Arboviruses associated with neurological disease in animals in South Africa and their zoonotic potential in humans

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Background: Zoonotic arboviruses in the families flaviviridae, togaviridae and bunyaviridae may cause severe neurological disease in humans and animals but are rarely reported in Africa. We recently described West Nile (WNV), wesselsbron and Shunivirus in several fatal cases of neurological disease in horses and WNV in undiagnosed neurological disease in humans in South Africa. This prompted us to investigate the epidemiology over 5 years using horses and other species with neurological disease as sentinels to detect arbovirus activity. Serosurveys was undertaken in high risk humans.

Methods & Materials: Blood, brain and spinal cord specimens from 700 horses and 135 postmortem specimens from several wildlife species and livestock with nervous signs before death were collected and screened by PCR from 2008-2013 for flaviviridae and specifically for WNV, Wesselsbronvirus, Shunivirus, Equine Encephalitis (EEV), Sindbis, Middelburgvirus, equine herpes virus and African horse sickness virus (AHSV). Histopathology was carried out on fatalities. Sera from 123 equine, state and wildlife veterinarians were screened by neutralisation assays for WNV and Shunivirus.

Results: WNV was identified in 1-17% of horses with neurological signs, (fatality rate, 39%), in cattle and a fatal case in a giraffe. Wesselsbronvirus was detected in 2 neurological horses, 1 being fatal. Shunivirus was identified in 0-10% of horses, 53% that were fatal, while Middelburg virus, was identified in 2-16% of cases in horses, 39% being fatal. Sindbisvirus, EEV and AHSV was detected in a few neurological cases in horses without co-infections. Shuni and Middelburgvirus were also detected in the brains of several rhinoceros, warthogs, buffalo and crocodiles. Sindbisvirus and EEV were detected in the brains of 1 rhinocerous each. Horse cases were detected across the country while wildlife cases occurred in reserves in the Limpopo, Northern Kwazulu Natal, North-West and Gauteng Provinces in late summer and autumn.

Conclusion: WNV and Shunivirus seropositivity in veterinarians were 7.9 and 3.9% respectively.

Background: Infection with arthropod-borne pathogens is a leading cause of febrile illness in humans across the globe. These include the mosquito-borne arboviruses such as Rift Valley fever, West Nile, Sindbis and chikungunya viruses, which are endemic to South Africa. They can cause influenza-like symptoms, rash, and occasionally chronic sequelae and death. Rickettsiae are transmitted to humans by the bite or crushing of infected ticks from various species. Typical features of tick bite fever include the presence of a black skin lesion, enlarged lymph nodes, fever, severe headache and rash, and may occasionally lead to death.

Methods & Materials: We collected 226 blood samples (sera) from volunteers in the southern and central region of Kruger National Park in May and November 2013. Serology tests were performed to detect antibodies to Rift Valley fever, West Nile, Sindbis and chikungunya viruses, and tick-borne bacteria (Rickettsiae).

Results: The median age of adult respondents (N=200) was 42 (inter-quartile range(IQR):33-49) years. Of those that disclosed their gender, 71% (131/184) were males and 29% (53/184) females. Of 185 respondents, 64 (35%) were general workers, 43 (23%) were rangers/field guides, 50 (27%) scientific/veterinary staff and 28 (15%) belonged to other categories. The average time working in KNP was 10 (IQR:4-21) years. From the completed analysis of 92 samples collected in May 2013, evidence of recent exposure to Shunivirus was found in a general worker with over 20 years service at the Park. West Nile antibodies were found in a female who had lived in the Park for the past 10 years. A total of 61 out of 92 (66%) persons tested positive for Rickettsiae, meaning that they were infected with tick bite fever in the past.

Conclusion: Rickettsiae exposure was expected to be common in KNP’s sampled population, parallel to the distribution of Amblyomma ticks in northern-eastern South Africa and high antibody prevalence reported in humans throughout tick-endemic areas of Africa. Rather surprisingly, arbovirus exposure was rare, despite for example up to 21% Rift Valley fever seropositivity reported in KNP’s buffaloes. Estimation of the extent of exposure to arthropod-borne
viruses and other pathogens in KNP’s staff requires implementation of a long-term surveillance programme.

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Diversity of novel arenaviruses in South Africa

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Background: Arenaviruses are hosted by Murid rodents in the Murinae and Sigmodontinae subfamilies. They are divided into two serocomplexes; Tacaribe containing viruses hosted by New World rodents, and the Lassa-Lymphocytic choriomeningitis complex containing various arenaviruses hosted by African rodents, the ubiquitous LCMV and Dandenong virus from Australia. While non-pathogenic in rodents, some arenaviruses are highly pathogenic for human beings: In Africa, these are Lassa fever virus hosted by Mastomys natalensis and endemic to West Africa and Lujo virus with an unknown host that emerged in Zambia and caused a nosocomial outbreak in South Africa.

Methods & Materials: In this study South African rodents were screened for arenaviral RNA sequences. A total of 1648 small mammals representing 16 different genera were trapped at various locations in 8 provinces in South Africa between 2008 and 2012. RNA extracted from lung tissue was tested using broadly reactive RT-PCR assays targeting the L gene. The positive samples were amplified using PCR assays targeting the GPC and NP genes, which were then used for phylogenetic analysis.

Results: Thirteen novel arenavirus sequences were identified in four different rodent species indigenous to Southern Africa. Phylogenetic analysis showed that these arenaviruses belong to different clades; 3 outliers of the Lassa-Mopeia clade and 10 forming a monophyletic clade with Merino Walk virus, only very recently described from Myotomys unisulcatus from the Eastern Cape.

Conclusion: A surprisingly high diversity of arenaviruses was detected in this study and further characterisation efforts are ongoing. The identification of arenaviruses by our group and others in Southern Africa suggests that they are more widely distributed than previously thought. The distribution and pathogenicity (or not) for humans is yet to be defined. The outbreak of Lujo virus in 2008 is a reminder that there may be horrible surprises out there.

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Cancer risk evaluation: Preliminary analysis of inflammatory biomarkers in farmers exposed to zoonotic agents

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Background: The majority of emerging diseases are zoonoses and surveillance programs of animals for zoonotic pathogens are a global challenge. Notably, previous studies of individuals occupationally exposed to animals have suggested an increased risk of cancer, especially hematopoietic malignancies, which may be related to their exposure to a variety of infectious agents, showed an elevated risk of lung cancer for poultry exposure in subjects ever had direct contact with chicken blood. Cancer risk associated with specific types of farm animals or specific etiologic agents have not been clearly described. It is hypothesized that chronic infection by several infectious agents are associated with human cancer risk and co-morbidities.

The aim of the present study was to organize a cohort study with the long range goal to analyse the association between infectious agents and bio-molecular markers in a fraction, randomly selected, of both human and animal samples derived from a cohort of farmers.

Methods & Materials: Subjects included in the cohort are 12,600 workers from 4311 farms located in Catania and Ragusa area. The total number of 7,754,704 animals raised in the farms comprises poultry, cattle, sheep and hogs. Peripheral blood samples from both workers and animals were collected during the health survey program, according to the current rule. For the risk evaluation (odds ratio), all workers, with a history of chronic diseases or positive for infectious agents were considered as “cases”, the remaining subjects were considered as “controls”.

Results: Preliminary data on the analyses of biomarkers, such as interleukin-6, interferon-alpha, beta, osteopontin and matrix metalloproteinase-9, performed by standard procedures in a group of human samples, reveals higher levels of these markers in subjects with a history of pulmonary and liver chronic diseases compared with other workers. Intriguingly, the history of such infections may be associated with work exposure.

Conclusion: The identification of bio-markers and zoonoses could contribute to translational research by supporting clinicians for the development of tailored therapies. Furthermore, early detection of infectious agents could be of great relevance to public health in terms of: a) reduction of infection diseases and infection-related diseases; b) reduction of costs associated to patients’ monitoring.

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