Ecology and eco-epidemiology of *Culicoides*-borne diseases:

what have we learnt and where do we go from here?

Kate R. Searle[1], Alberto Allepuz[2], Sebastian Napp[3], Steven White[1], Simon Carpenter[4], Miguel Miranda[5], Claire Garros[6,7], Rene Bødker[8], Assane Gueye Fall[9], Bethan V. Purse[1] and Catherine Cêtre-Sossah[6,7]

[1] NERC Centre for Ecology and Hydrology, UK
[2] Centre de Recerca en Sanitat Animal (CReSA) - Institut de Recerca i Tecnologia Agroalimentàries (IRTA) / Autonomous University of Barcelona, Spain
[3] Centre de Recerca en Sanitat Animal (CReSA) - Institut de Recerca i Tecnologia Agroalimentàries (IRTA), Spain
[4] The Pirbright Institute, UK
[5] University of the Balearic Islands, Spain
[6] CIRAD, Montpellier, France
[7] INRA, Montpellier, France
[8] National Veterinary Institute, Danish Technical University, Denmark
[9] Institut Sénégalais de Recherches Agricoles, Sénégal

INTRODUCTION

Culicoides biting midges are abundant haematophagous flies capable of acting as vectors for arthropod-borne viruses of veterinary and medical importance. The past two decades have witnessed dramatic changes in the epidemiology of *Culicoides*-borne viruses, including the emergence of exotic viruses in northern temperate regions, increases in global disease incidence and enhanced virus diversity in tropical zones. The drivers of these changes may include altered climate, land use, trade and animal husbandry practices. During this time, several new *Culicoides* species and new wild reservoir hosts have been implicated in disease transmission, highlighting the dynamic nature of pathogen-vector-host interactions and the importance of understanding how the wider natural and agricultural environment influences these processes. Throughout EDENext, the *Culicoides* group has sought to enhance the evidence base for effective disease policy and management by engaging in a wide range of empirical and model-based research activities. Here, we summarise the main findings of the *Culicoides* group in relation to vector behaviour, life history traits, population dynamics and phenology. We then discuss how this enhanced understanding has stimulated a suite of methods aimed at understanding the consequence of midge vector biology for *Culicoides*-borne disease spread and control across Europe. Finally, we identify the key knowledge gaps hindering further refinement of models and improved implementation of disease policy.

VECTOR BEHAVIOUR, LIFE HISTORY TRAITS, POPULATION DYNAMICS AND PHENOLOGY

In response to the recent emergence of bluetongue virus (BTV) and Schmallenberg virus (SBV), researchers within EDENext have studied the behaviour, life history, phenology and population dynamics of *Culicoides* species across Europe and North Africa.

The EDENext *Culicoides* group collaborated with international colleagues outside the project to provide a comprehensive review of the bionomics of *Culicoides* in relation to the transmission of *Culicoides*-borne diseases (Purse et al. 2015). This review focused on potential vector species worldwide and key elements of vectorial capacity, and assessed the sensitivity of *Culicoides* life cycles to abiotic and biotic factors. It also considered the implications for designing control measures and understanding

the impacts of environmental change in different ecological contexts. Finally, critical geographical, biological and taxonomic knowledge gaps were prioritised. These knowledge gaps, along with others identified within the EDENext *Culicoides* group, are discussed in the conclusions of this article.

A critical aspect of providing an evidence base for informing disease management and policy involves assessing how aspects of *Culicoides* vector capacity vary across Europe and North Africa. The EDENext *Culicoides* group assessed vector competence for several *Culicoides* vectors and viruses, for instance investigating the competence of *C. imicola* with three BTV serotypes (BTV-8, BTV-4 and BTV-1). The EDENext *Culicoides* group developed standardised methods for comparative estimates of vector susceptibility to infection and onward transmission potential. One aspect of this work involved significant advances in feeding techniques for North-Western Palaearctic species (*C. imicola,* Obsoletus group species) thereby targeting key potential vectors of orbiviruses. Our group developed novel methods using the Hemotek feeder to improve comparison between sites and countries, allowing the first multi-country comparison of feeding rates in *Culicoides*. These methodological advances were transferred to Senegal and applied to *C. oxystoma,* an important Afrotropical vector species. Importantly, these methodological advances in feeding methods allowed crucial vector competence assessments, standardised across countries, which can be used in disease transmission studies, bionomics and genomics experiments, thereby comprising a vital prerequisite for understanding of *Culicoides*-borne arboviruses.

To investigate existing prevalence of key *Culicoides*-borne arboviruses across our study region, the EDENext *Culicoides* group screened field collected individual *Culicoides* from virus epidemic areas. In Denmark, 200 pools of *Culicoides* (comprising 10 to 50 individuals) were screened for BTV and 300 for SBV in 2012, demonstrating high prevalence between July to September across the whole country (Rasmussen et al. 2014). Similarly, in France pools of 50 *Culicoides* were screened for SBV across the national entomological surveillance network in 2011; and in Senegal seroprevalance was assessed for nine serotypes of African Horse Sickness (AHSV), demonstrating seroprevalance of 65% for AHSV-9 and 60% for AHSV-2 based on serosurveys of donkeys in 2008 (Fall et al. 2015).

Key life history parameters were also empirically assessed for *Culicoides* vector species. Parallel studies in France, Spain and the UK investigated how fecundity, oogeneis and survival within vector species varied in relation to environmental parameters. Understanding how these life history traits vary by species and environment is a key component to building population and epidemiological models capable of predicting how orbiviruses may spread across landscapes in diverse geographic regions.

Night trapping in Denmark.
© Carsten Kirkeby, Danish Technical University

The *Culicoides* group also combined *Culicoides* surveillance data from the UK and Spain to develop two modelling frameworks for predicting the maximum abundance and seasonal abundance of five key *Culicoides* vector taxa (*Culicoides obsoletus* complex*, C. pulicaris, C. imicola, C. impunctatus, C. newsteadii*) for sites across Western Europe. The first method estimated the effect of environmental covariates (wild and domestic host densities, meteorological variables and land cover) on the maximum abundance of *Culicoides* at each site. This modelling framework was used to generate predictions at new sites across Western Europe falling into similar environmental spaces as those used to train the model. The second method estimated the seasonal abundance of *Culicoides* (*C. obsoletus* complex*, C. pulicaris, C. imicola,*

C. impunctatus, C. newsteadii) using the surveillance datasets from the UK and Spain containing weekly trapping data from the UK and Spain for approximately 400 sites spanning multiple years from 2005 to 2010. This modelling methodology was used to generate predictions of seasonal activity at new locations across Western Europe, properly accounting for uncertainty in model fitting and parameter estimates using Bayesian methods. In western Senegal, the seasonal dynamics of *Culicoides* vector species were empirically assessed and modelled. The temporal dynamics of the four dominant species, including *C. imicola* and

C. oxystoma, were analysed over 12 months (July 2011-July 2012) using horse-baited and light traps. A cross-sectional study was also conducted with the national veterinary services in Senegal to model the spatial distribution of key vector species across 108 sites and 36 districts (Diarra et al. 2014).

In the UK, the EDENext *Culicoides* group used five years of *Culicoides* surveillance data from national surveillance activities to assess how the phenology of individual species varied geographically and seasonally (Searle et al. 2014). This work demonstrated significant inter-specific differences in *Culicoides* adult phenology, the most notable of which related to the seasonal activity of one species, *Culicoides scoticus,* being approximately eight weeks shorter than that of *Culicoides obsoletus*. The work also demonstrated important species-specific differences in the length of the seasonal vector-free period (SVFP), which were related to host density and local variation in landscape habitat. There was evidence that the current treatment of *Avaritia Culicoides* as a single group in surveillance work inhibits understanding of environmentally-driven spatial variation in species phenology and hinders the development of models for predicting the SVFP from environmental factors.

Importantly, the study demonstrated that active surveillance of haematophagous female *Culicoides* vector populations cannot currently be replaced using remote environmental models of abundance. This failure was most likely related to the diverse ecology of species conflated within this taxonomic grouping. It was found that the timing of the end of the season was the most difficult to forecast, and should perhaps be treated with more caution by policy makers than the beginning of the season, because it varies widely between species, years and locations in response to environmental heterogeneity. To rectify this concern, the study recommended that intensive trapping should take place across a range of climatic zones with species-level identification of *Culicoides* females wherever feasible to facilitate more accurate detection and understanding of the start of the vector-free period in temperate zones.

SPATIO-TEMPORAL DYNAMICS OF VBD SPREAD AND CONTROL

Within EDENext, several group members studied the effect of direct control methods on *Culicoides*. Most of the common insecticidal products authorised in the EU for use in domestic animals are based on pyrethroids. The products are used topically as pour-on solutions, which provide efficacy for at least 4-8 weeks. Other insecticides are used for dipping or spraying. To date, few attempts have been conducted to apply chemical control of *Culicoides* spp. in an area-wide approach and there is currently no veterinary product on the European market authorised for the control of *Culicoides*.

Within EDENext, we conducted coordinated research at laboratory and field level for developing standardised control methods for *Culicoides*. The main aim of this work has been to provide an evidence base for the development of control practices that will reduce the rate of biting by *Culicoides* on animals. As a result of this work, our group has provided data on resistance to insecticides, such as permethrin and deltamethrin, for *C. obsoletus* and *C. imicola* from France, Spain, Senegal and South Africa (Venail et al. 2011, 2015a, b, c). Field evaluation of the efficacy of the ZeroFly® mosquito net (a commercial deltamethrine-impregnated net) was conducted in Senegal. This experiment demonstrated that the number of *Culicoides* captured (all species combined) was not significantly different between control and treatment nets showing that the impregnated net had no significant effect on the capture, instantaneous and delayed mortality rates of *Culicoides*. However, results did vary by *Culicoides* species, with larger bodied species suffering a greater negative impact than smaller species.

The group also assessed the impact of vector control using a UK data-driven *Culicoides* mechanistic population model to explore the impact of vector control on seasonal vector abundance. This mechanistic simulation population model for *Culicoides obsoletus* explicitly links environmental variation with life-history processes, such as development time, fecundity and mortality, and predicts the seasonal abundance of adults. Mechanistic vector dynamics are important as they affect disease seasonality and are affected by environmental variability. Building upon this model framework, the group investigated the efficiency of alternative timing and intensity of vector control; focusing on a control strategy that targets host-seeking adults that come into contact with insecticide, akin to the insecticide-treated nets used for the protection of sheep and cattle and evaluated by the CBD group in Del Rio et al. (2014).

This work demonstrated that for significantly high control efficiencies (a combination of mortality rate of insects and the proportion of the population that contact the treated hosts), we may eradicate the *Culicoides* population. However, for low control efficiencies, there is only a small reduction of the average annual *Culicoides* abundance. Importantly, for intermediate control efficiencies the average annual *Culicoides* abundance was markedly reduced but the peak autumn abundance was greatly increased. This was because pre-adults were released from density-dependent competition due to the decrease in population in the spring peak. As a consequence, although the control strategy had a desirable effect on the whole, the increase in peak autumn abundance may have dramatic consequences for the reproductive value for a disease (R_0, the number of new disease cases generated by one case) in this warmer period and the prevalence and seasonal persistence of midge-borne diseases.

This work also considered the seasonal timing of vector control. Here, the insecticide was present at a constant level for only a limited time in the year (50 days) and the starting day in the year at which the control window started was varied. When the control window was centred over the spring abundance peak (approximately 100th starting day), the spring peak was reduced. However, the knock-on effect was a markedly increased peak in autumn abundance. Furthermore, the mean abundance throughout the year also increased. This was in contrast to windows centred over the autumn peak, which markedly reduced the midge population in the control year but also reduced the subsequent spring population, because fewer individuals entered diapause.

In summary, vector control strategies with differing efficiencies and timing may either successfully reduce the vector population, or deleteriously increase the vector population. Control efforts should concentrate on reducing the autumn peak, as this will reduce the midge population at the peak, as well as on average through the year and in subsequent years. However, the precise timing is crucial for effective control, especially if the strategy is used year upon year, since late autumn control may increase subsequent late summer populations. Key knowledge gaps in predicting the outcome of vector control include the proportion of the adult population visiting the treated hosts in different ecological settings, the rate of decay of impact of nets or treatments on mortality as well as diapause triggers and levels of density dependent competition.

In other work, our group also considered the design of optimal prevention and control strategies for *Culicoides*-borne diseases. The independent or combined impact of vaccination and vector control on the reproductive value for a disease (R_0) will vary seasonally, geographically and between years with different meteorological conditions. EU Member States are presently not able to determine the necessary protective levels of vaccination cover, vector control efforts or movement restrictions based on objective calculations of R_0 or derived indicators for each country or ecosystem. Calculations of R_0 for within- and between-herd spread will allow stakeholders and Member States to adjust the intensity of preventive strategies as well as outbreak control measures to the predicted level of transmission in each country or ecosystem at various meteorological conditions.

Within EDENext, we have developed statistical and mechanistic simulation spread models simulating virus transmission within herds and between herds to facilitate the development of optimal prevention and control strategies. We developed a set of representative outbreak scenarios for various geographical areas, seasons and meteorology including average weather conditions and 90% extremes. This set of outbreak scenarios was used to run different spread models and formed the basis of the model comparison. We then identified spatial and temporal levels of vaccination and vector control that were necessary to keep R_0 below one, thereby preventing spread of the disease, which have been presented as guidelines for control of *Culicoides*-borne diseases in Europe.

Our group also assessed the effectiveness of alternative vaccination strategies on the potential spread of the Schmallenberg virus (SBV) at the landscape scale in Scotland. Here a stochastic mathematical model of SBV spread was used to identify the optimal method for deployment of a vaccine, under different regimes of current and changing temperatures, and in scenarios where *Culicoides* show a marked or no preference for feeding on cattle over sheep. Targeting strategies for vaccination included ad hoc strategies, where farmers could voluntarily take up vaccination, versus tactical strategies such as vaccination of only cattle, only breeding replacement stock, or only in the south of the country to break the transmission chain and reduce overall disease spread (Bessell et al. 2014). This analysis demonstrated that vaccine impact was optimised by targeting high risk areas in the south of Scotland, or by vaccinating only cattle. Importantly, given the recent rise in average temperatures experienced in the UK, the results demonstrated that at higher than average temperatures, and hence increased transmission potential, the relative impact of vaccination was considerably enhanced. Vaccine impact was also enhanced if vectors fed preferentially on cattle. These findings are of considerable importance when planning control strategies for SBV and also have important implications for management of other arboviruses such as bluetongue virus. Critically, environmental determinants and feeding preferences should be researched further to inform development of effective control strategies.
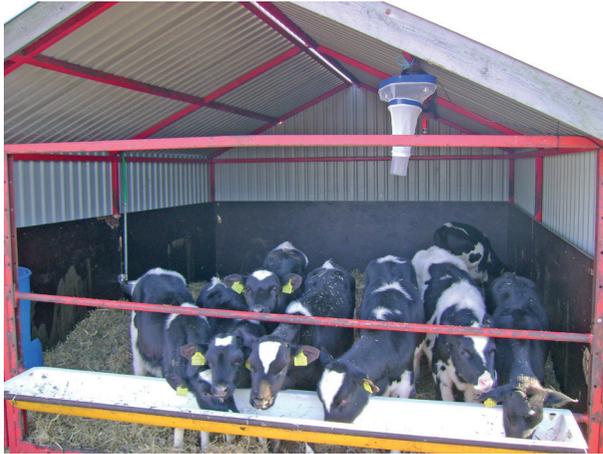
Trapping in Denmark. © René B Bødker, Danish Technical University

In other group work, we developed a spread model (Græsnøll et al. 2014) and used it to compare different preventive vaccination strategies for bluetongue virus (BTV) in Denmark, determining that the most cost-effective vaccination strategy was to vaccinate cattle on pastures (Græsbøll et al. 2014). The group also undertook an intensive study in Denmark to quantify dispersal of vectors between farms (Kirkeby et al. 2013), a key parameter in *Culicoides*-borne disease spread models. In France, Pioz et al. (2014) investigated the effect of vaccination on the propagation of bluetongue virus based on data from the 2008 bluetongue virus serotype 1 of southwest France. The average estimated velocity across the country, despite restrictions on animal movements, was 5.4km/day, which is very similar to the velocity of spread of the bluetongue virus serotype 8 epizootic in France. In comparison to municipalities with no vaccine coverage, the velocity of BTV-1 spread decreased by 1.7km/day in vaccinated municipalities. Our findings emphasise the importance of vaccination in limiting disease spread across natural landscapes. Environmental factors, specifically those related to *Culicoides* abundance and activity, were found to be good predictors of the velocity of BTV-1 spread, indicating that these variables need to be adequately accounted for when evaluating the role of vaccination on bluetongue spread.

The CBD group also collaborated with the EMIDA VICE project to model the monthly mean abundance of *Culicoides* vectors across Western Europe. *Culicoides* surveillance data from Norway, Sweden, Denmark, Germany, Poland, Austria, Switzerland, France and Spain were used to generate the monthly outputs. These predictions were then used to generate monthly Europe-wide R_0 prediction. These outputs showed R_0 values exceeding '1' for most of Europe for several months a year, suggesting that prevention and control of transmission can only be achieved when peak transmission intensity is reduced by more than 90%. As such, reductions in vector abundance or vector contact in the magnitude of 50% will only have limited impact in most areas during the peak transmission period in Europe. In

REFERENCES

Bessell et al. (2014). Impact of temperature, feeding preference and vaccination on Schmallenberg virus transmission in Scotland. *Scientific Reports* 4: 5746.

Del Río R, Barceló C, Lucientes J, Miranda MA (2014). Detrimental effect of cypermethrin treated nets on *Culicoides* populations (Diptera; Ceratopogonidae) and non-targeted fauna in livestock farms. *Veterinary Parasitology* 199: 230–234.

Diarra M, Fall M, Fall AG, Diop A, Seck MT, Garros C, Balenghien T, Allène X, Rakotoarivony I, Lancelot R, Mall I, Bakhoum MT, Dosum AM, Ndao M, Bouyer J and Guis H (2014). Seasonal dynamics of *Culicoides* (Diptera: Ceratopogonidae) biting midges, potential vectors of African horse sickness and bluetongue viruses in the Niayes area of Senegal. *Parasites & Vectors* 2014, 7:147 doi:10.1186/1756-3305-7-147.

Fall M, Diarra M, Fall AG, Balenghien T, Seck MT, Bouyer J, Garros C, Gimonneau G, Allène X, Mall I, Delécolle J-C, Rakotoarivony I, Bakhoum MT, Dusom AM, Ndao M, Konaté L, Faye O and Baldet T (2015). *Culicoides* (Diptera: Ceratopogonidae) midges, the vectors of African horse sickness virus – a host/vector contact study in the Niayes area of Senegal. *Parasites & Vectors* 8:39.

Græsbøll K, Enoe C, Bødker R, Christiansen LE (2014). Optimal vaccination strategies against vector-borne diseases. *Spatial and Spatio-temporal Epidemiology* 11, 153-62.

Kirkeby C, Bødker R, Stockmarr A, Lind P, Heegaard PMH (2013). Quantifying Dispersal of European *Culicoides* (Diptera: Ceratopogonidae) Vectors between Farms Using a Novel Mark-Release-Recapture Technique. *PLoS One*. Doi: 10.1371/journal.pone.0061269.

Pioz M, Guis H, Pleydell D, Gay E, Calavas D, Durand B, Ducrot C and Lancelot R (2014). Did Vaccination Slow the Spread of Bluetongue in France? *PLoS One*. 2014; 9(1): e85444. Doi: 10.1371/journal.pone.0085444.

Purse BV, Carpenter S, Venter GJ, Bellis G and Mullens BA (2015). Bionomics of Temperate and Tropical *Culicoides* Midges: Knowledge Gaps and Consequences for Transmission of Culicoides-Borne Viruses. *Annual Review of Entomology* 60:20.1–20.20.

Rasmussen LD, Kirkeby C, Bødker R, Kristensen B, Rasmussen TB, Belsham GJ and Bøtner A (2014). Rapid spread of Schmallenberg virus-infected biting midges (*Culicoides* spp.) across Denmark in 2012. *Transboundary Emerging Diseases* 61(1):12-6. Doi: 10.1111/tbed.12189.

conclusion, the reduction in transmission intensity needed for prevention and control of *Culicoides*-borne bluetongue virus and Schmallenberg virus in European ruminants is likely to be so high that for all practical purposes vaccination is currently the only practical solution.

CONCLUSIONS

Research on *Culicoides* lags behind that on most other vector groups, primarily because of their minute size, the lack of colonies of primary vector species and, until recently, the relatively low impact of *Culicoides*-borne animal disease agents in developed countries.

Our work has identified techniques that can reduce *Culicoides* survival and their attack rates on livestock, however these techniques work best for species whose ecology has been well characterised. Moreover, because many regions have multiple potential vectors with widely varying ecologies and diverse habitats, many of these techniques will only be effective locally and are unlikely to control widespread outbreaks.

Long-term *Culicoides* surveillance using trapping is currently restricted to BTV-epidemic regions of Western Europe. This surveillance can track the seasonal abundance and distribution of *Culicoides* vectors aiding in the demarcation of seasonally vector free areas for relaxation of animal trade and movement restrictions. However, the current treatment of the *Avaritia Culicoides* as a single group inhibits understanding of environmentally driven spatial variation in species phenology and hinders the development of models for predicting seasonally vector free periods from environmental factors. As such, we recommend that *Culicoides* surveillance methods should be adapted to focus on concentrated assessments of species-specific abundance during the start and end of seasonal activity in temperate regions to facilitate refinement of ruminant movement restrictions, thereby reducing the impact of *Culicoides*-borne arboviruses.

There remain key knowledge gaps in relation to vectoral capacity parameters for *Culicoides*. These parameters have complex interactions that ultimately determine how diseases are spread. Improved mathematical models must be developed with empirically derived parameters from laboratory and natural environments, and confronted with empirical data from disease outbreaks to create robust and accurate models for predicting virus transmission and spread.

Finally, we know that the landscape context alters disease transmission and spread, particularly through its influence on the abundance and interactions of *Culicoides* vectors with wild and domestic hosts. For instance, short-range upwind flight of *Culicoides* has been shown to be critical in explaining epidemic spread, however an understanding of how short-range midge movements are modulated by landscape and topography is currently lacking. Similarly, recent work has implicated wild ruminant hosts as playing an important role in disease spread, however little is known about how the abundance of wild ruminants affects the behaviour and distribution of vectors.

20/07/2015   16:31

Searle et. al. 2014. Drivers of *Culicoides* phenology: how important is species-specific variation in determining disease policy? *PLoS One*, November 11, 2014. Doi: 10.137.

Venail R, Mathieu B, Setier-Rio ML, Borba C, Alexandre M, Viudes G, Garros C, Allene X, Carpenter S, Baldet T (2011). Laboratory and Field-Based Tests of Deltamethrin Insecticides Against Adult *Culicoides* Biting Midges. *Journal of Medical Entomology*, Volume: 48, Issue: 2, Pages: 351-357. Doi: 10.1603/ME10178.

Venail R, Fall M, del Río R, Talavera S, Labuschagne K, Balenghien T, Garros C, Côtre-Sossah C, Miranda M, Pagès N, Venter G, Carpenter S and Baldet T (2015a). Insecticide susceptibility of *Culicoides* biting midges (Diptera: Ceratopogonidae): a multicentric study using a modified WHO standard assay, *Parasites & Vectors*, in review.

Venail R, Lhoir J, Rakotoarivony I, Allène X, Setier-Rio ML, Scheid B, Gardes L, Gosset A, Lancelot R, Gimonneau G, Garros C, Balenghien T, Carpenter S and Baldet T (2015b). Insecticide susceptibility of *Culicoides* biting midges (Diptera: Ceratopogonidae) in France, *PLoS One*, in review.

Venail R, Balenghien T, Carpenter S and Baldet T (2015c). Assessment of the efficiency of insecticide formulations against *Culicoides* biting midges (Diptera: Ceratopogonidae) using a novel approach. *Vet Parasitology*, in review.