Serological evidence for Parapoxvirus infection in chamois from the Tyrol regions of Austria and Italy

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Summary
Orf-virus (ORFV) is a parapoxvirus that infects small ruminants worldwide causing sporadic zoonotic infections, mainly transmitted by direct contact with sheep and goats. Following an ORFV case in a hunter of Alpine chamois (*Rupicapra rupicapra*), who did not report previous contact to domestic animals, a serological survey in Western Austria was conducted to assess the seroprevalence of ORFV in this species. In addition, this study also tested blood/tissue samples of chamois from different areas of the adjacent province of Bolzano/Northern Italy for antibodies against ORFV using immunofluorescence and ELISA. The observed seropositivity rates in the chamois tested on the Austrian and Italian side of the Alps were 23.5% and 9.5%, respectively, with a combined 95% confidence interval ranging from 0.0678 to 0.238. Although the prevalence was significantly lower than the one observed in Austrian sheep flocks, this study provided the first evidence that parapoxviruses have spilled over into chamois populations to a significant degree in the Tyrol regions of Austria and Italy.

*Evidenza sierologica di Parapoxvirus in camosci (*Rupicapra rupicapra*) in Tirolo (Austria e Italia)*

Parole chiave
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Riassunto
ORF-virus (ORFV) è un Parapoxvirus che colpisce i piccoli ruminanti ed è diffuso in tutto il mondo, può causare infezioni zoonotiche sporadiche negli esseri umani, che sono trasmesse principalmente attraverso il contatto diretto con pecore e capre. L'indagine sierologica in camosci provenienti dalla zona colpita in Austria occidentale descritta in questo studio è stata condotta a seguito di un caso di ORFV in un cacciatore di camosci delle Alpi (*Rupicapra rupicapra*), che non aveva avuto contatti precedenti con animali domestici. Nell'ambito dello stesso studio, campioni di sangue e di tessuto di camosci di diverse aree della provincia adiacente Bolzano (Nord Italia) sono stati testati per anticorpi ORFV con immunofluorescenza ed ELISA. I tassi di sieropositività osservati nei camosci testati è risultato del 23,5% nell'area austriaca e del 9,5% nel versante italiano delle Alpi, con un intervallo di confidenza del 95% compreso tra i valori 0,0678 e 0,238. Sebbene la prevalenza sia significativamente inferiore a quella osservata in greggi di pecore austriache, i dati relativi a questo studio forniscono la prima prova che Parapoxvirus interessa ad un livello significativo le popolazioni di camosci presenti nelle regioni del Tirolo austriaco e italiano.

Orf-virus (ORFV) is a member of the *Poxviridae* family, genus *Parapoxvirus*, causing contagious ecthyma (also known as scabby/sore mouth disease) in small ruminants and it is spread worldwide. The morbidity in sheep and goats is high whereas the mortality is low. Animals can be infected repeatedly due to the insufficient immune response caused by immune-evasive peculiarities of the virus. Orf-virus also causes zoonotic infections in humans following unnoticed scarification and direct contact with infected animals or virus-contaminated surfaces (Haig, 2006).

Farmers and veterinarians are the main risk groups for human ORFV infections. However, children infected while visiting petting zoos and infections due to meat processing during food preparation have been reported (Centers for Disease Control and Prevention 2012). The infection in humans is mostly benign, self-limited and usually restricted to a few painful sores on the hands and resolves in 2 months. Fever and more widespread infections are rare and mostly associated with immunosuppressive conditions (Larcher *et al.* 2009, Lederman *et al.* 2007). Sequence data suggest that there are no distinct strains or ORFV variants showing an enhanced virulence or capacity to infect humans (Scagliarini *et al.* 2004).

In 2012, a case of ORFV infection in a hunter without a history of contact with domestic animals has been reported (Kitchen *et al.* 2014). The virus was identified by electron microscopy and the diagnosis confirmed by semi-nested PCR (Inoshima *et al.* 2001) and subsequent DNA sequencing. The sequence, which aligned with European ORFV sheep strains, was deposited in Genbank under the accession number HE996965[1]. As this patient had been hunting Alpine chamois (*Rupicapra rupicapra*), we decided to perform a small epidemiological survey concerning this species. Blood/tissue-fluid samples were extracted by centrifugation from fresh tissue samples (lung, liver, kidney) provided by the local hunter's community. Samples were collected from 17 chamois of the suspected area of the index case, i.e. the Sellrain valley of North Tyrol, Austria. Subsequently, 42 samples of extracts of lung tissue from chamois, collected in the same year (2012), were obtained from 9 areas of the neighboring Italian province of Bolzano.

The samples were centrifuged at 13.000 x g and tested for parapoxvirus specific antibodies using indirect immunofluorescence (IF) as recently described (Kitchen *et al.* 2014, Larcher *et al.* 2009). In brief, Vero (African green monkey kidney) cells were infected with laboratory ORFV BO15 strain (Cottone *et al.* 1998). The infected cells were spotted on 12 well microscopic slides (Thermo Scientific, Portsmouth, NH, USA), air-dried and fixed with ice cold methanol/acetone (1:1). The slides were then vacuum-sealed and stored at -80°C until use. Dilutions of animal blood/tissue fluids and secondary antibodies were performed in phosphate buffered salt solution (PBS) containing 10% bovine serum. Antibodies in chamois samples were detected with a mixture of FITC-labeled anti-goat (from KPL, Gaithersburg, MD, USA) and anti-sheep antiserum (from DAKO, Glostrup, Denmark), both raised in rabbit, at the working dilution suggested by the manufacturer (1:100). Sheep sera from different areas of Tyrol and Styria (Austria) served as positive and negative controls using FITC labeled anti-sheep rabbit serum alone. For better contrast in microscopy, the cells on the IF microscopic slides were counter-stained with a 1:1000 dilution of a 1% (w/v) Evans blue stock solution before mounting with 80% glycerol/PBS under glass cover slides (Kitchen *et al.* 2014). A repeatedly observed fluorescence titer of >1:40 was considered positive.

Additionally, ultracentrifuge concentrated ORFV laboratory BO15 strain, which had been heat inactivated and treated with detergent following published protocols, was used in enzyme-linked immune-sorbent assay (ELISA) as described (Chin and Petersen 1995).

As shown in Table I, 4 (23.5 %) of the 17 chamois (95% Confidence Interval (CI) = 0.0956 – 0.4726) from the Austrian North Tyrol were seropositive, whereas 4 (9.5 %) of the 42 animals from the Italian province of Bolzano tested positive (95% CI = 0.0376 – 0.2354). Sixteen of the 17 Austrian chamois came from the Sellrain valley, where the human index case occurred, and 3 of them tested positive. An additional positive animal was detected in the Austrian Karwendel mountains North of Innsbruck. This is of interest as 5 samples from Alpine ibex (*Capra ibex*) from the same Karwendel mountains were serologically negative for ORFV (not shown).

In contrast to North Tyrol, where mainly the location of the human index case was tested, the samples from South Tyrol originated from 9 areas as listed in Table I. The majority of seropositve chamois was detected in the Zillertaler mountains and 1 case occurred in the Ötztaler mountains, all close to the Austrian-Italian border (Figure 1). In the Ötztaler mountains infection of sheep had been reported in previous years including human cases (Larcher *et al.* 2009).

In game animals, parapoxviruses had been detected sporadically by molecular methods and some of these sequences had been included in Genbank (Scagliarini *et al.* 2011). But so far, no studies concerning the seroprevalence of parapoxviruses

[1] www.ncbi.nlm.nih.gov/genbank/.

Table I. *Number of chamois positive for antibodies against parapoxvirus from different regions (Austrian and Italian) of the Tyrol alpine area (titer >1:40 in indirect immunofluorescence).*

Area	Location	No. animals tested	No. positive
North Tyrol	Sellrain valley	16	3
North Tyrol	Karwendel	1	1
North Tyrol	**Summary**	**17**	**4**
South Tyrol / Alto Adige	Mühlwald	2	0
South Tyrol / Alto Adige	Lappach	5	2
South Tyrol / Alto Adige	Moos	5	1
South Tyrol / Alto Adige	Pfunders	5	0
South Tyrol / Alto Adige	Vahrn	5	0
South Tyrol / Alto Adige	Mühlbach	5	0
South Tyrol / Alto Adige	Terenten	5	1
South Tyrol / Alto Adige	Vintl	5	0
South Tyrol / Alto Adige	Trens	5	0
South Tyrol / Alto Adige	**Summary**	**42**	**4**



Figure 1. *Geographic location of seropositive chamois samples.* Positive samples detected are from the Sellrain Valley, and the Karwendel mountains in North Tyrol and the Zillertaler Alps (right) and Ötztaler Alps (left) of the South Tyrol/Alto Adige area.

in wild animals have been performed in Central Europe. Even in domestic animals, parapoxviruses are often ignored due to the mild symptoms of the infection. No national incidence/prevalence data of ORFV in sheep or goats are available for Austria or Italy. Therefore, we also tested domestic animals and found a rather high seroprevalence of parapoxviruses in Austrian sheep: 24 (63%) of 38 sheep from Styria and Tyrol were seropositive (95% CI = 0.4729 – 0.7662). This rate was significantly higher than the rate observed in goats ($p < 0.0001$ in Fishers exact test): only 5 (7%) of 71 goats had detectable antibodies against parapoxvirus (95% CI = 0.0304 – 0.1545). Whether this reflects a lower susceptibility for ORFV infection, a weaker immune response or regional differences in herding of goats remains speculative. However, the lower seropositivity rate of 10-20% of the tested chamois seems plausible given the rather solitary living of these animal, the infrequent contact with sheep on high mountain pastures as well as the presumably low risk of virus transmission via salt licks.

With the exception of variola, poxviruses often have a broad host tropism. The spreading of parapoxviruses to animals other than the common ruminant hosts has also been observed, especially in countries with a high density of sheep. In New Zealand, for instance, infection of cats with ORFV has been recently described (Fairley *et al.* 2008). Parapoxviruses have also been found in red deer (*Cervus elaphus*), which is considered a separate species: i.e. parapoxvirus of red deer New Zealand (PVNZ) type (Robinson and Mercer 1995). Surprisingly, recently a PVNZ related strain has also been detected in red deer of Northern Italy (Scagliarini *et al.* 2011). Cattle might also be a source of infection for red deer as suggested by the detection of a strain related to pseudocowpox virus (PCPV) in American deer hunters (Roess *et al.* 2010). PCPV and ORFV also could cross the species barrier to reindeer (*Rangifer tarandus*), as severe outbreaks with both viruses have been described in those animals in Finland (Tikkanen *et al.* 2004). A similar situation seems to exist in Japanese serows (*Capricornis crispus*), goat/antelope-like mammals, in which bovine papular stomatitis virus (BPSV) as well as ORFV related strains have been found (Inoshima *et al.* 2001).

This report is the first to highlight that ORFV related parapoxviruses may have infected a significant number of wild animals in different areas of the Alpine mountains. Game animals, including chamois, could in return also serve as reservoir for infection of domestic animals and even zoonotic infections.

As poxviruses cause more severe disease in immunocompromised persons (Huemer *et al.* 2007, Larcher *et al.* 2009, Lederman *et al.* 2007), patients with autoimmune disease, rheumatoid arthritis, organ transplantation, taking chemotherapy for cancer, corticosteroids, or other immunosuppressive drugs, are advised to avoid direct contact not only to the known domestic reservoirs of parapoxviruses like sheep or goats, but also to wild animals or raw meat of game animals potentially susceptible to parapoxvirus infection.

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