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Research Article

Listeria monocytogenes, Yersinia enterocolitica and *Salmonella enteritidis* in Quail Eggs

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Abstract: The aim of this study was to determine the presence of *Listeria monocytogenes*, *Yersinia enterocolitica* and *Salmonella enteritidis* in 123 liquid whole quail eggs.

The method suggested by USDA-FSIS was used for the isolation and identification of *L. monocytogenes*. *S. enteritidis* was identified and sero-grouped by co-agglutination test and slide agglutination test. *Y. enterocolitica* was isolated in Trypticase-Soy Broth, with bile-oxalate-sorbose medium for enrichment. Both enrichment cultures were plated on Yersinia Selective Agar Base containing cefsulodin, irgasan and novobiocin (CIN Agar).

No Yersinia was detected among the natural flora of the quail eggs. Seven (5.69%) of 123 whole quail eggs were determined to have *S. enteritidis* and 5 of them (4.06%) were found to have *L. monocytogenes*. There were significant differences between the 3 groups of bacteria ($P < 0.05$).

Key Words: *Listeria monocytogenes*, *Yersinia enterocolitica*, *Salmonella enteritidis*, quail egg.

Bıldırcın Yumurtalarında *Listeria monocytogenes, Yersinia enterocolitica* ve *Salmonella enteritidis* Varlığı

Özet: Çalışmada 123 tane bıldırcın yumurtasında *Listeria monocytogenes*, *Yersinia enterocolitica* ve *Salmonella enteritidis* varlığı araştırılmıştır.

Listeria monocytogenes izolasyon ve identifikasyonunda USDA-FSIS tarafından önerilen metot uygulanırken, *Salmonella enteritidis* koaglütinasyon ve lam aglütinasyon testleri ile tanımlanmıştır. *Yersinia enterocolitica* varlığı Trypticase-Soy Broth, bile-oxalate-sorbose besi yerinde ön zenginleştirme ve cefsulodin, irgasan, novobiosin içeren Yersinia Selective Agar (CIN Agar)'da araştırılmıştır. Çalışma sonucunda 123 bıldırcın yumurtasında *Yersinia enterocolitica* tespit edilemezken, 7 örnekte (% 5,69) *Salmonella enteritidis*, 5 örnekte (% 4,06) *Listeria monocytogenes* tespit edilmiştir. Örneklerde gözlenen bakteriler arasındaki ilişki istatistiki açıdan farklı bulunmuştur ($P < 0,05$).

Anahtar Sözcükler: *Listeria monocytogenes*, *Yersinia enterocolitica*, *Salmonella enteritidis*, bıldırcın yumurtası

Introduction

The consumption of raw or slightly cooked eggs (in mousse, mayonnaise, beverages and other foods) makes it necessary to incorporate hygiene and disinfecting measures in the technology of fresh egg production. Several pathogenic organisms have been isolated from the surface of chicken egg shells, among them are *Listeria monocytogenes* (1), *Yersinia enterocolitica* (2), *Escherichia coli* O157:H7 (3) and *Campylobacter jejuni* (4). Both eggs and foods containing them can be important routes for *Salmonella enteritidis*, *S. typhimurium* and *S. heidelberg* (5-7). The ingestion of homemade mayonnaise prepared with fresh eggs contaminated with Salmonella sp. caused enteric disorders in a group of subjects in San Luis, Argentina (8), and transovaric contamination with Salmonella is also possible (9).

L. monocytogenes is a ubiquitous, Gram-positive, psychrotrophic, food-borne pathogen and is consequently of major concern in the food industry. Listeria spp. have been isolated from egg wash water and from the outer surface of the eggshell (10), and Listeria spp., including *L. monocytogenes*, have been isolated from raw liquid whole egg (LWE) (11). Moore and Madden (1) found that 72% of raw blended egg samples were positive for Listeria spp., of which 37.8% were identified as *L.*

monocytogenes. *L. monocytogenes* has been reported to survive in frozen liquid egg products and powdered egg products for up to 180 days (12). Such findings raise concern regarding the safety of commercial egg products.

Y. enterocolitica, an emergent food-borne pathogen widely distributed in nature, in animals, and in aquatic reservoirs, can produce enterocolitis, mesenteric lymphadenitis and therminal ileitis with or without pseudo-appendicitis symptoms or other extra intestinal manifestation (13). The presence of *Y. enterocolitica* in different foods has been demonstrated in our laboratory (14,15).

The aim of this study was to determine the presence of *L. monocytogenes*, *Y. enterocolitica* and *S. enteritidis* in liquid whole quail eggs.

Materials and Methods

Quail eggs: Between January and December 2000, 123 quail egg samples were obtained at random from local supermarkets in the city of Kahramanmaraş. The eggshell surfaces were disinfected with 70% alcohol and the residual alcohol was removed by flaming the shell. Eggshell membranes were aseptically removed with a peeling action from the inside of the shell.

Culture method for isolation and identification of *L. monocytogenes*: The liquid whole quail egg was transferred aseptically in University of Vermont Medium Modified Listeria Enrichment Broth (UVM, Difco 0223) and pre-enriched at 30 ºC for 20-24 h and then 0.1 ml of the pre-enriched culture was transferred to Fraser Broth (Difco 0219) at 35 ºC for 24-48 h. After selective enrichment, samples were streaked onto Modified Oxford Agar (MOX Agar, Difco) and incubated at 35 °C for 24-48 h. The plates were examined for the presence of Listeria colonies. Five of the suspected Listeria colonies, which were brown-greenish and surrounded by a black halo, were transferred to Trypticase Soy Agar supplemented with 0.6% yeast extract (TSA-YE, Difco) and incubated at 30 ºC for 24-48 h. The following characteristics were used to confirm isolates on MOX Agar as *L. monocytogenes*; presence of catalase, hemolysis on horse blood Columbia agar bilayer plates (Remel, Lenexa, KS, USA), fermentation of xylose and rhamnose, oxidase, and umbrella-shaped growth in motility in SIM Medium (Sulfur Reduction Test, Indole Production, Motility) (Oxoid, CM 435). Gram staining was also performed on the doubtful colonies (16). The method is illustrated in Figure 1.

Culture method for isolation and identification of *S. enteritidis*: Both the egg yolk and liquid egg of the quail eggs were pre-enriched in phosphate buffered saline (PBS) at 37 °C for 18-24 h and 1 ml of the pre-enriched culture was then transferred to 9 ml of Selenite Cystine (SC) broth at 35 ºC for 18-24 h. After selective enrichment, samples were streaked onto MacConkey Agar (Difco), which was used as the distinctive medium and incubated at 35 ºC for 18-24 h. Salmonella-Shigella Agar (SS Agar, Difco) was used as selective medium and incubated at 37 ºC for 18-24 h. The plates were examined for the presence of suspected Salmonella colonies. Typical Salmonella colonies were selected and incubated in Lassen's medium, glucose phosphate broth, KCN broth, nutrient gelatin Simmon's citrate agar, malonate broth and carbohydrate fermentation broth (17-19). Gram staining was performed on the doubtful colonies determined on MacConkey Agar to be colorless and translucent and on SS Agar to be translucent.

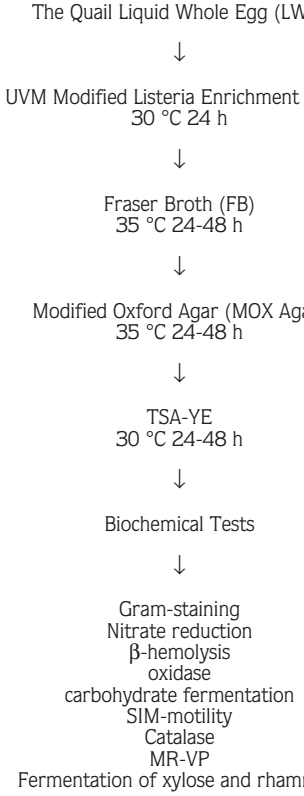

Figure 1. Isolation and identification of *L. monocytogenes*.

Pure cultures of Gram negative rods were streaked onto MacConkey Agar and were selected and serologically tested using Bacto-Salmonella O Antisera set A-I (Difco Lab. Detroit, MI, USA), and tested with polyvalent O and then Salmonella strains were sero-grouped by co-agglutination and slide agglutination tests. Slide agglutination tests with group antisera Salmonella B, C1, C2, D1 group antisera were also used. In addition, sero-types were fixed with type specific antisera (19). The method is shown in Figure 2.

Culture method for isolation and identification of *Y. enterocolitica*: Each of the samples were aseptically taken into Trypticase-Soy Broth (TSB) and incubated at 22 ºC for 24 h. One milliliter of this pre-enrichment culture was then added to 9 ml of bile-oxalate-sorbose (BOS) medium and incubated at 22 ºC for 5 d. A further sample of each was added to TSB and incubated at 4 ºC for 21 d. Both enrichment cultures were plated on Yersinia Selective Agar Base (Oxoid, CM 653) (Schieman) containing the Selective Supplement (SR 109, Oxoid) (CIN/Cefsulodin-Irgasan-Novobiocin). CIN Agar plates were incubated at 25 ºC for 24-48 h in an aerobic atmosphere. Organisms capable of fermenting mannitol produce a localized pH drop around the colony, which, followed by absorption of the neutral red, imparts a red color to the colony. Due to the localized pH drop, a zone of precipitated bile may also be present. Colonies of organisms that do not metabolize mannitol to acid end products remain colorless and translucent and colonies showing 'bull's-eye' morphology on this agar were removed for further testing. All isolates were screened using Kligler Iron Agar (KIA, Oxoid) and Christensen's Urea Media (Difco) in Andrade's Peptone Water (Oxoid) containing 1% (w/v) mannitol. Only those isolates that produced an acid butt and alkaline slope with no gas or hydrogen sulfide on KIA (incubated at 35 ºC for 24 h), were urea positive (incubated at 28 ºC for 48 h), and produced acid with very little or no gas from mannitol (incubated at 28 ºC for 48 h) were retained for additional tests such as Gram staining, catalase, ornithine decarboxylase, motility at 25 ºC and 37 ºC, oxidase, lysine decarboxylase, phenylalanine deaminase, arginine dihydrolase, and Simmon's citrate, and carbohydrate fermentation tests were also performed (20-22). The method is illustrated in Figure 3.

Statistical Analysis: The data were analyzed using the chi-square test. The statistical package SPSS Base 7.5 for Windows was used (SPSS Production Facility Release 7.5, SPSS Inc., 1995) (23).

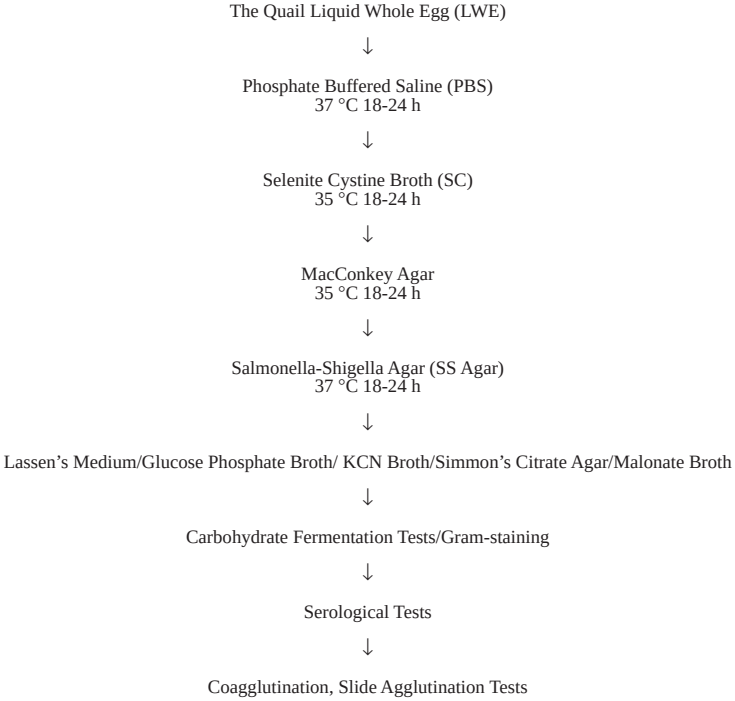

Figure 2. Isolation and identification of *S. enteritidis*.

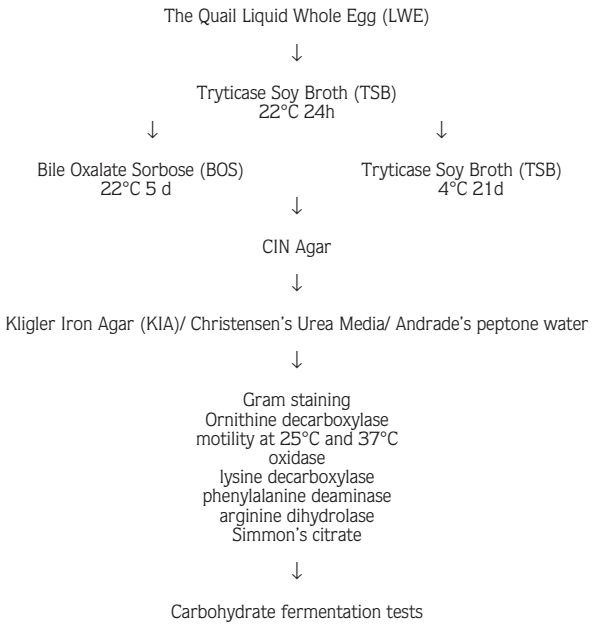

Figure 3. Isolation and identification of *Y. enterocolitica*.

Results

The method suggested by the United States Department of Agriculture Food Safety and Inspection Service (USDA-FSIS) was used for the isolation and identification of *L. monocytogenes*. *S. enteritidis* was identified and sero-grouped by co-agglutination tests and slide agglutination tests. *Y. enterocolitica* isolation was performed as illustrated in Figure 3. At the end of the study no strains of Yersinia were detected by the culture techniques employed. Seven (5.69%) of 123 liquid whole quail eggs were determined to have *S. enteritidis* and 5 of them (4.06%) were found to have *L. monocytogenes*. There were significant differences between the 3 groups of bacteria ($P < 0.05$).

The results are shown in the Table.

Discussion

Several recent publications have demonstrated the inability of current U.S. and Canadian pasteurization standards to provide a sufficient margin of safety for the destruction of *L. monocytogenes* in liquid egg products (24,25). Liquid egg products are used in a wide variety of foods that may or may not receive an additional heat treatment, and this could ultimately lead to contamination of the final product with *L. monocytogenes*. Looking at the seasonal distribution of contamination with *L. monocytogenes* and *S. enteritidis*, it was noted that there was no significant difference between months and that there was not an important relation from the statistical point of view ($P > 0.05$) (25). In this study 5 (4.06%) of 123 liquid whole quail eggs were determined to have *L. monocytogenes*.

Even though the original population of *S. enteritidis* in liquid egg seems to be low, there is potential for these populations to increase to levels capable of causing disease. Temperature abuse of the egg product can lead to higher numbers of organisms that may not be completely eliminated by current pasteurization protocols. *S. enteritidis* survived best in 5% NaCl + 5% sucrose egg yolk, followed by egg yolk + 10% NaCl, egg yolk and whole egg and, lastly, egg white. The increased resistance of *S. enteritidis* in salted egg yolk products has been documented previously by other scientists (24,26).

In this study no strains of Yersinia were detected by the culture techniques employed. Seven (5.69%) of 123 liquid whole quail eggs were determined to have *S. enteritidis*. In Argentina, Favier et al. (27) divided 128 fresh chicken eggs into 4 groups. From the non-inoculated/non-treated group (normal flora) one strain of *Y. enterocolitica* O:9 was isolated by surface enrichment and no Salmonella strains were detected. This difference may be caused by local factors.

Table. *L. monocytogenes*, *Y. enterocolitica* and *S. enteritidis* in Quail Eggs.

Product	Material no.	*L. monocytogenes* Positive sample a	*Y. enterocolitica* Positive sample b	*S. enteritidis* Positive sample a
Whole Quail Egg	123	5	-	7

$X^2 = 6.5$, $X^2_{2,0.05} = 5.99$, $X^2_{2,0.05} = 9.21$

From the results of the experiment it was established that care and cleanliness in food handling and preparation are important. This study confirmed that eggs are a significant reservoir of food-borne pathogens such as Salmonella and Listeria. New on-farm initiatives in food safety, such as the application of hazard analysis critical control point systems, will help reduce the risk of the food-borne transmission of pathogens. Eggs can be part of a healthy diet. However, they are perishable just like raw meat, poultry, and fish. To be safe, they must be properly refrigerated and cooked. Proper refrigeration, cooking, and handling should prevent most egg safety problems.

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