In vivo and in vitro propagation, transmission and epidemiology of Toggenburg Orbivirus (TOV)

J. Planzer[1], C. Kaufmann[2], G. Worwa[1], M.A. Hofmann[1], E. Brugnera[3], R. Weilenmann[3], T. Vanzetti[4], S. Casati[5], H.P. Schwermer[6], A. Reber[6], V. Chaignat[1], B. Thür[1]
[1]IVI, CH-3147 Mittelhäusern, [2]Institute of Parasitology, University of Zurich, CH-8057 Zurich [3]Institute of Veterinary Pathology, University of Zurich, CH-8057 Zurich, [4]Ufficio Veterinaria Cantonale, CH-6500 Bellinzona, [5]Laboratorio Cantonale, CH-6500 Bellinzona, [6]Federal Veterinary Office, CH-3003 Bern

Key words
Toggenburg Orbivirus, TOV, transmission, in vitro isolation, experimental infection, goats, epidemiology

Aim of the study
In vitro propagation of TOV on cell cultures and by feeding of possible vectors of TOV. Investigation of cell tropism by analysing tissue from infected goats. Investigation of the transmission of TOV (excretion, intrauterine transmission). Evaluation of epidemiological situation in Ticino and determination of the natural vector.

Material and methods
Saanen goats were inoculated with TOV-containing blood from field cases from Ticino, Grison and Germany. Tissues from the goats was investigated using in situ hybridisation and immunohistochemistry to determine the cell tropism of TOV. All excretions and foetuses were investigated by real-time RT-PCR (rPCR) for TOV. Blood from infected animals was inoculated on cell cultures as primary and secundary goat cells and different insect cell lines. Intracerebral inoculation of newborn mice was performed. To enhance infectivity of TOV several modified protocols, e.g. pretreatment of the virus and lipofection were applied. Blood feeding of Culicoides nubeculosus was done. TOV serologically positive herds in Ticino were investigated for circulating virus, additionally blood from cattle and sheep in these herds was analyzed for TOV. Caught insects (mainly Culicoides spp.) from Ticino (trapped near TOV-infected goat herds) were analyzed by rPCR for TOV. Material from goat experiment was used to establish and validate TOV-specific rPCR.

Results and significance
Although all infected goats developed viremia, no viral RNA could be detected in excretion/secretion sample and the sentinel animal remained uninfected, therefore it seems unlikely that TOV is transmitted by direct animal contact. No intrauterine infection of fetuses was seen. In tissue from infected goats, TOV-specific reaction using in situ hybridisation and immunohistochemistry was too weak to determine cell tropism. Isolation of TOV on cells, attempts to infect C. nubeculosus by feeding TOV-positive blood and the intracerebral inoculation of newborn mice were unsuccessful. The results suggest that TOV requires yet unknown factors for in vitro replication. TOV could not be found in caught Culicoides from Ticino, therefore it is not proven that these insects are the natural vectors. Beside TOV-positive goats also antibody positive sheep but no cattle were found in Ticino. Using newly developed TOV-specific diagnostic tools, TOV was detected in goats from Germany and Italy so it seems that TOV could have a broad distribution in Europe. Thus isolation of this virus could also be relevant for other laboratories. Even if we did not achieve to grow the virus, new experiments could be based on results of our attempts. As we could not see any clinical sings and in experimentally and field infected goats it seems that TOV is not a big issue concerning clinical disease in infected animals.

Publications, posters and presentations
Planzer, J.; et al. In vivo and in vitro propagation and transmission of Toggenburg Orbivirus (submitted).
Hofmann, M.A., et al. (2010) Detection of Toggenburg orbivirus by a segment 2-specific quantitative RT-PCR. J. Virol. Meth. 165, 325-329.
Chaignat, V.; et al. (2010) Occurrence and spatial distribution of Toggenburg orbivirus in Switzerland. Small Ruminant Res. 93, 157-164.

Project *1.09.01*

Project duration *January 2009 – April 2010*