Seroprevalence, geographic distribution and risk factor analysis of *Borrelia burgdorferi* sensu lato in naturally exposed dogs of Iran

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Lyme borreliosis is a worldwide tick-borne zoonotic disease caused by spirochetes of the *Borrelia burgdorferi* sensu lato group. The aims of the current study were to determine seroprevalence of Lyme borreliosis caused by *B. burgdorferi* sensu lato among dogs and to analyze its environmental risk factors in Iran. Blood samples were collected from 273 asymptomatic dogs in three northern Caspian provinces of Iran, known habitats of *Ixodes ricinus* ticks. Enzyme-linked immunosorbent assay (ELISA) and Western blot were used as screening and confirmatory tests, respectively. For evaluation of environmental risk factors, a geographic information system (GIS) was utilized. A logistic regression model was developed to analyze multiple risk factors associated with seropositivity. Of 273 serum samples from the studied area, 22 (8.1%) showed antibodies against *B. burgdorferi* sensu lato. Out of the seven protein bands analyzed by Western blot, OspC, flagellin and OspA were found at highest proportions. Dogs living in forested areas, at altitudes ≤ 200 m, with tick infestation and between 3 and 5 years of age exhibited higher risk of infection than other dogs (P < 0.05). The majority (97.3%) of ticks collected from the sampled dogs were identified as *Rhipicephalus* spp., while only 2.7% were *I. ricinus*.

Key words: Lyme borreliosis, *Borrelia burgdorferi*, dog, Iran, *Ixodes ricinus*.

INTRODUCTION

Lyme borreliosis is a widespread zoonotic disease caused by spirochetes of the *Borrelia burgdorferi* sensu lato complex and is transmitted by *Ixodes ricinus* and *Ixodes persulcatus* ticks in Europe and Eurasia, respectively (Burgdorfer et al., 1982; Greene and Straubinger, 2006; Ulrich et al., 2003). Birds, mice, dormice, voles, deer, western gray squirrels, as well as lizards serve as reservoirs of *Borrelia* and some of these hosts may have a role in introducing the ticks and *B. burgdorferi* to new locations (Leonhard et al., 2010; Nadelman and Wormser, 1998). Due to phenotypic and genotypic heterogeneity of *B. burgdorferi*, 18 geno-species have been described under the name *B. burgdorferi* sensu lato in the world such as *B. burgdorferi*, sensu stricto, *Borrelia garinii* and *Borrelia afzelii* (Baranton et al., 1992; Canica et al., 1993; Gem and Humair, 2002; Rudenko et al., 2009). Since *Borrelia*-infected dogs can produce antibodies that persist up to 2 years and are exposed to the outdoor environment...
Figure 1. Geographical distribution of canine *B. burgdorferi* sensu lato serological status overlaid on elevation map of three Caspian provinces of Iran.

frequently, it has been assumed that dogs can be used as sentinel animals for the estimation of the risk of Lyme borreliosis for humans (Goossens et al., 2001; Guerra et al., 2001).

Diagnosis of canine borreliosis can be made based on evidence of tick exposure in an endemic area, compatible clinical symptoms, detection of antibodies in blood serum, explicit response to antibiotics, and exclusion of other diseases (Fritz and Kjemtrup, 2003; Skotarczak, 2002; Speck et al., 2007). Enzyme-linked immunosorbent assay (ELISA) and immunofluorescence assay (IFA) have been used as screening tests for detection of antibodies against *B. burgdorferi* sensu lato and immunoblotting procedure to confirm positive results (Guerra et al., 2000, 2001; Lindenmayer et al., 1990).

Numerous studies have been focused on the seroprevalence against *B. burgdorferi* among dogs in different parts of the world including Americas (Joppert et al., 2001; Wright et al., 1997), Europe (Goossens et al., 2001; Merino et al., 2000; Pejchalová et al., 2006), and Asia (Arashima, 1991). To the best of our knowledge, only a few works have been done in the Middle East (Baneth et al., 1998; Bhide et al., 2008; Icen et al., 2011); however, knowledge about its epidemiology in Iran is absent.

As the risk of Lyme disease for dogs and humans in an area is directly associated with the presence of infected ticks (Guerra et al., 2001), three northern Caspian provinces of Iran (Guilan, Mazandaran and Golestan) were selected for this study, based on known presence of *I. ricinus* (Greene and Straubinber, 2006; Nabian et al., 2007, Rahbari et al., 2007).

The aims of the current study were to determine seroprevalence against *B. burgdorferi* sensu lato among dogs, to describe its spatial distribution and to analyze its environmental risk factors in the mentioned areas. This survey is the first study regarding Lyme borreliosis in dogs carried out in Iran.

**MATERIALS AND METHODS**

**Area of the study**

Three northern Caspian provinces, namely Guilan, Mazandaran and Golestan, were selected for this study (Figure 1). These provinces, located between the Caspian Sea and the Alborz Mountains, are covered by forests, mountains and sea shores, have temperate climate and three different geographical zones: plain moderate, mountainous and semi-arid. Climatological conditions for Guilan, Mazandaran and Golestan Provinces, respectively, are as follows: mean annual temperature: 16.6, 17.2 and 18.5°C, relative humidity: 82, 82 and 71%, and precipitation: 1438, 952 and 446 mm.

**Sample population**

On the basis of seroprevalence reported from the Middle East (Israel: 10%, Baneth et al., 1998; West of Turkey: 23.2%, Bhide et al., 2008; East of Turkey: 0.0%, Icen et al., 2011), a sample size of 273 dogs was determined, in order to detect a presumptive prevalence of 10 ± 3.56% at 95% confidence level, using Win Episcope 2.0 software.
Blood samples were randomly collected from 273 asymptomatic, large crossbreed dogs in Caspian provinces of Iran [Guilan (n = 91); Mazandaran (n = 91) and Golestan (n = 91)]. All dogs were kept in the open air by the owners, for herding or guarding. The samples were obtained from five counties in various geographical and climatological areas of each province (Figure 4). 6 ml of blood were collected from the cephalic vein in vacutainer tubes (BD Diagnostics, Franklin Lakes, NJ, USA); serum was separated for serological assays. The owners were asked to complete a questionnaire for each dog. Any ticks observed on dogs during blood sampling were collected and kept for further identification.

Questionnaire

A questionnaire was designed to collect information on the sampled dogs including: gender, age (1 and 2, 3 to 5 and ≥ 6 years), history of tick exposure and tick control program. Dogs younger than one year of age were not included in the study. None of the sampled dogs were imported or had travel history. Since no vaccination program against Lyme disease of dogs is running in the country, none of the sampled dogs had been vaccinated.

Geospatial analysis

The residential locations of dogs were entered into a Geographic Information System (GIS), using ArcGIS 9.3 software (ARC/INFO, ESRI, Redlands, CA) to create a georeferenced database (Figure 1). Locations of B. burgdorferi seropositive and seronegative dogs were overlaid on an elevation map of Guilan, Mazandaran and Golestan Provinces, obtained from the National Cartographic Center of Iran. Seroprevalence of canine B. burgdorferi sensu lato in the selected localities was also analyzed (Figure 4).

The land cover/use was defined in two ways: land cover/use 1 (agriculture, forest, grassland and urban) and land cover/use 2 (forest versus agriculture, grassland and urban). Since plain moderate and semi-arid areas were located at ≤ 200 m and mountainous areas at ≥ 200 m, the areas in this study were divided into two categories: low (≤ 200 m) and high altitude (> 200 m).

Laboratory tests

ELISA

IgG class antibodies against B. burgdorferi sensu lato were detected qualitatively by a commercial ELISA kit (Novartec Immunodiagnostica, Germany) containing VlsE antigens. VlsE is an outer surface lipoprotein of B. burgdorferi that masks the presence of Borrelia from the immune system through an elaborate gene conversion mechanism and allow it to escape attack (Eicken et al., 2002). The specificity and sensitivity of the ELISA test, according to the manufacturer, were > 95 and 93.3%, respectively. The optical density was recorded at 450 nm; absorbance values 10% below the cut-off were considered negative, while absorbance values 10% over the cut-off were considered positive, according to the manufacturer’s instructions. Samples with an absorbance value of 10% above or below the cut-off (so called grey zone) should have been tested again after two weeks; however, since re-sampling was not feasible for this study, they were considered as positive and confirmed by Western blot technique.

Western blot

Western blot analyses were performed on ELISA positive and grey zone sera using a commercial Western blot assay (MegaBlot; IgG; MegaCor Diagnostik GmbH, Austria). Furthermore, 15 ELISA negative samples were used as negative controls. This kit is a qualitative assay for the detection of B. burgdorferi sensu lato specific IgG antibodies in canine serum or plasma using proteins derived from B. garinii (strain VS102). The sensitivity and specificity of this test are estimated to be 90% and 95% respectively, according to the results obtained in dogs from Austria (Leschnik et al., 2010). Proteins derived from B. garinii are separated based on their molecular weights in seven bands for scoring: p100 (93 kDa), p58 (58 kDa), flagellin (41 kDa), BmpA (39 kDa), OspA (32.5 kDa), OspC (22 and 23 kDa) and p18 (18 kDa). Visualization of specific protein bands indicates the presence of serum IgG antibodies against B. burgdorferi sensu lato-derived antigens. Samples were classified as positive or negative in accordance with the scoring system established by the manufacturer. Accordingly, the samples were considered as > 95% naturally infected if at least 2 positive bands among p100, BmpA, OspC or p18 bands were seen.

Statistical analysis

To measure the frequency of B. burgdorferi sensu lato in different populations, descriptive analysis was performed on all variables. Initially, pair wise correlations were studied between each independent risk factor (variable) and seropositivity as well as between each variable with Pearson’s chi-square test or the Fisher’s exact test, as appropriate. Correlation between any two variables was checked by Spearman’s test and only one of the correlated variables was kept in the model if the p-value of coefficient was small (P < 0.05).

Variables having an association with Wald’s P < 0.2 were entered into a multivariable logistic regression analysis and only variables with Wald’s P < 0.05 were included in the final model. The outcome variable was positive (1) or negative (0) serological status. All statistical analyses were performed using the analytical software package SPSS® 16.0 for Windows® (SPSS Inc., Chicago, IL, USA). Potential confounders were considered with causal diagram and their effects on other variables in the model were checked; none were found. Furthermore, one-level interactions were checked but they were not significant. In binary logistic regression, the overall model fit was evaluated using log likelihood ratio test. The area under the receiver-operating characteristic curves (AUROC) corresponding to the model was also calculated as test of model fit which usually range between 0 and 1. The cut off values were defined based on both highest sensitivity and specificity values.

Based on the literature, odds ratios can be interpreted as ratios of two probabilities only if the disease or condition is rare with incidence less than 5% (Dohoo et al., 2003). Therefore, the odds ratios received from the logistic regression using the well known relationship between odds and probabilities [probability equals odds divided by (1 + odds)] and comparing the calculated probabilities to disease frequency in the comparison group, were transferred into probabilities.

RESULTS

Screening by ELISA showed that 16 of 273 samples (5.9%) were positive, most of them (13/16; 81.3%) being confirmed by Western blotting, while 18 samples (6.6%) were in grey zone following the ELISA test, with 9 of them (50%) confirmed as positive by Western blotting (Figure 2).

Since no vaccination program against canine Lyme disease is running in Iran, all 22 positive samples
confirmed by Western blot were considered as naturally infected. Based on our immunoblot patterns (Figure 3), the frequencies (%) of seven analyzed protein bands were as follows: flagellin (96%), OspC (96%), OspA (73%), BmpA (55%), p58 (50%), p100 (41%) and p18 (9%) (Figures 2 and 3).

The overall seroprevalence of *B. burgdorferi* sensu lato in dogs from three Caspian provinces of Iran was 8.1% (22/273) (95% CI, 5.4 to 12%); Table 1 shows seroprevalence in different dog categories.

Among fifteen counties selected for the study, the highest values were detected in Gonbad-e Qabus (5/10; 50%), Ramian (4/17; 23.5%) and Kordkuy (3/13; 23.1%) counties (P < 0.05).

A total of 76 dogs (76/273; 27.8%) were found infested with ixodid ticks, of which 13 (17.1%) proved seropositive. *Rhipicephalus* spp. ticks comprised 97.3% (144/150) of total tick infestation of sampled dogs (adult *Rhipicephalus sanguineus*: 70.7% (106/150); *Rhipicephalus turanicus*: 17.3% (26/150); *Rhipicephalus bursa*: 1.3% (2/150) and *Rhipicephalus* spp. nymphs: 8% (12/150)), while *I. ricinus* represented only 2.7% (4/150) of the infesting ticks. According to the questionnaire, tick control program was apparently being conducted
Table 1. Seroprevalence of *B. burgdorferi* sensu lato in different categories for 273 dogs in the study area. Positivity was defined with both the screening (ELISA) and confirmation tests (Western blot).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Category</th>
<th>Number(%) of dogs examined</th>
<th>Number(%) of positive dogs</th>
<th>95% CI for percentage in previous column</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Lower</td>
</tr>
<tr>
<td>Gender</td>
<td>Female</td>
<td>56 (20.5)</td>
<td>6 (10.7)</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>217 (79.5)</td>
<td>16 (7.4)</td>
<td>4.6</td>
</tr>
<tr>
<td>Age (year)</td>
<td>1 - 2</td>
<td>104 (38.1)</td>
<td>1 (0.96)</td>
<td>0.2</td>
</tr>
<tr>
<td></td>
<td>3 - 5</td>
<td>111 (40.7)</td>
<td>17 (15.3)</td>
<td>9.8</td>
</tr>
<tr>
<td></td>
<td>≥ 6</td>
<td>58 (21.2)</td>
<td>4 (6.9)</td>
<td>2.7</td>
</tr>
<tr>
<td>Geographical area (province)</td>
<td>Golestan</td>
<td>91 (33.3)</td>
<td>20 (22)</td>
<td>14.7</td>
</tr>
<tr>
<td></td>
<td>Mazandaran</td>
<td>91 (33.3)</td>
<td>2 (2.2)</td>
<td>0.6</td>
</tr>
<tr>
<td></td>
<td>Guilan</td>
<td>91 (33.3)</td>
<td>0 (0)</td>
<td>0</td>
</tr>
<tr>
<td>Tick existence</td>
<td>Yes</td>
<td>76 (27.8)</td>
<td>13 (17.1)</td>
<td>10.3</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>197 (72.2)</td>
<td>9 (4.6)</td>
<td>2.4</td>
</tr>
<tr>
<td>Altitude (m)</td>
<td>≤ 200</td>
<td>156 (57.1)</td>
<td>18 (11.5)</td>
<td>7.4</td>
</tr>
<tr>
<td></td>
<td>&gt; 200</td>
<td>117 (42.9)</td>
<td>4 (3.4)</td>
<td>1.3</td>
</tr>
<tr>
<td>Land cover/use 1</td>
<td>Agriculture</td>
<td>163 (59.7)</td>
<td>16 (9.8)</td>
<td>6.1</td>
</tr>
<tr>
<td></td>
<td>Forest</td>
<td>36 (13.2)</td>
<td>6 (16.7)</td>
<td>7.9</td>
</tr>
<tr>
<td></td>
<td>Grassland</td>
<td>69 (25.3)</td>
<td>0 (0)</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Urban</td>
<td>5 (1.8)</td>
<td>0 (0)</td>
<td>0</td>
</tr>
<tr>
<td>Land cover/use 2</td>
<td>Forest</td>
<td>36 (13.2)</td>
<td>6 (16.7)</td>
<td>7.9</td>
</tr>
<tr>
<td></td>
<td>Others(</td>
<td>237 (86.8)</td>
<td>16 (6.7)</td>
<td>4.2</td>
</tr>
</tbody>
</table>

* CI = confidence interval

Table 2. Risk factors in the logistic regression model for *B. burgdorferi* sensu lato seropositivity among dogs in the study area.

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>Beta</th>
<th>Wald's P-value*</th>
<th>Odds ratio</th>
<th>95 % CI for odds ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age 1: (3-5 versus 1-2 years)</td>
<td>3.205</td>
<td>0.003</td>
<td>24.645</td>
<td>3.075</td>
</tr>
<tr>
<td>Age 2: (≥ 6 versus 1-2 years)</td>
<td>2.701</td>
<td>0.021</td>
<td>14.892</td>
<td>1.509</td>
</tr>
<tr>
<td>Forest versus agriculture/grassland/urban</td>
<td>1.496</td>
<td>0.013</td>
<td>4.465</td>
<td>1.366</td>
</tr>
<tr>
<td>Tick existence</td>
<td>1.791</td>
<td>0.000</td>
<td>5.994</td>
<td>2.199</td>
</tr>
<tr>
<td>Constant</td>
<td>-5.961</td>
<td>0.000</td>
<td>1.000</td>
<td>16.337</td>
</tr>
</tbody>
</table>

* P < 0.05.

Based on our results the significant risk factors for canine *B. burgdorferi* sensu lato seropositivity in Caspian provinces of Iran were: age (≥ 3 years), habitat (forest) and presence of ticks (Table 2). Despite living at low altitudes (≤ 200 m) was not significant in binary logistic regression model (P=0.074) but it was significant in Pearson chi-square analysis (3.4 times more risk of infection for low altitudes, P<0.05).

The difference between log likelihoods with intercept only and with predictors for the model was 34.681 (P<0.0001), indicating that the overall fit of the model was significant. The AUROC for model was 0.822, which means that in 82% of all possible pairs of subjects where
one is seropositive and the other seronegative, this model will allocate a higher probability to the seropositive subject. The specificity and sensitivity with cut off 0.087 in the model was 82.9% and 68.2, respectively.

Dogs between 3 and 5 years old, with almost 25 times higher odds ratio and 19% probability, had nearly 20 times higher risk for seropositivity and dogs ≥ 6 years, with almost 15 times higher odds ratio and 13% probability, had nearly 13 times higher risk for seropositivity than 1 and 2 years old dogs (Table 2). Dogs living in forested areas with 4.5 times higher odds ratio (Table 2) and 24% probability had around 3.6 times higher risk of Lyme borreliosis than those living in other three land cover/uses (Table 2). Additionally, dogs with visible ticks were at higher risk for seropositivity and while having almost 6 times higher odds ratio, had nearly 5 times higher probability of infection.

DISCUSSION

Canine Lyme borreliosis has been reported from many countries in Americas, Europe, Asia and Middle East (Baneth et al., 1998; Bhide et al., 2008; Goossens et al., 2001; V. Guerra et al., 2001; Joppert et al., 2001; Merino et al., 2000; Arashima, 1991; Salinas-Melendez et al., 1999; Kybicova et al., 2009). Based on the literature, this is the first study to investigate the presence of B. burgdorferi antibodies in dogs from Iran; regarding Lyme disease in humans, there is only one case report in a child from Iran (Tabatabaie and Siadati, 2006).

The overall seroprevalence of 8.1% in this study is in agreement with the earlier reports of 9.7% from Brazil (Joppert et al., 2001) and 10% from Israel (Baneth et al., 1998) using ELISA or IFA techniques as screening assays and Western blot as confirmatory test. However, our seroprevalence was higher than the prevalence reported from Germany (IFA, 5.8%) (Kasbohrer and Schonberg, 1990), Sweden (ELISA, 3.9%) (Egenwall et al., 2000), East of Turkey (ELISA, 0.0%) (Icen et al., 2011), and Bolivia (ELISA, 0.0%) (Ciceroni et al., 1997) but lower than the seropositivity reported from Japan (ELISA, 27.3%) (Arashima, 1991), West of Turkey (enzyme-linked protein A/G assay, 23.2%) (Bhide et al., 2008) and the Netherlands (ELISA, 17%) (Goossens et al., 2001).

Regarding Western blot analysis, based on the literature, the actual measured size of the bands varies in different investigations because of varied techniques and strain differences; after natural exposure to B. burgdorferi, antibodies develop to proteins in the range of p58 (58 kDa), flagellin (41 kDa), periplasmic protein (39 kDa), and OspC (22 kDa) (Greene and Straubinber, 2006). Reactivity, particularly to OspA (31 kDa) and OspB (34 kDa), possibly OspF (28 kDa), and 93 kDa bands occurs in vaccinated or parenterally inoculated dogs, but is normally absent or occurs (rarely) very late in naturally infected dogs (Greene and Straubinber, 2006). In our

Figure 4. Seroprevalence (%) of canine B. burgdorferi sensu lato in 15 selected localities from three Caspian provinces of Iran.
study, we found strongest antibody responses against flagellin, OspC and OspA (Figure 3), whereas Guerra et al. (2000) and Leschnik et al. (2010), who reported similar values for flagellin, showed quite lower values for OspC and OspA. The latter study in Austria was conducted using the same Western blot kit (MegaBlot) as we used. OspA band was found in a relatively high proportion of our Western blot positive dogs (73%). This is in contrast with results obtained by Greene and Straubinber (2006), who mentioned it should not be normally detected in naturally infected dogs, and Guerra et al. (2000) or Leschnik et al. (2010), who detected it at low levels in dogs with natural infection (17 and 2% early infection (EI) - 28% late infection (LI) in USA and Austria, respectively). BmpA (39 kDa) was found in our study in 55% of positive dogs, while other authors reported an occurrence of 88% (Guerra et al., 2000) and 26% (EI) - 33% (LI) (Leschnik et al., 2010). p58 and p18 bands, which were found in 50 and 9% of our positive cases, respectively, while being detected in 84 and 64% of cases, respectively, by Guerra et al. (2000), have not been detected at all by Leschnik et al. (2010). We found p100 band (93 kDa) in 41% of our positive cases while Guerra et al. (2000) and Leschnik et al. (2010) have detected it in 94 and 48% (EI) - 70% (LI) of their cases, respectively.

In the binary logistic regression model several risk factors were found significant for Lyme borreliosis seropositivity in dogs (Table 2). Based on our findings, dogs ≥ 3 years in general had significantly higher seroprevalence than younger dogs (Table 2) which is in agreement with the earlier reports demonstrating higher risk of infection for older dogs (Goossens et al., 2001; Lindenmayer et al., 1991). However, dogs between 3 to 5 years had the highest seroprevalence in our study (Table 2), whereas seroprevalence in dogs ≥ 6 years was intermediate between the other two categories. Lindenmayer et al. (1991) and Goossens et al. (2001) reported relatively stable rather than increasing seroprevalence after the age of 24 months, emphasizing that yearly re-infection of dogs is necessary to maintain seropositivity (Goossens et al., 2001; Hovius et al., 1999).

There was a significant and expected association between tick infestation and seropositivity in our study (Table 2). Based on the literature, I. ricinus and I. persulcatus are the primary vectors of B. burgdorferi in Europe and Eurasia, respectively (Greene and Straubinber, 2006). While I. ricinus is reported from north of Iran (Greene and Straubinber, 2006; Nabian et al., 2007; Rahbari et al., 2007) and is the major known vector present in neighboring Turkey (Gem and Humair, 2002), there has been no report of B. burgdorferi isolation from this tick in Iran. In our study as described before, 76 dogs (27.8%) were found being infested with ixodid ticks, of which Rhipicephalus spp. comprised 97.3%, but I. ricinus only 2.7% of the total tick infestation. While R. sanguineus has not yet been identified as a vector of borreliosis, its finding as the main infesting tick in general (70.7%) and the only isolated tick in Gonbad-e Qabus, where the highest seroprevalence among the counties was recorded, is consistent with other reports from Mexico and Brazil in which this species has been found as the main and second most common tick present on dogs with B. burgdorferi seropositivity, respectively (O’Dwyer et al., 2004; Tinoco-Gracia et al., 2009).

Whether this finding is due to missing tiny I. ricinus ticks in case of a concurrent R. sanguineus infestation in Borrelia-positive dogs or shows possible role of R. sanguineus in transmission of B. burgdorferi is not clear for us and needs further studies.

Tick control program has been apparently conducted only in Guilan Province; whether this program has influenced our results in Guilan Province with zero tick infestation and negative serologic tests is not known.

Dogs that lived in forested areas had higher risk of seropositivity than those living in other habitat types. Forested areas contain trees, shrubs, leaf litter and low strata vegetation creating a more suitable habitat for I. ricinus ticks than other areas (Gern, 2002; Guerra et al. 2001). However, we have detected only four (2.7%) I. ricinus ticks in two forested areas of Ramian and Chalus; one possible reason for this may be missing these ticks due to their very small size during the collection process.

Relative humidity of more than 80% is an important factor for the activity and survival of free-living I. ricinus (Gern, 2002). This is easily provided in most studied areas in this survey.

Our finding that dogs living at lower altitudes are at higher risk of exposure to B. burgdorferi than those living at higher altitudes is consistent with the findings of Lindenmayer et al. (1991) and Jouda et al. (2004), who detected lower survival and infectivity among ticks at higher altitudes. The presence of I. ricinus at high altitudes is limited by delayed development of ticks due to colder temperature (Jouda et al., 2004). The detrimental effects of cold are accumulative and exposure for one month to only −10°C has been shown to be lethal for a high proportion of unfed nymphs and diapausing engorged larvae and nymphs of I. ricinus (Gray et al., 2009; Knulle and Dautel, 1997). Accordingly, high altitudes, colder temperatures and hard winters in mountainous regions of three Caspian provinces of Iran (Figure 1) may affect and decrease I. ricinus survival and consequently the risk of exposure to B. burgdorferi among dogs.

In conclusion, seroprevalence of 8.1% for B. burgdorferi sensu lato in dogs from three northern Caspian provinces of Iran suggests that local veterinarians, especially in the Golestan Province, should pay attention to this disease in their clinical practice and consider it within the differential diagnoses. Lyme disease should also be taken more into account in humans living in these regions. Additionally, it is recommended to perform further studies including
detection of *I. ricinus* ticks in vegetation and animals, especially domestic and wild canids, together with molecular detection of *B. burgdorferi* sensu lato from isolated Ixodid ticks in suspected areas, particularly in Golestan Province of Iran.

Having better knowledge about the geographic distribution of Lyme disease and related environmental risk factors obtained through this survey, the development of programs for prevention and control of canine and human population in Iran is emphasized.

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