

extended territory in Romania including the anthropic ecosystems where the highest risk of transmission to humans exists. The permanent surveillance of WNV circulation and the implementation of integrated mosquito control programmes in the key areas at risk in Romania are adequate decisions for public health. Work supported by funding from FP6 of EU (Contract No. 010284/2004) and from BIOTECH Research Programme of the Ministry of Research, Romania (Contract No. 92/2006).

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49.020

Proteases of *Blastocystis hominis* in Mexican children

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Background: The prevalence of *Blastocystis hominis* is increasing in Mexican children. However, it is found in both symptomatic and healthy individuals, and hence its pathological potential is still controversial. Proteases have been described in protozoan pathogen participating in host parasite interactions, inducing the immune evasion mechanism, in adhesion on substrate and in other metabolic events.

Objective: In this study was analyzed the protease activity of *B. hominis* isolates from Mexican children with and without gastrointestinal symptoms.

Materials and Methods: Four isolates of *B. hominis* from two children with gastrointestinal symptoms and two asymptomatic were cultured in diphasic eggs slant medium, supplemented with 10% inactivated bovine serum in axenic conditions. The protein concentration of parasitic lysates was estimated by Bradford method. The samples were analyzed using 10% SDS-PAGE gels copolymerized with 1% gelatin. After electrophoresis, gels were immersed for one hour in 2.5% Triton X-100 at ambient temperature and washing three times with distilled water. Gels were incubated in activity buffer at pH 5.0 and 7.0 at 37 °C for 24 h and washing newly. Finally the gels were Coomassie Brilliant blue stained.

Results: In SDS-PAGE-gelatin gels protease activity can be seen as clearing areas. Three bands between 30–50 kDa and two bands between 70 and 100 kDa. The 70 kDa band was present in all the children isolates. Protease activity of *B. hominis* was highest at pH 7.0.

Conclusions: Proteases profile from *B. hominis* not showed significant differences among symptomatic and asymptomatic children.

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Clinical and Serological Evidence of Lassa Fever in Edo State, Nigeria

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Since Lassa fever was first discovered thirty-nine years ago, it has remained a major public health problem in Nigeria; due to the endemic circulation of the virus and to a number of other prevailing factors. Its clinical manifestations are indistinguishable from many other febrile illnesses. This, coupled with lack of appropriate diagnostic facilities directly reflects in poor recognition and mismanagement of disease. In the main areas of its endemicity, the available means of diagnosis is based on clinical features such as; presence of muscle ache, sore throat, swelling in the neck region, nausea, vomiting, chest, abdominal pain, and signs of haemorrhage. We made an attempt to assess the burden of Lassa virus infection in one of the endemic areas with high clinical suspicion, by testing for Lassa virus specific immunoglobulin M and G (IgM and IgG). Using the traditional indirect fluorescent-antibody (IFA), twenty-seven patients out of the seventy-one suspected cases seen in 2006 and 2007 were tested. Out of these cases 5 (19%) were positive for IgM while 6 (33%) tested positive for IgG. We assume that this survey yielded some false-negative results due to the low sensitivity of the IFA assay employed. However, findings indicate that Lassa fever is still widespread in Nigeria but remains a neglected and unrecognized cause of most human fevers. It also stresses the need for clinicians and public health officials to remain alert to endemic infectious diseases of this nature and to institute appropriate measures to promptly identify cases; protect healthcare workers and care-givers. Also as early laboratory confirmation of infection is paramount both for therapeutic intervention and prevention of nosocomial spread, laboratory infrastructure; technical expertise and research capacity must be improved in endemic areas in order to positively influence Lassa fever surveillance, clinical case management and the development of effective approaches to control.

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A Rubber-degrading Organism Growing from a Human Body

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Introduction: Patients with hematological malignancies are susceptible to unusual infections because of the use of broad-spectrum anti-infective agents and their immunocompromised state.

Case: A 17 yr old lady with Undifferentiated Myeloblastic leukemia (AML - M1 type) on Cytarabine and Mitoxantrone, presented to the hospital with 4 days of fever, rhinorrhea

and productive cough. One month earlier she developed *Rhodococcus equi* bacteremia. Examination did not show any source of infection. Laboratory exam, WBC of 300. Chest x ray showed right lower lobe consolidation in the lung which was confirmed on CT scan. She was started on Vancomycin and Ceftazidime. Ambisome was also added for aspergillus coverage. All the cultures remained sterile and an echo done did not show vegetations. Bone marrow aspirate and biopsy done to rule out recurrence of leukemia showed only hypocellular marrow. The cultures continued to be negative until 4 weeks after admission when the blood grew a Gram-positive weakly acid-fast organism identified by Center for Disease Control (CDC) to be *Gordonia polyisoprenivorans*.

Discussion: *Gordonia polyisoprenivorans* is a ubiquitous environmental aerobic Actinomycetes belonging to the family of Gordoniaceae in the order Actinomycetales. Most of the 21 identified species are typically gram positive, catalase positive, weakly acid fast, thin beaded coccobacilli. *G. polyisoprenivorans* was first described in 1999 as a rubber-degrading bacteria isolated from stagnant water inside a deteriorated automobile tire. Extensive literature review has only identified 2 case reports of bacteremia due to this unusual organism. The 2 earlier case reports highlighted patients with hematological diseases (one with bone marrow transplant and the other with Osler-Weber-Rendu and myelodysplastic syndrome). Our patient although is free from leukemia, is similar in being neutropenic. We believe that hematologically immunocompromised patients with broad-spectrum antibiotics and long term central catheters select the possibility of infection with *G. polyisoprenivorans*.

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Spatio-Temporal and Molecular Analyses of a DENV3 Outbreak Show the Dynamics of Dengue Infection (Viral Spread)

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Background: Dengue fever (DH) and dengue hemorrhagic fever (DHF) are important public health issues. Brazil was responsible for 60% of the worldwide dengue cases in 2006 and the virus is endemic in the country. Molecular epidemiology of dengue viruses in endemic areas, associated with spatio-temporal tracking may help to understand the dissemination patterns of viral lineages.

Methods: We followed an outbreak in the city of S.J. Rio Preto - Brazil in 2006. Blood samples from patients with DF and DHF symptoms were collected and tested by RT-PCR. Sequences of NS5 gene from viral genomic RNA were amplified and DENV-3 positive samples were sequenced and compared to other reference sequences for phylogenetic reconstruction. Phylodynamics of DENV-3 was inferred using MCMC-based Bayesian method under the assumption of the

relaxed molecular clock. We also generated matrices of distances between samples to analyze the spatio-temporal dispersion pattern and applied a customized algorithm to obtain the putative spatio-temporal pattern of spread of the disease.

Results: For this analysis we generated a 399 nucleotide-long dataset with 134 taxa by aligning the 82 sequences with 52 reference sequences. DENV-3 samples were closely related to strains circulating in Martinique and in Brazil. Sixty samples formed a monophyletic group, representing lineage 1; 22 samples formed lineage 2. The basic reproductive rate (R0) was 3.765 for lineage 1 and 3.093 for lineage 2. Preliminary studies indicated that both lineages split 1 to 3 years before the last collected sample. They propagated in different regions of the city, North-Western (lineage 1) and South-Eastern (lineage 2).

Conclusion: Our results indicated that spatial analysis associated with molecular epidemiology are important tools to enhance the understanding of the viral strain spread patterns and may be paramount in monitoring and predicting the occurrence of severe forms of dengue. We plan to account in the future for other factors modulating dengue virus dispersion such as climate, adult mosquito density and biology, density of human population and socio-economical aspects.

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49.024

Synchronous Seasonal Activity of *Ixodes ricinus* Immature Stages in Brateiu, Sibiu County, Romania - Implications for TBE Outbreaks

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Background: The two most important tick-borne infections of humans in Europe are tick-borne encephalitis and Lyme borreliosis. While Lyme borreliosis occurs extensively throughout Europe, TBE is far more focal in its distribution suggesting that it requires more narrowly defined conditions for maintenance. In Romania the largest TBE outbreak occurred in Brateiu, Sibiu County. The aim of this study was to define those abiotic and biotic factors that could favor a TBE outbreak.

Methods: *Ixodes ricinus* (the main vector for TBEV) ticks were collected monthly, for two years, from six sites in Romania (including Brateiu) using the flagging method. The questing ticks' activity was estimated by reporting the collected tick number to 100 m². Seasonal dynamics of ticks was compared between the sites. Environmental data were collected during the sampling period, including climate data like temperature, humidity, precipitations etc.

Results: Analyzing the ticks' dynamics we noticed that in Brateiu the immature stages were synchronous for a few months, while in other sites they were not. Thus, in Brateiu approximately 75% of the immature were active during July and August, while in the other sites there was an approximately two months delay of larvae peak activity to that of nymphs activity. Tick numbers were positively correlated to temperatures and humidity from the regional weather stations.