 were isolated.

No clinical signs were seen in the dogs during the trial. Rectal temperatures of all dogs remained within the normal range ($39 \pm 0{\cdot}5$ °C).

Duration of faecal shedding of Yersinia enterocolitica

The duration of faecal shedding by the dogs inoculated orally (1–6) varied (Table 2), ranging from 7–23 days (median 10·5 days).

Five of the six in-contact dogs (7–12) excreted the organism (Table 2). One dog was positive on one occasion only (dog 9) and two were still positive at the end of the experiment (dog 7, 26 days shedding; dog 11, 8 days shedding).

At no time was the experimental organism detected in the faeces of the control animals (13–14). In addition, dog 12 was culture negative throughout the trial.

In three dogs the organism was cultured for a longer period using cold enrichment than using direct culture. These were dog 1 (days 22–23), dog 7 (days 24–30), dog 10 (days 20–21) (Table 2).

The numbers of *Y. enterocolitica* in faeces collected from the floor of the pen housing Group I dogs were between 10^4 and 10^5 cfu/gm on each occasion.

Period from challenge to first isolation of yersiniae

Y. enterocolitica biotype 4 was isolated from the faeces of all six dogs on the day following experimental inoculation of the test organism. For the animals in-contact with the inoculated dogs, the time to the first faecal isolation of *Y. enterocolitica* was 5 days (dogs 7, 8 and 10), 9 days (dog 11) and 12 days (dog 9). The median time from exposure to first isolation from in-contact dogs was 5 days.

Table 2. *Duration of faecal shedding of* Y. enterocolitica *biotype 4, serotype 0:3*

	Group											
	I†				II				III		IV	
Dog...	1	2	3	4	5	6	7	8	9	10	11	12
Day*												
1–4	+	+	+	+	+	+	−	−	−	−	−	−
5–7	+	+	+	+	+	+	+‡	+‡	−	−	−	−
8–9	+	+	+	+	−	+	+	+	−	−	−	−
10–11	+	+	+	−	−	−	+	+	−	−	−	−
12	+	+	+	−	−	−	+	+	−	+	−	−
13–18	+	−	−	−	−	−	+	+	−§	+§	−	−
19	+	−	−	−	−	−	+	+	+	+	−	−
20–21	+	−	−	−	−	−	+	+	−	Φ	−	−
22	Φ	−	−	−	−	−	+	+	−	−	−	−
23	Φ	−	−	−	−	−	+	+	−	−	+	−
24–30	−	−	−	−	−	−	Φ	−	−	−	+	−
Total	23	12	12	9	7	9	26	19	1	10	8	0

* Day 1 is the first day after inoculation.

† +, Isolation by direct plating and cold enrichment; Φ, Isolation following cold enrichment; −, Culture negative.

‡ Dogs transferred to Group III on day 7.

§ Dogs transferred to Group IV on day 14.

Pharyngeal carriage

Y. enterocolitica biotype 4 was isolated from the pharynx of two dogs inoculated orally; dog 2 from day 2–7 and dog 3 on the first day only.

DISCUSSION

Prior to the start of the trial, faecal samples were cultured from all the experimental dogs and a range of yersinia strains were isolated. Although some were considered environmental in origin. (*Y. enterocolitica* biotype 1a and *Y. intermedia*), others were potential human pathogens (*Y. enterocolitica* biotypes 3, 0:5, 27 and 2, 0:9 and *Y. pseudotuberculosis*). Human infections in New Zealand are usually associated with *Y. enterocolitica* biotype 4, 0:3, however, the other two potentially pathogenic bioserotypes isolated from the dogs are being isolated more frequently from people [17]. The detection of biotype 2, 0:9 in two of the dogs was of interest since this strain has not been isolated from pigs in New Zealand and the source of human infection has not yet been identified. It is worth noting that our dogs were all 6-months of age at the time of the trial and sampling was carried out in winter. Fukushima and coworkers [18], isolated a range of yersinia strains from healthy dogs in Japan more frequently in pups less than 1 year of age and during the colder months. Similarly, Kaneko and coworkers [9] also noted that the highest prevalence of yersinia carriage was in the 5 to 6-month-old age group.

No clinical signs of disease were evident in our dogs and this is consistent with the findings in the majority of surveys in which *Yersinia* spp. were isolated only from apparently healthy subjects [4, 7, 8, 9, 18]. Reports of clinical yersiniosis in dogs, which occurs sporadically, suggest that underlying stress factors may precipitate the development of disease, as they do in other animal species [19].

The duration of excretion is very important since long-term shedding increases the chance of dissemination of the organisms. Faecal shedding of *Y. enterocolitica* varied considerably within our experimental dogs, from 0–26 days, (median duration 9·5 days). It is not known whether this was continuous colonisation or reinfection from the environment although every effort was made to reduce environmental exposure by thorough daily cleaning of the kennels. All pathogenic *Yersinia* spp. share common virulence-associated outer membrane proteins that induce a serological response [20], therefore the variation in faecal shedding could have been associated with the previous immune status of each of the dogs. However, we found no correlation between pre-trial carriage of potentially virulent yersiniae and duration of shedding of the challenge organism.

The period from challenge of the dogs to isolation of the organism from the faeces varied with the method of exposure. Animals that were inoculated orally all excreted the organism after 24 h. However, the first animals that were in-contact with the artificially infected dogs did not excrete detectable numbers of organisms for 5 days. This period was extended further with subsequent groups, the mean period from challenge to isolation for all dogs being 7·2 days. This suggests a progressively lower infectious dose for the in-contact dogs although the numbers of *Y. enterocolitica* excreted in the faeces was not determined in dogs in Groups II to IV. Fukushima and coworkers [18], found up to 10^7 cfu per gm from the intestinal contents of asymptomatic dogs carrying *Y. enterocolitica* biotype 4, 0:3. However, since the minimum infectious dose for *Y. enterocolitica* in dogs is not known the significance of these figures remains unclear.

Pharyngeal carriage of *Y. enterocolitica* biotype 4, 0:3 is common in pigs and the tonsils are believed to constitute the primary reservoir tissue [21], thus the possibility of a similar nidus in dogs was of interest. Pharyngeal carriage was detected, however only one (No. 2) of two dogs carried the organism in the pharynx for an appreciable length of time (6 days). This could be due to the method of sampling employed since yersiniae are believed to colonize the tonsillar crypts in low numbers which means swabbing of the pharynx in live dogs may not be a particularly sensitive method of detection.

In conclusion, the results of our study indicate that dogs may act as a possible source of *Y. enterocolitica* infection for humans since transmission occurs readily between dogs and faecal excretion of the organism can last for several weeks, leading to contamination of the environment. In addition, pharyngeal carriage is possible and thus licking could conceivably transmit the organism. Infants and young children are probably most at risk of infection from canine sources as they are more likely to spend time on the ground and to come into contact with dog faeces. As *Y. enterocolitica* infections are most prevalent in young children [22], the possible role of dogs in the epidemiology of human yersiniosis should not be discounted.

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