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Short communication

# The loop-mediated isothermal amplification assay for rapid diagnosis of *Babesia canis canis* infections in dogs

Ł. Adaszek<sup>1</sup>, M. Jankowska<sup>2</sup>, M. Kalinowski<sup>1</sup>, T. Banach<sup>1</sup>, D. Wulupek<sup>3</sup>,  
S. Winiarczyk<sup>1</sup>

<sup>1</sup> Department of Epizootiology and Infectious Diseases, Faculty of Veterinary Medicine,  
University of Life Sciences, Głęboka 30, 20-612 Lublin, Poland

<sup>2</sup> Department of Biotechnology and Food Microbiology, Faculty of Food Science and Nutrition,  
University of Life Sciences, Wojska Polskiego 48, 60-627 Poznań, Poland

<sup>3</sup> Department and Clinic of Animal Internal Diseases,  
University of Life Sciences, Głęboka 30, 20-612 Lublin, Poland

## Abstract

The aim of this study was to use a rapid and easy DNA-based test, the loop-mediated isothermal amplification (LAMP), for diagnosis of *Babesia canis canis* infections in dogs. 10 DNA samples of 18S RNA-A and 10 DNA samples of 18S RNA-B of *B. canis canis* were used in the study. LAMP method could successfully detect DNA in all examined samples down to 0.1 pg dilution. Obtained results suggest that this method has high specificity and sensitivity and can be applied in analytical laboratories in diagnosis of canine babesiosis.

**Key words:** *Babesia canis canis*, dogs, loop-mediated isothermal amplification (LAMP)

## Introduction

Canine babesiosis is a common and clinically significant tick-borne disease caused by hematozoan parasites of the genus *Babesia*. *Babesia canis canis* is the protozoic species that causes most often babesiosis in dogs. In previous studies two strains of parasites within this species were detected, which have different molecular structures of 18S RNA gene and virulence (Adaszek et al. 2009).

Standard diagnosis of babesiosis is the identifica-

tion of *Babesia* parasites in Giemsa-stained thin-film blood smears examined by microscopy. However, the detection of *Babesia* parasites is difficult in dogs with unapparent or chronic infections since the level of parasitemia is very low (Muller et al. 2010). Therefore, the development of a highly specific and sensitive system for the diagnosis of *B. canis* infection is required. The aim of this study, was to use a rapid and easy test – loop-mediated isothermal amplification (LAMP) for diagnosis of *Babesia canis canis* infections in dogs.

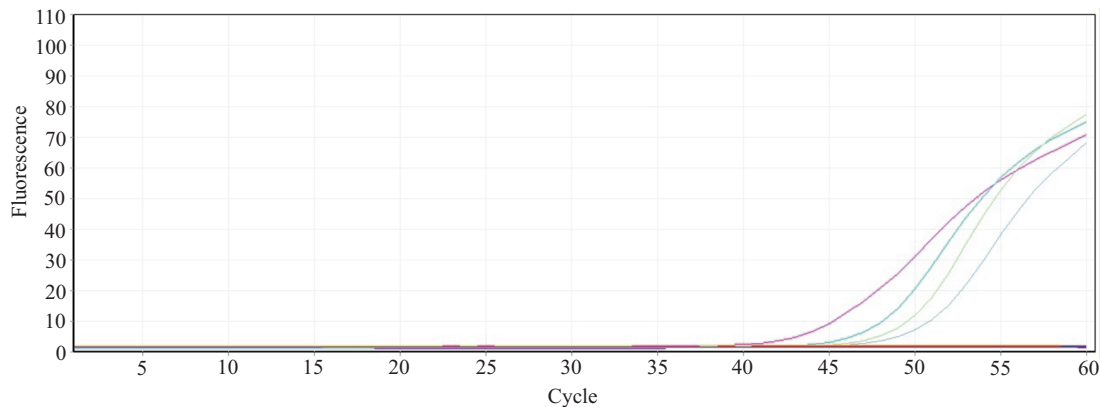


Fig 1. Amplification curves of serially diluted DNA samples: curve violet-DNA in concentration 100 ng/μl, curve dark green DNA in concentration 10 ng/μl, curve light green DNA in concentration 1 ng/μl, and curve grey DNA in concentration 0,1 ng/μl.

## Materials and Methods

The *B. canis canis* 18S RNA gene (genbank accession no. EU622792.1) was used to construct LAMP primers using the Primer Explorer Program (<http://primerexplorer.jp/e/>). To assess the analytical and diagnostic sensitivity of LAMP test DNA of 10 strains of *B. canis canis* 18S RNA-A and 10 strains of 18S RNA-B, isolated from the blood of dogs with clinical babesiosis, were subjected to this reaction. The strain of protozoa was determined based on the sequence analysis of the amplicons obtained in previously conducted traditional PCR.

The LAMP reaction was performed in a final volume of 12.5 μl which contained Isothermal LAMP Mastermix (Novazym, Poland), 10 μM each FIP and BIP primers, 2.5 μM each F3 and B3 primers, and 1.5 μl (135 ± 23 ng/μl) of target DNA. The mixture was incubated at 61, 62, 63, 64, 65°C for 60 min using a conventional thermocycler (Biometra Gottingen, Germany) as well as Real-Time PCR Corbett apparatus and then heated at 80°C for 5 min to terminate the reaction. The specificity of LAMP primers was examined by testing 100 ng/μl of DNA of *B. canis canis* and deionized water as a negative control. To determine the analytical sensitivity of the LAMP assay, tenfold dilutions were made from 100 ng/μl of *B. canis canis* DNA which served as templates for the LAMP reaction. The LAMP products were subjected to electrophoresis on a 2% agarose gel containing 0,5 μg/ml ethidium bromide and visualized under ultraviolet light.

The diagnostic sensitivity was defined as the ratio of the number of true positive results in LAMP to the sum of true positives and false-negatives (ISO 13843, 2000).

## Results and Discussion

Out of 20 tested DNA samples, the presence of the *Babesia* genetic material was detected in all 10 18S RNA-A samples and in all 10 18S RNA-B samples (diagnostic sensitivity – 100%), whereas no DNA of *Babesia* was detected in the negative control.

The reaction could successfully take place in temperatures ranging from 61°C to 65°C. The strongest signal in electrophoresis was obtained for samples that were incubated at 65°C for 45 min and this temperature was chosen as the best reaction condition. Under these conditions, LAMP primers specifically produced positive LAMP amplicons of typical ladder patterns from DNA of *B. canis canis* while there were no products in negative control (deionized water). Using serially diluted DNA samples, LAMP could successfully detect parasite DNA down to 0.1 pg dilution. These results were confirmed by Real-Time PCR technique (Fig. 1).

It is generally considered that LAMP is highly sensitive method, being able to detect DNA extracted from 50 *Babesia*-infected red blood cells (Iseki et al. 2007). In our study, the sensitivity of LAMP was tested by subjecting serially tenfold diluted DNA of *B. canis canis* 18S RNA-A and 18S RNA-B to LAMP. Obtained results showed that this is a good method for the detection of *B. canis canis* infections and it is less time-consuming than standard PCR. The diagnostic sensitivity, reported as 100% on a cohort of 20 dogs, confirms that the test is appropriate for the diagnosis of the disease (ISO 13843, 2000, Müller et al. 2010).

In summary, the LAMP method presented in this study is sufficiently robust for detection of infections by *B. canis canis*. The isothermal conditions required

for the DNA polymerase make the use of a thermocycler dispensable, reducing overall cost. This method has a great potential as a test that can be applied in analytical laboratories.

### References

- Adaszek Ł, Winiarczyk S, Skrzypczak M (2009) The clinical course of babesiosis in 76 dogs infected with protozoan parasites *Babesia canis canis*. *Pol J Vet Sci* 12: 81-87.
- Iseki H, Alhassan A, Ohta N, Thekiso OM, Yokoyama N, Inoue N, Nambota A, Yasuda J, Igarashi I (2007) Development of a multiplex loop-mediated isothermal amplification (mLAMP) method for the simultaneous detection of bovine *Babesia* parasites. *J Microbiol Methods* 71: 281-287.
- ISO/TR13843 (2000) Water quality-Guidance on validation of microbiological methods. International Organization for Standardisation, Geneva, Switzerland.
- Mosqueda J, Olvera-Ramirez A, Aguilar-Tipacamu G, Canto GJ (2012) Current advances in detection and treatment of babesiosis. *Curr Med Chem* 19: 1504-1518.
- Müller H, Aysul N, Liu Z, Salih DA, Karagenc T, Beyer D, Kullmann B, Ahmed JS, Seitzer U (2010) Development of a loop-mediated isothermal amplification (LAMP) assay for rapid diagnosis of *Babesia canis* infections. *Transbound Emerg Dis* 57: 63-65.