

Prevalence, serotypes and antimicrobial resistance patterns of *Salmonella* isolates from apparently healthy camels in Canary Islands (Spain)

María Teresa Tejedor-Junco^{1*}, Margarita González¹, Noé Francisco Rodríguez² and Carlos Gutiérrez²

¹Laboratory of Microbiology, Clinical Sciences Department, University of Las Palmas de Gran Canaria, P.O. BOX 550, 35080 Las Palmas de Gran Canaria, Canary Islands, Spain.

²Animal Pathology Department, Veterinary Faculty. University of Las Palmas de Gran Canaria, 35413 Arucas, Las Palmas, Canary Islands, Spain.

Abstract

The prevalence, serotypes and antimicrobial resistance patterns of *Salmonella* strains isolated from apparently healthy camels in Canary Islands (Spain) were determined. A total of 52 camels from 3 different farms were tested for the carriage of *Salmonella* spp. in their faeces. *Salmonella* was detected in 9 (17.3 %) of the samples. All of the isolates were characterized as *Salmonella enterica* subsp. *enterica* serotype Frintrop. Feed (oat, alfalfa, wheat straw and maize) and water were analyzed for the presence of *Salmonella*. *Salmonella enterica* subsp. *enterica* serotype Limete was isolated from water, while all feed samples tested negative. All isolates were susceptible to all of the tested antimicrobial agents, which included ampicillin, amoxicillin/ clavulanic acid, tetracycline, enrofloxacin, chloramphenicol, nalidixic acid, piperacillin and trimethoprim-sulfamethoxazole. These results suggest that some sanitary measures should be taken for veterinarians and animal handlers in order to minimise the potential risk of acquiring *Salmonella* zoonoses from Camels.

Key words: Camel; *Salmonella*; serotypes; antimicrobial resistance.

* Corresponding author: Email: mtejedor@dcc.ulpgc.es

1. Introduction

Non-typhoid *Salmonella* are considered as one of the most important zoonotic pathogens globally. Numerous species of *Salmonella* are carried in the intestine of warm and cold-blooded vertebrates, from where they are shed into the environment (Geue and Löschner, 2002; Seepersadsingh and Adesiyun, 2003). Human cases of *Salmonellosis* are often associated with food transmission and typically manifest as gastrointestinal illness. However, infection in people can also occur following exposure to asymptomatic-carrier and clinically infected animals (Schott *et al.* 2001; Wright *et al.* 2005).

The continuing emergence of antimicrobial resistant *Salmonella* in animals represents a further 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 (Fey *et al.* 2000; Angulo *et al.* 2004). Zoonotic infections, resulting from antimicrobial resistant *Salmonella*, are a potential risk to those with routine or occupational exposure to infected animals, including animals serving recreational purposes (Ward *et al.* 2005).

Numerous authors have reported *Salmonella* infection in camels in different parts of the world, including Spain (Wernery, 1992; Moore *et al.*, 2002; Wernery and Kaaden, 2002; Molla *et al.*, 2004). In Gran Canaria, we recently isolated a penicillin-resistant strain of *Salmonella enterica* subsp. *enterica* serotype Newport from an abscess occurring in a camel being used for recreational purposes (Tejedor-Junco *et al.* 2009). Considering the potential

public health risks associated with such a finding, we decided to analyze the prevalence, serotypes and antimicrobial resistance phenotypes of *Salmonella* in recreational camels located in Gran Canaria (Canary Islands, Spain).

2. Material and methods

2.1. Samples

Faecal samples were taken from 52 apparently healthy camels from three different farms located in the south of Gran Canaria (Canary Islands, Spain). These animals are used for recreational purposes and there is movement of animals between farms. Samples of feed (oat, alfalfa, wheat straw and maize) and water were taken at the different farms.

2.2. Culture and identification

Five hundred milligrams of each faecal sample were inoculated in 10 ml of Selenite broth (Difco, MO USA). After incubation at 37°C for 24h, 5 µl of Selenite broth (Difco) were plated onto *Salmonella-Shigella* Agar (SS Agar, Difco, MO, USA) and incubated at 37°C for 24h. Colonies that were suspected to be *Salmonella* (lactose negative with H₂S production) were biochemically identified using API 20E system (BioMérieux, France).

Samples of feed were processed adding 25 g of each to 225 ml of Buffered Peptone water (BPW, Difco, MO USA) and homogenised in a Stomacher. After incubation at 37°C for 24h, 100 µl were added to 10 ml of Rapaport Vassiliadis broth (RV), incubated at 42°C for 24 h and then plated on SS Agar and incubated at 37°C for 24h. Colonies that were suspected to be *Salmonella* (lactose negative with H₂S production) were biochemically identified using API 20E system (BioMérieux, France).

For the detection of *Salmonella* in samples of water, 25 ml of water were added to 100 ml of Buffered Peptone water (BPW, Difco, MO USA) and incubated at 37°C for 24h. Then 100 ml were filtered and filter membranes were placed onto Selenite broth (Difco) and incubated at 37°C for 24h. After this, 5 µl of Selenite broth (Difco) were plated onto Agar SS at 37°C for 24 h. Lactose negative with H₂S positive colonies were identified using API 20E system (BioMérieux, France).

To confirm the identification and determine serotypes, strains from all origins were sent to Laboratorio Central de Veterinaria from Ministerio de Medio Ambiente y Medio Rural y Marino (Algete, Madrid, SPAIN). Serotypes were determined following the Kaufmann-White scheme by slide agglutination test using O and H polyvalent antisera (Difco).

2.3. Antimicrobial susceptibility tests

Antimicrobial susceptibility was determined using disk-diffusion method on Müller-Hinton agar (Difco) following the National Committee on Clinical Laboratory Standards Guidelines (NCCLS, 1999). The following antimicrobial disks (Difco, concentrations in µg) were used: ampicillin (10), amoxicillin/ clavulanic acid (20/10), tetracycline (30), enrofloxacin (5), chloramphenicol (30), nalidixic acid (30), piperacillin (100) and trimethoprim-sulfamethoxazole (1.25/23.75).

3. Results and Discussion

A total of 52 apparently healthy camels from 3 different farms in Gran Canaria were tested for the presence of *Salmonella* spp. in faeces. *Salmonella* was detected in 9 (17.3 %) of the samples. All of the isolates were characterized as *Salmonella enterica*

subsp. *enterica* serotype Frintrop. This prevalence is in line the findings of Molla *et al.* (2004), which describe the prevalence of *Salmonella* in the faeces (15.1%) and mesenteric lymph nodes (15.9%) of apparently healthy camels slaughtered in Ethiopia (Molla *et al.*, 2004). In the United Arab Emirates however, Wernery (1992) found the prevalence of *Salmonella* in camels to be less than five percent.

Chronic carriers of *Salmonella* are not only a threat for themselves and other animals, but also present a human health hazard if used as food animals or if immuno-compromised persons are in contact with them (Van Immerseel *et al.* 2004). All of the animals studied were asymptomatic carriers of *Salmonella*. Carrier animals are known to introduce *Salmonella* to, and maintain it within herds of animals (Cummings *et al.*, 2009).

The literature shows that feed and water are also common sources of *Salmonella* infection for animals (Donaghy and Madden, 1993; Koyuncu and Haggblom, 2009). In this study, feed (oat, alfalfa, wheat straw and maize) and water were analyzed for *Salmonella*. *Salmonella enterica* subsp. *enterica* serotype Limete was isolated from water samples, but all feed samples were negative. Given that we isolated *Salmonella enterica* subsp. *enterica* serotype Limete from water samples, but not faecal samples, it is reasonable to speculate that there is another source causing water contamination. Water could be contaminated by faeces of animals not tested or not present on-farm at the time of sampling.

All of our isolates from faeces were *Salmonella enterica* subsp. *enterica* serotype Frintrop. Several authors have reported the carriage of several different *Salmonella* serotypes in Camels, however in these cases animals came from several distinct herds (Wernery,

1992; Molla *et al.*, 2004). Wernery (1992) described serotype Frintrop as the second most frequent (18.8%) in United Arab Emirates after serotype Saintpaul (41.8%). Molla *et al.* (2004) also detected serotype Saintpaul in faecal samples (33.3%), however they did not describe serotype Frintrop. Isolation of one unique serotype suggests a single source of contamination, but this source was not identified.

All of the isolates (from faeces and water) were susceptible to all antimicrobial agents tested. This is despite the fact that our research group has previously reported the discovery of an antimicrobial resistant strain of *Salmonella* in a camel in Gran Canaria (Tejedor-Junco *et al.* 2009). It appears that antimicrobial resistant *Salmonella* are more prevalent in other animal species, like cattle, sheep, goats and pigs (B. Molla *et al.* 2006, W. Molla *et al.* 2006, Zewdu and Cornelius, 2009). The therapeutic use of antimicrobials is lower in camels than other animals. Furthermore, no antimicrobials compounds are used as prophylactics or growth promoters in the camels sampled in this study. These factors are likely to be limiting the development of antimicrobial resistance in strains from these animals.

The presence of *Salmonella* in animal faeces constitutes a public health hazard, especially for infants, the elderly and for immuno-compromised persons (Woodward *et al.* 1997, Van Immerseel *et al.* 2004, Rodríguez *et al.* 2006). People working with carrier animals, like the camels described in this study, are potentially at risk of zoonotic infection (Wright *et al.* 2005). As such it is recommended that some sanitary measures be taken for veterinarians and animal handlers in order to minimise the risk of acquiring *Salmonella* infections.

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