Grubich P. Y., Candidate of Veterinary Science,
Kurman A. F., Ph.D. Candidate of Biological Science,
Lepeta L.V., Researcher,
Parkhomenko E. A., Junior researcher
Institute pig breeding and agro-industrial production NAAS of Ukraine, Poltava

DEVELOPMENT PCR TEST SYSTEM FOR SPECIFIC DETECTION OF BACTERIA BABESIOSIS ANIMALS

Reviewer - Candidate of Veterinary Science I. N. Ksonz

The system of oligonucleotide primers that allow PCR amplified in section 18S rRNA gene 6 species of the genus Babesia. The article presents the features and design of primers tested multiplexed PCR test systems for the identification of the genus Babesia. Yznacheni in amplified fragment length - From 299 to 2 5 8 pairs for Babesia canis, Babesia divergens, Babesia caballi, Babesia major, Babesia bovis. Studied 342 blood samples from different animal species and is 100% coincidence with the results of microscopic studies.

Keywords: *babezioz, diagnosis, test-system identification, primers, nucleotides.*

Statement of the problem. Babesiosis in Ukraine is common among pet seasonal disease and is ranked by the number of cases and the severity of one of the leading parasitic diseases. It is known that each species babesiosis is a kind of parasite. But long species identification was performed only on morphological characters of the pathogen, which varied within wide limits. However, is not completely clear whether the cause of the disease in other species, the agent other species.

Now is poorly understood belonging to the genus Babesia parasite of certain, specific properties of pathogens and pathogenesis of the disease, in particular, mechanisms that inhibit the development of the parasite in the body. There are no effective means of specific prophylaxis of babesiosis and chemotherapy are extremely toxic, with severe side effects.

Analysis of the main research and publications on this issue. Prepared by orystannya molecular genetic techniques has allowed to make a clear classification of family *Piroplasmida* mammals and divide them into four primary groups, namely *genera Babesia, Theileria, Cytauxzoon* and *Babesia microti* [1]. In world practice used babezy means of identification that are based on molecular genetic methods, but they allow you to specify only certain types babezy [2, 3, 4]. In Ukraine these features was developed.

The aim of our work was to develop a system for identifying babezy based on molecular genetic features and design of PCR diagnostic test kits.

Methods research. With MEGA [5] determined the conservative and variable within the genus Babesia, plot 18S rRNA gene. Conservative sequences were used to design PCR test system which allows amplified sequence dilyan at k 18S rRNA gene 6 species of the genus *Babesia: Babesia canis, Babesia divergens, Babesia caballi, Babesia major, Babesia bovis*. Variable region 18S rRNA gene were used for the development of species-specific oligonucleotide primers as well allow posts to identify three species of the genus *Babesia*, namely: *Babesia canis, Babesia divergens* and *Babesia bovis*.

Structure of oligonucleotide primers used were determined from the equation of programs and FastPCR [6]. Options for designing primers were as follows: length 18 to 24 nucleotides, annealing temperature from 58 ° C to 63 ° C. As a result, the system was designed oligonucleotide primers (*Babesia* sp.): direct and inverse BSPF BSPR, which theoretically would allow amplified in the PCR section 18S rRNA gene 6 species of the genus Babesia: *Babesia canis, Babesia divergens, Babesia caballi, Babesia major, Babesia bovis.* Nucleotide sequences of primers are given in Table 1.

For species identification was developed multiplexed system with two lines BSPF, BCANF, and three reverse BSPR, BBOVR, BDIVR. Pairs of primers and the length of PCR products, allowing species-identification Three species of the genus *Babesia* are given in Table 3. This system is also used oligonucleotide primers for the general definition of the genus Babesia, and specificity was determined with different primer pairs.

Table 1. Name, nucleotide sequence and characteristics of primers for multiplexed PCR diagnostic test system of the genus Babesia

Num.	Primer	Sequence (5* -3*)	Length (bp)	Annealing temperature (˚ C)	CG (%)
1	BCANF	gtgacccaaaccctcaccaga	21	59.0	57.1
2	BSPF	ccattggagggcaagtctggt	21	59.4	57.1
3	BDIVR	tcccaaaacceaactecaatctcc	24	59.9	50
4	BBOVR	ccaaagtcaaccaacggtacgaca	24	59,3	50
5	BSPR	acgaatgcccccaaccgtt	19	59.6	57.9

Bold nucleic acids from biological material was carried out using reagent "Chelex-100," according to the JMA 85.2-37-206:2004. Biomaterials are samples of venous blood from different animal species. Amplification was performed on a programmable thermostat TERTSYK-2 (DNA Technologies, Russia) using a set of agents' TAPOTYLY "(HosNYY Genetics microorganisms, Russia). PCR products were analyzed in 8% polyacrylamide and 2% agarose gels. As molecular weight markers using DNA plasmid *pBR*322, Hydrolyzed endonuclease *Msp*I and *pU*C19, Hydrolyzed endonuclease *Msp*I. Visualization of amplification products was performed by ethidium bromide staining of gels and photographing the trans-porthole in ultraviolet light.

Studies. For different members of the genus *Babesia* by different lengths of DNA fragments amplified - from 299 to 268 base pairs, which were due to slightly different content is conservative areas of 18S rRNA gene.

In order to diagnose as many members of the genus *Babesia*, was used pair of primers BSPF / BSPR. Nucleotide sequence of these oligonucleotide primers given in Table 1. This system allows the primers in the PCR amplified area of a certain size 18S rRNA gene representatives of six species of the genus *Babesia*, (*Babesia* sp.) Table 2.

Table 2. Length PCR products members of the genus Babesia, allowing amplified using primer pair BSPF / BSPR (Babesia sp.)

	View	Most international database	of PCR product (bp)
1	Babesia canis	EU711061	298 bp
	Babesia canis	FJ200218	298 bp
	Babesia canis	EU 622793	298 bp
2	Babesia divergens	EU182595	298 bp
	Babesia divergens	DQ866843	298 bp
	Babesia divergens	DQ866844	298 bp
3	Babesia caballi	EU888901	287 bp
	Babesia caballi	EU642514	287 bp
4	Babesia major	EU622 907	287 bp
	Babesia sp. CS	EU622 824	285 bp
5	Babesia bigemina	DQ785311	284 bp
6	Babesia bovis	FJ426364	268 bp
	Babesia bovis	EF458215	270 bp
	Babesia bovis	EF458214	267 bp
	Babesia bovis	EF643475	268 bp
	Babesia bovis	EF643473	268 bp
	Babesia bovis	EF643466	268 bp
	Babesia bovis	AY150059	267 bp
	Babesia bovis	L19078	267 bp
	Babesia bovis	EU407240	267 bp
	Theileria equi	EU888906	Not amplified

Pairs of primers and the length of PCR products, allowing species-identification species *Babesia canis, Babesia divergens* and *Babesia bovis* are shown in Table 3.

Table 3. Pairs of primers and the length of PCR products, allowing species-identification Three species of the genus Babesia.

Num.	A pair of primers	View	Length of PCR product (bp)	Notes
1	BCANF / BSPR	Babesia canis	325	Species-specific
2	BSPF / BBOVR	Babesia divergens	146	Species-specific

3	BSPF / BDIVR	Babesia bovis	233	Species-specific

Checking on clinical biomaterials diagnostic PCR test systems showed high specificity amplification plot conservative 18S rRNA gene of the genus Babesia and specific areas of 18S rRNA gene type Babesia canis. Section amplification products shown in Fig. 1.

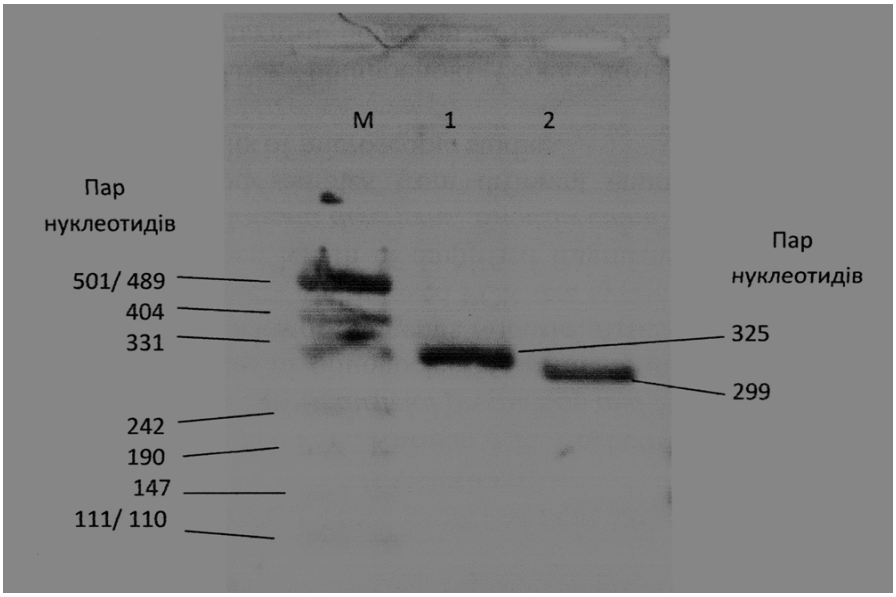

Figure 1. Electrophoretic separation in 2% agarose gels, PCR products: M - molecular weight marker pUC 19 / Msp I, 1 - PCR product specific section 18S rRNA gene Babesia canis, 2 - PCR product of the conservative section 18S rRNA gene of the genus Babesia.

Test multiplexed PCR test systems was carried out in laboratory conditions by examining blood samples of animals, including diagnosis of babesiosis on which was installed using the microscope. Overall studied 342 blood samples. Data on plant species biological samples is shown in Table 4.

Table 4. Number of samples tested by PCR for the animal species

Type of animal	Number of samples tested	Number of positive samples	The key to the results of microscopy,%
Dog	168	112	100
Horse	92	58	100
Cattle	67	41	100
Small Cattle	8	1	100
Man	7	0	100
Total	342	212	

As can be seen from the table babezy genetic material was detected in 100% of blood samples where babeziyi were detected by microscopy.

Conclusions and prospects for research. Thus designed, constructed and tested multiplexed PCR test system for species identification of three species of the genus *Babesia*. Dimensions amplified fragments of 18S rRNA gene plots obtained by experimental verification PCR test system coincided with dimensions that theoretically predicted.

Using oligonucleotide primers BSPF, BSPR to amplify conservative section 18S rRNA gene, which theoretically allow for general identification of the genus *Babesia*. With a size of PCR fragments also make preliminary identification. 299 bp (*Babesia canis, Babesia divergens*), 285p.n. (*Babesia caballi, Babesia major*), 268 bp (*Babesia bovis*).

Multiplex PCR test system for species identification of the genus Babesia can continue to use both in research and for diagnosis of babesiosis animals.

BIBLIOGRAPHY

1. Babesia microti-group parasites compared phylogenettcally by complete sequencing of the CCTeta gene in 36 isolates / [R. Nakajima, M. Tsuji, K. Oda, A. Zamoto-Niikura et all] / / J. Vet. Med. Sci. - 2009. - V.71 (l). - P. 55-68.

2. Specific and Highly Sensitive Primers for PCR Detection of Babesia bovis / [Nutcha Patarapadungkit, Suporn Nuchadomrong, Nison Sattayasai, Patchima Indrakamhang et all] / / ScienceAsia. - 2004. - V 30. - R. 67-73.

3. The use of different diagnostic tools for Babesia and Theileria parasites in cattle in Menofia, Egypt / [Mohamed Nayel, Khaled Mohamed El-Dakhly, Mahmoud Aboulaila, Ahmed Elsify et all] / / Parasitology Research. - 2012. - V. 111. - R. 1019-1024.

4. PCR-based detection of Babesia bovis and Babesia bigemina in their natural host Boophilus microplus and cattle / [T. Oliveira-Sequeira, M. Oliveira, Jr. J. Araujo, A. Amarante] / / Int. J. Parasitol. - 2005. - V. 35. - 105-111.

5. *Kumar S.* MEGA3: Integrated software for Molecular Evolutionary Genetics Analysis andsequence alignment / S. Kumar, K. Tamura, M. Nei / / Briefings In Bioinformatics. - 2004. - Vol. 5, № 2. - R. 150-163.

6. "FastPCR" [electronic resource] - System Requirements: Microsoft Windows 7/Vista/2003/XP/2000 (32-Bit (x86) or 64-Bit), 2 GB RAM, 1280x800 minimum screen resolution. - Mode of access: http://primerdigital.com/fastpcr.html