Posters

005

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 250kbp 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 micro-evolution 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.

021

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

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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 IC₅₀ of diminazene acetate obtained by fluorescence and microscopy did not differ significantly with 5% HCTs for B. caballi and T. equi. Likewise, the IC₅₀ values of luteolin, pyronaridine tetraphosphate, nimobilide, 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 IC₅₀ 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 IC₅₀ 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.

036

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 hemoproteozoon of the genus Babesia and Theileria. Equine pathogenic species are Babesia caballi and Theileria equi. Theileria genus differs from Babesia genus for a pre erthrocytic 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 lymphopenia, 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 babesicides 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 lymphosarcoma, 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 vertebrae, in the 17th 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 Universitário 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 17th intercostal space region. A
A 30 mm x 0.8 mm needle was introduced in a 90° angle. Blood sample was collect in syringe containing ACD anticoagulant. Splenic blood smears were made and stained by modified Romanowsky method. Blood smears were then analyzed by optic microscopy. Inclusions of T. equi were observed in four of five blood smears of admitted horses (80%) and in four of five of healthy animals (80%). No B. caballi were observed. Total prevalence was 80%, when healthy and admitted horses were evaluated together. All the admitted horses had no clinical signs of acute piroplasmosis. We can conclude the apparently healthy horses and admitted horses presented T. equi chronic/latent infection, since, normally, animals are able to eliminate B. caballi infection, a prevalence of 0% of infected animals was expected.

040
Common infectious diseases of working donkeys: their epidemiological and zoonotic role

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Over 38% of the world equine population (114 million) is made up of donkeys and more than 97% are found in developing countries and are specifically kept for work. Despite their significant contribution to the national economy, the attention given to study the infectious diseases of working donkeys is minimal. To address this The Donkey Sanctuary (DS) has been conducting studies in collaboration with Addis Ababa and Nairobi Universities, Central Veterinary Research Laboratory (CVRL) in Dubai and the Trypanosomosis Research Centre (TRC) in Kenya. These studies have shown a high prevalence of some important infectious diseases. Helminthosis: Helminth infection profiles of working donkeys living in semi-arid or tropical conditions are often very different from those of equids in temperate climates. They are often diagnosed with a high worm burden or faecal egg count irrespective of their age. The high level of age-independent infection may show that donkeys either do not develop protective immunity or that they might have become immuno-compromised, consequent upon the stress of their work intensity and/or undernourishment and general poor husbandry. Trypanosomosis: Although there is a general belief that donkeys are more resistant, trypanosomosis has been shown to cause severe clinical disease in working donkeys. Epidemiological studies in Ethiopia and Kenya have shown that the prevalence of trypanosomosis was as high as 65%, often with mixed infections of two or more species. In both countries T. congolense was the predominant species followed by T. brucei and T. vivax; often associated with anaemia and poor body condition. Trypanosomosis is claimed by local farmers as the major health constraint of donkeys in both countries. Recent serological studies by the DS in collaboration with the CVRL showed a sero-prevalence of 1.1% (n=662) T. equiperdum in Ethiopia. Piroplasmosis: Equine piroplasmosis is one of the most significant tick-borne diseases of donkeys in Ethiopia and Kenya. Recent studies in Ethiopia in collaboration with CVRL showed sero-prevalence of 53.3% to 58% T. equi and 13.2%–13.3% B. caballi (n=15–395) Most of the cases were associated with anaemia. Similar studies in Kenya reported only T. equi with a sero-prevalence of 81.2% (n=314).

Viral and bacterial diseases: A recent study in Ethiopia in collaboration with CVRL showed a sero-prevalence of 8.5% (n=165) AHS, 84.6% (n=104) EHV-4, 20.2% (n=104) EHV-1, 0.5% (n=662) glanders and 0.2% (n=657) EIA. Similar study made in Kenya also showed a sero-prevalence of 35.2% (n=398) AHS. Donkeys showing typical clinical signs of AHS were noted in Kenya and Ethiopia.

Fig.1. Suspected cases of AHS in Kenya (a) and Ethiopia (b).

Although no epidemiological studies are available, cases of tetanus, strangles, rabies, anthrax and dermatophilosis are common occurrences in donkeys. These studies highlight how important infectious diseases in donkeys are and the need to consider them in overall epidemiological studies and for sound control and prevention strategies.