

METHODOLOGY ARTICLE

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A novel PCR-based point-of-care method enables rapid, sensitive and reliable diagnosis of *Babesia gibsoni* infection in dogs

I-Li Liu^{1†}, Nai-Yu Chi^{2†}, Chia-Ling Chang², Ming-Long Hung², Chun-Ta Chiu³ and Hui-Wen Chen^{3*} 

Abstract

Background: *Babesia gibsoni* (*B. gibsoni*) is an intraerythrocytic protozoan parasite of dogs that causes fever and hemolytic illness. A timely diagnosis is essential for the disease management.

Results: Here, we report a QubeMDx PCR system which enables a rapid, sensitive and reliable diagnosis of *B. gibsoni* near the dog patient. Within 30 min, this diagnostic assay was able to detect as low as 0.002% parasitemia of the dog blood. Using clinical samples, this new assay was validated to demonstrate 100% agreement with real-time PCR.

Conclusions: This novel diagnostic method provides a reliable point-of-care test to assist in the identification of *B. gibsoni*.

Keywords: *Babesia gibsoni*, Point-of-care, QubeMDx PCR system, Real-time PCR

Background

Babesia gibsoni (*B. gibsoni*) is a widespread tick-transmitted protozoan blood parasite that was first identified in 1910 in dogs and jackals from India and now prevalent in many regions, including Taiwan [1, 2] and other Asian countries such as Japan [3], Korea [4], China [5], Bangladesh [6], India [7] and Malaysia [8]. *B. gibsoni* infection is frequently found in companion dogs and presents a serious clinical problem in the world. The clinical severity of *B. gibsoni* infection in dogs is age-dependent and potentially life-threatening with clinical symptoms ranging from remittent fever and severe hemolytic illness. Therefore, the diagnosis of this disease and the detection of dogs that carry this blood parasite are very important [9–12].

Generally, the standard method for definitive means of diagnosis of babesiosis is microscopic examination of blood smears following Giemsa-staining for visual

identification of parasites. Although this microscopic method is an inexpensive diagnostic test, the detection of *B. gibsoni* in erythrocytes on blood smears is sometimes difficult and may be error-prone. As *B. gibsoni* is a small and pleomorphic organism (1–3 µm in diameter), unlike the large, paired and teardrop-shaped *Babesia canis*, a highly experienced diagnostic skill is required to distinguish it from artifacts under the microscope, and it becomes even more challenging to find the parasites when an infected dog has low levels of parasitemia [12]. On the other hand, the molecular diagnosis detecting the DNA of *B. gibsoni* in canine blood samples by polymerase chain reaction (PCR) offers high degrees of detection sensitivity and specificity. Various conventional [13], nested [14] and real-time [15] PCR assays detecting *B. gibsoni* infection in dogs have been developed. However, these assays mostly require a relatively expensive thermo cycler and well-trained personnel, which may delay results and add costs. As a result, a rapid, affordable and user-friendly platform is needed for a point-of-care (POC) detection of *B. gibsoni* in daily veterinary

* Correspondence: winnichen@ntu.edu.tw

[†]I-Li Liu and Nai-Yu Chi contributed equally to this work.

³Department of Veterinary Medicine, National Taiwan University, 1 Sec 4 Roosevelt Rd, Taipei, Taiwan

Full list of author information is available at the end of the article



practice to facilitate animal care and disease management.

The recently developed QubeMDx (Credo Biomedical Pte. Ltd., Singapore) is a portable diagnostic system (12.1 × 10.9 × 14.7 cm, H × W × D) using reaction tubes prefilled with fluorescence-based reagents to facilitate the real-time PCR detection process within 15 min. The programmed device allows users to perform four different targeted DNA or RNA molecular diagnosis in a given round of reaction with minimal training. The purpose of the present study is to evaluate the QubeMDx PCR system as a practical and accurate method for the diagnosis of *B. gibsoni* infection in dog by using clinical canine blood samples and comparing to a standard real-time PCR assay.

Results

As illustrated in Fig. 1a, the DNA extraction can be completed within 8 min. The resultant DNA solution was subsequently assayed using the QubeMDx PCR device (Fig. 1b). After 15-min reaction time, the test result for *B. gibsoni* was displayed in the panel A, either positive (+) or negative (-) on the screen, and the test validity (internal control) was displayed in the panel B (Fig. 1c). Evaluated using 28 reference test (real-time PCR)-positive clinical samples, the sensitivity of the QubeMDx PCR was 100%. The specificity was also

100%, calculated using 27 reference test-negative samples. The *Kappa* value was 1, indicating highly reproducible results between these two methods. Furthermore, among different levels of parasitemia analyzed from 28 positive dog samples (Table 1), the QubeMDx PCR system successfully detected the *B. gibsoni* ranging from 94 to 398,000 parasites/μL of blood (0.002 to 8% of parasitemia). Importantly, no discrepancy between these two methods was found among these clinical samples containing low levels of parasitemia (16/28) (< 5000 parasites/μL of blood or parasitemia < 0.1%). Using ten-fold serially diluted blood sample (dog #25), the QubeMDx PCR was shown to be able to detect 0.00576%, but not 0.000576% of parasitemia (Fig. 2). Collectively, DNA rapid extraction and QubeMDx PCR allow for detection of the *B. gibsoni* DNA with a sensitivity limit in between 0.000576 and 0.002% of parasitemia. The analytical specificity of the QubeMDx PCR for *B. gibsoni* detection was further assessed using blood samples positive for *Anaplasma platys*, *Ehrlichia canis* or *Babesia canis*. As indicated in Additional file 1: Table S1, no false positive detection results were obtained from canine blood samples infected with other three pathogens.

Discussion

In this study, a novel and reliable POC diagnostic method for *B. gibsoni* detection was reported. By

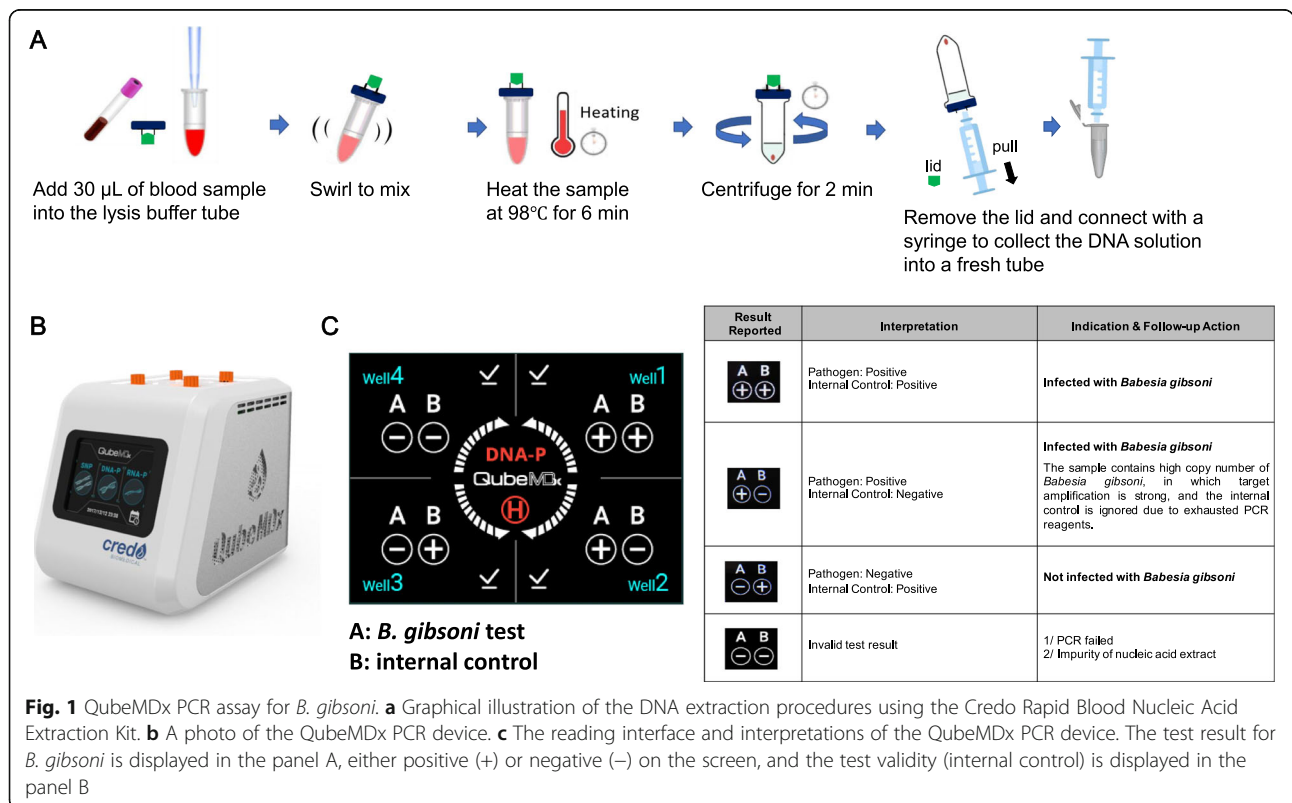
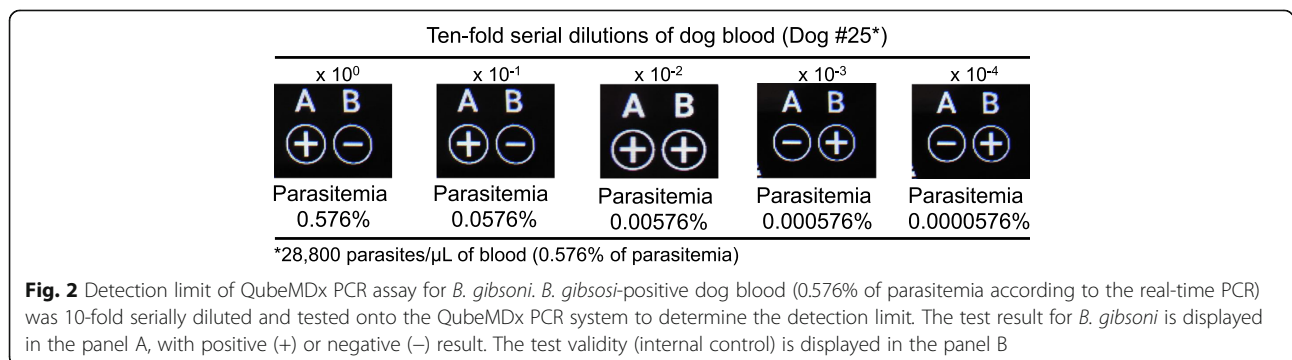


Table 1 *B. gibsoni*-positive dog blood samples included in this study

| No. | Age (yr) | Sex | Breed | PCV ^a (%) | Test results | | Parasites/ μL of blood ^b |
|-----|----------|-----|---------------------|----------------------|--------------|---------------|----------------------------------------|
| | | | | | QubeMDx | Real-time PCR | |
| 1 | 8 | M | Mixed | n/a | + | + | 94 |
| 2 | 7 | M | n/a | 32.3 | + | + | 98 |
| 3 | 12 | F | Mixed | 28.8 | + | + | 103 |
| 4 | 13 | M | Mixed | 21.3 | + | + | 188 |
| 5 | 4.3 | F | Mixed | 50.5 | + | + | 249 |
| 6 | 1.7 | M | Miniature Schnauzer | 23.6 | + | + | 261 |
| 7 | 14 | F | Mixed | 27.3 | + | + | 422 |
| 8 | 9 | F | Mixed | 31.8 | + | + | 798 |
| 9 | 2 | F | Mixed | n/a | + | + | 1030 |
| 10 | 5 | M | Poodle | 37.8 | + | + | 1150 |
| 11 | 8 | M | Mixed | 48.7 | + | + | 1290 |
| 12 | 6 | F | Mixed | n/a | + | + | 1360 |
| 13 | 5 | M | Poodle | 37.7 | + | + | 1870 |
| 14 | 6 | F | Mixed | n/a | + | + | 2180 |
| 15 | 5 | F | Mixed | n/a | + | + | 3610 |
| 16 | 9 | M | Labrador Retriever | 25.9 | + | + | 3840 |
| 17 | 4 | F | Mixed | 43.9 | + | + | 5410 |
| 18 | 6 | M | Poodle | 47.3 | + | + | 8140 |
| 19 | 3 | M | Poodle | 14.6 | + | + | 8140 |
| 20 | 2 | F | Mixed | 51.2 | + | + | 8760 |
| 21 | 0.2 | F | Mixed | 30.3 | + | + | 10,100 |
| 22 | 12 | F | Miniature Schnauzer | 16.7 | + | + | 10,800 |
| 23 | 9 | F | Mixed | n/a | + | + | 13,700 |
| 24 | 6 | M | Corgi | 10.5 | + | + | 14,200 |
| 25 | 2 | M | Pomeranian | 40.6 | + | + | 28,800 |
| 26 | 12 | M | Dachshund | 16 | + | + | 30,400 |
| 27 | 0.5 | M | Bichon Frisé | 18.6 | + | + | 149,000 |
| 28 | 10 | M | Maltese | 14.6 | + | + | 398,000 |

^aPacked cell volume

^bParasites was determined by real-time PCR and calculated from the standard curve constructed with the plasmid DNA according to Matsuu et al., 2005 [15]



coupling the Credo Rapid Blood Nucleic Acid Extraction Kit and the QubeMDx PCR system, the entire diagnostic procedures for *B. gibsoni* can be completed within 30 min in clinic, where veterinarians primarily rely on microscopic examination as the diagnosis method. The simplified assay procedures and saved time from laboratory identification may expedite the prescription medications and lead to effective disease management for the affected dogs.

The detection limit of diagnostic tests is a critical parameter for accurate and timely pathogen identification. It has been suggested that *B. gibsoni* infection with low parasitemia (0–0.75%) is easily misdiagnosed as immune-mediated hemolytic anemia and sometimes inappropriately treated with immune suppressive drugs [16, 17]. For dogs with babesiosis, either during the early or chronic stages of infection or under medical treatment, the parasite numbers in the blood may be low. Previous experimental infection study has shown that, not until 10 days after infection, dogs inoculated with *B. gibsoni* revealed a significantly decreased level of packed cell volume (PCV) (< 37%) or with a parasitemia greater than 0.1% [15]. By using this sensitive QubeMDx PCR system, the detection limit of 0.002% of parasitemia (94 parasites/ μ L of blood) allows for early diagnosis of infected dogs. Although greater detection limit was previously demonstrated by the real-time PCR (9 parasites/ μ L of blood) [15], recombinase polymerase amplification-lateral flow (LF-RPA) dipstick method (0.5 parasites/ μ L of blood) [5], loop-mediated isothermal amplification (LAMP)(0.0000135% of parasitemia) [18], the differences can be attributed to the use of rapid DNA extraction procedures in the QubeMDx system, leading to a compromised DNA yield.

In addition to a low detection limit, a 100% of sensitivity and specificity for *B. gibsoni* detection was achieved by this QubeMDx PCR based on real-time PCR confirmed positive/negative samples, while previously 86.67 and 57.33% of positive rate were obtained by the LF-RPA dipstick method [5] and the LAMP method [18], respectively, evaluated from clinically suspected dogs. Furthermore, the internal control included in each reaction of QubeMDx PCR provides an additional source for quality control. Taken together, compared to other available molecular methods, QubeMDx PCR system is a reliable POC method with advantages of rapidity and reaction simplicity.

Conclusions

This reported QubeMDx PCR system offers a rapid (< 30 min), sensitive, and effective diagnosis for *B. gibsoni* in clinical infections. The present point-of-care testing allows for treatment prescription in the early

stages of the infection, facilitating the recovery of affected dogs.

Methods

A total of 57 EDTA-anticoagulated canine whole blood samples were collected at the National Taiwan University Veterinary Hospital. For the reference test of *B. gibsoni* detection, total DNA was extracted from 100 μ L of blood sample with the DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. A real-time PCR for *B. gibsoni* detection was performed to define the infection status and the standard curve was constructed with plasmid DNA for the conversion of parasitemia [15]. Through the reference test, 28 samples were defined as positive and used for the sensitivity evaluation for QubeMDx PCR system, whereas other 29 samples were negative and used for the specificity determination. In addition, the detection limit of the QubeMDx PCR system was determined using serially-diluted dog blood from one of the real-time PCR-positive blood sample. Furthermore, six canine whole blood samples diagnosed as *Ehrlichia canis*, *Anaplasma platys* or *Babesia canis* positive by PCR or real-time PCR [19–21] were also included in this study to evaluate the specificity of the QubeMDx PCR system.

To conduct the QubeMDx PCR assay, DNA was prepared by using the Credo Rapid Blood Nucleic Acid Extraction Kit (Credo Biomedical Pte. Ltd.). Briefly, 30 μ L of blood sample was added to the tube containing the lysis buffer and the mixture was heated at 98 °C for 6 min. After the heating, the reaction mix was fractionated via centrifugation, and total DNA in the supernatant was collected to a fresh tube. The resultant DNA solution was subsequently assayed using the QubeMDx *Babesia gibsoni* Detection Kit (Credo Biomedical Pte. Ltd.) according to the manufacturer's instructions. Briefly, 30 μ L of the DNA solution was added to the reaction tube prefilled with lyophilized reagents containing primers and fluorogenic probes targeting the p18 gene of *B. gibsoni* as well as PCR essential reagents and internal controls. Tubes were then loaded onto the QubeMDx PCR device, and the fluorescent signals during amplification were detected and processed by a built-in algorithm. The video demonstrating assay procedures was filmed and can be found in the following link: <https://www.dropbox.com/s/nbdt9ryvuubxsea/QubeMDx.mp4?dl=0>.

Supplementary information

Supplementary information accompanies this paper at <https://doi.org/10.1186/s12917-019-2181-5>.

Additional file 1: Table S1. Analytical specificity results of the QubeMDx PCR for *B. gibsoni*.

Abbreviations

B. gibsoni: *Babesia gibsoni*; LAMP: Loop-mediated isothermal amplification; LF-RPA: Recombinase polymerase amplification-lateral flow; PCR: Polymerase chain reaction; PCV: Packed cell volume; POC: Point-of-care

Acknowledgments

Not applicable.

Authors' contributions

ILL, MLH and HWC designed the study. NYC, CLC, MLH and CTC performed the experiments. ILL, MLH and HWC analyzed the data. ILL, MLH and HWC wrote the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

The data used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

Samples used in this study were derived during medical practice in the National Taiwan University Veterinary Hospital (NTUVH) and the use of samples has been approved by dog owners and NTUVH with written consent. The ethics approval was waived according to the "Guideline for the Care and Use of Laboratory Animals" issued by Council of Agriculture Executive Yuan, Taiwan on June 22nd of 2018 and the official notice made by the Ministry of Health and Welfare, Taiwan on July 5th of 2012 (document number: 1010265083).

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Author details

¹Institute of Veterinary Clinical Science, National Taiwan University, Taipei, Taiwan. ²Credo Biomedical Pte. Ltd., Singapore, Singapore. ³Department of Veterinary Medicine, National Taiwan University, 1 Sec 4 Roosevelt Rd, Taipei, Taiwan.

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