

Original Article

Detection of *Leishmania infantum* Infection in Reservoir Dogs Using a Multiepitope Recombinant Protein (PQ10)

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ABSTRACT

Mediterranean visceral leishmaniasis is a zoonotic disease caused by *Leishmania infantum* and transmitted by the bites of infected female sand flies. Iran is one of the endemic areas of this disease. Dogs and canines are the major reservoir hosts of *Leishmania infantum* in the new and old world, including Iran. If visceral leishmaniasis is left untreated, it may result in a 90% mortality rate. The identification and elimination of infected dogs are efficient ways to control this disease. The diagnostic methods used to identify these animals cannot yield 100% detection. Therefore, in the present study, we used a multiepitope recombinant protein (PQ10) to distinguish between symptomatic and asymptomatic infections caused by *Leishmania infantum* in animal reservoirs (dogs). The coding sequence of PQ10 recombinant protein was sub-cloned in pET28 expression vector and was commercially synthesized by GENERAY Biotechnology, China. In the following process, sequencing was performed with plasmid-specific primers and followed by the expression, optimization of expression. The purified recombinant protein was confirmed using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and Western blot. The efficacy of recombinant PQ10 protein was evaluated by an indirect enzyme-linked immunosorbent assay (ELISA) test using 150 dog sera (25 symptomatic positive sera, 25 asymptomatic positive sera, 80 negative sera, and 20 sera of dogs with other infectious diseases). Direct agglutination test (DAT) as the standard method was used to compare and determine specificity and sensitivity. The results indicated that the sensitivity and specificity of ELISA using infected dog sera were 94% and 86%, respectively. Positive and negative predictive values for dog sera were reported as 87.03% and 93.47%, respectively. This protein was able to identify 92% of asymptomatic dogs with visceral leishmaniasis. The results showed that the recombinant protein PQ10 is able to identify positive cases of canine visceral leishmaniasis, especially asymptomatic cases

Keywords: Visceral leishmaniasis, *Leishmania infantum*, PQ10, Recombinant protein, Serodiagnosis, Dog, Iran

Détection de l'Infection à *Leishmania infantum* chez les Chiens Réservoirs en Utilisant une Protéine Recombinante Multiépitopique (PQ10)

Résumé: La leishmaniose viscérale méditerranéenne est une maladie zoonotique causée par *Leishmania infantum* et transmise par les piqûres de phlébotomes femelles infectées. L'Iran est l'une des zones où cette maladie est autochtone. Les chiens et les Canidés sont les principaux hôtes réservoirs de *Leishmania infantum* dans le nouveau et l'ancien monde, y compris l'Iran. Si la leishmaniose viscérale n'est pas traitée, elle peut entraîner un taux de mortalité de 90%. L'identification et l'élimination des chiens infectés représentent des moyens efficaces pour contrôler cette maladie. Les méthodes de diagnostic utilisées pour identifier les animaux infectés ne peuvent pas permettre une détection à 100%. Par conséquent, dans cette étude, nous avons utilisé une protéine recombinante multiépitopique (PQ10) afin de distinguer les infections symptomatiques et

asymptomatiques causées par *Leishmania infantum* dans les réservoirs animaux (chiens). La séquence codante de la protéine recombinante PQ10 a été sous-clonée dans le vecteur d'expression pET28 et a été synthétisée commercialement par GENERAY Biotechnology, Chine. Dans le processus suivant, le séquençage a été effectué avec des amorces spécifiques du plasmide et suivi par l'expression et l'optimisation de l'expression. La purification de la protéine recombinante a été confirmée par électrophorèse sur gel de sodium dodécyl sulfate-polyacrylamide (SDS-PAGE) et Western blot. L'efficacité de la protéine PQ10 recombinante a été évaluée par un dosage d'immunoabsorption par enzyme (la méthode immuno-enzymatique ELISA) utilisant 150 sérums de chien (25 sérums positifs symptomatiques, 25 sérums positifs asymptomatiques, 80 sérums négatifs et 20 sérums de chiens atteints d'autres maladies infectieuses). Le test d'agglutination directe (TAD) été utilisé comme méthode standard pour comparer et déterminer la spécificité et la sensibilité. Les résultats ont indiqué que la sensibilité et la spécificité de l'ELISA pour identifier les sérums de chiens infectés étaient de 94% et 86%, respectivement. Les valeurs prédictives positives et négatives pour les sérums de chiens ont été rapportées à 87,03% et 93,47%, respectivement. Cette protéine a permis d'identifier 92% des chiens asymptomatiques atteints de leishmaniose viscérale. Les résultats ont montré que l'ELISA à base de protéine recombinante PQ10 est capable d'identifier les cas positifs de leishmaniose viscérale canine, en particulier les cas asymptomatiques.

Mots-clés: Leishmaniose viscérale, *Leishmania infantum*, PQ10, Protéine recombinante, Sérodiagnostic, Chien, Iran

INTRODUCTION

Visceral leishmaniasis is a neglected and poorly reported disease caused by *Leishmania infantum* and transmitted by the bites of infected female sandflies (Desjeux, 2004). Canine leishmaniasis was first described by Nicolle and Comte (1908) in Tunisia. Domestic dogs (*Canis familiaris*) which are the main reservoir hosts for *L. infantum* play a peculiar role in the transmission cycle of visceral leishmaniasis (Travi et al., 2018). Sand fly vectors could get infected by both symptomatic and asymptomatic dogs (Gradoni et al., 1987; Molina et al., 1994). The close contact between dogs and human populations increases the risk of parasite transmission from dogs to sand flies and then to humans (Miro et al., 2008). Canine leishmaniasis is responsible for a wide variety of clinical manifestations ranging from mild common skin diseases to severe forms characterized by keratoconjunctivitis, cachexia, alopecia, anorexia, weight loss, and death (Costa et al., 2003; Ordeix et al., 2005; Farahmand et al., 2015). Although visceral leishmaniasis is endemic in northwestern and southern

areas of Iran accounting for 100-300 new cases per year (Edrissian, 1996; Edrissian et al., 1998; Mohebbali et al., 2006), it has been sporadically reported in humans (Mohebbali, 2013). For serodiagnostic purposes, different numbers of *Leishmania* antigens, such as A2 antigen from amastigote forms of *Leishmania infantum*, Ecto-Nucleoside Triphosphate Diphosphohydrolase NTPDase-2, *Leishmania* amastigote-specific protein, and Synthetic Peptides have been investigated (Faria et al., 2011; Akhoundi et al., 2013; de Souza et al., 2013; Martins et al., 2013; Fonseca et al., 2014). Studies indicated that high values of sensitivity and specificity are very essential for these antigens. Nonetheless, high sensitivity is a priority for screening tests, while high specificity assumes more importance in the case of confirmatory tests (Faria et al., 2015). Faria et al. (2015) noted that high sensitivities and specificities in these tests can be achieved using multiepitope proteins, which have been shown to be of great help for the diagnosis of canine visceral leishmaniasis. In the present study, we developed and evaluated a multiepitope recombinant protein (PQ10) for the detection of *Leishmania*

infantum infections in asymptomatic and symptomatic dog reservoirs in the endemic area of visceral leishmaniasis in Iran. This recombinant protein was constituted by ten antigenic peptides with epitopes previously identified and demonstrated good results in the diagnosis of Canine Visceral Leishmaniasis by enzyme-linked immunosorbent assay (ELISA) with an accuracy up to 0.94 (Costa et al., 2011; Faria et al., 2015).

MATERIAL AND METHODS

Dog sera collection. Dog sera that were confirmed as positive by the direct agglutination test (DAT) for visceral leishmaniasis were prepared as follows: for asymptomatic serum samples (Group A, n=25), blood samples were taken from Kordan region of Alborz Province, an endemic area for visceral leishmaniasis. On the other hand, the symptomatic serum samples (Group B, n=25) were obtained from the different endemic regions of Iran collected in the Parasitology Laboratory, School of Public Health, Tehran University of Medical Sciences during the research period. The dog sera that were confirmed as negative for visceral leishmaniasis were prepared as follows: 50 serum samples were collected from healthy dogs that were living in visceral Leishmaniasis endemic areas in Iran (group C). These negative sera were used to define a cut-off value. Moreover, 20 serum samples were collected from dogs with other infectious diseases (Canine distemper virus, Leptospirosis, and Parvovirus infection) that were living in non-*Leishmania* endemic areas in Iran (group D). In total, 30 of the other negative samples and 20 of the serum samples from dogs with other infectious diseases were used to calculate specificity. All negative samples were confirmed by DAT for the absence of anti-*Leishmania* antibodies.

Design of synthetic gene constructs. The PQ10 multiepitope synthetic gene was designed according to Faria et al. (2015). Initially, 10 coding sequences of antigenic peptides (Faria et al., 2011) were joined resulting in PQ10. A flexible linker (Gly-Ser-Gly-Ser-

Gly) coding sequence was used as a spacer between epitope sequences (Robinson and Sauer, 1998). NdeI and NotI restriction sites were added to the 5' and 3' ends of the synthetic gene to aid in cloning, respectively. For affinity purification of recombinant protein, a 6xHIS tag coding sequence was added upstream of the stop codon of the synthetic gene. The sequence was codon-optimized for *Escherichia coli* expression. Three-dimensional structure of PQ10 protein was predicted by I-TASSER (Iterative Threading ASSEMBLY Refinement) server (<https://zhanglab.ccmb.med.umich.edu/ITASSER/>). The *PQ10* gene was commercially synthesized by GENERAY Biotechnology, China. The synthesized gene was cloned into the NdeI and NotI restriction sites of a pET28 expression vector resulting in pET-PQ10. Sequence analysis of the cloned fragments was performed for the confirmation of the correct fusion and orientation of the insert.

Expression, production, purification, and confirmation of recombinant protein. Recombinant plasmid was transformed to *E.coli BL21 DE3* expression host, and protein expression was carried out by inoculating 500 ml Luria Bertani medium containing 0.05 mg/ml kanamycin with an overnight bacterial culture. All cultures were incubated on a rotary shaker at 180 rpm at 37 °C and were grown until they reached an optical density(OD) of 0.6 at 600 nm. Thereafter, the expression of PQ10 recombinant protein was induced by the addition of 1 mM IPTG (isopropyl-Beta-D-thiogalactopyranoside) to the medium on a rotary shaker (180 rpm) at 37 °C. The induction time was 5 h and the expression levels were assessed at one-hour intervals of induction. The induced samples (soluble and insoluble protein) were screened and analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (mod.VSTS-3000; AKHTARIAN) with 12.5% resolving gel, followed by Coomassie Brilliant Blue G-250 staining. The cultures (200 ml of 4-hour induced) were pelleted, and the purification of PQ10 protein was performed under

denaturing conditions according to the manufacturer's instructions (The QIA expressionist TM, QIAGEN). The eluted proteins were analyzed by SDS-PAGE and quantified by the Bradford method. The western blot analysis was performed on purified protein samples. Proteins were electrotransferred to nitrocellulose membranes (Sigma-Aldrich®) in a semi-dry transfer cell at 15 volts/overnight (mod WB-1100, AKHTARIAN). The detection of antigens was performed by an indirect antibody immunoassay using anti-dog IgG (whole molecule)-HRP (from rabbit) (Riz Pad tan Parse, Iran) diluted 1:3000 in PBS with 0.05% Tween20 (PBS-T) and DAB staining.

Checkerboard titration. The best concentrations of the antigen, sera, and conjugates were optimized for the immunoassay by checkerboard titration. The Maxisorp microplate (Nunc, Denmark) was coated with antigen at the concentrations of 0.06, 0.125, 0.25, 0.5, 1, 2, 4, 8, 16, 32 and 64µg/ml. These plates were incubated with pools of sera from dogs infected with *L.infantum* and uninfected animals at different dilutions (1/50, 1/100, 1/200, 1/400, 1/800, 1/1600, 1/3200). Antibody binding was detected using anti-dog IgG-HRP (from Rabbit) (Sigma, reference A6792) in different dilutions (1/5000, 1/10000, 1/20000).

Immunoassays with dog sera. ELISA procedures were carried out to evaluate the antigenicity of multiepitope PQ10 protein. Maxisorp microplate (Nunc, Denmark) was coated overnight with 2 µg/ml PQ10 protein diluted in 0.1 M carbonate buffer (pH 9.6) at 4 °C. After three washes in PBS-0.05 %Tween-20(PBST) (PBS: 10.14 mM Na₂HPO₄ ; 1.37 mM KH₂PO₄; 146 mM NaCl; 2.64 mM KCl, pH 7.4, containing 0.05%Tween20), the wells were blocked with 200 µl/well 1% Bovine Serum Albumin (BSA) in PBS at 37°C for 2 h. The serum samples which were diluted 1:100 in PBS-0.05 %Tween-20 containing 0.5% BSA were added and incubated at 37 °C for 1 h. After three washing steps, the microplate was incubated with anti-dog IgG-HRP (from Rabbit) (Sigma, reference A6792) and diluted 1:10000 in PBS at 37 °C for 1 h. After washing five times, reactions were

developed with Tetra Methyl Benzidine (TMB) (Biologend, reference 421101), and the plates were incubated for 20 min in a dark room. Reactions were stopped with 2 M H₂SO₄, and microplate was read at 450 nm in a DYNEX (MRX II) ELISA reader.

Direct Agglutination Test (DAT) assay: The principal procedure for preparing the DAT antigen was as follows: initially, the mass cultivation of promastigotes of *L. infantum* Lon49 (Iranian strain) were performed in RPMI1640 medium (supplemented with 10% fetal bovine serum) and the promastigotes were then centrifuged (4000 g) at 4 °C for 10 min. After washing (x5) with Locke's solution, the parasites were trypsinized and then fixed with 2% formaldehyde. Finally, the fixed parasites were stained with Coomassie Brilliant Blue and resuspended in citrate saline containing 0.4% formaldehyde and stored until use (el Harith et al., 1989; WHO, 1996). For performing the test, the serums were initially screened by 1: 80 dilution. The samples with titers 1: 80 were further diluted to obtain end-point titers of 1: 20480. Antigen control well (antigen only), as well as negative and positive control sera (the positive serum was prepared from dogs with *L. infantum* infection from the endemic areas confirmed by microscopy, culture, and DAT titers of 1: 20480), were used in all procedures. The highest dilution at which agglutination was still visible, in comparison to negative control wells which had clear blue dots, was regarded as the cut off titer (WHO, 1996).

Statistical analysis. The cut-off value of PQ10 protein was calculated as the mean values + 3SD of the OD value of the negative sera that were obtained from healthy dogs. Consequently, OD values higher than the mean value + 3 SD+10% were considered positive. The sensitivity, specificity, PPV, and NPV were calculated as follows: Sensitivity=TP/(TP + FN)×100%, specificity=TN/(TN +FP)×100%, PPV= TP/ (TP + FP) × 100%, NPV = TN / (TN + FN) × 100% (Tjitra et al., 1999). The obtained data were analyzed in SPSS software (version 25). A p-value less than 0.05 was considered statistically significant.

RESULTS

Constructing molecular sequences, recombinant protein expression, and confirmation of protein production. A sequence that was codon-optimized (Faria et al., 2015) for PQ10 protein expression in *Escherichia coli* was commercially synthesized successfully (Figure 1) and confirmed by sequencing with PET-28 vector-specific primers. The PQ10 multiepitope protein was successfully expressed by *E. coli* BL21 DE3 strain under the conditions described above in materials and methods. The three-dimensional structure of PQ10 protein that was predicted by I-TASSER server is displayed in Figure 2. SDS-PAGE analysis of *E. coli* BL21 (DE3), which was transformed with pET28- PQ10 and induced with IPTG, demonstrated the expected 21.4 kDa band of PQ10 protein. Figure 3 illustrates the expression of PQ10 induced by IPTG 1mM at different times. There was a difference among the cells induced within the interval of 1-5 hours in terms of the quantitative expression of the protein. The maximized protein expression was 4 h after induction. PQ10 recombinant protein was mainly observed in the insoluble fraction of the cell lysate. Figure 3 shows PQ10 protein in cell lysate and its purified form at the expected sizes (21.4 kDa). The concentration of the purified PQ10 protein was calculated at 150µg/ml using the Bradford assay. Western blotting was utilized to examine the affinity of purified PQ10 protein against dog sera. Western blots of purified PQ10 demonstrated that recombinant protein reacted with dogs' sera (Figure 4).

Checkerboard titration:

Immunoassays with dog sera. The standardization assay was performed to determine the best concentration of the PQ10, as well as the best dilutions of the primary and secondary antibodies. It was observed that the best results were obtained by coating the wells with the PQ10 recombinant protein at 2µg/ml concentration, with the dog sera diluted at 1/50 and the conjugate diluted at 1/10000 (Figure 5, 6).

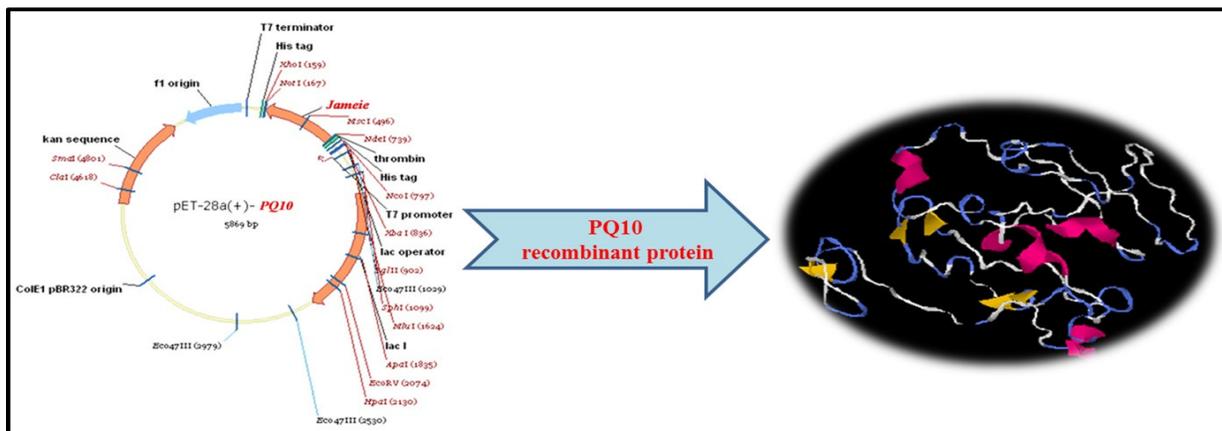
Sensitivity and specificity determination of PQ10-Indirect ELISA. As illustrated in Table 1, 6% of 50 confirmed subjects, which were infected by *Leishmania infantum*, did not react with PQ10 antigen, while 14% of 50 healthy subjects reacted with PQ10. The PQ10 displayed reactivity with one sample from healthy sera and cross-reaction was observed with six samples collected from other infectious diseases. The results of antibody detection by DAT and PQ10-Indirect ELISA testing are compared in Table 1. The PQ10-Indirect ELISA test was sensitive (94%) and specific (86%) for the diagnosis of canine visceral leishmaniasis, with a PPV and an NPV of 87.03 and 93.47%, respectively. In addition, as confirmed by DAT, PQ10 multiepitope protein was able to detect 92% of asymptomatic and 96% of symptomatic infected dogs. From among 20 serum samples that were collected from dogs with other infectious diseases, six samples from dogs with Leptospirosis, parvovirus infection, and distemper infection were false positive. The kappa index was calculated for PQ10 antigen to find the level of agreement with DAT. This index was calculated at 0.800 yielding excellent agreement with DAT. The Youden's index is a single statistic that captures the performance of a dichotomous diagnostic test. The Youden's J index for PQ10-ELISA test was calculated at 0.8. The McNemar test yielded a value of 0.344; therefore, considering a 95% confidence interval, it can be concluded that there is no significant difference between the results of DAT and PQ10-ELISA in the diagnosis of dogs infected with *Leishmania infantum*.

DISCUSSION

Canine visceral leishmaniasis should be promptly diagnosed even on the basis of only a few or a single clinical sign to enhance the prediction of disease and avoid both dog and human transmission and unnecessary euthanasia of apparently positive dogs (false positive dogs) (Gharbi et al., 2015). Due to the high proportion of asymptomatic dogs and the absence of obvious clinical signs, the diagnosis of infected dogs

hinges upon laboratory diagnostic test support (Ribeiro et al., 2018). Most antileishmanial drugs are toxic; therefore, an ideal diagnostic test should be able to differentiate between acute disease and asymptomatic

infection (Srivastava et al., 2011). Nonetheless, there exists no method with a satisfactory visceral leishmaniasis diagnosis efficiency. The accurate methods which are cost-effective and easy to use in the



1. Graphical abstract
2.

Table 1. Diagnostic performance of anti-PQ10 indirect ELISA assay and comparison between ELISA using recombinant protein PQ10 (cut-off=0.701) and DAT result

Direct Agglutination Test	Positive ELISA result		Negative ELISA Result		Total	
	Number	Percentage	Number	Percentage	Number	Percentage
DAT Positive sera	47	94 †	3	6	50	100
DAT Negative sera	7	14	43	86 ‡	50	100

PQ10 indirect ELISA had (94%) sensitivity and ‡ (86%) specificity for *Leishmania infantum*

DAT: direct agglutination test

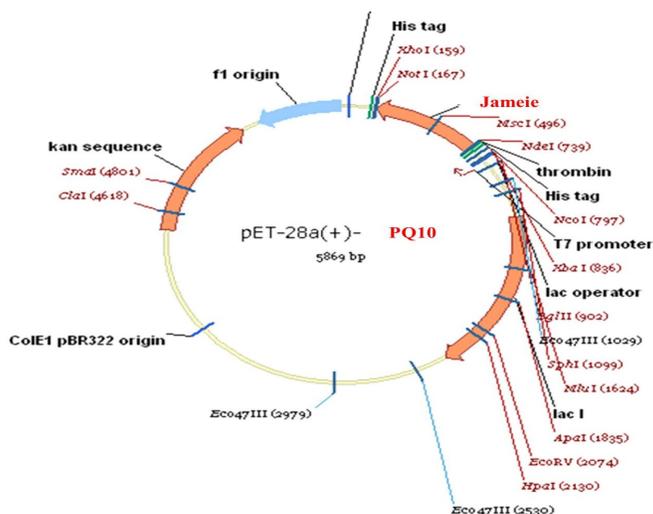


Figure 1. Schematic image of pET-28-PQ10 structure

field are crucial for the diagnosis and control of this disease (Mary et al., 1992). Direct methods of diagnosis, such as microscopy examination, culture, or the inoculation of hamsters with biopsy materials, have serious limitations for the detection of parasites in dogs. Consequently, anti-*Leishmania* antibodies are usually employed as a marker of infection (Dye et al., 1993; Quinnell et al., 1997). The immunodiagnostic assays

have been used as efficient alternatives to the abovementioned tests (Srivastava et al., 2011; Ehab Kotb et al., 2014). Serological methods are powerful tools for the diagnosis of visceral leishmaniasis (Faria et al., 2015). In order to make a diagnosis of visceral leishmaniasis, several serological techniques, such as DAT, indirect immunofluorescence test (IFAT), and ELISA, have been broadly developed based on the

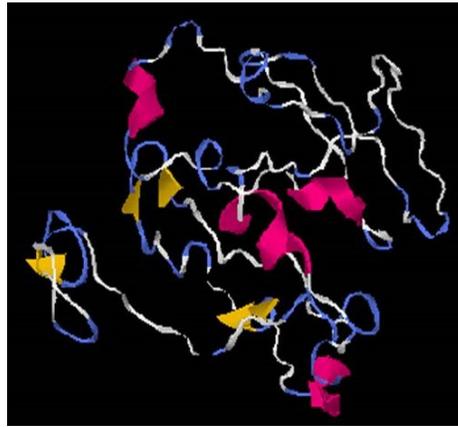


Figure 2. Three-dimensional structure of PQ10 protein

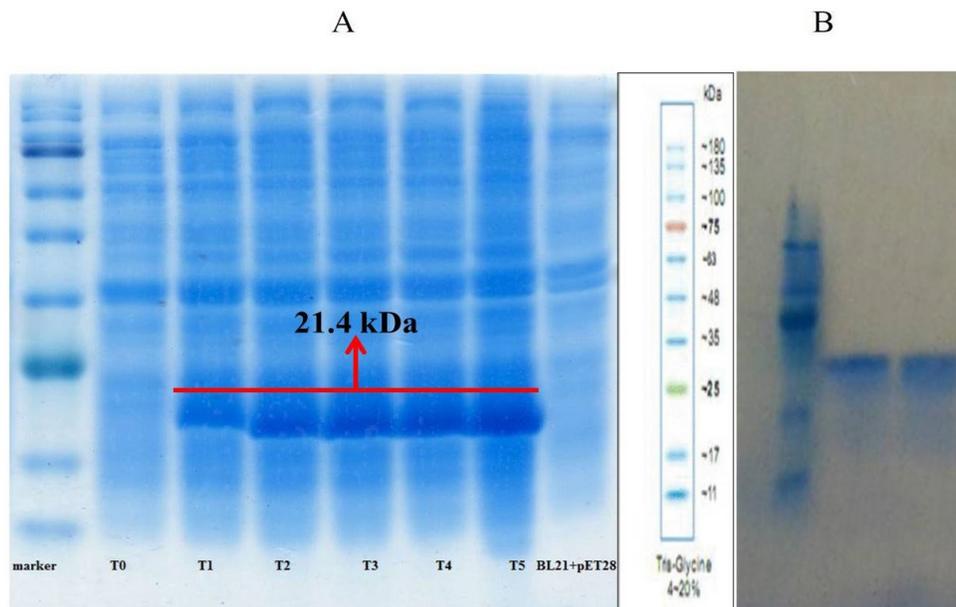


Figure 3. Cell lysate fractions from IPTG-induced bacterial cultures in different times (A), T0: Non-induced BL21-pET28-PQ10; T1: 1-hour induced BL21-pET28-PQ10; T2: 2-hour induced BL21-pET28-PQ10; T3: 3-hour induced BL21-pET28-PQ10; T4: 4-hour induced BL21-pET28-PQ10; T5: 5-hour induced BL21-pET28-PQ10 and BL21-pET28 as negative control and purified PQ10 protein (B), showing bands in the expected size (21.4 kDa) for the PQ10 recombinant protein. 12% SDS-PAGE gel stained with Coomassie blue-stained. M: molecular weight marker in kDa.

immunologic response, (Seyyedtabaei et al., 2017). DAT has long incubation times, and some level of expertise is required to run and read the test. IFAT is a diagnostic method commonly used for canine visceral leishmaniasis detection. Nonetheless, this technique has some serious limitations, such as cross-reactivity with other diseases and low sensitivity in the identification of asymptomatic dogs (Mancianti et al., 1995). ELISA has been used as an appropriate diagnostic method for almost all infectious diseases, including Leishmaniasis (Sundar and Rai, 2002). This technique requires a highly specific antigen to capture a specific antibody (Sakkas et al., 2016). In contrast to IFAT, ELISA is readily conformable to large-scale seroepidemiological studies and it has been shown to be as sensitive and specific as IFAT (Evans et al., 1990; Paranhos-Silva et al., 1996). On the other hand, it was reported that ELISA has cross-reactivity with other canine infectious diseases (Roffi et al., 1980; Mancianti et al., 1996). Several identified *Leishmania* antigens have been used for the development of new enzymatic immunoassays (Faria et al., 2015). For instance, to detect subclinical and clinical infections, crude promastigote antigens are highly sensitive but have lower specificity, compared to those techniques that use other antigens (Gomez-Ochoa et al., 2003; Rodriguez-Cortes et al., 2010). Since recombinant proteins can be easily absorbed onto several scaffold surfaces and yield reproducible results, they are considered good candidate antigens for field diagnosis (Pattabhi et al., 2010; Santarem et al., 2010; Venturin et al., 2015; Lauricella et al., 2016). Among the recombinant proteins, rK39 was the most successful antigen which has good efficacy to detect active canine visceral leishmaniasis and has displayed high sensitivity (90-100%) in studies conducted in the Mediterranean basin and South America. Nevertheless, this protein has less sensitivity and specificity for the detection of asymptomatic canine visceral leishmaniasis, in contrast to other serological methods (Badaró et al., 1996; Ozensoy et al., 1998; Rhalem et al., 1999; Scalone et al., 2002; Gomez-Ochoa et al., 2003; Mettler et al., 2005; Santarem et al., 2010). rKLO8 and rK26

are other antigens that could enhance the diagnostic accuracy of canine visceral leishmaniasis. The individual use of these two antigens indicated lower sensitivity and specificity (rKLO8, 68% and 92%, respectively; rK26, 77% and 91%, respectively), compared with their combination which exhibited higher sensitivity (85%) and specificity (93%) (Martínez Abad et al., 2017). Chimeric multi-epitope proteins were designed to improve the detection of asymptomatic infected dogs. In the current study, we have also produced a multiepitope recombinant antigen. According to previous studies (Faria et al., 2015), the use of these antigens offers some advantages, including low cost, easy automation in ELISA tests, and the ability to detect circulating antibodies in the early phase of infection. In our study, the cut-off value was calculated by adding three standard deviation values to the mean absorbance of 50 negative serum samples from healthy dogs. It provided the values of 94% and 86% for sensitivity and specificity, respectively. A sensitive and specific antigen for the detection of asymptomatic infections would be highly desirable since the important limitation in visceral leishmaniasis control is the inability to identify asymptomatic infections. The observation of false positives related to the cross-reaction samples (six samples from dogs with leptospirosis, distemper infection, and parvovirus infection) raises the suspicion of cross-reaction with other infectious diseases. The PQ10 multiepitope protein was able to detect 92% of asymptomatic infected dogs indicating its advantage in performing control programs. The epidemiological, clinical, and laboratory aspects of canine visceral leishmaniasis are very variable. Therefore, complete diagnosis and then treatment and control of this disease, especially due to the lack of more effective drugs and vaccines, is difficult (Ribeiro et al., 2018). Consequently, we used a recombinant synthetic protein to improve dog visceral leishmaniasis serodiagnosis. The obtained results denoted that PQ10 could be of great help for the diagnosis of asymptomatic dogs; moreover, it is useful in the early phase of infections.

As a final note, it is necessary to perform more studies in the different target population of dogs in the endemic

area of disease to find the accurate efficiency of this recombinant protein.

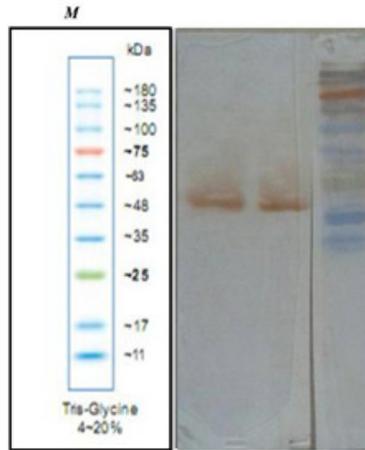


Figure 4. Nitrocellulose membrane after western blotting with anti-dog IgG. M: Molecular marker in kDa

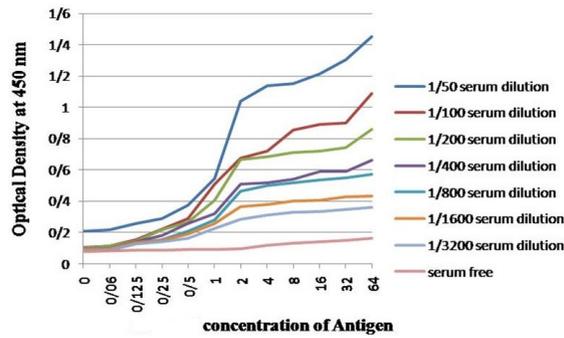


Figure 5. Checkerboard titration of different dilution of positive serum and different concentration of PQ10 antigen

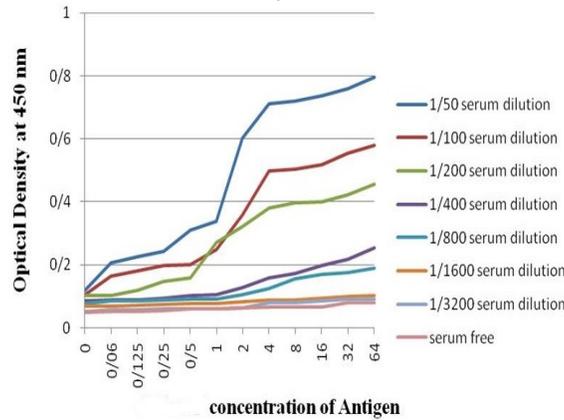


Figure 6. Checkerboard titration of different dilution of negative serum and different concentrations of PQ10 antigen

Research Highlights

- PQ10 is a suitable antigen for the detection of Visceral Leishmaniasis.
- PQ10 could be of great help for the diagnosis of asymptomatic infected dogs; moreover, it is useful in the early phase of infections.
- PQ10 multiepitope protein was able to detect 92% of asymptomatic infected dogs that is its advantage in performing control programs.

Ethics

Informed written consents were obtained from all participants, and all clinical investigations were conducted according to the principles expressed in the Declaration of Helsinki. The project was approved by the Research Advisory Committee of Tarbiat Modares University based on the Specific National Ethical Guidelines for Biomedical Research issued by the Research and Technology Deputy of Ministry of Health and Medicinal Education of Iran.

Conflict of Interest

The authors declare that they have no conflict of interest.

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Authors' Contribution

F. Jameie and A. Dalimi conceived the study and designed the study protocol. F. Jameie managed the sample collection and performed the experiments. A. Dalimi was supervisor and corresponded the work. M. Pirestani and M. Mohebbali were advisors. F. Jameie and A. Dalimi drafted the manuscript. All authors read and approved the final manuscript.

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