Characterisation of *Salmonella* Frintrop isolated from dromedary camels *(Camelus dromedarius).*

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Abstract

Different studies have reported the prevalence and antibiotic resistance of *Salmonella* in dromedary camels and its role in camelid-associated salmonellosis in humans, but little is known about the epidemiology of *Campylobacter* in dromedaries. Here, we investigate the prevalence, genetic diversity and antibiotic resistance of *Campylobacter* and *Salmonella* in dromedary camels (*Camelus dromedarius*). A total of 54 individuals were sampled from two different dromedary farms located in Tenerife (Canary Islands, Spain). While all the samples were *Campylobacter*-negative, *Salmonella* prevalence was 5.5% (3/54) and the only serovar isolated was *S.* Frintrop. Pulsed-field gel electrophoresis analysis revealed a low genetic diversity, with all isolates showing a nearly identical pulsotype (similarity > 95%). Our results indicate that dromedary camels could not be a risk factor for *Campylobacter* human infection, but seems to be a reservoir for *Salmonella* transmission. Since camel riding has become one of the main touristic attractions in several countries and its popularity has increased considerably in recent years, a mandatory control, especially for zoonotic pathogens such as *Campylobacter* and *Salmonella,* should be implemented.

Keywords: Antimicrobial resistance; *Campylobacter*; dromedary; genetic diversity; PFGE; *Salmonella*

1. Introduction

Campylobacter and *Salmonella* are widely recognised as one of the most important zoonotic pathogens with economic impact in animals and humans. There are roughly 5.5 million gastrointestinal cases worldwide, with *Campylobacter* and *Salmonella as* the main pathogens of these disease outbreaks. In the United States, both pathogens are a significant public health concern, and cause around 1.2 million illnesses and 450 deaths every year (WHO, 2018b). In Europe, campylobacteriosis and salmonellosis are responsible for 246,571 and 91,857 confirmed cases of illnesses in humans, respectively (EFSA and ECDC, 2019). These pathogens constitute an important government concern, and monitoring the disease has become one of the main challenges in most European countries (EFSA and ECDC, 2019; FAO/WHO, 2009). To our best knowledge, no previous studies on *Campylobacter* in dromedary camels have been carried out in Europe. Even so, dromedaries have been identified as reservoirs of *Salmonella* and other zoonotic infections, forming a potential hazard for public health, especially in vulnerable patients such as infants, young children, the elderly or immunocompromised adults (Münch *et al.*, 2012; Raufu *et al.*, 2015).

In recent years, dromedary camel riding has become one of the main tourist attractions in several countries, and its popularity has increased considerably in recent years (Fernández, 2015). The most important dromedary population in the EU is in the Canary Isles (Spain) (Mentaberre *et al.*, 2013). After Spain joined the European Union (EU) and adopted the same animal health legislation, the imports of dromedary camels from Africa stopped completely. Since 1989, the Canary Isles is the only region that provides dromedary camels in the EU (Mentaberre *et al.*, 2013; Fernández, 2015;). These animals could constitute a source of zoonotic agents, such as *Campylobacter* and *Salmonella*, to the rest of the EU. The risk of

transmission might be particularly high during stressful long-term movements and recreational activities, when the bacterial shedding in faeces increases.

The emergence of antimicrobial resistant bacteria (AMR), including *Campylobacter* and *Salmonella*, in animals represents an important risk to public health. This is largely due to the potential for such microorganisms to contribute to antimicrobial therapy failure and the increased severity of associated infections (Tejedor-Junco *et al.*, 2010). Some authors have reported *Salmonella* infection in camels in different parts of the world with a resistant strain of *Salmonella* ser. Newport from an abscess occurring in a camel used for recreational purposes (Wernery, 1992; Moore *et al.*, 2002; Molla *et al.*, 2004).

Considering the potential public health risks associated with *Campylobacter* and *Salmonella*, the aims of this work were to investigate *Campylobacter* and *Salmonella* presence in dromedary camels (*Camelus dromedarius*) in the Canary Isles and determine the genetic diversity and antibiotic susceptibilities of the isolates.

2. Material and Methods

All animals were handled according to the principles of animal care published by Spanish Royal Decree 53/2013 (BOE, 2013 ; BOE = Official Spanish State Gazette).

2.1 Study location

The dromedary camels (*Camelus dromedarius*) investigated in this study belonged to the only two dromedary farms located in Tenerife (Canary Is., Spain). Each individual was randomly selected from each farm.

85 **2.2 Sample collection**

86 Rectal samples from each individual were collected using sterile swabs (Cary Blair sterile
87 transport swabs, DELTALAB®, Barcelona, Spain,) for *Campylobacter* isolation. In addition,
88 faeces from each individual were collected directly from the rectum and placed into sterile
89 plastic pots for *Campylobacter* and *Salmonella* isolation. To determine the sanitary status of
90 the animals, blood samples were taken from the jugular vein (about 5mL) and the levels of
91 lymphocytes, basophils, eosinophils, monocytes, and leucocytes were analysed. All samples
92 were transported to the laboratory under refrigerated conditions and analysed within 24 h of
93 collection.
94
95 **2.3 *Campylobacter* spp isolation and identification**
96 *Campylobacter* isolation and confirmation was performed following the ISO 10272:2006
97 recommendations (Annex E). Faecal samples were pre-enriched in 1:10 vol/vol Bolton broth
98 (CM0983, Oxoid, Dardilly, France) and then preincubated at 37 ± 1ºC for 5 ± 1 h, followed
99 by incubation at 41.5 ± 1ºC for 43 ± 1 h. Afterwards, 100 µL sample was cultured on two
100 selective agar plates mCCDA, (mCCDA, Oxoid, Dardilly, France) and Preston agar, (AES
101 laboratories®, Bruz Cedex, France) and incubated at 41.5 ± 1ºC for 44 ± 4 h. However, rectal
102 swabs were harvested onto mCCDA and Preston, and incubated under the same conditions
103 as faecal samples. All samples were incubated under microaerophilic conditions (84% N_2,
104 10% CO_2 and 6% O_2) (CampyGen, Oxoid). *Campylobacter*-like colonies were purified on
105 blood agar and identified to species level on the basis of standard procedures comprising tests
106 for hippurate and indoxyl acetate hydrolysis, catalase production, and susceptibility to

cephalothin and nalidixic acid.

2.4 *Salmonella* spp isolation and characterisation

Samples were analysed according to ISO 6579-1:2017. Firstly, faeces samples were pre-enriched 1:10 (vol/vol) in buffered peptone water 2.5% (BPW, Scharlau®, Barcelona, Spain) and incubated at 37 ± 1ºC for 18 ± 2 h. After incubation, the pre-enriched samples were transferred onto Semi-Solid Modification Rappaport Vassiliadis agar plate (MSRV, Difco®, Valencia, Spain), and incubated at 41.5 ± 1ºC for 24-48 h. The resulting culture was used to streak Xylose–Lysine–Desoxycholate (XLD, Liofilchem, Valencia, Spain) and ASAP (ASAP, bioMérieux, Madrid, Spain) agar plates, and incubated at 37 ± 1ºC for 24 h. Next, five typical colonies were streaked onto pre-dried nutrient agar plates (Scharlab®, Barcelona, Spain) at 37 ± 1ºC for 24 ± 3 h and confirmed as *Salmonella* spp. using the API (API-20®, bioMérieux, Madrid, Spain) biochemical test. All confirmed isolates were serotyped according to the Kauffman-White scheme (Grimont & Weill, 2007) at the Laboratori Agroalimentari (Cabrils, Spain) of the Departament d'Agricultura, Ramaderia, Pesca i Alimentació.

2.5 Molecular typing

Genotyping of *Salmonella* isolates was performed by pulsed-field gel electrophoresis (PGFE) according to the PulseNet standardised protocol (www.pulsenetinternational.org). Genomic DNA of the isolates was digested with Xbal restriction enzyme (Roche Applied Science, Indianapolis, IN), and the resulting PFGE band patterns were analysed using Fingerprinting II v3.0 software (Bio-Rad, Hercules, CA, USA). Similarity matrices were calculated using

the Dice coefficient and cluster analysis was performed by the unweighted-pair group method with arithmetic mean (UPGMA). A cut-off of 90% was used for determination of the different profiles.

2.6 Antimicrobial susceptibility testing

AMR susceptibility of *Salmonella* isolates was tested according to the European Committee on Antimicrobial Susceptibility Testing guidelines (Matuschek, Brown, & Kahlmeter, 2014). The source for zone diameters used for interpretation of the test was http://www.eucast.org/clinical_breakpoints/. *Salmonella* strains were inoculated onto Mueller-Hinton agar (Scharlab, S.L., Barcelona, Spain) to form a bacterial lawn, the antibiotic discs were added and plates were incubated at 37 ± 1ºC for 24 h. The antibiotics selected were those set forth in Decision 2013/653 (EU, 2013), including two quinolones: ciprofloxacin (CIP, 5 mg) and nalidixic acid (NAL, 30 mg); three b-lactams: ampicillin (AMP, 10 mg), cefotaxime (CTX, 30 mg), and ceftazidime (CAZ, 30 mg); one phenicol: chloramphenicol (CHL, 5 mg); one potentiated sulfonamide: trimethoprim-sulfamethoxazole (SXT, 1.25/23.75 mg); one polymyxin: colistin (CST, 10 mg); one macrolide: azithromycin (AZM, 15 mg); one glycylcycline: tigecycline (TGC, 15 mg); one aminoglycoside: gentamycin (GEN, 10 mg); and one pyrimidine: trimethoprim (TMP, 5 mg).

2.7 Statistical analysis

A generalised linear model with a binomial probability distribution and a logit link function was used to compare the isolation of *Campylobacter* and *Salmonella* in dromedary samples (faces and swabs). For this analysis, the error was designated as having a binomial

distribution and the probit link function was used. Binomial data for each sample were assigned as 1 if *Campylobacter* and *Salmonella* were isolated or as 0 if not. A *P value* <0.05 was considered statistically significant. Data are presented as least squares means ± standard error of the least squares means. All statistical analyses were carried out using a commercially available software program (SPSS 16.0 software package; SPSS Inc., Chicago, IL, 2002).

3. Results

A total of 54 individuals were sampled in this study. According to the blood parameters obtained, all dromedary camels tested were within the reference parameters (Farooq, Samad, Khurshid, & Sajjad, 2011). The results are shown in Table 1.

None of the 54 swabs and faeces samples analysed were positive for *Campylobacter* spp. On the contrary, *Salmonella* was isolated from 5.5% (3/54) of the samples collected and all isolates were identified as serovar Frintrop.

Regarding antimicrobial susceptibility, all *Salmonella* isolates were pansusceptible to the antimicrobials tested. Moreover, the PFGE analysis revealed a low genetic diversity among isolates, with a single pulsotype identified with a similarity > 95% (Figure 1).

4. Discussion

Since Spain joined the EU and established the same health legislation, Canary Is. is the only region that provides dromedary camels within the EU (Mentaberre *et al.*, 2013; Fernández, 2015). Moreover, dromedary camel riding has become one of the most important tourist attractions in several countries, and its popularity has increased considerably in recent years

(Fernández, 2015). Therefore, the sanitary status of these animals should be assessed, especially for zoonotic pathogens such as *Campylobacter* and *Salmonella*. This study demonstrates dromedaries as *Salmonella* reservoirs and a potential risk factor for *Salmonella* infection, but not for *Campylobacter*.

Campylobacter is a leading foodborne zoonosis worldwide, widespread in nature. It colonises the intestinal mucosa of most warm-blooded hosts, including all food-producing animals and humans (Facciolà *et al.*, 2017). However, few studies identify *Campylobacter* spp in dromedary camels as a potential zoonosis (Rahimi *et al.*, 2017; Gwida *et al.*, 2019). In the present study, *Campylobacter* was not detected in any of the samples collected. One reason that could explain this fact is that *Campylobacter* detection is highly dependent on the sampling and culture method procedure (Marin *et al.*, 2013). This could be due to a lack of appreciable faecal material from rectal swabs. Nevertheless, in our study both samples analysed (rectal swabs and faeces) were negative for *Campylobacter* detection. Even though molecular techniques have demonstrated advantages over classical microbiological *Campylobacter* isolation, both methods showed a high level of agreement, especially faecal samples (Ugarte-Ruiz *et al.*, 2012). Therefore, if the bacteria had been present in the samples collected, it is unlikely that we would not have been able to isolate it from any of the samples analysed. Thus, results of this study show that dromedary camels do not seem to be a reservoir for *Campylobacter.*

The frequency of *Salmonella* among Canarian dromedaries in this study was moderate (5,5%) and consistent with that noted by other authors (Mohamed and Suelam, 2010; Raufu *et al.*, 2015), who reported a *Salmonella* prevalence of 5,6% and 6%, respectively. Nevertheless, diverse occurrence of this pathogen has been reported in camels in the literature; some

198 authors showed a low presence of *Salmonella* (Wernery, 1992), while others reported a
199 medium or high prevalence in captive dromedaries (Moore *et al.*, 2002; Molla *et al.*, 2004;
200 Tejedor-Junco *et al.*, 2010; Münch *et al.*, 2012). As in this study, salmonellosis in
201 dromedaries is generally asymptomatic, although clinical *Salmonella* infections have been
202 reported with symptoms that included epiphora, anorexia, muscle twitching and lateral
203 recumbency (Nour-Mohammadzadeh *et al.*, 2010). In addition, controlling *Salmonella*
204 infections in camels should be taken into account, as it has been shown that *Salmonella* could
205 be the cause of co-infections such as clostridia or theileriosis diseases (Abdelwahab *et al.*,
206 2019). Regarding *Salmonella* serovars isolated, ser. Frintrop, was identified in all positive
207 camels. This is one of the main *Salmonella* serovars described in dromedaries and may be
208 host adapted to camels (Wernery, 1992; Molla *et al.*, 2004; Tejedor-Junco *et al.*, 2010;
209 Münch *et al.*, 2012). Although this is an uncommon serovar in other animal species, it may
210 constitute a threat to camels and other animal species that are in contact with humans. The
211 isolation of a single *Salmonella* serovar and all isolates belonging to the same genotype
212 suggests a single source of infection.
213 Emergence of antibiotic resistance is of worldwide concern, as it reduces the therapy options
214 in human and veterinary medicine. Thus, the increasing trends of resistance to critical
215 antimicrobials (WHO, 2018a) that have been reported in the last decade for *Salmonella* and
216 other zoonotic bacteria is of concern (EFSA & ECDC, 2015). It is believed that antibiotic
217 resistance is promoted by the use of antimicrobial drugs in livestock animals (Landers, Cohen,
218 Wittum, & Larson, 2012). However, in this study, none of the *Salmonella* isolates were
219 resistant to any antimicrobial drug tested. This result is consistent with those published by
220 Münch *et al.* (2012), where all *S.* Frintrop serovars were susceptible to all antimicrobial

agents tested. Antimicrobial resistant *Salmonella* seems to be more prevalent in other livestock animals such as pigs or poultry (Tejedor-Junco *et al.*, 2010).

Animal movements through European countries, and in this case particularly of dromedaries, could pose a serious threat, as they could contribute to the spread of *Salmonella* resistant strains and therefore increase the risk of human infection. Hence, biosecurity safety protocols should be applied for the movement of dromedaries and other animals among different countries. In particular, care must be taken during recreational activities, where animals could come into close contact with children, elderly and immunocompromised people (Wright *et al.*, 2005; Tejedor-Junco *et al.*, 2010).

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Conflicts of interest

The authors declare no conflicts of interest.

244 **Ethical Statement**

245 All animals were handled according to the principles of animal care published by Spanish
246 Royal Decree 53/2013 (BOE, 2013 ; BOE = Official Spanish State Gazette).

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248 **Data availability statement**

249 All data relevant to the study are included in the article or uploaded as supplementary
250 information. All individual data that underline the results reported in this article have been
251 shared.

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343

344 **Table 1**. Mean (± SEM) white blood cell values in female and male camels

345

	Female		Male	
Parameters	**Value**	**Reference †**	**Value**	**Reference †**
Total leucocyte count (10^3/µl)	10.15 ± 0.71	12.97 ± 0.99	10.63 ± 0.8	12.38 ± 0.97
Neutrophils (%)	40.88 ± 1.49	43.60 ± 1.30	42.8 ± 1.7	44.70 ±1.4
Lymphocytes (%)	44.88 ± 1.36	48.60 ± 1.50	41.14 ± 1.72	47.50 ±1.4
Eosinophils (%)	9.03 ± 1.11	7 ± 0.39	10.1 ± 1.17	7.20 ± 0.4
Monocytes (%)	2 ± 0.45	1 ± 0.10	3.47 ± 0.68	1.20 ± 0.10
Basophils (%)	<0.1	<0.1	<0.1	<0.1

346 †Farroq et al., 2011.
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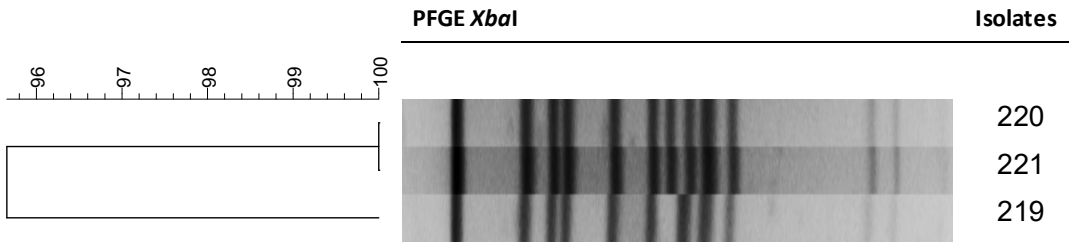

Figure 1. PFGE dendrogram of XbaI patterns of *Salmonella* Frintrop isolates from dromedaries.