Survey of Theileria, Babesia and Anaplasma Infections of Cattle and Ticks from Sivas Province of Turkey*

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Summary: This study was carried out to investigate the presence and distribution of Theileria, Babesia and Anaplasma species in cattle and their ticks from Sivas province. A total of 314 EDTA-blood samples and 610 ticks were analysed. A part of 18S and 16SrRNA genes of Theileria/Babesia/Anaplasma species were amplified from the genomic DNAs extracted of the blood samples and tick pools by with polymerase chain reaction (PCR). A total of 14 probes (two catchall, two genera and ten species-specific) were bound on a membrane and then the PCR products were tested by reverse line blot (RLB) assay. The partial sequences of the 18S and 16S rRNA genes of representative positive samples were determined. According to the results of the blood and tick samples analysed by RLB and sequencing, T. buffeli (GenBank accession number: KJ183080), A. centrale (KJ183082), A. marginale (KJ183083), A. bovis (KJ183084), one Babesia genotype (Babesia sp. Sivas, KJ183081) and one Anaplasma genotype (Anaplasma sp. Sivas, KJ210855) were detected. Babesia sp. Sivas were found to be 99% identical with B. occultans, Babesia sp. Sivas were found to be 99% identical with Anaplasm sp. Clone 7 and A. bovis, respectively. Overall prevalences of Theileria and Anaplasma infections in cattle were found to be 5.10% and 11.15% by RLB, respectively. This study is the first molecular survey on species of Theileria, Babesia and Anaplasma in cattle and ticks from Sivas.

Key words: Anaplasma, Babesia, cattle, Theileria, tick, Sivas

Introduction

Theileria, Babesia and Anaplasma species are transmitted by ixodid ticks and infect domestic and wild animals throughout the world. The diseases cause important economic losses in livestock industry. Some of the species of these genera have zoonose potential and known as emerging infectious diseases (Inci et al., 2013; Inci et al., 2016; Uilenberg, 1995; Uilenberg, 2001).

Genetically and pathogenetically different six Theileria species have been found in cattle. Theileria annulata and T. parva are the most common pathogen Theileria species of cattle. These two species cause lympho-proliferative disease with high mortality. T. sergenti/buffeli/orientalia group, T. mutans, T. velfiera and T. tauri/taurotrag are known as lower pathogenic or apathogenic species (Uilenberg, 1995). Babesia bovis, B. bigemina, B. divergens and B. major are caused bovine babesiosis. Bovine babesiosis is an important livestock problem in tropical and subtropical regions (Uilenberg, 1995). Bovine anaplasmosis is caused by Anaplasma marginale, A. centrale, A. phagocytophilum and A. bovis (Inokuma, 2007). A. marginale is the most important species among these
species and causes clinical infections characterized with anemia and jaundice. *A. centrale* causes mild infections in cattle (Dumler, 2001). *A. phagocytophilum* (compiled from previously known as *Ehrlichia phagocytophila*, *E. equi* and human granulocytic ehrlichiosis agent) is a causative agent of tick-borne fever (TBF) in cattle. TBF characterized by high fever, depression, decreased milk production and reduced fertility (Pusterla et al., 1997; Woldehiwet and Scott, 1993). Although Sivas has a suitable climate for presenting Ixodid ticks, there is not available re-
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et al., Theileria, Babesia and Anaplasma infections in Sivas... Erciyes Üniv Vet Fak Derg 2020;17(1): 32-38.
Table 2. Theileria, Babesia and Anaplasma genera and species specific primers and probes

<table>
<thead>
<tr>
<th>Primers</th>
<th>Sequences (5'-3')</th>
</tr>
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<tbody>
<tr>
<td>RLB-F2</td>
<td>GACACAGGGAGGTAGTGACAAG</td>
</tr>
<tr>
<td>RLB-R2</td>
<td>biotin-CTAAGAAATTTACCTCGAGACTGT</td>
</tr>
<tr>
<td>16S8FE</td>
<td>GGA ATT CAG AGT TGG ATC ATG GCT CAG</td>
</tr>
<tr>
<td>B-GA1B</td>
<td>biotin-CGGGATCCTGGGACTTTCTTCT</td>
</tr>
</tbody>
</table>

**Primer or probe**
- Catchall (Theileria spp.+Babesia spp.)
- Theileria spp.
- Theileria annulata
- Theileria buffeli/orientalis
- Babesia spp.
- Babesia bigemina
- Babesia bovis
- Babesia major
- Babesia divergens
- Catchall (Anaplasma spp.+Ehrlichia spp.)
- Anaplasma centrale
- Anaplasma marginale
- Anaplasma (E.) bovis
- Anaplasma (E.) phagocytophylum

**Probes**
- Amino-TAATGGTATAGG(A)G(C)GTTG
- Amino-TGATGGAAATTTAAC(T)CTTTCA
- Amino-CTCTGGGGGGTCGTGTCA
- Amino-GGCTTATTCGGWTTGATTTT
- Amino-GGTAAATGGTATAGGAAA
- Amino-CTGTTTCGCGTATAATTGAG
- Amino-TCCGACTTTGTTGTTGT
- Amino-GACGCTATAAGTGTCGAG
- Amino-GGG GGA AAG ATT TAT CGC TA
- Amino-TCG AAG GGA CCA TAC GC
- Amino-AAG GGA CCA TAC GC
- Amino-GTA GCT TGC TAT GGG GAC A A
- Amino-TTG CTA TRA AGA ATA RRT AGT GG

**DNA sequencing of 18S and 16S rRNA genes**

The hypervariable V4 region of 18S rRNA gene of *Theileria-Babesia* species and V1 region of 16S rRNA gene of *Anaplasma* species were sequenced. The PCR products were purified from 1.6% agarose gel after electrophoresis with a commercial kit (Wizard SV gel and PCR clean-up system, Promega, Madison, WI, USA). The purified PCR products were sequenced by a commercial company (Iontek, Istanbul, Turkey).

**Results**
Prevalence of the tick-borne haemoparasites detected with the microscopic examination and RLB results of cattle from Sivas are given in Table 3. of 314 blood samples, 5.10% (16/314) were found as positive by microscopic examination, whereas 16.24% (51/314) were found as positive by reverse line blotting (Table 3).

Table 3. Microscopic examination and reverse line blotting results of cattle investigated for tick-borne haemoparasites (n: 314)

<table>
<thead>
<tr>
<th>Examination</th>
<th>Total infection (%)</th>
<th>Single infection (%)</th>
<th>Mixed infection (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microscopic</td>
<td>16 (5.10)</td>
<td>15 (4.78)</td>
<td>1 (0.32)</td>
</tr>
<tr>
<td>Reverse line blotting</td>
<td>51 (16.24)</td>
<td>45 (14.33)</td>
<td>1 (0.32)</td>
</tr>
</tbody>
</table>

Total prevalence of tick-borne haemoparasites in the cattle was found as 16.24% by RLB. Prevalence of Anaplasma spp. was 11.15%, whereas prevalence of T. buffeli was 5.10%. The most abundant Anaplasma species was identified as A. centrale (6.69%) followed by A. marginale (4.14%), A. bovis was detected in only one sample (0.32%). On the other hand, T. annulata, B. bigemina, B. bovis, B. divergens, B. major and A. phagocytophilum were not detected in the cattle. Single infection any of Anaplasma species and Anaplasma spp. amplified by PCR were used in RLB and hybridised onto the membrane with specific oligonucleotide probes. All the PCR positive samples gave positive signals with their complementary probes. The reverse line blot assay revealed that T. buffeli, Babesia sp., A. centrale, A. marginale, A. bovis and Anaplasma sp. existed in the cattle and their tick (Table 3 and Table 4). Representative samples were chosen and sequenced. BLAST similarity searches showed that sequence of T. buffeli

Table 4. Tick pools used in reverse line blotting and results of reverse line blotting

<table>
<thead>
<tr>
<th>Tick species</th>
<th>ntp</th>
<th>np</th>
<th>Theileria +Babesia positive</th>
<th>Anaplasma +Ehrlichia positive</th>
<th>A. centrale</th>
<th>A. marginale</th>
</tr>
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<tbody>
<tr>
<td>Hyalomma marginatum Rhipicephalus turanicus Rhipicephalus bursa Boophilus annulatus Hyalomma excavatum Dermacentor marginatus Haemaphysalis sulcata</td>
<td>211</td>
<td>20</td>
<td>5</td>
<td>1</td>
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<td></td>
<td>209</td>
<td>14</td>
<td>-</td>
<td>7</td>
<td>1</td>
<td>5</td>
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<td>86</td>
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<tr>
<td>Total</td>
<td>610</td>
<td>53</td>
<td>6</td>
<td>8</td>
<td>1</td>
<td>5</td>
</tr>
</tbody>
</table>

ntp: ticks number used in pools, np: tick pool numbers
Theileria, Babesia and Anaplasma are among the most important tick-borne diseases of domesticated animals. These infections, which are common in tropical and subtropical regions including Turkey, cause significant economic losses in animal husbandry (Dumler et al., 2001; Inci et al., 2013; Kocan et al., 2000; Uilenberg, 2001). Theileria, Babesia and Anaplasma species have morphological and biological differences as well as their pathogenicity. Comparative studies conducted at the molecular level in recent years have shown that there are significant genetic differences between species and there are differences even among the isolates of the same species (Altay et al., 2007; Ciloglu et al., 2018; Dumler et al., 2001; Duzlu et al., 2011).

Reverse line blotting, in addition to allowing the identification of new species or genotypes, has high specificity and sensitivity (Altay et al., 2008a; Gubbels et al., 1999; Nagore et al., 2004; Oura et al., 2004). In this study, using RLB method: the presence of T. buffeli, A. centrale, A. marginale and A. bovis was detected in cattle and ticks from Central Anatolian region of Turkey. In addition, two new isolates, one of which was in Babesia genus and the other in Anaplasma genus, were found in the region. There are molecular-based studies showing the presence of new tick-borne parasite isolates in Turkey (Altay et al., 2008a; Ica et al., 2007b). In this study, two catchall (Theileria + Babesia spp. and Anaplasma + Ehrlichia spp.), two strains (Theileria spp. and Babesia spp.) and 10 species specific probes (T. annulata, T. buffeli, B. bigemina, B. bovis, B. divergens, B. major, A. centrale, A. marginale, A. bovis and A. phagocytophilum) were used in RLB (Table 3). Six Babesia spp. probe positive and 2 Anaplasma + Ehrlichia spp. probe samples did not give signals to the species-specific probes in RLB. The Babesia sp. Sivas (KJ183081) was found 99% to be similar to the DNA sequences of Babesia sp. Kashi (AY726557) and Babesia sp. Kayseri (EF434766). The sequence of Anaplasma-Ehrlichia genus probe positive sample was similar 99% to Anaplasma sp. Clone A7 (AY851664) and 98% to A. bovis (JN558822). The genotypes detected in the study were named Babesia sp. Sivas and Anaplasma sp. Sivas and deposited in GenBank with the accession numbers; KJ183081 and KJ210855, respectively.

During the survey, 87 (27.70%) of the 314 cattle were found to be infested with Ixodid ticks. A total of 1212 ticks were collected from the cattle. Seven different tick species were identified from the cattle. The most prevalent tick species was H. marginatum (38.04%) followed by R. turanicus (30.20%), R. bursa (13.86%). The other tick species detected from the cattle were Boophilus annulatus (12.05%), Hyalomma excavatum (4.78%), Dermacentor marginatus (0.82%) and Haemaphysalis sulcata (0.25%) (Table 1).

Discussion and Conclusion

Theileriosis, babesiosis and anaplasmosis are among the most important tick-borne diseases of domesticated animals. These infections, which are common in tropical and subtropical regions including Turkey, cause significant economic losses in animal husbandry (Dumler et al., 2001; Inci et al., 2013; Kocan et al., 2000; Uilenberg, 2001). Theileria, Babesia and Anaplasma species have morphological and biological differences as well as their pathogenicity. Comparative studies conducted at the molecular level in recent years have shown that there are significant genetic differences between species and there are differences even among the isolates of the same species (Altay et al., 2007; Ciloglu et al., 2018; Dumler et al., 2001; Duzlu et al., 2011).

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There are many molecular-based studies aimed at detecting the presence and prevalence of theileriosis, babesiosis, anaplasmosis in cattle and ixodid ticks from Turkey (Aktas et al., 2011; Altay et al., 2008a; 2014; Ica et al., 2007a; 2007b; Yildirim et al., 2013). However, there is not any molecular-based research on tick-borne parasites in cattle in Sivas. This study is the first investigation of the parasites in cattle and their ticks from Sivas in the Central Anatolia Region of Turkey using RLB and DNA sequencing. In this study, 314 cattle were examined with RLB, the prevalences of T. buffeli, A. centrale, A. marginale and A. bovis were determined as 5.10%, 6.69%, 4.14%, respectively. The results showed that tick-borne diseases prevalence is lower than in the other parts of Turkey. The main reason for this is that there are climatic and geographical differences in the region located in the continental climate zone.

It is important to diagnose disease in vector ticks, to understand their epidemiology and to develop appropriate control strategies. T. lestoquardi in H. anatolicum (Kirvar et al., 1998), T. annulata in Hyaloma sp. (d’Oliveira et al., 1997), B. bigemina and B. bovis in B. microphilus (Oliveira-Sequeira et al., 2005), T. ovis and B. ovis in R. bursa (Aktas et al., 2006; Altay et al., 2008c) were detected with PCR and DNA sequencing. In the study, H. marginatum, H. excavatum, R. bursa, R. turanicus, Hae. sulcata, B. annula-


Ica A, Inci A, Yildirim A. Parasitological and molecular prevalence of bovine Theileria and Babesia species

References


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