

## Bluetongue disease vectors (*Culicoides* spp.): Definition of parameters which determine 'vector-free periods/areas' in Switzerland

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### Key words

Biting midges, vectors, bluetongue disease, vector-free periods/areas, climate zones, Switzerland, identification, MALDI-TOF MS, real-time PCR, breeding habitat.

### Aims of the study

Biting midges (*Culicoides* spp.) are incriminated as the biological vectors of viruses of veterinary relevance (bluetongue, African horse sickness, epizootic hemorrhagic disease, Toggenburg orbivirus) with current or potential occurrence in Europe. The aims of the study were to determine the spatio-temporal distribution of biting midges in Switzerland and to develop molecular techniques (real-time PCRs, MALDI-TOF mass spectrometry) to efficiently and reliably identify these tiny insects.

### Material and methods

*Culicoides* biting midges were caught with UV-light traps at 12 stations representing the climate zones of Switzerland once weekly over 3 years. The midges were morphologically classified into Obsoletus group, Pulicaris group and others for monitoring purposes. Species identification was achieved by analysing slide-mounted specimens. Genetic analyses by PCR/sequencing were done at the mtCOI and the rDNA loci.

### Results and significance

Biting midges were abundant at all 12 climate zones, the highest number (about 60'000 per season) were observed in lowlands (Dittingen/BL) with species of the Obsoletus group prevailing, but considerable numbers (12'000) mainly from the Pulicaris group were also collected at the highest site (Juf/GR, 2100 m asl). Vector-free periods in all three years were similar, stretching from week 51 to week 18 (e.g. 18 December 2009 to 27 April 2010). Genetic analyses revealed the presence of novel species in the alpine zone. Real-time PCR primers/probes were designed to distinguish 10 Swiss species and *C. imicola* (France), and the specificities and sensitivities of the assays were thoroughly evaluated. MALDI-TOF biomarker mass sets were determined for 12 Swiss species and for *C. imicola*. Field-collected specimens were correctly identified using this reference database. MALDI-TOF MS was further applied to also identify the larval stages of ceratopogonids but also of mosquitoes. Thus, real-time PCRs and MALDI-TOF MS assays were established for rapid and reliable identification of important species of biting midges.

### Publications, posters and presentations

Kaufmann, C. et al. (2011) Evaluation of matrix-assisted laser desorption/ionization time of flight mass spectrometry for characterization of *Culicoides nubeculosus* biting midges. Med. Vet. Entomol. 25: 32-38.

Wenk, C.E. et al. (2011) Molecular characterisation of Swiss Ceratopogonidae (Diptera) and evaluation of real-time PCR assays for the identification of *Culicoides* biting midges. Vet. Parasitol. (accepted).

Kaufmann, C. et al. (2011) Identification of field-caught *Culicoides* biting midges using matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF MS) (submitted).

Steinmann, I. et al.: Evaluation of matrix-assisted laser desorption/ionization time of flight mass spectrometry for the identification of ceratopogonid and culicid larvae (in preparation).

Kaufmann, C. et al.: *Culicoides* biting midges: suitability and abundance maps of Switzerland based on 3 years monitoring (in preparation).

**Project 1.08.10**

**Project duration February 2008 – July 2011**