

GOATS – SOURCE OF *LISTERIA MONOCYTOGENES****Snežana Ivanović, Oliver Radanović, Ivan Pavlović, Jadranka Žutić***Veterinary Institute of Serbia, Belgrade, Republic of Serbia*
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Listeriosis is the infectious disease of cattle, sheep, goats, pigs, dogs, cats ... and humans. In goats, the disease is manifested in the form of abortion, encephalitis and septicemia. The cause is *Listeria monocytogenes*, widespread in the nature and a relatively resistant microorganism. *Listeria* comes in the environment by excretes of sick humans and animals as well as of clinically healthy organisms. This microorganism can be found in waste and water, soil, plants, feedstuffs, walls and floors of the buildings where animals live, various kinds of foods of plant and animal origin, as well as in animals and people. After infection, sometimes there are no clinical symptoms and goats come to the slaughterhouse as clinically healthy animals (latent infection). When the goats come to the slaughterhouse, it is not necessary the transmission of the cause to meat, but it can be transferred to the equipment, walls, floors, aprons and boots. In our study, twenty clinically healthy goats, slaughtered in the slaughterhouse, were tested – one hyperemic uterus and meat of all goats. Floor swabs (from nine different locations) and swabs from the carriage bottom (20 swabs) in which intestines and uterus were placed were taken three days after slaughter. Uterus and carriage swabs were tested according to the method RES, chapter 2.10.14.B.1. and meat by ISO 11290 / 1:1996 (E). From the uterus, which was slightly hyperemic *Listeria monocytogenes* was isolated from slightly hyperemic uterus and from five samples of floor and three samples of the carriages. It was not isolated from meat samples. These results indicate the possibility of indirect transmission of this bacterium to meat.

Key words: goat; *Listeria monocytogenes*; meat; disease; humans

КОЗИ – ИЗВОР НА *LISTERIA MONOCYTOGENES*

Листерииозата е инфективно заболување кај говедата, овците, козите, свињите, мачките... и луѓето. Кај козите болеста се манифестира во форма на абортус, енцефалитис и септикемија. Причинител е *Listeria monocytogenes*, широко распространет во природата и релативно отпорен микроорганизам. Листериија доаѓа во животната средина преку екскрети од болни луѓе и животни, но и од клинички здрави организми. Овој микроорганизам може да се најде во отпад и во вода, почва, растенија, хранителни сировини, сидовите и подовите на објектите каде живеат животни, во различни видови на храна од растително и животинско потекло, како кај животните и кај луѓето. По инфекцијата понекогаш не се манифестираат клинички симптоми и козите доаѓаат во клиницата како клинички здрави животни (латентна инфекција). При пристигнувањето на козите во клиницата не доаѓа секогаш до пренесување на причинителот во месото, но може да се пренесе на опремата, сидовите, подовите, кецелите и чизмите. Во рамките на нашата студија беа тестирани дваесет клинички здрави кози заклани во клиница – еден хиперемичен утерус и месо од сите кози. Брисевите од подот (од девет различни локации) и брисевите од дното на транспортните камиони (20 брисеви) во кои беа сместени цревата и утерусот беа земени три дена по колењето. Брисевите од утерусот и транспортниот камион беа тестирани според методот RES, оддел 2.10.14.B.1, и месото според ISO 11290/1 : 1996 (E). Од утерусот, кој беше слабо хиперемичен, беше изолирана *Listeria monocytogenes*. Овој микроорганизам беше изолиран од слабо хиперемичен утерус и од пет мостри од подот и три мостри од транспортниот камион. Не беше изолиран од примерците од месо. Овие резултати укажуваат на можноста за индиректна трасмисија на оваа бактерија во месото.

Клучни зборови: коза; *Listeria monocytogenes*; месо; болест; луѓе

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INTRODUCTION

Listeriosis is an acute infectious disease of various domestic and wild animals and humans. Clinically it is manifested as meningoencephalitis or septicemia with localization in different organs. In females the most frequent localization is in uterus. There is often seasonal appearance of this disease in the period from December to May, probably as a consequence of changes in the external temperature.

The cause of disease is *Listeria monocytogenes*. As an ubiquitous microorganism it is widely distributed in the nature. It is isolated from 42 kinds of domestic and wild mammals, 22 species of birds, fish, crabs and insects. Also, this cause is identified in the samples of meconium, faeces, waste water, water, silage and other feed-stuffs, milk, cheese and meat. *Listeria* comes in the environment by excreted of sick humans and animals as well as of clinically healthy organisms. Some researches indicate that 1 to 10% of healthy people and 11 to 52% of healthy animals can have *L. monocytogenes* in intestines. Researches also showed that the presence of pathogens on animal carcasses is linked with fecal contamination before and during slaughter (Bunčić, 1991).

As the main source of *Listeria monocytogenes* is signed stored feed – silage (Fenlon, 1985; Wiedmann, 1997). After per os infection, intestinal mucosa is a common way for the spread of *Listeria monocytogenes* in the whole organism.

Listeria monocytogenes is a small, motile, non-sporulating Gram-positive bacillar to coccobacillar bacterium. It is a high resistant microorganism. The optimal growth temperature is 30–37 °C, but it can actually grow at a temperature of 4–44 °C (Holt et al., 1994). Infection in animal occurs mostly in the sub clinical form, but sporadically it can have epidemic character. Incubation may be short, only one day and for the encephalitis form for 2–3 weeks. Disease lasts 1–4 days in goats and sheep (Roberts, 2003), however in cattle it can be longer.

Clinical symptoms of listeriosis in animals are encephalitis, septicemia, and abortion, particularly in sheep, goats and cows. The septicemic form is relatively rare and it usually occurs in young animals. In this form there are conspicuous depression, inappetence, fever and death. The encephalitis form is sometimes described as "moving in a circle" – turning in one direction, and it is more common in ruminants. This form of disease

is characterized by depression, anorexia, animals are leaning on the wall or turned by face to the wall. There is also unilateral facial paralysis. Abortion is almost regular (after 7 months of pregnancy in cows and sheep at 12 weeks) (Hird, 1990; Walker, 1993). In one flock, there is usually only one clinical form of listeriosis. In addition to the above mentioned symptoms, it is described ophthalmitis in cattle and sheep (Walker, 1993, 1993a). Mastitis *L. monocytogenes* in ruminants rarely occurs, while the gastro-intestinal infections may occur sometimes in sheep (Clark, 2004). In pigs, the primary is septicemia, and encephalitis and abortion rarely occur. Listeriosis in birds can be the result of the secondary infection as well as various forms of inadequate condition or, for example, the appearance of listeriosis after salmonella infection (Wesley, 1999).

Although *Listeria monocytogenes* is a pathogen that occurs in animals, described a lot of years ago, Schlech et al. (1983) described that this pathogen occurs in people as well. Humans are mostly infected by contaminated food by *L. monocytogenes*, and it is most often transmitted by cheese, especially if it is made from unpasteurized milk, inadequate cooked and roasted meat, brain, pig tongue in gel and boiled sausages. Food in the fridge, which is not treated by heat before consumption can be the risk of the development of infection. This microorganism can survive at the fridge temperature and can be multiplied as well (Schlech 1983).

Listeria monocytogenes causes an invasive and non-invasive form of infection. According to the literature data, invasive listeriosis is rare and occurs once in 2–9 millions of years. It is one of the strong infections transmitted by food that is in 30% cases lethal. Listeriosis is a characteristic phenomenon in immunocompromised persons (cancer, alcoholics, diabetes, HIV positive, the elderly, pregnant women and infants) (Rocourt, 1997; Swami-Nathan, 2001). As a consequence of infection CNS disease, septicemia, abortion, infection of infants may occur. Incubation in invasive listeriosis is different from one day to several weeks. *L. monocytogenes* can also cause contact dermatitis, which is described in the veterinarians and farmers.

Humans can carry *Listeria monocytogenes* in the intestines, without clinical symptoms. Despite intensive research on *L. monocytogenes* and listeriosis, minimum infectious dose that leads to diseases of people and indicative as individual for each person is not yet known (Autio, 2003).

Different countries have adopted different measures of precautions for the presence of *L. monocytogenes* in food. Thus, because of the frequency of epidemics and of specific characteristics of the pathogen the U.S. and New Zealand have defined so-called "zero tolerance" for the presence of *L. monocytogenes* in ready to eat food. Food is considered as infected if the sample of 25 g has *L. monocytogenes*.

The EU supports this normative of risk assessment for fresh cheese and pasteurized milk (must not be found in the 25 g of a sample), as well as for other milk products (absence in 1 g), in Australia for the majority of food ready to eat. Italy, mainly uses the principle of "zero tolerance" to assess the validity of food hygiene for *L. monocytogenes* (Vesković, 2005).

MATERIAL AND METHODS

In our study we used twenty clinically healthy goats, slaughtered in the slaughterhouse, for examination swab of mucosa and tissue of uterus of one goat, that was lightly edemic and hyperemic, and meat of all goats. After three days of slaughter, we took floor swabs (from ten different places) and the bottom of the carriages for intestines (20 swabs). Swabs were transferred directly to the surface of solid medium: agar with 10% sheep's blood, Columbia agar (HiMedia) and MacConkey agar (Biomedics) and they are incubated under aerobic conditions at a temperature of 37°C for 24 hours. For enrichment, a sample of uterine tissue was homogenized in 10% suspension of brain-heart broth (BHI, Biomedics). Suspension was refrigerated at the temperature of +4 ° C to +8 ° C (cold enrichment). The third day, suspension was transferred to the blood agar, which was incubated at the same conditions.

For the examination of biochemical activities (oxidase, VP, urea, indol, glucose, salicin, esculin, manitol, nitrate reduction, ramnose and xylose) commercial tests (HiMedia) were used. We also did the catalase test (catalase color ID, bioMerieux), mobility of culture in the 25 ° C (API M Medium, bioMerieux) and the CAMP test.

For the purpose of checking identification BBL Crystal G / P ID kit (Becton Dickinson) was applied. 25 g of meat samples were placed in 225 ml of UVM-1 broth (primary selective enrichment), homogenized and incubated for 20 to 24 hours at the temperature of 30 °C. By 0.1 ml of

the incubated broth it was shifted in the Fraser broth (selective secondary enrichment) by the addition of 0.1 ml of the appropriate additives (ferriammoniumcitrat and acriflavine) and incubated for 24 to 48 hours at the temperature of 37 °C. As there was no broth change, the medium was considered as negative and further investigations were not carried out.

RESULTS AND DISCUSSION

In our investigations, from the uterus which was slightly hiperemic we isolated *Listeria monocytogenes*.

Grown colonies on the blood agar were small, identical in shape, surrounded by a narrow zone β hemolysis (Fig. 1). On Columbia agar has also pure culture in small, transparent, grayish colonies (Fig. 2).

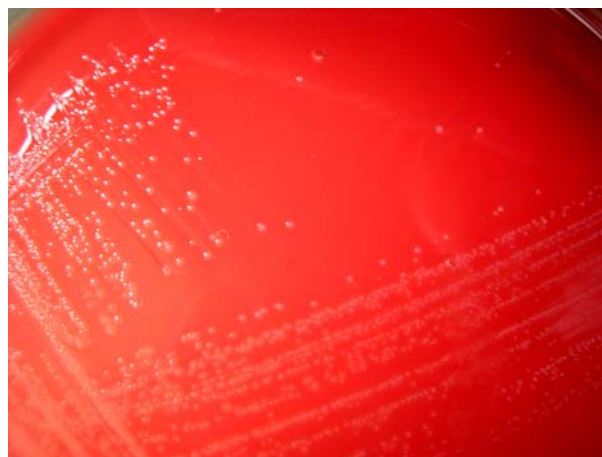


Figure 1. *Listeria monocytogenes* – colonies on sheep blood agar, surrounded by a zone of β -haemolysis

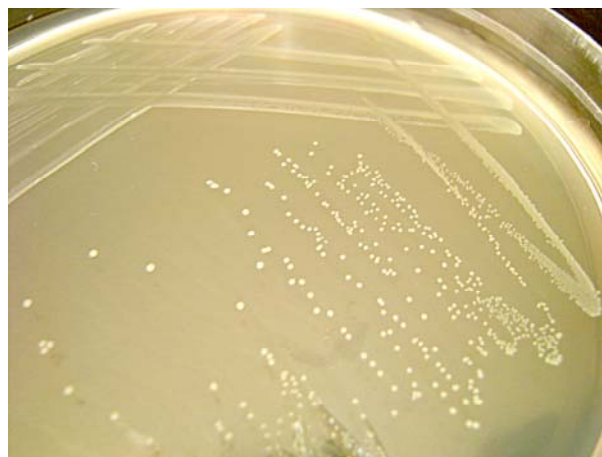


Figure 2. *Listeria monocytogenes* – colonies on Columbia agar

In microscopic preparations done from the culture, the Gram-positive individual, less coccobacillar forms in short chains or short sticks, which have their position up similar forms in letters V and L, were established (Fig. 3). The same cultural and microscopic characteristics were established in the culture isolated from the brain-heart broth.

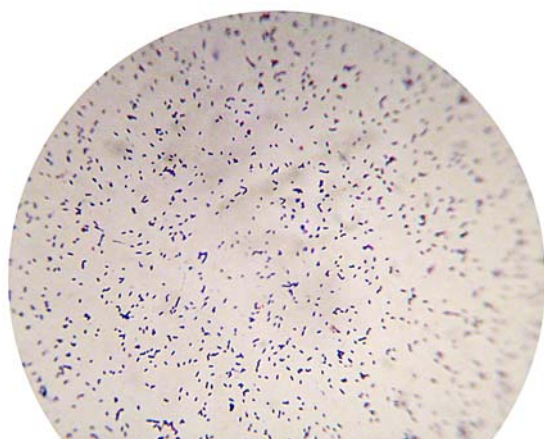


Figure 3. *Listeria monocytogenes* in a Gram-stained from a culture (Gram stain $\times 1000$)

Table 1 presents the results of the investigation. Grown colonies showed hemolysis on the blood agar, positive catalase and negative oxidase reaction. The culture was movable on the 25°C. Reactions to the VP, polychrome, glucose, salicin, rhamnase were positive with urea, indol, manitol, xylose negative. The culture did not cause reduction of nitrate. The positive CAMP test was established with *Staphylococcus aureus* and negative with *Rhodococcus equi*.

Table 1

Cultural-physiological and biochemical properties of isolated culture Listeria monocytogenes

Biochemical properties	Reaction	Sacharide	Reaction	Sacharide	Reaction
Hemolysis	+	Nitrate reduction	-	Raffinose	-
Mobility at 25°C	+	VP	+	Salicin	+
Catalase	+	Urease	-	Manitol	-
Oxidase	-	Indol	-	Rhamnase	+
CAMP (with <i>S. aureus</i>)	+	Esculin	+	Xilose	-
CAMP (with <i>R. equi</i>)	-	Glukose	+	Nitrate	-

On the basis of the results of the analysis, the culture was identified as *Listeria monocytogenes*,

which is in accordance with its properties described by Quinn (2002) and Bergey's (Garrity, 2005). The identification was confirmed by BBL Crystal ID kit GP. In all twenty meat samples the presence of pathogens was not determined, but it is isolated from the 5 floor swabs and 3 carriage swabs.

It is said that many animal species are susceptible to *Listeria*, but after infection it is not necessary to develop the symptoms and mainly these animals come to the slaughterhouse as healthy animals (latent infection).

When animals come to the slaughterhouse, they do not obligatory transmit the cause to the meat, but it can be transmitted to the equipment, walls, floors, aprons and boots. There are many equipment in the slaughterhouse such as conveyors, machines for refrigeration and freezing, machines items for the removing of skin, equipment for cutting, machines for filling and packaging. All such equipment is complex, with slit and accessible places for impurities but inaccessible for washing, cleaning and disinfection. Therefore, it is not surprising if *L. monocytogenes* is found in the equipment that has been washed and disinfected and later contaminates meat and meat products. Some strains of *L. monocytogenes* are resistant to the equipment and can survive several months or years (Rij, 2003).

The results of our examination are in accordance with the information in available literature, and indicate the possibility of indirect transmission of *Listeria monocytogenes* to meat. The environment in which *Listeria monocytogenes* was established, deduction recalls the fact that this bacterium is able to form biofilm. Besides the characteristics of substrates, such as roughness, the presence of conditional layer, etc., they are important factors for the initial binding of bacteria. Also, the connection between bacteria and substrates is stronger during the time, making the process irreversible. It is well known the advantage of life in the biofilm, in terms of increased resistance of bacteria, not only to the antibiotics but also to disinfectants and detergents. While some biocids are 100% effective in the destruction of suspended cells, their efficiency is reduced drastically, or does not exist in cells linked to biofilm. All of this has special significance and dimension of the whole technological process of production of quality and safe food (Moretro et al., 2004).

In the purpose of this investigation and its origin in goat uterus, we conducted epidemiologi-

cal questionnaire. During the survey, we did not find out data about the health status of goats and their healthy condition, as well as data about eventual abortions. Limiting factors are represented as restraint and the subjectivity of the owners, and the fact that the goats were originated from three raising places.

CONCLUSION

1. Goats, as well as other types of animals can be carriers of *Listeria monocytogenes* that have in some stages of infection, no clinical manifestations of the disease. In our case, *Listeria monocytogenes* was established in the uterus, which was confirmed by the knowledge about the tendencies of this kind of bacteria for the localization in particular tissues, primarily uterus.

2. The proved presence of *Listeria monocytogenes* in the uterus is indisputable and opens the possibility of contamination with these microorganisms, almost the whole area in the slaughterhouse, primarily the working surfaces, walls, accessories and tools.

3. *Listeria monocytogenes* is a zoonotic microorganism and its presence in slaughterhouses milieu presents a potential risk for the health of workers, but also the possibility of contamination of meat and meat products that have to be distributed in the market places.

4. Its presence on the floor and the carriages, confirms the ability of survival of pathogens in the conditions prevailing in the slaughterhouse ambient, particularly due to the ability of *Listeria monocytogenes* to make the biofilm.

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