



Vector competence of *Culicoides* biting midges from Switzerland for African horse sickness virus and epizootic haemorrhagic disease virus, and establishment of a novel arbovirus sentinel system

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Key words

African horse sickness virus, epizootic haemorrhagic disease virus, *Culicoides* biting midges, vector competence, Swiss Plateau, pre-alpine area, sentinel arbovirus kit

Aim of the study

The study was performed to investigate the vector competence of two *Culicoides* populations from Switzerland (one collected in the Swiss Central Plateau, Zürich area, 650 m asl; mainly *Obsoletus* complex species *C. obsoletus* and *C. scoticus*; the other in the pre-alpine area, Juf, Grisons, 2100 m asl; overwhelmingly *C. grisescens*) for two isolates each of African horse sickness virus (AHSV, serotypes 4 and 9) and epizootic haemorrhagic disease virus (EHDV, serotypes 6 and 7).

The second aim was to evaluate whether a passive virus surveillance system developed to investigate the circulation of mosquito-transmitted pathogens ('sentinel mosquito arbovirus capture kit') could be adapted for the use with biting midges. In this approach, insects are collected in traps where they survive for extended periods and repeatedly feed on sugar-impregnated, nucleic acid preserving cards (FTA cards), thereby depositing saliva onto the cards that can be investigated for the presence of pathogens.

Material and methods

Culicoides were fed on bovine blood spiked with virus and incubated at one of two different fluctuating temperature regimes, corresponding to normal or exceptionally hot summer conditions at the Swiss Central Plateau (average temperatures of 22 °C or 26 °C ± 6 °C). After the 8-day incubation period, midges were dissected and heads were examined for the presence of viral RNA using RT-qPCR. Positive heads were further tested for the presence of live virus through virus isolation on Vero cells. Thoraxes were analysed by MALDI-TOF mass spectrometry for species determination.

The feeding rates of laboratory-reared *C. nubeculosus* on FTA cards impregnated with coloured honey or invert sugar was examined as well as the possibility of detecting virus applied directly to FTA cards by RT-qPCR. Additionally, *Culicoides* were intrathoracically injected with virus and then exposed to FTA cards to determine whether virus was deposited on FTA cards through saliva. Latin square comparisons of standard UV light traps (220 V) vs. blue LED light traps (6 V), as well as of green vs. blue LED traps were performed. Several prototypes of arbovirus sentinel traps were designed and evaluated in the field.

Results and significance

A total of 13'337 midges from the Swiss Plateau and 1'924 midges from the pre-alpine site were exposed to infectious blood meals. Feeding rates ranged from 16 - 40% and survival rates from 13 - 44%. The number of *Culicoides* with fully disseminated infections was 14 (6 *C. obsoletus*, 8 *C. scoticus*), all originating from the Swiss Plateau. Dissemination rates between 1 - 5% were observed. There were positive individuals for each of the four virus strains, but for AHSV-9 and EHDV-7 only when incubated at the high fluctuating temperature regime. Viable virus of all four strains was demonstrated with head homogenates from 8 of the 14 specimens through virus isolation. Thus, very similar to the results of an earlier study from our group with bluetongue virus (serotypes 1, 4, 8), biting midges from the Swiss Plateau altitude are competent vectors for both AHSV and

EHDV under summer conditions whereas *Culicoides* from the pre-alpine area cannot be considered suitable vectors.

Feeding rates between 70 - 95% were observed for *C. nubeculosus* when allowed to feed on sugar-impregnated FTA cards. Virus (AHSV-4) could be detected until a titre of $10^{2.75} \log_{10} \text{TCID}_{50}/\text{ml}$ and for at least 4 weeks of storage at 25 °C when applied directly onto an FTA card. Intrathoracic injection of *Culicoides* was repeated numerous times, however, due to the extremely low survival rates, it was not possible to expose enough virus-injected *Culicoides* to FTA cards in order to validate the method. In a comparison of standard UV light traps and blue LED traps, the traps performed comparably with regard to number of *Culicoides* caught. A comparison of blue and green LED lights showed that blue was considerably more effective. Three different potential traps were constructed and set up in the field. Due to the late time point in the season, *Culicoides* catches were low and the experiments were inconclusive with regard to capture effectiveness, FTA card feeding rate and survival rate of *Culicoides* in the traps. Overall, the approach seems promising and will be pursued, focusing on improving the technique of intrathoracic injections into these tiny insects.

Publications, posters and presentations

Maurer, LM, Paslaru A, Torgerson P, Veronesi E, Mathis A. 2021. Vector competence of *Culicoides* biting midges from Switzerland for African horse sickness virus and epizootic haemorrhagic disease virus. Manuscript in preparation (under revision by external co-authors), to be submitted to peer-reviewed scientific journal.

“Vector competence of *Culicoides* from Switzerland for African horse sickness virus (AHSV) and epizootic haemorrhagic disease virus (EHDV), and establishing a novel arbovirus sentinel assay”. Swiss Vector Entomology group, annual scientific meeting; Basel, 16/17 January 2020.

“Vector competence of *Culicoides* from Switzerland for African horse sickness virus (AHSV) and epizootic haemorrhagic disease virus (EHDV), and establishing a novel arbovirus sentinel assay”. Seminar of the Institute of Parasitology, University of Zürich, 16 March 2021.

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