Results: The presence of *Campylobacter spp.* in bulk milk was microbiologically confirmed. 3% of individual milk samples and 10% of fecal samples were positive for *Campylobacter spp.* PCR-tests of all the isolates confirmed the presence of *C.jejuni*. No environmental swab was positive for *Campylobacter spp.*, excluding the suspect of biofilm formation in milking unit.

Conclusion: Consumption of unpasteurized milk can be a health threat. Bacterial contamination of raw milk can occur even under optimal hygiene conditions, and changes in bovine bacterial shedding or inadequate hygiene during milk collection might contribute to outbreak occurrence.

The importance of this pathogen for public health justifies and requires further investigation.

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Evidence of hantavirus infection in South Africa

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Background: Hantaviruses are hosted by rodents from different subfamilies and shrews from the *Crocidurinae* subfamily. Hantaviruses are responsible for two types of disease in human beings: Haemorrhagic fever with renal syndrome (HFRS) in Asia and Europe, and Hantavirus cardiopulmonary syndrome (HCPS) in the Americas. As the first African hantavirus, Sangassou virus was identified from the rodent *Hylomyscus simus* in Guinea in 2006, followed by Tanganya virus in Guinea and Azagny virus in Cote d'Ivoire, both from shrews. There is evidence of human infections occurring.

Our study aims to identify hantaviruses in Southern African small mammals and detect evidence of infection in human beings.

Methods & Materials: Total RNA was extracted from the lungs of rodents and shrews trapped in the Western and Northern Cape Provinces between 2007 and 2012 and screened for hantavirus RNA by RT-PCR using Pan-hantavirus primers. Human sera left over after routine biomedical laboratory testing at four laboratories in the Western Cape province were screened for hantavirus IgG antibodies by in-house ELISA using recombinant nucleocapsid proteins from Dobrava and Puumala viruses. Reactive specimens underwent a confirmatory algorithm using more specific assays: mAb-capture IgG and IgM ELISA, Western blot, indirect immunofluorescence test and focus reduction neutralisation test (FRNT).

Results: Of 2544 small mammal specimens screened for hantavirus genome by RT-PCR so far all had negative results. Of 1442 human sera screened for hantavirus IgG antibodies so far, 210 were reactive and underwent confirmatory testing through which fourteen samples were confirmed true-positive, resulting in a prevalence of 1% in the tested population.

Conclusion: Our inability so far to identify a hantavirus does not mean that these viruses do not occur in South Africa. Apart from their host species specificity, highly localised distribution patterns and enormous seasonal and interannual fluctuations are well-known phenomena. A greater diversity of species from different biomes will be screened. The seroprevalence of 1% in humans is comparable to prevalences reported inform known endemic areas in Europe. Sera will be collected from rural laboratories to give a more complete picture of human hantavirus antibody prevalence.

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Massive proliferative cutaneous lesions associated with Poxviridae and Papillomaviridaeviral species in ruminants

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Background: The families *Papillomaviridae* and *Poxviridae* include a number of species affecting different mammal species. Among *Poxviridae*, the genus *Parapoxvirus* and *Orthopoxvirus*, include viruses causing skin lesions in ruminants as well as in humans. These viruses infect via damaged skin and give rise to pustular lesions of the skin and occasionally the buccal mucosa. The family *Papillomaviridae* (PV) now comprises 29 genera among which several viral types infect ruminants. During the last few years, massive cutaneous lesions had been reported in cows from Sicily, our study was aimed at identifying the epitheliotropic viruses responsible of the disease that had been generically defined "papillomatosis".

Methods & Materials: Proliferative lesions from 13 cows, were submitted for histopathological and virological investigations (isolation, PCR, electron microscopy). Rolling circle amplifications have been carried out to identify circular DNA viruses. Different sets of primers had have been used to identify bovine papillomavirus types and PCRs, followed by mini- array, were performed to detect zoonotic poxviruses. Rolling circle amplified products were subsequently analysed by restriction enzyme reactions, while PCR amplified products were purified and sequenced.

Results: Our analyses showed that in many cases, the proliferative lesions were the result of *Papillomaviridae* and *Poxviridae* co-infection. *Delta*-papillomavirus types, BPV-1 and 2, responsible of fibropapillomas were detected in few cases, while most of the animals showed to be infected with *Xi*-papillomavirus BPV. Interestingly, the mini-array assays revealed that most of the animals (11/13) were also co-infected with *Parapoxviruses*, Pseudocowpoxvirus or Bovine Papular Stomatitis virus.

Conclusion: The skin is the largest interface between the internal environment and the external world, representing the ecological niche for different epitheliotropic viruses. Our results show that the so-called "papillomatosis" can be the result of viral multiple infections and that histopathology and electron microscopy alone cannot be conclusive in most of the cases. It is known that BPV can induce papillomas and fibropapillomas, which can evolve to malignant lesions, usually when influenced by environmental co-factors. These data might suggest that BPV malignant lesions can be also the result of multiple infections with epitheliotropic viruses such as poxviruses that can be transmitted to humans.

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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 sentinals 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 flavi and alphaviruses and specificly for WNV, Wesselsbronvirus, Shunivirus, Equine Encephalosis (EEV), Sindbis, Middelburgvirus, equine herpes virus and African horse sickness virus (AHSV). Histopathology were 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. Wesselsbron virus was detected in 2 neurological horses, 1 being fatal. Shunivirus was identified in 0-10% of horse, 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 summar and autumn.

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

Conclusion: WNV, Shunivirus, Middelburgvirus, sindbis and Wesselsbron virus are all potential zoonotic viruses and caused severe and fatal neurological disease in horses and several wildlife species. Exposure to WNV and Shunivirus was confirmed in humans and should be investigated in febrile and neurological disease in hospitals.

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A serological study of exposure to arthropod-borne pathogens in humans working and living in Kruger National Park, South Africa

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Background: Infection with arthopod-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 Sindbis virus 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: Rick*ettsia* 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