Serological survey of tularemia among butchers and slaughterhouse workers in Iran

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Background: Tularemia is a zoonotic disease caused by the Gram-negative bacterium Francisella tularensis. Human infections often occur through manipulation of infected animals or animal carcasses.

Methods: In this study, we determined the tularemia seroprevalence in butchers and slaughterhouse workers in 10 counties of Sistan and Baluchestan Province in Iran.

Results: A mean seroprevalence of 6.5% for IgG antibodies against F. tularensis was seen. The highest seropositivity rates were observed in the counties of Zabol and Nikhshahr. There was no difference in the seroprevalence rates between butchers and slaughterhouse workers (p = 0.25).

Conclusion: These data suggest that tularemia is endemic in Sistan and Baluchestan Province in Iran.

Keywords: Francisella tularensis, Iran, Seroprevalence, Sistan and Baluchestan Province, Tularemia, Zoonosis

Introduction

Francisella tularensis is the etiological agent of tularemia. There are two subspecies of F. tularensis, namely F. tularensis subspecies tularensis (type A strain) commonly found in North America and F. tularensis subspecies holarctica (type B strain) found in the whole northern hemisphere. Type B strains are responsible for almost all tularemia cases in Europe and Asia, and are usually associated with less severe symptoms and lower mortality rates as compared to type A strains. F. tularensis has a large animal reservoir, which includes many terrestrial mammals (especially rodents and lagomorphs) and arthropods (especially ticks). Human infections usually occur through direct contact with infected animals or animal carcasses. Alternatively, infection may occur via tick (or other arthropod) bites or after exposure to a contaminated environment. The clinical manifestations of tularemia may vary from an asymptomatic infection to a severe and possibly fatal disease. Six clinical forms are classically recognized: ulceroglandular, glandular, oculoglandular, oropharyngeal, pneumonic and typhoidal.

In Iran, antibodies against F. tularensis were detected in domestic animals (cattle and sheep) in the northwest, and in a porcupine in the southeast (Zabol county) in 1973. F. tularensis antibodies were also detected in an Afghan hedgehog in the same year. The first human case of tularemia in Iran was reported in the city of Marivan, southwest Kurdistan (western Iran) in 1980. In a recent study (2011–2012) among different groups in western Iran, the rate of tularemia seroprevalence was 14.4%. In this study, 16% of the butchers were seropositive for tularemia. The aim of the study was to determine the seroprevalence of F. tularensis in southeastern Iran and to establish a baseline for surveillance of the disease in the country. Because anti-F. tularensis antibodies were previously reported in domestic animals, sera from butchers and slaughterhouse workers were studied. These occupational groups may develop tularemia after contact with blood or tissues from animals infected with F. tularensis or healthy carriers of this bacterium, or via the bite of infected ticks during slaughtering operations.

Materials and methods

The study area

This cross-sectional study was carried out in Sistan and Baluchestan Province, in southeastern Iran, in 2011. This province
is bordered by the Oman Sea to the south, Afghanistan and Pakistan to the east, the South Khorasan province to the north, and the provinces of Kerman and Hormozgan to the west (Supplementary Figure 1). It has a dry, arid to semiarid climate.

Sampling
In this study, 184 serum samples were collected from 120 butchers and 64 slaughterhouse workers from 10 counties of Sistan and Baluchestan Province (including Zahak and Zabol in the north, Iranshahr, Zahedan and Khash in the center and Chabahar, Sarbaz, Saravan and Konarak in the south of the province; Table 1). All official slaughterhouses in this province were recruited and participants were selected randomly from among the employees. All participants were male. The median (interquartile range) age and length of employment were 34 (25–45) years and 8 (3–15) years, respectively. The inclusion criteria were being over range) age and length of employment were 34 (25–45) years and employees. All participants were male. The median (interquartile range) age and length of employment were 34 (25–45) years and workers (9.4%). The prevalence observed in butchers (5.0%) and slaughterhouse workers (9.4%). The F. tularensis seroprevalence rates were not correlated to age (p=0.94) and length of employment (p=0.19) of participants.

Serological tests
Collected sera were tested for the presence of IgG antibodies against F. tularensis, using a commercial ELISA kit (Virion/Serion, Würzburg, Germany) according to the manufacturer’s instructions (positive cut-off titer >15 U/mL, borderline cut-off titers 10–15 U/mL). Serum samples with positive or borderline results for F. tularensis were further tested against Brucella antigen because of the possibility of serological cross-reactions between both antigens. We used a locally prepared antigen and a standard tube agglutination test elaborated by the Pasteur Institute of Iran (Tehran).

<table>
<thead>
<tr>
<th>Region</th>
<th>No. tested (seropositivity %)</th>
<th>County</th>
<th>No. tested (seropositivity %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>North</td>
<td>43 (9.3)</td>
<td>Zabol</td>
<td>38 (10.5)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Zahak</td>
<td>5 (0)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Zahedan</td>
<td>70 (8.8)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Iranshahr</td>
<td>14 (0)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Khash</td>
<td>12 (8.3)</td>
</tr>
<tr>
<td>South</td>
<td>45 (2.2)</td>
<td>Chabahar</td>
<td>10 (0)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sarbaz</td>
<td>5 (0)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Saravan</td>
<td>10 (0)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Nikshahr</td>
<td>10 (10.0)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Konarak</td>
<td>10 (0)</td>
</tr>
<tr>
<td>Total</td>
<td>184 (6.5)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Statistical analysis
Data were analyzed with SPSS statistical software, 16th version (SPSS Inc, Chicago, IL, USA). Logistic regression and χ² tests were used to compare the variables; p-values less than 0.05 were considered statistically significant. ArcGIS software version 9.3 (ESRI, Redlands, CA, USA) was used for mapping the results.

Ethical considerations
The study was approved by the ethics committee of Pasteur Institute of Iran.

Results
In total, 12 samples (6.5%, 95% CI 3.58–10.82%) had positive IgG titers and 19 samples (10.3%) had borderline IgG titers against F. tularensis antigen. Only two (6%) of the 31 sera positive or borderline for F. tularensis antibodies also displayed antibodies against Brucella abortus antigen. The highest F. tularensis seroprevalence was observed in Zabol (10.5%) and Nikshahr (10.0%), which are northern and southern counties, respectively. However, the mean seropositivity rates progressively declined from the northern (9.3%) to the central (6.3%) to southern (2.2%) regions of Sistan and Baluchestan Province, although these differences were not statistically significant (p=0.37). We found no statistically significant difference (p=0.25) between the F. tularensis seroprevalence observed in butchers (5.0%) and slaughterhouse workers (9.4%). The F. tularensis seroprevalence rates were not correlated to age (p=0.94) and length of employment (p=0.19) of participants.

Discussion
Our study shows the presence of IgG antibodies against F. tularensis in 6.5% of the 184 healthy butchers and slaughterhouse workers investigated in Sistan and Baluchestan province, in south-eastern Iran. The ELISA test we used for screening of anti-F. tularensis IgG antibodies is considered to have a specificity rate higher than 95%, close to the 98.1% specificity reported for the western blot technique.11 False positive results for tularemia serology have been mainly observed in brucellosis patients, owing to serological cross reactions between F. tularensis and Brucella spp. antigens.12 However, in this study, only 6.5% of serum samples positive for anti-F. tularensis antibodies also displayed anti-Brucella sp. antibodies. Thus, it is highly probable that positive F. tularensis ELISA tests found in butchers and slaughterhouse workers represent true tularemia infections. Because the seropositive participants were asymptomatic, positive antibody titers probably indicated past tularemia infections. It is also possible that they were recent but asymptomatic because not all infections with Francisella result in symptoms. Moreover, we did not find an increase in tularemia seroprevalence in older patients as reported in other studies,4 which suggests that tularemia has recently emerged or re-emerged in the studied province. Altogether, our data suggest that tularemia is currently endemic in Sistan and Baluchestan Province, which warrants further investigations in the general population and in domestic animal herds to which the two occupational groups studied are exposed.
In recent years, there have been no official reports of human tularemia cases in Sistan and Baluchestan Province. However, the clinical manifestations of tularemia in humans are vary and often unspecific. Infections caused by type B *F. tularensis*, found in Europe and Asia, are usually of mild to moderate severity, with mortality rates lower than 1%. The more frequent clinical forms of tularemia may be easily confused with other diseases, such as tuberculosis for chronic lymphadenopathies or Streptococcus pyogenes infection for pharyngitis. Also, significant antibody titers are usually detected 2 to 3 weeks only after the onset of symptoms, and may persist for months. Thus, serological tests are often negative at the time patients seek medical attention, and a positive test does not necessarily reflect acute and/or recent infection. Thus, tularemia may be an underdiagnosed disease in Iran.

In the 1970s, antibodies against *F. tularensis* were found in wild animals in Zabol county in the northern region of Sistan and Baluchistan province, and it was subsequently expected that the seroprevalence of tularemia would be higher in this region. In the present study, the seroprevalence of tularemia in the northern region was higher than in the central and the southern regions of this province. This suggests that tularemia endemic foci may predominate in the northern part of Sistan and Baluchistan Province, and thus the presence of unrecognized tularemia cases should be carefully investigated in this area. However, a seroprevalence of 10% was also found in the southern county of Nikhshahr, suggesting an extended tularemia endemic area in south-eastern Iran.

The risk of tularemia in butchers and slaughterhouse workers could have been better evaluated if we had included a control group. It is recommended that for similar studies a group from the general population be recruited as a control group. Another limitation of this study was not having access to the medical records of the study participants to tentatively correlate their past or present clinical manifestations with positivity of tularemia serology.

**Conclusions**

Our results emphasize the need to improve the knowledge of the clinical manifestations and diagnosis of tularemia among health-care workers, to better clarify the real impact of the disease in the studied occupational groups, but also in other high-risk groups (such as veterinarians and hunters) and in the general population. Further work is also needed to clarify the current reservoirs and modes of transmission of *F. tularensis*. In the meantime, because our data indicate that butchers and slaughterhouse workers may be exposed to *F. tularensis* infection during their work, it is recommended that these employees be tested for tularemia at the time of recruitment and then on a regular basis during their active period. They should also use personal protective equipment to prevent such contaminations.

**Supplementary data**

Supplementary data are available at Transactions Online (http://trstmh.oxfordjournals.org/).

**Authors’ contributions:** MMG and EM conceived the study; MMG, MRS and EM designed the study protocol; SE, BE, MRS and EM carried out the clinical assessment; MM, FBA and EM analysed and interpreted the data. SE and EM drafted the manuscript; all authors critically revised the manuscript for intellectual content. All authors read and approved the final manuscript. SE, MM and EM are guarantors of the paper.

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**Competing interests:** None declared.

**Ethical approval:** The study was approved by the ethics committee of Pasteur Institute of Iran.

**References**