



Experimental determination of humane endpoints in infection studies with rainbow trout (*Oncorhynchus mykiss*)

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Key words

Infection experimentation, bacterial disease, rainbow trout, surrogate endpoints, video evaluation

Aim of the study

To meet the refinement point of the 3R principle in animal experimentation application of “humane” or surrogate endpoints is a requirement of many agencies. However, for fish hardly any data on such endpoints are published. The aim of the present project was therefore to identify visually perceptible clinical signs that allow timely distinction between survivors and non-survivors to be used as a surrogate for death as endpoint in infection experiments with rainbow trout.

Material and methods

Batches of marked young of the year rainbow trout were infected either with *Aeromonas salmonicida* (i.p.) or with *Yersinia ruckeri* (immersion). Uninfected controls were included. The course of the infection until death or recovery of the animals was followed by video. Typical signs of the infection were then identified in a first step. In a second in-depth evaluation, these signs were analyzed for their suitability as early predictors of death and survival. The data were statistically evaluated for specificity and sensitivity to predict the death of the experimental animals.

Results and significance

While signs for a highly debilitated state of the animals, like inability to hold upright position or being passively dragged by the water current in the tanks, had both a high sensitivity and specificity in predicting the death of the fish, they proved to be not suitable as they appeared only shortly before death and thus did not reduce the overall time that the animals spent in the experiment. In contrast, anorexia was identified as a promising predictor in regards to sensitivity and timely identification of non-survivors. A further promising sign in fish infected with *A. salmonicida* was a change in body shape although more difficult to discriminate. The evaluation of the material demonstrated, that there are considerable differences in the course of infections depending on the pathogens applied. Another finding was a strong social aggression between tank mates in the given setup, which proved to be a major obstacle for the determination of robust criteria. In order to identify individual fish the group size had to be limited to ten animals per tank which allowed single fish to become dominant despite limited space available thus possibly altering the sequence of disease signs. Using single fish in tanks with an otherwise unchanged setup might have resulted in a clearer outcome. Nevertheless the method using evaluation of video material showed to be highly suitable and allowed to establish a first set of potential surrogate endpoints. However, these will have to be tested in infection experiments under different conditions, in particular husbandry conditions adapted to the behavior of the test animals in order to reduce social aggression to a minimum.

Publications, posters and presentations

Keeling, C.M. (2017) Video-based identification of surrogate endpoints in experimental bacterial infections of rainbow trout (*Oncorhynchus mykiss*). Vet. Med. Dissertation Vetsuisse Faculty Bern

Keeling, C.M.; von Siebenthal, B.; Wahli, T. (2016). Experimentelle Ermittlung von Abbruchkriterien im Infektionsversuch mit der Regenbogenforelle (*Oncorhynchus mykiss*). XVI. Gemeinschaftstagung der Deutschen, Österreichischen und Schweizer Sektionen der European Association of Fish Pathologists (EAFP), 5.-7.10.2016, Graz, Österreich (Presentation).

Keeling, C.M.; von Siebenthal, B.; Wahli, T. (2016). Experimentelle Ermittlung von Abbruchkriterien im Infektionsversuch mit der Regenbogenforelle (*Oncorhynchus mykiss*). Tagungsband der XVI. Gemeinschaftstagung der Deutschen, Österreichischen und Schweizer Sektionen der European Association of Fish Pathologists (EAFP), 5.-7.10.2016, Graz, Österreich (Extended summary of presentation) (In press).

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