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Isolation and Characterization of *Campylobacter* spp. from Domestic Animals and Poultry in South of Iran

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Abstract: A total of 455 domestic animals (cow, horse and camel) and poultry from south of Iran were surveyed for fecal carriage of *Campylobacter* spp. Out of all collected fecal samples, the highest isolation rate of *Campylobacter* was recorded among poultry (35%), followed by horse (27%) and cow (21%) while, lowest isolation rate was recorded among camel. Of the 85 *Campylobacter* strains isolated, 76 were classified as catalase positive *Campylobacter*. Out of them, high frequency of occurrence was belonged to *Campy. jejuni*. Furthermore, catalase positive *Campylobacter* spp. were isolated from all the sources of investigation, other than camel. The results obtained from biotyping of the isolates indicated *Camp. lari* biotype I followed by *Camp. jejuni* and *Camp. coli* biotypes I existed in high frequency; while *Camp. jejuni* biotype II and untypable *Campylobacter* existed in low frequency. Overall, domestic animals and poultry other than camels are vehicle of *Campylobacter* in the area of investigation therefore, the people who living in this area may be infected via feces of domestic animals and poultry.

Key words: *Campylobacter*, isolation, animals, poultry, Iran

INTRODUCTION

The genus *Campylobacter* comprises a group of closely related gram-negative bacteria, which primarily colonize the gastrointestinal tracts of a wide variety of host species.

The natural habitat of most *Campylobacter* spp. is the intestine of birds and other warm-blooded animals, including seagulls and several other wild birds (Kapperud and Rosef, 1983). Some of these bacteria are commensals, but many, particularly *Campylobacter jejuni* and its close relatives, are enteric pathogens of humans and domestic animals. *Camp. jejuni* and *Camp. coli* are common cause of human acute bacterial enteritis worldwide (Notermans, 1994). Although spiral bacteria in the feces were first described in 1880, the role of *Campylobacter* as a cause of enteric disease in man was not fully recognized until the development of isolation methods and selective media during 1970 (Penner, 1988).

It is widely assumed that campylobacteriosis is primarily a food-borne disease. Because, the infective dose of *Campylobacter* is very small; it has been estimated that 500 cells of *Camp. jejuni* can cause human illness (Black *et al*., 1988). This means that even very small number of *Campylobacter* cells in water or food may be a potential health hazard.

Several studies in developed regions, such as Europe and the United States, have reported that the incidence of disease associated with *Campylobacter* is as high as 1% of the population per annum. Extensive epidemiologic investigations have been done in those countries to identify sources of contamination and routes of transmission to humans to facilitate control efforts (Pattison, 2001).

Extensive studies in the United States (Tauxe, 1992) have suggested that a major source of human infection is consumption of contaminated poultry meat. Other identified food vehicles of campylobacters in developed countries are including unpasteurized milk, undercooked meats, mushrooms, hamburger, cheese, pork, shellfish and eggs. In developed countries, risk factors associated with foods include occupational exposure to farm animals, consumption of raw milk or milk products and unhygienic food preparation practices (Alterkruse *et al*., 1999).

However Baserisalehi *et al*. (2006) opined that having difficulty in *Campylobacter* detection cause deficiency in the accurate information concerning *Campylobacter* infection in developing countries, but *Campylobacter* infection is hyper endemic in developing countries. For instance, campylobacters were isolated from 40 and 77% of retail poultry meat sold in Bankok, Thailand and Nairobi, Kenya, respectively (Rasrinaul *et al*., 1988;

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Osano and Arimi, 1999). *Camp. jejuni* was also isolated from poultry meat samples and poultry edible organs in India (Khanna *et al*., 1996). Adegbola *et al*. (1990) in Nigeria reported that *Campylobacter* strains isolated from human and chickens were phenotypically and genotypically correlated, therefore poultry could be considered as reservoir of campylobacteriosis.

Based on foregoing evidence and to achieve information regarding existence of campylobacters in the geographical area of investigation, the present study was conducted to survey frequency of occurrence of campylobacters from domestic animals (cow, horse and camel) and poultry in south of Iran.

MATERIALS AND METHODS

Sampling sites and samples collection: Fecal samples from domestic animals and poultry were collected in south of Iran (Fars and Boshehr states) within six months during 2006.

In all 455 fecal samples were collected from healthy domestic animals (cow, horse and camel) and Poultry from different farms in Fars and Boshehr states, Iran. The samples were collected from each animal using sterile stick and polyethylene bag and transferred to the laboratory within one hour of sampling. The samples were subjected to detection of *Campylobacter* immediately upon arrival in the laboratory. Time of sampling varied from 8 am to 5 pm during the animal grazing and feeding.

Sample processing and isolation: The preT-KB method was used for isolation of campylobacters (Baserisalehi *et al*., 2004). One gram of the collected feacal samples were emulsified in sterile phosphate-buffered saline (pH = 7.0, 0.1 M) at 10% (w/v) concentration. The suspension was centrifuged at 8500 rpm for 10 min, followed by holding at room temperature. After 10-15 min a loopful of supernatant was withdrawn and spread onto the KB medium. The plates were incubated at 37°C for 48 h under microaerophilic conditions and examined daily for 5 days.

Phenotypic identification of *Campylobacter* spp.: All presumptive campylobacters subjected to Gram staining, oxidase and catalase tests, microscopic examination of wet mount under dark field and phase contrast microscope. The isolates exhibiting characteristic motility of *Campylobacter* were characterized using standard *Campylobacter* phenotypic identification tests recommended by Atabay and Corry (1997). These tests included H_2S by lead acetate strip, nitrate reduction, growth in 1% glycine and 3.5% NaCl, growth at different temperatures (25, 37 and 42°C), hippurate hydrolysis, indoxyl acetate hydrolysis, urease production, resistance to nalidixic acid (30 μg) and cephalothin (30 μg).

Additional tests for identification of campylobacters were alkaline phosphotase production and Glucose fermentation.

Table 1: Biotypes of thermophilic *Campylobacter* spp.

	Camp. jejuni				*Camp. coli*		*Camp. lari*
Test	I	II	III	IV	I	II	I
Hypurate hydrolysis	+	+	+	+	-	-	-
Rapid H_2S test	-	-	+	+	-	-	+
DNA hydrolysis	-	+	-	+	-	+	-

* Based on Lior scheme (1984); + Positive; - Negative

Biotyping of thermophilic *Campylobacter* spp.: Biotyping of thermophilic *Campylobacter* isolates was carried out using Lior scheme (1984). According to the biotyping scheme, *Camp. jejuni*, *Camp. coli* and *Camp. lari* were divided into seven biotypes based on three tests viz., hippurate, rapid H_2S and DNase. *Camp. jejuni* comprise four biotypes, *Camp. coli* two biotypes and *Camp. lari* one biotype (Table 1). Therefore, in order to know occurrence of different biotypes of thermophilic *Campylobacter*, the thermophilic isolates from fecal samples were biotyped using hydrolysis of hippurate, rapid production of H_2S and deoxyribonuclease enzyme production (DNase) tests.

RESULTS

Isolation frequencies of campylobacters from fecal samples: Fecal samples were collected from a total of 455 cow, horse, camel and poultry and analyzed for detection of *Campylobacter*. Of all, 85 samples were positive for *Campylobacter*. Therefore, it can be concluded that approximately one-fifth of the samples (18.7%) were harboured *Campylobacter*. The results obtained from the present study indicated that *Campylobacter* occurred with different levels in almost all of the sources of investigation. As seen in the Table 2, the frequency of occurrence of *Campylobacter* in poultry was relatively high and in camel was relatively low. Therefore, it can be concluded that cow, horse and poultry are reservoir of

Table 2: Isolation rates of *Campylobacter* spp. from feces of domestic animals and poultry

Samples	Total No. of samples tested	No. of positive sample for *Campylobacter*	Occurrence of isolates (%)
Camel	145	3	2.0
Horse	78	21	27.0
Cow	121	26	21.0
Poultry	111	35	31.0
Total	455	85	18.7

campylobacters hence; these bacteria may enter the environment of investigation through the feces of domestic animals and poultry with different levels.

Occurrence of catalase positive and negative campylobacters in domestic animals and poultry: A total of 85 *Campylobacter* isolates from different sources were tested for catalase production. The results indicated that majority of the *Campylobacter* isolates from different sources other than camel were catalase positive. However, catalase negative *Campylobacter* strains were not recovered from horse, but all isolated strains of *Campylobacter* from camel were catalase negative (Table 3).

Identification and biotyping of catalase positive thermophilic campylobacters: In all 85 catalase positive and negative campylobacters were subjected to tests recommended by Atabay and Corry (1997). As shown in Table 4 frequency of occurrence of *Camp. jejuni* in all sources was high followed by unidentified *Campylobacter* whereas, isolation frequencies of *Camp. lari* and *Camp. coli* was found to be similar. Although, *Camp. jejuni* and *Camp. coli* were isolated from all sources other than camel, *Camp. lari* was detected neither camel nor horse. However, all *Campylobacter* isolates from camel were catalase negative, isolation rates of them from horse was nil. In addition, the characterization of catalase negative campylobacters isolates indicated that all of the catalase negative *Campylobacter* isolates were *Campylobacter sputorum* while, high isolation rate was recorded *Campylobacter sputorum* var *sputorum* and low isolation rate was recorded *Campylobacter sputorum* var *bubulus* (Table 4).

Biotyping: The catalase positive *Campylobacter* spp. were biotyped by the extended scheme of Lior (1984). The results indicated that most of the *Campylobacter* biotypes existed in all of the sources other than camel. This result illustrated that the frequency of occurrence of *Camp. lari* biotype I in the area of investigation was relatively high followed by *Camp. jejuni* and *Camp. coli* biotypes I. Therefore, the present work clearly showed that the area of investigation was harboured different biotypes of *Campylobacter*.

As shown in Table 5 the highest isolation rate of *Camp. jejuni* biotype I was relatively recorded among poultry and cows, while isolation rate of *Campylobacter* biotypes I and III among horse was similar. Furthermore, *Camp. jejuni* biotypes II and III, IV were not recovered from cow and poultry, respectively. Although rate of existence of *Camp. coli* biotype I in cow and horse was relatively high, frequency of occurrence of *Camp. coli* biotype I and II in poultry was similar. Besides, our data illustrated that *Camp. lari* biotype I was detected from poultry as well as cow, while it was absent in the other sources. The results obtained from this study demonstrated however, untypable *Campylobacter* was detected from fecal samples of cow and horse, but it was not detected from fecal samples of poultry.

Table 3: Occurance of catalase positive and catalase negative **Campylobacter* in fecal samples

Samples	No. of positive samples tested	(%) *Campylobacter* catalase	
		Positive	Negative
Camel	3	0	3
Horse	21	21	0
Cow	26	25	1
Poultry	35	30	5
Total	85	76	9

*Weak catalase positive campylobacters were placed in the catalase negative campylobacters category

Table 4: Prevalence of existing *Campylobacter* spp. in fecal samples of domestic animals and poultry

Sample source	Frequency of occurrence of					
	Camp. jejuni	*Camp. coli*	*Camp. lari*	*Camp. sputorum* var *sputorum*	*Camp. sputorum* var *bubulus*	Unidentified *Campylobcter*
Camel (n* = 3)	0 (0)	0 (0)	0 (0)	3 (100)	0 (0)	0 (0)
Cow (n* = 26)	8 (30)	4 (15)	3 (11)	3 (11)	2 (7)	6 (23)
Horse (n* = 21)	10 (47)	5 (23)	0 (0)	0 (0)	0 (0)	6 (28)
Poultry (n* = 35)	6 (17)	4 (11)	10 (28)	5 (14)	3 (8)	7 (20)
Total	24 (28)	13 (15)	13 (15)	11 (13)	5 (6)	19 (22)

The Figures in the parentheses represent percentage of isolates of each species/group; *Number of *Campylobacter* isolates

Table 5: Occurrence of biotypes* of catalase positive thermophilic *Campylobacter* spp. in environmental samples

Sample source	No. of Isolates	*Camp. jejuni*				*Camp. coli*		*Camp. lari*	
		I	II	III	IV	I	II	I	Untypable
Camel	0	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Cow	15	3 (20)	0 (0)	2 (13.3)	2 (13.3)	4 (26.6)	0 (0)	3 (20)	1 (6.6)
Horse	15	3 (20)	1 (6.6)	3 (20)	2 (13.3)	4 (26.6)	1 (6.6)	0 (0)	1 (6.6)
Poultry	20	4 (20)	0 (0)	1 (5)	0 (0)	2 (10)	2 (10)	11 (55)	0 (0)
Total	50	10 (20)	1 (2)	6 (12)	4 (8)	10 (20)	3 (6)	14 (28)	2 (4)

*Biotying according to Lior scheme (1984); The figures in the parentheses represent percentage of isolates of each biotype

In general, frequency of occurrence of *Camp. jejuni* biotype I (20%) was relatively high, while, biotype II (2%) was relatively low. Amongst *Camp. coli* isolates, biotype I (20%) existed in most frequently. Although, *Camp. lari* and untypable *Campylobacter* were not fitted for typing according to Lior scheme, our observations indicated these bacteria existed in this geographical area.

DISCUSSION

Infection with campylobacters is established zoonoses and the organisms can be transmitted to human being via food (meat and milk), water and through contact with farm animals and pets. A number of potential risk factors associated with *Campylobacter* infection include inadequately cooked chicken, domestic pets such as cat and dogs, raw milk, untreated water, poor food hygiene and handling practices (McMahon and Mahmood, 1993). In order to ascertain the likely sources of *Campylobacter* it is necessary to characterize strains, which are commonly isolated from food chain and environment and to identify these strains in the human infections.

Campylobacters after entry into the environment use its particular characteristics viz., unique metabolism along with complete citric acid cycle, complex and highly branched respiratory chain and great regulatory functions enable them to survive and colonize a number of environments in addition to the mammalian or avian gut (Kelly, 2001).

Poultry, especially broiler chickens, are some of the most important sources of *Campylobacter* infection in humans and the water supply has been shown to be a prominent factor in colonization of campylobacters in chickens (Kapperud *et al*., 1993). In addition, distribution of *Campylobacter* species in chicken and lamb was similar to that seen in humans, suggesting that both of these food sources play a significant role in human infection.

Based on foregoing evidence, domestic animal and poultry could be considered as a link between natural habitat of campylobacters and human being. Therefore, to determine possibility of dissemination of campylobacters and estimate their frequency of occurrence in domestic animals and poultry the present study was conducted to isolate *Campylobacter* spp. from fecal samples of domestic animals and poultry. Then Thermopilic *Campylobacter* isolates were subjected for biotyping using Lior schemes. The results obtained from the present study indicated that all sources of survey were contaminated with different levels of campylobacters. According to our observations the major vehicle of campylobacters in this area was relatively poultry and minor vehicle was camel. Several studies parallel to our finding have shown that poultry is a major source of *Campylobacter* and chicken meat is predominantly associated with *Campylobacter* infection in man (Harrios *et al*., 1986; Humphery *et al*., 1993).

As seen in the Table 2 and 3 frequency of occurrence of catalase positive campylobacters as well as catalase negative campylobacters in poultry was relatively high. Besides, catalase positive campylobacters and catalase negative campylobacters were not isolated from fecal samples of camel and horse, respectively.

However, *Campylobacter* were isolated from all sources of investigation, but isolation frequencies of them in camel were rare. To find out the reason concerning to low frequency of occurrence of campylobacters in camel, it must be noted that camels are adapted to harsh environments such as desert, whereas campylobacters are very sensitive to adverse environmental conditions viz., dryness and low water activity. Besides, methanogenic bacteria are common residents of the digestive tracts of ruminant animals such as cattle, sheep, buffalo, camel and goats (Johnson and Johnson, 1995), whereas population of these bacteria in intestinal tracts of camels due to their diet is not as much as the other ruminant animals. Hence, low populations of metanogenic bacteria in the rumen of camel cause accumulation of H_2 and therefore survival of sensitive bacteria such as campylobacters quickly affected by high concentration of H_2. Hence, based on our finding camels could not be considered as a vehicle of campylobacters.

The Lior schemes were used for biotyping of *Campylobacter* strains isolated in this study. Of the 50 strains of Thermophilic *Campylobacter* tested, 20 strains of *Campylobacter coli* and *Campylobacter jejuni* were belonged to biotype I.

The most prevalent of *Campylobacter* isolates biotypes I was associated with *Campylobacter lari* and less prevalent was related to *Campylobacter jejuni* biotype II followed by untypable *Campylobacter*. Therefore, based on foregoing evidence probably most of campylobacteriosis in this geographical area is related to Thermophilic *Campylobacter* biotypeI. Mégraud and his colleagues (1987) after biotyping of *Campylobacter* strains isolates using Lior schemes reported *Campylobacter coli* and *Campylobacter jejuni* biotype I was the most prevalent (48.2%) of campylobacters in the France. Although, their geographical area of investigation was differ to our investigated area, their result was parallel to our finding indicating high frequency of occurrence of *Campylobacter* biotype I in both areas.

In addition, the results obtained in this study have clearly illustrated that some species of the catalase negative campylobactes existed in the environment of this geographical area. However, *Campylobacter* species other than Thermophilic catalase positive campylobacters named non-pathogenic *Campylobacter*, but recently several studies reported that these organisms also have potential of causing illness in man (Ie Roux and Lastovica, 1998; Amisu *et al*., 2001).

Overall, although, Coker *et al*. (2000) and Raji *et al*. (2000) stated chicken, goat, sheep and pig are major vehicle of *Camp. jejuni* and *Camp. coli* in developing countries, we believed that climate and relative humidity affected the population of campylobacters in the environment. Therefore population of campylobacters in the environment is depended on the weather status of the countries. On the other hand, existence of campylobacters in the intestinal tract of animals depended on their diet and intestinal tracts conditions.

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