Investigation of Coxiella burnetii antibodies in sheep in Aydin region, Turkey

S. KILIC¹, S. PASA²*, C. BABUR¹ and M.B. OZLEM²

SUMMARY

The present study was carried out on sheep to determine the prevalence of Coxiella burnetii. Serum samples were collected randomly from 100 sheep, in five different locations in West Turkey and were investigated for IgG antibodies against C. burnetii phase II antigen by indirect immunofluorescent antibody test (IFAT). History of the animals such as age, breed, tick control and abortion were recorded. A total of 3 (3 %) analyzed sera had antibodies to C. burnetii. This study revealed the presence of C. burnetii in sheep in the region of Aydin, Turkey.

Keywords : Coxiella burnetii - Q fever - Sheep - Indirect Immunofluorescent Antibody Test (IFA).

Introduction

Q fever is a zoonosis caused by obligate intracellular bacteria Coxiella burnetii (C. burnetii). The disease is endemic worldwide, occurring in different geographic regions and climatic zones except in New Zealand [15, 21].

Characteristics of the epidemiology of C. burnetii are the many reservoirs and multiple infection cycles. The principal vectors of C. burnetii are ticks, which transmit the agent to wild animals (causing wildlife coxiellosis) or to domestic animals (creating the livestock reservoir of C. burnetii) [17, 19]. The most important reservoirs in nature are small wild rodents, but infection has been identified in a wide variety of other animals, including arthropods, ungulates, ruminants, marsupials, birds, and fish [22, 31, 35].

Animals typically acquire C. burnetii through exposure to other infected animals, either by direct contact with parturition products or by inhalation of infectious aerosols. Although most animals have persistent, relatively asymptomatic infection, abortion in sheep and goats, and infertility in cattle has been associated with chronic C. burnetii infection [1, 2, 6, 7, 19, 23]. Infected animals produce antibodies, but are unable to eliminate the pathogen. Infected female animals shed enormous numbers of bacteria through their decidua (amniotic fluids and placenta) at parturition and intermittently in various secretions and excreta into the environment, where very resistant organism remain viable over long periods of time in harsh environmental conditions, dust, wool, and dried secretions [21, 31].

Since isolation and identification of the causative agent is time-consuming, hazardous, expensive, and available only in reference laboratories, diagnosis of Q fever relies mainly on the detection of specific antibodies to C. burnetii [14,18]. A wide variety of serological techniques including Microagglutination (MA), Radioimmunoassay (RIA), Indirect hemolysis test, Indirect immunofluorescent assays (IFA), Complement fixation test (CFT), Enzyme-Linked Immunosorbent Assays (ELISA), Dot immunoblotting and Western blotting (WB) have been used for detecting C. burnetii antibodies. The techniques most commonly used include CFT, IFA, and ELISA [2, 9, 28, 34]. Since IFA is both sensitive and highly specific and to date no significant cross-reaction with known cases of illness caused by various rickettsia, it is regarded as the reference technique for Q fever diagnosis [10, 13, 14, 21].

C. burnetii has been known to occur in Turkey since 1948 [27]. Q fever in humans and animals is known to be endemic, and disseminated throughout Turkey. However, the importance of these data has not been evaluated from the point of view of public health yet.

Since domestic farm animals are the major source of the infection, it is of interest to define the prevalence of specific antibodies in these species. To our knowledge, seroprevalence of Q fever in sheep in Aydin province located in the west of Turkey has not been reported in the literature so far. The aim of the present study was to determine the prevalence of Q fever in sheep in Aydin region.
Materials and Methods

All the sheep were White Karaman breeds, 1 to 2 years old, female and non-pregnant. One hundred blood samples were obtained from apparently healthy sheep herds, which were approximately 60 sheep in size, and each one of these herds were represented with 20 randomly selected samples. The herds were located in the city center of Aydin, and its four districts; Cine, Yenipazar, Kocarli and Germencik. The blood sampling was carried out between March and April 2002 (Figure 1).

The herds were selected randomly from a list provided by the Turkish Ministry of Agriculture. The breed, age, sex, number of birth, abortion history, tick infestation and tick control of the study populations were registered for each animal on the basis of information gained from the owner. In addition, animals and the housing areas were examined for existence of ticks. The serum was separated 24 h after sampling and stored at -20°C until tested.

Serum samples were analysed at the Department of Communicable Diseases Research of Refik Saydam National Hygiene Center (RSNHC) in Ankara.

The presence of IgG antibodies to *C. burnetii* was measured by indirect immunofluorescent antibody technique (IFA) using commercial slides coated with *C. burnetii* phase II, Nine Mile strain (Vircell SL Granada, Spain). IFA was performed according to the instructions of the manufacturer. For the detection of antibodies against *C. burnetii*, sera were initially screened at a 1:64 dilution in a phosphate buffered saline (PBS) with anti-sheep IgG marked with fluorescein isothiocyanate conjugate (Sigma F 7634, USA). Positive and negative controls were run with each test. Considering the relative little information about interpretation of the serology of *C. burnetii* infection in sheep, particularly diagnostic titres as in humans, we considered sheep with result ≥ 1:64 for phase II antibodies as positive sheep. Serial twofold dilutions of sera positive at this solution were tested to determine end titres.

Results

Three of 100 sheep sera (3%) were seropositive in the IFA test at titre 1:64 for *C. burnetii*. Sheep came from two different herds from two provinces (Cine and Germencik). Geographical location-related prevalence is presented in Table I.

Infected sheep were 2 years of age. No tick on the animals and in housing areas has been determined in the examinations. No pathological history (abortion, premature parturition and birth of stillborn) was observed in these three seropositive sheep when the samples were drawn. In addition, no pathological history for seropositive sheep was reported by the owners.

<table>
<thead>
<tr>
<th>Location</th>
<th>No. of sera tested</th>
<th>No. of positives</th>
<th>(%) positives</th>
</tr>
</thead>
<tbody>
<tr>
<td>City Center</td>
<td>20</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Cine</td>
<td>20</td>
<td>2</td>
<td>10</td>
</tr>
<tr>
<td>Kocarli</td>
<td>20</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Yenipazar</td>
<td>20</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Germencik</td>
<td>20</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>TOTAL</td>
<td>100</td>
<td>3</td>
<td>3</td>
</tr>
</tbody>
</table>

Table I. — *C. burnetii* IgG antibodies to phase II in sheep as determined by IFAT in Aydin region, Turkey.

Discussion

Serologic studies suggest that infection may be widespread in the world, lack of clinical data and diagnosis renders it impossible to estimate the true incidence of the disease in animals and particularly in humans [17, 31]. The prevalence of *C. burnetii* infection in animals varies widely with species tested, year, geographic location, assay type, and criteria used to define positive results [1, 19, 22]. Seropositivity to *C. burnetii* in healthy sheep has been variously reported to be 3.5 % in the Netherlands [16], 2 to 30 % in Italy and 10 to 20 % in Spain [36], 10 % in Mexico [30], and 62 % in Sudan [29].
Q fever was first recognised in Turkey when a small outbreak occurred in rural community in 1947 due to the fact that infectious dust produced by shearing of sheep whose wool was presumably contaminated with the bacteria was suspected as a possible source of transmission [26]. Since the importance of Q fever became apparent, serologic surveys have been performed to trace the prevalence of the disease in both humans and animals. Results of several seroprevalence studies, using CFT conducted in humans and animals suggest that \textit{C. burnetii} infection has been widespread in Turkey. The results of surveys performed in sheep are summarised in table II. As shown in table II, the prevalence of antibody to \textit{C. burnetii} in healthy sheep in Turkey varies widely, between 2.7% and 22.1%. This small survey indicates that three of 100 randomly selected sheep (3%) in Aydin region had serological evidence of previous \textit{C. burnetii} infection. The seroprevalence rate in the present study is consistent with the results of previous serological studies performed by PAYZIN [27], ATILLA [4] and ATUN [5]. On the other hand, our prevalence result is lower than those reported by TURTIN [37], ALPAR [3], LELOGLU [20], OZYER [25] and CETINKAYA \textit{et al} [8]. The reasons for these discrepancies may be explained by spatial, temporal, strain, and many other factors determining the prevalence of Q fever in sheep as well as possible differences among laboratories and testing procedures.

Turkey can be distinguished into seven geographical areas whose climate depends on the geographical locations and geographical variations may be responsible for the difference in the prevalence figures of serological studies. Since the study areas, the city center Aydin and its districts, were adjacent to each other and they have similar weather and husbandry conditions, there was no evidence of any such association in this study. However, there were significant differences arose from geographic locations and also husbandry condition, between the present study and the results of studies conducted in other parts of Turkey. A study by CETINKAYA [8] in Elazig found that 10.5% of 411 sheep had antibodies to \textit{C. burnetii} phase II using IFA. This higher prevalence rate may be related to geographical variation and economy of this region. Elazig is located in mountainous region of Eastern Turkey where the economy depends largely on agriculture and animal husbandry and a considerable proportion of the population is employed in this sector, whereas Aydin in the west of Turkey has a mix of agriculture and developed industry. With regard to the role of economical conditions in the epidemiology of Q fever among different areas, lower economical status in eastern Turkey can cause inadequate animal husbandry condition that may result in spreading much the agent in the flocks.

Although ticks have been suggested to play a role in the epidemiology of \textit{C. burnetii}, this is hardly likely in western Turkey. In the east of Turkey, ticks have been reported to be widespread and \textit{C. burnetii} has been isolated from \textit{Dermacentor} and \textit{Ornithodorus} species [33]. Although tick-borne disease caused by \textit{Babesia ovis} has been reported in sheep in the west of Turkey [32], there is no report on tick species and isolation of \textit{C. burnetii} from ticks in Aydin region. Therefore, it is not possible to explain the role of ticks in \textit{C. burnetii} infection between the west and east of Turkey in this respect.

The prevalence of Q fever was higher in females than in males, because females are more susceptible to Q fever than males. In addition, pregnancy is an important parameter in occurrence of Q fever and the higher seroprevalence rate of disease have been reported in sheep during pregnancy [7, 19, 25]. In Turkey, parturition takes place in the spring and the prevalence of \textit{C. burnetii} infection could be expected to be higher in this season. In this study, the samples were taken in spring from non-pregnant sheep in which no reproductive condition, between the present study and the results of studies conducted in other parts of Turkey. A study by CETINKAYA [8] in Elazig found that 10.5% of 411 sheep had antibodies to \textit{C. burnetii} phase II using IFA. This higher prevalence rate may be related to geographical variation and economy of this region. Elazig is located in mountainous region of Eastern Turkey where the economy depends largely on agriculture and animal husbandry and a considerable proportion of the population is employed in this sector, whereas Aydin in the west of Turkey has a mix of agriculture and developed industry. With regard to the role of economical conditions in the epidemiology of Q fever among different areas, lower economical status in eastern Turkey can cause inadequate animal husbandry condition that may result in spreading much the agent in the flocks.

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<table>
<thead>
<tr>
<th>Reference</th>
<th>Survey Location</th>
<th>Diagnostic test (Cut-off titre)</th>
<th>No. of animals</th>
<th>Prevalence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Payzin [27]</td>
<td>Central Anatolia</td>
<td>CFT (≥1:64)</td>
<td>49</td>
<td>4.1</td>
</tr>
<tr>
<td>Atun [5]</td>
<td>Southwestern Turkey</td>
<td>CFT (≥1:16)</td>
<td>974</td>
<td>2.7</td>
</tr>
<tr>
<td>Turtin [37]</td>
<td>Central Anatolia</td>
<td>CFT (≥1:16)</td>
<td>164</td>
<td>9.1</td>
</tr>
<tr>
<td>Alper [3]</td>
<td>Western Turkey</td>
<td>CA (titre not specified)</td>
<td>1128</td>
<td>10</td>
</tr>
<tr>
<td>Leologlu [20]</td>
<td>Eastern Turkey</td>
<td>CA (≥1:16)</td>
<td>456</td>
<td>22.1</td>
</tr>
<tr>
<td>Ozyer [25]</td>
<td>Southeastern Turkey</td>
<td>CFT (≥1:8)</td>
<td>75 (unaborted)</td>
<td>78.6</td>
</tr>
<tr>
<td>Cetinkaya [8]</td>
<td>Eastern Turkey</td>
<td>IFA (≥1:80)</td>
<td>411</td>
<td>10.5</td>
</tr>
</tbody>
</table>

TABLE II. — Epidemiologic studies on Q fever in sheep in Turkey.
problems and abortions have been occurred. Although there have been a significant number of abortions in domestic animals in Turkey, there is no study on reproductive problems and the causes of abortions in ruminants in Aydin in the literature so far. Since the time of sample collection, history of abortion and pregnancy were not indicated that in other studies, it is not possible to explain the differences in the seroprevalence rates in this respect. Lower seroprevalence rate obtained in the present study may be due to partly to the small number of the animals examined and the size of sheep herds. Although serological evidence of the infection has been demonstrated in sheep, this small sample size probably makes it of little importance as a cause of Q fever in this region.

It is difficult to compare the results of this survey with previous serological studies in our country owing to assay type and different criteria used. It should be stressed that in most of previous studies CFT was used to detect C. burnetii antibodies (Table II). CFT has been shown to be less sensitive than IFA and ELISA and particularly in diagnostic IgM specific serology [9, 13, 28]. It has been established that IFAT is considered to be more sensitive for the diagnosis of the disease and to offer more accurate results [10, 13, 14, 34]. In addition, there is not currently an universal consensus of appropriate antibody cut-off level to use for sero-epidemiologic surveys as the cut-off level is dependent on the antigen preparation being used and the population studied [8, 24, 36]. We choose a higher cut-off antibody titre of ≥1:64 because the prevalence rate in sheep in Aydın region was unknown. Therefore, it is difficult to appreciate sensitivity and specificity with our cut-off criteria.

Interpretation of results of serological studies in animals should focus on the immunological response to C. burnetii and the relationship between infection and excretion of bacteria. C. burnetii undergoes phase variation in which antigenic transition occurs from the wildtype phase I to avirulent phase II during successive passages in embryonated eggs or in cell cultures [17, 21, 31]. Serologic assay may detect antibodies to phase II and phase I C. burnetii. Phase II antibodies are more prevalent during in acute infection, while chronic infection is characterised by a predominantly phase I antibody response [11, 14]. In contrast, the importance of the development of antibody responses to phase I and phase II and stage of infection has not been well evaluated in animals [1, 12, 22]. Serologic test are not useful tools in order to determine which animal represents a current risk for transmission, as animals may seroconvert without shedding or remain seropositive long after the acute infection has resolved. Conversely, some animals may pose a risk for infection prior to the development of antibodies by shedding the bacteria and some infected animals never seroconvert [1, 7, 12, 19]. In the absence of longitudinal and repeated sampling, seroprevalence studies do not provide information on incidence of current infection in humans or animals, or indicate whether an animal is infectious. Instead, the results of the studies can only be interpreted in animals as evidence of previous infection (past exposure) [19, 22, 30].

In conclusion, the results of this study confirm the presence of anti-C. burnetii antibodies in sheep in the region of Aydın, Turkey. The data obtained from this study may be useful for reference in further studies in the region of Aydın. Further studies in collaboration between veterinary and medical services on Coxiella infection in both domestic animals and humans are needed to elucidate the epidemiology of Q fever in Turkey.

References


