2011 International Conference on Food Engineering and Biotechnology
IPCBEE vol.9 (2011) © (2011)IACSIT Press, Singapore

A 3-year study of Escherichia coli O157:H7 in cattle, camel, sheep, goat, chicken and beef minced meat

Sima Hajian[1], Ebrahim Rahimi[1], Hasan Mommtaz[2]

[1] Membership of Young Research Club, Islamic Azad University, Shahrekord Branch, Shahrekord, Iran.

[2] Department of Microbiology, College of Veterinary Medicine, Islamic Azad University, Shahrekord Branch, Shahrekord, Iran.

Abstract. *Escherichia coli* O157: H7 is recognized as and important cause of diarrhea, hemorrhagic colitis and hemolytic-uremic syndrome worldwide. Meat, meat products, dairy products, vegetables and drinking water contaminated with animal feces are probably the major sources of the *E. coli* O157: H7 infection. The aim of the present study was to investigate the prevalence of *E. coli* O157: H7 in raw meat samples of in Iran. From April 2006 to November 2009, 484 raw meat samples were collected from cattle (n= 90) camel, (n= 75), sheep (n= 60), goat (n= 60), chicken (n= 82) and minced beef (n=117). Bacterial DNA extraction was performed after an enrichment step in a broth was followed by PCR. Twenty-three (4.8%) of 484 samples were positive for *E. coli* O157. Most of the *E. coli* O157 strains were isolated from cattle and beef minced meat samples. Of 23 *E. coli* O157 isolates, 8 were serotype O157: H7 and 15 were serotype O157: NM. The prevalence of this organism was different during different seasons. The highest incidence of *E. coli* O157:H7 occurred in summer and fall seasons. To our knowledge, this is the first report of the isolation of *E. coli* O157: H7 from retail raw camel meat in Iran. The data reported in this study provides some useful baseline in formation for future research.

Keywords: Escherichia coli O157: H7, raw meat, chicken meat, minced meat

1. Introduction

There are four major pathogens that have frequently been associated with meat and meat products including *Salmonella* spp., *Campylobacter* spp., *Listeria monocytogenes*, and *Escherichia coli* O157:H7. These organisms have been linked to a number of cases of human illness [1]. One of the most significant food-borne pathogens that has gained increased attention in recent years is *E. coli* O157:H7. Typical illness as a result of an *E. coli* O157:H7 infection can be life threatening, and susceptible individuals show a range of symptoms including hemolytic colitis, hemolytic-uremic syndrome, and thrombotic thrombocytopenic purpura [2]. Domestic and wild animals are the sources of *E. coli* O157, but ruminants are regarded as the main natural reservoirs. Sporadic cases and outbreaks of human diseases caused by *E. coli* O157 have been linked to ground beef, raw milk, meat and dairy products, vegetables, unpasteurized fruit juices and water [3, 4]. Infections can also be acquired by direct contact with animals and by person-to-person spread [2, 5]. Currently, there is limited information regarding the prevalence of *E. coli* O157:H7 in animal meat in Iran. Therefore, this study was conducted to determine the contamination rate of *E. coli* O157:H7, from retail raw beef, camel, sheep, goat, chicken, and beef minced meat using PCR in Three cities of Iran.

2. Materials and methods

2.1. Sample collection and preparation

From April 2006 to November 2008, a total of 484 raw meat samples from beef (n = 90), camel (n = 75), sheep (n = 60), goat (n = 60), chicken (n= 82) and beef minced (n= 117) meat were purchased from randomly selected retail outlets in Isfahan, Shahrekord and Yazd, Iran. All samples were placed in separate sterile plastic bags to prevent spilling and cross contamination and were immediately transported to the laboratory in a cooler with ice packs.

2.2. Isolation of E. coli O157:H7

Twenty-five g of each sample were homogenized in 225 mL trypton soya broth supplemented with novobiocin (20 mg/L) and incubated at 37 °C for 18-24h.

Then the enrichment samples were streak onto levine eosin methylene blue agar and sorbitol McConkey agar plates supplemented with cefexime (0.5 mg/L) and potassium tellurite (2.5 mg/L) and incubated as above. Suspected colonies were confirmed by TSI agar and IMViC tests [6]. Sorbitol negative colonies were reported as E. coli O157: H7 with PCR assay by using the O-antigen encoding region of O157 gene and flagellar H7 gene (fli C) generic primers as described previously [7, 8]**.**

2.3. Statistical analysis

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

3. Results

Table 1 show the prevalence of *E. coli* O157 and *E. coli* O157:H7 isolated from beef, camel, sheep, goat and minced beef meat in Isfahan, Shahreekord and Yazd, Iran. In this study, 23 of 484 meat samples (4.7%) were found to be contaminated with *E. coli* O157. The highest prevalence of *E. coli* O157 was found in beef minced meat (11.1%), followed by beef meat (8.9%), goat meat (1.7%), and camel meat (1.3%). PCR products are shown in Figs.1 and 2. There were significant differences ($P<0.05$) in the level of contamination with *E. coli* O157 between different meat samples; however, no significant differences ($P>0.05$) were found between lamb meat, goat meat, camel meat and chicken meat. No significant differences in the prevalence rates ($P>0.05$) were observed between meat samples isolated in Isfahan, Shahr-e Kord and Yazd. The highest prevalence of *E. coli* O157 occurred in fall (9.1%) followed by spring (5.7%). The prevalence rates of *E. coli* O157 in summer and winter were 2.5% and 1.6%, respectively.

TABLE 1. PREVALENCE OF ESCHERIOSHIA COLI O157 ISOLATED FROM BEEF, CAMEL, SHEEP, GOAT, CHICKEN AND BEEF MINCED MEAT

Meat sample	No. of samples	No. (%) of positive *E. coli* O157 :H7 samples	No. (%) of positive *E. coli* O157 :NM samples
Beef	90	2 (2.2)	6 (6.7)
Camel	75	1 (1.3)	0 (0.0)
Sheep	60	0 (0.0)	0 (0.0)
Goat	60	0 (0.0)	1 (1.7)
Chicken	82	0 (0.0)	0 (0.0)
Beef minced	117	5 (4.3)	8 (6.8)
Total	484	8 (1.7)	15 (3.1)

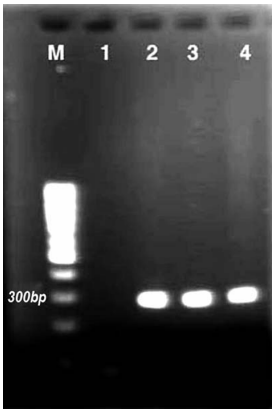

Figure 1. PCR products of the samples for O157 gene (Column M: 100 bp DNA ladder, SM 0241, Fermentas Co.); Column 1: negative control, Column 2: positive control, Columns 3 and 4: positive samples).

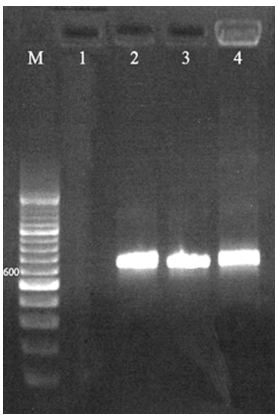

Figure 2. PCR products of the samples for Flagellar H7 gene (Column M = 100 bp DNA ladder, SM 0321, Fermentas Co.); Column 1: negative control, Column 2: positive control, Columns 3 and 4: the positive samples).

4. Discussion

Human infections of *E. coli* O157:H7 have mostly been recognized to be from food products with animal origin [4]. Cattle have been implicated as the principal reservoir of *E. coli* O157:H7 [3]. Many studies determined the prevalence of *E. coli* O157:H7 on cattle carcasses which were from 0.0% to 27.8% (up to 68% in heifers) [1, 3, 4]. The prevalence value reported in the presented in Iran (2.2%) is lower than Netherland (10.4%) [9] and England (13.4%) [3]. Direct comparison of results is difficult due to differences in the study methodologies, such as the type of slaughtering, improved enrichment and isolation procedures, differences in sample size, the type of sample and how and when it was collected [10]. While there is some evidence that *E. coli* O157:H7 may be increasingly common in beef production systems the detection of higher proportions of *E. coli* O157:H7 in more recent studies is more probably associated with the wider use of more sensitive detection methods such as IMS [3].

Of the 117 minced beef samples collected over a 3-year period, a total of 5 (4.3%) were positive for *E. coli* O157:H7. In comparison to other countries, the prevalence reported in this study is higher than in previous studies i.e. 0.17% [11] and 1.4% [12], similar to a Swiss study (2.3%) [13] and lower than reported in a study in Argentina (3.8%) [14].

Due to relative increase in the consumption of camel meat in Iran, we decided to determine the prevalence of *E. coli* O157: H7 in the camel carcasses. The results of this study showed that 1.7% of camel carcasses were positive for *E. coli* O157:H7. The present study demonstrated that the prevalence of *E. coli* O157:H7 was lower than that observed on beef. The study suggests that camel meat may not be a significant source of *E. coli* O157: H7 that have seen in other meat industries but monitoring program and inspection are necessary for preventing outbreaks of food-borne diseases.

The contamination rate of chicken meat samples observed in this study was in agreement with those reported by Jo et al. (2004) in Korea. However, Abdul-Raouf et al. (1996) reported a higher prevalence of *E. coli* O157:H7 in chicken carcasses in Egyption (4.0%) [15]. Also, in a study conducted in Argentina, *E. coli* O157:H7 were isolated from 10.3% of chicken meat samples (14).

The results of this study showed that sheep and goat meat are not an important source for *E. coli* O157:H7 infection. In one study conducted in the Shiraz, Iran, 19 *E. coli* O157:H7 isolates were recovered form 159 sheep meat samples [16]. Similarly, in a study in Ethiopia, Hiko et al. (2008), found a prevalence rate of *E. coli* O157:H7 of 2.5% and 2.0% in sheep and goat meat samples, respectively (17). Also, the prevalence of *E. coli* O157:H7 spp. in retail sheep and goat meat was reported to be 0.77%-7.3% in the Italy (18), 4.0% in Egyption (15), 1.5% in USA (19), 0.5% in Australia (20).

In this study the highest prevalence of *E. coli* O157:H7 was found on meat sampled in fall and spring, which is in agreement with finding of previous studies on beef that reported peak prevalence occurs in early fall (10).

The current study is the first report on the prevalence of *E. coli* O157:H7 on camel and goat carcasses in Iran. Our findings provide some baseline information regarding the prevalence of *E. coli* O157:H7 isolated from animal meat that could be used in future studies. As a result, the most important practice that should be considered in animal slaughtering are cleaning dirty animals before slaughtering, skinning while being on the rail, separating carcasses from each other and avoiding contact between the external surface of the hide and carcasses. Hygiene measures must be sufficient to prevent from contamination via hands, knives, saws, equipments, and clothing.

5. Acknowledgment

The authors would like to thank Abbas Doosti, Majid Riahi, Mohsen Permoradian, Mohammad Reza Nazem, Mohammad Ali Rashidfarokhi, Mohammad Reza Jafarian and Manocher. Momeni for the sincere help in performing technical parts of the project.

6. References

[1] R.H. Madden, W.E. Espie, L. Moran, J. McBride, and P. Scates. Occurrence of E. coli O157:H7, Listeria monocytogenes, *Salmonella* and *Campylobacter* spp. on beef carcasses in Northern Ireland. *Meat Sci.* 2001, **58**: 343-346.

[2] A. Caprioli, S. Morabito, H. Brugère, and E. Oswald. Enterohemorrhagic *Escherichia coli*: emerging issues on virulence and modes of transmission. *Vet. Res*. 2005, **36**: 289–311.

[3] P.A. Chapman, C.A. Siddons, A.T. Cerdan-Malo, and M.A. Harkin. A 1-year study of *Escherichia coli* O157 in cattle, sheep, pigs and poultry. *Epidemiol. Infect*. 1997, **119**: 245-250.

[4] M.Y. Jo, J.H. Kim, J.H. Lim, MY. Kang, H.B. Koh, Y.H. Park , and et al. Prevalence of characteristics of *Escherichia coli* O157 from major food animals in Korea. *Int. J. Food Microbiol.* 2004, **95**: 41-49.

[5] S. Cho, F. Diez-Gonzales, C.P. Fossler, SJ. Wells, C.W. Hedberg, J.B. Kaneene, and et al. Prevalence of Shiga toxin-encoding bacteria and Shiga toxin-producing *Escherichia coli* isolates from dairy farms and county fair. *Vet. Microbiol*. 2006, **118**: 289–298.

[6] S. Stampi, A. Caprioli, G. De Luca, P. Quaglio, R. Sacchetti, and F. Zanetti. Detection of *Escherichia coli* O157 in bovine meat products in northern Italy. *Int. J. Food Microbiol.* 2004, **90**: 257- 262.

[7] V.P. Gannon, S. D'Souza, T. Graham, R.K. King, K. Rahn, and S. Read. Use of the flagellar H7 genes as a target in multiplex PCR assays and improved specificity in identification of enterohemorrhagic *E. coli* strains. *J. Clin. Microbiol.* 1997, **35**: 656-662.

[8] A.W. Paton, and J.C. Paton. Detection and characterization of shiga toxigenic *Escherichia coli* by using multiplex PCR assays for *stx1, stx2, eaeA,* enterohemorrhagic *E. coli hlyA, rfbO111, rfbO157 . J. Clin. Microbiol.* 1998, **36**: 598-602.

[9] A.E. Heuvelink, J.T.M. Zwartkruis-Nahuis, R.R. Beumer, and E. De Boer. Occurrence and survival of verocytotoxin-producing *Escherichia coli* O157 in meats obtained from retail outlets in The Netherlands. *J. Food Prot.* 1999, **62**: 1115–1122.

[10] CM. Bryane, I. Erol, J.E. Call, C.W. Kaspar, D.R. Burge, C.J. Hiemke, et al. Characterization of *E. coli* O157:H7 from downer and healthy dairy cattle in the upper Midwest region of the United States. *Appl. Environ. Microbiol*. 2003, **69**: 4683-468.

[11] L. Walsh, D. Dooge, and C. Hill. Screening for *Escherichia coli* O157:H7 in Irish ground beef using two commercial detection systems. *Irish Vet. J. Incorporating Irish Vet. Times.* 1997, **50**: 111–115.

[12] E.A. Duffy, K.E. Belk, J.N. Sofos, S.B. Levalley, M.L. Kain, and J.D. Tatum. Microbial contamination occurring on lamb carcasses processed in the United States. *J. Food Prot.* 2001, **64**: 503-508.

[13] K. Fantelli, and R. Stephan. Prevalence and characteristics of shigatoxin-producing *Escherichia coli* and *Listeria monocytogenes* strains isolated from minced meat in Switzerland. *Int. J. Food Microbiol.* 2001, **70**: 63–69.

[14] I. Chinen, J.D. Tanoro, E. Miliwebsky, L.H. Lound, G. Chillemi, and S. Ledri. Isolation and characterisation of *Escherichia coli* O157:H7 from retail meats in Argentina. *J. Food Prot*. 2001, **64**: 1346–1351.

[15] U.M. Abdul-Raouf, M.S. Ammar, and L.R. Beuchat. Isolation of *Escherichia coli* O157:H7 from some Egyptian foods. *Int. J. Food Microbiol.* 1996, **29**: 423-426.

[16] S. Shekarfroush, Y. Tahamtan, and A. Pourbakhsh. Detection and frequency of Stx2 gene in *Escherichia coli* O157 and O157:H7 strains isolated from sheep carcasses in Shiraz-Iran. *Pak. J. Bioch. Sci.* 2008, **11**: 1085-1092.

[17] A. Hiko, D. Asrat, and G. Zewde. Occurrence of *Escherichia coli* O157:H7 in retail raw meat products in Ethiopia. *The J. Infect. Dev. Countries.* 2008, **2**: 389-393.

[18] A. Battisti, S. Lovari, A. Franco, A. Di Egidio, R. Tozzoli, A. Caprioli. Prevalence of *Escherichia coli* O157 in lambs at slaughter in Rome. central Italy. *Epidemiol. Infect.* 2006, **134**: 415-419.

[19] M.P. Doyle, and J.L. Schoeni. Isolation of *Escherichia coli* O157:H7 from retail fresh meats and poultry. Appl. Environ. *Microbiol.* 1987, **53**: 2394-2396.

[20] D. Phillips, D. Jordan, S. Morris, I. Jenson, and J. Sumner. Microbiological quality of Australian sheep meat in 2004. *Meat Sci.* 2006, **74**: 261-266.