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SUBMITTED BY
Susan Tschitschmann
Research Center Borstel
Parkallee 22,
D-23845 Borstel
Germany
stschitschmann@fz-borstel.de

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Identification and Characterisation of Potential Attenuation Markers of *Theileria annulata* Live vaccines

S. Tschitschmann, B. Kullmann, D. Beyer, J. Ahmed, U. Seitzer

Prolonged culturing of *Theileria*-infected cells leads tothe attenuation of the parasite virulence. The aim of the study is to investigate whether epigenetic silencing plays a role in this process. In Plasmodium, it has been shown that heterochromatin formation is initiated by the histone deacetylases Sir2 A and B (*PfSir2A* and *PfSir*2B), in associationwith other epigenetic regulators, thus leading to gene silencing. Specifically, Sir2 promotes a restricted expression of the *var* gene family, which are important for pathogenicity in Plasmodium (reviewed by Cui and Miao, 2010). Based on the similarity between these two apicomplexan parasites, we assumed that a similar mechanism involving Sir2 could exist in *Theileria*. Sequence analyses revealed that the TaSir2 is an about 3kb big gene. The resulting protein sequence is predicted to harbor all characteristical features of Sir2-like proteins. Furthermorethe bovine proteome has for every orthologous human Sirtuin one Sirtuin. In this context, we speculated that bovine Sir2 and /*Theileria*/ Sir2 may promote silencing of virulence genes in /*Theileria*/, thus mayplay a role in attenuation. We investigated whether bovine Sir2 might be up-regulated in highly passaged, attenuated cells. We were able to detect bovine Sir2 in /*T. annulata*/ infected cells using Western Blot and immunofluorescence staining. Western Blot analysis showed that passaging of /*Theileria annulata* /infected//cells seems not to have any impact on the bovine Sir2 expression level. Furthermore, the characterisation of the bovine Sir2 was attempted by carrying out a set of immunofluorescence experiments, in which anti-human Sir2 antibody (detects bovine Sir2) and anti-TaSP-antibody (detects the parasite /Ta/SP protein on the schizont membrane) were used. Bovine Sir2 staining could be demonstrated in few schizonts of /*Theileria*/ infected cells. Interestingly, these schizonts were indeed undergoing differentiation to merozoites. This finding led to the assumption that bovine Sir2 may play a role during merogony in /*Theileria*/.Moreover, we propose epigenetic regulation might be involved in stage differentiation and possibly attenuation, due to the detection of histone methylation markers in/*Theileria*/ infected cells implying the presence of epigenetic machinery.

Cui L, Miao J. (2010). Chromatin-Mediated Epigenetic Regulation in the Malaria Parasite Plasmodium falciparum. Eukaryot Cell 9(8):1138--1149.

SUBMITTED BY
Zati Vatansever
Kafkas University
Kars - Turkey
Zativet@Gmail.Com

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CCHF Infection Prevalence in Field Collected Ticks in Turkey

Zati Vatansever, Munir Aktas, Esin Guven, Nurettin Canakoglu, Engin Berber, Aykut Ozdarendeli

Host-seeking ticks were collected in 8 villages during June 2010 in CCHF highly endemic area of Turkey. Crop fields, where human activities were concentrated, were selected with the aim of collecting active host-seeking Hyalomma ticks. Collection was made by using human volunteers, where one collector spending 15 minutes sitting/standing by the bushy border of the field in order to attract hiding ticks. Sampling was done between 10 and 12 in the morning and 14 and 16 hours in the evening for total of 4 hours per day. Approaching or already crawling ticks were collected into vials, kept alive in a cool place at aprox. 85%RH and later were transferred to the laboratory. Ticks were identified at species level and were further crushed and stored in liquid nitrogen and later tested byantigen capture ELISA developed by Fırat University, Turkey.

A total of 814 host-seeking ticks were collected during 7 days of study period. The mean collection number was 15.65 ticks/person/hour. Off the collected ticks, 790 (97.05%) were *Hyalomma marginatum* and 20 (2.47%) *H. aegyptium*. Also 4 individuals of *Rhipicephalus turanicus* were found crawling on collectors, probably acquired from vegetation. All ticks were individually subjected to agELISA and 47 (5.77%) were found positive. Off positive ticks 3 (6.39%) were H. aegyptium, while the rest were H. marginatum (93.6%). Prevalence of infected ticks among 8 localities varied between 2.2% and 11.9%. A total of 10 CCHF cases were diagnosed in the sampled localities in 2010.

In our previous study (2008) in the same area mean tick number was 4.67 ticks/person hour and infection prevalence in ticks was 16.43%, varying between 6.06% and 29.86%. The number of CCHF cases in the study cites was 18 in 2008.

Although the number of CCHF cases looks like correlated with the infection prevalence in ticks, this correlation is not consistent at the locality level.