Iranian J Publ Health, Vol. 37, No.4, 2008, pp.96-102 **Original Article**

Detection of *Brucella* by Peripheral Blood PCR and Comparison with Culture and Serological Methods in Suspected Cases

B Kazemi [1,2]***, SA Yousefi Namin*** [3]***, M Dowlatshahi*** [3]***, M Bandepour*** [1]***, F Kafilzadeh*** [3]***, L Gachkar*** [4]***, F Mahmoudinejad*** [3]***, A Samarghandi*** [1]***, M Mardani*** [4]

[1]*Cellular and Molecular Biology Research Center, Shahid Beheshti University, M. C., Tehran, Iran*
[2]*Dep. of Parasitology and Mycology Shahid Beheshti University, M.C., Tehran, Iran*
[3] *Islamic Azad University, Jahrom, Iran*
[4]*Infectious Disease and Tropical Medicine Research Center, Shahid Beheshti University, M.C., Tehran, Iran*

(Received 30 Apr 2008; accepted 27 Oct 2008)

Abstract

Background: Brucellosis is a zoonotic disease and its symptoms and appearances are not exclusive in human and its traditional diagnosis is based on blood culture and serological methods. For more sensitive and specific detection, the PCR method is recommended.
Methods: One hundred four blood samples were gathered from suspicious patients with brucellosis different ages form Kermanshah, Mazandaran, Khorassan and Hormozgan provinces, and were examined by Rose Bengal, anti globulin, culture and PCR methods.
Results: Seventy three samples were positive by PCR method, 15 samples were positive by cultured method and 84 samples were positive by serological methods.
Conclusion: PCR method is sensitive and specific for diagnosis of *Brucella* from peripheral blood in suspected cases.

Keywords: *Brucella, Culture and serological methods, PCR*

Introduction

Brucellosis is a zoonotic disease of wild and domestic animals which also infect humans (1, 2). Farmers, veterinarians, laboratory personnel and abattoir workers are subject to be infected by *Brucella* (1, 3). Childhood brucellosis in the United States is now an imported disease (4).
Considering that disease symptoms are not specified, by epidemiological evidence of the disease about his or her contact with animals, consumption of non pasteurized dairy products, residence in enzootic regions and hematological evidence such as anemia, leucopenia and thrombocytopenia, brucellosis could be diagnosed. Laboratory diagnosis is based on positive blood culture and serological tests. Diagnosing of chronic disease is difficult (5) and usually the blood culture is negative (6, 7). Despite false positive and negative in serological tests and cross reactions with other gram negative bacteria, using molecular methods such as PCR or ELISA is recommended (8, 9). Fulfillment of PCR is most quick and irritable method for detection of *Brucella* by amplification of bacterial genome in blood sample, bone marrow, mucus or CSF (10-12). It should be reminded that PCR is not a routine method in diagnostic most laboratories in Iran. Despite a few studies has done in this field (13), it is necessary to have more studies in order to identify its power for diagnosing disease. PCR is considered as gold standard and there are some studies about comparison between PCR and culture and serological test for diagnosis of *brucella* in blood and other tissues (14, 15).
This research has performed and designed to determine PCR power for detecting *Brucella* from

***Corresponding author:** Tel/fax: +9821 22439956, E-mail: bahram_14@yahoo.com

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peripheral blood and compared to culture and serological methods in suspected patients.

Materials and Methods

Sampling

Each patient signed consent and then 104 blood samples were gathered from suspicious patients with brucellosis with different ages from Kermanshah, Mazandaran, Khorassan Razavi and Hormozgan provinces. Eight ml blood (5 ml for culture and serology and 3 ml mixed with EDTA for extracting DNA) were taken from suspected cases. Samples with EDTA were transferred to freezer and samples relevant to culture were kept for 3 months at 37º C in microaerophilic condition.

DNA extraction and PCR amplification

Blood DNA was extracted by boiling method and PCR reaction was performed using Nested PCR: Nest 1 primers (Bruc 1F 5` – ATA GCT GGT CTG AGA GGA TGA TCA G – 3` and Bruc 1R 5` – TTC GGG TAA AAC CAA CTC CCA TGG – 3`) were amplified 1100 bp and Nest 2 primers (Bruc2 F 5` - ATA TTG GAC AAT GGG CGC AA – 3` and Bruc2R 5` - AGC GAT TCC AAC TTC ATG CA -3`) were amplified 958 bp of *brucella* 16S rRNA gene. PCR reaction was included 2 µL of DNA (containing 100 ng), 150 nM dNTP, and 40 Pico moles each of forward and reverse primers, 1.5 mM MgCI2, 1 X PCR buffer and 1.25 units of Taq DNA polymerase in 30 µL final volume. PCR amplification was performed by following parameters: denaturing at 94º C for 30 sec., annealing at 62º C for 30 sec. and extension at 72º C for 30 sec. These processes were repeated for 30 cycles. Reaction was settled at 94º C and 72º C for 5 min before and after PCR cycling, respectively. Second PCR reaction was made in 50 µL volume, and its parameters were also like first PCR except what annealing temperature was made in 47º C (16).

Detection of PCR product

PCR product was electrophoresed on 1% agarose gel and DNA band was observed by UV Translluminator after ethidium bromide staining (17).

Serological methods

Blood serums were separated and serological diagnostic tests were performed by Rose Bengal method and Wright Agglutination test by kits prepared of Pasture Institute of Iran (18, 19).

Culture

After transferring blood samples to laboratory, samples that were considered for culture, they were kept in period of 3 weeks at 37° C inside of crystal container containing medium for culturing liquid of Soya bean casein medium or Tripton Soya Broth with microaerophilic condition. For making microaerophilic condition, candle and gas pack were used. After 3 weeks, each sample was cultured on two agar plates. One of the plates was kept in aerobic condition and another one in microaerophilic condition at 37° C, and after 48 h their results were obtained (20).

Results

From 104 suspected patients, 43 were male. From 84 patients infected by brucellosis (diagnosed by Wright test) 40 were male. Difference faced between sex and infection by brucellosis was not considerable.

In this research, 104 blood samples were examined in suspected cases of brucellosis. Seventy three cases were positive by PCR method (Fig. 1), 15 cases by culture and 84 cases by serological methods. Distribution of samples under study based on PCR results, culturing and serology have shown in Tables 1-3.

Most of infected cases with brucellosis were (concerning on results obtained from PCR) in age group between 30 to 39 yr and the least infected cases with brucellosis were in age group of 1 to 9 year. Between age groups and infection to brucellosis, considerable difference was not faced (Table 4).

PCR product was sequenced and diagnosed as *Brucella melitensis* biovar Suis (deposited to GenBank at accession no. (DQ377361).

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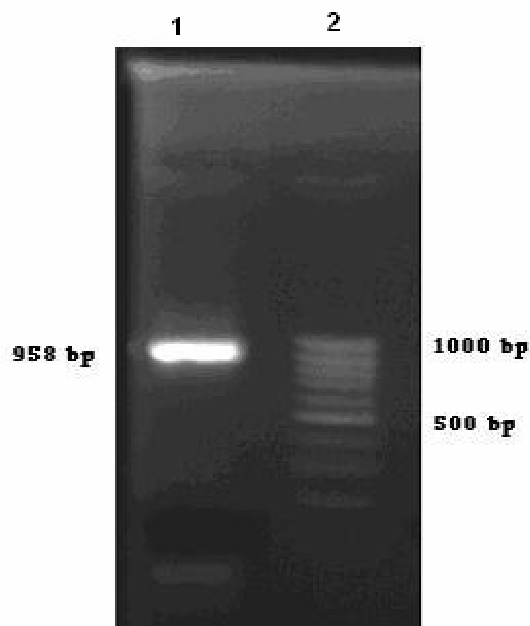

Fig. 1: Electrophoresis of *Brucella* PCR product on 1% agarose gel

Lane 1: 985 bp as PCR product of *Brucella* 18 S rRNA gene
Lane 2: 100 bp DNA ladder marker

Table 1: Ferequency of *brucella* positive and negative cases diagnosed based on peripheral blood PCR and culture methods

Culture \ PCR	Positive	Negative	Total
Positive	15	o	15
Negative	58	31	89
Total	73	31	104

Sensitivity, specificity, positive prediction value and negative prediction value of blood culture in comparing with PCR were calculated 20, 100, 100 and 8.34, respectively.

Table 2: Ferequency of *Brucella* positive and negative cases diagnosed based on peripheral blood PCR and serological (Wright analyze) methods

Serology \ PCR	Positive	Negative	Total
Positive	73	11	84
Negative	0	20	20
Total	73	31	104

Sensitivity, specificity, positive prediction value and negative prediction value of serology in comparing with PCR were calculated 100, 64.5, 86.9 and 100 respectively.

Table 3: Ferequency of *Brucella* positive and negative cases diagnosed based on blood culture and serological (Wright analyze) methods.

Serology \ Culture	Positive	Negative	Total
Positive	15	69	84
Negative	0	20	20
Total	15	89	104

Sensitivity, specificity, positive prediction value and negative prediction value of serology in comparing with blood culture were calculated 100, 5.22, 9.17 and 100 respectively.

Table 4: Frequency of brucellosis according to age (diagnosed by PCR method)

Age (year) \ PCR results	Positive		Negative		Total	
	Frequency	Percent	Frequency	Percent	Frequency	Percent
1-9	4	3.8	2	1.9	6	5.7
10-19	8	7.6	3	2.8	11	10.5
20-29	16	15.3	2	1.9	18	17.3
30-39	25	24	4	3.8	29	27.8
40-49	16	15.3	5	4.8	21	20.1
50-59	9	8.6	3	2.8	12	11.5
More than 60	6	5.7	1	0.9	7	6.7
Total	84	80.7	20	19.2	104	100

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Discussion

Brucellosis is a disease of wild and domestic animals that could be transferred to human by direct or indirect contact with infected animals (1, 2). Symptoms and signs of human brucellosis are not specific (1, 3, 4). This disease is prevalent in Mediterranean regions (21, 22), India (23), Arabian Peninsula and some parts of Mexico, Latin America and southern America (6, 24). Exact diagnosis of brucellosis is not just based on clinical symptoms, because it will be considered in differential diagnosis of other diseases such as malaria, typhoid and leptospirosis. Therefore defining organism in culture or identification of organism by serological and molecular methods for confirming clinical diagnosis is necessary (25, 26).

We used 104 suspicious blood samples from Mashhad (Khorasan Razavi Province), Bandar Abbas (Hormozgan Province), Kermanshah (Kermanshah Province) and Tonekabon (Mazandaran Province) for diagnosis of *brucella* by PCR, blood culture and serological (Wright and Rose Bengal) methods. Fifteen samples were grown on culture medium, 84 samples were positive by Wright method and 73 samples by PCR method.

Considering brucellosis in different ages indicate that most infections are in ages 30 to 39 (24%) and after that (15.3%) related to ages 20 to 29 and 40 to 49 yr. These age groups include active age groups. These peoples are settled in different manners in animal husbandry, dairying, working at home and have connection with livestock and products of livestock. At all there is not considerable difference in outbreak of disease in adults and children. Therefore, there is not any rational relation ship between age and having brucellosis. But, Cetinkaya et al. considered brucellosis serologically and indicated that there is relationship between age, sex and positivity (27).

Roushan et al. diagnosed brucellosis in Iran by Rose Bengal method and reported that 62.5% were positive. These cases were followed by 2ME and Wright methods. They considered cut-off for 2ME equivalent to 1/160 and for Wright test equivalent to 1/320 and 37.7% became positive (28). There are some investigations on diagnosis of Brucellosis by PCR method (11, 12, 25, 29-34). Elfaki et al. diagnosed many positive brucellosis by agglutination tests, while there were 40% and 70% positive by culture and PCR methods. They believe that producing antibody against *Brucella* is not related to disease condition and for following disease have to use blood culture and PCR (32). Salari et al. considered 792 cases for brucellosis with serological method and they believe that outbreak of disease in men is more than women (35). Karimi et al. considered brucellosis outbreak in 415 healthy people including butchers and slaughterers by serology method and confirmed contribution of job in this disease (36). By considering brucellosis epidemiology that was made by Hassanjani Roushan et al. in Babol city, highest risk factor was from using of dairy products (fresh cheese). In aforementioned study, job (such as veterinarian) is not introduced as risk factor. Infection level was higher in rural areas and men were infected more than women (37).

In consideration of job groups, most infection is between housekeeping and animal husbandry jobs, since these jobs (in villages) have direct contact with livestock and livestock products. Women housekeepers in village are subject to have connection with livestock because of daily activities and even some times they attempt to help animals to born and without usage of gloves they remove aborted fetus from their wombs by hand.

One of target genes for determining bacterial identification is 16S rRNA which in this study has used. This gene has high endurance. DNA Sequences in separate types of one genus, they have just small difference with each other.

In conclusion the PCR method is more sensitive and specific than culture and serology for diagnosis of *Brucella* from peripheral blood in suspected cases.

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Acknowledgments

This study was supported by Vice Chancellor for Research of Shahid Beheshti University, M C, (project No: 1079) and was done in Cellular and Molecular Biology Research Center. Here with, authors of research appreciating relevant responsible men.
The authors declare that they have no conflict of interests.

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