OCCURRENCE OF *LISTERIA SP.* IN RAW POULTRY MEAT AND POULTRY MEAT PRODUCTS

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Abstract

The aim of the study was to determine the occurrence of *Listeria* sp. in poultry meat and poultry meat products and estimate the prevalence of *L. monocytogenes* strains producing listeriolysin O. No *listeria* strain was detected in any of 50 examined heat-treated products. These bacteria were present in 43 out of 70 samples of raw poultry meat and 6 of *Listeria* strains were defined as *L. monocytogenes*. The presence of *hlyA* gene coding listeriolysin O production was found in all the isolated *L. monocytogenes* strains.

**Key words:** poultry meat, food contamination, *Listeria*, PCR.

Listeric infections caused by micro organisms of the genus *Listeria* occur worldwide and in variety of animals and man. It is 70 years since *Listeria monocytogenes* has been recognised as an animal pathogen (10). Just in recent time *L. monocytogenes* has been considered as a pathogen transmitted by food. Since the beginning of 80’s after several foodborne listeriosis outbreaks in Canada (coleslaw salad contaminated with listeria, 1981) and the United States (*listeriae* in soft cheese, 1985) WHO has decided to find this pathogen to be a severe threat of food and consumer’s safety (14). Both in humans and animals the majority of listeriosis cases are caused by the species *L. monocytogenes* whereas *L. ivanovii* is an occasional cause of abortion in sheep and cattle but rarely causing human infection (10). The identification of virulence determinants have shown that both *L. monocytogenes* and *L. ivanovii* have a group of genes which allow intracellular invasion and multiplication. The infection of the pathogenes is determined by specific proteins in their external membrane such as internalin A and B. Crucial to the virulence of *L. monocytogenes* is its ability to escape from macrophages by lysis of the phagosomal membrane and penetration into the cytoplasm. This process is mediated by the secretion of a haemolysin (listeriolysin O), which is one of 22 members of the cholesterol-dependent family of cytolsins secreted by Gram-positive bacteria and is coded by the *hlyA* gene (13).

Except for the major outbreaks, which involved hundreds of individuals, the incidence of listeriosis is difficult to determine. Estimates range from <2 to 12 per million of population. The Center for Disease Control has estimated 1700 cases per year in the USA. Listeriosis cases in Africa, Asia and South America are rarely reported (9). Recent studies have confirmed the presence of *L. monocytogenes* in a wide variety of foodstuffs. Milk (mainly unpasteurised), dairy products (especially soft ripened cheeses), poultry meat and products and raw vegetables are considered to be the most frequently contaminated with listeriae (2). Contamination of foodstuffs occurs mainly after cooking process as a result of cross-contamination. The presence of microorganisms in ready to eat food is a result of lack of hygiene because *L. monocytogenes* was noticed in slaughter animals and human faeces (5). It is estimated that ca. 5-9% of healthy people may be carriers of *Listeria monocytogenes* but among workers of slaughter-houses it can even amount 16%. The heating processes as cooking, pasteurisation should eliminate listeriae.

The goal of this study was to determine the occurrence of *Listeria* sp. in raw poultry meat and poultry meat products and estimate the presence of *L. monocytogenes* strains producing listeriolysin O.

**Material and Methods**

One hundred and forty three samples of poultry meat products from supermarkets in Wrocław were examined: 70 samples of raw poultry meat (chicken parts), 23 of poultry minced meat and 50 of poultry meat heat-treated products such as pate, sausages, etc.

Samples of poultry meat products and minced meat were examined according to PN-EN ISO 11290-1 method, using half-Fraser and Fraser Broth, Oxford and PALCAM agar as culture media.
The raw chicken part was treated as a sample. It was immersed in buffered peptone water and next incubated for 4 h at 37°C. After this time the part was taken away and the remaining buffered peptone water was incubated at the same temperature during next 16 h. Afterwards, 10 ml of this incubated medium was inserted into 90 ml of Fraser Broth (Merck), incubated for 48 h at 30°C and next the broth was cultured on Oxford and PALCAM Agar (BTL). After 48 h of incubation at 37°C the colonies morphologically resembling Listeria were submitted to confirmatory examinations using Gram staining, catalase test, evaluation of haemolysis type and motility at 25°C and 37°C. The strains expressing these standard features were tested for their biochemical activity using API Listeria test (BioMerieux) and API LAB Plus bacterial computer identification programme. Similar procedure of bacteria identification was applied for poultry meat products and minced meat. The isolated strains of listeria were also examined according to PCR technique.

Total DNA was isolated using a method described by Agersborg et al. (1). Sample preparation and DNA extraction procedures for polymerase chain reaction performed in order to identify L. monocytogenes strains were as follows. The bacteria from 1 ml of the overnight culture were centrifuged and the pellet was resuspended in 100 µl of distilled water, then 100 µl of 2% Triton X-100 was added. The contents were incubated at room temperature for 10 min and the tubes were boiled for next 10 min. Following incubation, the tubes were centrifuged for 5 min at 13,000 × g. DNA containing supernatant was used in PCR. The primer pairs described by Choi and Hong (4) flanking 636 bp region of listeriolysin O gene and 421 bp fragment of 16S rRNA gene were used. Enumeration of L. monocytogenes strains isolated from poultry meat was carried using competitive PCR. The PCR was performed in total volume of 25 µl. The reaction mix contained 50 mM KCl, 10 mM Tris-HCl, 1.5 mM MgCl₂, 0.2 mM of each dNTP, 30 pmole of each primer, 1 µl of DNA solution, and 1 U of recombinant Taq DNA Polymerase (Fermentas). There were performed thirty five cycles of 94°C for 45 s, 55°C for 45 s and 72°C for 45 s using a T3 thermal cycler (Biometra). A 10 µl aliquot of PCR product was resolved on 1.5% agarose gel (Prona).

**Results**

No listeria strains were found in all 50 examined samples of poultry meat heat-treated products. These bacteria were present in 36 samples of raw chicken parts (51.4%) and in 7 samples of poultry minced meat (30.4%). L. monocytogenes was also detected in these samples (5 and 1 sample, respectively) (Fig.1). The predominant species among the isolated Listeria strains was L. innocua which was demonstrated in 27 samples (63%). Moreover, L. welshimeri (7 samples - 16%), L. grayi (2 samples - 5%) and L. seeligeri (1 sample - 2%) were also recognised (Fig.2).
The presence of hlyA gene coding LLO production was found in all isolated *L. monocytogenes* strains (Fig. 3). In contrast, this gene was not detected in other *Listeria* sp. including *L. innocua*, *L. seeligeri*, *L. welshimeri* and *L. grayi*. Lanes 1-5 represent the PCR products obtained with primers for 636 bp fragment of hlyA gene. Lanes 6-10 represent the PCR products obtained with primers for 421 bp fragment of 16S rRNA gene. Lanes: 1 and 6 *L. innocua*, lanes: 2 and 7 *L. welshimeri*, lanes 3 and 8 *L. grayi*, lanes 4 and 9 *L. seeligeri*, lanes 5 and 10 *L. monocytogenes*. M -100 bp DNA ladder.

The examination of the isolated strains of *L. monocytogenes*, using the traditional microbiological method with API test and PCR technique, were similar except for 2 cases which gave different results in API method than in PCR technique. Obviously, PCR technique was more relevant and precise for microbiological diagnostics and its results were accepted.

**Discussion**

The lack of listeria isolation from poultry meat heat-treated products suggests that thermal treatment used in processing procedures was effective because listeriae are usually killed after heating at 67.5°C for 60-70 s.

The investigations of poultry meat confirmed the results obtained by other authors. Similar percentage (60%) of contamination of chicken raw meat with listeriae reported Kwiatek (8). But on the other hand, in our study there was reported the higher percentage of isolation of *L. innocua* than *L. monocytogenes* – 63% and 14% of all isolated strains, respectively. The considerably higher level of contamination of poultry raw meat from supermarkets in Spain was reported by Capita *et al.* (3). Listeriae were found in as much as 95% of examined carcasses and 32% of them were recognised as *L. monocytogenes* and 66% as *L. innocua*. Vitas *et al.* (16) reported 36.1% positive samples of raw poultry in their survey carried out in Northern Spain.
The surveys of chicken carcasses and poultry raw meat from shops in Finland proved that L. monocytogenes was present in 62% of examined samples (11). Similar researches in Belgium reported that 38.2% of raw chicken carcasses were contaminated with this pathogen (15). In the investigations of poultry meat in Nordic countries it was demonstrated that L. monocytogenes was present in 22.2% of examined samples but the L. innocua was the dominated bacterium (6).

In the official surveillance of raw minced poultry meat in Japan there was indicated the presence of L. monocytogenes in 37% of examined samples (7). A polymerase chain reaction assay targeting the gene encoding listeriolysin O is one of the very specific methods of the detection of L. monocytogenes. This gene and its product: thiol-activated, cholesterol-binding cytolysin listeriolysin O was found only in L. monocytogenes strains. But there are also other cholesterol-dependent cytolysins which are specific for other Listeria strains, for example L. ivanovii produces ivanolysin, L. seeligeri seeligerolysin. These cytolysins are coded by different genes (12).

The high percentage of the isolation of Listeria sp. from raw poultry meat is a real threat for consumer’s health, thus the possibility of contamination of poultry meat products should be taken into consideration during its processing and control procedures introduced with HACCP system implementation.

References