

# Prevalence of *Campylobacter* species in milk and dairy products in Iran

E. RAHIMI<sup>1\*</sup>, S. SEPEHRI<sup>2</sup> AND H. MOMTAZ<sup>3</sup>

<sup>1</sup> Department of Food Science and Technology, College of Agriculture, Shahrekord Branch, Islamic Azad University, Shahrekord, Iran.

<sup>2</sup> Graduated Student of Food Science and Technology, College of Agriculture, Shahrekord Branch, Islamic Azad University, Shahrekord, Iran.

<sup>3</sup> Department of Microbiology, College of Veterinary Medicine, Shahrekord Branch, Islamic Azad University, Shahrekord, Iran.

\*Corresponding author: ebrahimrahimi55@yahoo.com

## SUMMARY

The objective of this study was to determine the prevalence of *Campylobacter* spp. in milk and dairy products in Isfahan and Chaharmahal va Bakhtyari provinces, Iran. From July 2011 to July 2012, a total of 552 of various milk and dairy products were obtained from randomly selected retail stores. In this study, 13 of 552 samples (9.3%) were contaminated with *Campylobacter*. The highest prevalence of *Campylobacter* spp. was found in raw cow milk (6.25%), followed by traditional cheese (5.0%), and traditional butter (4.0%). The most prevalence *Campylobacter* species isolated from samples was *Campylobacter jejuni* (76.92%); the remaining isolates were *C. coli* (23.08%). Susceptibilities of 13 *Campylobacter* isolates were determined for ten antimicrobial drugs using the disk diffusion assay. Resistance to nalidixic acid was the most common finding (46.15%), followed by resistance to tetracycline (38.46%), and ciprofloxacin (30.77%). All *Campylobacter* isolates were susceptible to amoxicillin, chloramphenicol, and gentamicin. To our knowledge, the present study is the first report of the isolation of *Campylobacter* spp. from raw milk and dairy products in Iran. The results of this study indicate the potential risk of infection with *Campylobacter* in people consuming raw and unpasteurized milk and dairy products.

**Keywords:** *Campylobacter*, raw milk, Pasteurized milk, dairy products, antimicrobial resistance

## RÉSUMÉ

### Prévalence de *Campylobacter* dans le lait et les produits laitiers en Iran

L'objectif de cette étude était de déterminer la prévalence de *Campylobacter* spp. dans le lait et les produits laitiers dans les provinces de Isfahan et Chaharmahal va Bakhtyari, en Iran. De Juillet 2011 à Juillet 2012, un total de 552 prélèvement ont été obtenus à partir de magasins sélectionnés de manière aléatoire. Dans cette étude, 13 des 552 échantillons (9,3%) étaient contaminés par *Campylobacter*. La plus forte prévalence de *Campylobacter* spp. a été trouvé dans le lait cru de vache (6,25%), suivi du fromage traditionnel (5,0%), et di beurre traditionnel (4,0%). La plupart des espèces de *Campylobacter* était *Campylobacter jejuni* (76,92%), les isolats restants étaient *C. coli* (23,08%). La susceptibilité des 13 isolats de *Campylobacter* a été déterminée pour dix antimicrobiens par la méthode de diffusion sur disque. Une résistance à l'acide nalidixique était la plus fréquente (46,15%), suivie d'une résistance à la tétracycline (38,46%) et la ciprofloxacine (30,77%). Tous les isolats étaient sensibles à l'amoxicilline, le chloramphénicol et la gentamicine.

**Mots-clés:** *Campylobacter*, lait, fromage, produits laitiers, résistance, antimicrobiens, antibiotiques, Iran

## Introduction

*Campylobacter* is the leading cause of zoonotic infections in many countries, and the public health burden of Campylobacteriosis is increasing [10], of the 17 species within the genus *Campylobacter* [7], *C. jejuni* and *C. coli* are the most important from a food safety point of view [29]. Disease caused by *Campylobacter* usually manifests as diarrhoea, fever, malaise and severe abdominal pain. However, it may lead to Guillain-Barre syndrome or Miller Fisher syndrome, which is a serious neurological disease with symptoms that include flaccid paralysis [24]. More recent studies suggest that *C. jejuni* infections can lead to inflammatory bowel disease such as Crohn's disease [10].

The consumption and/or handling of raw or undercooked poultry or other meats were identified as important risk factors for *Campylobacter* infection in sporadic cases, while consumption of milk and water were usually found to be the cause of outbreaks of infection [14, 15]. Cross contamination of ready to eat foods during food preparation as well as direct contact with animals have been identified [12, 30].

Most patients with *Campylobacter* infections have a self-limited illness and do not require antimicrobial drugs except in cases with severe or prolonged symptoms, or in immunocompromised patients [16]. The use of antimicrobial agents in food animals has resulted in the emergence and dissemination of antimicrobial-resistant bacteria, including antimicrobial-resistant *Campylobacter*, which has potentially serious impact on food safety in both veterinary and human health [28]. Although *Campylobacter* with resistance to antimicrobial agents has been reported worldwide [13, 28], the situation seems to deteriorate more rapidly in developing countries, where there is widespread and uncontrolled use of antibiotics [9].

Currently, there is limited information regarding the prevalence and antimicrobial susceptibility patterns of *Campylobacter* in raw milk and dairy products in Iran. The present study was conducted to determine the prevalence and antimicrobial resistance of *Campylobacter* spp. isolated from retail raw milk, pasteurized, and traditional and industrial dairy products in Isfahan and Chaharmahal va Bakhtyari, Iran.

## Materials and Methods

### SAMPLE COLLECTION

From July 2011 to July 2012, total of 552 samples of various milk and traditional and commercial dairy products were obtained from randomly selected retail stores located in 9 major cities of Isfahan and Chaharmahal va Bakhtyari provinces, Iran (Table II). These cities are the most prominent national cultural and tourist center **located** in the center of **the country**. From each city 25-35 samples (0.5-1 kg or litter / sample) were purchased monthly. Kashk and doogh are two popular dairy products in Iran that are available both

as traditional and commercial products. Kashk is prepared by prolonged boiling yogurt and doogh which is also called yogurt soda is prepared by beating unflavored yogurt until smooth, and then diluting with water to a consistency similar to whole milk. Traditional dairy products in Iran are produced in small productive centers mostly located in urban areas and distributed unpacked. These products may be produced from unpasteurized milk.

All samples were immediately transferred to the food microbiology laboratory, Islamic Azad University of Shahrekord Branch, in portable insulated cold-boxes. The samples were analyzed on the day they were collected.

| Organism                    | Primer  | PCR product (bp) | Sequence   |
|-----------------------------|---------|------------------|--|
| <i>Campylobacter</i> spp.   | 16SrRNA | 857              | 5' ATC TAA TGG CTT AAC CAT TAA AC 3'<br>5' GGA CGG TAA CTA GTT TAG TAT T 3'      |
| <i>Campylobacter jejuni</i> | mapA    | 589              | 5' CTA TTT TAT TTT TGA GTG CTT GTG 3'<br>5' GCT TTA TTT GCC ATT TGT TTT ATT A 3' |
| <i>Campylobacter coli</i>   | ceuE    | 462              | 5' AAT TGA AAA TTG CTC CAA CTA TG 3'<br>5' TGA TTT TAT TAT TTG TAG CAG CG 3'     |

TABLE I: Primers for polymerase chain reaction (PCR) amplification of campylobacterial DNA for identification DNA

| Type of food                    | No. of samples | No. of <i>Campylobacter</i> spp. (%) | No. of <i>C. jejuni</i> (%) | No. of <i>C. coli</i> (%) |
|---------------------------------|----------------|--------------------------------------|-----------------------------|---------------------------|
| Raw cow milk                    | 80             | 5 (6.25)                             | 4 (5.0)                     | 1 (1.25)                  |
| Pasteurized cow milk            | 30             | -                                    | -                           | -                         |
| Raw sheep milk                  | 60             | 1 (1.67)                             | 1 (1.67)                    | -                         |
| Raw goat milk                   | 60             | 2 (3.33)                             | 2 (3.33)                    | -                         |
| Raw camel milk                  | 37             | -                                    | -                           | -                         |
| Cheese                          | 80             | 3 (3.75)                             | 2 (2.50)                    | 1 (1.67)                  |
| Commercial cheese               | 30             | -                                    | -                           | -                         |
| Traditional cheese <sup>a</sup> | 60             | 3 (5.0)                              | 2 (3.33)                    | 1 (2.0)                   |
| Ice cream                       | 50             | 1 (2.0)                              | -                           | 1 (2.0)                   |
| Commercial ice cream            | 15             | -                                    | -                           | -                         |
| Traditional ice cream           | 35             | 1 (2.86)                             | -                           | 1 (2.86)                  |
| Yogurt                          | 35             | -                                    | -                           | -                         |
| Commercial yogurt               | 10             | -                                    | -                           | -                         |
| Traditional yogurt              | 25             | -                                    | -                           | -                         |
| Doogh <sup>b</sup>              | 30             | -                                    | -                           | -                         |
| Commercial doogh                | 10             | -                                    | -                           | -                         |
| Traditional doogh               | 20             | -                                    | -                           | -                         |
| Butter                          | 40             | 1 (2.50)                             | 1 (2.50)                    | -                         |
| Commercial butter               | 15             | -                                    | -                           | -                         |
| Traditional butter              | 25             | 1 (4.0)                              | 1 (4.0)                     | -                         |
| Kashk <sup>c</sup>              | 50             | -                                    | -                           | -                         |
| Commercial doogh                | 15             | -                                    | -                           | -                         |
| Traditional doogh               | 35             | -                                    | -                           | -                         |
| Total                           | 552            | 13 (2.36)                            | 10 (1.81)                   | 3 (0.54)                  |

<sup>a</sup>Made from raw sheep or cow milk.

<sup>b</sup>A dairy product prepared by beating unflavored yogurt until smooth, and then diluting with water to a consistency similar to whole milk; it is also called yogurt soda.

<sup>c</sup>A dairy product prepared by prolonged boiling yogurt.

TABLE II: Prevalence of *Campylobacter* spp. in milk and dairy products in Iran

## MICROBIOLOGICAL ANALYSIS

The samples were processed immediately upon arrival using aseptic techniques. Of each milk or dairy product samples, 25 g/mL was homogenized and transferred to 225 mL of Preston enrichment broth base containing *Campylobacter* selective supplement IV (HiMedia Laboratories, Mumbai, India) and 5% (v/v) defibrinated sheep blood. After inoculation at 42 °C for 24 h in a microaerophilic condition (85% N<sub>2</sub>, 10% CO<sub>2</sub>, 5% O<sub>2</sub>), 0.1 mL of the enrichment was then streaked onto *Campylobacter* selective agar base (HiMedia Laboratories, Mumbai, India) supplemented with an antibiotic supplement for the selective isolation of *Campylobacter* species (HiMedia Laboratories, Mumbai, India) and 5% (v/v) defibrinated sheep blood. The composition of *Campylobacter* selective agar base (per liter of medium) was follows: proteose peptone (15.0 g), Liver digest (2.5 g), Yeast extract (5.0 g), Sodium chloride (5.0 g) and Agar (12.0 g). After incubation at 42 °C for 48 h under the same condition, one presumptive *Campylobacter* colony from each selective agar plate was subcultured and identification of presumptive *Campylobacter* species was performed using standard microbiological and biochemical procedures including Gram staining, production of catalase, oxidase, hippurate hydrolysis, urease activity, indoxyl acetate hydrolysis, and susceptibility to cephalotin [2, 30].

## DNA EXTRACTION AND PCR CONDITIONS

Only *Campylobacter* isolates identified by bacteriological methods were tested by PCR. The PCR procedures used in this study have been described previously [4]. Briefly, 1 mL of pure culture of *Campylobacter* was centrifuged at 13000 g for 5 min at room temperature. The DNA was then extracted using a genomic DNA purification kit (Fermentas, GmbH, Germany, K0512) according to the manufacturer's protocol. Three genes selected for the identification of the *Campylobacter* spp., *C. jejuni*, and *C. coli* were the 16S rRNA gene [17], the *mapA* gene [26], and the *ceuE* gene [8], respectively. The sequences of the three sets of primers used for gene amplification are presented in Table I. Amplification reactions were performed in a 30 µL mixture containing 0.6 U Taq polymerase (Fermentas, GmbH, Germany), 100 µmol l<sup>-1</sup> of each dNTP, 0.11 µmol l<sup>-1</sup> of MD16S1 and MD16S2 primers, and 0.42 µmol l<sup>-1</sup> of MDmapA1, MDmapA2, COL3 and MDCOL2 primers in the Fermentas buffer (Fermentas, GmbH, Germany). Amplification reactions were carried out using a DNA thermal cycler (Master Cycle Gradient, Eppendorf, Germany) with the following program: one cycle of 10 min at 95 °C, 35 cycles each consisting of 30 s at 95 °C, 1 min and 30 s at 59 °C, 1 min at 72 °C and a final extension step of 10 min at 72 °C. The amplification generated 857 bp, 589 bp, and 462 bp DNA fragments corresponding to the *Campylobacter* genus, *C. jejuni* and *C. coli*, respectively. *C. coli* (ATCC 33559) and *C. jejuni* (ATCC 33560) were used as the positive controls and DNase free water was used as the negative control. The PCR products were stained with 1%

solution of ethidium bromide and visualized under UV light after gel electrophoresis on 1.5% agarose.

## ANTIMICROBIAL SUSCEPTIBILITY TESTING

One strain from each *Campylobacter*-positive sample was selected for susceptibility tests. Antimicrobial susceptibility testing for 172 *C. jejuni* and 15 *C. coli* isolated strains were performed by the Kirby-Bauer disc diffusion method using Mueller-Hinton agar (HiMedia Laboratories, Mumbai, India) supplemented with 5% defibrinated sheep blood, according to the Clinical Laboratory Standards Institute [3]. The following antimicrobial impregnated disks (HiMedia Laboratories, Mumbai, India) were used: nalidixic acid (30 µg), ciprofloxacin (15 µg), erythromycin (15 µg), tetracycline (15 µg), streptomycin (30 µg), gentamicin (10 µg), amoxicillin (30 µg), ampicillin (10 µg), chloramphenicol (30 µg), and enrofloxacin (10 µg). After incubation at 42 °C for 48 h in a microaerophilic atmosphere, the susceptibility of the *Campylobacter* spp. to each antimicrobial agent was measured and the results were interpreted in accordance with interpretive criteria provided by CLSI [3]. *Staphylococcus aureus* and *Escherichia coli* were used as quality control organisms in antimicrobial susceptibility determination.

## STATISTICAL ANALYSIS

Data were transferred to a Microsoft Excel spreadsheet (Microsoft Corp., Redmond, WA, USA) for analysis. Using SPSS 16.0 statistical software (SPSS Inc., Chicago, IL, USA), a chi-square test and fisher's exact two-tailed test analysis was performed and differences were considered significant at values of  $P < 0.05$ .

## Results

In the present study, a total of 552 samples of various commercial and traditional milk and dairy products were tested for *Campylobacter* species (Table II). Using cultural techniques, 13 of 552 samples (2.36%) were positive for *Campylobacter* spp. No *Campylobacter* spp. was isolated from pasteurized milk, camel milk and commercial dairy product samples and all yogurt, doogh, and kashk samples were negative for *Campylobacter* spp. In contrast, 5 raw cow milk (6.25%), 1 raw sheep milk (1.67%), 2 raw goat milk (3.33%), 3 traditional cheese (5.0%), 1 traditional ice cream (2.86%), and 1 traditional butter (4.0%) samples were contaminated with *Campylobacter* spp. There were not significant differences ( $P > 0.05$ ) in the level of contamination with *Campylobacter* between different milk and traditional dairy product samples. The most prevalence rate of *Campylobacter* species isolated from samples was *C. jejuni* (76.92%); the remaining isolates were *C. coli* (23.08%). All 13 *Campylobacter* isolates identified as *C. jejuni* and *C. coli* by using conventional bacteriological methods were also positive using the PCR assay. No significant differences in the prevalence rates were observed between milk and dairy product samples isolated in Isfahan and Chaharmahal va Bakhtyari.

| Antimicrobial agent | <i>Campylobacter</i> spp.<br>(N = 13) | <i>Campylobacter jejuni</i><br>(N = 10) | <i>Campylobacter coli</i><br>(N = 3) |
|---------------------|---------------------------------------|---|--------------------------------------|
| Amoxicillin         | 0 (0.0%)                              | 0 (0.0%)                                | 0 (0.0%)                             |
| Ampicillin          | 1 (7.69%)                             | 1 (10.0%)                               | 0 (0.0%)                             |
| Chloramphenicol     | 0 (0.0%)                              | 0 (0.0%)                                | 0 (0.0%)                             |
| Ciprofloxacin       | 4 (30.77%)                            | 3 (30.0%)                               | 1 (33.33%)                           |
| Enrofloxacin        | 1 (7.69%)                             | 0 (0.0%)                                | 1 (33.33%)                           |
| Erythromycin        | 1 (7.69%)                             | 0 (0.0%)                                | 1 (33.33%)                           |
| Gentamicin          | 0 (0.0%)                              | 0 (0.0%)                                | 0 (0.0%)                             |
| Nalidixic acid      | 6 (46.15%)                            | 5 (50.0%)                               | 1 (33.33%)                           |
| Streptomycin        | 1 (7.69%)                             | 1 (10.0%)                               | 0 (0.0%)                             |
| Tetracycline        | 5 (38.46%)                            | 4 (40.0%)                               | 1 (33.33%)                           |

TABLE III: Antibiotic resistance profiles of *Campylobacter* strains isolated from milk and traditional dairy products in Iran.

The resistance pattern of *Campylobacter* isolates to 10 antimicrobial agents tested in this study is shown in Table III. Overall, 9 of 13 *Campylobacter* isolates (69.23%) were resistant to one or more antimicrobial agent. Three strains (23.07%) were resistant to single antibiotic and 4 strains (30.77%) showed resistance to two antimicrobial agents. Multiresistance which was defined as resistance to three of drug tested was found in only 7.69% of *Campylobacter* strains. Resistance to nalidixic acid was the most common finding (46.15%), followed by resistance to tetracycline (38.46%), and ciprofloxacin (30.77%). All *Campylobacter* isolates were susceptible to amoxicillin, chloramphenicol, and gentamicin.

The highest prevalence of *Campylobacter* spp. occurred in summer (4.44%) followed by spring (1.46%). The prevalence rates of *Campylobacter* spp. in fall and winter were 2.11% and 0.72%, respectively. No significant differences in the prevalence rates of *Campylobacter* spp. were observed for milk and dairy product samples taken in different seasons in Isfahan and Chaharmahal va Bakhtyari.

## Discussion

Milk and dairy products have been previously reported as vectors in the transmission of *Campylobacter* spp. [18]. Consumption of raw milk, inadequately pasteurized milk, and cheese contaminated with *Campylobacter* was shown to be responsible for six enteric infection outbreaks reported in England and Wales since 1981 [5, 19].

In the present study, No *Campylobacter* spp. was isolated from 115 pasteurized milk and commercial dairy product samples, although 13 of the 437 raw milk and traditional (3.0%) dairy product samples were positive for *Campylobacter* spp. and the difference was statistically significant ( $P < 0.05$ ). This result is in agreement with the results reported by SALIHU *et al.* [23] from Nigeria, EL-SHAROUD [6] from Egypt and WHYTE *et al.* [30] from Ireland. The high occurrence of *Campylobacter* spp. in traditional dairy products could be due to environmental contamination with infected animal wastes or unsanitary food production and

storage practices. However, this could be also due to the use of unpasteurized milk.

Among all the various milk and dairy products tested in this study, traditional cheese (made from raw sheep or cow milk) and raw milk samples had the highest prevalence rate of *Campylobacter* spp. Although the prevalence of *Campylobacter* spp. may vary in different dairy products, it has been shown that *Campylobacter* isolates can be found more frequently in raw milk samples and soft cheeses [6, 12, 23]. In a study in Pakestan, *Campylobacter* spp. were detected in 10.2% and 11.7% of raw milk and cheese samples [12]. In a study in Egypt, 2 of 50 raw milk samples (4.0%) and 4 of 38 fresh domiati cheese samples (11.0%) were positive for *Campylobacter* spp., in which *Campylobacter* isolates recovered from these two product were all identified as *C. jejuni* [6]. Also, WHYTE *et al.* [30] reported that 1 of 62 raw milk samples were positive for *C. coli*. Similarly, HUMPHREY and HART [11] and ROHRBACH *et al.* [22] recovered *Campylobacter* from raw milk with prevalence up to 12.3%.

The results of this study show that camel milk is not an important source for *Campylobacter* infection. No previous report could be found on the occurrence of *Campylobacter* spp. on the camel milk. Methanogenic bacteria are common residents of the digestive tract of ruminant animals [14]. However, population of these bacteria in intestinal tract of camels due to their diet is not as much as the other ruminants. It seems that the low populations of heterogenic bacteria in the rumen of camel cause an accumulation of  $H_2$  which affects survival of sensitive bacteria such as *Campylobacter* spp. In a study on feces samples from camels (*Camelus dromedarius*) only 2.0% of feces were positive for *Campylobacter* spp. [1]. Also, absence of *Campylobacter* in camel milk samples in this study may be a result of good hygienic practices of milking. However, further intensive prevalence studies on camel milk and other dairy products will be needed.

In the present study, no *Campylobacter* isolate was detected in yogurt, doogh, and kashk samples and only 1 traditional

butter samples were positive with *Campylobacter* spp. These results are consistent with those reported by EL-SHAROUD [6]. The absence of *Campylobacter* spp. in yogurt, doogh, and kashk samples and low prevalence of *Campylobacter* spp. in butter found in this study could possibly be accounted for by the acidity of these products; however, it could also be due to the boiling stage performed during the processing of these products.

In this study, *C. jejuni* was the most prevalence *Campylobacter* species recovered from milk and dairy product samples. *Campylobacter jejuni* has been reported to be the most frequent species recovered from food of animal origin [6, 12, 18]. Variation in the prevalence of *Campylobacter* isolates from raw milk and traditional dairy product samples reported in other studies may be a result of different sampling techniques employed, seasonal effects [25] and/or laboratory methodologies employed in different studies (bacteriological and biochemical testing vs. polymerase chain reaction assays).

The results of antimicrobial susceptibility testing in the present study indicate that there is a high resistance of *Campylobacter* spp. to tetracycline, ciprofloxacin, and nalidixic acid. These results are comparable to those reported by other investigators [21, 27, 28, 31]. Erythromycin is one of the first choice of treatment for man and high resistance rate to erythromycin by *C. coli* observed in this study is comparable with the results reported in several other studies including PAYOT *et al.* [20].

The results of antimicrobial resistance found in this study are correlated to antibiotics that are being used to treat infection in food animals in Iran. Due to the high number of antimicrobial-resistant isolates, we recommend that *in vitro* antimicrobial susceptibility testing of *Campylobacter* be performed and appropriate treatment be instituted especially for those cases of food borne campylobacteriosis with severe or prolonged symptoms or in immunocompromised patients.

In conclusion, the presence of *Campylobacter* spp. in a variety of raw milk and traditional dairy products indicate the potential risk of infection with *Campylobacter* in people consuming raw milk, unpasteurized milk, or traditional dairy products in Iran. To our knowledge, the present study is the first report of the isolation of *Campylobacter* spp. from raw milk and dairy products in Iran.

## Acknowledgements

The authors would like to thank Amir Shakerian, Manochehr Momeni and Majed Riahi for the sincere help in performing technical parts of the project.

## References

1. BASERISALEHI M., BAHADOR N., KAPADNIS B. P.: Isolation and characterization of *Campylobacter* spp. from domestic animals and poultry in south of Iran. *Pak. J. Biol. Sci.*, 2007, **10**, 1519-1524.
2. BOLTON F. J., WAREING D. R., SKIRROW M. B., HUTCHINSON D. N.: Identification and biotyping of *Campylobacter*. In: Identification Methods in Applied and Environmental Microbiology. BOARD G. R., JONES D., SKINNER F. A. (Eds.), Society for Applied Microbiology, Technical Series 29, Blackwell Scientific Publications, Oxford, 1992, pp. 151-161.
3. CLINICAL AND LABORATORY STANDARDS INSTITUTE (CLSI): Performance Standards for Antimicrobial Disk Susceptibility Tests, Approved standard-Ninth Edition (M2-A9). Clinical and Laboratory Standards Institute, Wayne, PA., 2006.
4. DENIS M., SOUMET C., RIVOAL K., ERMEL G., BLIVET D., SALVAT G., COLIN P.: Development of a m-PCR for simultaneous identification of *Campylobacter jejuni* and *C. coli*. *Lett. Appl. Microbiol.*, 1999, **29**, 406-410.
5. DJURETIC T., WALL P. G., NICHOLS G.: General outbreaks of infectious intestinal disease associated with milk and dairy products in England and Wales: 1992 to 1996. *Commun. Dis. Rep. CDR Rev.*, 1997, **7**, 41-45.
6. EL-SHAROUD W. M.: Prevalence and survival of *Campylobacter* in Egyptian dairy products. *Food Res. Int.*, 2009, **42**, 622-626
7. FOSTER G., HOLMES B., STEIGERWALT A. G., LAWSON P. A., THORNE P., BYRER D. E., *Campylobacter insulaenigrae* sp. Nov., isolated from marine mammals. *Int. J. System Evol. Microbiol.*, 2004, **54**, 2369-2373.
8. GONZALEZ I., GRANT K. A., RICKARDSON P. T., PARK S. F., COLLINS M. D.: Specific identification of the enteropathogens *Campylobacter jejuni* and *Campylobacter coli* using PCR test based on the *ceuE* gene encoding a putative virulence determinant. *J. Clin. Microbiol.*, 1997, **35**, 759-763.
9. HART C. A., KARIUKI S.: Antimicrobial resistance in developing countries. *BMJ*, 1998, **317**, 647-650.
10. HORROCKS S. M., ANDERSON R. C., NISBET D. J., RICKE S. C.: Incidence and ecology of *Campylobacter jejuni* and *coli* in animals. *Food Microbiol.*, 2009, **15**, 18-25.
11. HUMPHREY T. J., HART R. J. C.: *Campylobacter* and *Salmonella* contamination of unpasteurised cow's milk on sale to the public. *J. Appl. Bacteriol.*, 1988, **65**, 463-467.
12. HUSSAIN I., MAHMOOD M. S., AKHTAR M., KHAN A.: Prevalence of *Campylobacter* species in meat, milk and other food commodities in Pakistan. *Food Microbiol.*, 2007, **24**, 219-222.
13. ISENBARGER D. W., HOGE C. W., SRIJAN A., PITARANGSI C., VITHAYASAI N., BODHIDATTA

- L., HICKEY K. W., CAM P. D.: Comparative antibiotic resistance of diarrheal pathogens from Vietnam and Thailand, 1996–1999. *Emerg. Infect. Dis.*, 2002, **8**, 175–180.
14. JOHNSON K. A., JOHNSON D. E.: Methane emission from cattle. *J. Anim. Sci.*, 1995, **73**, 2483-2492.
  15. JORGENSEN F., BAILEY R., WILLIAMS S., HENDERSON P., WAREING D. R., BOLTON F. J., FROST J. A., WARD L., HUMPHREY T. J.: Prevalence and numbers of *Salmonella* and *Campylobacter* spp. on raw, whole chickens in relation to sampling methods. *Int. J. Food Microbiol.*, 2002, **76**, 151-164.
  16. KETLEY J. M.: Pathogenesis of enteric infection by *Campylobacter*. *Microbiol.*, 1997, **143**, 5-21.
  17. LINTON D., LAWSON A. J., OWEN R. J., STANLEY J.: PCR detection, identification to species level and fingerprinting of *Campylobacter jejuni* and *Campylobacter coli* direct from diarrheic samples. *J. Clin. Microbiol.*, 1997, **35**, 2568-2572.
  18. PARK S.: The physiology of *Campylobacter* species and its relevance to their role as foodborne pathogens. *Int. J. Food Microbiol.*, 2002, **74**, 177–188.
  19. PEBODY R., RYAN M., WALL P.: Outbreaks of *Campylobacter* infection: Rare events for a common pathogen. *Commun. Dis. Rep. CDR Rev.*, 1997, **7**, 33–37.
  20. PAYOT S., DRIDI S., LAROCHE M., FEDERIGHI M., MAGRAS C.: Prevalence and antimicrobial resistance of *Campylobacter coli* isolated from fattening pigs in France. *Vet. Microbiol.*, 2004, **101**, 91-99.
  21. RAHIMI E., AMERI M., KAZEMAINI H. R.: Prevalence and antimicrobial resistance of *Campylobacter* species isolated from raw camel, beef, lamb, and goat meat in Iran. *Foodborne Pathog. Dis.*, 2010, **7**, 443-447.
  22. ROHRBACH B. W., DRAUGHON F. A., DAVIDSON P. M., OLIVER S. P.: Prevalence of *Listeria monocytogenes*, *Campylobacter jejune*, *Yersinia enterocolitica* and *Salmonella* in bulk tank Milk: risk factors and risk of human exposure. *J. Food Protect.*, 1992, **55**, 93-97.
  23. SALIHU M. D., JUNAIDU A. U., MAGAJI A. A., RABIU Z. M.: Study of *Campylobacter* in raw cow milk in Sokoto state, Nigeria. *Br. J. Dairy Sci.*, 2010, **1**, 1-5.
  24. SMITH J.: *Campylobacter jejuni* infection during pregnancy: Long-term consequences of associated bacteremia, Guillain-Barre syndrome, and reactive arthritis. *J. Food Prot.*, 2002, **65**, 696-708.
  25. SOPWITH W., ASHTON M., FROST J. A., TOCQUE K., O'BRIEN S., REGAN M., SYED Q.: Enhanced surveillance of *Campylobacter* infection in the North West of England 1997-1999. *J. Infect.*, 2003, **46**, 35-45.
  26. STUCKI U. R. S., JOACHIM F., NICOLET J., BURNENS A. P.: Identification of *Campylobacter jejuni* on the basis of a species gene that encodes a membrane protein. *J. Clin. Microbiol.*, 1995, **33**, 855-859.
  27. TAREMI M., SOLTAN-DALLAL M. M., GACHKAR L., MOEZARDALAN S., ZOLFAGHARIAN K., REZA ZALI M.: Prevalence and antimicrobial resistance of *Campylobacter* isolated from retail raw chicken and beef meat, Tehran, Iran. *Int. J. Food Microbiol.*, 2006, **108**, 401-403.
  28. VAN LOOVEREN M., DAUBE G., DE ZUTTER L., DUMONT J. M., LAMMENS C., WIJDOOGHE M., VANDAMME P., JOURET M., CORNELIS M., GOOSSENS H.: Antimicrobial susceptibilities of *Campylobacter* strains isolated from food animals in Belgium. *J. Antimicrob. Chemother.*, 2001, **48**, 235-240.
  29. WESLEY I. V., WELLS S. J., HARMON K. M., GREEN A., SCHROEDER-TUCKER L., GLOVER M., SIDDIQUE I.: Fecal shedding of *Campylobacter* and *Arcobacter* spp. in dairy cattle. *Appl. Environ. Microbiol.*, 2000, **66**, 1994-2000.
  30. WHYTE P., MCGILL K., COWLEY D., MADDEN R. H., MORAN L., SCATES P., CARROLL C., O'LEARY A., FANNING S., COLLINS J. D., MCNAMARA E., MOORE J. E., CORMICAN M.: Occurrence of *Campylobacter* in retail foods in Ireland. *Int. J. Food Microbiol.*, 2004, **95**, 111-118.
  31. YILDIRIM M., ISTANBULLUOGLU E., AYVALI B.: Prevalence and antibiotic susceptibility of thermophilic *Campylobacter* species in broiler chickens. *Turk. J. Vet. Anim. Sci.*, 2005, **29**, 655-660.