Seroprevalence of Q fever in dairy cattle in the Konya province, Turkey

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SUMMARY

The present study was conducted to determine the seroprevalence of Q fever among the dairy cattle in the Konya province, Central Anatolia, Turkey. Specifically, sera samples were collected from 322 dairy cattle and the presence of IgG antibodies occurred against Coxella burnetii phase II was identified by an indirect immunofluorescence assay (IFA) test using the 1:32 dilution as positive threshold. A total of 40 cows (seroprevalence: 12.4%) gave positive results. No association was evidenced between the seropositivity and reproductive disorders or the tick presence. These results indicate that C. burnetii was implanted in the dairy cattle in both the Konya province and Central Anatolia, Turkey.

Keywords: Coxella burnetii, Q fever, dairy cows, Indirect Immunofluorescent assay, Konya, Turkey.

Introduction

The term, “Q fever” was introduced by Edward Holbrook Derrick in 1937 in Australia in order to define an inflammatory disease appearing in slaughterhouse workers [10]. Bringing the first letter of the word “query,” which means suspicious, and the word “fever,” which indicates a high body temperature, together, the disease has been called “Q fever.” Q fever, caused by Coxella burnetii (C. burnetii), is a worldwide zoonosis disease [31]. C. burnetii is a common microorganism in all regions of the world, with a broad reservoir that includes wild and domestic mammals, fowls, and arthropods such as ticks [2]. It is a compulsory, intracellular, small (0.2-0.4 / 0.4-1.0 μm), pleomorphic, immobile bacteria with neither a flagella nor a capsule. They are relatively resistant to environmental conditions because of their spore-like structure [25].

Infection in animals may arise from ticks, as well as direct contact with contaminated materials (e.g., aerosol inhalation, contaminated pasture grazing, eating contaminated hay or fodder) [1, 22, 36]. Most of the infected animals appear to be asymptomatic, although clinical symptoms, including abortion, metritis, mastitis, infertility, stillbirth and calves weak at birth, may be present [2]. Humans are mainly infected either by inhalation of contaminated dust, or through the processing of (or consumption of) raw animal products [31].

Three different laboratory methods are employed for the diagnosis of coxiellosis: the isolation of bacteria from clinical samples, their direct identification via molecular and immunohistochemical techniques, and displaying antibodies thereof. However, the laboratory diagnosis for Q fever is essentially based on the determination of antibodies using serological methods [21]. A diverse range of serological techniques, including Radioimmunoassay (RIA), Microagglutination (MA), Enzyme-Linked Immunosorbent Assays (ELISA), Indirect Haemolysis Test (IHLT), Indirect Immunofluorescent Assays (IFA) test, Complement Fixation Test (CFT), Dot Immunoblotting, and Western Blotting (WB), have been used for detecting C. burnetii antibodies [6, 9]. IFA test is considered as the reference method in the serological diagnosis of Q fever [6, 13].

Although C. burnetii has been known to be present in Turkey since 1948 [30], a limited number of investigations have been performed with cattle. To date, no report on the Q fever in cattle was available in the Konya area. The aim of the study was to define the seroprevalence of Q fever in cattle in the Konya province, Turkey.

Materials and Methods

ANIMALS AND BLOOD SAMPLING

This study was conducted in a recently installed dairy farm in the Konya province in the Central Anatolia Region, with a total 322 Holstein Friesian dairy cows from different farms in the region. Abortion, dead, or weak offspring, as well as the
infertility history of the study populations, were recorded for each animal in terms of information acquired from the farm owner. Additionally, the animals and housing regions were both examined for the presence of ticks.

Blood samples were collected from the cows (3-5 years old) by the jugular vein puncture in serum tubes (Vacutainer tube with clot activator, Becton Dickinson Co. USA). After clotting at room temperature for 1 hour, blood samples were centrifuged at 1 500g, for 15 minutes at room temperature. Sera were carefully harvested and stored at -20°C until assayed.

SEROLOGICAL ASSAY

Serum samples were analyzed at the Department of Communicable Diseases Research of Refik Saydam National Hygiene Center (RSNHC) in Ankara, Turkey. The presence of phase II IgG antibodies to C. burnetii was measured with the Indirect Immunofluorescent Antibody (IFA) test, using commercial slides coated with C. burnetii phase II, Nine Mile strain (Vircell SL, Granada, Spain) and following the manufacturer’s recommendations. All serum samples that reacted at a cut-off dilution of ≥ 1:32 or more were considered as positive.

Results

Forty of the 322 cattle sera (12.4%) were seropositive in the IFA test at titre ≥ 1:32 for C. burnetii (Table I).

No pathological history, abortion, dead or weak offspring or infertility was observed in these forty seropositive cattle when the samples were drawn. Additionally, no ticks were observed either on the animals or in housing areas upon examination.

<table>
<thead>
<tr>
<th>Turkish regions</th>
<th>Positive results (number) - Seroprevalence (%)</th>
<th>Diagnostic test (Cut-off titre)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>East Anatolia</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>32/234 – 13.7%</td>
<td>MA (≥1.16)</td>
<td></td>
<td>23</td>
</tr>
<tr>
<td>41/262 – 15.6%</td>
<td>CFT (≥1.16)</td>
<td></td>
<td>23</td>
</tr>
<tr>
<td>24/416 – 5.8%</td>
<td>IFA (≥ 1.80)</td>
<td></td>
<td>7</td>
</tr>
<tr>
<td>12/53 – 22.6%(^1)</td>
<td>ELISA</td>
<td></td>
<td>33</td>
</tr>
<tr>
<td>10/177 – 5.6%(^2)</td>
<td>ELISA</td>
<td></td>
<td>33</td>
</tr>
<tr>
<td>15/92 – 16.3%</td>
<td>ELISA</td>
<td></td>
<td>8</td>
</tr>
<tr>
<td>Marmara</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3/275 – 1.1%</td>
<td>CFT (≥1.16)</td>
<td></td>
<td>4</td>
</tr>
<tr>
<td>35/96 – 36.5%(^3)</td>
<td>ELISA</td>
<td></td>
<td>28</td>
</tr>
<tr>
<td>4/52 – 7.7%(^4)</td>
<td>ELISA</td>
<td></td>
<td>28</td>
</tr>
<tr>
<td>128/1593 – 8.0%</td>
<td>ELISA</td>
<td></td>
<td>37</td>
</tr>
<tr>
<td>Aegean</td>
<td></td>
<td></td>
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<tr>
<td>85/391 – 21.7%</td>
<td>MA (≥1.32)</td>
<td></td>
<td>14</td>
</tr>
<tr>
<td>6/138 – 4.3%</td>
<td>PCR</td>
<td></td>
<td>20</td>
</tr>
<tr>
<td>Central Anatolia</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>53/208 – 25.5%</td>
<td>CFT (≥1.16)</td>
<td></td>
<td>3</td>
</tr>
<tr>
<td>Mediterranean</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0/53 – 0.0%</td>
<td>MA (≥1.16)</td>
<td></td>
<td>5</td>
</tr>
<tr>
<td>17/32 – 53.1%</td>
<td>IFA (≥1.16)</td>
<td></td>
<td>11</td>
</tr>
<tr>
<td>41/92 – 44.6%(^3)</td>
<td>CA (≥1.20)</td>
<td></td>
<td>29</td>
</tr>
<tr>
<td>37/318 – 11.6%(^2)</td>
<td>CA (≥1.20)</td>
<td></td>
<td>29</td>
</tr>
</tbody>
</table>


\(^1\) including only cows with abortion; \(^2\) including cows without abortion; \(^3\) including cows with fertility problems; \(^4\) including healthy cows.

Discussion

Q fever is a common disease in most parts of the world with the exception of Antarctica and New Zealand [15]. The epidemiology of coxiellosis varies from country to country. Given an approximately 50% infection rate of C. burnetii in cattle globally, it has primarily been detected in the dairy cattle rather than in the beef cattle [1]. Q fever presence in Turkey was first revealed in 1946-1947 [30]. Subsequently, various studies in human and veterinary medicine have been conducted in order to determine its geographical distribution and prevalence in Turkey [18]. However, to our knowledge, this is the first reported seroprevalence investigation on C. burnetii in dairy cattle in the Konya province in the Central Anatolia Region, Turkey.

Q fever in humans and farm animals is known to be endemic and dispersed throughout Turkey [17]. The seroprevalence rates in cattle were reported to greatly fluctuate between 0.0% and 53.3% in the various regions of Turkey [18] (Table II). Some previous results [8, 23] were similar with the coxiellosis seroprevalence reported in the present study whereas some others were higher [3, 11, 14, 28, 33] or lower [4, 5, 7, 20, 37]. These discrepancies may be explained by geographic and climatic varieties, size of sampling populations, definition of

| Cattle | Positive results (number) Seroprevalence (%) Diagnostic test (Cut-off titre) |
|--------|---------------------------------------------------------------|------------------------|
| 322    | 40                                                            | IFA                    |
|        | 12.4% (≥1.32)                                                 |                        |

IFA: Indirect Immunofluorescent Assay.

TABLE I: Seroprevalence of C. burnetii infection in a cattle farm in Konya province, in Central Anatolia region, Turkey.

TABLE II: Seroprevalence rates (%) of the C. burnetii infection in various Turkish regions (Revised from Kilic and Celebi, 2008 [18]).
the cow population (with or without abortion and fertility problems), assay type, or criteria used to cut-off positive values. In addition, the seropositivity in the current study and previous ones performed in the same region but in different provinces [3, 11], indicated that coxiellosis is not a new disease but a common problem in cattle in Central Anatolia.

The signs of Q fever in cattle include abortion, dead or weak offspring, retained placenta, metritis, and infertility [22, 25]. However, *C. burnetii* infections are generally asymptomatic [22]. Therefore, it does not appear possible to diagnose the disease from clinical findings only. The IFA test, employed in the present study, is considered to be the reference method in serologically diagnosing Q fever [6, 13]. An appropriate antibody cut-off level to use for sero-epidemiologic investigations, either regionally or globally has not been reported. For instance, antibody titres above 1:8 [16, 24], 1:16 [11, 32, 35], 1:32 [19, 34], or 1:50 [26], were accepted as positive threshold in various seroepidemiological studies. Since the prevalence rate of the disease in cattle was unknown in Konya, a relatively high cut-off antibody titre level of ≥ 1:32 was used in the present study.

*C. burnetii* occurs in two antigenic phases: I and II. This antigenic difference is important in diagnosis [13]. Although phase II antibodies are more prevalent during an acute infection, chronic infection is characterized by a predominantly phase I antibody response [12, 13]. Nonetheless, antibodies to both phase I and II antigens have been known to persist for months, or even years, following the initial infection [13]. In the present study, anti-phase II antibody titres were utilized in order to determine seropositivity. There was no clinical finding (e.g., abortion, dead or weak offspring, and infertility) related to disease in seropositive animals in the present study. Additionally, no tick was found either on cattle or in housing areas upon examination. Therefore, this seropositivity may not be a sign of acute infection; it may only be interpreted as evidence that cattle were exposed to infection in the past.

Since domestic farm animals are the major source of the infection, it is of interest to define the specific antibody prevalence among these species [17]. Epidemiological data indicate that dairy cows are chronically and more frequently infected than sheep and can be the most significant source of human infection [25]. In studies performed with farmers in various regions of Turkey, a seropositivity of 10.2% to 51.8% has been determined [7, 27, 29, 33]. In the present study, the seropositivity of 12.4% seems to be a significant risk factor for the farmers who come into contact with those animals.

Consequently, it has been concluded that the 12.4% seropositivity obtained in this study appears to be an important health problem for the farm used in this study, the region’s cattle, and individuals coming into contact with these cattle. In addition, the study supplements the data acquired from previous studies to identify the coxiellosis seroprevalence globally, and in Turkey.

References

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