

PRESENCE OF *Listeria monocytogenes* AT GOATS

S. Ivanović, M. Žutić, I. Pavlović

Veterinary Institute of Serbia, Belgrade, Republic of Serbia

Corresponding author: snezaivanovic@gmail.com

Original scientific paper

Abstract: Listeriosis is the infectious disease of numerous mammals' species, including cattle, sheep, goat, pigs, and humans. Cause is *Listeria monocytogenes*, widespread in the nature and relatively resistant microorganism. *Listeria* come in 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 live animals, various kinds of foods of plant and animal origin, as well as in animals and people. In goats, the disease is manifested in the form of abortion, encephalitis and septicemia. 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 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 were placed intestines and uterus were taken three days after slaughter. Uterus and carriage swabs were tested according to method RES, chapter 2.10.14.B.1. and meat by ISO 11290 / 1:1996 (E). From the uterus, which was slightly hyperemic it was isolated *Listeria monocytogenes* 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

Introduction

Listeriosis is acute infectious disease of various domestic and wild animals and humans. Clinically is manifested as meningoenzephalitis 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 December-May, probably as a consequence of changes in external temperature.

Cause of disease is *Listeria monocytogenes*. As ubiquitous microorganism 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 meconium, faeces waste water, water, silage and other feedstuffs, milk, cheese and meat.

Listeria come in 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 (Buncic, 1991).

As the main source of *Listeria monocytogenes* is signed stored feed – silage (Fenlon 1985; Wiedmann, 1997). After per os infection, intestinal mucosa is 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 high resistant microorganism. Optimal growth temperature is 30-37°C, but 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 can has epidemic character. Incubation may be short, only one day and for encephalitis form for 2-3 weeks. Disease lasts 1-4 days in goats and sheep (Roberts, 2003), however in cattle can be longer.

Clinical symptoms of listeriosis in animals are encephalitis, septicemia, and abortion, particularly in sheep, goats and cows. Septicemic form is relatively rare and usually occurs in young animals. In this form there is the conspicuous depression, inappetence, fever and death. 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, that 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 occurs 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 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 of heat, can be risk for the development of infection. This microorganism can survive at fridge temperature and can be multiplied as well (Schlech, 1983).

Listeria monocytogenes causes invasive and non-invasive form of infection. According to the literature data, invasive listeriosis is a rare and occurs once in 2-9 millions yearly. 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; Swaminathan 2001). As a consequence of infection may occur CNS disease, septicemia, abortion, infection of infants etc. 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, is not yet known minimum infectious dose that leads to diseases of people and indicative as individual for each person (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 been 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*.

EU supports this normative of risk assessment for fresh cheese and pasteurized milk (must not be found in the 25 g of 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).

Materials and Methods

In our study, twenty clinically healthy goats, slaughtered in slaughterhouse, we used 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 refrigerated at the temperature +4 ° C to +8 ° C (cold enrichment). The third day, suspension was transferred on 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) were used commercial tests (HiMedia). We also did catalase test (catalase color ID, bioMerieux), mobility of culture in the 25°C (API M Medium, bioMerieux) and CAMP test.

For the purpose of checking identification, was applied BBL Crystal G / P ID kit (Becton Dickinson). 25 g of meat samples placed in 225 ml of UVM-1 broth (primary selective enrichment), homogenized and incubated for 20 to 24 hours at a temperature of 30°C. By 0.1 mL of incubated broth shifted in Fraser broth (selective secondary enrichment) by the addition of 0.1 mL of the appropriate additives (feriamoniumcitrat and acriflavine) and incubated for 24-48 hours at the temperature of 37°C. As there was no change broth, medium was considered as negative and further investigations are not carried out.

Results and Discussion

In our investigations, from the uterus, which was slightly hiperemic we isolated *Listeria monocytogenes*. Grown colonies on blood agar were small, identical in shape, surrounded by a narrow zone β hemolysis (Figure 1). On Columbia agar has also pure culture in a small, transparent, grayish colonies (Figure 2).

In microscopic preparations done from the culture, established 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 (Figure 3). The same cultural and microscopic characteristics were established in the culture isolated from the brain-heart broth.

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



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

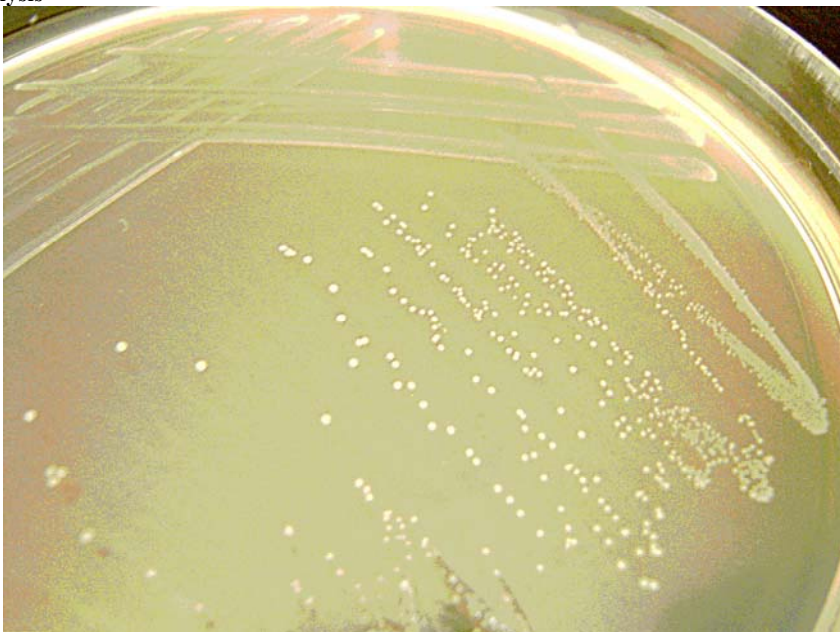


Figure 2. *Listeria monocytogenes*- colonies on Columbia agar

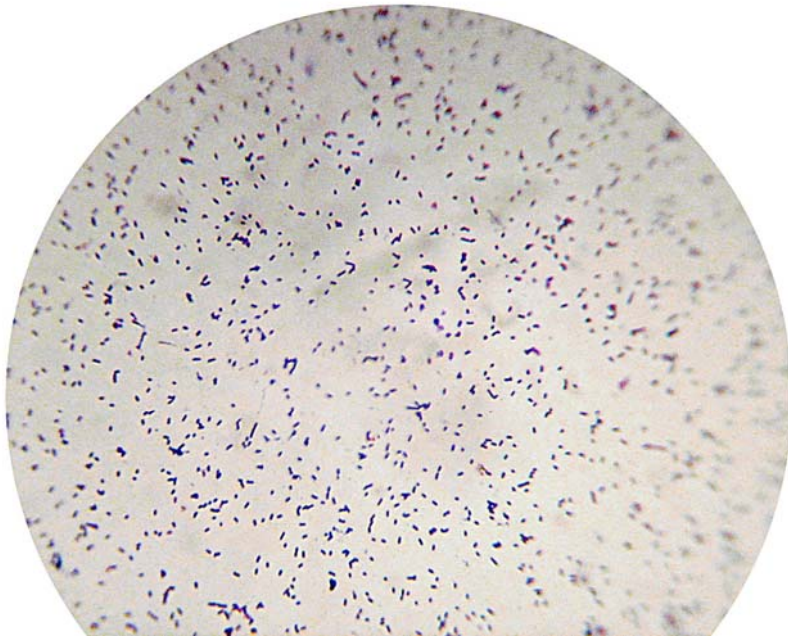


Figure 3. *Listeria monocytogenes* in a Gram-stained from a culture (Gram stain x 1000)

On the basis of the results of analysis, the culture was identified as *Listeria monocytogenes*, which is in accordance to its properties described by *Quinn, (2002)* and *Bergey's (Garrity, 2005)*. Identification was confirmed by BBL Crystal ID kit GP.

In all of twenty meat samples did not determine the presence of pathogens, but it is isolated from the 5 floor swabs and 3 carriage swabs. But 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 slaughter, they do not obligatory transmitted the cause to the meat, but it can be transmitted to the equipment, walls, floors, aprons and boots. There is much equipment in the slaughterhouse like: conveyors, machines for refrigeration and freezing, machines for the removing of skin, equipment for cutting, machines for filling and packaging. All such equipment is complex, with a slit and accessible places for impurities but inaccessible for washing, cleaning and disinfection. Therefore, it is not surprising if *L.monocytogenes* found in the equipment that has been washed and disinfected and

later contaminates meat and meat products. Some strains *L. monocytogenes* are resistant to the equipment and can survive several months or years (Rij, 2003).

Table 1 Cultural-physiological and biochemical properties of isolated culture *Listeria monocytogenes*

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

The results of our examination are in accordance to the informations in available literature, and indicate the possibility of indirect transmission of *Listeria monocytogenes* to meat. The environment in which it was established *Listeria monocytogenes*, 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. 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 good 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 epidemiological questionnaire. During the survey, we did not find out the 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

- 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 disease. In our case, *Listeria monocytogenes* was established in the uterus, which was confirmed knowledge about the tendencies of this kind of bacteria for the localization in particular, tissues, primarily uterus.

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

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

- The 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.

Acknowledgment

The research was done within the project TP 20005, "Improving of productive performances and quality of goat and kid meat in the ecological system of rising", funded by the Ministry of Science of the Republic of Serbia

Zastupljenost *Listeria monocytogenes* kod koza

S. Ivanović, M. Žutić, I. Pavlović

Rezime

Listerioza je infektivna bolest velikog broja sisara uključujući goveda, ovce, koze i ljude. Uzročnik oboljenja, *Listeria monocytogenes* je relativno rezistentna bakterija široko rasprostranjena u prirodi gde dospeva sekretima klinički obolelih ali i klinički zdravih životinja. Nalzi se u vodi, zemlji, na biljkama, po zidovima, podovima objekata za držanje životinja, u hrani životinjskog i biljnog porekla kao i kod ljudi i životinja. Kod koza, bolest se manifestuje abortusima, encefalitima i septikemijom. Po infekciji koza, klinička slika ponekad izostaje tako da na klanje odlaze životinje sa latentnom infekcijom. Po klanju, nije nužno da se infekt nađe u mesu ali zato kontaminira zidove, podove

i opremu u klanicama. U našoj studiji mikrobiološki je nakon klanja ispitano meso i uterusu 20 klinički zdravih koza. Istovremeno, metodom briseva, ispitane su podne površine u klanici sa 9 mesta i kolica kojima se prenose unutrašnji organi. Brisevi su obrađivani metodom navedenom u RES, poglavlje 2.10.14.B.1. a meso metodama navedenim u ISO 11290 / 1:1996 (E). Iz jednog uterusa koji je bio hiperemičan izolovana *Listeria monocytogenes* je kao i sa 5 uzoraka briseva poda i 3 brisa iz kolica. *Listeria monocytogenes* nije ustanovljena u uzorcima mesa. Ovi rezultati ukazuju na mogućnost indirektnog prenosa *Listeria monocytogenes* na meso.

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Received 3 June 2010; accepted for publication 15 June 2010