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Pet Dogs and Chicken Meat as Reservoirs of *Campylobacter* spp. in Barbados

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***Campylobacter* spp. are the second most common pathogen isolated from stools of patients with gastroenteritis in Barbados. The aim of this study was to identify reservoirs of *Campylobacter* and the likely source(s) of human infection. Fecal specimens from 596 animals and 311 samples of animal food products were analyzed for the presence of *Campylobacter* spp. by standard culture techniques. Isolates were characterized by conventional phenotypic tests, confirmed by latex agglutination and PCR with genus-specific primers, and identified by the use of species-specific primers. High isolation rates were obtained for chickens (94.2%), pigs (90.5%), dogs (46.9%), cats (37.3%), and wild birds (39.3%). *Campylobacter* was also recovered from monkeys (17.1%) and sheep (4.2%) but not from cows. Chicken meat was frequently contaminated with *Campylobacter* (58.4%), but its recovery from other animal food products was rare. *Campylobacter jejuni* was the most commonly identified species in humans (63.6%), chickens (86.6%), dogs (51.5%), and chicken meat (79.8%). Porcine isolates were predominantly *C. coli* (98.4%), while cats harbored mainly *C. upsaliensis* and *C. helveticus*. Wild birds alone carried urease-positive thermophilic campylobacters. *C. jejuni* and *C. coli* isolates from different sources were compared with isolates from humans by randomly amplified polymorphic DNA typing with the primers OPA 11 and HLWL 85. Genotyping revealed similarities between isolates from chicken meat and those from humans and could not distinguish between two clinical isolates and four canine strains. Our results suggest that dogs are significant reservoirs of *Campylobacter* and contribute to human enteric infections and that chicken meat is a likely vehicle for the transmission of campylobacters to humans.**

Campylobacter jejuni is the leading bacterial cause of diarrhea in the developed world (3, 74), and in developing countries, it is associated mainly with illness in children (11, 65; S. N. Workman, G. E. Mathison, and M. C. Lavoie, submitted for publication). In industrialized countries, sporadic cases of human infection are generally attributed to the consumption of raw or undercooked poultry meat (13, 70), whereas outbreaks have been traced to contamination of the water supply (2, 76, 86) and the consumption of contaminated raw milk (6, 10, 19, 43, 72). Natural reservoirs for *Campylobacter* include chicken and other poultry, wild birds, pigs, dogs, cats, sheep, and cows, among others (82, 84). *Campylobacter* species have also been recovered from the feces of exotic pets such as turtles (33), hamsters (22), and monkeys (89).

C. jejuni is carried by most of these animal reservoirs and is the predominant species isolated from chickens and cattle. However, some *Campylobacter* species tend to be associated with particular animal hosts. *C. coli*, *C. hyointestinalis*, and *C. mucosalis* are usually isolated from the intestines of pigs (25, 53, 69). *C. upsaliensis* and *C. helveticus* are predominantly associated with dogs and cats (5, 9), *C. fetus* subsp. *fetus* usually colonizes the intestinal tracts of cattle and sheep (53), and *C. lanienae* is associated with the feces of cattle (40). Wild birds are a large reservoir of *Campylobacter* spp., including urease-positive thermophilic campylobacters, *C. jejuni*, and *C. lari* (34, 42, 57).

The incidence of human *Campylobacter* enteritis in Barbados has increased over the past few years (S. N. Workman, S. Sobers, G. E. Mathison, and M. C. Lavoie, submitted for publication). To date, there has been no investigation of the source(s) of human *Campylobacter* infections on the island. The present study was performed in order to identify local animal reservoirs and to assess the level of contamination of animal food products. The chief animal reservoirs of *Campylobacter* spp. were chickens, dogs, pigs, cats, and wild birds, and chicken meat was the only food product that was frequently contaminated. The identification of isolates to the species level and randomly amplified polymorphic DNA (RAPD) typing of isolates revealed that the most likely sources of human campylobacteriosis are dogs and chicken meat. This is the first step in an effort to devise strategies for the control of human *Campylobacter* infection on the island.

MATERIALS AND METHODS

A total of 596 animals and 311 animal food products were analyzed for the presence of *Campylobacter* spp. Specimens were collected between January 2002 and April 2004.

Testing of animals. Food animals were tested for *Campylobacter* spp. either while being reared on the farm or upon delivery to the abattoir for slaughter. On farms, fresh feces were collected from pigs, cows, and sheep, placed in 50-ml Corning Falcon tubes, and transported to the laboratory on crushed ice for analysis. One gram of feces was suspended in 9 ml of buffered peptone water, and one loopful of the fecal suspension was plated onto cefoperazone amphotericin teicoplanin (CAT) agar (*Campylobacter* blood-free agar base [Oxoid Ltd., Basingstoke, United Kingdom] supplemented with CAT selective supplement [Oxoid Ltd.]) and modified cefoperazone charcoal deoxycholate agar (mCCDA) (*Campylobacter* blood-free agar base [Oxoid Ltd.] supplemented with CCDA

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selective supplement [Oxoid Ltd.]). Plates were incubated at 37°C for up to 7 days in a microaerobic atmosphere (6% O_2, 14% CO_2) generated with the CampyGen system (Oxoid Ltd.) At the abattoir, rectal swabs were taken from animals prior to evisceration. Swabs were streaked onto CAT and mCCDA and incubated as described for fecal samples. In total, 61 cows, 74 pigs, and 71 sheep were tested.

Rectal swabs were obtained from 130 healthy and diarrheic dogs and 51 cats from the local Royal Society for the Prevention of Cruelty to Animals (RSPCA), a private veterinary clinic, and various households. Cloacal swabs were obtained from 69 broiler chicks on the farm or at slaughter and from 61 wild birds at a recreational shooting swamp. The wild birds tested included both migratory and resident birds representing the following species: Lesser yellowlegs (*Tringa flavipes*), "pika"/Greater yellowlegs (*Tringa melanoleuca*), snipe (*Gallinago gallinago*), "que"/stilt sandpiper (*Micropalama himantopus*), chirp (not identified), cattle egret (*Bubulcus ibis*), ramier (*Columba squamosa*), golden plover (*Pluvialis dominica*), blue-winged teal (*Anas discors*), nit/semipalmated sandpiper (*Calidris pusilla*), and local ducks (*Anas* sp.). Samples from wild birds were collected immediately postmortem.

Rectal swabs were also obtained from nine mongooses which were trapped for an independent study being carried out by the Centre for Resource Management and Environmental Studies of the University of the West Indies and from 70 vervet monkeys (*Cercopithecus aethiops sabaeus*) which were processed by the Barbados Primate Research Centre and Wildlife Reserve for export to be used for vaccine production.

All specimens were transported to the laboratory on crushed ice and stored at 4°C until further processing. Samples were processed as soon as possible and always within 24 h of collection. Swabs were plated onto CAT and mCCDA selective media and incubated at 37°C for up to 7 days in a microaerobic atmosphere generated with a CampyGen system (Oxoid Ltd.).

Testing of animal food products. Various cuts of a range of fresh meats were purchased from 10 retail outlets on the island, ranging from small meat shops to large supermarkets. Samples were rinsed in 50 or 100 ml (depending on the size of the sample) of sterile maximum recovery diluent (0.1% [wt/vol] peptone, 0.9% [wt/vol] sodium chloride) for 2 min with agitation. Two milliliters of the rinse was added to 18 ml of cefoperazone charcoal deoxycholate broth (CCDB) and pre-enriched for 3 h at 37°C. Eighty microliters of reconstituted CCDA supplement (Oxoid Ltd.) was added to the broth for selective enrichment at 37°C for a further 21 h. Enrichments were streaked onto CAT and mCCDA plates and further incubated for up to 7 days.

Additional testing of meat was conducted at a local abattoir. Immediately after slaughter of the animal and just prior to evisceration, the area around the hind legs of the carcass was wiped with a sterile cotton pad which was moistened with sterile maximum recovery diluent. Pads were placed in CCDB and transported to the laboratory on ice. Upon arrival, they were pre-enriched, enriched, and plated as described for the rinses. In total, 77 chicken, 78 pork, 56 beef, 50 lamb, and 20 turkey samples were analyzed.

Fresh, raw goat's milk and fresh, pasteurized cow's milk were also analyzed for *Campylobacter* contamination. Goat's milk is marketed on a very small scale, so testing was conducted on a monthly basis, depending on availability. Overall, 10 samples of goat's milk and 20 samples of cow's milk were analyzed. The pH of the milk was adjusted to above 8.0 by using sterile sodium hydroxide, and 50-ml samples were centrifuged at 20,000 × *g* for 40 min. The fat layer was removed from the surface and the supernatant was discarded. The pellet was resuspended in 10 ml of CCDB, transferred to 90 ml of CCDB, and pre-enriched at 37°C for 4 h, followed by selective enrichment at 42°C. After 24 and 48 h of enrichment, a loopful of broth was plated onto CAT agar and incubated at 37°C for up to 7 days.

Testing of water. Tap water was tested on a monthly basis. Local tap water is chlorinated by the Barbados Water Authority and stored in reservoir tanks across the island prior to distribution to residences and businesses. Four-liter samples were collected from domestic pipes (one located on the university campus and another located at a household in the parish of Christ Church, representing desalinated tap water and tap water from groundwater supplies, respectively) and stored on ice until further processing. The tap was turned on for 3 min before collection of the sample. Five milliliters of sterile 1 M sodium thiosulfate solution was added to each liter of chlorinated tap water to neutralize the chlorine prior to filtration through a 0.22-μm-membrane filter. The filter was placed in a MacCartney bottle containing 20 ml of CCDB, pre-enriched initially at 30°C for 3 h and then at 37°C for 2 h, and selectively enriched at 42°C. Loopfuls of enrichment were plated on CAT agar after 24 and 48 h of enrichment and incubated for up to 7 days in a microaerobic atmosphere. Water from communal standpipes located on the roadside in two parishes on the island was also analyzed as described for tap water.

Confirmation of isolates. Suspected colonies were purified on Columbia blood agar base (Oxoid Ltd.) supplemented with 5% (vol/vol) sterile laked horse blood (Oxoid Ltd.) and confirmed as *Campylobacter* based on their characteristic cell morphology and motility, catalase and oxidase tests, and latex agglutination in an INDXCampy (jcl) culture confirmation test (PanBio INDX, Inc., Baltimore, Md.). Confirmed isolates were stored for further characterization in brain heart infusion broth (Difco, Becton Dickinson and Company, Sparks, Md.) containing 20% glycerol, initially at −20°C and subsequently at −70°C.

Crude lysates of the isolates were prepared by suspending 10-μl loopfuls of 24- to 48-h bacterial growth in 500 μl of sterile distilled water and boiling them for 10 min. Cellular debris was removed by centrifugation at 14,000 × *g* for 5 min in a benchtop microcentrifuge. The supernatants were transferred to sterile microcentrifuge tubes and stored at −20°C for PCR analysis.

PCR with genus-specific primers was used for further confirmation of the isolates since the latex agglutination kit was not designed to detect all species of *Campylobacter*. The C412F/C1228R primer set developed by Linton et al. (47) was used to amplify an 816-bp fragment of the rRNA gene of *Campylobacter* spp. Briefly, PCR amplification was performed in a 25-μl volume containing 20 mM Tris-HCl (pH 8.4), 50 mM KCl, 2.5 mM $MgCl_2$, a 200 μM concentration of each deoxynucleoside triphosphate, a 0.4 μM concentration of each primer, 0.625 U of recombinant *Taq* DNA polymerase (Invitrogen Life Technologies, Carlsbad, Calif.), and 2.5 μl of crude DNA extract. The mixture was cycled through the following temperature profile in a Techne Progene thermocycler (Techne Ltd., Cambridge, United Kingdom): 30 cycles of 94°C for 1 min, 55°C for 1 min, and 72°C for 1 min. PCR products were visualized in ethidium bromide-stained 1% agarose (Ultrapure; Invitrogen) gels and viewed on a UV transilluminator.

Identification of isolates to the species level. The hippurate hydrolysis test was used to identify *C. jejuni* among confirmed isolates. A small quantity of 24-h growth was suspended in 400 μl of filter-sterilized 0.1% (wt/vol) sodium hippurate solution and incubated at 37°C for 2 h. Two hundred microliters of ninhydrin reagent (3.5% [wt/vol] ninhydrin in a 1:1 mixture of acetone and butanol) was added and incubated for a further 10 min. The development of a purple-violet color identified the isolate as *C. jejuni*. Hippurate hydrolysis-positive isolates were confirmed as *C. jejuni* by PCR with the cj hip primer pair (46).

Suspected *C. upsaliensis* or *C. helveticus* isolates, detected by a weak or negative catalase test, were confirmed by PCR with the CHCU146F/CU1024R and CHCU146F/CH1371R species-specific primers, respectively (39). All other isolates were subjected to PCR with primers specific for *C. coli* (cc asp primers) (46), *C. fetus* (CFCH57F/CF1054R), *C. lari* (CL594F and CL1155R), and *C. hyointestinalis* (CFCH57F/CH1344R) (47). The PCR mixtures and cycling conditions for species-specific PCRs were the same as those described for the genus-specific primers, but with different annealing temperatures as follows: 60°C for *C. coli*, *C. upsaliensis*, and *C. helveticus*; 64°C for *C. lari*; 65°C for *C. hyointestinalis* and *C. fetus*; and 66°C for *C. jejuni*.

Urease-positive thermophilic campylobacters (UPTC) were identified based on a negative hippurate hydrolysis test, the production of catalase and urease, growth at 42°C, and a failure to be amplified with any of the species-specific primers.

Statistical analysis. The chi-square test (P values of <0.05 indicated significance, and P values of <0.01 indicated a high level of significance) was used to test the association between the prevalence of *Campylobacter* and animal characteristics. Some of the data were tested by Fisher's exact two-tailed test because of their small numbers. Statistical calculations were performed by using the VassarStats website for statistical computation (http://faculty.vassar.edu/lowry/VassarStats.html) and the GraphPad QuickCalcs free online calculator for scientists (http://www.graphpad.com/quickcalcs/index.cfm) (accessed May to June 2004).

Molecular typing of isolates. Isolates of *C. jejuni* and *C. coli* from different sources were selected for genotyping and comparisons with clinical isolates obtained from the stools of patients with diarrhea in a previous study (Workman et al., submitted). Isolates were subtyped by RAPD PCR analysis using the primer OPA 11 (5′-CAA TCG CCG T-3′) based on the method of Hernandez et al. (36), with a few modifications. Template DNAs were prepared as described by Miwa et al. (55). For each isolate, a suspension of bacterial cells was prepared in sterile distilled water, and the cell density was adjusted to that of a McFarland no. 1 standard. The suspension was boiled for 10 min, and cell debris was pelleted by centrifugation at 14,000 × *g* in a benchtop centrifuge for 1 min. The supernatant was used as the template DNA. The RAPD reaction mixture consisted of 50 mM KCl, 10 mM Tris-HCl (pH 8.4 at 25°C), 2.5 mM $MgCl_2$, 0.1% Triton X-100, a 200 μM concentration of each deoxynucleoside triphosphate, 0.3 μM of the primer, 2.5 U of *Taq* DNA polymerase (Invitrogen), 2.5 μl of the template DNA, and sterile distilled water to a final volume of 25 μl. The mixture was cycled through the following temperature profile in a Techne Genius thermocy-

TABLE 1. Frequency of isolation of *Campylobacter* spp. from animals and animal food products

Sample type	No. of samples	No. of *Campylobacter* positive samples (%)
Animals		
Dogs	130	61 (46.9)
Cats	51	19 (37.3)
Cows	61	0 (0)
Pigs	74	67 (90.5)
Sheep	71	3 (4.2)
Wild birds	61	24 (39.3)
Chickens	69	65 (94.2)
Monkeys	70	12 (17.1)
Mongooses	9	0 (0)
Animal food products		
Chicken	77	45 (58.4)
Pork	78	3 (3.9)
Beef	56	1 (1.8)
Lamb	50	0 (0)
Turkey	20	1 (5)
Goat's milk	10	0 (0)
Cow's milk	20	0 (0)

cler (Techne Ltd., Cambridge, United Kingdom): an initial denaturation step at 94°C for 1 min; 45 cycles of 94°C for 1 min, 36°C for 1 min, and 72°C for 2 min; and a final elongation step at 72°C for 5 min. Some of the isolates from different sources which gave similar banding patterns with OPA 11 were also genotyped by use of the random primer HLWL 85 (5′-ACA ACT GCT C-3′) (50) for further discrimination. The PCR mixture and amplification conditions were the same as those described for OPA 11. The amplified DNA fragments were electrophoresed in ethidium bromide-stained 1.5% (wt/vol) agarose gels (Ultrapure agarose; Invitrogen). A 1-kbp Plus DNA ladder (Invitrogen) was used as a size marker for the PCR products. Gels were photographed by using a Kodak Digital Science electrophoresis documentation and analysis system (model 120; Eastman Kodak Co., N.Y.) and were interpreted visually.

RESULTS

Two hundred forty-nine (41.8%) of the animals tested carried *Campylobacter* spp. *Campylobacter* was isolated from chickens, dogs, cats, pigs, sheep, wild birds, and monkeys (Table 1). High isolation rates were obtained for chickens (94.2%), pigs (90.5%), dogs (46.9%), wild birds (39.3%), and cats (37.3%). All broiler chicks older than 3 weeks carried *Campylobacter*. *Campylobacter* was not recovered from the feces of cows or mongooses. Primary isolation plates that were streaked with suspensions of cow and sheep feces generally became covered with fungal growth (an *Aspergillus* sp.) by the fourth day of incubation.

Contamination of chicken meat was common (58.4%), but the isolation of *Campylobacter* from other meats and animal food products was rare (Table 1). Samples of tap water and water from standpipes were consistently negative for *Campylobacter*.

Four hundred sixty-two isolates were recovered from animals and animal food products during this study. Twenty-six (5.6%) of the isolates lost viability before they could be further characterized and thus could not be identified, and another 31 (6.7%) isolates were not identified by any of the procedures outlined above (Table 2). There were 13 cases of infection or contamination with more than one *Campylobacter* species, including 2 in chicken meat, 3 in broiler chicks, 1 in cats, and 7 in wild birds.

C. jejuni was the most commonly identified species isolated from dogs, chickens, and chicken meat (Table 2). Pigs carried *C. coli* almost exclusively, *C. upsaliensis* was only isolated from dogs and cats, and *C. helveticus* was recovered from cats alone (Table 2). UPTC strains were harbored exclusively by several wild birds. Table 3 shows the isolation frequencies and distributions of *Campylobacter* species among different wild bird species. The carriage rates were significantly higher for migrating birds than for resident birds ($\chi^2 = 12.438$; $P = 0.0004$).

The carriage of *Campylobacter* in dogs was generally asymptomatic, but there was no significant difference in carriage rates between diarrheic and nondiarrheic dogs ($\chi^2 = 0.299$; $P = 0.5844$). *Campylobacter* was more common in young dogs. The average age of *Campylobacter*-positive dogs was 17.6 months, in contrast to 50.0 months for *Campylobacter*-negative dogs. Over 70% of *Campylobacter*-positive dogs were under 1 year old, and 32.8% were younger than 9 weeks old. The number of *Campylobacter*-positive dogs that were younger than nine weeks was significantly higher than that of *Campylobacter*-negative dogs in the same age group ($\chi^2 = 10.855$; $P = 0.0010$). Likewise, the majority of dogs of less than 1 year old were carriers ($\chi^2 = 7.107$; $P = 0.0077$). The rate of isolation was significantly higher for dogs from the RSPCA than for dogs from other sources ($\chi^2 = 10.218$; $P = 0.0014$). There was a high level of carriage in stray dogs that were up for adoption (77.3%) and a significantly lower isolation rate from household dogs (23.7%) ($\chi^2 = 6.143$; $P = 0.0132$).

TABLE 2. Species distribution of *Campylobacter* isolates

Source (*n*)	No. of isolates (%)					
	C. jejuni	*C. coli*	*C. upsaliensis*	*C. helveticus*	UPTC	Unidentified
Humans[a] (44)	28 (63.6)	14 (31.8)	0	0	0	2 (4.5)
Chicken meat (94)	75 (79.8)	13 (13.8)	0	0	0	6 (6.4)
Pork (3)	0	1 (33.3)	0	0	0	2 (67.7)
Beef (1)	1 (100)	0	0	0	0	0
Dogs (66)	34 (51.5)	5 (7.6)	3 (4.5)	0	0	24 (36.4)
Cats (20)	2 (10)	0	6 (30)	10 (50)	0	2 (10)
Pigs (122)	0	120 (98.4)	0	0	0	2 (1.6)
Sheep (3)	0	1 (33.3)	0	0	0	2 (66.7)
Wild birds (42)	12 (28.6)	3 (7.1)	0	0	19 (45.2)	8 (19.1)
Chickens (97)	84 (86.6)	10 (10.3)	0	0	0	3 (3.1)
Monkeys (13)	4 (31.8)	2 (15.4)	0	0	0	7 (53.8)

[a] Data from Workman et al. (submitted).

TABLE 3. Carriage of *Campylobacter* species by different wild bird species

Common name (species) (*n*)	No. of positive samples (%)	Species carried (no. of isolates)
Resident birds		
Cattle egret (*Bubulcus ibis*) (2)	1 (50)	*C. jejuni* (1), unidentified (1), mixed infection (1)
Ramier (*Columba squamosa*) (2)	0 (0)	
Duck (family Anatidae) (20)	0 (0)	
Migrating birds		
Lesser yellowlegs (*Tringa flavipes*) (13)	9 (69.2)	*C. jejuni* (5), *C. coli* (1), UPTC (4), unidentified (2), mixed infection (3)
Greater yellowlegs (*Tringa melanoleuca*) (3)	3 (100)	*C. coli* (1), UPTC (2)
Golden plover (*Pluvialis dominica*) (3)	1 (33.3)	UPTC (1)
Blue-winged teal (*Anas discors*) (2)	1 (50)	Unidentified (1)
Semipalmated sandpiper (*Calidris pusilla*) (3)	1 (33.3)	*C. coli* (1)
Common snipe (*Gallinago gallinago*) (1)	1 (100)	*C. jejuni* (1), UPTC (1)
Stilt sandpiper (*Micropalama himantopus*) (4)	4 (100)	*C. jejuni* (2), UPTC (3)
"Chirp" (not identified) (8)	3 (37.5)	*C. jejuni* (1), UPTC (2)

Fifty percent of dogs diagnosed with parvovirus infection were positive for *Campylobacter*, and it was observed that coinfection was associated with a more severe disease and a poor prognosis. There was no significant difference in carriage rates between male and female dogs ($\chi^2 = 1.065$; $P = 0.320$).

There was no statistically significant association between the age, origin, or any other characteristic of cats and their *Campylobacter* status.

The results of RAPD subtyping of *C. jejuni* and *C. coli* isolates from various sources with the random primer OPA 11 are shown in Fig. 1 to 3. All isolates from wild birds, monkeys, cats, and pigs were distinct from human strains. The OPA 11 fingerprints of two human *C. jejuni* strains were identical to those of four canine isolates (Fig. 1, lanes 10, 11, and 16 to 18; only three of the canine isolates are represented), and a human *C. jejuni* isolate was identical to one from chicken meat (Fig. 1, lanes 3 and 27). The OPA 11 profiles of another five human *C. coli* isolates were indistinguishable from those of five isolates from chicken meat (Fig. 3, lanes 2 to 6 and 14 to 18). When these like strains were analyzed with the HLWL 85 primer, only two clinical *C. jejuni* isolates were identical to four strains from dogs (Fig. 4, lanes 9 to 14).

DISCUSSION

Samples from a variety of animals and food products were analyzed for the presence of *Campylobacter* spp. Of the animals tested, chickens had the highest prevalence of *Campylobacter* (94.2%), and all negative chicks were under the age of 3 weeks. It has long been recognized that there is a high prevalence of *Campylobacter* in broiler chicks. *Campylobacter* is considered part of the normal flora of the gut of broiler chicks, and they generally become colonized at 2 to 3 weeks of age (60). Furthermore, with intensive chicken farming, one positive bird in a flock soon spreads the infection to the entire flock, so it is likely that birds from the same pen will have the same *Campylobacter* status. Conversely, a low isolation rate for broiler chicks (36%) was found in Denmark (63). For that study, only one sample was collected from each flock analyzed,

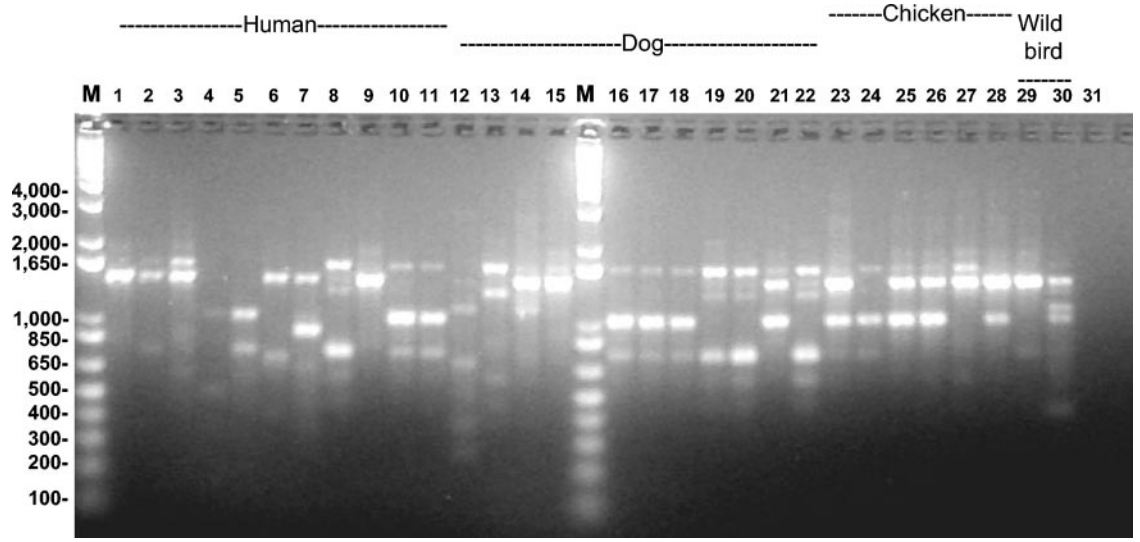

FIG. 1. RAPD profiles of representative *C. jejuni* isolates, using the random primer OPA 11. Lanes M, 1-kb Plus ladder; lanes 1 to 11, isolates from patients with diarrhea; lanes 12 to 22, isolates from dogs; lanes 23 to 28, isolates from chickens; lanes 29 and 30, wild bird isolates; lane 31, negative control (sterile distilled water).

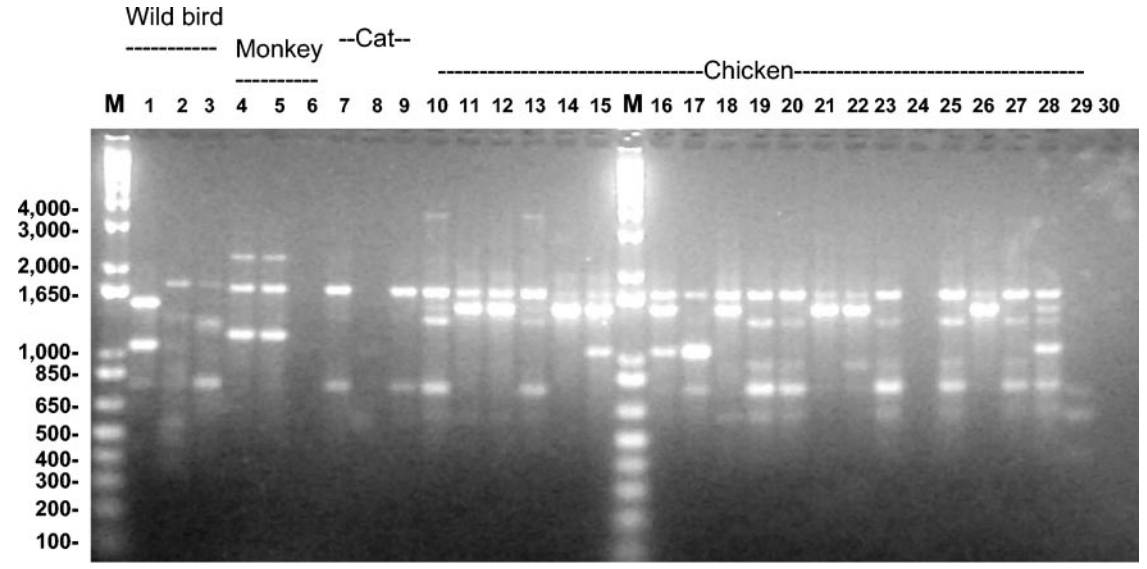

FIG. 2. RAPD profiles of representative *C. jejuni* isolates, using the random primer OPA 11. Lanes M, 1-kb Plus ladder; lanes 1 to 3, isolates from wild birds; lanes 4 to 6, isolates from monkeys; lanes 7 to 9, isolates from cats; lanes 10 to 29, isolates from chickens; lane 30, negative control (sterile distilled water).

so the resulting prevalence rate is a measure of the number of flocks that were contaminated rather than the number of birds. An investigation of fewer birds on a larger number of local broiler farms may yield a different prevalence rate and give a better indication of the overall prevalence of *Campylobacter* in broiler chicks on the island.

The high prevalence of *Campylobacter* in pigs in our study is consistent with it being part of the normal flora of swine. Young et al. found that from birth, pigs are very susceptible to colonization by *Campylobacter*, and they reported carriage rates of 57.8% and 100% for newborn and weaned piglets, respectively (91). *C. coli* was the most common species isolated from pigs. This is consistent with the findings of most investigations of the carriage of campylobacters in swine (53, 63, 69, 75). Nevertheless, there are also reports of a high prevalence of *C. jejuni* in pigs (32, 91).

The isolation rate for dogs in our study was comparable to that obtained in Sweden (56%) (18) but was much higher than that obtained in a study done in Argentina (17%) (48). Like other investigators, we found a high carriage rate for asymptomatic dogs (61), and in accordance with many others, there was no significant difference in isolation rates between healthy and diarrheic dogs (48, 66, 73, 77). In contrast, Fleming (21) and Nair et al. (59) found a higher prevalence in diarrheic dogs, and Burnens et al. noted that this relationship only held true for younger dogs (9).

Consistent with most studies, we found that *Campylobacter* was more common in young dogs (1, 18, 59, 88). We also noted

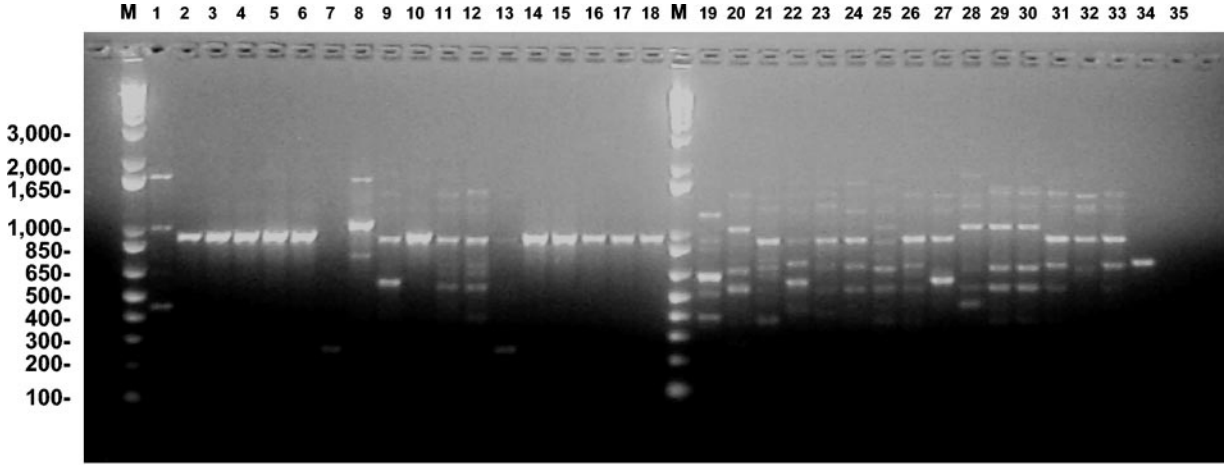

FIG. 3. RAPD profiles of representative *C. coli* isolates, using the random primer OPA 11. Lanes M, 1-kb Plus ladder; lane 1, isolate from a wild bird; lanes 2 to 8, isolates from humans; lane 9, isolate from a dog; lanes 10 to 18, isolates from chickens; lanes 19 to 34, isolates from pigs; lane 35, negative control (sterile distilled water).

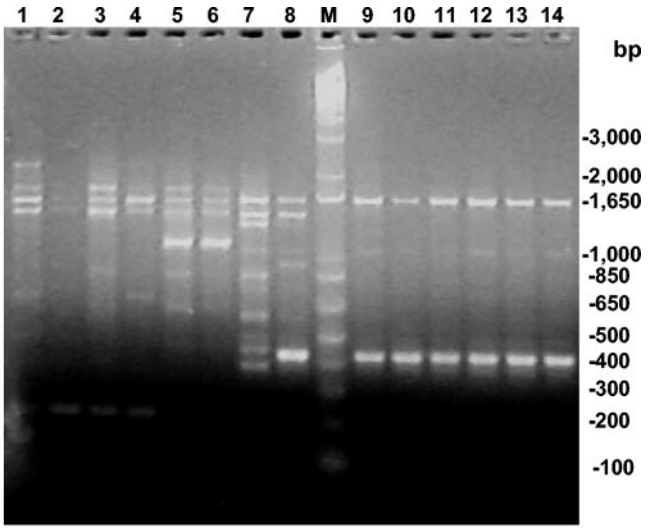

FIG. 4. HLWL 85 RAPD profiles of isolates with identical OPA 11 profiles. Lane M, 1-kb Plus ladder; lanes 1 to 6, *C. coli* isolates from humans and chickens; lanes 7 and 8, *C. jejuni* isolates from a human and a chicken; lanes 9 to 14, *C. jejuni* isolates from humans and dogs.

a significantly higher incidence in stray dogs than in household dogs, and we propose that stray dogs may have more exposure to environmental sources of *Campylobacter* than do household pets. Similarly, Simpson et al. (81) and Fernandez and Martin (20) found that campylobacters were isolated more frequently from stray dogs than from pet dogs. This might also explain the low prevalence obtained by Lopez et al., as their investigation was limited to domestic animals (48).

Our results imply that there is a relationship between canine parvovirus infections and the carriage of *Campylobacter*, with coinfection apparently affecting the disease severity and prognosis. Intestinal colonization with *Campylobacter* may predispose dogs to infection with parvovirus, or a secondary infection with *Campylobacter* may occur in dogs infected with parvovirus and may increase the severity of the primary infection. Such an association was also suggested by Sandstedt and Wierup (78) and Olson and Sandstedt (66).

In our study, *Campylobacter* was carried by 37.3% of cats, which is a higher isolation rate than that for domestic cats in Argentina (48) and for cats in Norway (77). The species identified among our feline isolates were expected, as cats are established reservoirs of *C. jejuni*, *C. upsaliensis*, and C. *helveticus* (5, 9, 23, 24, 29). Such a high frequency of infection with *C. helveticus* (50%), however, could not be found in the literature. As reported by several other groups, there was no significant difference in carriage between healthy and diarrheic cats (9, 21, 77), and there was no difference in prevalence between household cats and cats from the RSPCA that were up for adoption.

The failure to recover *Campylobacter* from cows and the low isolation rate from sheep were unexpected. Our data are comparable with those of Rosef et al., who isolated *Campylobacter* from 0.8% of cows and 8.1% of sheep (75). However, there are several reports of higher isolation rates from cattle, ranging from 5% to 89.4% (38, 39, 53, 63, 83), and from sheep (53, 73). The frequent overgrowth of our primary isolation plates with *Aspergillus* before the end of the 7-day incubation period may well have hindered the detection of *Campylobacter* strains present in cow or sheep feces, resulting in an underestimation of the carriage rate. The incorporation of an alternative antifungal agent such as cycloheximide or Saproxin (Randolph Biomedical) into the primary isolation medium may aid in decreasing such saprophytic growth and allow the emergence of *Campylobacter* strains that require incubation for more than 4 days. Initially, a membrane filtration isolation method was used in conjunction with mCCDA and CAT media. However, this was discontinued in the interest of economy when there was no difference in isolation rates between the two methods. PCR detection of *Campylobacter* directly from feces may give a better indication of the carriage rate in cows (40).

There are reports of seasonal variations in shedding by cows and sheep and also of age-related changes in colonization (27, 62, 83). More frequent sampling throughout the year may give a better indication of the prevalence of *Campylobacter* in these animals. Higher prevalence rates in cattle (>80%) are obtained when PCR-based methods are used (41, 90), highlighting the difficulty encountered in comparing results from studies using different detection methods.

The diversity of *Campylobacter* species isolated in this study may have been restricted by the use of the CampyGen gas-generating system, as it does not create a hydrogen-enriched atmosphere, which is required by species such as *C. sputorum*, *C. concisus*, *C. mucosalis*, *C. hyointestinalis*, and some strains of *C. upsaliensis*. This may also have contributed to our low isolation rates from sheep and cows.

Several studies have indicated that poultry meat is often contaminated with *Campylobacter*, with isolation rates reaching as high as 100% (79). Our recovery rate (58%) was comparable with those reported for France (58%) (26) and The Netherlands (61%) (12) but slightly higher than the rates in Spain (49.5%) (14) and Germany (45.9%) (4) and lower than the prevalence rates in Japan (67.9%) (87) and the United States (98% and 70.7%) (85, 93). There were fewer investigations of contamination of turkey meat in the literature. Our isolation rate from turkeys (5%) was lower than those reported for the United States (14%) (93) and Italy (20%) (92) but similar to that reported for turkey carcasses from Poland (3%) (44).

Based on the isolation rates from local cows and sheep, the low recovery rates from beef and lamb were expected. However, pork meat was infrequently contaminated, despite the very high prevalence of *Campylobacter* in pigs. Hygiene practices at the slaughterhouse and processing methods can explain these low recovery rates. There is less chance of spillage of intestinal contents onto the meat surface during the processing of these meats than during the evisceration of chickens. Furthermore, chilling of carcasses prior to delivery to the butcher would significantly reduce *Campylobacter* levels (67). The frequency of contamination of red meats with *Campylobacter* spp. is customarily very low, and similar prevalence rates were obtained in the United States (31, 93), Japan (87), and Italy (92).

The frequency of isolation of *Campylobacter* from wild birds was high (39.3%), particularly among migratory birds, yet the species most commonly identified were different from the species found in clinical isolates. Furthermore, the *C. jejuni* and *C. coli* strains from wild birds were genotypically distinct from

clinical strains. Therefore, these birds are not a likely source of human infection in Barbados. In spite of this, they are significant reservoirs of UPTC, among other species, and can transmit campylobacters over long distances both within the island and across international borders.

Few studies have found a similarity between strains isolated from birds and clinical isolates when molecular techniques have been used to compare strains (7, 8), except for isolates from birds that are closely associated with human activities (8). Resident birds such as the sparrow, pigeon, ground dove, and black bird (common names), which have closer contact with humans than the birds that we examined, should be tested so that their contribution to human infection can be determined. Interestingly, *C. jejuni* was carried by one of two cattle egrets tested. These resident birds frequent farmlands in search of food and may come in contact with farm animals. Their role in the transmission of campylobacters within the island is worthy of investigation.

Based on their very low *Campylobacter* isolation rates, milk (pasteurized and raw), pork, beef, and lamb are not likely sources of human infection on the island. Likewise, contamination of the local water supply was not detected, and although more extensive testing of the water supply is necessary, it is not likely that this is a major source of human infection. This contrasts with the situation in another Caribbean island, Curaçao, where there is evidence that contamination of the water supply during the rainy season is responsible for outbreaks of *Campylobacter* enteritis (17). In Barbados, there is no association between human cases of *Campylobacter* enteritis and the rainy season (S. N. Workman et al., submitted for publication).

In our study, strains of *C. helveticus* were restricted to cats, and *C. upsaliensis* was only isolated from cats and dogs. This suggests that dogs and cats are the most likely sources of human infection with *C. upsaliensis*, which is consistent with the general consensus that these species are almost exclusive to these animals (29). The proportion of positive dogs that carried *C. upsaliensis* (4.5%) was lower than expected based on isolation rates from other studies (18, 30, 77). However, it was comparable with the carriage rate obtained in a study in Brazil (56).

We recorded the carriage of multiple *Campylobacter* species by broiler chicks, wild birds, cats, and chicken meat, albeit infrequently. This is significant, as it implies that with a single exposure to such reservoirs or vehicles of infection, more than one species can be transmitted, thus complicating efforts to trace human infection to a particular reservoir. Furthermore, it underscores the importance of characterizing several presumptive colonies from each specimen and using culture techniques that allow for the isolation of multiple species.

Taking into consideration the species recovered from patients with diarrhea and those recovered from animals and animal food products, chicken meat, chickens, and dogs are the most likely sources of *C. jejuni* infection, pigs are the most probable source of *C. coli* infection, and dogs and cats are the most likely source of infection with *C. upsaliensis*. However, a RAPD analysis of strains from these potential sources showed that very few of them were related to clinical strains.

The overwhelming majority (77.4%) of our *C. coli* isolates were obtained from pigs, suggesting that they are the most probable source of human infection with *C. coli*. Although pigs are a large reservoir of *C. coli*, RAPD profiles of porcine isolates were distinct from those of *C. coli* isolates from patients with diarrhea. Some of the *C. coli* isolates from chicken meat gave OPA 11 fingerprints which were identical to those of human *C. coli* isolates, although their HLWL 85 profiles were distinguishable. Thus, chicken meat appears to be a more likely vehicle of transmission of *C. coli* to humans than pigs or pork. Despite this observation, pigs remain a risk factor for *C. coli* infection in individuals who come into close contact with them.

Our data suggest that chicken meat is a likely vehicle for the transmission of *Campylobacter* to humans. RAPD fingerprinting of chicken isolates revealed that *C. coli* isolates from certain brands of chicken meat were related to human isolates, although they were not genetically identical. In addition, the high frequency of contamination of chicken meat with *C. jejuni* (the species that is most commonly isolated from patients with enteritis) shows its potential as a source of human infection, although poultry and human *C. jejuni* strains gave different RAPD patterns.

The high incidence of *Campylobacter* in dogs and the predominance of *C. jejuni*, including strains that were identical to human isolates, suggest that dogs are a more important source of *C. jejuni* enteritis than chickens. Furthermore, although the prevalence of *Campylobacter* was lower in household pets than in stray dogs, the canine strains which were genetically related to human isolates were isolated from household dogs attending a veterinary clinic for the treatment of various ailments. Similarly, Torre and Tello found that biotypes recovered from dogs were the same as those associated with human cases of *Campylobacter* enteritis (88). More recently, Siemer et al. evaluated the genetic relatedness of isolates with human and animal origins by using the amplified fragment length polymorphism (AFLP) technique, and they showed that dogs should be considered a source of human infection in Denmark (80).

RAPD subtyping with OPA 11 has been recognized as a useful tool for the typing of *Campylobacter* strains isolated during epidemiological studies (36) and has been used to detect DNA polymorphisms in *C. jejuni* and *C. coli* strains from human feces, seawater, poultry meat, and broiler chicks (36, 55, 68). Other random primers have been used by several other investigators to establish relationships between *Campylobacter* strains from different sources (37, 49, 54, 64). We chose this method because it was the most cost-effective typing technique available in our laboratory. Other more discriminatory techniques such as multilocus sequence typing (52), ribotyping (51), pulsed-field gel electrophoresis macrorestriction profiling (58), AFLP (15, 16), and combinations of these (35, 71) have been used to compare the genotypes of *Campylobacter* strains isolated from various reservoirs with those of isolates from patients with *Campylobacter*-associated diseases. The application of one or more of these other tools may assist with clarifying the relationships between the strains which we found to be closely related by RAPD analysis.

Sequencing of the 16S rRNA gene sequences amplified by genus-specific PCR may allow for better characterizations of some our unidentified isolates (28, 45), particularly the UPTC strains and perhaps those strains which lost viability. Furthermore, it could also be used for molecular typing instead of RAPD analysis. However, we do not have access to an automated sequencer and our lab is not fully equipped for manual

sequencing. Hence, we opted for a RAPD-PCR method which had been successfully used by several other research groups and was within our limited budget.

Our findings suggest that public health protection measures to control human *Campylobacter* enteritis in Barbados should focus on preventing transmission from dogs and chicken meat to humans. This may involve reducing the contamination of chicken meat and encouraging adequate hand washing after contact with pet dogs. Barbadians are in the habit of cooking meat thoroughly and tend not to consume raw meats. Furthermore, chicken is generally treated with acidic lime juice, salt, and various spices during preparation, all of which are expected to reduce the levels of contaminating campylobacters. We therefore believe that cross-contamination in the kitchen is a more likely route of transmission than the consumption of chicken meat. A more extensive investigation of the role of dogs and chicken meat in the transmission of *Campylobacter* species to humans is warranted.

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