A New Specific Sequence to Distinguish B.canis From Other Brucella by PCR

YinBo YE
Shenyang Agricultural University
JiangHua YANG
Shenyang Agricultural University
DongLiang LI
Beijing Animal Disease Prevention & Control Center
LiHua HAO
Division of the Standards, China Institute of Veterinary Drug Control
Zhao ZHANG
Shenyang Agricultural University
SiYao MEI
Shenyang Agricultural University
Huan ZHANG
Shenyang Agricultural University
FangYuan DU
Shenyang Agricultural University
BaoShan LIU
Shenyang Agricultural University
ZeLiang CHEN (zeliangchen@yahoo.com)
Shenyang Agricultural University

Research Article

Keywords: B.canis, Brucellosis, other Brucella, PCR, specific sequence

Posted Date: April 22nd, 2021

DOI: https://doi.org/10.21203/rs.3.rs-413085/v1

Abstract

Background: Brucellosis is a zoonotic disease worldwide. The increasing number of pet dogs has raised new risk of people getting canine *brucella* with absent or mild symptom. Besides, the canine brucellosis can be caused by other *brucella* species, so their infection could be omitted by the PCR method. The present PCR methods can only detect canine *brucella*, by which cases infected with other *brucella* would appear negative. It's an urge to develop a specific PCR assay for detecting canine Brucellosis, Whether the pathogen was *B.canis* or any other *Brucella*.

Resaults: a differential sequence of *B.canis* were found by genome comparison analysis and were analyzed by BLAST. Then a PCR method was established using specific primers in the sequence and tested for clinical application. It could detect canine brucellosis caused by *B.canis* or other *Brucella* species with 310-bp and 413-bp product, respectively. The developed PCR method had specificity for non-*brucella* and a sensitivity of 100 copies of *Brucella* DNA. The detection accuracy verified with spiked samples was 95.5% (21/22) for *B.canis* and 100% (22/22) for other *brucella*.

Conclusions: The study found a specific sequence of *B.canis* and developed a PCR detection method to detect canine brucellosis caused by *B.canis* or other *Brucella* species. The method established in this study will more comprehensively detect the pathogen of canine brucellosis and provide important methods and means for preventing and controlling this disease.

Introduction

The genus *Brucella* is one kind of the most widespread bacteria, inducing Brucellosis. They have been gradually expanded by discovering strains from wildlife animal species, such as amphibians and fish[1]. Four major pathogenic *Brucella* species causing disease in humans are *B.abortus* (cattle, buffalo), *B.melitensis* (goats, sheep, camels), *B.suis* (pigs), and *B.canis* (Dogs)[2]. Annually more than 500,000 new brucellosis cases are diagnosed worldwide[3].

As the primary pathogen of brucellosis in dogs, *B.canis* can be transmitted to humans by infected dogs or their secretions. Unlike the infection by other *Brucella* species, the infection symptoms with *B.canis* are absent or mild[4]. However, endocarditis or meningitis may develop in some cases[5]. So, the human infection of *Brucella* canine remains a concern, especially as pets are being raised in large numbers.

The isolation and culture are the most accurate method for brucellosis detection and species identification by amino sugar quinovosamine assay[6]. However, they are time-consuming and need to be performed in a laboratory with biosafety level III. Its effectiveness is affected by the infected animal's bacteremia level[7]. Molecular biological detection technology has the characteristics of safety and reliability, high sensitivity, strong specificity and simple operation, and has begun to be popularized and applied to detect *Brucella* [8, 9].

Now there were a variety of PCR methods for detecting canine *Brucella*[10, 11], such as bruce-ladder[11] and multilocus variable-number tandem-repeat (VNTR) analysis (MLVA) procedures[12]. Most of them distinguished *B.canis* by multiple amplification products, which required a lot of time to remember each Brucella species' amplified bands. More to the point, it needed high quality and concentration genomic DNA, limiting the choice of clinical specimens[13].

In 2014, a PCR method for only detecting *B.canis* was established based on the 12bp deletion of BCAN_B0548 region 530056 site in chromosome II of *B.canis* ATCC 23365[13]. However, it cannot detect other *Brucella* species and omit probably the *brucella* infection by other *Brucella* species[14].

In this study, we found a specific sequence of *B.canis* by comparing the genomes of *B.canis* and *B.melitensis* and BLASTing in the GeneBank, which is reverse complementary in other *Brucella* species. PCR of three primers designed based on this sequence can specifically detect canine *Brucella* and other *Brucella*, which simplified the clinical identification and diagnosis of canine brucellosis and is very conducive to its prevention and control to reduce human infection.

Materials And Methods

Strains and DNA extraction

All the strains used in this experiment were listed in Table 1. The DNA of *B.abortus* strains 2308, *B.melitensisbv.* 1 str. 16M and *B.canis* strain RM6/66 were donated by the China Institute of Veterinary Drug Control. *B.melitensis* strains M5, *B.abortus* strain A19 and *B.suis* strain S2 were purchased from Tecon Biology Co. Ltd, Xinjiang. Caine Vanguard® Plus 5-CVL vaccine and feline Fel-O-Vax® PCT vaccine (Zoetis, USA) were purchased from a local pet hospital. Other common bacterial strains are preserved in this laboratory. Their genetic DNA was extracted with MiniBEST Bacteria Genomic DNA Extraction Kit (Takara, Dalian, China) According to the manufacturer's instructions and measured with an ultraviolet spectrophotometer. The copies of the genome were counted online on the website http://scienceprimer.com/copy-number-calculator-for-realtime-pcr. The extracted DNA was stored at -20°C until use. 86 Cannie blood samples were collected in the pet hospitals in Shenyang. Their DNA was extracted with the MiniBEST Whole Blood Genomic DNA Extraction Kit (Takara, Dalian, China) and stored at -20°C. Taq PCR MasterMix was purchased from Vazyme Biotech Co., Ltd, Nanjing, China.

Screening of the specific sequence of *B.canis*

Genome sequences of *B.canis* and other species were downloaded from the NCBI database. Sequences were analyzed by the multiple genome alignment software Mauve 20150226 (The Darling lab at the University of Technology Sydney). The list of gaps and single nucleotide polymorphisms (SNPs) of alignment results of chromosomes 1 and 2 were exported. BLAST was used to compare the candidate gaps of the two strains for the specific differential sequences of the *B.canis*. The potential sequences in both genomes of *B.canis* and *B.melitensis* were aligned by the software DNAMAN 7.

primer design

Primer-blast on the NCBI website was used to design the primers and make the appropriate adjustments to obtain specific primers. The designed primers were synthesized by Sangon Biotech Co., Ltd (Shanghai, China).

Optimization of the PCR Amplification condition

In the PCR reaction, 2×Taq PCR MasterMix ten μL, each primer (10 μM) 1 μL and five μL water were mixed in the 200 μL PCR tube. Then one μL *Brucella* DNA template or one μL distilled water were added as templates or negative control. The PCR amplification conditions were as follows: pre-denaturation at 94°C for 5 minutes, then 35 cycles at 95 °C for 15 s, 60 °C for 15 s and 72 °C for 30 s, followed by a final extension at 72 °C for 10 min. The PCR amplification products were identified by electrophoresis in 1.5% agarose gel.

For determining better amplification conditions, PCR reaction was optimized by annealing in temperature 60°C, 64°C, 68°C, 72°C. The optimum annealing temperature was determined according to the stray band's existence and the amplified band's brightness.

Specificity and sensitivity of the assay

The DNA template was diluted from 10^4 to 1 copies/μL with sterilized distilled water. One μL diluted DNA was used as the template in PCR amplification. Electrophoresis was performed to determine the sensitivity of the PCR assay.

For verifying the PCR assay's specificity, DNAs of other *Brucella* strains and bacteria and vaccine in Table 1 were used as templates for PCR amplification under the best annealing temperature. The amplification products were analyzed in a 1.5% electrophoresis gel and observed under ultraviolet light.

Detection and verification

For evaluating the clinical efficacy of the established PCR method, 66 DNAs from *B.canis*-negative blood samples were spiked double-blindly with the DNA of *B.canis* and other *Brucella* were tested. The number of samples spiked with positive brucella DNAs was listed in Table 3. The spiked samples were detected by the developed PCR and the results were analyzed by 1.5% electrophoresis gel.

Results

Analysis of the genome

Genome sequences of *B.canis* strain RM6/66 (NZ_CP007758.1, NZ_CP007759.1) and *B.melitensisbv.* 1 str. 16M (NZ_CP007763.1, NZ_CP007762.1) strains were downloaded from the NCBI database. Chromosome I (NZ_CP007758.1, NZ_CP007763.1) and chromosome II (NZ_CP007759.1, NZ_CP007762.1) sequences of *B.canis* strain RM6/66 and *B.melitensisbv.* 1 str. 16M were analyzed by the multiple genome alignment software Mauve 20150226, respectively. Compared with 16M, both chromosomes I and II of RM6/66 genome had gene rearrangement (Fig. 1A, 1B), but the frequency of chromosome II rearrangement was higher than that of chromosome I.

The list of gaps and single nucleotide polymorphisms (SNPs) of alignment results of chromosomes 1 and 2 were exported from the mauve program. There are 204 gaps, 231 inserts and 5143 SNPs in chromosome I, while there are 140 gaps, 169 inserts and 3113 SNPs in chromosome II (Fig. 1C). Chromosome 1 is more diverse than Chromosome 2.

BLAST was used to compare the candidate gaps of the two strains for the specific differential sequences of the *B.canis*. It was found that a 132bp gap at 943403rd site on *B.canis* chromosome 1 corresponds to the same gap at 303367th site on *B.melitensis* chromosome 1 (Fig.2A). By aligning, the gap sequence in both genomes of *B.canis* and *B.melitensis* has a very low similarity (Fig.2B). Further analysis found that they are reverse complementary sequences (Fig.2C). The gap and adjacent sequence's alignment showed good specificity in BLAST, only having a high similarity and high score with *B.canis* (Fig.2D). It was suggested that this sequence is a characteristic of *B.canis* and can be potential for identifying *B.canis*.

Primer design

Three primers (Table 2) in the region 943226-943581 of chromosome I of *B.canis* strain RM6/66 were designed by the online primers design programs Primer-BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi). The position of each primer was shown in figure 2C. PCR with these three primers should amplify 310bp and 413bp fragments using *B.canis* and other *Brucella* species as templates, respectively.

Optimization of the PCR Amplification condition

For determining better amplification, annealing temperature in PCR was optimized. The results showed that evident PCR bands could be seen at annealing temperature 60 °C to 68 °C (Fig.3A). However, the amplification band at 64 °C was the brightest and the amplification effect was the highest. So, 64 °C was used as the reaction temperature in the subsequent specificity and sensitivity tests.

The sensitivity of the assay

For determining the sensitivity of the PCR method, it was executed using the gradient dilution DNA of *B.canis* strain RM6/66 as a template. The results showed that the reaction solution with 10^4-10^2 copies of DNA showed PCR amplification bands in the electrophoresis (Fig. 3B), indicating that the established PCR assay could be detected a minimum of 100 copies of *Brucella* DNA.

The specificity of the assay

Respectively using DNA of the *B.canis* strain RM6/66, strain A19, *B.suis* strain S2, *B.melitensis* strain 16M, *B.abortus* strain 2308, *B.suis* strain 1330, Vanguard® Plus 5-CVL vaccine, Fel-O-Vax® PCT vaccine (Zoetis, USA) and other bacterial as a template, PCR assay was conducted with best annealing temperature 64°C. The results show that the reaction product with the *B.canis* DNA and other *brucella* DNA existed a specific 310bp and 413bp band in the electrophoresis, respectively (Fig. 3C). At the same time, the reaction products with other DNA are no specific bands. It showed that the established PCR detection method had excellent specificity. Meantime, it can not only detect *Brucella* but also distinguish *B.canis* from other *Brucella* species.

Detection and verification

For verifying the efficacy of the PCR assay, 66 DNAs of clinical blood samples spiked randomly with the DNA of *B.canis* or other *Brucella* were tested (Fig. 4). The result showed that the developed assay detected 43 of the 44 spiked samples, with a detection accuracy of 95.5% (21/22) for *B.canis* and 100% (22/22) for other *Brucella*, respectively (Table 3). These results indicated that the developed assay had a good feature for the detection of *B.canis* or other *Brucella*.

Discussion

The current study aimed to develop a specific PCR assay for detecting canine Brucellosis, Whether the pathogen was *B.canis* or any other *Brucella*. Firstly, a differential sequence of *B.canis* was found by genome comparison analysis and was analyzed by BLAST. Then a differential PCR method was established using specific primers in the sequence and tested for clinical application. The developed PCR method had good specificity and sensitivity and can be used to detect canine brucellosis caused by various *Brucella*, which is conducive to the prevention and control of canine brucellosis and the protection of human safety.

With the development of sequencing, databases, and networking technologies, vast amounts of genomic and sequence data are now available at individual terminals. How to make good use of these data has become an essential aspect of improving research efficiency. Desktop or web bioinformatics software addresses this problem very well. Multiple Genome Alignment Software Mauve can quickly analyze rearrangements, insertions, deletions and changes between genome sequences and determine the differences between genes from a macro and micro perspective[15, 16]. BLAST is a general alignment procedure that compares sequences similar to target sequences in Genebank databases to discover target sequences' specificity[17]. The combination of the two software can be used to screen target sequences. Primer-Blast is a procedure for Primer design and comparison, which can effectively analyze the specificity of primers [18]. The combined use of these software can effectively improve the specificity and success rate of PCR, which is widely used[19]. The same process was used in this study and satisfactory results were obtained.

Molecular biological detection of *Brucella* is abundant[9, 20, 21]. However, there were a few PCR methods for detecting canine *Brucella*[10, 11]. Bruce-ladder assay is a common method[11], which distinguished *B.canis* by multiple amplification products. However, it required much time to remember each *brucella* species' amplified bands and high quality and concentration genomic DNA limiting clinical specimens' choice[13]. A PCR method for only detecting *B.canis* was established in 2014, which was based on the 12bp deletion of chromosome II of *B.canis* ATCC 23365 and had a detection limit of 3×10^3 colony-forming units (CFU)[13]. However, the canine brucellosis can be caused by other *Brucella* species[14], so their infection could be omitted by the PCR method. In this study, a *B.canis* specific sequence was found, which showed reverse complementary in other *Brucella*. The established PCR based on the specific sequence can detect *B.canis* and other *Brucella* species simultaneously without amplifying other common bacteria and viruses. Its detection limit was 100 copies of the *B.canis* genome, higher than the above PCR method.The PCR assay was evaluated with spiked DNA samples. Of 66 DNA samples, the accuracy was 97.5% (43/44). It might be a low concentration that one sample was identified as a false negative. This work could give a more convenient verification for PCR method without comparative standard methods due to the spiked samples.

B.canis mostly cause canine brucellosis. However, other commonly exposed *brucella* strains, such as *B.suis*, *B.abortus* and *B.melitensis*, can also cause it[14]. Unlike *B.canis*, they have high pathogenicity to humans. People who contact with dogs infected these *Brucella* are at high risk. Therefore, it is necessary to strengthen the detection of brucellosis. The method established in this study will more comprehensively detect the pathogen of canine brucellosis and provide important methods and means for preventing and controlling the disease.

In conclusion, the study found a specif sequence of *B.canis* and developed a PCR detection method to detect canine brucellosis caused by *B.canis* or other *Brucella* species. It will serve for the prevention and control of canine brucellosis to reduce human brucellosis.

Declarations

Ethics approval and consent to participate

The experiment was licensed by the Institutional Animal Care and Use Committee of Shenyang Agricultural University (IACUC Issue No.2020070307). Informed consent was obtained from owners for animal sample collection. All methods were carried out in accordance with relevant guidelines and regulations.

Consent for publication

Not applicable.

Availability of data and materials

The datasets generated and/or analysed during the current study are available in the NCBI database(https://www.ncbi.nlm.nih.gov/nuccore/NZ_CP007758.1;https://www.ncbi.nlm.nih.gov/nuccore/NZ_CP007759.1;https://www.ncbi.nlm.nih.gov/nucc

Competing interests

The authors declare that they have no competing interests.

Funding

This work was supported by National Key Research and Development Program Projects of China (2017YFD0500901, 2017YFD0500305), the National Key Program for Infectious Diseases of China (2018ZX10101002-002), the State Key Program of National Natural Science of China (U1808202), NSFC International (regional) cooperation and exchange program (31961143024), Major science and technology projects of Inner Mongolia of China.

Authors' contributions

BS LIU and ZL CHEN conceived the study. YB YE and JH YANG directed the study. DL LI and LH HAO analyzed the data. Z ZHANG and SY MEI performed the PCR. H ZHANG and FY DU wrote the manuscript.

References

1. Cloeckaert A, Vergnaud G, Zygmunt MS. Omp2b Porin Alteration in the Course of Evolution of Brucella spp. Front Microbiol. 2020;11:284. https://doi.org/10.3389/fmicb.2020.00284.
2. Kyebambe PS. Acute brucella meningomyeloencephalo-spondylosis in a teenage male. Afr Health Sci. 2005;5(1):69-72-.
3. Pisarenko SV, Kovalev DA, Volynkina AS, Ponomarenko DG, Rusanova DV, Zharinova NV, Khachaturova AA, Tokareva LE, Khvoynova IG, Kulichenko AN. Global evolution and phylogeography of Brucella melitensis strains. BMC Genomics. 2018;19(1):353. https://doi.org/10.1186/s12864-018-4762-2.
4. Yüksekkaya S, Aras Z, U?an US. Investigation of Brucella canis Seroprevalence in Brucellosis Suspected Cases. Mikrobiyol Bul. 2013;47(1):152-7-.
5. Sayan M, Erdenli? S, Etiler N. Investigation of Brucella canis seropositivity by in-house slide agglutination test antigen in healthy blood donors. Mikrobiyol Bul. 2011;45(4):655-63-.
6. Ohishi K, Abe E, Amano M, Miyazaki N, Boltunov A, Katsumata E, Maruyama T. Detection of serum antibodies to Brucella in Russian aquatic mammals. J Vet Med Sci. 2018:-.
7. Camargo-Castañeda AM, Stranahan LW, Edwards JF, Garcia-Gonzalez DG, Roa L, Avila-Granados LM, Hensel ME, Arenas-Gamboa AM. Characterization of epididymal and testicular histologic lesions and use of immunohistochemistry and PCR on formalin-fixed tissues to detect Brucella canis in male dogs. J Vet Diagn Invest. 2021:1040638720986883. https://doi.org/10.1177/1040638720986883.
8. Ren H, Yang M, Zhang G, Liu S, Wang X, Ke Y, Du X, Wang Z, Huang L, Liu C, Chen Z. Development of a rapid recombinase polymerase amplification assay for detection of Brucella in blood samples. Mol Cell Probes. 2016;30(2):122-4. https://doi.org/10.1016/j.mcp.2016.02.007.
9. Bhat IA, Mashooq M, Kumar D, Varshney R, Rathore R. Development of probe based real time loop mediated isothermal amplification for detection of Brucella. J Appl Microbiol. 2018:-.
10. Hinić V, Brodard I, Thomann A, Cvetnić Z, Makaya PV, Frey J, Abril C. Novel identification and differentiation of Brucella melitensis, B. abortus, B. suis, B. ovis, B. canis, and B. neotomae suitable for both conventional and real-time PCR systems. J Microbiol Methods. 2008;75(2):375-8. https://doi.org/10.1016/j.mimet.2008.07.002.
11. López-Goñi I, García-Yoldi D, Marín CM, de Miguel MJ, Barquero-Calvo E, Guzmán-Verri C, Albert D, Garin-Bastuji B. New Bruce-ladder multiplex PCR assay for the biovar typing of Brucella suis and the discrimination of Brucella suis and Brucella canis. Vet Microbiol. 2011;154(1-2):152-5. https://doi.org/10.1016/j.vetmic.2011.06.035.
12. Daugaliyeva A, Sultanov A, Usserbayev B, Baramova S, Modesto P, Adambayeva A, Acutis PL, Peletto S. Genotyping of Brucella melitensis and Brucella abortus strains in Kazakhstan using MLVA-15. Infect Genet Evol. 2018;58:135-44-.
13. Kang SI, Lee SE, Kim JY, Lee K, Kim JW, Lee HK, Sung SR, Heo YR, Jung SC, Her M. A new Brucella canis species-specific PCR assay for the diagnosis of canine brucellosis. Comp Immunol Microbiol Infect Dis. 2014;37(4):237-41. https://doi.org/10.1016/j.cimid.2014.07.003.
14. Philippon A, Roumy B, Renoux G. [A case of canine brucellosis due to Brucella abortus]. Bull Acad Vet Fr. 1969;42(9):923-8.
15. Darling AC, Mau B, Blattner FR, Perna NT. Mauve: multiple alignment of conserved genomic sequence with rearrangements. Genome Res. 2004;14(7):1394-403. https://doi.org/10.1101/gr.2289704.
16. Rissman AI, Mau B, Biehl BS, Darling AE, Glasner JD, Perna NT. Reordering contigs of draft genomes using the Mauve aligner. Bioinformatics. 2009;25(16):2071-3. https://doi.org/10.1093/bioinformatics/btp356.
17. Camacho C, Coulouris G, Avagyan V, Ma N, Papadopoulos J, Bealer K, Madden TL. BLAST+: architecture and applications. BMC Bioinformatics. 2009;10:421. https://doi.org/10.1186/1471-2105-10-421.
18. Ye J, Coulouris G, Zaretskaya I, Cutcutache I, Rozen S, Madden TL. Primer-BLAST: a tool to design target-specific primers for polymerase chain reaction. BMC Bioinformatics. 2012;13:134. https://doi.org/10.1186/1471-2105-13-134.
19. Baoshan L, Yinbo Y, Jingbo Z, Yi Z, Jianghua Y, Dawei C, Chi M, Donghai Y, Bohan Y, Rongnian Z, Sheng F, Jun Z, Han X, Chen Z. Combined nucleic acid assays for diagnosis of A19 vaccine-caused human brucellosis. Transbound Emerg Dis. 2020. https://doi.org/10.1111/tbed.13685.
20. Pal D, Boby N, Kumar S, Kaur G, Ali SA, Reboud J, Shrivastava S, Gupta PK, Cooper JM, Chaudhuri P. Visual detection of Brucella in bovine biological samples using DNA-activated gold nanoparticles. PLoS One. 2017;12(7):e0180919-0180919.
21. Hasanzadeh M, Babaie P, Mokhtarzadeh A, Hajizadeh N, Mahboob S. A novel DNA based bioassay toward ultrasensitive detection of Brucella using gold nanoparticles supported histidine: A new platform for the assay of bacteria in the cultured and human biofluids with and without polymerase chain reactions (PCR). Int J Biol Macromol. 2018;120(Pt A):422-30-.

Tables

Table 1 Bacteria strains tested in this study.

Bacterial species		Strain	Source
Brucella abortus		A19	Tecon Biology CO. Ltd
Brucella suis		S2	Tecon Biology CO. Ltd
Brucella melitensis		M5	Tecon Biology CO. Ltd
Brucella canis		RM6/66	CVCC
Brucella abortus		2308	CVCC
Brucella suis		1330	CVCC
Brucella melitensis		16M	CVCC
Salmonella enteritidis		CVCC3949	CVCC
Shigella dysenteriae		CVCC1881	CVCC
Pasteurella multocida		CVCC1676	CVCC
Streptococcus hemolyticus		CVCC1886	CVCC
Clostridium perfringens type C		CVCC1147	CVCC
Staphylococcus aureus		CVCC4098	CVCC
Proteus mirabillis		CVCC1969	CVCC
Candida albicans		CVCC3597	CVCC
Streptococcus pyogenes		CVCC1930	CVCC
Streptococcus pneumoniae		CVCC4105	CVCC
Campylobacter jejuni		CVCC3883	CVCC
listeria monocytogenes		CVCC3763	CVCC
pseudomonas aeruginosa		CVCC3795	CVCC
Escherichia coli		DH5a	Our laboratory
Vanguard® Plus 5-CVL vaccine	Canine distemper virus	Snyder Hill	Zoetis, USA
	Canine adenovirus type 1		
	Canine adenovirus type 2	Manhattan	
	Canine parainfluenza virus	NL-CPI-5	
	Canine parvovirus	NL-35-D	
	Canine coronavirus	NL-18	
	Leptospira canicola	C-51	
	Leptospira icterohemorrhaiae	NADL	
Fel-O-Vax® PCT vaccine	Feline Rhinotracheitis virus	605	Zoetis, USA
	Feline Calicivirus	255	
	Feline Panleukopenia virus	Cu-4	

CVCC: Chinese veterinary Culture collection center.

Table 2 Primers and probes used in this study

Primer name	Sequence	Length	Tm	Amplicon length
BSU1	GCAGGTCGTTACCGTCGATC	20	60.86	
BCD	CAATATCCGCAACGCCTCTTG	21	60.00	310
BSD2	CATCAAGCCGCATCGCAGC	19	62.80	413

Table 3 Detection of spiked DNA samples from clinical blood specimen

	Spiked number	Developed PCR	Accuracy
B.*canis*	22	21	95.5%
Other *brucella*	22	22	100%
Negative	22	22	100%
Total	66	65	98.8%

Figures

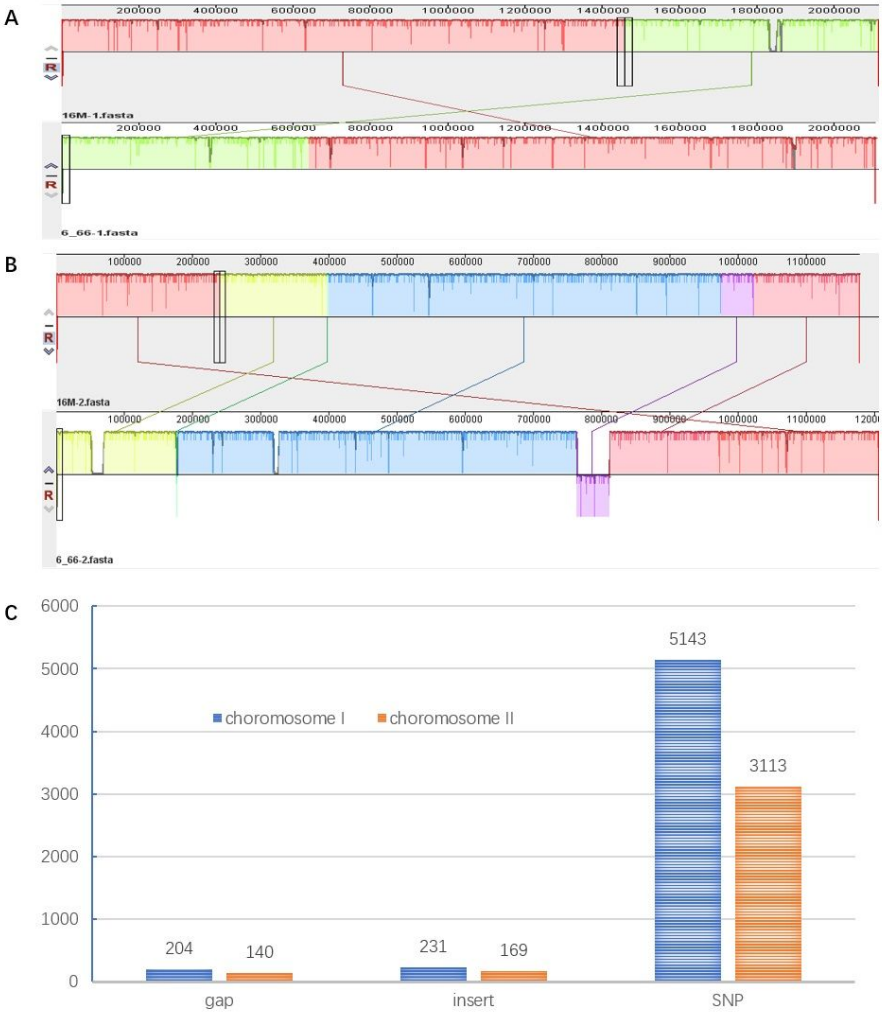

Figure 1

genome analysis of the B.canis strain RM6/66 and B.melitensis 2308. A. the alignment of the chromosome I. B. the alignment of the chromosome II. C. differential statistics of the B.canis strain RM6/66 genomes in comparison to B.melitensis 2308 genome.

Figure 2

The analysis of the specific sequence in the B.canis. A. the region of the specific sequence in the genome alignment. B. the alignment of the specific sequences in B.canis and the corresponding sequence in B.melitensis. The red box represents the sequences in C. Red sequences represent the designed primers. C. the alignment of the specific sequence in B.canis and corresponding reverse-complementary sequence in B.melitensis. D. BLAST result of the specific sequence.

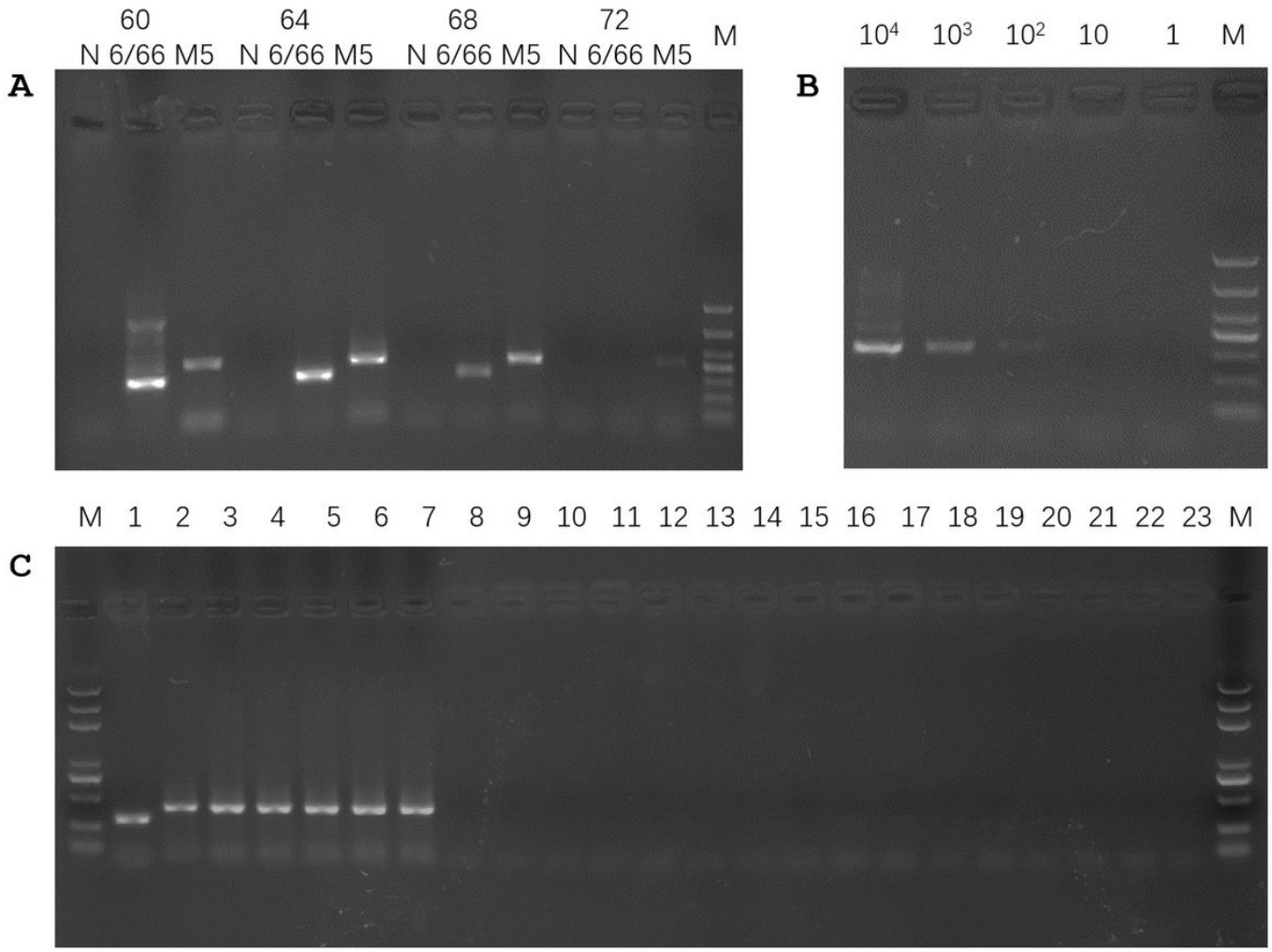

Figure 3

PCR assay based on the specific sequence. A. Annealing temperature optimization. M, DL1000 DNA Marker. B. Sensitivity assay. M, DL1000 DNA Marker. C. Specificity assay. M, DL2000 DNA Marker; 1, B.canis strain RM6/66; 2, B.abortus strain A19; 3, B.suis strain S2; 4, B.melitensis strain M5; 5, B.abortus strain 2308; 6, B.suis strain 1330; 7, B.melitensis strain 16M; 8, Vanguard® Plus 5-CVL vaccine; 9, Fel-O-Vax® PCT vaccine (Zoetis, USA); 10, E.coli; 11, Salmonella enteritidis; 12, Shigella dysenteriae; 13, Pasteurella multocida; 14, Streptococcus hemolyticus; 15, Clostridium perfringens type C;16, Staphylococcus aureus; 17, Proteus mirabillis; 18, Candida albicans; 19, Streptococcus pyogenes; 20, Streptococcus pneumoniae; 20, Campylobacter jejuni; 21, Listeria monocytogenes; 22, Pseudomonas aeruginosa; 23, negative contral.

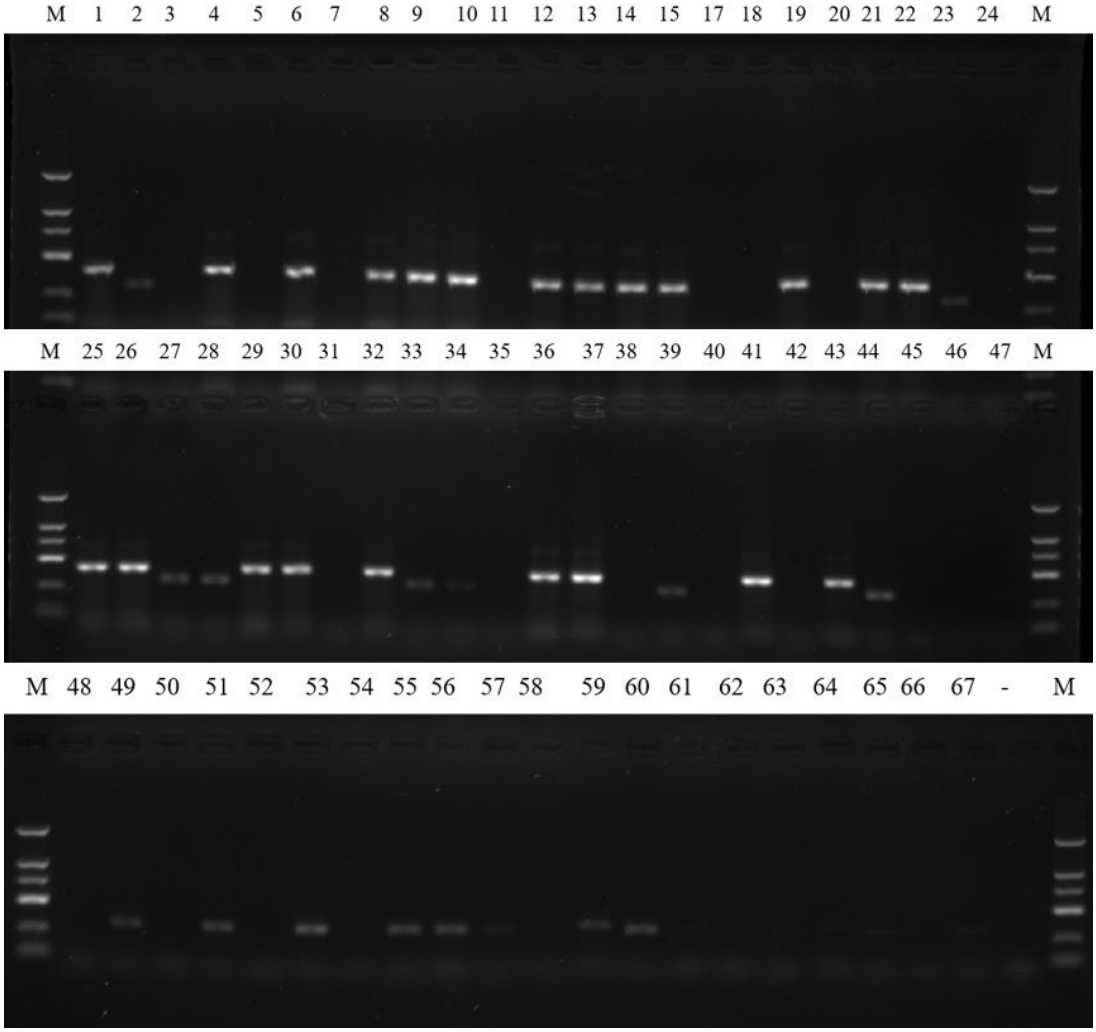

Figure 4

Detection on spiked samples with developed PCR. M, DL2000 DNA Marker; 1-67, DNA samples, NO. 16 missed.