Bluetongue, Schmallenberg - what is next?
Culicoides-borne viral diseases in the 21st Century

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BMC
Veterinary Research

REVIEW   **Open Access**

Bluetongue, Schmallenberg - what is next? *Culicoides*-borne viral diseases in the 21st Century

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Abstract

In the past decade, two pathogens transmitted by *Culicoides* biting midges (Diptera: Ceratopogonidae), bluetongue virus and Schmallenberg virus, have caused serious economic losses to the European livestock industry, most notably affecting sheep and cattle. These outbreaks of arboviral disease have highlighted large knowledge gaps on the biology and ecology of indigenous *Culicoides* species. With these research gaps in mind, and as a means of assessing what potential disease outbreaks to expect in the future, an international workshop was held in May 2013 at Wageningen University, The Netherlands. It brought together research groups from Belgium, France, Germany, Spain, Switzerland, United Kingdom and The Netherlands, with diverse backgrounds in vector ecology, epidemiology, entomology, virology, animal health, modelling, and genetics. Here, we report on the key findings of this workshop.

Keywords: *Culicoides*, Midge, Schmallenberg virus, Bluetongue virus, Emerging disease, Ecology

Introduction

In 2011, a novel Orthobunyavirus, provisionally named Schmallenberg virus (SBV), was first detected in cattle blood samples and subsequently in the brains of stillborn, malformed lambs [1]. During the summer of that year, SBV infections caused reductions in milk yield, diarrhoea and fever in adult cattle in Germany and the Netherlands [2]. Later, the involvement of the virus in congenital malformation was demonstrated [3], which remains SBVs primary clinical impact, in addition to evidence of infection in Deer [4] and Camelids [5]. Prior to this incursion, outbreaks of several strains of bluetongue virus (BTV) occurred within the same geographic region [6,7], although it remains uncertain whether the two viruses shared the same route of entry into Europe [8]. Despite both viruses being transmitted by *Culicoides*, clear differences in their pattern of spread have become apparent, as well as differences in their apparent ecology. During a workshop organized in May 2013 at Wageningen University, The Netherlands, these issues were addressed

under five different themes: (1) Virus-vector interactions; (2) Sampling methods of *Culicoides*; (3) Diagnostics of *Culicoides* and SBV; (4) Ecology of *Culicoides* and SBV in Europe and (5) Modelling of *Culicoides*-borne disease spread in Europe.

Virus-vector interactions

The vast majority of detailed studies of *Culicoides*-arbovirus interactions have been conducted using laboratory lines of *C. sonorensis* and *C. nubeculosus* infected with either BTV or the closely related African horse sickness virus (AHSV) [9-11]. Within these restricted models of infection, a series of barriers to dissemination of arboviruses within *Culicoides* have been demonstrated, either directly through immunochemistry, or indirectly from time-series virus infection titres [12,13]. While superficially similar to those barriers defined for mosquitoes, a key apparent difference lies in the lack of salivary gland barriers of infection and release [10,12,14]. This observation has recently been confirmed in *C. sonorensis* infected with SBV [15] and allows evidence of infection in the head of field-collected *Culicoides* to be inferred as being fully disseminated [16,17]. Another relevant characteristic observed in *C. sonorensis* is the 'leaky gut phenomenon,'

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which is especially seen when reared at higher temperatures [18]. It results in higher infection rates and indicates that successful dissemination and transmission of a virus is temperature-dependent.

Genetic factors underlying vector competence of *C. sonorensis* for BTV have been explored in the USA and appear to involve a multi-locus determination of susceptibility to fully disseminated infection [19]. These pioneering studies are now being revisited in light of the development of next generation sequencing technology using a systems-based approach of comparative genomics and contemporary genetic mapping strategies. Understanding genetic inheritance of vector competence for BTV is an important driver of the first *de novo* full genome sequence project for the *Culicoides* genus currently being conducted by The Pirbright Institute and the European Bioinformatics Institute (as part of the *Culicoides* Genomics and Transcriptomics Alliance). The production of an accurately annotated genome for *C. sonorensis* will trigger major future advances in comparative genomics in European species, as has been seen in other arthropod vector groups [20]. Understanding the factors driving vector competence of *Culicoides* from the point of view of SBV are likely to be aided by the development of reverse genetics, allowing detailed investigation of infection in the insect host [21].

Following the identification of SBV as a disease-causing agent, several European countries possessing on-going vector surveys were able to identify various *Culicoides* species as potential vectors through the screening of late summer midge pools for the presence of SBV [16,17]. In nine locations in northern Belgium, pools consisting of the heads of *C. obsoletus*, *C. dewulfi* and *C. chiopterus* from August 2011 were found to be positive for SBV [17]. No positive pools were found in the south of Belgium, correlating with a low seroprevalence rate in sheep and cattle at the end of the first vector season. In The Netherlands, individuals of the *Obsoletus* complex (*C. scoticus* and *C. obsoletus*) and *C. chiopterus* were found positive, with estimated field infection rates of 0.56 and 0.14%, respectively. All *C. dewulfi* and *C. punctatus* samples were SBV-negative. The high viral load (as derived from Ct-values in quantitative PCR) and relatively high proportions of infected midges as compared to BTV infection rates could explain the rapid and efficient transmission of SBV in comparison to BTV. Furthermore, the fact that the PCR C_t values found in the heads of midges matched closely those obtained from their associated abdomens makes it more certain that SBV had replicated to transmissible levels in these midges, and supports the contention that two species of the Obsoletus Complex, along with *C. chiopterus*, act as natural vectors for SBV [22].

In France, the first cases of SBV were detected in January 2012 and the disease has since spread throughout the country, including to the island of Corsica. Retrospective studies on *Culicoides* from the national surveillance network also implicated *C. chiopterus* as putative vector. In addition, positive pools of *C. dewulfi* and *C. pulicaris* were found (C. Garros, pers. comm.). Interestingly, one positive pool of *C. nubeculosus* was also detected. This species is the only European midge species that has been continuously kept and reared under laboratory conditions [23], but its abundance in France and other northern European countries is relatively low and a recent study of a colony line has demonstrated a low vector competence for SBV [15]. Laboratory studies where explicit estimates of vector competence for SBV can be made for field populations to infer likely influence on dispersal rate are now a priority.

Sampling methods of *Culicoides*

Adult *Culicoides* are at present most commonly collected in the field using Onderstepoort Veterinary Institute (OVI) light-suction traps that utilise a UV light source to attract Insects. A key criticism of their use is that it remains unclear why *Culicoides* are attracted to light and catches have been repeatedly demonstrated to be unrepresentative of collections made on hosts at the same site [24]. In addition to light-suction traps, unbaited suction traps are also used such as the 11 metre suction trap network maintained as a national capability by the Rothamsted Insect Survey [25]. Studies from Belgium revealed that, during the same year, two Rothamsted suction traps collected 21 and 25 different *Culicoides* species, respectively. One OVI trap and 88 emergence traps located in the first site with a Rothamsted suction trap collected 17 and 12 species, respectively. Using sheep as bait for trapping, approximately 50% of the midges captured at a site in The Netherlands were *C. chiopterus* and around 40% were *C. obsoletus* [26]. Interestingly, *C. obsoletus* was by far the most common species found around horses. The average successful *Culicoides* feeding rate was 35–40%. *Culicoides* activity was greatest around sunset, less at sunrise and they were only rarely trapped in the afternoon or at night on hosts [27].

Adult sampling can also be conducted using emergence trapping from sites of larval development. In studies from Germany, 22 *Culicoides* species were collected from emergence traps, including species of the Obsoletus complex occurring in Germany (plus *C. dewulfi*) and four species of the *Culicoides* subgenus Pulicaris group (*C. pulicaris*, *C. punctatus*, *C. newsteadi*, *C. deltus*). Depending on the substrate onto which the emergence trap was placed, abundance per 48 hours of trapping ranged from very few to up to 2,000 individuals. These results demonstrated that the most important habitat types for *Culicoides* are bog land (primarily for the Obsoletus complex), wet forest areas (especially for Pulicaris complex) and animal faeces. It was noted that emergence traps on dung caught a greatest abundance of *Culicoides* in March and April, when

emergence commonly occurs from these overwintering habitats. Dung containing hay (from dung heaps) was found to be highly productive as a *Culicoides* larval habitat and also more important than loose animal droppings.

The OVI light-suction trap remains a standard tool for *Culicoides* detection that has a significant role in the detection of vector-low periods allowing animal movement restrictions to be reduced. However, as *Culicoides* ultimately find their hosts based on odours, it was concluded that more research effort should be put into the development and addition of semiochemicals as lures to trapping systems. Ideally, such trapping systems would be sensitive in low *Culicoides* density situations, thus making them more suitable for surveillance during winter periods.

Diagnostics of *Culicoides* and SBV

Accurate morphological identification of *Culicoides* is considered challenging, requiring significant expertise and is restricted to relatively few entomologists in Europe. Online resources for identification (e.g. www.culicoides.net; http://avabase.cirad.fr/) and taxonomic training [28] may reduce this problem. Some morphologically similar species of *Culicoides* can also now be accurately identified to species level based on DNA marker regions such as the CO-I and ITS-2 [29,30]. High-throughput systems for this have been developed that are being applied to large-scale surveillance sampling in France [31]. As an alternative to these molecular tools, matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF MS) has gained interest for high throughput identification of microorganisms and has been established to identify adults and larvae of *Culicoides* [32,33]. This protein-profiling approach revealed an accuracy of 98.9% when using 1,200 randomly selected *Culicoides* specimens. Whether this technology will become available at large scale for surveillance programs remains to be seen, especially in the light of the rapidly decreasing costs of DNA sequencing.

Sequence comparisons of SBV isolates from different hosts have revealed high variability in the M-region of the virus genome. This was especially apparent between an ovine brain isolate and other lamb brain isolates and cattle blood isolates. This variability may be due to adaptation to host or vector tissues and indicates variation within the epidemic. The origins of atypical variants remain to be investigated. Reverse genetics approaches have been and are being developed to identify cellular pathways involved in vector competence with selected virus mutants. Besides these molecular approaches, classic transmission experiments, where potential hosts are exposed to infected insects and naive blood-feeding insects are exposed to infected hosts remain essential for assessing the role of vectors in the natural transmission cycle.

Ecology of *Culicoides* and SBV in Europe

The capability of *Culicoides* species to overwinter and serve as reservoir for new infections during the next year is of relevance to the transmission of both BTV and SBV. Until quantitative evidence is provided otherwise, possible overwintering strategies in the vector, host or via alternative transmission pathways should all be considered. The detection of SBV RNA in field caught nulliparous *Culicoides* in Poland [34] is an important epidemiological finding and resembles a previous study that detected BTV in *C. sonorensis* larvae in the USA [35]. This implies that transovarial transmission could represent an alternative means of overwintering for SBV. Confirmation and isolation of live virus from adults is required, however, given the repeated failure of previous studies to replicate this phenomenon in *C. sonorensis* under laboratory conditions with BTV [10,36].

In The Netherlands, Denmark and Switzerland, first trials were performed with honey-baited FTA cards that were deployed in the vicinity of *Culicoides* traps. The goal of this was to detect the circulation of SBV at an early time of the year in a given area. Based on the expectoration of virus in the saliva onto the FTA preservation cards, trials using this technique in Australia have been able to successfully recover arboviruses from collected mosquitoes [37]. At present, this approach is being refined for virus surveillance in *Culicoides* although initial studies in the laboratory appear to show that these are unlikely to be effective [15].

Host preference of *Culicoides* is of significant importance in determining the probability of arbovirus transmission. Recent studies from France using sticky traps on animals have shown a clear preference of *Culicoides* midges (*C. obsoletus s.s.*, *C. dewulfi* and *C. scoticus*) to bite horses over sheep, chicken, goats or cattle, even after correcting for body surface or weight) [38]. *Culicoides* midges could be taken from a layer of petroleum jelly on panels applied to livestock and were still useful for molecular biology purposes [30]. Catch rates from an OVI trap used in parallel to the sticky traps on animals over-estimated the biting rate of *C. obsoletus* and under-estimated the biting rate of *C. dewulfi* on a horse. This supports earlier findings demonstrating that OVI traps are not representative of actual feeding events on a host [24]. Although most biting midges were captured outdoors, *C. obsoletus* showed some degree of endophagy. An overall increase of endophagy was observed in autumn. Again, the use of semio-chemicals in traps was recommended for surveillance purposes. Despite the limitations of the current trap types, data from traps remain useful for modelling purposes.

Modelling of *Culicoides*-borne disease spread in Europe

Predicting the spread of *Culicoides*-borne diseases is of great importance for preventing and reducing disease

burdens. In the case of BTV, a trend-surface analysis coupled to a simultaneous autoregressive model has been used to assess the wave-front velocity at which BTV spread in France [39,40]. In the context of restricted animal movement, the wave-front velocities of BTV-1 and BTV-8 were similar: 5.4 and 5.6 km per day, respectively. Ecological factors associated with vector abundance and activity (meteorologically related variables and elevation), as well as with host availability, were the most important drivers of this spread. The spread of SBV was probably faster than of BTV, but in this case there were no animal movement restrictions.

Other models, such as atmospheric dispersion models, have been employed to simulate vector movement and the risk of wind-borne vector introduction. These are highly effective at predicting incursions of infected *Culicoides* across water bodies and have been used to both predict movement and also in retrospective analyses of spread [41]. Modelling movement of *Culicoides* over land has been more challenging due to the difficulties of both predicting behaviour at a farm level and the greater complexity of air turbulence over landscape. Currently studies are focussing on the use of mark recapture techniques to define *Culicoides* dispersal at a local scale [42]. These studies are known, however, to invariably violate a rule of dispersal studies in significantly influencing the behaviour of released insects due to inflicting physical damage. An alternative approach may lie in the use of genetic or genomic markers to track population movement.

A third approach of modelling BTV spread is currently using Random Regression Forest modelling. 'Random forests' is an ensemble of decision trees using 74 variables (such as land cover, human population density and water capacity). Modelling efforts thus far have shown that the probability of vector occurrence is a good predictor of abundance at the used spatial resolution of 5 × 5 km. Abundance of *C. imicola* is mostly driven by rain, whereas *C. obsoletus* is more affected by temperature. The proposed methodology can be used as an input to TIR models and R_0 models and will become available to the general public as part of the VECMAP^{TM} software.

Conclusions

There was a general consensus within the group that future studies should move beyond simple monitoring of adult *Culicoides* populations and address fundamental aspects of vector competence in particular, as it has been shown that *Culicoides* species and populations contribute differentially to disease epidemiology. With respect to surveillance, a key question was to what degree a sampling strategy should reflect activity of *Culicoides* on a host. While a non-representative, but very sensitive surveillance method (e.g. the use of OVI light-suction traps) might be suitable for defining vector-free periods (as defined by EU

legislation), the degree to which these data could be used as a proxy for biting rate in modelling of transmission was not well understood. The proliferation of trap types used for surveillance in Europe was seen as presenting difficulties in maintaining a coherent understanding of *Culicoides* abundance and distribution, as in other vector groups. Besides these limitations in the surveillance of adult *Culicoides* populations, the group concluded that data on the larval biology and ecology of midge species, e.g. on their preferred breeding habitats and substrates, is sparse and limits our options for efficient and targeted vector management.

To answer the question posed in the title, it was concluded that the emergence of novel viruses cannot be predicted except in the very broadest sense. Assessments of potential outbreak risks in Europe for other known *Culicoides*-borne viruses, such as African horse sickness virus, are still in their infancy [43]. A key current question is whether SBV and the BTV-8 strain shared the same route of entry into northern Europe and if so whether this route remains open [8]. The incursions of Schmallenberg and bluetongue have led to studies that have generated fundamental insights into the ecology of *Culicoides*-borne diseases. In order to retain this capacity under the budget constraints likely to be imposed on research in Europe, transnational funding was seen as being highly appropriate. A model in this respect is the current EDENnext project (http://www.edenext.eu/), which has brought together a vast range of expertise from 48 institutes across Europe to understand vector-borne disease epidemiology.

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