RESEARCH ARTICLE

Investigations on *Listeria* spp. in partridge (*Alectoris chukar*) meat

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Özet

Uçar G, Telli N, Tekinşen KK, Telli AE, Kahraman HA. Kınalı keklik (*Alectoris chukar*) etinde listeria türlerinin varlığının araştırılması. **Eurasian J Vet Sci, 2014, 30, 1, 20-24**

Amaç: Bu araştırmada, keklik etlerinde *Listeria* spp. ve patojen tür olan *Listeria monocytogenes*'in varlığı araştırılarak insidensinin ve dolayısıyla halk sağlığı bakımından öneminin ortaya konması amaçlandı.

Gereç ve Yöntem: Araştırmada, 10 erkek ve 10 dişi olmak üzere 20 adet keklik eti kullanıldı. Numuneler, keklik yetiştiriciliği yapılan özel bir çiftlikten temin edildi. Kanatlı kesiminin yapıldığı özel bir kesimhanede kesilip aseptik şartlarda soğuk koşullar altında laboratuara getirilip analize alındı. *Listeria* spp.'nin izolasyon ve identifikasyonunda Food and Drug Administration (FDA) tarafından bildirilen yöntem kullanıldı.

Bulgular: İncelenen 20 keklik numunesinden bir erkek ve bir dişi olmak üzere 2'sinin (%10) *Listeria* spp. ile kontamine olduğu tespit edildi. Kontamine iki numuneden izole edilen 10 izolatın 5'i (%50) *L. innocua*, 4'ü (%40) *L. grayi* ve 1'i (%10) *L. welshimeri* olarak identifiye edildi.

Öneri: Elde edilen bulgular, keklik etinin halk sağlığı açısından risk oluşturabileceğini ve bazı önlemlerin alınması gerektiğini ortaya koymaktadır. Bu amaçla, özellikle yetiştirme ve kesimhane işletmelerinin hijyen kalitesinin yükseltilmesi, çapraz bulaşmaların önlenmesi ve tüketim aşamasına kadar soğuk zincirin sağlanmasının önem arz ettiği kanaatine varılmıştır.

Anahtar kelimeler: *Listeria* spp.,keklik, halk sağlığı.

Abstract

Ucar G, Telli N, Tekinsen KK, Telli AE, Kahraman HA. Investigation on *Listeria* spp. in partridge (*Alectoris chukar*) meat. **Eurasian J Vet Sci, 2014, 30, 1, 20-24**

Aim: In this study, it was aimed that to determine the presence of *Listeria* spp. and especially *Listeria monocytogenes* in partridge meats for putting forth the importance on public health.

Materials and Methods: The survey included 10 male and 10 female partridge carcasses. Samples were obtained from a private farm which performs partridge breeding. After the cutting of partridges under aseptic conditions in a private slaughterhouse, samples were brought in to the laboratory and analyzed. Food and Drug Administration (FDA) method was used for the isolation and identification.

Results: Two of the (10%) samples analyzed were found to be contaminated with *Listeria* spp. 10 isolates collected from the contaminated samples. These samples identified as *L. innocua* (5, 50%), *L. grayi* (4, 40%) and *L. welshimeri* (1, 10%).

Conclusions: As a result, findings showed us partridge meat may be a public health risk and preventive measures should be taken. For this purpose hygienic conditions have to be proven on breeding and slaughtering enterprises, prevent cross- contamination and kept the cold chain until consumption.

Keywords: *Listeria* spp., partridge, public health.

Introduction

There are six species known to be in *Listeria* genera. These species are *L. monocytogenes*, *L. ivanovii*, *L. innocua*, *L. welshimeri*, *L. seeligeri* and *L. grayi* (Holt et al 1994, Hitckins 2003). Although *L. monocytogenes* is associated with listeriosis cases in humans, *L. ivanovii* and *L. seeligeri* were found to be sparsely. Serology divides *L. monocytogenes* into 13 serotypes on the basis of somatic and flagellar antigens. Eventhough serovar 4b predominates in most of cases, there appears to be an even distribution of serovars 1/2a, 1/2b (Hitckins 2003, Koçan ve Halkman 2006, Erol 2007).

L. monocytogenes is capable of growing and reproducing under aerobic, microaerobic and facultative anaerobic conditions. Though the microorganism can be grow at a large scale of pH conditions, pH 6.0-8.0 is optimum. It can grow up under large scale of temperature rates (-0.1-45°C) but their optimum growth temperature is 37 degrees celsius (Gailunas 2003, Rocourt and Buchrieser 2007, Wagner and Mclauchlin 2008). Soil, water, air, plant, feed, fertilizer, animal, human, sewage waste, processing equipment, ingredients, additives and packaging materials are considered as potential sources of *L. monocytogenes* (Banwart 1983, Erol 2007). Survival of *L. monocytogenes* is in soil for 2-6 months, in milk for 12 months, in sheep faeces for 3 months, in cattle faeces for 16 months and in some foodstuffs for 5-26 months (WHO/FAO 2004, Koçan and Halkman 2006, Sauders and Wiedmann 2007).

Human listeriosis is classified as invasive and noninvasive forms. Noninvasive listeriosis is a form of mild disease course of febril gastroenteritis. The invasive form of the disease in predisposed individuals, characterized by high mortality rates, and the incubation period is extended to 90 days (Painter ve Slutsker 2007). Certain factors in predisposed individuals to infection with *L. monocytogenes*, such as neonates, pregnancy, leukemia, Hodgkin's disease, diabetes mellitus, alcoholism or cirrhosis and immunosuppressive or cytostatic therapy. The main forms of invasive listeriosis and clinical findings are; acute septic form (new-born listeriosis), the central nervous system form (meningitis, encephalitis, encephalomyelitis), glandular form (lymphadenitis), the local form (cutaneous listeriosis, conjunctivitis) and chronic septic form (endocarditis, abscesses) (Erol 2007).

With the eye and skin contact directly, such as the risk of contamination from food, animals, insects, plants and soil can be transmitted to human beings. Consumption of contaminated foods is the most common way (Erol 2007). *L. monocytogenes* is common (ubiquiter) in nature but the presence of food increases the possibility of contamination (Uhitil et al 2004). It's revealed that *L. monocytogenes* is a food pathogen after epidemics in the United States (US) and Canada resulted with deaths in the 1980s (Samelis and Metaxopoulos 1999, Hibi et al 2006). Food-borne listeriosis is less common than salmonellosis, campylobacteriosis and other food-borne infections, but can lead to more serious consequences clinically (FDA 2003, Koçan and Halkman 2006). Among foods that cause infections, raw or pasteurized milk, ice cream,

Table 1.Some biochemical and other characteristics of isolates.

Isolates	β-hemoliz	Motility on microscope	CAMP (S. aureus)	CAMP (R. Equi)	Motility on SIM medium	Catalase	Oxsidase	Glucose	Esculine	Mannitol	Maltose	Xylose	Ramnose	MR-VP	NO_2	Type of Listeria
M1	-	+	-	-	+	+	-	+	+	+	+	-	-	+/+	-	*L. grayi*
M2	-	+	-	-	+	+	-	+	+	-	+	-	+	+/+	-	*L. innocua*
M3	-	+	-	-	+	+	-	+	+	-	+	-	+	+/+	-	*L. innocua*
M4	-	+	-	-	+	+	-	+	+	-	+	+	+	+/+	-	*L. welshimeri*
M5	-	+	-	-	+	+	-	+	+	-	+	-	+	+/+	-	*L. innocua*
F1	-	+	-	-	+	+	-	+	+	+	+	-	-	+/+	-	*L. grayi*
F2	-	+	-	-	+	+	-	+	+	-	+	-	+	+/+	-	*L. innocua*
F3	-	+	-	-	+	+	-	+	+	-	+	-	+	+/+	-	*L. innocua*
F4	-	+	-	-	+	+	-	+	+	+	+	-	-	+/+	-	*L. grayi*
F5	-	+	-	-	+	+	-	+	+	+	+	-	-	+/+	-	*L. grayi*

M: Male partridge, F: Female partridge, +/+ : positive/ positive reaction.

raw vegetables and fruits, fermented meat products, raw or cooked meat, poultry meat, raw or smoked fish, shellfish, fresh cheeses produced from milk heat-treated, soft cheeses, ready to eat foods, heat-treated ham, salami and a variety of sausages can be considered (Molla et al 2004, Berktaş et al 2006, Sergelidis and Abrahim 2009).

Although poultry listeriosis was first described in 1932 by TenBroeck, microorganism has been identified for the first time in poultry in 1935. After determination of listeriosis in poultry, chickens have been considered as a general host. Listeriosis is often seen in sporadic cases in domestic poultry (Ryser 2007). Wild and domestic birds may be asymptomatic carriers. It has been reported that *L. monocytogenes* was found in many poultry species' (eg, chickens, turkeys, pheasants, crows, ducks, partridges, eagles, parrots, canaries, pigeons, starlings, gulls) stool samples. This situation plays an important role in the contamination of the environment, and constitutes a source for cases of listeriosis in humans (Sauders and Wiedmann 2007, Wesley 2007). Skovgaard and Morgen (1988) and Kalender (2003) reported that the faeces of healthy chickens may be asymptomatic carriers so they carry microorganisms. Poultry often become infected by contaminated soil and faeces (Wesley 2007).

Cutting process and presentation for consumption of poultry consist of many stages (Efe 2005). *L. monocytogenes* contamination of poultry meat occurs during the cutting, hair removal, flaying the skin and other processing stages (Ryser 2007, Sauders and Wiedmann 2007). It has been reported that contamination at poultry meat processing stages is the primary cause of the listeriosis cases in humans (Sauders and Wiedmann 2007).

Partridge is often grown for the purpose of hunting and conservation of wild life. Partridges are alternative to chicken meat because of the delicious flavour so there is a tendency for intensive farming in recent years (Çetin and Kırıkçı 2000). Partridge is the common name of Alectoris and Perdrix birds genera in Phasinidae family (Özçelik 1995). Partridge breeds that present in Turkey are chukar partridge (A. chukar), rock partridge (A. graeca), freckle partridge (Perdix perdix) and sand partridge (Ammoperdix griseogularis) (Kiziroğlu 1983). However, the red-legged partridge is considered to be the partridge type adapted to commercial production with highest level (Çetin and Kırıkçı 2000).

High-protein and low-fat ratio of partridge meat foodstuffs are important in terms of nutrition and diet. However, some processes such as the cutting process, the pH value, redox potential and temperature as well as nutritional composition with partridge meats (Özek 2001) suitable for food contamination and makes a favorable environment for the growth of microorganisms (Erol 2007).

The works about determination of nutrition, maintenance and optimum environmental conditions in Turkey and encouraging intensive farming of partridge and this increasing production will enable the food-stuffs to be more economical. In this way, with the increased production of partridge foodstuffs, these products could become more economical. This will contribute to transform into a sector in partridge breeding (Woodard 1982 and Özek 2001).

In this study it's aimed that to determine the presence of *Listeria* spp. and especially *Listeria monocytogenes* in partridge meats for put forth the importance on public health.

Materials and Methods

The survey included 10 male and 10 female partridge carcasses. Samples were obtained from a private farm which perform partridge breeding. Food and Drug Administration (FDA) method (Hitckins 2003) was used for the isolation and identification. Samples were kept under cold chain at transport and storage stages and brought to the laboratory. 25 g of sample was taken aseptically into sterile pouches. 225 mL of *Listeria* Enrichment Broth was added and homogenized with homogenizer for two minutes. After homogenization of samples they were transferred to Pyrex bottles. Listeria Enrichment Broth enrichment was performed at 30°C for 4 hours. Listeria Enrichment Broth selective supplement was added for selective enrichment for 44 hours. Oxford agar was drawn after enrichment, and were incubated at 35°C for 48 hours under aerobic conditions. Five of the suspected colonies were selected which are black-green and 2-3 mm in diameter halo. Then the colonies were drawn to Tyriptic soy agar with yeast extract and incubated at 30°C for 24-48 hours. Colonies which grown in Tyriptic Soy Agar with yeast extract were analyzed in terms of whether typical Listeria colonies. For this purpose, Gram stain, catalase, oxidase and sulfate Indole Motility medium motion tests were applied. Hemolysis of blood agar, nitrate reduction test, MR-VP test, carbohydrate fermentation tests and Christie Atkins Munch Peterson (CAMP) test were used for the purpose of identification at species level.

Results

Listeria spp. was found to be positive in one male and one female partridge sample out of 20 samples totally. Five isolates from contaminated samples with Listeria were analyzed in order to determine the type of Listeria. 10 isolates studied, were identified as 5 (50%) *L. innocua*, 4 (40%) *L. grayi*i and 1 (10%) *L. welshimeri* (Table 1). When contamination rates were evaluated taking into account the sex discrimination, one of the male partridge sample was contaminated with (10%) Listeria spp. Isolates obtained from male partridge were identified as one of *L. grayi*, three of *L. innocua* and one

of *L. welshimeri*. One of the female partridge samples was contaminated with (10%) Listeria spp., while three isolates *L. grayi* and two of them were identified as *L. innocua*.

Discussion

In recent years, development of methods for isolation and identification of *L. monocytogenes* and results of epidemiological studies have shown us the importance of *L. monocytogenes* in foodborne infections and intoxications as well as Clostridium perfiringens, Salmonella spp. and *Staphylococcus aureus* (Şireli and Erol 1999). Every year in European Union countries and the United States approximately 1558 listeriosis cases were recorded and 450 people died according to the data of European Food Safety Authority (EFSA) (Roasto ve ark 2010).

According to the data of Center for Disease Control and Prevention (CDC) in 1999, *L. monocytogenes* is the second most important foodborne pathogen with 20% mortality rate (FDA 2003). However, it is reported that in 25 gr of ready-to-eat foods *L. monocytogenes* should not exist (Official Journal 2011). With respect to the findings of the study, it's concluded that the samples were in accordance with the criteria identified in the notification. In the studies that investigate the presence of *L. monocytogenes* in poultry meat observed that contamination levels were various. Fallah et al (2012), have found that raw, ready to cook and ready to eat poultry meat and poultry meat products were contaminated with Listeria spp (33.3%) in Iran markets. Researhers identified the isolates as 46.3% of *L. innocua*, 38.8% of *L. monocytogenes*, 9.7% of L. ivanovi and 5.2% of *L. seeligeri*. El- Malek et al (2010), have determined that 50 pieces of chicken meat and their products for sale were contaminated with Listeria spp. in markets in Egypt. When the findings of our study were compared with others, level of partridge meats' contamination rates with Listeria spp. were lower than Fallah et al (2005) and El-Malek et al (2010) 's chicken meat and products that detect Listeria spp. rates. The results obtained were similar to Fallah et al (2012)'s findings about the dominant species of Listeria. In this context, *L. innocua* was the dominant species in both studies. Barbalho et al (2005), have investigated the presence of Listeria spp. in carcass samples at various stages of the process line, the personels' hands and hand gloves in operation line and cooler water samples in an industrial poultry processing plant. The researchers have examined through the process of sampling line including 66 of carcasses, 37 of staff and 18 of water samples for 9 times for 121 samples totally. In the study, *L. innocua* was isolated from 90,3 % of water samples. And the contamination level of personnel hand and hand gloves with Listeria spp. were 46%. Contamination rates of samples taken from carcasses at the stages of shedding of blood, hair excoriation and flaying of skin were found to be 33,3 %. Contamination rates were relatively lower at the boiling stage (16.7%), increased at stage of flaying the inner skin (50%) and the highest level were obtained after packaging (76.2%). The researchers have suggested that the cause of the increase in the rate of contamination with Listeria spp., is due to the characteristics of Listeria species ubiquiter so they are widespread in the environment and not to take effective measures at process line from raw material to the final product.

It's thougt to be there is a relationship between cases of listeriosis in humans with lack of hygienic conditions in large capacity of breedinghouse and slaughterhouses. When ubiquiter and psychrotrophic characteristics of Listeria spp. are evaluated it's considered that there is a risk of partridge meat on public health.

Conclusions

Based on the preceding discussion, it was shown in practice how important it is to improve the hygienic conditions of slaughterhouses and intensive breedinghouses. It's thought that to prevent contamination via environment, to prevent cross contamination, to supply cold chain until consumption stage, to keep an eye on thawing times of frozen foods and to apply adequate heat treatment before consumption for keeping from emergence of listeriosis cases.

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