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IDENTIFICATION AND CHARACTERISATION OF CAMPYLOBACTER SPECIES FROM CAMEL (*Camelus dromedarius*) IN AL-AHSA PROVINCE OF SAUDI ARABIA

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ABSTRACT

The present research project was aimed to isolate and characterise *Campylobacter* spp. from camels in the Al-Ahsa province. A total of 500 faecal samples were collected from camels of different farms. These samples were processed microbiologically to isolate *Campylobacter* spp. using standard culture techniques. Isolates were characterised by conventional phenotypic tests, confirmed by latex agglutination and with the commercial VITEK 2 system (bioMérieux, France).

Out of the 500 faecal samples examined, 25 (5%) yielded *Campylobacter* spp. Characterisation of the isolates showed 19 (3.8%) to be *Campylobacter jejuni*, 5 (1%) *C. coli* and 1 (0.2%) *C. lari*. These thermophilic *Campylobacter* spp. were subjected to biotyping. *C. jejuni* biotypes belonged to biotypes I (57.9%), II (31.6%), III (10.5%), IV (0), while biotypes *C. coli* to I (80%), II (20%) and biotypes *C. lari* to I (100%). Isolation of these species and biotypes may indicate that camels are a reservoir for human infection and the importance of these foodborne pathogens on Public Health is discussed. Conclusions were drawn and recommendations were suggested.

Key words: Camel, *Campylobacter* spp., characterisation, public health, Saudi Arabia

Members of the genus Campylobacter are a group of closely related Gram negative curved rods which primarily colonise the gastrointestinal tract of a wide range of hosts. *Campylobacter* spp. naturally inhabit the intestines of birds and warm-blooded animals including humans.

Characterisation of *Campylobacter* species from some animal species have been documented worldwide, but very little information about *Campylobacter* findings in camelids is available. Such information is completely lacking in the Kingdom of Saudi Arabia.

A study was conducted in Iran (Rahimi *et al*, 2010) to determine the prevalence and antimicrobial resistance of *Campylobacter* spp. isolated from retail raw meats of camel, beef, lamb and goat in Iran. In this study, 6.9% meat samples were contaminated with *Campylobacter* spp. out of which 0.9% were of camel meat.

The present study was aimed to investigate the frequency of *Campylobacter* species among apparently healthy camels in Al-Ahsa Province.

Materials and Methods

Sample Collection

Faecal samples (n=500) from camels were collected in Al-Ahsa Province (Eastern Region of the Kingdom of Saudi Arabia) over a period of nine-months. These were collected with sterile forceps, inserted into polyethylene bags and were immediately transferred to the laboratory.

Sample Processing and Isolation

One gram of faecal sample was emulsified in phosphate buffer saline (pH = 7.0, 0.1 M) at a 10% concentration. The suspension was centrifuged at 8000 rpm for 10 minutes. One loopful of the supernatant was plated onto modified cefoperazone charcoal deoxycholate agar (Oxoid, CM739 plus SR155). Plates were incubated at 37°C for up to 7 days in a microaerophilic atmosphere in CampyGen system (Oxoid, CN0035A). Suspicious colonies were purified on Columbia blood agar base (Oxiod, CM331) plus horse blood (Oxoid, SR0048).

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Phenotypic identification of *Campylobacter* spp.

Campylobacter suspicious colonies were stained with Gram's method and tested by catalase and oxidase tests. Wet mounts were prepared and examined under dark field microscope. The isolates exhibiting characteristic motility of Campylobacter were further characterised using latex agglutination with Campylobacter dryspot kit (Oxoid, DR0150M).

Confirmed isolates were then suspended in proteose peptone (1% [W/V])-glycerol (15% [V/V]) and stored at -70° C for subsequent species identification and biotyping. The isolates were identified to species level using the standard Campylobacter phenotypic identification tests as recommended by Atabay and Corry (1997). The hippurate hydrolysis test was used to identify *C. jejuni* among the confirmed isolates. A small quantity of 24h growth culture was suspended in 0.4ml of 0.1% (W/V) sodium hippurate (Sigma) solution and incubated at 37°C for 2h; 0.2ml of 2% ninhydrin solution (Sigma) was added and incubated for further 15min. The development of a purple-violet colour indicated the isolate to be *C. jejuni*.

Identification of isolates was also confirmed using VITEK 2 system (bioMérieux Vitek Co, France). After presumptive identification, isolates were applied in the NH cards inside the VITEK 2 system and incubated for six hours according to instructions of the manufacturer.

Bio-typing of *Campylobacter* spp.

Biotyping of the thermophilic Campylobacter (*C. jejuni*, *C. coli* and *C. lari*) was carried out as described by Lior (1984). According to the biotyping scheme, *C. jejuni*, *C. coli* and *C. lari* were divided into seven biotypes using sodium hippurate hydrolysis, rapid production of H_2S and deoxyribonuclease enzyme production (DNase) tests. *C. jejuni* comprised four biotypes, *C. coli* two biotypes and *C. lari* one biotype (Table 1).

Results

Isolation Frequency of *Campylobacter* spp. from Camel

Out of the 500 faecal samples, 25 were positive for Campylobacter (5%). *C. jejuni* was with 19 (3.8%) the most commonly isolated species. Other *Campylobacter species* in this study were *C. coli* 5 (1 %) and *C. lari* 1(0.2%) (Table 2).

All tested isolates in the present study were catalase positive.

Table 1. Biotypes of thermophilic *Campylobacter* spp*.

Test	*C. jejuni* I	*C. jejuni* II	*C. jejuni* III	*C. jejuni* IV	*C. coli* I	*C. coli* II	*C. lari* I
Hippurate hyd.	+	+	+	+	-	-	-
H_2S test	-	-	+	+	-	-	+
DNA hydroly.	-	+	-	+	-	+	-

* Lior (1984).

Table 2. Isolation rate of *Campylobacter* spp. from faecal samples of camels in Al-Ahsa province.

Campylobacter species	Isolated Numbers and Rate (%)
C. jejuni	19 (3.8 %)
C. coli	5 (1 %)
C. lari	1 (0.2%)
Total	25 (5%)

Biotyping of *Campylobacter* spp.

The biotyping of the thermophilic *Campylobacter* spp. revealed that *C. jejuni*, biotype I was cultured in 57.9% and *C. coli* biotype I in 80% of cases (Table 3).

Table 3. Biotypes of thermophilic *Campylobacter* species isolated from camel faecal samples in Al-Ahsa Province.

Campylobacter Species	Biotypes	Numbers	Percentage
C. jejuni	I	11	57.9%
C. jejuni	II	6	31.6
C. jejuni	III	2	10.5
C. jejuni	IV	0	0
C. coli	I	4	80
C. coli	II	1	20
C. lari	I	1	100

Discussion

Studies on the presence of *Campylobacter* species in camelids worldwide are rare. To-date, there is no investigation of *Campylobacter* spp. in dromedaries in Al-Ahsa Province.

The prevalence of Campylobacter in dromedary faecal samples was 5% in Al-Ahsa Province. This finding may indicate that camel may act as a reservoir for human and animal infection. A study in Iran reported a 20% faecal prevalence of *Campylobacter* spp in dromedaries (Baserisalehi *et al,* 2007). This result is more or less matching the findings of the present study and the difference in the rate may be explained by the different species isolated in both the studies. Other investigators reported a higher

prevalence rate (11.3%) in Nigerian camels (Salihu *et al,* 2009). This discrepancy may be attributed to the different climatic conditions between Nigeria and Saudi Arabia. *Campylobacter* spp. are fragile organisms and may be affected by adverse climatic conditions which may interfere with isolation rate.

The most common *Campylobacter* species isolated in the present study was *C. jejuni* with 19 isolates (3.8%). This is in agreement with the work of Salihu *et al* (2009) who reported *C. jejuni* to be the most common isolate in their study. *Campylobacter* species in the present study are *C. coli* with 5 isolates (1 %) and *C. lari* with 1 isolate (0.2%). In a study in southern Iran Baserisalehi *et al* (2007) cultured only *C. sputorum* subsp. *sputorum* in camel faecal samples. A recent study in Iran which investigated prevalence of *Campylobacter* spp in retail raw meats including camel meat, reported *C. jejuni* to be the most prevalent species followed by *C. coli* (Rahimi *et al,* 2010). Eviscerated carcasses at slaughter are frequently contaminated with intestinal contents.

Baserisalehi *et al* (2007) reported that all Campylobacter isolates from camel faecal samples were catalase negative, while in our study all the strains were catalase positive. This may be explained by the difference in species isolated in both the studies. Yet, further bacteriological investigations on more isolates of *Campylobacter* spp are needed.

The common *C. jejuni* biotype isolated from camel in the present study is biotype I, which accounts for 57.9% of total *C. jejuni* isolates. Furthermore, *C. coli* biotype I was the most common biotype of *C. coli* constituting 80% of the total *C. coli* isolates. The presence of these biotypes in dromedaries is of serious public health concern, as studies revealed that the frequently isolated *C. jejuni* and *C. coli* biotypes from human were biotype I (Varoli *et al,* 1991; Lior, 1984; Pezzotti *et al,* 2003; Skirrow, 1998). Public health concern was further stressed as *C. jejuni* and *C. coli* were reported from camel meat (Rahimi *et al,* 2010). Campylobacteriosis is a established zoonosis and it can be transmitted to humans via food (meat and milk), water and by contact with farm animals. Moreover, epidemiological data have provided strong evidence that animals and food products of animal origin are the main reservoirs for human infection (Kist, 1983; Newell, 1982; Skirrow, 1982). Hence, Campylobacter enteritis constitutes a zoonosis of major concern in public health and, indeed, has been shown to be a greater problem than salmonellosis in several countries (Blaser *et al,* 1983; Newell, 1982; Skirrow, 1982; Svedhem and Kaijser, 1980).

Conclusion

C. jejuni biotype I and *C. coli* biotype I were the most prevalent biotypes.

The isolation of Campylobacter species and identification of *C. jejuni* biotype I and *C. coli* biotype I from camels in the present study, is important as these biotypes have been demonstrated to cause diseases in humans. Therefore, the present study showed that camels in Al-Ahsa Province could be a reservoir of human infection with *Campylobacter* spp. There is need for further studies to highlight the precise role of the camel and camel products like meat and milk in the transmission of this important foodborne pathogen. An epidemiological investigation would be important to carry out molecular characterisation of *Campylobacter* spp. from camels and humans to detect any correlation between them.

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