Posters

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Microevolution of *Burkholderia mallei* studied during experimental infection within its natural host

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Background and objectives: Glanders is a notifiable epizootic disease caused by *Burkholderia mallei*. The infection mainly affects horses and donkeys, but sporadic cases in humans have been reported. The pathogen is a host-adapted lineage of *B. pseudomallei* and developed by genome reduction, rearrangements and elimination of prophages. Both species are recognized biothreat agents. Materials and methods: Donkeys and goats were infected intranasally with *B. mallei* strain Dubai7 and monitored for clinical signs of illness. Subsequent genomic analyses comprised the initial strain used for infection and 47 isolates that have been re-isolated either from lesions or carcasses of 9 experimentally infected animals. Whole genome sequencing (WGS) was applied to selected strains using PacBio RS II and Ion PGM platforms. Results: All typical manifestations of the disease like mucopurulent nasal discharge and pneumonia were observed (Fig. 1). We found 30 closely related but different clusters by MLVA23-typing, suggesting genomic alterations within repeat regions. By WGS extensive deletions of up to 250k bp with the involvement of IS elements as well as a series of single and multiple nucleotide exchanges were determined. Conclusion: This study provides insights into microevolution of a zoonotic pathogen with a narrow ecological niche within its natural host. Our findings reveal the enormous structural flexibility of the genome, challenge the meaning of *in-vitro* studies, and will have a strong impact on bioforensics.

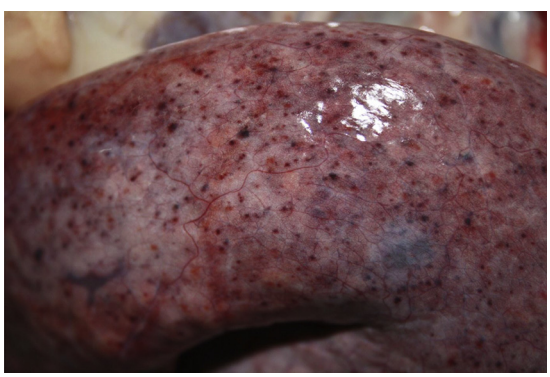

Figure 1. Multiple subpleural pyogranulomatous nodules of various sizes in a donkey lung 23 days p.i.

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Optimization of a fluorescence-based assay for mass drug screening against *Babesia* and *Theileria*

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A rapid and accurate assay for evaluating novel compounds on a large scale is required for the development of new chemotherapy for piroplasmosis. In the present study, we evaluated the usefulness of a fluorescence-based assay for determining the efficacies of drugs in *in vitro* cultures of *Babesia caballi* and *Theileria equi*. Three different hematocrits (HCT), 2.5%, 5%, and 10%, were used for *in vitro* screening assay without daily replacement of the medium. Five % of HCT was the best for *B. caballi* and *T. equi* by high-throughput screening assay. The IC_{50}s of diminazene aceturate obtained by fluorescence and microcopy did not differ significantly with 5% HCTs for *B. caballi* and *T. equi*. Likewise, the IC_{50} values of luteolin, pyronaridine tetraphosphate, nimbolide, gedunin, and enoxacin did not differ between the two methods. Furthermore, using this high-throughput screening assay, we evaluated the inhibitory effects of 400 anti-malarial compounds (200 drug-like and 200 probe-like) against *B. caballi* and *T. equi* from the Open Access Malaria Box. Fifty-three, 66 and 11 compounds showed strong inhibitory effects with nanomole levels of IC_{50} against *T. equi* and *B. caballi*, and both protozoan parasites, respectively. Among them, two compounds were identified with mean selectivity indices (SI) greater than 250 and IC_{50}s ranged from 71 to 480 nM for *T. equi* and *B. caballi*. In conclusion, our fluorescence-based assay uses low HCT and does not require daily replacement of culture medium, is highly suitable for *in vitro* large-scale drug screening against *B. caballi* and *T. equi*.

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Chronic piroplasmosis diagnosis in healthy and admitted at a teaching veterinary hospital horses by splenic puncture

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Piroplasmosis is one of the most important tick-borne diseases. It's endemic at American countries. The disease is caused by hemoprotozoan of the genus *Babesia* and *Theileria*. Equine pathogenic species are *Babesia caballi* and *Theileria equi*. *Theileria* genus differs from *Babesia* genus for a pre erithrocytic phase in vertebrate host (usually in lymphocytes). The disease is characterized by fever, anemia, depression, ataxia, anorexia, weakness, epiphora, mucous nasal secretion, oedema, jaundice and hemoglobinuria. Clinical signs may be unspecific and variable. Most common hematological changes are anemia, thrombocytopenia and hemoglobin concentration decrease, neutropenia and limphopenia, decrease in plasmatic fibrinogen, seric iron, phosphorus and increase of seric bilirubin. Parasitemia by *B. caballi* may get to 1% instead, *T. equi* parasitemia will get to 7% been considered a more severe disease leading to severe anemia and death. Besides conventional treatment with babesicids drugs, animals with severe disease may get support treatment and dietetic supply in order to proper recovery. Chronically infected horses may or may not present clinical sign of the disease. Splenic puncture technique has been described and used since the 1950's in humans to diagnose different hematological illness such as limphosarcoma, leukemia, polycitemia vera, Gaucher's disease. In human medicine, reference values for different types of cells recovered by splenic puncture are well established. Miranda et al. (2014) say that the best place to do equids splenic puncture is approximately ten centimeters below vertebral transverse processes of lumbar vertebras, in the 17^{th} intercostal space. Splenic blood smears are been used as an efficient diagnostic method to chronic/latent disease by *B. caballi* and *T. equi*. The aim of this study was to evaluate presence of *T. equi* and/or *B. caballi* in splenic blood of five animals admitted at Centro Univesitário de Itajubá's Veterinary Medicine Teaching Hospital, and five healthy animal located in the municipality of Natércia, Minas Gerais, Brazil. Splenic puncture was performed as described by Miranda et al. (2014), for this, animal were properly restrained, than surgical antisepsis was performed in 17^{th} intercostal space region. A