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Research Article

Occurrence of Campylobacterin Poultry Meat and Edible Offal's in the northwest of Iran

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ABSTRACT:
The Campylobacter jejuni and Campylobacter coli are the most Common Cause of foodborne bacterial gastroenteritis in both developed Countries worldwide aim of this study was to detect and determine the Seasonal Prevalence of Campylobacter jejuni and Campylobacter coli in Poultry Meat and Edible Offal's Using mPCR Assay. To conduct the study, a total of 552 chicken samples including meat (138 samples), liver (138 samples), gizzard (138 samples) and hearts (138 samples) were randomly collected from poultry slaughterhouses at West Azerbaijan province from April 2014 to September 2014. Based on the culture tests, 208 samples (37.7%) were infected with Campylobacter species. The highest range of Campylobacter species outbreaks was observed in poultry liver (49.2%), followed by gizzard (42.8%), heart (33.3 %) and meat (25.4%). Among the isolated Campylobacter, the Jejuni type was the most prevalent (78.4%) and the rest were of Coli type (21.6%). All 208 species of Campylobacter isolated as Jejuni and Colitypes from culturing were also approved by multiplex polymerase chain reaction test (m.PCR). The results of the study pinpointed to the chicken edible offal importance as a potential source of bacterial Campylobacter infections.

KEYWORDS: Campylobacter, Poultry Meat, Edible Offal's, m-PCR

[I] INTRODUCTION

Campylobacteriosis, serving as an important common disease, shoulders a major role in infectious gastroenteritis in humans and is considered as the world's first cause of gastroenteritis followed by dysentery and diarrhea and other complications such as meningitis, Guillain Barre Syndrome and Cholecystitis [18, 1]

Digestive disorders and diarrhea caused by the bacterial Campylobacter is among the common illnesses primarily in developing countries, and 5 to 15 percent of diarrhea in these countries are instigated by the bacteria, and the prevalence of the bacteria as a cause of diarrhea in our country has been reported to be from 2 to 10 % [11], and even the primary cause of death in developed countries, especially among children under 5 in the United States, was attributed to such bacteria in that two million cases of bacterial infection are reported each year [18].

The bacteria which was first discovered in the nineteenth century by Theodor Escherichia a gram-negative, spiral-shaped or curved, growing and multiplying at Microaerophilic conditions at a temperature of 42 ° C, and below 25 ° C, it stops growing and multiplying but does not disappear [18]. The thermophilic Campylobacter species, such as Jejuni and Coli, have an important role in the development of Campylobacteriosis becoming more prominent in the food infection as the spread of Campylobacter contamination in food has become very important in recent years going even further than Salmonella [18].Studies show that the bacterium originates mainly from food with animal resource. Moreover, raw milk, meat and poultry, untreated surface water and edible fungi are the sources of infections with Campylobacteriosis disease being common in spring and summer. Infections with Campylobacter have been reported in several countries in broiler chickens, so that the bacteria survived during the slaughtering process and were transmitted to uninfected poultry organs[18].

Due to its high sensitivity and detection speed, the PCR method has the potentiality to determine if there is even a bacterium per ml and reaches the response in a very short time. Many studies have examined the prevalence of the bacteria in meat and poultry as well as diarrheal samples [15]. The consumption of half-baked meat and the related products serves as the main source of human infection although other livestock meat and milk products are potential sources of the disease. There are reports on the prevalence of Campylobacter among live birds and animals and different kinds of food from all over the world revealing upon a wide range of results. Previous reports highlighted the infection in chickens from zero to 100% which was up to 60% for the cows. The prevalence of the infection in poultry given to the market have been reported to be100%and lower incidences of other animals meat were also given [7,22]. Although there are many studies casting light on the prevalence of Campylobacter species among poultry in Iran [16,17,21,23] and other countries including Korea [10], Japan [22], Canada [24], Ireland [25], Pakistan [12] Belgium [9], little data existed on the poultry meat bi-products infection including liver, gizzard and heart in Iran, therefore, the study aimed at evaluating the contamination of these products.

[II] MATERIALS AND METHODS

To conduct the study, a total of 552 samples including chicken meat (138 samples), liver (138 samples), gizzard (138 samples) and hearts (138 samples) were randomly collected from poultry slaughterhouses at West Azerbaijan province from April 2014 to September 2014and were transferred to the laboratory for analysis aiming at investigating the presence of Campylobacter species in cold temperature. To determine the phenotype of bacteria, 10 grams of the sample was added to a bag with one hundred ml broth Preston (Preston enrichment broth base, himedia, Mumbai, India, m899) containing five percent defibrinated sheep blood for enrichment. The bags were incubated for 24 hours at 42 ° C in carbon dioxide presence. Then, the liquid in the bag was removed by a sterile loop and cultured on specific Campylobacter selective Agar (Himedia, Mumbai, India, m994) as a separate line to obtain single separated colonies. Then, it was incubated for 48 hours (Co2 10% v / v) at 42 ° C. After the initial plate analysis, the suspected colonies were stained with Gram's stain and upon the curved bacteria resolution, the re-culturing was also applied. Upon obtaining the colonies, the biochemical tests including catalase, Indoxyl acetate hydrolysis were used and in order to differentiate between the two species Jejuni and Coli hippurate hydrolysis test was conducted, which was positive for Jejuni [1].To investigate the Campylobacter genotypic, DNA extraction and Multiplex PCR method were applied. Using the culturing method and DNA extraction kit

(Cina Gen, Iran), the confirmed colonies DNA were extracted according to the Kit manufacturer's instructions. The PCR test in this study followed Denis et al. (1999) procedure [3] For PCR reaction, the reaction final volume of 25 microlitres was considered including 20 ng DNA template, 2 mM MgCl2, 25 pmol of each primer, a single Taq polymerase enzyme and 200 mM mixed dNTP. The size of PCR products corresponding to each sampleis given below. To confirm the presence of the multiplied sample, 20 microlitres of PCR was placed on electrophoresis with1/5 percent agarose gel containing ethidium bromide in the presence of 100 bp DNA marker in constant voltage of 80 volts.

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Gene	Primer consequence	Type of product
16SrRNA	MD16S1 Upper Primer 3 ATC TAA TGG CTT AAC CAT TAA AC5 MD16S1 Lower Primer 3GGA CGG TAA CTA GTT TAG TAT T 5	857bp for Campylobacter genus
mapA	MDmapA1 upper Primer 3CTA TTT TAT TTT TGA GTG CTT GTG 5 MDmapA2 Lower Primer 3GCT TTA TTT GCC ATT TGT TTT ATT A5	589bp for C.jejuni
CeuE	COL3 Upper Primer 3AAT TGA AAA TTG CTC CAA CTA TG5 MDCOL2 Lower Primer 3TGA TTT TAT TAT TTG TAG CAG CG5	462bp for C.coli

Table 1: sequences of primers used for detecting Campylobacter and the related Jejuni and Coli species

[III] RESULTS

The current study aimed at evaluating Campylobacter phenotypic and genotypic outbreaks in chicken meat and its edible offal at West Azerbaijan province, Iran. To conduct the study, a total of 552 samples including chicken meat (138 samples), liver (138 samples), gizzard (138 samples) and hearts (138 samples) were isolated as Jejuni and Coli types from culturing were also approved by multiplex polymerase chain reaction test (m.PCR). A statistically significant difference ($P < 0.05$) was observed in Campylobacter species outbreak in meat samples taken in summer (40/9 %). The results are presented in the following tables:

Table 2. The contamination of chicken meat and its related edible offal to Campylobacter and its species

Positive Number and percentage of campylobacter coli infection	Positive Number and percentage of campylobacter jejuni infection	Positive Number and percentage of campylobacter infection	number	samples
(%8/6) 3	(%91/4) 32	(%25/4) 35	138	Meat
(%10/3) 7	(%89/7) 61	(%49/2) 68	138	Liver

(% 10/2) 6	(% 89/8) 53	(% 42/8) 59	138	gizzard
(% 8/7) 4	(% 91/3) 42	(% 33/3) 46	138	hearts

Table 3: Monthly contamination of chicken meat and its related edible offal to Campylobacter and its species

Positive Number and percentage of campylobacter coli infection	Positive Number and percentage of campylobacter jejuni infection	Positive Number and percentage of campylobacter infection	Number	months
(%20/7) 6	(%79/3) 23	29(%31/6)	92	March
(%21/9) 7	(%78/1) 25	(%34/8) 32	92	April
(%23/5) 8	(% 76/5) 26	(%37) 34	92	May
(%20) 7	(% 80) 28	(%38) 35	92	June
(%21/6) 8	(% 78/4) 29	(%40/2) 37	92	July
(%22) 9	(%78) 32	(%44/6) 41	92	August

Table 4: the seasonal contamination of chicken meat and its related edible offal to Campylobacter and its species

Positive Number and percentage of campylobacter coli infection	Positive Number and percentage of campylobacter jejuni infection	Positive Number and percentage of campylobacter infection	Number of samples	Season
(%22/1) 21	(%77/9) 74	(%34/4) 95	276	Spring
(%21/2) 24	(%78/8) 89	(%40/9) 113	276	summer
(% 21/6) 45	(% 78/4)163	(% 37/7) 208	552	Total

[IV] DISCUSSION

The given result shows 208 samples (37/7 %) were infected with Campylobacter species. Previous studies in Iran have reported the contamination level to the bacteria for different cities as Isfahan with 56/1 percent [17], Shahrekord with 47 percent [16], Tehran with 63/2% and 49/5%[21] and Mashhad with 76 % [8].

The outbreak of Campylobacter species in chicken meat in other countries also suggests a contamination level of30 to 90 percent. The contamination levels in different countries were as follows: Turkey with92/8%[26], Korea with68/3% [10], Canada with 62/4% [24], Japan with 60% [22], Ireland with 49/90% [25] and Pakistan with 48% [12]. Despite having many studies reporting the prevalence of Campylobacter species contamination in poultry, there are few studies highlighting on the edible offal contamination to the bacteria.

A similar study conducted byRahimiin2006 and 2008 casted light on the prevalence of Campylobacter in the chicken liver marketed in Isfahan. Accordingly, the contamination of 205 samples under study was reported to be 49/3%, and the livercontamination for the, chicken, turkey and ostrich were49/3%, 40% and / 16/7%, respectively [17]. Also, Shakerian et al (2004) in a study on evaluating the Campylobacter contamination in poultry liver in Shahrekord indicated that 259 samples of 400 samples (64/8%) were infected with Jejuni Campylobacter [20].The report by Gafir et al (2007) suggests that contamination of broiler chicken liver distributed in Belgium capital from 1997 to1998was about68/7%. The chicken liver infection rate in 1997 and 1998 were 61/7% (74 from 120) and 74/6% (106 from 143) [9]. Sallam et al (2007) reported the contamination of meat and edible offal for chicken from 40 to 77 for breast, thighs, wings, liver, gizzard and heart

as64/4%, 70%, 77/1%, 64%, 45% and 40%, respectively [19].

Suzuki andYammamato (2009) reported the contamination of chickenmeat, gizzard, liver and heart as 59%, 62/2%, 62/3% and 33/3, respectively [22]. In both studies similar to result of this study, the highest infection rates was in liver and the lowest was observed in heart. The reason could be due toliver greater contact area than the heart and its further manipulation.The differences between the results reported from different parts could be attributed to the poultry infection rates in different regions, the interval between studies, the difference in the killing and hygiene practices during different slaughtering phases, sampling seasons and sensitivity of testingmethods.

The results showed that among the isolated Campylobacter, the Jejuni type was the most prevalent (78/4 %) and the rest were of Coli type (21/6 %). Other studies have also shown that Jejuni type is the most common species in food withanimal origin [6,8,12,16,17,22].For example, in a study by Hussain et al (2007) the prevalence of Campylobacter species (Jejuni and Coli)in food samples with animal origin was70/6% and 29/4%, respectively. The same study reported the prevalence of Jejuni and Coli Campylobacter in chicken as, 72% and 28%, in sheep meat as 65% and 35% and in cow meat as 79% and 21%, respectively [12].

Another similar study in 2004 was conducted in Ireland by Whyte et al. focusing on Jejuni and Coli Campylobacter in food with animal origin which revealed the fact that the contamination level for Jejuni and Coli type were 3804 and 16/6, respectively. The prevalence of bacteria in chicken as 6/84 and 6/16% have been reported [25].

Moreover, evaluating the Campylobacter contamination in poultry meat samples in different season showed there existed a significant difference in contamination level in summer (50 /.> P) than in other seasons which was also confirmed by reports from other studies [24]. Such high prevalence could be attributed to high temperature creating favorable conditions for thebacteria growth of and transferring of infection by insects.The overall results of this study on chicken meat and its edible offal contamination to Campylobacter species showed that a relatively high number of samples especially the liver were infected with this pathogen. Therefore, in order to reduce contamination of chicken meat and its edible products to Campylobacter species and similar microorganisms, maintaining individual health, preserving sanitation in slaughterhouses, following HACCP principles in poultry chains, minimizingthe carcasses contact with the edible offal, minimizing the chicken carcasses contact and maintaining the least manipulation and drinking water in slaughtering process seem to be the most important. Also, maintaining hygiene practices in splitting, packaging, and transportationstages and maintainingthe cold conditionin meat preserving chainuntil being delivered to consumer serve as very important measures in reducing meat contamination to such pathogens.

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Fig. 1. Multiplex PCR amplicons on 1.5% agarose gel. Lane M: 100 bp ladder; Lane: 1, 2, 3 - C. coli; Lane: 4, 5, 6 - C. jejuni