Evaluation and application of detection methods for anti-virus antibodies in the blood of rainbow trout (Oncorhynchus mykiss)

My Klenk (previously Khong Thi)[1], Helmut Segner[1], Thomas Wahli[1]
[1]Institut of Animal Pathology, Centre for Fish- and Wildlife Health, University of Bern, CH-3012-Bern

Key words
VHSV, Infection model, serologic methods, ELISA, SNT

Aim of the study
The proof of the presence of antiviral antibodies in the serum of fish can be an indication for the health status of a fish population. However, at present, there are no validated serological methods available. In particular comparative data on specificity and sensitivity of different methods are missing. Precondition for a comparison of different methods is a reproducible and reliable virus infection model. Such a model for fish has not been described to date. The aim of the present work was therefore to compare a) different infection models of rainbow trout with the virus causing viral haemorrhagic septicaemia (VHSV) and b) different serological methods to test sera of rainbow trout (Oncorhynchus mykiss) for the presence of anti VHSV antibodies.

Material and methods
360 rainbow trout were randomly attributed to 6 equal groups. A control group (G1) was injected with PBS. Fish of four groups were infected with equal doses of VHSV by different methods: i.p once (G3), i.p twice (repetition 25 days after first injection) (G4), i.p and rectally (G5) and rectally only (G6). To test for specificity fish of a last group (G2) were injected intraperitoneally (i.p.) with the bacterium causing redmouth disease, Yersinia ruckeri. From all groups 15 fish were sampled 5 and 10 days after initial infection. Fish organs were checked for the presence of VHSV by means of RT-PCR and cell-culture. 72 days post first infection (p.i.) the sera of all surviving fish were sampled to test for the presence of anti VHSV antibodies. For these tests the following three methods were used: indirect ELISA, indirect capture ELISA and serum neutralization test (SNT).

Results and significance
In none of the groups VHSV could be re-isolated from all fish.No virus positive fish were found in fish injected with PBS or Yersinia ruckeri. A significant correlation between percentage of virus-positive fish, appearance of clinical symptoms and mortality could not be established in any of the groups. The antibody levels in the experimentally infected fish were clearly higher in groups G3 and G4 (i.p.-injection) than in group G6 (rectally infected). A booster by repeated i.p. injection (G4) resulted in an increased antibody titer, but not in an increased number of sero-positive fish. The results of the three antibody detection methods coincided at a level of 70% of the sera. In the remaining 30% of sera a partly strong divergence was recorded. This divergence could result from methodical shortcomings in the test methods themselves, but there might also be differences in the reaction pattern and functionality of the fish antibodies. None of the methods revealed anti VHSV antibodies in Y. ruckeri infected fish indicating that all methods were specific. No positive results were obtained with sera from The results of this study demonstrate that the actual state of the serological techniques do not yet allow the use of these methods for the demonstration of VHS-carrier fish in routine diagnostics.

Publications, posters and presentations
XI. Gemeinschaftstagung der Deutschen, Österreichischen und Schweizer Delegation der EAFP „Serologische Studien zu verschiedenen Infektionsmodellen und zur Dynamik von VHS- und IHN-Infektionen in Regenbogenforellen (Oncorhynchus mykiss)“ (Poster-Präsentation)
Klenk, M. (2008) Serologische Studien von verschiedenen VHSV-Infektionsmodellen in Regenbogenforellen. Dissertation, Vetsuisse Fakultät, Universität Bern.

Project *1.05.07*

Project duration *Juni 2005 – Juni 2008*