

1-1-2007

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ÖZBEY, GÖKBEN; KÖK, FİLİZ; and MUZ, ADİLE (2007) "Isolation of Salmonella spp. in Camel Sausages from Retail Markets in Aydın, Turkey, and Polymerase Chain Reaction (PCR) Confirmation," *Turkish Journal of Veterinary & Animal Sciences*: Vol. 31: No. 1, Article 11. Available at: <https://journals.tubitak.gov.tr/veterinary/vol31/iss1/11>

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Isolation of *Salmonella* spp. in Camel Sausages from Retail Markets in Aydın, Turkey, and Polymerase Chain Reaction (PCR) Confirmation

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Received: 27.09.2005

Abstract: A total of 100 camel sausage samples from different retail markets in Aydın, located in southwest Turkey, were examined for the presence of *Salmonella* spp. by culture method. After sausage samples were pre-enriched in buffered peptone water and incubated 18-20 h at 37 °C, they were transferred to Rappaport-Vassiliadis broth and Selenite broth, respectively, and incubated at 42 and 37 °C, respectively. After 24 and 48 h of incubation, one loopful from each of the enriched broths was streaked onto plates of Salmonella-Shigella agar and xylose lysine deoxycholate agar and incubated at 37 °C for 24 h.

A 572-bp fragment of the 16S rRNA gene was amplified using 16SF1 and 16SIII primers by polymerase chain reaction (PCR) for the identification of suspicious isolates. In the examination of the 100 camel sausage samples by culture and PCR, 7 (7%) were identified as positive for *Salmonella* spp.

This study demonstrated a potential risk of acquiring salmonellosis due to the consumption of camel sausage in Turkey and that PCR, as utilized in this study, may be successfully used for rapid detection of *Salmonella* spp.

Key Words: Camel sausage, culture, PCR, *Salmonella* spp.

Aydın'daki Marketlerden Elde Edilen Deve Sucuklarından *Salmonella* spp.'nin İzolasyonu ve Polimeraz Zincir Reaksiyonu (PZR) ile Teyit Edilmesi

Özet: Bu çalışmada, Aydın ilindeki farklı marketlerden temin edilen toplam 100 deve sucuğu örneğinde kültür yöntemi ile *Salmonella* türlerinin varlığı araştırıldı. Sucuk örnekleri tamponlanmış peptonlu suda 37 °C'de 18 saat ön zenginleştirme işlemini takiben Rappaport Vassiliadis broth'ta 42 °C'de 24-48 saat inkube edilerek selektif zenginleştirme işlemi gerçekleştirildi. Zenginleştirme sıvı besiyerlerinden öze ile Salmonella-Shigella ve xylose lysine deoxycholate agara ekim yapıldı ve 37 °C'de 24 saat inkubasyona bırakıldı.

Şüpheli izolatların identifikasyonu için 16S rRNA geninin 572 bp'lik fragmenti 16SF1 ve 16SIII primerleri kullanılarak polimeraz zincir reaksiyonu (PZR) ile amplifiye edildi. Kültür ve PZR yöntemi ile incelenen 100 deve sucuğu numunesinden 7 (% 7) *Salmonella* spp. identifiye edildi.

Sonuç olarak, bu çalışma Aydın'daki marketlerden temin edilen deve sucuklarında *Salmonella* spp. varlığını ve PZR yönteminin *Salmonella* türlerinin kısa sürede saptanması için kullanılabilirliğini gösterdi.

Anahtar Sözcükler: Deve sucuğu, kültür, PZR, *Salmonella* spp.

Fermented, dry sausages are commonly eaten in many countries (1). The Turkish style of fermented sausage is a very popular meat product in Turkey, and similar

products are also produced in most Middle Eastern countries and Europe (2). Most often a mixture of beef and water buffalo meat are used to make fermented

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sausages in Turkey; however, fermented sausages made from camel meat are also consumed in some parts of Turkey.

Camel meat is a good source of meat in areas where the climate adversely affects other animals (3). Camel meat is an important source of protein (4) and contains 19.6 g of protein per 100 g of the raw product (5). It is also less expensive than sausages made from other meat. Cutting into strips and allowing it to dry can preserve camel meat. It is then preserved by putting the dried strips in clarified butterfat (6). The pH of this product is 6.00 ± 0.25 and the water activity is 0.852 ± 0.02 (7).

Salmonella spp., which causes poisoning, has been demonstrated to be transmitted in sausages prepared without starter culture and with a short fermentation period. Their survival in such products has been reviewed by Gaier (8).

The detection and identification of *Salmonella* spp. is time consuming to the food industry (9). To detect *Salmonellae* more rapidly, an alternative method to the conventional culture method was evaluated using polymerase chain reaction (PCR). PCR has been demonstrated to be a very specific and sensitive method for the detection of *Salmonellae* (10-12).

The aim of this study was to detect the occurrence of *Salmonella* spp. in 100 random camel sausage samples obtained from different retail markets in Aydin, Turkey and to confirm the identification of *Salmonella* spp. by PCR.

Twenty-five grams of sausage samples was placed in a stomacher bag containing 225 ml of buffered peptone water (BPW, Oxoid, Basingstoke, UK) and was treated in a Stomacher (Interscience, 78860 St Nom, France) for 2 min, then incubated for 18-20 h at 37 °C. Then 0.1 and 1 ml from the pre-enriched culture were transferred to Rappaport-Vassiliadis broth (Oxoid) and Selenite broth, respectively (Difco Laboratories, Detroit, MI, USA), and incubated at 42 and 37 °C, respectively. After 24 and 48 h of incubation, one loopful from each of the enriched broths was streaked onto plates of *Salmonella*-Shigella (SS) agar (Difco) and xylose lysine deoxycholate (XLD) agar (Difco), and incubated at 37 °C for 24 h. The plates were examined for the presence of typical *Salmonellae* colonies, i.e. transparent colonies with black centers on SS agar and red colonies with black centers on XLD agar (13). Suspected colonies were confirmed by conventional biochemical methods (14,15).

We transferred 10^8 CFU/ml of suspicious *Salmonella* spp. culture into an Eppendorf tube containing 300 µl of distilled water and mixed it thoroughly by vortexing. Lysis was accomplished by the addition of 300 µl of TNES buffer (20 mM Tris (pH 8.0), 150 mM NaCl, 10 mM EDTA, 0.2% SDS) and 200 µg/ml Proteinase K. The lysis mixture was incubated at 37 °C for 2 h and then boiled for 30 min. The DNA released was purified by phenol chloroform isoamyl alcohol extraction. Then, it was precipitated by absolute ethanol and 0.3 M sodium acetate at -20 °C for 1 h. The mixture was then centrifuged at 11,600 xg for 10 min and the upper phase was discarded. The DNA pellet was washed with 300 µl of 90% and 70% ethanol, respectively, each step followed by 5-min centrifugation. The pellet was air-dried, dissolved in 50 µl of distilled water, and stored at -20 °C until use as a template DNA for PCR.

The PCR primers were 16SF1 (5'-TGTTGTGGTTAATAACCGCA-3') and 16SIII (5'-CACAAATCCATCTCTGGA-3') (Promega, Madison, WI, USA) (16). These primers specifically amplify a 572-bp DNA fragment of the 16S rRNA gene from *Salmonella* spp.

Reference *S. Enteritidis* strain (kindly provided by Dr. A.A. Mohamed Hatha, Department of Biology, The University of the South Pacific, Private Mail Bag, Suva, Fiji) was used in PCR tests as a positive control. Positive control was used to confirm the PCR test; however, it was not used during isolation of samples.

The reaction mixture was prepared in a total volume of 50 µl containing 5 µl of 10 × PCR buffer (10 mM Tris-HCl (pH 9.0), 50 mM KCl, 0.1% Triton®, X-100), 5 µl of 25 mM MgCl₂, 250 µM of each deoxynucleoside triphosphate, 2 U of Taq DNA polymerase (Fermentas, Lithuania), 10 pg of each primer, and 5-µl samples of extracted bacterial DNA. PCR involved 35 cycles of denaturation (94 °C, 1 min), primer annealing (58 °C, 1 min), and primer extension (72 °C, 1 min). The primer extension step (72 °C, 10 min) followed the final amplification cycle (17). A Touchdown thermocycler (Hybaid, Middlesex, UK) was used for all experiments. PCR reaction products (15 µl) were analyzed by electrophoretic separation on 1.5% agarose gel stained with ethidium bromide. The gel was visualized by UV illumination and photographed with Polaroid film. The suspected *Salmonellae* colonies were identified as *Salmonella* spp. by conventional procedures.

In the PCR reaction with 16SF1 and 16SIII primers of the 16S rRNA gene, a product of 572 bp was amplified. No amplification products were obtained from negative controls (Figure).

Dry fermented sausage was frequently suspected in a number of outbreaks of salmonellosis in East Netherlands (18). Since foods of animal origin are frequently implicated in human infection, sensitive and specific detection methods are necessary for routine screening of *Salmonella* spp. contamination, both for food control and regulation (19).

Conventional methods for the detection of *Salmonellae* require multiple subculture steps followed by biochemical and serological confirmation, which may take up to 5-7 days (20). In addition, the sensitivity of the method may be lower than DNA-based detection protocols. This might, in part, be due to the inability of cultures to detect the sub-lethally injured or viable, but non-culturable, cells in foods. The primary advantages of PCR tests include high sensitivity and less time required to process samples in the laboratory, as compared to standard culture methods (21). However, one major drawback in the development and validation of most PCR assays is that, in general, PCR assays are evaluated by using pure cultures or artificially contaminated material, and are not carried out under controlled experimental

conditions of the host in whom the pathogen would ultimately be detected (22). In addition, the PCR method can be inhibited or its sensitivity severely reduced because of the complex nature of the sample matrices, such as inner organs, lymphoid tissue, and flesh (23).

In the present study, the primers 16SF1 and 16SIII derived from the 16S rRNA gene were used and we found that all isolates of *Salmonellae* identified by culture method gave positive bands by PCR. For the negative control, no product was amplified. *Salmonella* spp. have been isolated from different sausages in many studies. A study was conducted on the bacteriological status of fresh and baked pork sausages produced in Ibadan, Nigeria. *Salmonellae* were present in 42% of the fresh pork sausage samples and in 56% of the baked samples (24). Another study showed that *Salmonellae* were detected in 16 (11%) of the samples, both from traditional and modern producers (14).

In a study covering 40 sausage producing plants in 1969, the incidence rate of *Salmonellae* was 28.6% among the 566 samples examined (25). Ten years later, the overall incidence had decreased to 12.4% among 603 examined samples.

In Greece, Abraham et al. (26) performed microbiological investigations of fresh sausage

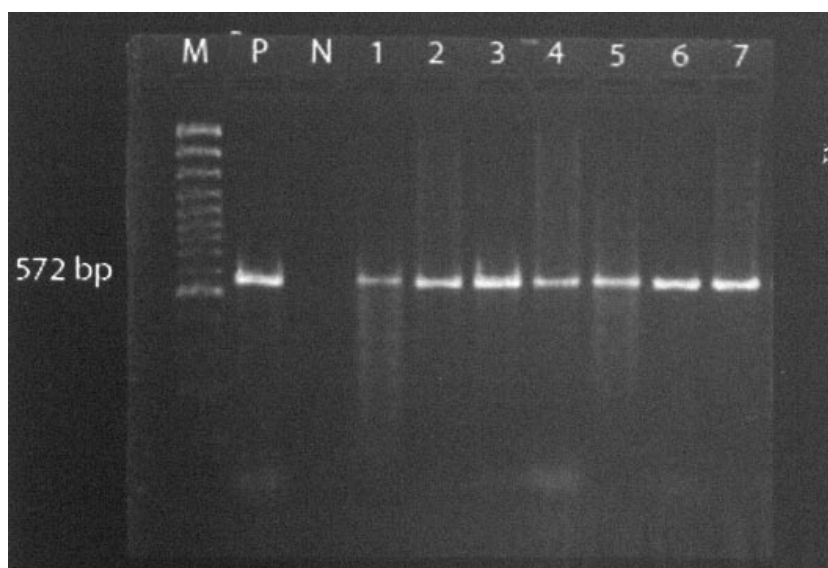


Figure. An agarose gel stained with ethidium bromide (0.5 µg/ml) with PCR products of *Salmonella* isolates (M: 100 bp DNA ladder, P: positive control, N: negative control, 1-7: *Salmonella* isolates).

(traditionally prepared) and they found *Salmonellae* in 20% of the samples studied (54% *S. Typhimurium* and 46% *S. Enteritidis*). Worcman-Barninka et al. (9) reported that *Salmonellae* were detected in 8 (22.8%) fresh pork sausage samples.

A study carried out by the PHLS Food Microbiology Research Unit in December 1996 and January 1997 found that 15 of 32 samples (47%) of low-priced sausages were contaminated with *Salmonellae* (27).

Mattick et al. (28) reported that *Salmonellae* were detected in 7.5% of frozen and 9.1% of refrigerated sausages (8.6% overall) they sampled. Durango et al. (29) showed that *Salmonellae* were isolated in 25% of sausages.

The present study found the presence of *Salmonella* spp. in camel sausages in Turkey for the first time. The study demonstrated a potential risk of acquiring salmonellosis due to the consumption of camel sausage in Turkey. It revealed the presence of *Salmonella* spp. in a significant proportion (7%) of samples of camel sausage on sale in different retail markets in Aydin province in southwest Turkey.

These studies highlight the need for improved strategies for food safety, in particular, appropriate hygienic precautions to avoid contamination during the manufacturing process and appropriate preservation techniques during storage and transport, all necessary to prevent the transmission of *Salmonella* spp. to consumers in Turkey and abroad. In addition, results of this study indicate that PCR may be successfully used as an alternative to conventional methods, both for identification and confirmation of *Salmonella* spp. detection. It was also indicated that PCR-based identification and *Salmonella* spp.-specific primers may give more rapid and sensitive results than conventional procedures. Further studies are required to improve control strategies for decreasing the prevalence of *Salmonella* spp. in camel sausages in Aydin.

Acknowledgment

We thank Dr. A. A. Mohamed Hatha, (Department of Biology, The University of the South Pacific, Private Mail Bag, Suva, Fiji) for supplying the *S. Enteritidis* strain.

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