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

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SALMONELLA CHARACTERIZATION IN DROMEDARY CAMELS

Abstract

Different studies have reported the prevalence and antibiotic resistance of *Salmonella* in dromedaries' 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 unique dromedary farms located in Tenerife (Canary Islands, Spain). Whilst all the samples were *Campylobacter*-negative, *Salmonella* prevalence was 5.5% (3/54) and the only serovar isolated was *S.* Frintrop. The pulsed field gel electrophoresis analysis revealed a low genetic diversity, with all isolates showing a nearly identical pulsotype (similarity > 95%). Our results indicate that dromedaries' camels could not be a risk factor for *Campylobacter* human infection, but seems to be a reservoir for *Salmonella* transmission. Since camel ride has become one of the main touristic attractions in several countries and its popularity has considerably risen in the last 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 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, campilobacteriosis 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

transmission might be particularly high during stressful long-term movements and recreational actions, 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 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*) at the Canary Is., and to 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 belong to the two unique dromedary farms located in Tenerife (Canary Is., Spain). Each individual was randomly selected from each farm.

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 ± 1ºC for 5 ± 1 h, followed by incubation at 41.5 ± 1ºC for 43 ± 1 h. Afterwards, 100 µL 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 ± 1ºC for 44 ± 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% N_2, 10% CO_2 and 6% 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 pro-duction, and susceptibility to

cephalothin and nalidixic acid.

2.4 *Salmonella* spp isolation and characterization.

Samples were analysed according to the 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, bioMerieux, Madrid, Spain) agar plates, and incubated at 37 ± 1ºC for 24 h. Next, 5 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 standardized 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 the 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 the 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 (European Union, 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 generalized linear model with a binomial probability distribution and a logit link function was used to compare the isolation of *Campylobacter* and *Salmonella* in dromedaries' 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

In this study a total of 54 individuals were sampled. According to the blood parameters obtained, all dromedary camels tested were within the reference parameters (Farooq, Samad, Khurshid, & Sajjad, 2011). The results are represented 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 unique pulsotype identified with a similarity > 95% (Figure 1).

4. Discussion

Since Spain joined de 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 ride has become one of the most important touristic attractions in several countries, and its popularity has increased considerably in the last years

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

Campylobacter is a leading foodborne zoonosis worldwide, widely spread in nature. It colonizes the intestinal mucosa of most warm-blooded host, including all food-producing animals and humans (Facciolà et al., 2017). However, few studies identify *Campylobacter* spp of dromedary camel as potential zoonoses (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 cloacal swabs. Nevertheless, in our study both samples analyzed (cloacal 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, specially 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 isolated it from any of the samples analysed. Thus, results of this study showed that dromedary camels seem not to be a reservoir for *Campylobacter*.

The frequency of *Salmonella* among Canarian dromedaries in this study was moderate (5,5%) and consistent with other authors (Mohamed and Suelam, 2010; Raufu et al., 2015), which 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 authors

showed a low presence of *Salmonella* (Wernery, 1992), whilst others reported a medium or high prevalence in captive dromedaries (Moore et al., 2002; Molla et al., 2004; Tejedor-Junco et al., 2010; Münch et al., 2012). As in this study, salmonellosis in dromedaries are generally asymptomatic, though clinical *Salmonella* infections have been described with symptoms that included epiphora, anorexia, muscle twitching, and lateral recumbence (Nour-Mohammadzadeh et al., 2010). In addition, controlling *Salmonella* infections in camels should be taken into account, as it has been shown that *Salmonella* could be the cause of co-infections as clostridia or theileriosis diseases (Abdelwahab et al., 2019). Regarding *Salmonella* serovars isolated, ser. Frintrop, was identified in all positive camels. This is one of the main described *Salmonella* serovar in dromedaries and may be host adapted to camels (Wernery, 1992; Molla et al., 2004; Tejedor-Junco et al., 2010; Münch et al., 2012). Although this in an uncommon serovar in other animal species, it may constitute a threat to camels and other species of animals that are in contact with humans. The isolation of a single *Salmonella* serovar and all isolates belonging to the same genotype suggests a single source of infection. Emergence of antibiotic resistance is of worldwide concern, since it reduces the therapy options in human and veterinary medicine. Thus, the increasing trends of resistance to critical antimicrobials (WHO, 2018a) that have been reported in the last decade for *Salmonella* and other zoonotic bacteria is of concern (EFSA & ECDC, 2015). It is believed that antibiotic resistance is promoted by the use of antimicrobial drugs in livestock animals (Landers, Cohen, Wittum, & Larson, 2012). However, in this study, none of the *Salmonella* isolates were resistant to any antimicrobial drug tested. This result is consistent to those published by Münch et al. (2012) were 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, and in this case particularly of dromedaries, through European countries could be a serious threat, since they could contribute to the spread of *Salmonella* resistant strains, and therefore increase the risk of human infection. Hence, safety biosecurity protocols should be applied for the movement of dromedaries and other animals among different countries. Especially, care has to be taken during recreational activities, where animals could be in close contact with children, ancient 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.

Ethical Statement

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).

Data availability statement

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

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Abstract

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

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, campilobacteriosis 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

transmission might be particularly high during stressful long-term movements and recreational actions, 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 resistant strain of *Salmonella* ser. Newport from an abscess occurring in a camel used for recreational purposes (Molla, Mohammed, & Salah, 2004; Moore et al., 2002; Wernery, 1992).

Considering the potential public health risks associated with *Campylobacter* and *Salmonella*, the aims of this study were to investigate the prevalence of *Campylobacter* and *Salmonella* in dromedary camels (*Camelus dromedarius*) at the Canary Is., and to 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 belong to the two unique dromedary farms located in Tenerife (Canary Is., Spain). Each individual was randomly selected from each farm.

2.2 Sample collection

Cloacal samples from each individual were taken using sterile cotton swabs (Cary Blair sterile transport 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 collected from the jugular vein (about 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 ± 1ºC for 5 ± 1 h, followed by incubation at 41.5 ± 1ºC for 43 ± 1 h. Afterwards, 100 µL 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 ± 1ºC for 44 ± 4 h However, cloacal swabs were directly streaked onto two selective agar plates (mCCDA and Preston,) and incubated as described above. All plates were incubated in a microaerobic atmosphere (84% N_2, 10% CO_2 and 6% O_2) generated in a gas charged incubator (CampyGen, Oxoid). Plates were examined for grey, flat, irregular and spreading colonies typical of *Campylobacter*. *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 pro-duction, and susceptibility to cephalothin and nalidixic acid.

2.4 *Salmonella* spp isolation and characterization.

Samples were analysed according to the 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, bioMerieux, Madrid, Spain) agar plates, and incubated at 37 ± 1ºC for 24 h. Next, 5 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 standardized 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 the

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 the 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 (European Union, 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 generalized linear model with a binomial probability distribution and a logit link function was used to compare the isolation of *Campylobacter* and *Salmonella* in dromedaries' 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

In this study a total of 54 individuals were sampled. According to the blood parameters obtained, all dromedary camels tested were within the reference parameters (Farooq, Samad, Khurshid, & Sajjad, 2011). The results are summarized 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 unique pulsotype identified with a similarity > 95% (Figure 1).

4. Discussion

Since Spain joined de EU and established the same health legislation, Canary Is. is the only region that provides dromedary camels within the EU (Fernández, 2015; Mentaberre et al., 2013). Moreover, dromedary camel ride has become one of the most important touristic

attractions in several countries, and its popularity has increased considerably in the last years (Fernández, 2015). Therefore, the sanitary status of these animals, especially for zoonotic pathogens, such as *Campylobacter* and *Salmonella* should be assessed. This study demonstrates dromedaries as *Salmonella* reservoirs and could be a risk factor of *Salmonella* infection, but not for *Campylobacter*.

Campylobacter is a leading foodborne zoonosis worldwide, widely spread in nature. It colonizes the intestinal mucosa of most warm-blooded host, including all food-producing animals and humans (Facciolà et al., 2017). However, few reports identify *Campylobacter* spp of dromedary camel as a human pathogen (Gwida, Zakaria, El-Sherbiny, Elkenany, & Elsayed, 2019; Rahimi, Alipoor-Amroabadi, & Khamesipour, 2017). In the present study, *Campylobacter* was not detected in any of the samples collected. As for *Salmonella* isolation, *Campylobacter* detection is likely to be highly dependent on the choice of an adequate sampling procedure combined with a sensitive culture method (Marin, Ingresa-Capaccioni, González-Bodi, Marco-Jiménez, & Vega, 2013). One possible reason for the lack of detectable *Campylobacter* from cloacal swabs is a lack of appreciable faecal material. Nevertheless, in our study neither cloacal swabs nor faeces were positive. Even if molecular methods have shown advantages over classical microbiological *Campylobacter* isolation, there is a high level of agreement between both methods, particularly with faecal samples (Ugarte-Ruiz et al., 2012). Consequently, if *Campylobacter* had been present in the samples taken, it seems highly unlikely that the bacteria would not have been isolated in any of the samples analysed. Thus, our results show that dromedary camels appear not to be a reservoir for *Campylobacter*.

The frequency of *Salmonella* among Canarian dromedaries in this study was moderate (5,5%) and consistent with other authors (Mohamed and Suelam, 2010); Raufu et al., 2015), which 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 authors revealed a low prevalence of *Salmonella* (Wernery, 1992), whilst others reported a medium or high prevalence in captive dromedaries (Moore et al., 2002; Molla et al., 2004; Tejedor-Junco et al., 2010; Münch et al., 2012). As in this study, the infections in dromedaries are usually asymptomatic, although clinical salmonellosis has been reported with symptoms that included epiphora, anorexia, muscle twitching, and lateral recumbence (Nour-Mohammadzadeh et al., 2010). In addition, controlling *Salmonella* infections in camels should be taken into account, as it has been shown that *Salmonella* could be the cause of co-infections as clostridia or theileriosis diseases (Abdelwahab et al., 2019). Regarding *Salmonella* serovars isolated, ser. Frintrop, was identified in all positive camels. This is one of the most frequently reported serovar in dromedaries (Wernery, 1992; Molla et al., 2004; Tejedor-Junco et al., 2010; Münch et al., 2012) and may be host adapted to camels (Münch et al., 2012b). Although this in an uncommon serovar in other animal species, it may constitute a threat to camels and other species of animals that are in contact with humans. The isolation of a single *Salmonella* serovar and all isolates belonging to the same genotype suggests a single source of infection.

Emergence of antibiotic resistance is of worldwide concern, since it reduces the therapy options in human and veterinary medicine. Thus, the increasing trends of resistance to critical antimicrobials (WHO, 2018a) that have been reported in the last decade for *Salmonella* and other zoonotic bacteria is of concern (EFSA & ECDC, 2015). It is believed that antibiotic

resistance is promoted by the use of antimicrobial drugs in livestock animals (Landers, Cohen, Wittum, & Larson, 2012). However, in this study, none of the *Salmonella* isolates were resistant to any antimicrobial drug tested. This result is consistent to those published by Münch et al. (2012) were 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, and in this case particularly of dromedaries, through European countries could be a serious threat, since they could contribute to the spread of *Salmonella* resistant strains, and therefore increase the risk of human infection. Hence, safety biosecurity protocols should be applied for the movement of dromedaries and other animals among different countries. Especially, care has to be taken during recreational activities, where animals could be in close contact with children, ancient 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.

Ethical Statment

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).

Data availability statement

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

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Table 1. Means (± SEM) white blood cells values in female and male camels

	Female		Male	
Parametres	**Value**	**Reference †**	**Value**	**Reference †**
Total leucocytic 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

†Farroq et al., 2011.

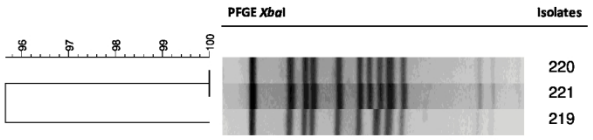

Figure 1. PFGE dendrogram of *Xba*I patterns of *Salmonella* Frintrop isolates from dromedaries.

209x297mm (200 x 200 DPI)