Training in fever case management and use of malaria rapid diagnostic testing kits improved fever case management in Uganda

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Background: In Africa, there is poor access to diagnostic tests; malaria is typically diagnosed clinically, though presumptive treatment results in significant overuse of antimalarials and delayed treatment of actual causes of fever. The WHO currently recommends antimaralial treatment for only laboratory confirmed malaria. Malaria rapid diagnostic tests (RDTs) may offer a reliable alternative, but effective training for health workers is a key challenge in RDT implementation. We tested the effectiveness of the training on use of RDTS in fever case management.

Methods: Clinicians at peripheral health centers without microscopy in two districts in a low endemic zone in Uganda were trained for two days and immediately followed up in their health facilities to observe performance and offer additional targeted on site training. Training covered clinical evaluation, selection of patients for RDT testing, performing and interpretation of RDT tests and treatment of patients with negative and positive RDT results. Data on practices in management of patients with suspected malaria before and after the training were collected and compared.Data on out patient consultations for 10 consecutive days in the pre and post training period was compared.

Results: Data revealed appropriate use of RDTs and improved fever case management; there was a reduction in proportion of patients; diagnosed as Malaria [61% to 26% ($p = 0.000$)] amongst the under fives and from 52.3% to 14.5% ($p = 0.000$)amongst adults above 5yrs, prescribed antimalarials from 97% to 80% ($p = 0.000$) among the under fives and from 94% to 86% ($p = 0.000$) among the above 5yrs, with malaria treated with antibiotics among those above 5yrs from 55% to 40% ($p = 0.000$), with malaria given both antimalarials and antibiotics from 63% to 47% ($p = 0.000$) amongst the under fives and from 46% to 29% ($p = 0.000$) amongst those above 5yrs. The training contributed to rational use of antimalarials; the proportion of patients with a negative RDT who received antimalarials in the two facilities 12% compared to 50-70% who receive antimalarials despite a negative blood smear in sites with microscopy.

Conclusion: The training in use of Malaria RDTs in fever case management substantially lead to rational use of antimalarials and antibiotics.

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73.001

Antibacterial effects of *Humulus lupulus L.* extract on topical staphylococcal infection in BALB/c Mice cornea

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Background: Staphylococcus aureus is an opportunistic pathogen and the most common cause of bacterial keratitis that can result in irreversible corneal scarring, a pathologic effect that reduces visual acuity and can lead to blindness. *Humulus lupulus L.* flower complex has multiple therapeutic properties such as antibiotic effects against some gram positive bacteria and fungi. In the present research, topical anti-staphylococcus aureus effects of hydroalcoholic extract of *Humulus lupulus L.* flowers in corneal infection induced by staphylococcus aureus were investigated in mice.

Methods: At first, staphylococcus aureus were inoculated into right cornea of animals under anesthesia by making parallel scars. One, three, five, seven and nine days post-inoculation, the eyes were observed carefully under microscope. In observations, eyes were scored according to the area and the degree of opacity. In order to assess the antibacterial effects of *Humulus lupulus L.*, the extract was administered in form of eye drop at 1%, 5% and 10% concentrations. Treatments were started twice daily as soon as the first opacity was observed and continued for one week. The first sign of corneal infection with opacity was observed after three days of bacterial inoculation as compared with the normal eye.

Results: The intensity of opacity was progressed time dependently in a manner that maximum opacity of the whole cornea was obvious in more than half of animals, after nine days. Administration of the *Humulus lupulus L.* extract 10% topically, reduced corneal opacity and consequently the infection. Introducing animal models of ocular diseases such as bacterial infection in cornea has special importance in ocular research.

Conclusion: Effective components existing in *Humulus lupulus L.* flower extract are mainly resins and essence which among them, resins has special importance and seems to be responsible for its antibiotic effects.

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73.002

Up regulation of IRF-2 in West Nile Virus infection: Implications for establishment of viremia in the brain leading to encephalitis

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Background: A proportion of patients afflicted with WNV can develop neuromuscular degenerative diseases and potentially fatal encephalitis. However, host immuno-

modulatory factors involved in the establishment of viremia and subsequent infiltration of cytotoxic immune cells is yet well understood. This study thus investigates the role of IRF-2, an attenuator of interferon (IFN) response, on the establishment of viremia and immune cell infiltration during WNV infection.

Methods: Real-time PCR, Western blot, and FACS analyses were used to study the regulation of various IRFs and downstream targets during WNV infection of an astrocytic cell line, A172. Regulation of IRF-2 at the cellular level was then studied using immunofluorescence microscopy. Subsequently, cell lines over-expressing, or with a knockdown of IRF-2 were infected to study the effect(s) that IRF-2 has on WNV production. Finally NK cell assay was performed to investigate the ability of NK cells to lyse these infected cells.

Results: The IRFs 1, 2, 3 and 7 are highly up regulated post-WNV infection. Expectedly, downstream gene targets like IFN-$, IL-12 and IL-6 are up regulated transcriptionally and translationally. In addition, concomitant increased mRNA expression of MHC Class I loading genes like TAP1, TAP2 and $2m with that of HLA-E, translated to an increased surface expression of HLA-E. Interestingly, FACS dot plot analysis revealed that expression of IRF-2 was insufficient to suppress HLA-E expression. Immunofluorescence microscopy further showed the surprising preferential enhanced expression of IRF-2 in the non-infected cells. Finally, infection of IRF-2 over-expressing cells resulted in increased virus production, while a reduction in virus titer was observed in the IRF-2 knockdown cells.

Conclusion: Our results show that IRF-2 is preferentially up regulated in the neighboring non-infected cells, possibly in a homeostatic fashion to regulate pro-inflammatory genes like IFN and cytokines in these cells. On the other hand, the activated activators overwhelm the attenuation effect(s) of IRF-2 in the infected cells. In these infected cells, the inhibitor of NK cell lysis, HLA-E, is expressed. Its expression thus protects the infected cells from the cytotoxic effects of NK cells. Predisposition of neighboring cells to NK cell lysis and/or subsequent infection thus contributes to overall WNV pathogenesis.

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73.003

Crimean-Congo hemorrhagic fever virus infects human hepatocytes and induces IL-8 secretion

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Background: Crimean-Congo hemorrhagic fever virus (CCHFV) is a very pathogenic tickborne virus member of the Bunyaviridae family and the Nairovirus genus. The knowledge of CCHFV pathogenesis is improving: recently, new target cells were identified. We and others had demonstrated that CCHFV is able to infect and partially activate monocytes derived dendritic cells and macrophages. During one retrospective study, it was shown that CCHFV was detected in the liver of infected patients.

Methods: Attempting to find other target cells to better understand the pathogenesis of CCHFV, we analyzed the host response induced by CCHFV in hepatocytes infected *in vitro* during a kinetic study.

Results: We noticed that while in HuH7 CCHFV infection elicited a cytopathogenic effect, no visible effect was seen in CCHFV infected HepG2. This intriguing feature led us to analyse the viral parameters expecting a differential cellular response. HuH7 and HepG2 both were shown to be permissive to CCHFV and to replicate the virus at a high load as monitored by plaque titration assay, genomic and anti-genomic strand quantification. The high secretion of IL-8 but no other inflammatory cytokines such as TNF-a, IL-1b indicated that CCHFV induced a response in both hepatocytes. Interestingly, no type I IFN was detected during the kinetic study. In spite of these similarities, we observed a pro-apoptotic CCHF effect more significant in Huh7 than in HepG2 cell lines.

Conclusion: We found that hepatocytes could be considered as CCHFV target cells that could be involved in the pathogenesis disorders. The high IL-8 production by infected hepatocytes associated to the pro-apoptotic effect likely contribute to the disease progress.

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73.004

The course of infection in respiratory infected chickens caused by avian influenza virus A/H5N1

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Background: High pathogenic avian influenza virus (AIV) is an object of research by many scientists in the world. This disease agent is capable of infecting a wide range of varieties of wild and domestic birds. Among the known pathways of any infection the most effective believe airborne and fecal-oral routes. It is believed that chickens infected by the fecal-oral route, i.e. through the gastrointestinal tract. Do not exclude also the aerosol route of transmission of the disease in chickens. In the present study infectious properties of various AIV strains and the degree of sensitivity to this pathogen of respiratory and gastric-intestinal tract of the chickens, as well as the dynamics of dissemination in their body were studied.

Methods: We used eight highly pathogenic AIV A/H5N1, isolated in Russia and CIS countries in chickens that are infected by aerosol, intranasal, intra gastric and oral methods.

Results: All studied AIV strains showed same high virulence for chickens (LD50 is 2—15 EID50) for aerosol challenge. When aerosol challenge sensitivity of these animals to AIV 30 times higher than in the intranasal, 500 times higher than with oral, and 10000 times higher than intra gastric method of infection, indicating a higher susceptibility to AIV of respiratory organs of chickens compared to gastrointestinal tract. Replication of the virus in the membrane of the nasal cavity has already recorded 18 hours after infection (a.i.). The second wave reproduction of the pathogen