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Detection of *Brucella melitensis* in Blood Samples Collected from Goats

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Abstract: PCR assays have been shown to be a promising option for the diagnosis of brucellosis. However, there is no study conducted in Malaysia to identify the brucellosis in goat's population. In this study three whole blood samples and sera were collected from goat's farm in Kedah state Malaysia which was suspected to have brucellosis. Serological and molecular detection of brucellosis have been done including RBPT, CFT, conventional PCR and Real time. The evaluation of each test have been discussed rather than the sensitivity and specificity of the each test which can be used in Malaysia national eradication programs. In conclusion, the combination between the serological test and molecular technique specially real time PCR depend on IS711 region in hypothetical protein is promising and can be reduced to false positive result which can cause heavy economical loss during controlling programs.

Key words: Goats, brucellosis, CFT, RBPT, real time PCR, PCR, diagnostic test validation

INTRODUCTION

Brucellosis is a worldwide re-emerging zoonotic infection that causes Malta fever in humans and high economic losses in animals (Al Dahouk *et al*., 2004). The disease that is transmitted to humans from infected animal reservoirs. Brucellosis has recently re-emerged as a zoonotic disease in Malaysia. The detection of high or rising titres of specific antibodies in the serum allows a tentative diagnosis of brucellosis. A variety of serological tests has been applied but at least two serological tests have to be combined to avoid false negative results. Sensitive and rapid serological tests have been introduced for the diagnosis of brucellosis in animal. In Malaysia, detection of *B. melitensis* in goats is performed using the RBPT and CFT assays.

Molecular techniques using PCR and Real-Time PCR (RT-PCR) have not been established to detect *B. melitensis* in clinical samples and their ability to detect the *Brucella* sp. that cause disease in populations of goats in Malaysia has not been investigated. Rapid and reliable, sensitive and specific, easy to perform and automated detection systems for *Brucella* sp. are needed urgently to allow early diagnosis, confirmation, detection and adequate antibiotic therapy. Real-time PCR assays using specific primers or probes meet all these requirements (Al-Dahouk *et al*., 2004). Bounaadja *et al*. (2009) concluded that RT-PCR assays are easy to use and they produce results faster than conventional PCR systems while reducing the risk of DNA contamination.

The IS711-based RT- PCR assay is specific and highly sensitive and appears to be an efficient and reproducible method for the rapid and safe detection of the genus *Brucella*. Hinic *et al*. (2009) presented results that indicate that the IS711 RT-PCR assay is a specific and sensitive tool for the detection of *Brucella* sp. infections. For this reason, the IS711 RT-PCR was employed in this present study as a complementary tool in screening of brucellosis and for confirmation of the diagnosis in doubtful cases. Genus-specific RT-PCR assays, e.g., based on the *bcsp31* gene will lead to an early diagnosis but for the purpose of epidemiological surveillance a species-specific RT-PCR derived from the conventional AMOS (Abortus Melitensis Ovis Suis)-PCR is necessary (Al-Dahouk *et al*., 2004). The sensitivity and specificity of the RBPT and CFT was evaluated and compared with PCR and RT-PCR in detection of brucellosis in blood samples from goats. Suitable sample is also important to ensure misdiagnosis of infection is avoided. The objectives of this study were:

- To determine the sensitivity and specificity of serological techniques in detection of *B. melitensis* in goats
- To determine the ability of molecular techniques in detection of *B. melitensis* in goats blood
- To compare the eligibility of serological techniques and molecular techniques in detection of *B. melitensis* infection in goats

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MATERIALS AND METHODS

Animal and study herds: About 288 blood samples were collected from goat in goat farm in Kedah state Malaysia. The goat populations in the farm were 600 head. The sample size required to estimate the prevalence of *B. melitensis* on the goat farm was determined using the method by Cannon and Roe.

Serum and whole blood samples: Blood samples of 10 mL were obtained using a sterile vacutainer tube from the jugular veins of the goats and were divided into two tubes, the first containing the anticoagulant EDTA, the other without anticoagulant for serum separation. The samples were kept on ice box with temperature approximately at 4°C upon transported them to the laboratory. Blood in plain tube was centrifuged at 6000 rpm for 5 min to obtained serum samples. The serum was kept at -20°C for serological tests and molecular works. Whole blood samples in EDTA tube was also kept at -20°C until used for molecular works.

Detection of *Brucella melitensis* infection by serological methods: The RBPT was carried out according to the method of Khairani-Bejo and Bahaman (2006) by the addition of 30 μL serum to 30 μL RBPT reagent; these were mixed together on a white plate using a tooth pick. A positive result was recorded within 3 min. A second untouched batch of serum was sent to Veterinary Research Institute VRI for CFT.

DNA extraction from blood and serum samples: The DNA purification was carried out using a MasterPure™ Complete DNA Purification Kit (Epicenter, USA).

DNA extraction from whole blood: The DNA from whole blood was extracted using a master Pure™ extraction kit (Epicenter®, USA). DNA extraction kit (Epicenter, USA). Briefly, 200 μL of whole blood samples in EDTA tube were transferred into a microcentrifuge tube and 600 μL of red cell lysis solution were added into it. The tube was inverted three times to mix the contents and the bottom of the tube was flicked to suspend any remaining material. Each sample was incubated at room temperature for 5 min; subsequently it was vortexed and mixed briefly. The samples were incubated at room temperature for an additional 5 min followed again by brief vortex mixing. The white blood cells were pelleted by centrifugation for 25 sec in a microcentrifuge. Most of the supernatant was removed, leaving approximately 25 μL of liquid which was vortexed and mixed to re-suspend the pellet. The white blood cells were re-suspended in 300 μL of tissue and cell lysis solution by pipetting the cells up and down several times; 1 μL of 5 μg μL^{-1} RNase A was added to the sample and mixed thoroughly. The samples were incubated at 37°C for 30 min. Finally, the samples were placed on ice for 3-5 min and then processed for DNA precipitation.

The MPC protein precipitation reagent (150 μL) was added to 300 μL of lysed sample and vortex mixed vigorously for 10 sec. The debris was pelleted by centrifugation for 10 min at ≥10,000×g in a microcentrifuge. If the resultant pellet was clear, small or loose, an additional 25 μL of MPC protein precipitation reagent was added, mixed and the debris was pelleted again. The supernatant was transferred to a clean microcentrifuge tube and the pellet discarded. Isopropanol (500 μL) was added to the recovered supernatant. The tube was inverted several times (30-40 times). The DNA was pelleted by centrifugation at 4°C for 10 min in a microcentrifuge. The isopropanol was poured off carefully without dislodging the DNA pellet. The sample was rinsed twice with 75% ethanol being careful not to dislodge the pellet. Centrifugation was repeated briefly if the pellet was dislodged. All of the residual ethanol was removed with a pipette. The DNA was re-suspended in 35 μL of TE buffer.

DNA extraction from sera: The DNA was extracted from serum samples using a master Pure™ DNA extraction kit (Epicenter®, USA). Briefly, 50 μL of each serum sample was transferred to an eppendorf tube. Proteinaes K (1 μL) was diluted in 400 μL of tissue lysis buffer then was added to the serum samples and incubated at 65°C for 15 min with vortex mixing every 5 min. The mixture was cooled to 37°C and 1 μL of RNAase A was added to the samples, mixed thoroughly and incubated on ice for 5 min. The MPC protein precipitation reagent (250 μL) was added and vortexed for 10 sec then centrifuged at = 10,000×g for 10 min. The supernatant was transferred to a new tube and the pellet was discarded. Isopropanol (600 μL) was added and mixed by inversion (30-40 times). The mixture was centrifuged at 4°C for 10 min; subsequently the isopropanol was poured off without dislodging the DNA pellet. The pellet was rinsed with 70% ethanol however, if the pellet was dislodged the centrifugation was repeated. The DNA was re-suspended in 35 μL of TE buffer and the purity and concentration were measured.

Measurement of DNA purity and concentration: The DNA purity and concentration was measured using a spectrophotometer (BioQuest, UK) according to the method described by Sambrook.

Detection of *B. melitensis* in blood and serum by conventional PCR: One set forward and reverse primers were selected to amplify target DNA for the detection of *B. melitensis* in blood and serum samples of goats. The forward Primer (P1) was derived from the 3' end of the genetic element IS711 whereas the reverse Primer (P2) was derived from signature sequences of *B. melitensis*. The primers P1 (5'-CATGCGCTATGT CTGGTTAC-3') and P2 (5'-AGTGTTTCGGCTCAGAATAATC-3') were used to amplify a 252 bp fragment (Redkar *et al*., 2001). The DNA amplification was obtained by added 24 μL of reaction mixture which contained 1 × buffer, 1.5 mM $MgCl_2$, 200 μM of each dNTPs, 25 μM of each primer and 2U of Taq DNA polymerase (Qiagen®, Germany) of into 1 μL of purified DNA. The PCR amplifications were performed in a thermorcycler (Mycylcer® Bio-Rad, USA). The primary PCR amplification consisted of an initial denaturation step at 95°C for 5 min; denaturation, anneling and extension for 34 cycles at 95°C for 1.15 min, 45°C for 2 min and 72°C for 2 min, respectively and a final elongation phase at 72°C for 5 min. The amplification was repeated on PCR products. Positive control derived from DNA of the *B. melitensis* 16 M reference strain and a reagent control consisting of all the PCR reagents without DNA were included to evaluate the success of amplification and the purity of the reagents. The product of the PCR was mixed with 1 μL of gel loading buffer and electrophoresed through a 2% agarose gel at 75 V for 1.30 h in 1×TBE buffer. A 100 bp molecular size marker (Promega®, USA) were run concurrently. The gel was then stained with ethidium bromide (0.5 μg mL^{-1}) and examined under UV light for the presence of a 252 bp band and photographed using a Bio-Rad® gel document system for documentation and determination of the expected band.

Melting curve analysis and evaluation of RT-PCR Standard curves: A suspension of genomic DNA from *B. melitensis* 16 M was prepared in sterile distilled water and titrated spectrophotometrically. Assuming a molecular mass for the *B. melitensis* genome of approximately 3 fg of DNA, this suspension contained approximately 6×10^5 genome copies per 5 μL DNA extract (Debeaumont *et al*., 2003). For each PCR assay, tenfold serial dilutions (10^{-1} to 10^{-7}) of this external standard were run in parallel with the serum samples to be tested and the logarithm 10 of the concentration of each dilution series was plotted versus the cycle number at which the fluorescent signal increased above a threshold value (Ct value).

Melting curves analysis: The melting curve analysis was performed after the amplification was completed by raising the incubation temperature from 65-95°C in 0.2°C increments with a hold of 1 sec at each increment. When the temperature reached the melting Temperature (Tm) of the amplified product there was a steep decrease in fluorescence as the product denatured to single strands and no SYBR Green I dye bound to the product. The SYBR Green I Fluorescence (F) was measured continuously during the heating period and the signal was plotted against Temperature (T) to produce a melting curve for each sample. The melting peaks were then generated by plotting the negative derivative of the fluorescence over temperature versus the temperature (-dF/dT versus T).

Detection of *B. melitensis* in blood and serum by RT-PCR: The tested samples were evaluated using the standard curve obtained from the amplified *B. melitensis* 16 M on the basis of the respective signatory Ct values generated. These Ct values were determined by running the RT-PCR using 1 μL of each sample of extracted DNA as a template, together with known standard PCR products in the same experimental run.

RESULTS

Detection of *B. melitensis* infection by serological methods: The RBPT and CFT were detected 23.3% (67/288) and 25.3% (73/288) of samples were positive to *B. melitensis*, respectively. The sensitivity and specificity of commercial RBPT and CFT was shown as in Table 1.

Detection of *B. melitensis* in blood by conventional PCR: Successful PCR amplification was achieved by amplification of the IS711 region of the hypothetical protein gene in *B. melitensis* and amplicon of 252 bp were demonstrated in Brucella-positive samples as shown in Fig. 1. Polymerase Chain Reaction (PCR) was detected 85 out of 288 (29.5%) blood samples were positive to *B. melitensis*.

Table 1: Sensitivity and specificity of RBPT and CFT results

Tests	CFT+	CFT-	Total
RBPT +	65	2	67
RBPT-	8	213	221
Total	73	215	288

Sensitivity of RBPT = True positives/total positives ×100 = 89.04%; sensitivity of CFT = True negatives/total negatives ×100 = 97.02%; specificity of RBPT = True negatives/Total negatives ×100 = 99.06%; specificity of CFT = True negatives/Total negatives x 100 = 96.38%

Fig. 1: Conventional PCR of blood samples subjected for IS711 region with amplicon size of 252 bp (white arrow) with annealing temperature at 72°C. Lane M: 100 bp ladder; lanes 1-26: samples 1-26; lane+ve: positive control and lane -ve: non-template control

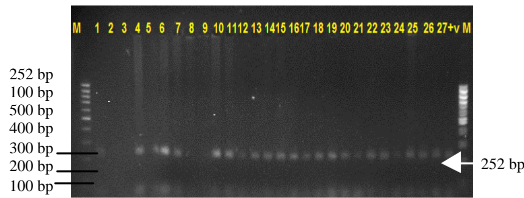

Fig. 2: Conventional PCR of serum samples subjected for IS711 region with amplicon size of 252 bp (white arrow) with annealing temperature at 72°C. Lane M: 100 bp ladder; lanes 1-27: samples 1-27; lane+ve: positive control

Melting curve analysis and evaluation of RT-PCR: Given that SYBR Green I dye binds to any dsDNA, the product specificity and the absence of non-specific amplification or primer-dimers was determined by melting curve analysis which was sometimes supported by gel electrophoresis. Melting curves were recorded by plotting the negative derivations of fluorescence with respect to temperature (-dF/dT vs temperature). As shown in Fig. 2, the Tm of specific PCR product, obtained using serially diluted *B. melitensis* 16 M to a dilution of 10^{-6} under the optimized protocol, recorded a single melting peak at a temperature of 82±0.5°C. In contrast, the Tm for DNA diluted to 10^{-7} was found to be 74°C. Failure to set the temperature at 79°C before the plate was read which was required to eliminate secondary structures associated with amplification was screened for by melting curve analysis. As shown in Fig. 3, the Tm for the PCR product was 82°C whilst the Tm for the primer-dimer product was 78°C.

Detection of *B. melitensis* in blood by RT-PCR: As shown in Fig. 3, the specificity of the SYBR Green I real-time PCR assay developed in this study was assessed. Specific amplification was detected from the *B. melitensis* positive samples in addition to the positive control and a standard curve with Ct values of 30.5±1.65 was generated whilst the No Template Control (NTC) showed negative amplification with no detectable Ct value. Signals specific

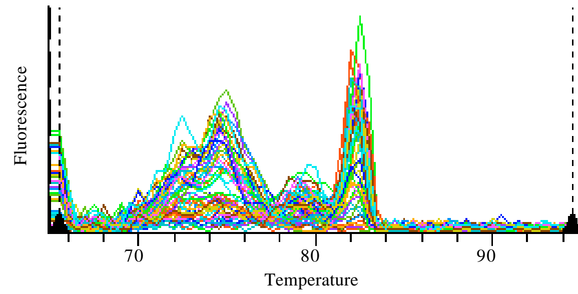

Fig. 3: Melting curve graph for samples from goat. Real-time PCR with DNA obtained from whole blood using primers specific for *B. melitensis* under optimized PCR conditions. The amplified product showed Tm at 82±0.5°C with no evidence of nonspecific products

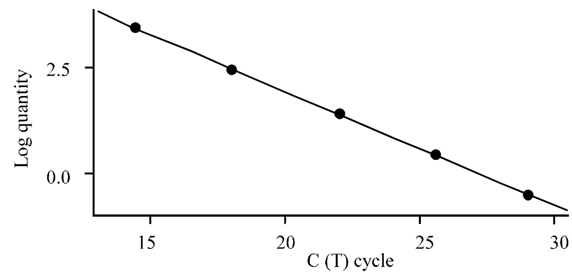

Fig. 4: C (T) cycle control graph y = -0.2721x + 7.39; r^2 = 0.999. Standard curve prepared from serial dilution of PCR product amplified from *B. melitensis* 16 M using IS711 specific primer

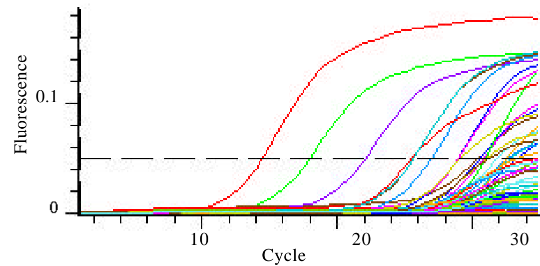

Fig. 5: Quantitation graph from goats 1-35. Real-time PCR for detection of *B. melitensis* using primers specific for *B. melitensis* IS711. Specific amplification was detected from whole blood samples with Ct values of 16.27±1.65 whilst the NTC showed negative amplification with no detectable Ct value

for *B. melitensis* had a characteristic Tm of 82±0.5°C whilst the Tm of the negative signal was ranged from 74°C to 78°C. Similar results were observed when the test was repeated three times (Fig. 4- 6).

The test samples containing an unknown quantity of DNA were assayed with the standards in the same

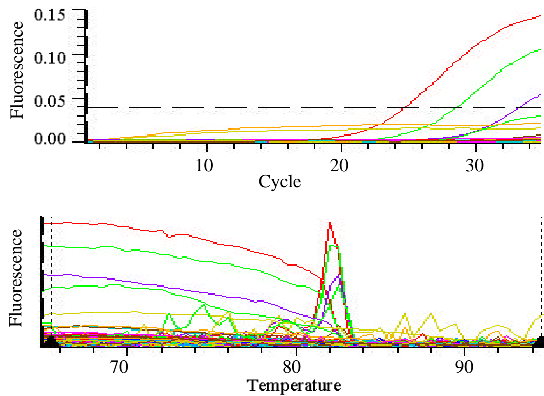

Fig. 6: Quantitation and melting curve graphs from serum goats. Real-time PCR for detection of *B. melitensis* using primers specific for *B. melitensis* IS711. Specific amplification was detected from serum samples with Ct values of 24.27±1.65 whilst the NTC showed negative amplification with no detectable Ct value

Table 2: Comparison of RBPT with RT-PCR, PCR and CFT

Parameters (%)	RBPT and CFT	RBPT and RT-PCR	RBPT and Conv. PCR	CFT and RT-PCR	CFT and Conv. PCR	RT- PCR Conv. PCR
Correlation	96.53	90.97	93.75	93.1	93.75	97.2
Discrepancy	3.47	9.03	6.25	6.9	6.25	2.8

Table 3: Comparison of CFT, RBPT, RT-PCR and PCR tests on 288 goat sera for detection of *B. melitensis* infection in goats

No. of goats.	CFT	RBT	Real-time PCR	Conv. PCR
65	+	+	+	+
5	+	-	+	+
3	+	-	+	-
2	-	+	+	+
13	-	-	+	+
5	-	-	+	-
195	-	-	-	-
288				

Table 4: Comparison of DNA extracted from serum samples and blood samples for detection of *B. melitensis* infection in goats

Parameters	Real-time PCR	Conventional PCR
Whole blood positive		
CFT positive	100% (50/50)	96 (48/50)
CFT negative	100% (50/50)	100% (50/50)
Serum samples		
CFT positive	100% (50/50)	90% (45/50)
CFT negative	98% (49/50)	100% (50/50)

Table 5: Sensitivity and specificity of RT-PCR and conventional-PCR results

Tests	Sensitivity	Specificity
RT-PCR	100.00	90.69
Conventional-PCR	95.89	93.02

experimental run. The standard curve (Fig. 3) constructed from the diluted standard template was then used to determine the target quantity in the unknown test samples by interpolation in a similar way to the use of molecular size standards to determine the molecular size of an unknown DNA band on an agarose gel. The evaluation of the real-time PCR in comparison with the serological tests and conventional PCR is shown in Table 2 and 3. About 50 samples positive on CFT and 50 negative on CFT were used to compare the sensitivity of tests on DNA extracted from whole blood and serum samples as shown in Table 4 and 5.

DISCUSSION

Brucellosis is a complicated disease in terms of diagnosis. No single serological test can be used for diagnosis of brucellosis in all stages of the infection. In many circumstances the RPBT may be negative especially in the early stages following infection or following the ingestion of colostrums from reactor dams; the CFT is often the last to become negative in the neonatal animal and after vaccination. The situation in which the CFT is negative but the RBPT positive is relatively common. It may arise following vaccination or after infection with other cross-reacting genera. Whole blood and serum samples are the easiest to use in terms of collection, handling and processing and pose lower risks especially serum samples therefore, it has been recommended to use such samples for the diagnosis of brucellosis. Moreover, the process of DNA extraction is easier for serum than for blood and tissues. Serum is a useful alternative to tissue samples for use in RT-PCR for animals with suspected brucellosis and negative or doubtful serological test results. Zerva *et al.* (2001) found that serum samples are best for the detection of brucellosis and the application of serum PCR for the diagnosis of infectious diseases has been reported by several investigators (Bougnoux *et al*., 1999; Morris *et al*., 1996; Murdoch *et al*., 1996). There is high correlation between the result of using of serum samples in both of conventional PCR and real-time PCR (94%) and have been showed that molecular technique is highly detectable for *Brucella melitensis* antigens in serum or whole blood.

However, serum samples are safer than whole blood samples and real-time PCR is low in hazard and real compared with conventional PCR. Serum showed highly detectable samples for *Brucella melitensis* Antigens in circulatory system of infected goats comparing with whole blood samples. The RBPT test is superior to all other laboratory techniques in terms of easy handling, rapidity and low cost however, it is limited in the diagnosis of early infection and cross reactivity with other bacteria such as *Yersinia enterocolitica* O:9 may give false positive results. A number of studies have revealed the effectiveness of the RBPT in the diagnosis of *B. melitensis* infection in sheep and goats although, some researchers reported low efficiency. Several studies have found that the RBPT detects infected animals earlier in the

immune response than the CFT and that the sensitivity was good. There is no doubt however that more research is required to standardize the RBPT preparations internationally (Young and Corbel, 2000). The CFT is the highest ranking of the confirmatory tests as recommended by the World Organization for Animal Health (OIE) and Food and Agriculture Organization (FAO). However, many samples appear to give a doubtful result. In addition, many factors prevent the use of CFT in the routine diagnosis of brucellosis, including the difficulty handling samples, lengthy procedure, requirement for highly experienced technicians and cross reactivity. The CFT, like the RBPT, shows poor ability to detect brucellosis in newly infected cases. The CFT test has a very low specificity when sera from sheep and goats vaccinated subcutaneously with Rev-1 are tested (Fensterbank *et al*., 1982) and cross reactivity with other bacteria means that it is affected by false positive serological reactions (Garin-Bastuji *et al*., 2006). Many researchers have used PCR as a diagnostic method for infection with brucellosis and it has high sensitivity which may reach 100% (Leyla *et al*., 2003).

Garin-Bastuji *et al*. (2006) reported that a PCR based on different molecular markers (16S rRNA, bscp31, IS 6501/711) was a valuable method for the detection of Brucella DNA in sheep and goats and provides a promising option. The technique has been used at different loci in the two chromosomes of *B. melitensis* either for diagnosis or characterization of the disease. Some researchers have used PCR to detect brucellosis in serum and whole blood samples and they have succeeded in amplifying the pathogens that were present in the blood stream. Polymerase chain reaction is high in sensitivity and specificity and it can detect infection at an earlier stage when compared with serological tests however, it is time consuming.

O'Leary *et al*. (2006) suggested there was no advantage in using PCR methods over standard serological and bacteriological methods in the detection of *B. melitensis* in bovine samples collected from whole blood or lymph nodes. However, molecular techniques have been shown to be more suitable, accurate, highly sensitive, rapid and simple to require small sample volumes to reduce the risks of handling tissues and more specific for the diagnosis of brucellosis from whole blood samples or from sera (Bounaadja *et al*., 2009; Debeaumont *et al*., 2003). The IS711 RT-PCR is rapid, easier than conventional PCR, more accurate, more sensitive and highly specific.

Real-time PCR is recommended for use as a confirmatory technique by Al Dahouk *et al*. (2004) and Bounaadja *et al*. (2009). High specificity test is needed to reduce the economical losses inherent in the control and eradication of *B. melitensis infection* in goat farms. The cost of the SYBR Green I dye is low compared with the cost of other techniques. The current study provided no evidence to support the use of specific serological tests to diagnose *B. melitensis* because of the inability of the available serological tests to diagnose brucellosis in the early stage of the disease when diagnoses depends mainly on detection of either I gM or I gG by antigens prepared previously. The use of more than one technique is recommended for the diagnosis of herds or individual animals that are suspected to be infected with brucellosis (Baum *et al*., 1995). The presence of anti-Brucella antibodies suggests exposure to *Brucella* sp. but it does not indicate which *Brucella* species induced production of those antibodies. Moreover, seropositivity does not necessarily mean that the animals have current or active infection at the time of sampling. The result showed high sensitivity and specificity of CFT and RBPT test (97.02, 89.04) and (99.38, 96.38), respectively.

Good correlation and low levels of discrepancy were seen between PCR and RT-PCR. These tests were followed by CFT and RBPT because of the similarity of the identification procedure whereas the worst correlation was shown between RBPT and real-time PCR.

A combination of a serological test and confirmation by a molecular technique especially RT-PCR is the best way to attempt control or eradication of *B. melitensis* infection on goat farms and accurate diagnosis of individuals infected with brucellosis. One single test is not sufficient to confirm the diagnosis of brucellosis and a combination of two tests should be performed, preferably the RBPT and real-time PCR.

The same suggestion was made by Abuharfeil and Abo-Shehada (1998) who compared three serological tests for the detection of *B. melitensis* infection in sheep. They suggested a combination of the RBPT and ELISA to achieve accurate diagnosis of brucellosis. The analytical sensitivity of the RT-PCR assay was higher than those of conventional PCR Procedures. Melting curve analysis increased the specificity of the assay by confirming that a positive fluorescence signal obtained during the real-time PCR was associated with an amplified product with a characteristic Tm.

The result of current study concluding that the sensitivity of CFT, RT-PCR and conventional PCR and RBPT were 97.02, 100, 95.89, 89.04 while the specificity were 96.38, 90.69, 93.02 and 99.06, respectively. The molecular techniques was higher in sensitivity and superior ability to detect of *Brucella melitensis* in goats blood comparing with other serological techniques which it was used to detected Abs against *Brucella melitensis* in goats blood samples. The existence of false-negative results, some in goats with positive CFT was surprising. Although, some of these false-negatives could result from the presence of inhibitors in the sample. The small

reaction volume used in the LC-PCR systems poses a potential disadvantage compared to conventional PCR since only a small volume of template can be added to the mastermix. The use of very small volume samples from goats with low concentrations of circulating *Brucella* sp. could result in an absence of target DNA in the sample tested. These findings suggest that increasing the sample volume may increase the sensitivity of the assay. New techniques allowing identification and sometimes quick typing of Brucella have been developed and are in use in certain diagnostic laboratories.

The essays were considered to have good specificity. The molecular diagnosis of brucellosis can become a clinical reality, studies aimed at harmonising the extraction and amplification protocols are required as well as verification of inter-laboratory reproducibility. However, the LC-PCR assay described in this study could be a practical and useful tool for the rapid diagnosis of goat's brucellosis. It is highly sensitive and specific is easy to perform and could provide results to a veterinarian in <2 h.

In addition, the risks to laboratory personnel associated with handling the microorganism are minimised. Therefore, as a general rule, prevention of human zoonotic brucellosis depends predominantly on the control of the disease in animals.

CONCLUSION

In the study, increasing of *B. melitensis* infection in goats farm a high prevalence of brucellosis requires the institution of national measures and there is a need to improve the diagnostic programme in order to assess the prevalence more accurately. It is widely recognized that it is necessary to undertake a national programme for the control and eventual eradication of the disease. The serological tests are more suitable as screening methods for use within a herd although, further serological tests with higher efficacy are still required. Moreover, improvements in the commercial RBPT and routine checks of the sensitivity and specificity of the commercial kit are required.

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