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Research Article

The Presence of *Yersinia enterocolitica* and Other Yersinia Species in Ground Beef in Aydın, Turkey

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Abstract: The objective of this study was to investigate the prevalence of *Y. enterocolitica* and other Yersinia species in raw ground beef samples and to determine their potential virulence. For this purpose, 61 ground beef samples purchased from 15 different butcher's shops and 3 supermarkets in Aydın province in Turkey were examined between June 2000 and January 2001. The presence of *Y. enterocolitica* and other Yersinia species was determined using 2 stage enrichment procedures including pre-enrichment (cold) and selective enrichment procedures. All positive cultures of *Y. enterocolitica* were also tested for markers of virulence including hydrolysis of esculin, fermentation of salicin, calcium binding, crystal violet and Congo red binding assays. Yersinia spp. were detected in 20 of 61 (32.8%) samples. Among the positive samples, 17 (27.9%) were identified as *Y. enterocolitica*, 2 (3.3%) as *Y. intermedia* and 1 (1.6%) as *Y. frederiksenii*. As a result of virulence marker tests, none of the *Y. enterocolitica* isolates were presumptively virulent.

Key Words: Ground beef, Yersinia spp., *Yersinia enterocolitica*, pathogenicity

Sığır Kıymalarında *Yersinia enterocolitica* ve diğer Yersinia Türlerinin Varlığı ve Patogenitesinin Belirlenmesi

Özet: Bu çalışma, Aydın ili'nde tüketime sunulan kıymalarda *Y. enterocolitica* ve diğer Yersinia türlerinin varlığını araştırmak ve izole edilen *Y. enterocolitica* suşlarının patojenitesini saptamak amacıyla planlandı. Bu amaçla, Haziran 2000 - Ocak 2001 tarihleri arasında 15 farklı kasap ve 3 süpermarketten 61 kıyma örneği alındı. İzolasyon amacıyla, soğukta önzenginleştirme ve selektif zenginleştirme işlemlerini kapsayan iki basamaklı zenginleştirme işlemi uygulandı. İzole edilen *Y. enterocolitica* suşlarının patojenitesini saptamak amacıyla eskülin hidrolizi, salisin fermentasyonu, kalsiyum bağlama, kristal violet ve Kongo red bağlama yöntemlerini kapsayan 5 farklı test yapıldı. Sonuçta, analiz edilen 61 kıyma örneklerinin 20'sinden (% 32,8) 3 farklı Yersinia türü izole ve identifiye edildi. Pozitif örnekler arasında 17 (% 27,9) *Y. enterocolitica*, 2 (% 3,3) *Y. intermedia*, 1 (% 1,6) *Y. frederiksenii* olarak identifiye edildi. Yapılan virulens marker testleri sonucu, izole edilen *Y. enterocolitica* izolatlarının hiçbirisinin şüpheli virulent suşlar olmadıkları bulundu.

Anahtar Sözcükler: Kıyma, Yersinia spp., *Yersinia enterocolitica*, patojenite

Introduction

Yersinia enterocolitica was first discovered by Schleifstein and Coleman in 1939 (1). *Y. enterocolitica* is known as a psychrotrophic waterborne and foodborne enteropathogen (2). Outbreaks of yersiniosis are commonly associated with food vehicles such as meat (particularly pork), milk, powdered milk, cheese, tofu and raw vegetables (3-5). The first and definitive food-associated outbreak of yersiniosis occurred in Oneida County, New York, where over 220 individuals (primarily school-age children) were stricken with acute gastroenteritis after the consumption of contaminated milk (6). *Y. enterocolitica* has been isolated from meat, chicken, vacuum packaged meat, pork, ham, drinking water, milk and oysters (7-14).

Y. enterocolitica is also isolated from human and animal infections. Yersinia species consist of pathogens and nonpathogenic spp. The virulence of *Y. enterocolitica* is associated with biogroup, serogroup and geographic distribution. *Y. enterocolitica* strains have been distributed in 5 biogroups according to their biochemical properties. Strains of biogroups 2, 3, 4 and 5 (except 3A and 3B) are restricted to a small number of serogroups (O:1; O:2; O:3; O:5,27; O:9) and are generally isolated

from a specific host. Strains of serogroups O:3; O:9; and O:5,27 are the major causes of human infections in Europe, Japan, southern Africa and Canada (15). Biogroup 1 should be subdivided according to the ecology of its members. It includes esculin-negative, pyrazinamidase-negative strains belonging to serogroups O:4; O:8; O:13a; O:13b; O:18; O:20; and O:21 that are pathogenic for humans and that have been isolated only in North America. It also contains pyrazinamidase-positive strains, generally esculin-positive, that are scattered among numerous serogroups and distributed world wide. These strains are ubiquitous in nature and are found in food, water, soil, and animal and human feces. They are not pathogenic for humans. Strains of biogroups 3A and 3B have nevertheless quite different ecological behavior, despite their high biochemical similarity to those of biogroups 3. These strains are found in water and are occasionally isolated from food, animals and humans. These environmental strains are devoid of any virulence-linked properties. The clinically important *Y. enterocolitica* serotypes are O:3; O:5,27; O:6,30; O:13,7; O:21; O:8; and O:9 (16-19). Pathogenic serotypes of *Y. enterocolitica* show a specific geographical distribution: O:8 and O:3 in the United States, O:3 in Canada, O:3 and O:9 in Europe, and O:3 in Japan and South Africa (19).

Y. enterocolitica is primarily a gastrointestinal tract pathogen with a strong propensity for extraintestinal spread under defined host conditions. It causes a broad range of diseases from acute bowel disease to extraintestinal manifestation such as reactive arthritis and uveitis. When it infects the gastrointestinal tract, acute enteritis with fever and watery, occasionally bloody diarrhea are observed, particularly in children. In young adults, acute terminal ileitis and mesenteric lymphadenitis mimicking acute appendicitis appear to be more common clinical symptoms (6,20).

Human pathogenic *Y. enterocolitica* synthesizes and secretes several outer membrane proteins (Yops), which play a major role in virulence (21,22). Genes encoding Yops are located on a 70 to 75-kb plasmid and are temperature and calcium regulated, and expressed maximally at 37 °C in response to the presence or absence of millimolar concentrations of calcium. In the absence of Ca^{++} at 37 °C, a set of plasmid-borne operons is induced (low calcium response), which is down regulated at 37 °C when Ca^{++} is present (22). Strains of all serotypes associated in human disease harbor a virulence-associated plasmid (O:3; O:8; O:9 etc.). It is associated with several properties that are strongly influenced by the growth temperature. These include a calcium requirement for growth at 37 °C, the capacity for Congo red intake, and autoagglutinability (5,21,23).

The serotyping and biotyping of isolates within *Y. enterocolitica* species can be helpful in determining whether they are potential pathogens. In the absence of the antisera to serogroup *Y. enterocolitica* isolates in routine microbiology laboratories, significance remains a function of assessing an isolate for plasmid-encoded virulence factors. These tests are indirect but simple markers of pathogenicity that can be determined in most laboratories and include autoagglutination, production of V (immunogenic protein) and W antigens (nonprotective lipoprotein) (24), serum resistance, calcium dependency for growth at 37 °C, Congo red and crystal violet binding tests, and even plasmid profiles (25,26). Other virulence assays include lethality for mice, production of conjunctivitis in guinea pigs (Sereny test) (27), absence of pyrazinamidase activity (28), hydrolysis of esculin (25 °C), and fermentation of salicin (35 °C) (29).

A few sero-epidemiological studies conducted in patients in Turkey showed that the occurrence of *Y. enterolitica* ranged between 1.35 and 2% (30-32). Although the occurrence of Yersinia species in meat has been investigated in several countries, there are very few reports about the incidence of *Y. enterocolitica* in different foodstufs in Turkey (14). Therefore, the aim of the present study was to determine the prevalence of *Yersinia* spp. in raw ground beef in Aydın, Turkey, and to determine the potential virulence of *Y. enterocolitica* isolates.

Materials and Methods

Sixty-one raw ground beef samples purchased from 15 different local butcher's shops and 3 supermarkets in Aydın province in Turkey were examined for the presence of *Y. enterocolitica* between June 2000 and January 2001. *Y. enterocolitica* and other Yersinia species were isolated using 2-stage enrichment procedures including pre-enrichment (cold) in Tripticase Soy Broth (TSB-Difco 0370-01-1) (23,33,34) and selective enrichment in Bile Oxalate Sorbose (BOS) Broth (34,35). Following the 2-stage enrichment procedures, alkaline post-enrichment treatment was performed as described by Aulisio et al.

(36). Then a loopful of each sample was streaked onto Cefsulodin-Irgasan-Novobiocin (CIN) agar (Oxoid-CM 653). Five presumptive Yersinia colonies from each sample were selected for further biochemical analyses (34,37). Standard oxidase, catalase, Gram staining, and other biochemical tests for the isolation and identification of Yersinia spp. were performed at 25 °C as shown in Figure. Then the biochemical results were assessed

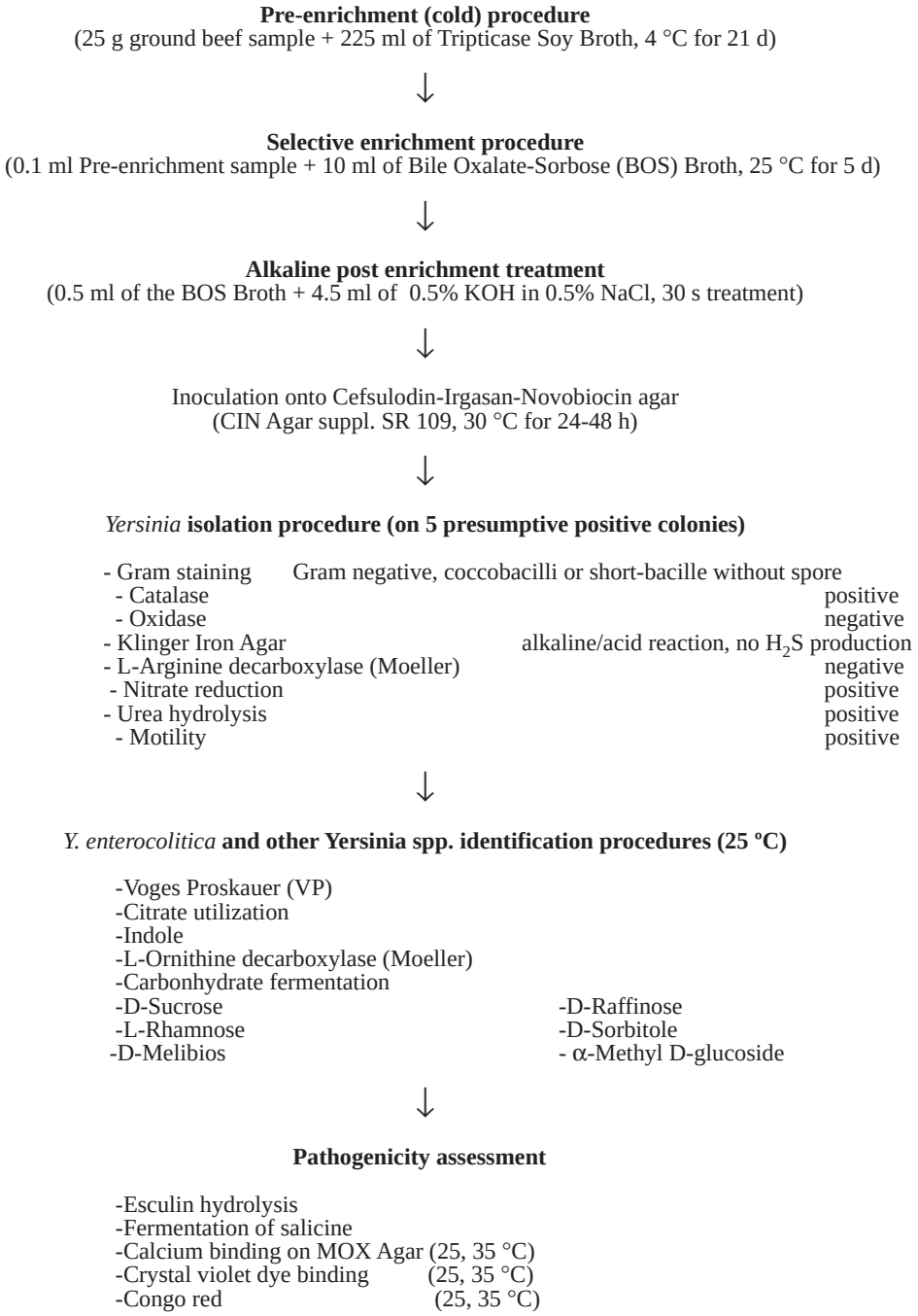

Figure. Isolation and Identification Procedure of *Y. enterocolitica* and other Yersinia spp. and Pathogenity Assessment

according to the Table for the identification of Yersinia species (34,37,38).

Finally, a pathogenicity assessment was carried out to detect the presence of virulence plasmid including esculine hydrolysis and fermentation of salicine (39), and other indirect but simple markers of pathogenicity including calcium binding on Magnesium Oxalate (MOX) Agar (at 25 °C and 35 °C) (3,23,40), crystal violet dye binding (at 25 °C and 35 °C) (26,41,42) and Congo red assays (at 35 °C and 25 °C) (26) were employed for this purpose (Figure).

Results

Yersinia spp. were recovered from 20 of 61 (32.8%) ground beef samples. *Y. enterocolitica* was the most frequently isolated Yersinia spp. It was found in 17 of 61 (27.9%) positive samples. The other 2 Yersinia spp. were *Y. intermedia* and *Y. frederiksenii* in 2 (3.3%) and 1 (1.6%) of 61 samples, respectively. All the positive cultures of *Y. enterocolitica* were also tested for markers of virulence. However, no *Y. enterocolitica* isolates were found to be presumptively virulent using esculine hydrolysis, fermentation of salicine, calcium binding, crystal violet dye binding or Congo red assays.

Discussion

Several studies have been conducted to isolate Yersinia spp. in ground beef and the isolation ratio was reported to be 9-99.2%. Among these studies, some generated lower isolation rates than the results of this study. For instance, Inoue and Kurose (43) and Leistner et al. (44) found that Yersinia spp. were recovered from 24 and 16% of samples, respectively. Hanna et al. (7) examined whole-sale cuts of vacuum-packaged fresh beef and reported that 10 out of 107 beef samples were positive for *Y. enterocolitica*. Similarly, Ibrahim and MacRae (45) examined 50 beef samples for Yersinia spp. and the isolation rate was 20%. There were 9 (18%) *Y. enterocolitica*, 2 (4%) *Y. frederiksenii*. The biotype of *Y. enterocolitica* was 1 A and serotypes O:5; O:7,13; O:4,33; O:7,8,19; O:7,13,19. Karib et al. (46) also reported that 4 out of 30 beef meat and 3 out of 20 ground beef samples were examined for Yersinia and *Y. enterocolitica* isolation rates were 13.3 and 15%, respectively. In another study by Falcao (47), 40 cultures of isolated ground beef and the distribution of Yersinia spp. were as follows: 9 *Y. enterocolitica*, 18 *Y. intermedia*, 3 *Y. frederiksenii* and 5 *Y. kristensenii* cultures, and 5 Yersinia NC. However, they reported that the virulence of the isolates was not determined.

Some researchers reported that even higher isolation ratios were detected compared to the results of this study. For instance, Warnken et al. (48) reported that Yersinia spp. were isolated in 4 out of 5 beef samples, whereas in ground beef 3 out of 5 samples were positive for Yersinia spp. Among the positive beef samples, 2 were identified as *Y. kristensenii*, 1 as *Y. frederiksenii* and 3 as *Y. intermedia*. In 6 Yesinia spp. isolates from ground beef, 2 were identified as *Y. enterocolitica*, 2 as *Y. kristensenii* and 2 as *Y. intermedia*. These researchers

Table. Biochemical differentiation of *Yersinia* spp. (34,37,38).

Test (25° C)	*Y. mollaretii*	*Y. bercovieri*	*Y . enterocolitica* Biogroups 1 through 4	Y. enterocolitica Biogroup 5	*Y. kristensenii*	*Y. frederiksenii*	*Y. intermedia*	*Y. aldovae*	*Y. rohdei* Biotype 1	*Y. rohdei* Biyotip 2
Voges Proskauer	-	-	V	+	-	+	+	+	-	-
Indol	-	-	V	-	V	+	+	-	-	-
Citrate	-	-	-	-	-	V	V	+	V	+
D-Sucrose	+	+	+	V	-	+	+	-	+	+
L-Rhamnose	-	-	-	-	-	+	+	+	-	-
D-Melibiose	-	-	-	-	-	-	+	-	+	-
α-Methyl-D-glucoside	-	-	-	-	-	-	+	-	-	-
D-Raffinose	-	-	-	-	-	-	+	-	+	-
D-Sorbitole	+	+	V	+	+	+	+	+	+	+
L-Ornithine decarboxylase	+	+	+	v	+	+	+	+	+	+

V: variable reaction, +: positive reaction, - : negative reaction

also reported that isolates were not pathogenic as in the present study. Although the results indicate a higher isolation ratio, the examination of a limited number of samples might have caused misinterpretation. It might also have been related to the efficiency of the detection method.

The differences between the findings of various authors and those of this study might be due to several factors such as isolation methods, number of analyzed samples, season, and geographical location. These factors may cause an increase or decrease in the prevalance of the Yersinia spp. For instance, the present study was carried out in Aydın province, where the climate is generally warm and humidity is high. It is known that the isolation ratio of *Y. enterocolitica* is higher in colder climates. In another example, Warnken et al. (48) studied only 5 ground beef samples and suggested it was too low a number of samples for a conclusion to be reached.

Numerous enrichment schemes have been described for the recovery of *Y. enterocolitica* from meat samples. These enrichment procedures include cold enrichment for up to a month, direct selective enrichment or 2-step pre-enrichment/selective enrichment procedures. It appears that some enrichment procedures are better for the recovery of pathogenic *Y. enterocolitica* than others, although recovery may be influenced by the type of meat product (49). For example, Jiang et al. (50) reported that the highest isolation rate was 32% for 4 °C/3-week enrichment, followed by 28% for 4 °C/2-week enrichment, 26% for 25 °C/24-h enrichment, 22% for 4 °C/1-week enrichment, and 10% for direct plating. Therefore, the isolation ratio may depend on the procedure. Even when using an enrichment and plating scheme to give good recovery for *Y. enterocolitica* from a particular meat product, considerable variations in recovery may be observed. Methods reported to provide improved recovery of pathogenic *Y. enterocolitica* in one part of the world may not work so well in another geographical area, possibly due to differences in levels of *Y. enterocolitica* and competing flora (49).

The recovery of pathogenic *Y. enterocolitica* is contingent upon a number of factors including the level of background flora on the product, the amount of background flora coming from enrichment and plating, the level of pathogenic *Y. enterocolitica* present in the sample, the numbers of non-pathogenic *Y. enterocolitica* and non-pathogenic Yersinia spp. present in the product, and the loss of virulence factors during enrichment and plating. Furthermore, a recovery method that gives good recovery of one particular serotype of pathogenic *Y. enterocolitica* may not suit another serotype (49).

Virulence in *Y. enterocolitica* is mediated by both chrosomal and plasmid-borne genes. Testing for markers of pathogenicity tests provides additional information. Markers are not perfectly correlated with pathogenicity but provide useful information under conditions where animal testing is undesirable or impractical. While chromosomal determinants are stable, plasmids containing virulence genes may be lost during culture and confirmational procedures. Temperatures above 30 °C are known to cause a loss of virulence plasmids in pathogenic *Y. enterocolitica*, but plasmid loss may also occur as a result of other less defined circumstances (49). However, in the absence of the antisera to serogroup *Y. enterocolitica* isolates in routine microbiology laboratories, these tests (esculine hydrolysis, fermentation of salicine, calcium binding, crystal violet dye binding and Congo red assays) have been shown to be reliable and easily performed for predicting the virulence of *Y. enterocolitica* isolates. These tests are indirect but simpler and quicker and an alternative to DNA hybrization or clinical assays, which are costly, complex and impratical for routine diagnostic use or for use in field laboratories (26,49).

The authors are not aware of any reports documenting foodborne illness associated with the consumption of ground beef products contaminated with *Y. enterocolitica*. In the present study, *Y. enterocolitica* was found in 27.9% of samples; but none of the *Y. enterocolitica* isolates were positive for virulence assays. This was in agreement with the results of several authors (43,46-48,51). However, Vishnubhatla et al. (52) reported that *Y. enterocolitica* was virulent in 30 of 50 ground beef samples. Because of the limited results of the present study, further investigations will be useful, like the one by Vishnubhatla et al. (52) which determines the extent to which virulent (plasmid-carrying) strains might be present in ground beef.

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