

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

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## SALMONELLA CHARACTERIZATION IN DROMEDARY CAMELS

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

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21 **Abstract**

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

36

37 **Keywords:** antimicrobial resistance; *Campylobacter*; dromedary; genetic diversity; PFGE;  
38 *Salmonella*

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## SALMONELLA CHARACTERIZATION IN DROMEDARY CAMELS

**1. Introduction**

*Campylobacter* and *Salmonella* are widely recognized as one of the most important zoonotic pathogens with economic impact in animals and humans. There are about 5.5 million of gastrointestinal cases worldwide, being *Campylobacter* and *Salmonella* the main pathogens of these diseases' outbreaks. In the United States, both pathogens are a significant public health concern, and cause about 1.2 million illnesses and 450 deaths every year (WHO, 2018b). In Europe, campylobacteriosis and salmonellosis are responsible for 246,571 and 91,857 cases of illnesses in humans confirmed, respectively (EFSA and ECDC, 2019). **These pathogens constitute an important government concern, and monitoring the disease has become one of the main dares in most industrialized 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, dromedary camels have been identified as reservoirs of *Salmonella* and other zoonotic infections, being 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 ride has become one of the main touristic attractions in several countries, and its popularity has considerably increased in the last years (Fernández, 2015). The Canary Islands (Spain) holds the most important dromedary camel population in the EU (Mentaberre et al., 2013). Since Spain joined de European Union (EU) and established the same animal health legislation, the imports of dromedary camels from Africa completely stopped. Nowadays since 1989 Canary Is., is the only region that provides dromedary camels in the EU (Mentaberre et al., 2013; Fernández, 2015;). Thus, these animals could be a source of zoonotic agents, such as *Campylobacter* and *Salmonella*, to the rest of the EU. The risk of

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6 63 transmission might be particularly high during stressful long-term movements and  
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8 64 recreational actions, when the bacterial shedding in faeces increases.

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10 65 The emergence of antimicrobial resistant bacteria (AMR), including *Campylobacter* and  
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12 66 *Salmonella*, in animals represents an important risk to public health. This is largely due to  
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14 67 the potential for such microorganisms to contribute to antimicrobial therapy failure and the  
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16 68 increased severity of associated infections (Tejedor-Junco et al., 2010). Some authors have  
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18 69 reported *Salmonella* infection in camels in different parts of the world resistant strain of  
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20 70 *Salmonella* ser. Newport from an abscess occurring in a camel used for recreational purposes  
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22 71 (Wernery, 1992; Moore et al., 2002; Molla et al., 2004).

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26 72 Considering the potential public health risks associated with *Campylobacter* and *Salmonella*,  
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28 73 the aims of this work were to investigate *Campylobacter* and *Salmonella* presence in  
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30 74 dromedary camels (*Camelus dromedarius*) at the Canary Is., and to determine the genetic  
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32 75 diversity and antibiotic susceptibilities of the isolates.

## 33 34 35 36 76 **2. Material and Methods**

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39 77 All animals were handled according to the principles of animal care published by Spanish  
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41 78 Royal Decree 53/2013 (BOE, 2013 ; BOE = Official Spanish State Gazette).

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### 44 45 46 80 **2.1 Study location**

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48 81 The dromedary camels (*Camelus dromedarius*) investigated in this study belong to the two  
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50 82 unique dromedary farms located in Tenerife (Canary Is., Spain). Each individual was  
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52 83 randomly selected from each farm.

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**2.2 Sample collection**

Cloacal samples from each individual were collected using sterile swabs (Cary Blair sterile transport cotton swabs, DELTALAB®, Barcelona, Spain,) for *Campylobacter* isolation. Also, faeces from each individual were collected directly from the rectum and placed into sterile plastic pots for *Campylobacter* and *Salmonella* isolation. In order to determine the sanitary status of the animals, blood samples were taken from the jugular vein (5mL) and the level of lymphocytes, basophils, eosinophils, monocytes, and leucocytes were analysed. All samples were transported to the laboratory under refrigerated conditions and were analysed within 24 h of collection.

**2.3 *Campylobacter* spp isolation and identification**

*Campylobacter* isolation and confirmation was performed following the ISO 10272:2006 recommendations (Annex E). Faecal samples were pre-enriched in 1:10 vol/vol Bolton broth (CM0983, Oxoid, Dardilly, France) and then preincubated at  $37 \pm 1^\circ\text{C}$  for  $5 \pm 1$  h, followed by incubation at  $41.5 \pm 1^\circ\text{C}$  for  $43 \pm 1$  h. Afterwards, 100  $\mu\text{L}$  sample was cultured on two selective agar plates mCCDA, (mCCDA, Oxoid, Dardilly, France) and Preston agar, (AES laboratories®, Bruz Cedex, France) and incubated at  $41.5 \pm 1^\circ\text{C}$  for  $44 \pm 4$  h. However, cloacal swabs were harvested onto mCCDA and Preston agar plates, and incubated as described above. Then, plates were incubated under microaerophilic conditions (84%  $\text{N}_2$ , 10%  $\text{CO}_2$  and 6%  $\text{O}_2$ ) (CampyGen, Oxoid). *Campylobacter*-like colonies were purified on blood agar and identified to species level on the basis of standard procedures comprising tests for Hippurate and indoxyl acetate hydrolysis, catalase production, and susceptibility to

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5 107 cephalothin and nalidixic acid.  
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10 109 **2.4 *Salmonella* spp isolation and characterization.**

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12 110 Samples were analysed according to the ISO 6579-1:2017. Firstly, faeces samples were pre-  
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14 111 enriched 1:10 (vol/vol) in buffered peptone water 2.5% (BPW, Scharlau®, Barcelona, Spain)  
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16 112 and incubated at  $37 \pm 1^\circ\text{C}$  for  $18 \pm 2$  h. After incubation, the pre-enriched samples were  
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18 113 transferred onto Semi-Solid Modification Rappaport Vassiliadis agar plate (MSRV, Difco®,  
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20 114 Valencia, Spain), and incubated at  $41.5 \pm 1^\circ\text{C}$  for 24-48 h. The resulting culture was used to  
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22 115 streak Xylose–Lysine–Desoxycholate (XLD, Liofilchem, Valencia, Spain) and ASAP  
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24 116 (ASAP, bioMérieux, Madrid, Spain) agar plates, and incubated at  $37 \pm 1^\circ\text{C}$  for 24 h. Next, 5  
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26 117 typical colonies were streaked onto pre-dried nutrient agar plates (Scharlab®, Barcelona,  
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28 118 Spain) at  $37 \pm 1^\circ\text{C}$  for  $24 \pm 3$  h and confirmed as *Salmonella* spp. using the API (API-20®,  
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30 119 bioMérieux, Madrid, Spain) biochemical test. All confirmed isolates were serotyped  
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32 120 according to the Kauffman-White scheme (Grimont & Weill, 2007) at the Laboratori  
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34 121 Agroalimentari (Cabrils, Spain) of the Departament d'Agricultura, Ramaderia, Pesca i  
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36 122 Alimentació.  
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44 124 **2.5 Molecular typing**

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46 125 Genotyping of *Salmonella* isolates was performed by pulsed-field gel electrophoresis (PGFE)  
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48 126 according to the PulseNet standardized protocol ([www.pulsenetinternational.org](http://www.pulsenetinternational.org)). Genomic  
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50 127 DNA of the isolates was digested with XbaI restriction enzyme (Roche Applied Science,  
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52 128 Indianapolis, IN), and the resulting PFGE band patterns were analysed using the  
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54 129 Fingerprinting II v3.0 software (Bio-Rad, Hercules, CA, USA). Similarity matrices were  
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6 130 calculated using the Dice coefficient and cluster analysis was performed by the unweighted-  
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8 131 pair group method with arithmetic mean (UPGMA). A cut-off of 90% was used for the  
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10 132 determination of the different profiles.

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12 13313 134 **2.6 Antimicrobial susceptibility testing**

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17 135 AMR susceptibility of *Salmonella* isolates was tested according to the European Committee  
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19 136 on Antimicrobial Susceptibility Testing guidelines (Matuschek, Brown, & Kahlmeter, 2014).  
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21 137 The source for zone diameters used for interpretation of the test was  
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23 138 [http://www.eucast.org/clinical\\_breakpoints/](http://www.eucast.org/clinical_breakpoints/). *Salmonella* strains were inoculated onto  
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25 139 Mueller-Hinton agar (Scharlab, S.L., Barcelona, Spain) to form a bacterial lawn, the  
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27 140 antibiotic discs were added, and plates were incubated at  $37 \pm 1^\circ\text{C}$  for 24 h. The antibiotics  
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29 141 selected were those set forth in Decision 2013/653 (European Union, 2013), including two  
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31 142 quinolones: ciprofloxacin (CIP, 5 mg) and nalidixic acid (NAL, 30 mg); three b-lactams:  
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33 143 ampicillin (AMP, 10 mg), cefotaxime (CTX, 30 mg), and ceftazidime (CAZ, 30 mg); one  
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35 144 phenicol: chloramphenicol (CHL, 5 mg); one potentiated sulfonamide: trimethoprim-  
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37 145 sulfamethoxazole (SXT, 1.25/23.75 mg); one polymyxin: colistin (CST, 10 mg); one  
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39 146 macrolide: azithromycin (AZM, 15 mg); one glycylicycline: tigecycline (TGC, 15 mg); one  
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41 147 aminoglycoside: gentamycin (GEN, 10 mg); and one pyrimidine: trimethoprim (TMP, 5 mg).

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43 14844 149 **2.7 Statistical Analysis**

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49 150 A generalized linear model with a binomial probability distribution and a logit link function  
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51 151 was used to compare the isolation of *Campylobacter* and *Salmonella* in dromedaries' samples  
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53 152 (faces and swabs). For this analysis, the error was designated as having a binomial



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6 153 distribution and the probit link function was used. Binomial data for each sample were  
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8 154 assigned as 1 if *Campylobacter* and *Salmonella* were isolated or as 0 if not. A *P value* <0.05  
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10 155 was considered statistically significant. Data are presented as least squares means  $\pm$  standard  
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12 156 error of the least squares means. All statistical analyses were carried out using a commercially  
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15 157 available software program (SPSS 16.0 software package; SPSS Inc., Chicago, IL, 2002).  
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### 19 159 3. Results

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21 160 In this study a total of 54 individuals were sampled. According to the blood parameters  
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23 161 obtained, all dromedary camels tested were within the reference parameters (Farooq, Samad,  
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25 162 Khurshid, & Sajjad, 2011). The results are represented in Table 1.

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28 163 None of the 54 swabs and faeces samples analysed were positive for *Campylobacter* spp. On  
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30 164 the contrary, *Salmonella* was isolated from 5.5% (3/54) of the samples collected and all  
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32 165 isolates were identified as serovar Frintrop.

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35 166 Regarding antimicrobial susceptibility, all *Salmonella* isolates were pansusceptible to the  
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37 167 antimicrobials tested. Moreover, the PFGE analysis revealed a low genetic diversity among  
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39 168 isolates, with a unique pulsotype identified with a similarity > 95% (Figure 1).  
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### 43 44 45 170 4. Discussion

46  
47 171 Since Spain joined de EU and established the same health legislation, Canary Is. is the only  
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49 172 region that provides dromedary camels within the EU (Mentaberre et al., 2013; Fernández,  
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51 173 2015). Moreover, dromedary camel ride has become one of the most important touristic  
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53 174 attractions in several countries, and its popularity has increased considerably in the last years  
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5 175 (Fernández, 2015). Therefore, the sanitary status of these animals, especially for zoonotic  
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8 176 pathogens, such as *Campylobacter* and *Salmonella* should be assessed. This study  
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10 177 demonstrates dromedaries as *Salmonella* reservoirs and could be a risk factor of *Salmonella*  
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12 178 infection, but not for *Campylobacter*.

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14 179 *Campylobacter* is a leading foodborne zoonosis worldwide, widely spread in nature. It  
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17 180 colonizes the intestinal mucosa of most warm-blooded host, including all food-producing  
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19 181 animals and humans (Facciola et al., 2017). However, few studies identify *Campylobacter*  
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21 182 spp of dromedary camel as potential zoonoses (Rahimi et al., 2017; Gwida et al., 2019). In  
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23 183 the present study, *Campylobacter* was not detected in any of the samples collected. One  
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25 184 reason that could explain this fact is that *Campylobacter* detection is highly dependent on the  
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27 185 sampling and culture method procedure (Marin et al., 2013). This could be due to a lack of  
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29 186 appreciable faecal material from cloacal swabs. Nevertheless, in our study both samples  
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31 187 analyzed (cloacal swabs and faeces) were negative for *Campylobacter* detection. Even  
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33 188 though molecular techniques have demonstrated advantages over classical microbiological  
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35 189 *Campylobacter* isolation, both methods showed a high level of agreement, specially faecal  
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37 190 samples (Ugarte-Ruiz et al., 2012). Therefore, if the bacteria had been present in the samples  
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39 191 collected, it is unlikely that we would not have been able to isolated it from any of the samples  
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41 192 analysed. Thus, results of this study showed that dromedary camels seem not to be a reservoir  
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43 193 for *Campylobacter*.

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45 194 The frequency of *Salmonella* among Canarian dromedaries in this study was moderate (5,5%)  
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47 195 and consistent with other authors (Mohamed and Suelam, 2010; Raufu et al., 2015), which  
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49 196 reported a *Salmonella* prevalence of 5,6% and 6%, respectively. Nevertheless, diverse  
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51 197 occurrence of this pathogen has been reported in camels in the literature; some authors

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5 198 showed a low presence of *Salmonella* (Wernery, 1992), whilst others reported a medium or  
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8 199 high prevalence in captive dromedaries (Moore et al., 2002; Molla et al., 2004; Tejedor-Junco  
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10 200 et al., 2010; Münch et al., 2012). As in this study, salmonellosis in dromedaries are generally  
11  
12 201 asymptomatic, though clinical *Salmonella* infections have been described with symptoms  
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14 202 that included epiphora, anorexia, muscle twitching, and lateral recumbence (Nour-  
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16 203 Mohammadzadeh et al., 2010). In addition, controlling *Salmonella* infections in camels  
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18 204 should be taken into account, as it has been shown that *Salmonella* could be the cause of co-  
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20 205 infections as clostridia or theileriosis diseases (Abdelwahab et al., 2019). Regarding  
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22 206 *Salmonella* serovars isolated, ser. Frintrop, was identified in all positive camels. This is one  
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24 207 of the main described *Salmonella* serovar in dromedaries and may be host adapted to camels  
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26 208 (Wernery, 1992; Molla et al., 2004; Tejedor-Junco et al., 2010; Münch et al., 2012). Although  
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28 209 this in an uncommon serovar in other animal species, it may constitute a threat to camels and  
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30 210 other species of animals that are in contact with humans. The isolation of a single *Salmonella*  
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32 211 serovar and all isolates belonging to the same genotype suggests a single source of infection.  
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34 212 Emergence of antibiotic resistance is of worldwide concern, since it reduces the therapy  
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36 213 options in human and veterinary medicine. Thus, the increasing trends of resistance to critical  
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38 214 antimicrobials (WHO, 2018a) that have been reported in the last decade for *Salmonella* and  
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40 215 other zoonotic bacteria is of concern (EFSA & ECDC, 2015). It is believed that antibiotic  
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42 216 resistance is promoted by the use of antimicrobial drugs in livestock animals (Landers, Cohen,  
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44 217 Wittum, & Larson, 2012). However, in this study, none of the *Salmonella* isolates were  
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46 218 resistant to any antimicrobial drug tested. This result is consistent to those published by  
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48 219 Münch et al. (2012) were all *S. Frintrop* serovars were susceptible to all antimicrobial agents  
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50 220 tested. Antimicrobial resistant *Salmonella* seems to be more prevalent in other livestock  
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6 221 animals such as pigs or poultry (Tejedor-Junco et al., 2010).  
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8 222 Animal movements, and in this case particularly of dromedaries, through European countries  
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10 223 could be a serious threat, since they could contribute to the spread of *Salmonella* resistant  
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12 224 strains, and therefore increase the risk of human infection. Hence, safety biosecurity  
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14 225 protocols should be applied for the movement of dromedaries and other animals among  
15  
16 226 different countries. Especially, care has to be taken during recreational activities, where  
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18 227 animals could be in close contact with children, ancient and immunocompromised people  
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20 228 (Wright et al., 2005; Tejedor-Junco et al., 2010).  
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37  
38 236 de Catalunya is also acknowledged.  
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### 46 239 **Conflicts of Interest**

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49 240 The authors declare no conflicts of interest.  
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### 53 242 **Ethical Statement**

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56 243 All animals were handled according to the principles of animal care published by Spanish  
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5 244 Royal Decree 53/2013 (BOE, 2013 ; BOE = Official Spanish State Gazette).  
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10 246 **Data availability statement**  
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12 247 All data relevant to the study are included in the article or uploaded as supplementary  
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14 248 information. All individual data that underline the results reported in this article have been  
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16 249 shared.  
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## SALMONELLA CHARACTERIZATION IN DROMEDARY CAMELS

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

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## SALMONELLA CHARACTERIZATION IN DROMEDARY CAMELS

21 **Abstract**

22 Different studies have reported the prevalence and antibiotic resistance of *Salmonella* in  
23 dromedaries' camels and its role in camelid-associated salmonellosis in humans, but little is  
24 known about the epidemiology of *Campylobacter* in dromedaries. Here we investigate the  
25 prevalence, genetic diversity and antibiotic resistance of *Campylobacter* and *Salmonella* in  
26 dromedary camels (*Camelus dromedarius*). A total of 54 individuals were sampled from two  
27 unique dromedary farms located in Tenerife (Canary Islands, Spain). Whilst all the samples  
28 were *Campylobacter*-negative, *Salmonella* prevalence was 5.5% (3/54) and the only serovar  
29 isolated was *S. Frintrop*. The pulsed field gel electrophoresis analysis revealed a low genetic  
30 diversity, with all isolates showing a nearly identical pulsotype (similarity > 95%). Our  
31 results indicate that dromedaries' camels could be a risk factor for *Salmonella* transmission,  
32 but do not seem to be a reservoir for *Campylobacter*. Since camel ride has become one of the  
33 main touristic attractions in several countries and its popularity has considerably risen in the  
34 last years, a mandatory control, especially for zoonotic pathogens, such as *Campylobacter*  
35 and *Salmonella* should be implemented.

36

37 **Keywords:** antimicrobial resistance; *Campylobacter*; dromedary; genetic diversity; PFGE;  
38 *Salmonella*

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## SALMONELLA CHARACTERIZATION IN DROMEDARY CAMELS

**1. Introduction**

*Campylobacter* and *Salmonella* are widely recognized as one of the most important zoonotic pathogens with economic impact in animals and humans. There are about 5.5 million of gastrointestinal cases worldwide, being *Campylobacter* and *Salmonella* the main pathogens of these diseases' outbreaks. In the United States, both pathogens are a significant public health concern, and cause about 1.2 million illnesses and 450 deaths every year (WHO, 2018b). In Europe, campylobacteriosis and salmonellosis are responsible for 246,571 and 91,857 cases of illnesses in humans confirmed, respectively (EFSA and ECDC, 2019). These zoonoses represent an important government concern and controlling the disease has become a vital challenge in most 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, dromedary camels have been identified as reservoirs of *Salmonella* and other zoonotic infections, being 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 ride has become one of the main touristic attractions in several countries, and its popularity has considerably increased in the last years (Fernández, 2015). The Canary Islands (Spain) holds the most important dromedary camel population in the EU (Mentaberre et al., 2013). Since Spain joined de European Union (EU) and established the same animal health legislation, the imports of dromedary camels from Africa completely stopped. Nowadays since 1989 Canary Is., is the only region that provides dromedary camels in the EU (Fernández, 2015; Mentaberre et al., 2013). Thus, these animals could be a source of zoonotic agents, such as *Campylobacter* and *Salmonella*, to the rest of the EU. The risk of

## SALMONELLA CHARACTERIZATION IN DROMEDARY CAMELS

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

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

72 Considering the potential public health risks associated with *Campylobacter* and *Salmonella*,  
73 the aims of this study were to investigate the prevalence of *Campylobacter* and *Salmonella*  
74 in dromedary camels (*Camelus dromedarius*) at the Canary Is., and to determine the genetic  
75 diversity and antibiotic susceptibilities of the isolates.

## 76 2. Material and Methods

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

### 80 2.1 Study location

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

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## SALMONELLA CHARACTERIZATION IN DROMEDARY CAMELS

**85 2.2 Sample collection**

86 Cloacal samples from each individual were taken using sterile cotton swabs (Cary Blair  
87 sterile transport swabs, DELTALAB®, Barcelona, Spain,) for *Campylobacter* isolation. Also,  
88 faeces from each individual were collected directly from the rectum and placed into sterile  
89 plastic pots for *Campylobacter* and *Salmonella* isolation. In order to determine the sanitary  
90 status of the animals, blood samples were collected from the jugular vein (about 5mL) and  
91 the level of lymphocytes, basophils, eosinophils, monocytes, and leucocytes were analysed.  
92 All samples were transported to the laboratory under refrigerated conditions and were  
93 analysed within 24 h of collection.

**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  $37 \pm 1^\circ\text{C}$  for  $5 \pm 1$  h, followed  
99 by incubation at  $41.5 \pm 1^\circ\text{C}$  for  $43 \pm 1$  h. Afterwards, 100  $\mu\text{L}$  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  $41.5 \pm 1^\circ\text{C}$  for  $44 \pm 4$  h. However, cloacal  
102 swabs were directly streaked onto two selective agar plates (mCCDA and Preston,) and  
103 incubated as described above. All plates were incubated in a microaerobic atmosphere (84%  
104  $\text{N}_2$ , 10%  $\text{CO}_2$  and 6%  $\text{O}_2$ ) generated in a gas charged incubator (CampyGen, Oxoid). Plates  
105 were examined for grey, flat, irregular and spreading colonies typical of *Campylobacter*.  
106 *Campylobacter*-like colonies were purified on blood agar and identified to species level on

## SALMONELLA CHARACTERIZATION IN DROMEDARY CAMELS

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5 107 the basis of standard procedures comprising tests for Hippurate and indoxyl acetate  
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8 108 hydrolysis, catalase production, and susceptibility to cephalothin and nalidixic acid.  
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12 110 **2.4 *Salmonella* spp isolation and characterization.**

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14 111 Samples were analysed according to the ISO 6579-1:2017. Firstly, faeces samples were pre-  
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16 112 enriched 1:10 (vol/vol) in buffered peptone water 2.5% (BPW, Scharlau®, Barcelona, Spain)  
17  
18 113 and incubated at  $37 \pm 1^\circ\text{C}$  for  $18 \pm 2$  h. After incubation, the pre-enriched samples were  
19  
20 114 transferred onto Semi-Solid Modification Rappaport Vassiliadis agar plate (MSRV, Difco®,  
21  
22 115 Valencia, Spain), and incubated at  $41.5 \pm 1^\circ\text{C}$  for 24-48 h. The resulting culture was used to  
23  
24 116 streak Xylose–Lysine–Desoxycholate (XLD, Liofilchem, Valencia, Spain) and ASAP  
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26 117 (ASAP, bioMérieux, Madrid, Spain) agar plates, and incubated at  $37 \pm 1^\circ\text{C}$  for 24 h. Next, 5  
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28 118 typical colonies were streaked onto pre-dried nutrient agar plates (Scharlab®, Barcelona,  
29  
30 119 Spain) at  $37 \pm 1^\circ\text{C}$  for  $24 \pm 3$  h and confirmed as *Salmonella* spp. using the API (API-20®,  
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32 120 bioMérieux, Madrid, Spain) biochemical test. All confirmed isolates were serotyped  
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34 121 according to the Kauffman-White scheme (Grimont & Weill, 2007) at the Laboratori  
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36 122 Agroalimentari (Cabrils, Spain) of the Departament d'Agricultura, Ramaderia, Pesca i  
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38 123 Alimentació.  
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43 125 **2.5 Molecular typing**

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45 126 Genotyping of *Salmonella* isolates was performed by pulsed-field gel electrophoresis (PGFE)  
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47 127 according to the PulseNet standardized protocol ([www.pulsenetinternational.org](http://www.pulsenetinternational.org)). Genomic  
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49 128 DNA of the isolates was digested with XbaI restriction enzyme (Roche Applied Science,  
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51 129 Indianapolis, IN), and the resulting PFGE band patterns were analysed using the  
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## SALMONELLA CHARACTERIZATION IN DROMEDARY CAMELS

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5 130 Fingerprinting II v3.0 software (Bio-Rad, Hercules, CA, USA). Similarity matrices were  
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8 131 calculated using the Dice coefficient and cluster analysis was performed by the unweighted-  
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10 132 pair group method with arithmetic mean (UPGMA). A cut-off of 90% was used for the  
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12 133 determination of the different profiles.  
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### 17 135 **2.6 Antimicrobial susceptibility testing**

19 136 AMR susceptibility of *Salmonella* isolates was tested according to the European Committee  
20  
21 137 on Antimicrobial Susceptibility Testing guidelines (Matuschek, Brown, & Kahlmeter, 2014).  
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23 138 The source for zone diameters used for interpretation of the test was  
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25 139 [http://www.eucast.org/clinical\\_breakpoints/](http://www.eucast.org/clinical_breakpoints/). *Salmonella* strains were inoculated onto  
26  
27 140 Mueller-Hinton agar (Scharlab, S.L., Barcelona, Spain) to form a bacterial lawn, the  
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29 141 antibiotic discs were added, and plates were incubated at  $37 \pm 1^\circ\text{C}$  for 24 h. The antibiotics  
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31 142 selected were those set forth in Decision 2013/653 (European Union, 2013), including two  
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33 143 quinolones: ciprofloxacin (CIP, 5 mg) and nalidixic acid (NAL, 30 mg); three b-lactams:  
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35 144 ampicillin (AMP, 10 mg), cefotaxime (CTX, 30 mg), and ceftazidime (CAZ, 30 mg); one  
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37 145 phenicol: chloramphenicol (CHL, 5 mg); one potentiated sulfonamide: trimethoprim-  
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39 146 sulfamethoxazole (SXT, 1.25/23.75 mg); one polymyxin: colistin (CST, 10 mg); one  
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41 147 macrolide: azithromycin (AZM, 15 mg); one glycylicycline: tigecycline (TGC, 15 mg); one  
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43 148 aminoglycoside: gentamycin (GEN, 10 mg); and one pyrimidine: trimethoprim (TMP, 5 mg).  
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### 51 150 **2.7 Statistical Analysis**

53 151 A generalized linear model with a binomial probability distribution and a logit link function  
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55 152 was used to compare the isolation of *Campylobacter* and *Salmonella* in dromedaries' samples  
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6 153 (faces and swabs). For this analysis, the error was designated as having a binomial  
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8 154 distribution and the probit link function was used. Binomial data for each sample were  
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10 155 assigned as 1 if *Campylobacter* and *Salmonella* were isolated or as 0 if not. A *P* value <0.05  
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12 156 was considered statistically significant. Data are presented as least squares means  $\pm$  standard  
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14 157 error of the least squares means. All statistical analyses were carried out using a commercially  
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16 158 available software program (SPSS 16.0 software package; SPSS Inc., Chicago, IL, 2002).  
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### 160 3. Results

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24 161 In this study a total of 54 individuals were sampled. According to the blood parameters  
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26 162 obtained, all dromedary camels tested were within the reference parameters (Farooq, Samad,  
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28 163 Khurshid, & Sajjad, 2011). The results are summarized in Table 1.

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31 164 None of the 54 swabs and faeces samples analysed were positive for *Campylobacter* spp. On  
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33 165 the contrary, *Salmonella* was isolated from 5.5% (3/54) of the samples collected and all  
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35 166 isolates were identified as serovar Frintrop.

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38 167 Regarding antimicrobial susceptibility, all *Salmonella* isolates were pansusceptible to the  
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40 168 antimicrobials tested. Moreover, the PFGE analysis revealed a low genetic diversity among  
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42 169 isolates, with a unique pulsotype identified with a similarity > 95% (Figure 1).  
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### 171 4. Discussion

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49 172 Since Spain joined de EU and established the same health legislation, Canary Is. is the only  
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51 173 region that provides dromedary camels within the EU (Fernández, 2015; Mentaberre et al.,  
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53 174 2013). Moreover, dromedary camel ride has become one of the most important touristic  
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## SALMONELLA CHARACTERIZATION IN DROMEDARY CAMELS

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5 175 attractions in several countries, and its popularity has increased considerably in the last years  
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8 176 (Fernández, 2015). Therefore, the sanitary status of these animals, especially for zoonotic  
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10 177 pathogens, such as *Campylobacter* and *Salmonella* should be assessed. This study  
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12 178 demonstrates dromedaries as *Salmonella* reservoirs and could be a risk factor of *Salmonella*  
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14 179 infection, but not for *Campylobacter*.  
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17 180 *Campylobacter* is a leading foodborne zoonosis worldwide, widely spread in nature. It  
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19 181 colonizes the intestinal mucosa of most warm-blooded host, including all food-producing  
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21 182 animals and humans (Facciola et al., 2017). However, few reports identify *Campylobacter*  
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23 183 spp of dromedary camel as a human pathogen (Gwida, Zakaria, El-Sherbiny, Elkenany, &  
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25 184 Elsayed, 2019; Rahimi, Alipoor-Amroabadi, & Khamesipour, 2017). In the present study,  
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27 185 *Campylobacter* was not detected in any of the samples collected. As for *Salmonella* isolation,  
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29 186 *Campylobacter* detection is likely to be highly dependent on the choice of an adequate  
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31 187 sampling procedure combined with a sensitive culture method (Marin, Ingesa-Capaccioni,  
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33 188 González-Bodi, Marco-Jiménez, & Vega, 2013). One possible reason for the lack of  
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35 189 detectable *Campylobacter* from cloacal swabs is a lack of appreciable faecal material.  
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37 190 Nevertheless, in our study neither cloacal swabs nor faeces were positive. Even if molecular  
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39 191 methods have shown advantages over classical microbiological *Campylobacter* isolation,  
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41 192 there is a high level of agreement between both methods, particularly with faecal samples  
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43 193 (Ugarte-Ruiz et al., 2012). Consequently, if *Campylobacter* had been present in the samples  
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45 194 taken, it seems highly unlikely that the bacteria would not have been isolated in any of the  
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47 195 samples analysed. Thus, our results show that dromedary camels appear not to be a reservoir  
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49 196 for *Campylobacter*.  
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## SALMONELLA CHARACTERIZATION IN DROMEDARY CAMELS

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5 197 The frequency of *Salmonella* among Canarian dromedaries in this study was moderate (5,5%)  
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7 198 and consistent with other authors (Mohamed and Suelam, 2010); Raufu et al., 2015), which  
8  
9 199 reported a *Salmonella* prevalence of 5,6% and 6%, respectively. Nevertheless, diverse  
10  
11 200 occurrence of this pathogen has been reported in camels in the literature; some authors  
12  
13 201 revealed a low prevalence of *Salmonella* (Wernery, 1992), whilst others reported a medium  
14  
15 202 or high prevalence in captive dromedaries (Moore et al., 2002; Molla et al., 2004; Tejedor-  
16  
17 203 Junco et al., 2010; Münch et al., 2012). As in this study, the infections in dromedaries are  
18  
19 204 usually asymptomatic, although clinical salmonellosis has been reported with symptoms that  
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21 205 included epiphora, anorexia, muscle twitching, and lateral recumbence (Nour-  
22  
23 206 Mohammadzadeh et al., 2010). In addition, controlling *Salmonella* infections in camels  
24  
25 207 should be taken into account, as it has been shown that *Salmonella* could be the cause of co-  
26  
27 208 infections as clostridia or theileriosis diseases (Abdelwahab et al., 2019). Regarding  
28  
29 209 *Salmonella* serovars isolated, ser. Frintrop, was identified in all positive camels. This is one  
30  
31 210 of the most frequently reported serovar in dromedaries (Wernery, 1992; Molla et al., 2004;  
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33 211 Tejedor-Junco et al., 2010; Münch et al., 2012) and may be host adapted to camels (Münch  
34  
35 212 et al., 2012b). Although this is an uncommon serovar in other animal species, it may  
36  
37 213 constitute a threat to camels and other species of animals that are in contact with humans.  
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39 214 The isolation of a single *Salmonella* serovar and all isolates belonging to the same genotype  
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41 215 suggests a single source of infection.

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43 216 Emergence of antibiotic resistance is of worldwide concern, since it reduces the therapy  
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45 217 options in human and veterinary medicine. Thus, the increasing trends of resistance to critical  
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47 218 antimicrobials (WHO, 2018a) that have been reported in the last decade for *Salmonella* and  
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49 219 other zoonotic bacteria is of concern (EFSA & ECDC, 2015). It is believed that antibiotic  
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## SALMONELLA CHARACTERIZATION IN DROMEDARY CAMELS

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5 220 resistance is promoted by the use of antimicrobial drugs in livestock animals (Landers, Cohen,  
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7 221 Wittum, & Larson, 2012). However, in this study, none of the *Salmonella* isolates were  
8  
9 222 resistant to any antimicrobial drug tested. This result is consistent to those published by  
10  
11 223 Münch et al. (2012) were all *S. Frintrop* serovars were susceptible to all antimicrobial agents  
12  
13 224 tested. Antimicrobial resistant *Salmonella* seems to be more prevalent in other livestock  
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15 225 animals such as pigs or poultry (Tejedor-Junco et al., 2010).  
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17 226 Animal movements, and in this case particularly of dromedaries, through European countries  
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19 227 could be a serious threat, since they could contribute to the spread of *Salmonella* resistant  
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21 228 strains, and therefore increase the risk of human infection. Hence, safety biosecurity  
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23 229 protocols should be applied for the movement of dromedaries and other animals among  
24  
25 230 different countries. Especially, care has to be taken during recreational activities, where  
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27 231 animals could be in close contact with children, ancient and immunocompromised people  
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29 232 (Wright et al., 2005; Tejedor-Junco et al., 2010).  
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243 **Conflicts of Interest**

244 The authors declare no conflicts of interest.

245

246 **Ethical Statment**

247 All animals were handled according to the principles of animal care published by Spanish

248 Royal Decree 53/2013 (BOE, 2013 ; BOE = Official Spanish State Gazette).

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250 **Data availability statement**

251 All data relevant to the study are included in the article or uploaded as supplementary

252 information. All individual data that underline the results reported in this article have been

253 shared.

254

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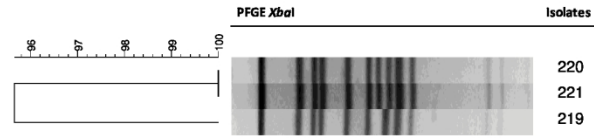
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**Table 1.** Means ( $\pm$  SEM) white blood cells values in female and male camels

Parametres	Female		Male	
	Value	Reference <sup>†</sup>	Value	Reference <sup>†</sup>
Total leucocytic count ( $10^3/\mu\text{l}$ )	$10.15 \pm 0.71$	$12.97 \pm 0.99$	$10.63 \pm 0.8$	$12.38 \pm 0.97$
Neutrophils (%)	$40.88 \pm 1.49$	$43.60 \pm 1.30$	$42.8 \pm 1.7$	$44.70 \pm 1.4$
Lymphocytes (%)	$44.88 \pm 1.36$	$48.60 \pm 1.50$	$41.14 \pm 1.72$	$47.50 \pm 1.4$
Eosinophils (%)	$9.03 \pm 1.11$	$7 \pm 0.39$	$10.1 \pm 1.17$	$7.20 \pm 0.4$
Monocytes (%)	$2 \pm 0.45$	$1 \pm 0.10$	$3.47 \pm 0.68$	$1.20 \pm 0.10$
Basophils (%)	$<0.1$	$<0.1$	$<0.1$	$<0.1$

<sup>†</sup>Farroq et al., 2011.



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

209x297mm (200 x 200 DPI)