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Severe cutaneous neoforations in animals caused by co-infection of orf virus and orthopoxvirus: A possible zoonosis?

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Background: Parapoxvirus - ORF virus (ORFV) is the etiological agent of contagious ecthyma, a zoonotic disease of small ruminants. Lesions are characterized by pustules and neoforations on the skin of the lips, tongue and mouth. Man can become infected by contact with animals. Lesions are observed in the skin of the hands. *Orthopoxvirus* has been associated with disease in domestic and wild animals. Little is reported on co-infection with other viruses. The authors describe a case of skin proliferative lesions in goats caused by ORFV and *Orthopoxvirus*. The presence of similar lesions in farmer's hands was also observed.

Methods & Materials: The authors observed lesions in ears, face and perineum in 4 month old kids. The farmer showed similar proliferative lesions in the dorsal part of the hands. Biopsies were collected by goats for histopathological and virological investigations (virus isolation, PCR, electron microscopy). Biomolecular methods were used for diagnosis of *Parapoxvirus*, *Orthopoxvirus* and *Papillomavirus*. Positive products of *Orthopoxvirus* PCR were used as templates in automated sequencing reaction. Obtained sequences were compared with sequences available on GenBank. Cell culture assays on Fetal Ovine Testis and inoculation of specific pathogen free (SPF) embryonated chicken eggs were carried out to test the infectivity both *Parapoxvirus* and *Orthopoxvirus*.

Results: Anatomic-pathological examination showed the involvement of skin and subcutaneous tissue. Ulcero-vesiculo-pustulo-proliferative lesions were observed. Hyperkeratosis, hyperplasia, acanthosis and ballooning degeneration were the main lesions observed in the epidermis. Diffuse non suppurative chronic infiltrate was detected in the dermis. Blood vessels were seen in the superficial dermis. Cell culture and SPF egg were negative. PCR for *Papillomavirus* was negative. Electron microscopy showed the presence of ORFV. PCR revealed the presence of *Parapoxvirus* and *Orthopoxvirus*. Phylogenetic analysis of *Orthopoxvirus* assigned the samples to *vaccinia virus* species.

Conclusion: Results show that the so-called "skin papillomatosis lesions" can be the results of virus co-infections. We demonstrated in our samples both *Parapoxvirus* and *Orthopoxvirus*. Further investigations should be lead to understand the mechanism of the presence of both Poxvirus. These results suggest to study

the skin lesions in humans in order to clarify the role of different epitheliotropic viruses.

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Integrated management of a human campylobacteriosis outbreak in South Tyrol, Italy

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Background: Campylobacteriosis is a prevalent foodborne disease in industrialized nations and is considered a major public health concern. In South-Tyrol (Italy), according to EFSA data, human campylobacteriosis increased from 2011 to 2012 of 13%, although data remain underestimated.

Although there are more than 20 different *Campylobacter* strains, *C.jejuni* and *C.coli* are the most important species associated with infections in humans.

Campylobacter spp. are capable of zoonotic transfer through the faecal-oral route. The main environmental niche is considered to be intestinal tract of fowl; thus consumption of undercooked poultry meat is considered a major risk factor for sporadic infections. Contrarily consumption of raw milk has been the most important source of campylobacteriosis outbreaks in the last 15 years. *Campylobacter* spp. may be present in raw milk contaminated with feces during the milking process or due to udder infection by *Campylobacter*.

Methods & Materials: During August 2013 symptoms referable to campylobacteriosis were described in 4 people in South-Tyrol. Laboratory diagnosis confirmed *Campylobacter* spp. in feces of 1 of those affected. As the consumption of raw milk has been supposed to be a common risk factor, the case has been reported to the Local Veterinary Service, which together with laboratory IZSVE-BZ, investigated outbreak's source.

Bulk tank milk samples, individual milk samples and feces from the 33 lactating cows, and environmental swabs of the milking parlour were collected.

Microbiology: Milk according to ISO10272-1:2006, feces to an in-house method, swabs to ISO18593:2004.

Molecular-biology: *Campylobacter* spp. was typed by PCR in-house method.

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 *Crociturinae* 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 in 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 Papillomaviridae viral 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 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.