The endemic status of *Anaplasma marginale* in cattle, in Turkey

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**ABSTRACT**

This study was designed to determine the prevalence and endemic status of *Anaplasma marginale* in cattle in the province of Konya. A total of 700 cattle were randomly selected, categorized in different age groups (i.e. 0–6, 7–12, 13–24, 25–36 and >36 months) and examined in Kadinhani, Cumra and Beysehir provinces and in Konya city center. The presence of *A. marginale* was diagnosed by microscopic examination of blood smears and competitive ELISA (cELISA). Parasites were observed in 11.29% of blood smears, and 31.86% of animals were positive for antibodies against *A. marginale*. The rates of seropositivity in successively older age groups were 19.1, 24.39, 33.14, 44.21 and 37.36% respectively. The endemic status of the disease was determined by calculating the inoculation rate (h) of cattle in each age group. The h value was detected to be lower than 0.005 and the endemic status of *A. marginale* was found to be unstable. If the cattle in Konya province were vaccinated when they are 9 to 12 months of age, they could be protected during the seasons when the disease is prevalent.

**Key words:** *Anaplasma marginale*, Cattle, Endemic status, Turkey.

**INTRODUCTION**

*Anaplasma marginale* is a causative agent of bovine anaplasmosis. It is seen in tropical and subtropical regions and is transmitted both biologically, by vector ticks, and mechanically. Many hematophagous diptera are implicated as mechanical vectors for transmission of the agent, such as *Stomoxys calcitrans*, *Haematobia irritans* and *Tabanus* spp. (Kocan et al. 2003). Other mechanical means of transmission involving blood-contaminated fomites, including needles, nose tongs, ear-tagging devices and castration instruments (de la Fuente et al. 2004, de la Fuente et al. 2005, Kocan et al. 2010), are very important in the epidemiology of anaplasmosis. In regions endemic for anaplasmosis, pathognomonic signs such as high fever, severe anemia and jaundice, weight loss, abortion, decreased milk production and death in animals over 2 years old are frequently seen (Kocan et al. 2010, Singh et al. 2014).

The diagnosis is based on clinical signs and microscopic examination of stained thin blood smears obtained from clinically infected animals during the acute phase of the disease. This method is not reliable for the detection of carriers and latently infected animals. In these situations, the infection is diagnosed by serology and molecular detection methods (Aubry and Geale 2011). The most accurate serological test is competitive ELISA (cELISA) (Knowles et al. 1996). This test uses a monoclonal antibody (MAb) specific for MSP5. It is very sensitive and specific for the detection of infected animals (Aubry and Geale 2011, Knowles et al. 1996, Torioni et al. 1998).

In regions endemic for anaplasmosis, control measures include arthropod control by application of acaricides, administration of antibiotics and vaccination (Kocan et al. 2003). The most reliable method of protecting animals from infection with the blood parasite is vaccination. To decide whether vaccination is necessary or not, the endemic status of the disease in a region should be determined. When the endemic status is stable, vaccination is not needed. However, when the endemic status in a region is unstable, vaccination is required. Vaccination reduces the economic effect of the disease by increasing the immunity of susceptible animals to field challenge. The endemic situation defines herd immunization. The rate of herd immunity is expressed by the term “inoculation rate” and is generally measured by serological tests. For calculation of the inoculation rate, the method developed by Mahoney and Ross (1972) is commonly used. If the inoculation rate of *Anaplasma* is high, young ruminants are protected by innate and colostral immunity; the clinical disease occurs at a limited rate, and endemic stability will be obtained. Conversely, if the inoculation rate is too low, clinical cases may occur because of endemic instability (Geleta 2005).

Turkey is located in the subtropical climate zone, where bovine anaplasmosis is an endemic disease. It is necessary to determine the endemic situation of the disease in this country as there is no published report available. The aims of this study were to determine the seroprevalence of *A. marginale* in cattle in the province of Konya, and to investigate whether anaplasmosis is endemically stable in the region.

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MATERIALS AND METHODS

Animals: This study was performed in Konya province, Turkey. A total of 700 cattle were randomly selected, categorized in different age groups (i.e. 0–6, 7–12, 13–24, 25–36 and >36 months) and examined in Kadinhani, Cumra and Beysehir districts and in Konya city center.

Microscopic examination of blood smears: Peripheral blood smears were obtained from the tip of one ear of each animal and stained with Giemsa.

Serum samples: Blood samples were collected from the 700 randomly selected cattle, categorized according to their age as follows: 0–6, 7–12, 13–24, 25–36 and >36 months. The samples were obtained by jugular vein puncture, stored at 4°C and then centrifuged at 4,000 rpm for 10 min on the same day. The serum samples were distributed in 1.5 µL aliquots and stored at −20°C until they were used in the cELISA.

cELISA: Serum samples were subjected to cELISA to investigate the presence of specific antibodies against A. marginale. The cELISA test was done according to the test procedure of the manufacturer (Anaplasma antibody test kit, cELISA, VMRD, Inc., USA).

Endemic status: In order to identify the endemic status of the region, inoculation rates of the cattle in each age group were calculated using the formula developed by Mahoney and Ross (1972).

the inoculation rate \( h = (-1) [\ln(1-I)] / t \),
where “I” is the proportion of animals infected and “t” the mean age of calves in days.

In endemically stable situations, the \( h \) value is less than 0.005. In endemically unstable situations, the \( h \) value is equal to or higher than 0.005.

RESULTS AND DISCUSSION

Based on microscopic and serological examinations, 79 (11.29%) cattle were found to be positive for A. marginale, and 223 (31.86%) cattle were found to be positive for A. marginale antibodies. A. marginale was detected microscopically at the lowest rate, in 7 (8.64%) of 81 cattle, in the group 0–6 months of age, and at the highest rate, in 22 (13.25%) of 166 cattle, in those aged 7-12 months. In microscopic examination 26 from 79 samples also positive (13.25%) of 166 cattle, in those aged 7-12 months. Inoculation rates of A. marginale were detected at the lowest rate 19.10% in the 0–6 months age group, and the highest rate was seen, in 44.21% in the group aged 25–36 months. The serology results were used to calculate inoculation rates. Inoculation rates were calculated for each age group to determine whether the endemic status of the region is stable or unstable (Table 1).

The rates of seropositivity in each of the age groups differed. As noted in Table 1, the \( h \) value was found to be lower than 0.005 in all groups. Therefore, the endemic status of A. marginale was determined to be unstable in the locations where the study was conducted.

Bovine anaplasmosis is endemic in tropical and subtropical areas and causes significant economic losses (Bowles et al. 2000, de la Fuente et al. 2004, de la Fuente et al. 2005). In many countries, the seroprevalence of A. marginale ranges between 1.45 and 98.2% (Chaudhri et al. 2013, Carrique et al. 2000, Lolli et al. 2016, Maharan et al. 2016, Ndou et al. 2010, Oliveria et al. 2011). In Turkey, the disease is the major health problem in dairy herds (Birdane et al. 2006, Coskun et al. 2012) and the prevalence ranges was determined at different rates (Aktas et al. 2011, Birdane et al. 2006, Cakmak 1990, Derinbay and Sevinc 2011, Duzgun et al. 1988, Gokce et al. 2013, Selcuk et al. 2015). In this study, the rate of seropositivity among all cattle was found to be 31.86%.

The methods employed to control anaplasmosis include chemotherapy, vaccination and tick control strategies. Vaccination is an economical and relatively effective way to control bovine anaplasmosis worldwide (Aubry and Geale 2011, Kocan et al. 2003). In regions where the disease is endemically instable, vaccination is an effective means of preventing outbreaks of anaplasmosis (Kocan et al. 2000, Kocan et al. 2003). However, vaccination against anaplasmosis is not carried out in Turkey.

One of the most important factors that affect the prevalence of bovine anaplasmosis is age (Aubry and Geale 2011). Animals over 2 years of age are usually affected by a peracute, fatal form of the disease (Ristic 1968). Calves from immune mothers receive protection that lasts until the animals are about 9 to 12 months of age (Carrique et al. 2000, Kocan et al. 2000, Taasi et al. 2002). If calves come into contact with an infested tick or other insect during this period of their life,

Table 1: The inoculation rates of A. marginale in cattle divided into different age groups

<table>
<thead>
<tr>
<th>Groups (month)</th>
<th>Number of examined animal</th>
<th>Mean age (day±SEM)</th>
<th>% Positivity (I)</th>
<th>Inoculation rate (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 - 6</td>
<td>81</td>
<td>51.91 ± 3.34</td>
<td>19.10</td>
<td>0.004</td>
</tr>
<tr>
<td>7 - 12</td>
<td>166</td>
<td>298.71 ± 4.14</td>
<td>24.39</td>
<td>0.0009</td>
</tr>
<tr>
<td>13 - 24</td>
<td>183</td>
<td>656.01 ± 8.23</td>
<td>33.14</td>
<td>0.0006</td>
</tr>
<tr>
<td>25 - 36</td>
<td>95</td>
<td>1069.62 ± 4.14</td>
<td>44.21</td>
<td>0.0005</td>
</tr>
<tr>
<td>&gt;36</td>
<td>175</td>
<td>2008.13 ± 53.39</td>
<td>37.36</td>
<td>0.0002</td>
</tr>
</tbody>
</table>
antibody titers will increase and they gain resistance against reinfection (Geleta 2005). Up to 9 to 12 months of age, the rate of seropositivity increases with age. After this age, it decreases gradually. To determine the endemic status of A. marginale in the Konya district of Turkey was one of the objectives of the present study. The inoculation rate of each group was calculated. The results indicate that the area is endemically unstable for A. marginale, because the h values calculated were lower than 0.005, which is the minimum level required for stability. If the cattle in Konya province were vaccinated when they are 9 to 12 months of age, they could be protected during the seasons when the disease is prevalent.

REFERENCES


CONCLUSION

In conclusion, this is the first study carried out in Konya province, in Turkey that this province is endemically unstable for anaplasmosis. To establish immunity, a vaccination protocol is required. And also similar studies should be performed in different parts of Turkey to prepare a local vaccine for this disease.

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