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## Isolation and detection of *Listeria monocytogenes* in poultry meat by standard culture methods and PCR

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**Abstract.** *Listeria* is the genus of a bacteria found in soil and water and some animals, including poultry and cattle. It can be present in raw milk and food made from raw milk. It can also live in food processing plants and contaminate a variety of processed meats. Microscopically, *Listeria* species appear as small, Gram-positive rods, which are sometimes arranged in short chains. In direct smears, they can be coccoid, so they can be mistaken for streptococci. Longer cells can resemble corynebacteria. Flagella are produced at room temperature but not at 37°C. Haemolytic activity on blood agar has been used as a marker to distinguish *Listeria monocytogenes* among other *Listeria* species, but it is not an absolutely definitive criterion. Further biochemical characterization is necessary to distinguish between the different *Listeria* species. The objective of this study was to detect, isolate and identify *Listeria monocytogenes* from poultry meat. Within a period of six months from January to June 2017, a total of 15 samples were collected. Three samples were positive for the presence of *Listeria monocytogenes*. Biochemical and microbiological tests as well as PCR technique using specific primers were used to confirm *L. Monocytogenes* in the samples.

### 1. Introduction

*Listeria monocytogenes* is recognized as an important foodborne pathogen in many industrialized countries. The consumption of food contaminated by *L. Monocytogenes* has been identified as the main transmission route for this pathogen in both humans and animals. In humans, listeriosis is a rare but serious illness that can lead to abortion or serious cases of meningitis or encephalitis, and even death [1]. Cases are observed especially in vulnerable and immunocompromised humans such as newborn infants, pregnant women, cancer or AIDS patients and the elderly. Because of the high fatality rate (20–30%), listeriosis ranks among the most frequent causes of death due to foodborne illnesses [2,3]. Since 2006, increasing numbers of listeriosis cases have been observed in several European Union countries, including France, predominantly in people of more than 60 years of age [4,5]. *L. Monocytogenes* is widely disseminated in the environment (soil, surface water, plants, and infected animals). The ubiquitous character of the pathogen inevitably results in the contamination of numerous food products (such as milk and dairy products, raw vegetables, meat and meat products and seafood). Poultry, poultry products, eggs and egg products have rarely been reported to be involved in *L. Monocytogenes* outbreaks [6]. In most studies, the contamination of poultry meat occurs during the



slaughtering and processing phases [7,8,9,10,11]. Very few studies have reported an incidence of *L. monocytogenes* at the farm level [12].

## 2. Materials and Methods

Isolation of *L. monocytogenes* was performed according to the ISO standard method [13]. A food sample of 25 g was added to 225 ml of half strength Fraser broth which is used as primary enrichment media, to obtain a 1:10 sample dilution. All samples were homogenized 30- 60 seconds and incubated at 30°C for 24 h  $\pm$  2 h. Pre-enrichment media may contain selective agents to inhibit the growth of competing microorganisms, but usually at lower concentrations than those used in selective enrichments.

From this primary enrichment, 0.1 ml was inoculated into 10 mL of Fraser Broth which is secondary enrichment medium, and incubated for 48 h at 37°C. A loopful of the Fraser Broth enrichment culture was streaked on the surface of different selective agar, ALOA agar and second Palcam agar, and incubated in an incubator at 37 or 35°C for 48  $\pm$  2 h. The selective enrichment culture is inoculated on to two selective agar media and incubated at 37 or 35°C for 48  $\pm$  2 h. Selective agar were observed for suspected colonies at 24 to 48 h of incubation. Characteristic colonies were purified on TSYEA for identification.

Two individual colonies of each suspect isolate were suspended in 50  $\mu$ l of DNA/RNA free water and heated at 95°C for 5 minutes. To demonstrate the *L. monocytogenes* genome, a commercial kit (TopTaq Master Mix Kit, Qiagen®, Germany) and primers (Lip1 gatacagaaacatcggttgcc and Lip2 gtgtaacttgatgccatcagg) and the thermal protocol described by Jofre *et al.*, [14] were used. 10  $\mu$ l of the PCR product was analysed by agar gel electrophoresis in 2% gel, with addition of ethidium bromide and visualized in UV transilluminator.

## 3. Results and Discussion

From a total of 15 poultry meat samples that were tested, 3 were positive for presence of *L. monocytogenes* and all 3 isolates were confirmed by PCR. Pure cultures were isolated from all three suspect poultry meat samples, and were shown by PCR to contain the marker for the *L. monocytogenes* genome (figure 1).



**Figure 1.** Agarose gel electrophoresis. M - molecular marker (100 bp), 1. *L. monocytogenes* positive sample (274 bp), 2. positive control, 3. negative control.

*Listeria* is not particularly resistant to disinfectants. In order to obtain optimal disinfection, it is thus necessary to respect cleaning and disinfection procedures. Moreover, the ability of *L. monocytogenes* to develop biofilms on a variety of surfaces makes disinfection treatment difficult [15].

The best way to avoid contamination would be to prevent biofilm formation in food industries by frequently disinfecting and cleaning surfaces [16]. This procedure should be sufficient to remove cells not yet strongly adhered, although any such cleaning can fail and not remove mature biofilms. To fulfil this purpose, the use of appropriate disinfectants is essential, and other strategies have been tested with this aim. Some examples are the use of ozone or acidic water, usually considered eco-friendly biocides, as they do not leave chemical residuals [17]. Besides these products, natural compounds extracted from bacteria or aromatic plants cultures and some GRAS (generally recognized as safe) ingredients have also been evaluated to eradicate biofilms [18,19].

#### 4. Conclusion

*L. monocytogenes* is considered as a highly pathogenic bacterium that, world-wide, contaminates a wide range of food products, and has a high mortality rate in infected patients. Our investigation showed *L. monocytogenes* was present in Serbian poultry meat. The hygienic status of the slaughterhouse and sanitary practices observed at the farm could be relevant for the *Listeria* status of poultry meat, although this data is not reported here. These factors have already been reported in studies related to *Salmonella* and *Campylobacter* in poultry flocks, but this is the first time this idea is presented for *L. monocytogenes* in Serbia.

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