

## SUMMARY

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### Characterization of Mutations in *Theileria annulata* Cytochrome b Gene in Association with Buparvaquone Resistance and the Detection of Prevalence of Buparvaquone Resistance in Infected Cattle in Aydın Region

Tropical theileriosis is a protozoal disease caused by *Theileria annulata* leading to serious economic losses due to high morbidity and mortality. Buparvaquone is the only drug currently used to treat cases of tropical theileriosis in the field. However, an increase in the rate of treatment failure cases of tropical theileriosis has been observed in recent years, raising the possibility of resistance developed against buparvaquone. according to this hypothesis, the present study aim to investigate the mutations in *Theileria annulata* cytochrome b gene in association with buparvaquone resistance and the detection of prevalence of buparvaquone resistance in infected cattle in Aydın region. To this end, susceptibility to buparvaquone was examined by comparing the proliferative index of infected cells, which were obtained from cattle with theileriosis before and/or after treatment, under titration of buparvaquone dose using the MTT assay. Cell lines identified as resistant or susceptible were then genotyped using satellite markers and the DNA sequence of the *T. annulata* cytochrome b gene determined. The results identified six nonsynonymous and six synonymous mutations. Two out of six nonsynonymous mutations were found to be located at putative buparvaquone binding regions of cytochrome b. These mutations result in amino acid substitutions from valine 135 to alanine or proline 253 to serine. Isolates possessing an identified mutation were cloned and tested with the MTT assay which indicated that the clones with either of the two mutations located in the binding regions are resistant to buparvaquone. To test for the frequency of these mutations in the field, an allele specific PCR was developed. The results demonstrated that the mutation V135A in 10 out of 168 *T. annulata* positive blood samples and two out of 127 *T. annulata* macroschizont infected cell lines, while the P253S mutation was detected in one out of 168 *T. annulata* positive blood samples and eight out of 127 cell lines. These data suggest that the two point mutations are candidates for generation of genetic resistance of *T. annulata* against buparvaquone.

**Keywords:** *T. annulata*, buparvaquone, resistance, cytochrome b gene.