Veterinary Microbiology 168 (2014) 124–130

Contents lists available at ScienceDirect

Veterinary Microbiology

journal homepage: www.elsevier.com/locate/vetmic

Genotypes and antibiotic resistance of canine *Campylobacter jejuni* isolates

Chantal Amar [a], Sonja Kittl [a], David Spreng [b], Andreas Thomann [a], Bożena M. Korczak [a], André P. Burnens [c], Peter Kuhnert [a,*]

[a] Institute of Veterinary Bacteriology, Vetsuisse Faculty, University of Bern, Bern, Switzerland
[b] Small Animal Clinic, Vetsuisse Faculty, University of Bern, Bern, Switzerland
[c] Unilabs, Bern, Switzerland

ARTICLE INFO

Article history:
Received 31 July 2013
Received in revised form 2 October 2013
Accepted 3 October 2013

Keywords:
Dog
Zoonosis
Prevalence
Multi locus sequence typing
Campylobacter
Antibiotic resistance

ABSTRACT

Campylobacter jejuni is the most important cause of bacterial gastroenteritis in humans. It is a commensal in many wild and domestic animals, including dogs. Whereas genotypes of human and chicken *C. jejuni* isolates have been described in some detail, only little information on canine *C. jejuni* genotypes is available. To gain more information on genotypes of canine *C. jejuni* and their zoonotic potential, isolates from routine diagnostics of diarrheic dogs as well as isolates of a prevalence study in non-diarrheic dogs were analyzed. Prevalence of thermophilic *Campylobacter* among non-diarrheic dogs was 6.3% for *C. jejuni*, 5.9% for *Campylobacter upsaliensis* and 0.7% for *Campylobacter coli*. The *C. jejuni* isolates were genotyped by multi locus sequence typing (MLST) and *flaB* typing. Resistance to macrolides and quinolones was genetically determined in parallel. Within the 134 genotyped *C. jejuni* isolates 57 different sequence types (ST) were found. Five STs were previously unrecognized. The most common STs were ST-48 (11.2%), ST-45 (10.5%) and ST-21 (6.0%). Whereas no macrolide resistance was found, 28 isolates (20.9%) were resistant to quinolones. ST-45 was significantly more prevalent in diarrheic than in non-diarrheic dogs. Within the common time frame of isolation 94% of the canine isolates had a ST that was also found in human clinical isolates. In conclusion, prevalence of *C. jejuni* in Swiss dogs is low but there is a large genetic overlap between dog and human isolates. Given the close contact between human and dogs, the latter should not be ignored as a potential source of human campylobacteriosis.

1. Introduction

In many industrialized countries *Campylobacter jejuni* is the most common cause of bacterial gastroenteritis in humans (European Food Safety Authority, 2013). Similar to other countries, in Switzerland *C. jejuni* is isolated in about 90% of human cases followed by *Campylobacter coli*, which accounts for the remaining 10% (Niederer et al., 2012). *Campylobacter lari*, *Campylobacter upsaliensis* and *Campylobacter fetus* are rarely found in humans and their occurrence may vary among different regions of the world (Moore et al., 2005). The main risk factors for human campylobacteriosis include consumption of contaminated foodstuffs, particularly undercooked chicken meat, raw milk or untreated water, as well as foreign travel and close contact with carrier animals, including pets (Moore et al., 2005; Mughini Gras et al., 2013; Ross et al., 2013). Source attribution studies clearly indicate chicken as the main reservoir with up to 80% of human cases linked to it (Mullner et al., 2009; Sheppard et al., 2009b; European

* Corresponding author at: Institute of Veterinary Bacteriology, Vetsuisse Faculty, University of Bern, Laenggassstr. 122, 3001 Bern, Switzerland. Tel.: +41 31 6312485; fax: +41 31 6312634.
E-mail address: peter.kuhnert@vetsuisse.unibe.ch (P. Kuhnert).

0378-1135/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.vetmic.2013.10.006

Food Safety Authority, 2011). *C. upsaliensis* and *C. jejuni* are the predominant species found in dogs (Wieland et al., 2005; Carbonero et al., 2012). Risk factors described for *C. jejuni* infection in dogs include age between seven and 12 months, male sex, living together with children, living in urban areas, being a stray dog, and contact with other dogs or birds (Hald and Madsen, 1997; Wieland et al., 2005; Tsai et al., 2007; Domingues et al., 2012; Carbonero et al., 2012). Dogs can be healthy carriers of *Campylobacter* spp., whereby animals under six month of age show a higher rate of carriage. Higher carriage rates were also observed in younger dogs with diarrhea, while in older dogs no difference in *Campylobacter* spp. shedding was reported between healthy and diseased animals (Burnens et al., 1992).

Multi locus sequence typing (MLST) is a standardized method, which has proven useful in epidemiological studies for *C. jejuni* and *C. coli* (Dingle et al., 2001; Korczak et al., 2009). It is highly reproducible, precise and can be compared between laboratories worldwide via public databases. For further differentiation, sequencing of the short variable region of the flagellin-encoding genes *flaA* or *flaB* can be used (Mellmann et al., 2004; Dingle et al., 2008; Korczak et al., 2009).

Macrolides and quinolones are mainly considered for therapy of severe *Campylobacter* infections, and increased antibiotic resistance of *C. jejuni*, especially to quinolones, has been observed worldwide (Alfredson and Korolik, 2007). The recognition of quinolone or macrolide resistant strains can be achieved in parallel to genotyping by sequencing *gyrA* and 23S rRNA gene fragments (Korczak et al., 2009). The point mutation C257T in *gyrA* leads to a high level of resistance to quinolones in *C. jejuni* and *C. coli*. Macrolide resistance is due to an A2075G or A2074G transition in the 23S rRNA gene (Alfredson and Korolik, 2007).

As currently little information on genotypes and antibiotic resistance of canine *C. jejuni/coli* isolates is available, we performed MLST, *fla*-typing and antibiotic resistance testing by sequencing partial *gyrA* and 23S rRNA genes on a large collection of canine *C. jejuni/coli* isolates from diarrheic and non-diarrheic dogs in Switzerland.

2. Materials and methods

2.1. Sampling and sample preparation

Fecal samples were collected from June to October 2012 in the Capital region of Bern, Switzerland, from 303 non-diarrheic pet household dogs. Only dogs with no gastrointestinal symptoms at the time of visit and not having received any antimicrobial treatment for at least two weeks prior to sampling were included in the study. Of the 303 dogs, 145 (48%) were sampled at the Small Animal Clinic, University of Bern, 35 at two pet obedience schools and a further 123 dogs were tested at six animal shelters. For each dog the date of sampling, age, sex and breed were recorded.

Samples were taken by rectal swabs (Transwab© Amies, Medical Wire & Equipment, United Kingdom) and kept at 4 °C, for no longer than 4 h before starting culture isolation. Swabs were then inoculated in 6 ml of Preston Campylobacter Selective Enrichment Broth (Oxoid, Pratteln, Switzerland) and incubated under microaerobic conditions at 41.5 °C for 24 h. A loopful of each enriched sample was streaked on two *Campylobacter* selective agar plates: mCCDA with antibiotic and antifungal agents (Oxoid) and Campylosel (BioMérieux, Geneva, Switzerland) and incubated under the same conditions for 48 h. Presumptive *Campylobacter* colonies were identified using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF) before sub cultivation for pure cultures on tryptone soy agar with 5% sheep blood (TSA; Becton Dickinson, Allschwil, Switzerland) for 24 h. For DNA template preparation, a few colonies from each plate were subsequently added to 500 μl of lysis buffer (0.1 M Tris–HCl, pH 8.5, 0.05% Tween 20, 240 μg/ml proteinase K) and incubated for 1 h at 60 °C followed by 15 min at 95 °C. The remaining colonies were transferred to freezing medium (T-soy-bouillon with 30% glycerin) and stored at −80 °C.

The set of 118 *C. jejuni* and *C. coli* isolates from dogs with diarrhea had been collected at the diagnostics unit (ZOBA) of the Institute of Veterinary Bacteriology Bern between 2003 and 2012. The strains were obtained from fecal specimens submitted by the veterinarian from individual dogs suffering from diarrhea. The isolates were stored at −80 °C, were grown on TSA under microaerobic conditions at 41.5 °C for 24 h and lysates were prepared as described above.

2.2. Multiplex PCR amplification and sequencing

A multiplex amplification and sequencing strategy according to Korczak et al. (2009) was used to genotype the strains. For MLST the sequences of internal fragments of seven housekeeping genes and in parallel partial *flaB*, 23S rRNA and *gyrA* gene sequences were determined for each isolate. The protocol was slightly modified according to Kittl et al. (2011).

2.3. Data analysis

The allele number, sequence type (ST), and clonal complex (CC) were determined with the online-based MLST application module for *Campylobacter* provided by SmartGene (Zug, Switzerland), which uses an integrated link to the PubMLST database (http://pubmlst.org/Campylobacter/). The PubMLST database was directly queried for determining *flaB* alleles. The 23S rRNA gene fragments were examined for point mutations A2075G and A2074G, which determine macrolide resistance and the *gyrA* fragments were screened for the C257T transition, which confers quinolone resistance.

Statistical analyses were performed with the NCSS 2008 software (NCSS, Kaysville, UT, USA) or STATA IC 12.1 (StataCorp LP, TX, USA). Differences between diarrheic and non-diarrheic dogs were analyzed with the level of significance set at $p < 0.05$ using Fisher's exact test (two tailed), Chi-square test or Armitage test for trend in proportions. The exact binominal method was applied for calculating the confidence intervals (CI).