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J Vet Res 61, 427-432, 2017
DOI:10.1515/jvetres-2017-0069

Detection of *Listeria* spp. and *Listeria monocytogenes* in biological samples by SYBR Green I and TaqMan probe-based real-time PCRs

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Received: August 14, 2017 *Accepted: December 8, 2018*

Abstract

Introduction: The aim of the study was the application and comparison of real-time PCR methods based on the fluorescence of SYBR Green I intercalating dye and TaqMan probes for the detection of the 23S rDNA gene of *Listeria* spp. and the *hly*A gene of *Listeria monocytogenes* in biological samples of the liver, brain, and blood. **Material and Methods:** Five strains of *L. monocytogenes* and single strains of each species *L. ivanovii*, *L. innocua*, *L. grayi*, *L. welshimeri,* and *L. seeligeri* were used for the experiments. Additionally, five strains of other species of bacteria were used for evaluation of the specificity of tests. In the first stage of the study SYBR Green I real-time PCRs, one allowing detection of the 23S rDNA gene and two based on the amplification the *hly*A gene, were performed. In the next part, three TaqMan probe-based real-time PCRs allowing confirmation of belonging to *Listeria* spp. and *L. monocytogenes* were conducted. **Results:** The observation of amplification curves in real-time PCRs enabled the detection of both genes. A high regression coefficient of 0.99 was found for all reactions. Specific amplification products were obtained for the 23S rDNA and *hly*A genes which confirm their belonging to *Listeria* spp. and *L. monocytogenes*, respectively. Other microbial species did not reveal real-time PCR products. **Conclusion:** Both real-time PCR methods for the detection of *Listeria* spp. and *L. monocytogenes* in biological samples demonstrated a significant sensitivity and high specificity.

Keywords: *Listeria monocytogenes*, real-time PCR, SYBR Green I, TaqMan probe.

Introduction

Among the species belonging to *Listeria*, only *Listeria monocytogenes* has epizootic and epidemiological significance. Infections caused by *L. ivanovii, L. innocua*, or *L. seeligeri* occur sporadically in humans and animals. Listeriosis was described for the first time in 1926 by Murray *et al.* (15) in rabbit. Nyfeldt was the first who isolated *L. monocytogenes* from a man in 1929 (9). Although Murray *et al.* (15) suspected the oral route of infection, a clear link of *Listeria* infections to contaminated food was not made until the occurrence of the 1980s outbreaks and sporadic cases of listeriosis in humans (13, 19, 21, 25). Food contaminated with *L. monocytogenes* is considered the main source of infection in humans and animals.

In animals listeriosis may occur as meningo-encephalitis or meningoencephalomyelitis. In cattle, the infection also involves spontaneous abortions, respiratory tract and udder inflammations but in suckling calves mostly takes the form of septicaemia. In sheep and goats, the clinical course of listeriosis is similar to that in cattle. In horses it occurs incidentally in adults and foals as encephalitis and septicaemia, respectively. Listeriosis in pigs occurs very rarely, predominantly in piglets which die due to septicaemia without the characteristic symptoms of the infection. The cerebral form is sometimes found in older animals. Outbreaks of listeriosis in cats and dogs are very rare. In domestic birds the infection occurs sporadically in the form of septicaemia and as an enzootic disease in acute or chronic form. Of fur animals, chinchillas were especially susceptible, demonstrating sometime enzootic disease with signs of septicaemia (2, 9, 13). The EFSA reports in 2015 show that in the European Union *Listeria* spp. and most of *L. monocytogenes* occur mainly in cattle, sheep, and goats. These infections were also reported in pigs, horses, broilers, cats, dogs, foxes, and other wild and zoo animals (7).

Pregnant women, newborns, elderly people, people with weak or suppressed immune system, and chronically ill are especially susceptible to listeriosis. The main clinical manifestations of listeriosis are meningoencephalitis, spontaneous abortions in pregnant women, and septicaemia. Listeriosis cases in humans are regularly listed in Europe, Australia, and USA. Although in most cases listeriosis occurs sporadically, the infection is characterised by high fatality rate (6, 13, 22, 24). In the European Union, 270 deaths among 2,206 cases of listeriosis were reported in 2015. The incidence rate was 0.46 per 100,000 population (7). In the same year, Poland reported 70 cases, with the incidence rate of 0.18 per 100,000 (16).

The application of molecular biology techniques for laboratory diagnosis improved the detection of the microorganisms. PCRs and real-time PCRs are rapid, sensitive, and specific methods. The aim of the study was application and comparison of real-time PCR methods based on the fluorescence of SYBR Green I intercalating dye and TaqMan probes for the detection of the 23S rDNA gene of *Listeria* spp. and the *hly*A gene of *L. monocytogenes* in experimentally contaminated biological samples (bovine liver, brain, and blood).

Material and Methods

Bacterial strains. The study involved five strains of *L. monocytogenes*: *L. monocytogenes* ATCC 7644, *L. monocytogenes* ATCC 13932, *L. monocytogenes* ATCC 19111, *L. monocytogenes* ATCC 19112, and *L. monocytogenes* ATCC 19115. To assess the specificity of the tests, the strains of other species of the genus *Listeria*, including single strains of *L. ivanovii* ATCC 19119, *L. innocua* ATCC 33090, *L. grayi* ATCC 19120, *L. welshimeri* ATCC 35897, and *L. seeligeri*, originating from the collection of the Department of Microbiology, National Veterinary Research Institute in Pulawy, Poland, were used. In addition, the experiments involved strains of other species, including *Bacillus cereus* ATCC 11778, *Staphylococcus aureus* ATCC 6538, *Escherichia coli* ATCC 25922, *Salmonella* Typhimurium ATCC 14028, and *Klebsiella pneumoniae* ATCC 13883.

Isolation of DNA. Each strain was streaked on TSYEA medium. Cultures were incubated for 24 h at 37°C. Then bacterial suspension with a density of 1 McF was prepared. Samples of 1 mL were centrifuged for 10 min at 13,000 rpm. The supernatant was removed and the pellet was used to contaminate bovine liver, brain, and blood samples. To isolate DNA, a DNeasy Blood and Tissue Kit (Qiagen, USA) was used.

The sensitivity and linearity of real-time PCRs were determined by 10-fold dilution technique. The initial concentration of *L. monocytogenes* ATCC 13932 was about 1.5×10^6 CFU/reaction.

SYBR Green I real-time PCR. Real-time PCR was performed in 25 µL QuantiTect SYBR Green PCR Kit (Qiagen) containing the appropriate concentration of each primer, and 5 µL of DNA. One pair of primers detecting the 23S rDNA gene of the genus *Listeria* (20) and two pairs of primers amplifying the *hly*A gene of *L. monocytogenes* (11, 17) were selected (Table 1). The primers were synthesised by Genomed (Poland). Primer concentrations of 0.1 µM, 0.25 µM, 0.5 µM, 1.0 µM, 1.5 µM, and 2.0 µM were evaluated to optimise the reaction. Reactions were run on the Rotor-Gene Q (Qiagen) and Rotor-Gene Q Series Software Version 2.0.2 with the following programme: 10 min at 95°C, and 40 cycles of 15 s at 95°C, 60 s at 60°C, and 90 s at 72°C. To confirm amplification specificity, the melting temperature of PCR products was determined by gradual raising the temperature of the reaction mixture from 55°C to 95°C and continuous fluorescence measurements.

TaqMan probe-based real-time PCR. Real-time PCR was performed in 25 µL QuantiTect Probe PCR Kit (Qiagen) containing the appropriate concentration of each primer and probe, and 5 µL of DNA. Based on the literature data (11, 17, 20), the sequences of three different pairs of primers and probes were selected (Tables 1 and 2). The primers and probes were also synthesised by Genomed (Poland). Primer concentrations of 0.1 µM, 0.25 µM, 0.5 µM, 1.0 µM, 1.5 µM, and 2.0 µM, and probe concentrations of 0.05 µM, 0.1 µM, and 0.2 µM were evaluated to optimise the reaction. The reactions profile was as follows: 2 min at 50°C, 10 min at 95°C, and 50 cycles of 15 s at 95°C and 60 s at 63°C.

Table 1. The characteristics of the primers for SYBR Green I and TaqMan probe-based real-time PCRs

	Primers	Sequence (5' – 3')	Concentration
23S rDNA gene	L23SQ-F	AGGATAGGGAATCGCACGAA	1.0 µM
	L23SQ-R	TTCGCGAGAAGCGGATTT	
	Lin23SQ-FR	TTCGCAAGAAGCGGATTTG	
*hly*A gene	hlyA-146-F	GGGAAATCTGTCTCAGGTGATGT	1.0 µM
	hlyA-146-R	CGATGATTTGAACTTCATCTTTTGC	
	hlyA-177-F	TGCAAGTCCTAAGACGCCA	0.5 µM
	hlyA-177-R	CACTGCATCTCCGTGGTATACTAA	

Table 2. The characteristics of the probes for TaqMan probe-based real-time PCRs

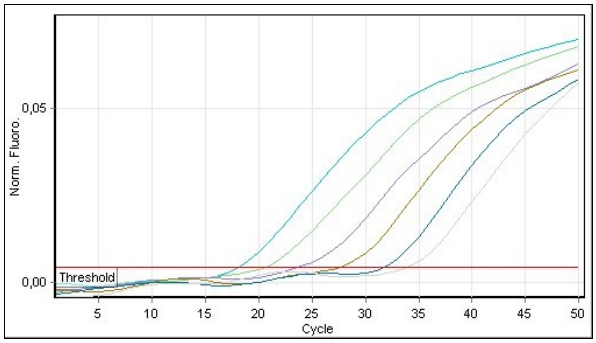
	Probes	Sequence	Concentration
23S rDNA gene	L23SQ-TM	5'-VIC-TCTCACACTCACTGCTTGGACGC-Tamra-3'	0.1 µM
*hly*A gene	hlyA-146-TM	5'-Fam -CAAAAATTCTTCCTTCAAAGCCGTAATTTACGG -Tamra-3'	0.2 µM
	hlyA-177-TM	5'Fam -CGATTTCATCCGCGTGTTTCTTTTCG -Tamra-3'	0.1 µM

Results

In the first stage, three SYBR Green I real-time PCRs were applied for the detection of the 23S rDNA gene of *Listeria* spp. and the *hly*A gene of *L. monocytogenes*. The influence of different primer concentrations was determined. The concentration for detecting the 23S rDNA gene with the L23SQ-F, L23SQ-R, and Lin23SQ-FR primers was set at the level of 1.0 µM. In the case of *hly*A gene detection with the hlyA-146-F and hlyA-146-R primers, the optimal concentration was 1.0 µM, whereas the concentration of 0.5 µM was selected for the hlyA-177-F and hlyA-177-R primers.

In the next stage, genus-specific SYBR Green I real-time PCR was performed using the L23SQ-F, L23SQ-R and Lin23SQ-FR primers with template DNA obtained from the liver, brain, and blood samples contaminated experimentally with 10 *Listeria* spp. strains. Ct values ranged from 17.47 to 22.8 for the liver, 14.93 to 21.13 for the brain, and 16.99 to 21.25 for blood samples. The specificity of amplification was confirmed by determination of the PCR product melting temperature. It was found that the melting temperature of the reaction products was about 80ºC. The sensitivity of the real-time PCR was 1.5×10^1 CFU/reaction for each type of biological samples (Fig. 1).

a)



b)

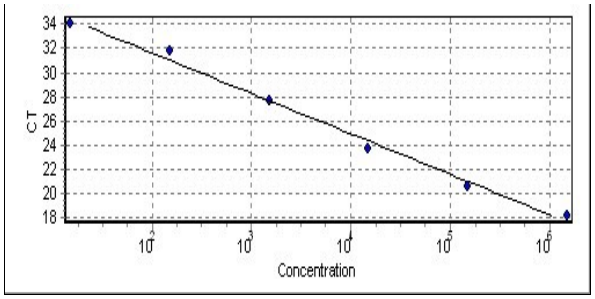
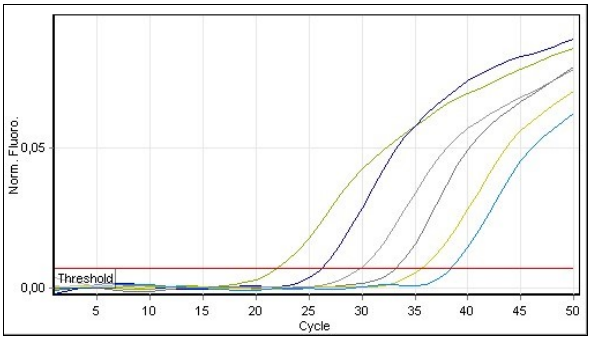


Fig. 1. Determination of the sensitivity (a) and linearity (b) of genus-specific SYBR Green I real-time PCR using L23SQ-F/R and Lin23SQ-FR primers for the detection of *L. monocytogenes* ATCC 13932 strain in the liver samples

Then, a species-specific SYBR Green I real-time PCR was applied using the hlyA-146-F and hlyA-146-R primers for biological samples contaminated with five *L. monocytogenes* strains. Ct values were from 12.59 to 18.77 for the liver, 11.89 to 16.99 for the brain, and 12.8 to 16.6 for blood samples. Specific amplification products dissociated at about 81ºC. The sensitivity was 1.5×10^1 CFU/reaction for all samples (Fig. 2).

a)



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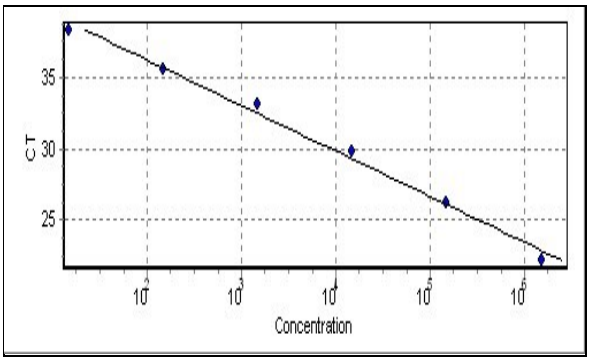


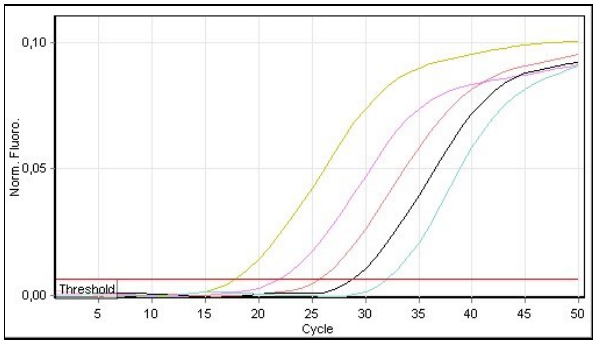
Fig. 2. Determination of the sensitivity (a) and linearity (b) of species-specific SYBR Green I real-time PCR using hlyA-146-F/R primers for the detection of *L. monocytogenes* ATCC 13932 strain in the brain samples

Amplification curves of biological samples contaminated with five *L. monocytogenes* strains were obtained with the hlyA-177-F and hlyA-177-R primers. Ct values ranged from 16.38 to 20.83 for the liver, 17.11 to 20.72 for the brain, and 17.72 to 20.13 for blood samples. It was shown that the melting temperature of the reaction products was about 76ºC. The sensitivity was established at 1.5×10^2 CFU/reaction for all samples (Fig. 3).

In the case of all SYBR Green I real-time PCRs, a high regression coefficient (0.99) was demonstrated for amplification of concentrations from 1.5×10^6 to 1.5×10^1 or 1.5×10^2 CFU/reaction, which indicates a high degree of reaction linearity (Figs 1b-3b).

In the second stage of the study, three TaqMan probe-based real-time PCRs were performed. It was found that in the case of TaqMan probe-based real-time PCR detecting the 23S rDNA gene with the L23SQ-F, L23SQ-R, and Lin23SQ-FR primers and L23SQ-TM probe, their optimal concentrations were 1.0 µM for primers and 0.1 µM for the probe. For detection the *hly*A gene with the hlyA-146-F and hlyA-146-R primers and hlyA-146-TM probe, the optimal concentrations were 1.0 µM for primers and 0.2 µM for the probe. In the case of the hlyA-177-F and hlyA-177-R primers and hlyA-177-TM probe, the concentration of 0.5 µM for primers and 0.1 µM for the probe were selected.

a)



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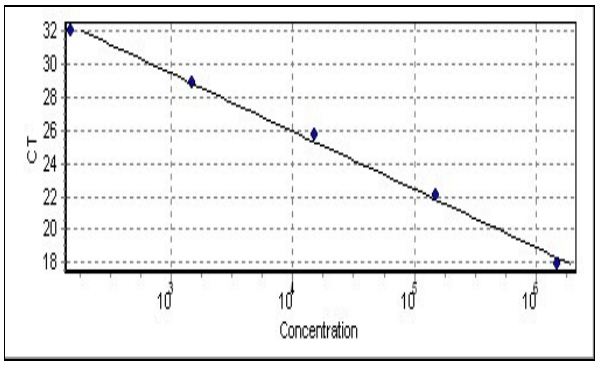
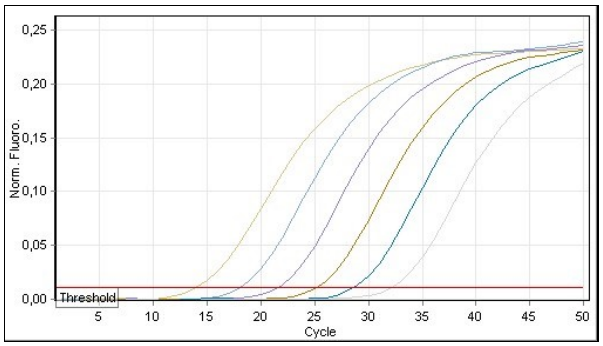


Fig. 3. Determination of the sensitivity (a) and linearity (b) of species-specific SYBR Green I real-time PCR using hlyA-177-F/R primers for the detection of *L. monocytogenes* ATCC 13932 strain in the blood samples

a)



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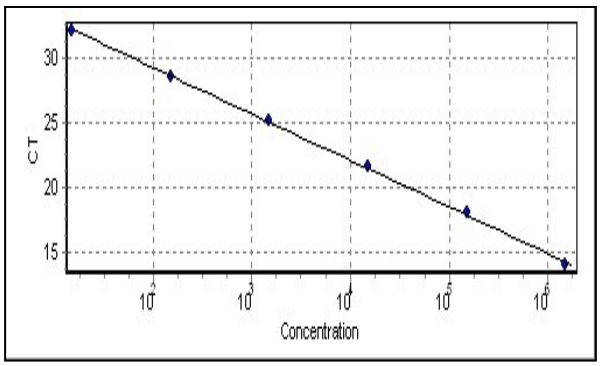
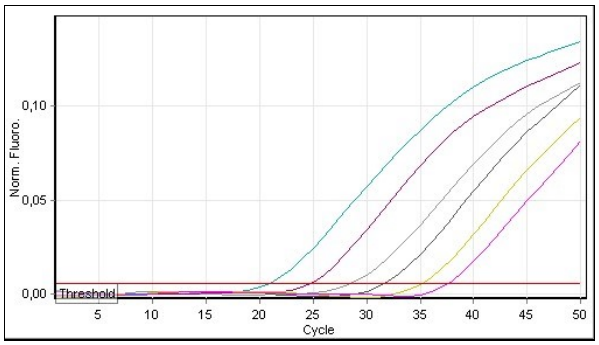


Fig. 4. Determination of the sensitivity (a) and linearity (b) of genus-specific TaqMan probe-based real-time PCR L23SQ-F/R and Lin23SQ-FR primers, and L23SQ-TM probe for the detection of *L. monocytogenes* ATCC 13932 strain in the liver samples

Then, a genus-specific TaqMan probe-based real-time PCR was applied using the L23SQ-F, L23SQ-R, and Lin23SQ-FR primers and L23SQ-TM probe with template DNA obtained from the liver, brain, and blood samples contaminated experimentally with ten *Listeria* spp. strains. Ct values were 13.88 to 20.04 for the liver, 15.03 to 17.71 for the brain, and 15.03 to 19.22 for blood samples. The same sensitivity of 1.5×10^1 CFU/reaction was found for each type of biological samples (Fig. 4).

Next, a species-specific TaqMan probe-based real-time PCR was performed using the hlyA-146-F and hlyA-146-R primers and hlyA-146-TM probe for biological samples contaminated with five *L. monocytogenes* strains. Ct values ranged from 17.68 to 22.97 for the liver, 18.7 to 21.66 for the brain, and 21.57 to 23.59 for blood samples. The sensitivity was 1.5×10^1 CFU/reaction for all samples (Fig. 5).

a)



b)

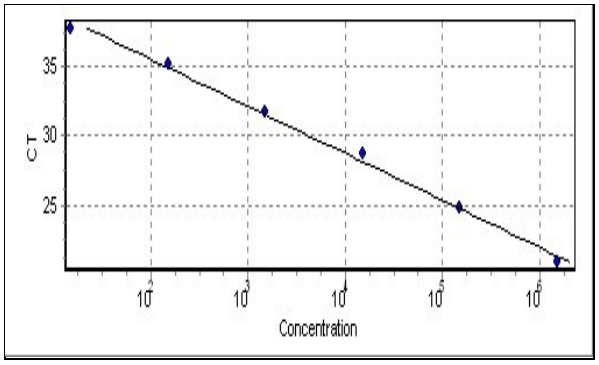


Fig. 5. Determination of the sensitivity (a) and linearity (b) of species-specific TaqMan probe-based real-time PCR using hlyA-146-F/R primers and hlyA-146-TM probe for the detection of *L. monocytogenes* ATCC 13932 strain in the brain samples

In the case of the hlyA-177-F and hlyA-177-R primers and hlyA177-TM probe obtained Ct values were 15.79 to 21.06 for the liver, 18.09 to 21.36 for the brain, and 18.62 to 21.1 for blood samples. The sensitivity of the TaqMan probe-based real-time PCR was 1.5×10^2 CFU/reaction for all samples (Fig. 6).

As in the case of SYBR Green I real-time PCRs, high regression coefficient was also achieved in TaqMan probe-based real-time PCRs with the use of 23S rDNA and *hly*A genes (Figs 4b-6b).

To demonstrate the specificity of SYBR Green I and TaqMan probe-based real-time PCR methods, the template DNA obtained from the liver, brain, and blood samples experimentally contaminated with five strains of *L. monocytogenes*, single strains of *L. ivanovii*,

L. innocua, *L. grayi*, *L. welshimeri*, and *L. seeligeri*, and other microbial species including *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli*, *Salmonella* Typhimurium, and *Klebsiella pneumoniae* was examined. All real-time PCRs were highly specific. The specific amplification products were obtained using the 23S rDNA and hlyA genes which identified the tested strains as *Listeria* spp. and *L. monocytogenes*. Isolates of other species failed to yield real-time PCR products.

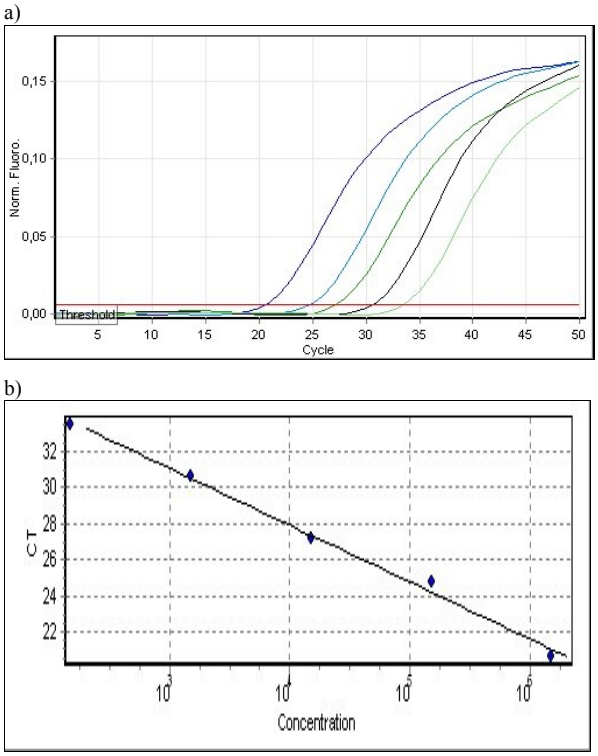


Fig. 6. Determination of the sensitivity (a) and linearity (b) of species-specific TaqMan probe-based real-time PCR using hlyA-177-F/R primers and hlyA-177-TM probe for the detection of *L. monocytogenes* ATCC 13932 strain in the blood samples

Discussion

Listeriosis is diagnosed on the basis of clinical symptoms and diagnostic tests. The conventional method of microbiological diagnostics comprises inoculation of biological material on selective media enabling growth of the bacteria, and identification of colonies based on morphological, physiological, and biochemical characteristics. Traditional bacteriological methods are, however, time-consuming because they may last up to a few days (1, 8, 22). *L. monocytogenes,* being a pathogen of humans and animals, requires faster and less laborious diagnostic methods such as real-time PCR (2, 5). For detection of real-time PCR products non-specific dyes and specific fluorophore-labelled oligonucleotide probes are used. The most commonly used non-specific dye is SYBR Green I, which, when bound to the double-stranded DNA, fluoresces by exposure to the light of an appropriate wavelength. TaqMan-type nucleotide probes are also commonly used. These are oligonucleotides fluorescently labelled with the reporter dye (FAM) and the quenching dye (TAMRA). In the presence of the product, the probe hybridises to it and then is hydrolysed in the presence of DNA polymerase elongating the starter. As a result, the fluorescence of FAM may be detected and measured because it is not suppressed by TAMRA. The intensity of the fluorescence is proportional to the amount of amplified product. Real-time PCR eliminates post-amplification steps such as gel electrophoresis and allows observing the results of the amplification in real time (10, 12). The optimisation of reaction conditions is very important for the efficiency of real-time PCR. The optimal concentration of pairs of primers and probes was selected by testing various concentrations of each primer and probe in SYBR Green I and TaqMan probe-based real-time PCR methods.

Several virulence-associated markers are used for the identification of *L. monocytogenes* in real-time PCR, but the *hly* gene, which encodes listeriolysin O, is one of the most frequently applied (11, 14, 17, 18, 20, 26). Hough *et al.* (11) achieved a detection limit 1.4×10^2 CFU in 25 g cabbage with the use of *hly* gene in TaqMan probe-based real-time PCR. Moreover, Portronieri *et al.* (18) detected 1×10^1 CFU in 10 g of yoghurt. In turn, Dadkhah *et al.* (4) obtained the detection limit of 1.58×10^3 CFU/mL in SYBR Green I real-time PCR using *iap* gene in nutrient broth and milk samples. Their results were similar to the TaqMan method, with the detection limit of 1.43×10^3 CFU/mL. The authors observed the same sensitivity of the two methods. In our study the same results of SYBR Green I and TaqMan probe-based real-time PCRs were obtained for different biological samples. In the case of both real-time PCRs detecting the 23S rDNA gene, the sensitivity was 1.5×10^1 CFU per reaction. The same level of sensitivity was achieved in methods detecting the *hly*A gene with hlyA-146-F and hlyA-146-R primers. Only in both real-time PCRs detecting the *hly*A gene with hlyA-177-F and hlyA-177-R primers, the sensitivity was observed at a slightly lower level of 1.5×10^2 per reaction. SYBR Green I and TaqMan probe-based real-time PCRs are equally useful for detection of *L. monocytogenes*, but the last method is more expensive because of the high cost of probe synthesis.

In the case of SYBR Green I real-time PCR with the use *hly*A gene in food samples, Traunšek *et al.* (26) achieved a regression coefficient higher than 0.98. In turn, Nogva *et al.* (17) obtained a regression coefficient higher than 0.993 in TaqMan probe-based real-time PCR using *hly*A gene in pure cultures, water, skim milk, and unpasteurised whole milk. Their results were comparable to those reported by Murphy *et al.* (14) in pure cultures (0.998) and Hough *et al.* (11) in cabbage (0.997). Moreover, Rodríguez-Lázaro *et al.* (20) achieved a regression coefficient above 0.995 in TaqMan probe-based real-time PCR using *hly* and the 23S rDNA genes in pure cultures. In our studies we obtained a high regression coefficient (0.99) in SYBR Green I and TaqMan probe-based real-time PCR with

the use the 23S rDNA and *hly*A genes, which indicated a high degree of reaction linearity.

Barkallah *et al.* (2) and Skerniškyté *et al.* (23) achieved 100% specificity in TaqMan probe-based real-time PCR using *hly* gene. Our results also demonstrated specificity reaching 100% in all SYBR Green I and TaqMan probe-based real-time PCRs with the use 23S rDNA and *hly*A genes.

In our studies real-time PCR methods based on the fluorescence of SYBR Green I intercalating dye and TaqMan probes, which were used for the detection of *Listeria* spp. and *L. monocytogenes* in biological samples, showed a significant sensitivity and high specificity. These methods improve the detection sensitivity in comparison to conventional PCR (3). In conclusion, real-time PCRs can be used as an alternative to classical methods for accurate, quick, and sensitive detection of contaminated samples.

Conflict of Interests Statement: The authors declare that there is no conflict of interests regarding the publication of this article.

Financial Disclosure Statement: This work was financed with the funds of the National Veterinary Research Institute in Pulawy, Poland.

Animal Rights Statement: None required.

References

1. Amagliani G., Omiccioli E., Brandi G., Bruce I.J., Magnani M.: A multiplex magnetic capture hybridisation and multiplex real-time PCR protocol for pathogen detection in sea food. Food Microbiol 2010, 27, 580–585.
2. Barkallah M., Gharbi Y., Hmani M., Mallek Z., Gautier M., Gdoura R., Fendri I.: Locked nucleic acid probe-based real-time PCR for the diagnosis of *Listeria monocytogenes* in ruminants. Mol Cell Probe 2016, 30, 138–145.
3. Budniak S., Kędrak-Jabłońska A., Szczawińska A., Reksa M., Krupa M., Szulowski K.: Comparison of two multiplex PCR assays for the detection of *Listeria* spp. and *Listeria monocytogenes* in biological samples. J Vet Res 2016, 60, 411–416.
4. Dadkhah H., Bassami M.R., Hashemi S., Shahraz F., Hosseini H., Karatzas K.A.G., Khaksar R.: Evaluation and comparison of SYBR Green I real-time PCR and TaqMan real-time PCR methods for quantitative assay of *Listeria monocytogenes* in nutrient broth and milk. Afr J Microbiol Res 2012, 6, 1908–1917.
5. Denis E., Bielińska K., Wieczorek K., Osek J.: Multiplex real-time PCRs for detection of *Salmonella*, *Listeria monocytogenes*, and verotoxigenic *Escherichia coli* in carcasses of slaughtered animals. J Vet Res 2016, 60, 287–292.
6. Drevets D.A., Bronze M.S.: *Listeria monocytogenes*: epidemiology, human disease, and mechanisms of brain invasion. FEMS Immunol Med Microbiol 2008, 53, 151–165.
7. European Food Safety Authority and European Centre for Disease Prevention and Control: The European Union summary report on trends and sources of zoonoses, zoonotic agents, and food-borne outbreaks in 2015. EFSA J 2016, 14, 4634, 1–231. doi10.2903/j.efsa.2016.4634.
8. Gasanov U., Hughes D., Hansbro P.M.: Methods for the isolation and identification of *Listeria* spp. and *Listeria monocytogenes*: a review. FEMS Microbiol Rev 2005, 29, 851–875.
9. Gray M.L., Killinger A.H.: *Listeria monocytogenes* and Listeric infections. Bacteriol Rev 1966, 30, 309–382.
10. Heid C.A., Stevens J., Livak K.J., Williams P.M.: Real time quantitative PCR. Genome Res 1996, 6, 986–994.
11. Hough A.J., Harbison S.A., Savill M.G., Melton L.D., Fletcher G.: Rapid enumeration of *Listeria monocytogenes* in artificially contaminated cabbage using real–time polymerase chain reaction. J Food Prot 2002, 65, 1329–1332.
12. Kubista M., Andrade J.M., Bengtsson M., Forootan A., Jonák J., Lind K., Sindelka R., Sjöback R., Sjögreen B., Strömbom L., Ståhlberg A., Zoric N.: The real-time polymerase chain reaction. Mol Aspects Med 2006, 27, 95–125.
13. Low J.C., Donachie W.: A review of *Listeria monocytogenes* and listeriosis. Vet J 1997, 153, 9–29.
14. Murphy N.M., McLauchlin J., Ohai C., Grant K.A.: Construction and evaluation of a microbiological positive process internal control for PCR-based examination of food samples for *Listeria monocytogenes* and *Salmonella enterica* Int J Food Microbiol 2007, 120, 110–119.
15. Murray E.G.D., Webb R.A., Swann M.R.B.: A disease of rabbits characterized by a large mononuclear leukocytosis, caused by a hitherto undescribed bacillus *Bacterium monocytogenes* (n.sp.). J Pathol 1926, 29, 407–439.
16. National Institute of Public Health and Chief Sanitary Inspectorate: Infectious diseases and poisonings in Poland 2015. Warsaw 2016, pp. 1–166.
17. Nogva H.K., Rudi K., Naterstad K., Holck A., Lillehaug D.: Application of 5'-nuclease PCR for quantitative detection of *Listeria monocytogenes* in pure cultures, water, skim milk, and unpasteurized whole milk. Appl Environ Microbiol 2000, 66, 4266–4271.
18. Poltronieri P., de Blasi M.D., D'Urso O. F.: Detection of *Listeria monocytogenes* through real-time PCR and biosensor methods. Plant Soil Environ 2009, 55, 363–369.
19. Rocourt J.: Risk factors for listeriosis. Food Control 1996, 7, 195–202.
20. Rodríguez-Lázaro D., Hernández M., Pla M.: Simultaneous quantitative detection of *Listeria* spp. and *Listeria monocytogenes* using a duplex real-time PCR-based assay. FEMS Microbiol Letters 2004, 233, 257–267.
21. Schlech W.F., Lavigne P.M., Bortolussi R.A., Allen A.C., Haldane E.V., Wort A.J., Hightower A.W., Johnson S.E., King S.H., Nicholls E.S., Broome M. D.: Epidemic listeriosis-evidence for transmission by food. N Engl J Med 1983, 308, 203–206.
22. Schuchat A., Swaminathan B., Broome C.V.: Epidemiology of human listeriosis. Clin Microbiol Rev 1991, 4, 169–183.
23. Skerniškyté J., Armalyté J., Kvietkauskaité R., Šeputiené V., Povilonis J., Sužiedélienė E.: Detection of *Salmonella* spp., *Yersinia enterocolitica*, *Listeria monocytogenes*, and *Campylobacter* spp. by real-time multiplex PCR using amplicon DNA melting analysis and probe-based assay. Int J Food Sci 2016, 51, 519–529.
24. Swaminathan B., Gerner-Smidt P.: The epidemiology of human listeriosis. Microbes Infect 2007, 9, 1236–1243.
25. Todd E.C.D., Notermans S.: Surveillance of listeriosis and its causative pathogen, *Listeria monocytogenes*. Food Control 2011, 22, 1484–1490.
26. Traunšek U., Toplak N., Jeršek B., Lapanje A., Majstorović T., Kovač M.: Novel cost-efficient real-time PCR assays for detection and quantitation of *Listeria monocytogenes*. J Microbiol Methods 2011, 85, 40–46.