Advances in control techniques for Culicoides *and future prospects*

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Summary
In most instances, vaccination is accepted to be the most effective method of preventing *Culicoides*-borne arbovirus transmission, as it has proven to be successful in large-scale campaigns. Under certain scenarios, however, vaccines require time to be developed and deployed or are not used due to financial, logistical or trade constraints. In the absence of vaccines, animal movement restrictions and techniques to reduce either the number of *Culicoides* biting livestock or their subsequent survival are the only responses available to prevent or reduce arbovirus transmission and spread. This review evaluates the progress made during the past 10 years in the development of *Culicoides* control techniques for this purpose and assesses their potential impact in reducing arbovirus transmission. In addition, the future prospects and challenges facing *Culicoides* control are examined and suggestions are made as to research directions and opportunities.

Progressi nelle tecniche di controllo di Culicoides *e prospettive future*

Parole chiave
Arbovirus,
Insetticida,
Integrated vector management,
Repellente,
Virus della Bluetongue.

Riassunto
La vaccinazione è considerata la misura più efficace per prevenire e controllare le malattie trasmesse da vettori così come dimostrato dal successo delle campagna vaccinali su larga scala. Tuttavia in alcuni casi lo sviluppo e l'uso dei vaccini può richiedere molto tempo o essere ostacolato da difficoltà finanziarie, logistiche o da regole commerciali. In assenza di vaccini, le restrizioni del movimento animale e le tecniche per ridurre sia il numero di *Culicoides* attivi sia la loro sopravvivenza sono gli unici metodi a disposizione per prevenire o ridurre la trasmissione e la propagazione di arbovirus. Questo articolo valuta il progresso fatto negli ultimi 10 anni delle tecniche per il controllo di *Culicoides* e il loro potenziale impatto nel ridurre la trasmissione da arbovirus. L'articolo esamina anche le prospettive future per migliorare le misure di controllo di *Culicoides* e suggerisce nuovi possibili indirizzi di ricerca.

Introduction

Biting midges of the genus *Culicoides* Latreille (Diptera: Ceratopogonidae) are small haematophagous insects, which biologically transmit arboviruses of veterinary and medical importance (Purse *et al.* 2015). Over the last decade, dramatic shifts in the distribution and economic importance of *Culicoides*-borne arboviruses have occurred. In particular, Bluetongue virus (BTV) caused unprecedented economically damaging outbreaks of Bluetongue (BT) across Europe (Carpenter *et al.* 2009). Additionally, novel *Culicoides*-borne arboviruses have been discovered and have proved capable of causing economically important outbreaks (Hoffmann *et al.* 2012). In this review, we examine the advances that have been made in *Culicoides* control in the last decade, updating the information presented at the 3rd International Symposium on Bluetongue, Taormina, Italy[1], and previous reviews (Carpenter *et al.* 2008a, MacLachlan and Mayo 2013). In addition, we evaluate the likely impact of control measures on arbovirus transmission and the future prospects for development of these techniques.

Within this review, the term 'vector control' refers to attempts to reduce *Culicoides*-borne arbovirus transmission via reducing vector-host contact. The more general term '*Culicoides* control' will be used less specifically and includes efforts to reduce biting nuisance to livestock, equines and/or humans. To reduce arbovirus transmission, vector control needs to be capable of having a significant effect on the basic reproduction number (R_0) of the relevant disease. The R_0 of a disease is defined as the mean number of secondary cases arising from the introduction of a single infected individual to a susceptible population. An arbovirus (or other pathogen) is able to spread in a host population only if $R_0 > 1$ (MacDonald 1952). A reduction in the R_0 for vector-borne diseases can be achieved through reducing the probability of transmission of the pathogen from vector-to-host and/or from host-to-vector (Gubbins *et al.* 2008).

Guidelines for the control of *Culicoides* for the prevention of arbovirus transmission of veterinary importance are laid out in the Office International des Épizooties (OIE) Terrestrial Animal Health Code (OIE 2014b). In addition, measures specific to African horse sickness virus (AHSV) have also been added to the recently included chapter on the 'High health status horse subpopulation', which addresses the international movement of horses for competition and racing (OIE 2014a). These chapters, however, only make broad recommendations regarding control measures. Due to this, specific reviews on *Culicoides* control are often commissioned at a transnational level, such as those prepared for the European Commission (EC) (European Food Safety Authority 2008), more specific guidance has been provided for some subject areas, such as the use of vector-protected establishments (European Commission 2012). In addition, there are a raft of national level risk assessment exercises that contain practical advice for farmers and veterinarians wishing to implement such techniques (Defra 2009, Defra 2012, Ministerio de Agricultura 2013).

Why do we need vector control when we have vaccination?

Vector control techniques are generally applied under two scenarios: (i) firstly, safe and efficacious vaccines for circulating arboviruses are not available. In this scenario, movement restrictions and vector control become the only available methods to reduce transmission and spread. In epidemic regions, vaccines to exotic strains and species of arbovirus may not be available as the new vaccines require time to developed, to be licensed and deployed as was the case for the control of BTV serotype 8 outbreak in Northern Europe (Carpenter *et al.* 2009). This scenario may also arise in endemic areas of circulation, like in the case of Oropouche virus (OROV) in Brazil, which causes epidemics of febrile illness in human populations (Carpenter *et al.* 2013). Alternatively, the concerned arbovirus may not be considered sufficiently pathogenic hence there will be limited commercial interest in vaccine development, but protection may still be required under certain scenarios such as export of livestock. This was the case observed during the initial discovery of several strains of BTV in Northern Europe which were subsequently declared as vaccine incidents (BTV-6, BTV-11) or as novel serotypes with apparently restricted host ranges and pathogenicity (BTV-25). (ii) In the second scenario, safe and efficacious vaccines are available, but are not economically viable to deploy. This scenario may result from trade issues regarding the use of vaccines, or more simply from farmers not being able to afford to purchase them. In subsistence, farming in particular the use of vector control plays a major role and is often based around traditional methods that are inexpensive to implement but entirely unquantified in effect.

Methods currently available for vector control can be divided into 4 broad categories (i) mechanical, (ii) chemical, (iii) biological, and (iv) genetic. In the following sections we discuss the advances in each of these types of control measures over the last decade, how they have been integrated into general programmes of control, and highlight the challenges facing their development and deployment in the field.

Mechanical control methods

Habitat modification

Habitat modification methods involve either the removal or alteration of habitats utilised by *Culicoides*, in order to reduce or eradicate larval populations. *Culicoides* larvae are generally semi-aquatic and have limited ability to survive periods of desiccation. Hence, targeting the development sites is more straightforward within relatively dry climates (Purse *et al.* 2015). Localised habitats utilised by *Culicoides* can include organically enriched soil kept wet by leaking taps and overflowing water troughs (e.g.

[1] MacLachlan N.J. & Pearson J.E. (eds). 2004. Bluetongue Proceedings. Proceedings of the 3rd International Symposium on Bluetongue, Taormina, Sicily, Italy, 2004. Part I. *Vet Ital*, **40** (3), 1-395.

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Culicoides imicola Kieffer, 1913) or cattle dung (e.g. *Culicoides brevitarsis* Kieffer, 1917). The majority of *Culicoides* are, however, habitat generalists utilising a diverse range of substrates and this results in limitations in the feasibility of attempting large-scale modifications in terms of economics and environmental impact. Difficulties also arise in regions where multiple vector species are active, for this broadens the range of habitats that require treatment.

Culicoides that directly or indirectly utilise animal dung as a larval development substrate are potentially the most straightforward target for elimination. For these species, it should be possible to employ simple and economically viable modifications to husbandry practices that reduce larval survival. Some evidence that this approach may be effective are provided by studies of the North American BTV vector *Culicoides sonorensis* Wirth and Jones, 1957. The larval ecology of *C. sonorensis* in dairy wastewater ponds was systematically characterised in a detailed series of studies based in California, which enabled specific targeting of habitats (Mullens and Rodriguez 1985, Mullens and Lii 1987, Mullens and Rodriguez 1988, Mullens 1989) and was later reviewed as part of an integrated control strategy (Mullens 1992). More recent studies have manipulated these habitats via removal of lagoons suitable for *C. sonorensis* development and examined the impact on adult populations (Mayo *et al.* 2014). However, this study demonstrated that the process of habitat elimination had a negligible impact on adult populations and subsequent on BTV transmission (Mayo *et al.* 2014). It was hypothesised that many more larval habitats were being utilised by *C. sonorensis* than previously thought and that dispersal of adults between farms may have been underestimated (Mayo *et al.* 2014).

In a similar study conducted in the United Kingdom, covering of muck heaps containing ruminant dung was also found to have no significant impact on *Culicoides* adult populations (Harrup *et al.* 2014), despite a large proportion of species using this habitat for larval development (Harrup *et al.* 2013). Studies in Germany also found that mechanical disruption of cow pats had no significant effect on the total number or species composition of *Culicoides* emerging from these larval development sites (Lühken *et al.* 2014). While the studies in California were focussed on a single species responsible for the vast majority of BTV transmission, the European epidemiological situation is highly complex, involving a wide-range of potential vectors with widely varying larval habitats. In addition, due to a lack of endemic arbovirus circulation in the study areas of Harrup and colleagues (Harrup *et al.* 2013) and Lühken and colleagues (Lühken *et al.* 2014), the UK and Germany respectively, it was not possible to infer potential impact of either technique on transmission in these areas.

To date, there has been no investigation into the impact of some of the more alternative uses of animal dung that exist and their impact on the local abundance of *Culicoides* resulting from changes in the availability or suitability of dung-based larval development habitats. For example, within the Indian subcontinent animal dung is utilised in rural areas both as a biomass fuel, a substitute for fossil fuels through the forming and subsequent burning of dung cakes (Figure 1a) (Mishra and Dikshit 2004), and for energy generation from methane capture from manure emissions via biogas digesters etc. (Tauseef *et al.* 2013) (Figure 1b). Interestingly, one of the few scenarios in which habitat modification may play a role in reducing arbovirus transmission is directly related to human activities. Local abundance of the Neotropical vector of OROV, *Culicoides paraensis* (Goeldi), 1905, is directly related to the availability of rotting banana stumps and cacao plant waste, which tends to accumulate in peri-urban areas contiguous with fruit production (Hoch *et al.* 1986, Mercer *et al.* 2003). Removal of this waste has been demonstrated to reduce the numbers of *C. paraensis* and may provide a straightforward intervention,

Figure 1. *Alternative manure management practices (**A**) preparation of dung cakes ready for use as a biomass fuel (Haryana, India), (**B**) biogas reactor (Tamil Nadu, India).*

if an economic value for the by-products of this removal process can be found it would negate the costs associated with the control measure and go some way to the implementation of a cost-neutral vector control system during major epidemics of OROV (Vasconcelos *et al.* 2009).

In addition to the uncertainty surrounding their impact on arbovirus transmission, the lack of data concerning habitat usage by juvenile *Culicoides* is also a major challenge for implementing habitat modification techniques, with only 13% and 17% of *Culicoides* species known as larvae or pupae, respectively (Borkent 2015). While these proportions are much greater for species of *Culicoides* that have been implicated as vectors of arboviruses, even the most carefully studied species, such as for example *C. sonorensis*, have been demonstrated in the past decade to be utilising previously unidentified habitats that are significant in transmission. This would suggest that habitat modification can only be recommended as a form of mitigation within wider integrated programmes of control.

Chemical control methods

Topical repellents

Since the last major review of techniques to interrupt arbovirus transmission, the use of topical repellents remains almost entirely restricted to equine hosts experiencing insect bite hypersensitivity and human hosts (Carpenter *et al.* 2008b). Topical repellents are frequently used by horse owners. However, there is little quantitative evidence of their efficiency in reducing *Culicoides* biting rates or the occurrence of equine seasonal recurrent dermatitis, and there is no evidence of their impact on reducing the incidence of equine arbovirus transmission. Lincoln and colleagues (Lincoln *et al.* 2015) found that a 9 mg/ml permethrin and 20 mg/ml *N,N*-diethyl-3-methylbenzamide (DEET) formulation containing piperonyl butoxide as a synergist (Flymax, Audevard Ltd, Clichy, France) applied to the neck, abdomen, flank, back, and croup of horses (0.6 ml per side per location) not to significantly affect the number of *Culicoides* collected in a ultraviolet (UV) light-suction trap located in the stable of treated in comparison to untreated horse.

This study, however, did not attempt to quantify vector-host contact rates beyond that inferred from light-suction trap data. Baker and colleagues (Baker *et al.* 2015) did find a potential anti-feedant effect of a commercially available 1% *w/w* Citriodiol formulation (NAF OFF Extra Effect, Greencoat Ltd t/a Natural Animal Feeds, Monmouth, UK) in laboratory assays using colony-reared *Culicoides nubeculosus* (Meigen), 1830, which is worthy of further investigation. No specific formulations of topical repellents for livestock have been developed or tested in response to the major BTV outbreaks in Europe due to concerns regarding withdrawal times for slaughter and the requirement for regular re application, which is not logistically feasible or economically viable in an agricultural setting.

Studies of repellent use on humans have progressed to find that, although topical applications of DEET remains the gold standard repellent against *Culicoides*, other active ingredients and formulations can be relatively effective against *Culicoides* (Carpenter *et al.* 2005). A novel approach involving the identification of volatiles that are naturally emanated from humans identified 6-methyl-5-hepten-2-one (6MHO) and geranylacetone [(*E*)-6,10-dimethylundeca-5,9-dien-2-one] as repellents for *Culicoides impunctatus* Goetghebuer, 1920 in laboratory-studies (Logan *et al.* 2009). This study shows that a 1:1 combination of 6MHO and geranylacetone produced a significant ($P \leq 0.001$) repellent effect (87%, 77.4% and 74.2% repellency) at 0, 1, and 2 hours post-application in the field, as measured by human landing catch studies. However, it is worth noticing that the effectiveness of the repellent effect rapidly declined from 74.2% to 31.6%, at 2 to 3 hours post-application and the repellent effect was not significantly ($P \geq 0.05$) better than that of DEET over the 3-hour period (Logan *et al.* 2009). This exciting area of research has not yet been applied to livestock species, but in the future it may yield alternatives to synthetic repellents (Logan *et al.* 2010).

Additionally, during the past decade research on repellents based on essential oils has also advanced, however these compounds suffer the same limitations as their synthetic alternatives for reducing biting rates on livestock with regard to limitations in the duration of their activity. In Northern Spain, a panel of 23 repellents was tested using a Y-tube olfactometer, which included 12 synthetic and 11 botanical active ingredients (González *et al.* 2014). The initial screening identified DEET, a mixture of fatty acids – (octanoic, nonanoic, decanoic) and a mix of lemon eucalyptus oil [*p*-menthane-3,8-diol (PMD) extracted from *Corymbia citriodora* (Hook) Hill and Johnson = *Eucalyptus maculata* var. *citriodora*] – as the one with the greatest repellent activity. There is, however, still much debate over the validity of studies monitoring *Culicoides* behavioural responses in Y-tube experiments with regard to how relevant these responses are to behaviours expressed in a field setting. Under field conditions, the mixture of fatty acids exhibited a significant ($P \leq 0.001$) repellence effect, equivalent to that provided by DEET, against the Obsoletus complex [*Culicoides obsoletus* (Meigen), 1818 and *Culicoides scoticus* Downes and

Kettle, 1952]. This was concordant with an earlier field study in South Africa in populations dominated by *C. imicola* (Venter *et al.* 2011). Interpretation of the repellent effects in these studies was hampered, however, by the fact that repellence was estimated in field trials utilising collections at UV light traps as a proxy for human/animal biting rates, significantly underestimating the complexity of interactions in repellent use.

In a study based in Australia, it was found that oil from Melaleuca ericifolia Smith at 5% *w/v* in 3 formulations significantly ($P \leq 0.001$) reduced landing rates on humans by *Culicoides ornatus* Taylor, 1913 and by *Culicoides immaculatus* Lee and Reye, 1953 for up to 3 hours post-application (Greive *et al.* 2010). This reduction was equivalent to the synthetic commercial repellent Off! Skintastic [SC Johnson, Wax, Lane Cove, NSW, Australia; 6.98% *w/v* DEET and 2.79 g/l di-*N*-propyl-isocinchomeronate (MGK-326)]. The commercial repellent lotion 'NO MAS' (Del Cielo, Salt Spring Island, BC, Canada) (16% PMD extracted from *Corymba citriodora*; and 2% lemongrass oil, extracted from *Cymbopogon citratus* Stapf and *Cymbopogon flexuosus* (Nees ex Steudel) Watson leaves), was also tested against *Culicoides pachymerus* Lutz, 1914 in Colombia using human landing catch tests (Santamaría *et al.* 2012). This repellent provided complete protection against biting of *C. pachymerus* up to 4 hours, and 99.5% protection up to 5 hours post-treatment (Santamaría *et al.* 2012). This topical repellent is of particular interest as it is currently available at low cost in both developed and developing countries with an estimated cost of $0.03 US dollars per person per day (Del Cielo 2013[2]).

The development and testing of cost-effective or ideally cost-neutral control measures are of particular importance as the majority of the economic and health burden of vector-borne diseases still lies in developing countries. Within these regions, the resources to prevent and respond to outbreaks are often limited or highly dependent upon the financial means of the individual. Interest has been shown in the use of products or parts of the Neem tree [*Azadirachta indica* A. Juss (Meliaceae)]. The burning of Neem leaves in livestock accommodation of subsistence farmers in rural India is a common practice in order to repel biting *Culicoides* within traditional livestock housing (Figure 2) and to limit BTV transmission and biting nuisance from other Diptera. There is, however, no quantitative data to support the use of this practice or the use of any other spatial repellents to reduce *Culicoides*-host contact rates. Nonetheless, 2% Neem-based topical formulations have been shown to exert both an anti-landing and anti-feeding effect on colony-reared *C. nubeculosus* and field-collected *C. impunctatus* (Blackwell *et al.* 2004). These formulations showed limited repellent properties in Y-tube olfactometer studies against Obsoletus complex *Culicoides* (González *et al.* 2014). Further investigations on their potential use as either topical or spatial repellents against *Culicoides* are required before their wide-spread use can be recommended.

Figure 2. *Traditional livestock housing within subsistence farming system in Tamil Nadu, Southern India.*

Topical adulticides

In contrast to the development and testing of topical repellents, the majority of research over the last decade on *Culicoides* control has focused on topical adulticides applied to livestock rather than equires, which were already widely use in the veterinary market and had a high degree of logistical convenience rather than novel compounds. The use of adulticides has 2 potential impacts on arbovirus transmission: (i) it can reduce successful blood-feeding rates of *Culicoides* through contact irritation, and (ii) it can lower the survival rates of *Culicoides*, which have been exposed. Within Europe, research has focussed primarily on the use of synthetic pyrethroid (SP) active ingredients in formulations, in part due to the widespread and on-going withdrawal of organophosphate (OP) active ingredients, due to the impact of their use on public health (Kamanyire and Karalliedde 2004). In laboratory-based studies, colony-reared *C. nubeculosus* (Venail *et al.* 2011, Venail *et al.* 2015) and field-collected *C. obsoletus* (Venail *et al.* 2011, Venail *et al.* 2015), and *C. imicola* (Del Río *et al.* 2014b, Venail *et al.* 2011, Venail *et al.* 2015) have all been found to be highly susceptible to SPs using modified World Health Organisation (WHO) susceptibility tests. Due to their availability in large numbers of known age and physiological state colonised lines of *Culicoides* (Nayduch *et al.* 2014) have become the model species for adulticide

[2] http://www.delcielo.net/characteristics/.

studies. However, due to the difficulties in colonising many of the key *Culicoides*-borne arbovirus vectors, e.g. *C. imicola* (Veronesi *et al.* 2009), there have been increased efforts to use field studies to investigate the effectiveness of insecticidal treatments.

The majority of studies conducted in the field have exposed *Culicoides* to hair or wool clippings taken from livestock species at known times after insecticide treatment. Several difficulties exist in translating the results of these studies to potential impact in the field and the level of standardisation achieved is generally poor. Specific issues are how, and for how long, *Culicoides* are exposed to the clippings, how long-term survival is measured and from what body area the hair or wool samples are initially taken. In addition, the low survival rate of field-collected *Culicoides* in the laboratory is a major issue and has led to reliance upon *C. nubeculosus* colony lines, which are known to be more susceptible to deltamethrin toxicity than field populations (Venail *et al.* 2011, Venail *et al.* 2015).

A commercially available formulation of deltamethrin (Butox® 7.5 Pour On: Intervet International B.V., Boxmeer, The Netherlands; 7.5% *w/v* deltamethrin) has been examined in a series of studies conducted in Germany (Mehlhorn *et al.* 2008, Schmahl *et al.* 2009 a, c) and France (Venail *et al.* 2011, Venail *et al.* 2015). The results from the first of these studies (Mehlhorn *et al.* 2008) are very difficult to interpret due to the anecdotal descriptions of experimental technique and a lack of clarity regarding sample sizes, quantitative mortality rates within control populations, breeds of animals used, and the quantity of hair or wool sampled. This was somewhat clarified by a very similar study that subsequently demonstrated that using a 2-minute feeding period, all exposed *Culicoides* died within 66 minutes, even when *Culicoides* were exposed to clippings taken 35 days following treatment (Schmahl *et al.* 2009a). This effect was only marginally reduced by exposure of sheep and cattle to tap water as a means of simulating rain (Schmahl *et al.* 2009c).

In similar, but more systematic trials colony-reared *C. nubeculosus* were exposed to fleece/hair clippings obtained from leg, belly, and back of sheep, cattle, and horses treated with a commercially available α-cypermethrin pour-on insecticide (Dysect Cattle Pour-On, Fort Dodge Animal Health, Southampton, UK) at 1.5% *w/v* α-cypermethrin 10 ml per cow; Dysect Sheep Pour On (Fort Dodge Animal Health, Southampton, UK) at 1.25% *w/v* α-cypermethrin 40 ml per sheep; Deosect Spray (Fort Dodge Animal Health, Southampton, UK), 5.0% *w/v* diluted to 0.1% *w/v* 500 ml per horse) (Papadopoulos *et al.* 2009, Papadopoulos *et al.* 2010). In this case, groups of 10 *C. nubeculosus* were allowed to walk over treated hair or wool within a petri dish environment for 3 minutes and then mortality was recorded at 1 hour post-treatment. Results demonstrated that treatment reliably killed close to 100% of *C. nubeculosus* for up to 21 days in cattle, 28 days in sheep, and 28 days on horses independent of where hair or wool was sampled from. In contrast, analysis of fleece clippings from sheep treated along the dorsal midline or flank with a 1.25% *w/v* high-*cis*-cypermethrin-based pour-on (Crovect, Elanco Animal Health, Basingstoke, UK) indicated that the formulation spreads poorly from its initial application site (Carpenter and Torr, unpublished data, Carpenter *et al.* 2013).

A field-based study later examined exposure by direct feeding of *C. nubeculosus* colony individuals on Arles Merino sheep sheared 15 days before being treated with Butox® 7.5 (Venail *et al.* 2011). An application of 10 ml was made from the head to tail along the back line, resulting in a theoretical coverage of ~71 mg/m^2 assuming equal spread across the sheep. Batches of *C. nubeculosus* were then fed on the inner thighs of sheep and then mortalities recorded at 1 and 24 hours post-exposure. Unlike the previous studies, the mortality rates recorded in this more realistic setting were disappointing with the duration of the lethal effect estimated to last just 10 days (Venail *et al.* 2011). Despite the fact that the colony *C. nubeculosus* used by Venail and colleagues (Venail *et al.* 2011) have previously been shown to be susceptible to the deltamethrin the active ingredient contained in Butox® 7.5. The poor performance of Butox® 7.5 during field trials was suggested to be due to the more realistic exposure of *Culicoides* to the insecticide and the limited spread of the active ingredient on the sheep, although differences in insecticide concentration on fleece were not quantified.

Direct-feeding or hair clipping trials have not been conducted for equines, however, Robin and colleagues (Robin *et al.* 2015) found an 'off-label' application of 10 ml 1% (*w/v*) deltamethrin (Coopers Spot On, Zoetis, London, UK) to the dorsal midline of horses produced no significant difference in the number of *Culicoides* or the number of blood-fed *Culicoides* collected in UV light-suction traps adjacent to treated horses in comparison to those located close to untreated horses. Coopers Spot On has previously been shown to cause high mortality rates in *C. nubeculosus* exposed to treated fleece in laboratory assays, but testing of fleece sample from treated sheep indicated that the formulation spreads poorly from its initial application site (Carpenter and Torr, unpublished data; Carpenter *et al.* 2013). Deltamethrin and other SP are generally considered primarily to be adulticides, however, the study design of Robin and colleagues (Robin *et al.* 2015) focuses on investigating the potential repellent effect of deltamethrin and does not attempt to investigate the impact that the deltamethrin may have on the survival rate of exposed *Culicoides*.

Research on permethrin-based insecticides has also been conducted in Europe. Schmahl and colleagues (Schmahl *et al.* 2009b) found that hair clippings from cattle treated with topical applications of Flypor® (Novartis Santé Animale, Huningue, France; 4% *w/v* permethrin, 40 ml per cow, 10 ml per sheep) caused mortality in field-collected *Culicoides* (species not stated) exposed to the treated hair clippings up to 35 days post-application. However, the authors for this study found that the time to 100% mortality following exposure to the treated hair varied significantly from between 45-55 minutes to 18 hours post-exposure. These results are comparable with the mortality time observed in deltamethrin studies performed in Germany (Mehlhorn *et al.* 2008, Schmahl *et al.* 2009 a, c).

In both the deltamethrin studies and the permethrin studies, a lack of clarity regarding sample sizes, quantitative mortality rates within control populations, breeds of animals used, and the quantity of hair or wool sampled limits the interpretability and scientific merit of these studies. A later study in the Netherlands using Tectonik (Virbac Animal Health, Bury St Edmunds, UK; 3.6% *w/v* permethrin applied at 0.1 ml/kg) resulted in an approximately 50% reduction in the number of *Culicoides* collected in a sheep-baited drop trap and a marginally reduced feeding rate in comparison to untreated sheep (Griffioen *et al.* 2011). A concern with the experimental design of this latter study was that, although breeds used are listed and efforts were made to standardise within-breed variation, it was not clear which breeds were used for the insecticidal trials. A second study, also conducted in the Netherlands, found the same permethrin treatment did not significantly ($P \geq 0.05$) reduce either the number of *Culicoides* collected or the feeding rate, when applied to horses (20 ml per horse applied along the dorsal midline) (de Raat *et al.* 2008).

A limited number of studies have investigated commercially available formulations of fenvalerate-based pour-on insecticides. Acadrex®60 (Novartis Santé Animale, Huningue, France; 6% *w/v* fenvalerate) and Arkofly® (Novartis Santé Animale, Huningue, France; 6% *w/v* fenvalerate applied diluted to 0.12% *w/v*) caused mortality in colony-reared *C. nubeculosus* exposed to fleece/hair clippings from treated sheep and cattle up to 28 days post-treatment (Schmahl *et al.* 2009b). Acadrex®60 and Arkofly® caused faster knockdown in *C. nubeculosus* as a result of exposure to treated sheep fleece in comparison to cattle hair, with Acadrex® more effective than Arkofly® when applied to sheep, but causing approximately equivalent mortality rates when applied to cattle. Without parallel assessments of seroconversion rates or anti-feedant effects it is difficult to evaluate how the time to mortality durations measured by Schmahl and colleagues (Schmahl *et al.* 2009b) and others may impact on transmission rates.

Impregnated ear tags

Insecticide-impregnated ear tags represent an attractive easy-to-use potentially long-lasting alternative to topical applications of repellents or insecticides. In North Germany the efficacy of placing 1 or 2 ear tags (Flectron® Flytags, Fort Dodge Animal health; 1,067 mg cypermethrin per ear tag) per animal in heifers and dairy cows was tested (Liebisch and Liebisch 2008). Following exposure to hair clipped from treated animals, 1 ear tag was found to provide a toxic effect to *Culicoides* for up to 14 days, while 2 tags provided a toxic effect for up to 21 days (Liebisch and Liebisch 2008). In a more integrated study, also based in Germany, the combined control measures of insecticide-treated ear tags (Auriplak Insecticidal Ear Tags, Battle, Hayward & Bower Ltd; 1,200 mg permethrin per tag) with topical applications of the insecticide Butox® 7.5 Pour-On (Intervet International B.V., The Netherlands; 7.5% *w/v* deltamethrin) were tested in a field scenario (Bauer *et al.* 2009). This combined treatment was not found to ($P \geq 0.05$) reduce significantly the total number of *Culicoides* in collections made using UV light traps or the number of engorged individuals as a proxy for vector-host contact.

Insecticide treated mesh and protective housing

The aim of vector-proofed accommodation is to reduce vector-host contact and therefore limit/prevent transmission of arboviruses. The degree of entry of *Culicoides* into stables has been shown to be proportional to the size of the entrance to the stable (Barnard 1997, Meiswinkel *et al.* 2000). Both in South Africa (Meiswinkel *et al.* 2000) and Switzerland (Lincoln *et al.* 2015), it was found that screening the windows and doors of stables dramatically reduces the number of *Culicoides* entering the stables. *Culicoides*, however, can easily pass through untreated insect screens, with mesh sizes > 1.6 mm^2. These screen have been found to reduce the entry rats of *Culicoides* by 56% (Porter 1959). Due to this, screens aimed at reducing *Culicoides* entry and exit from stables are usually treated with an insecticide with a strong residual effect (Defra 2009, World Animal Health Organisation 2014c).

Globally, OP treatments are often used on both metal and fabric screens and these have been reviewed without additional experimentation since

2008 (Carpenter *et al.* 2008a). Alternatives to the use of OPs include dedicated repellent compounds or SP insecticides, such as permethrin or deltamethrin, which are used frequently in personal protection mosquito nets globally (see Raghavendra *et al.* 2011 for review). Initial studies on anthropophilic *Culicoides* species (*Culicoides mississippiensis* Hoffman, 1926 *Culicoides furens* (Poey), 1853, and *Culicoides barbosai* Wirth and Blanton, 1956) indicated that lightweight polyester fine netting treated with either DEET or diisopentyl malate (DPM) at 1.25 mg/cm^2 could provide short-term area protection (4 and 5 days post-treatment respectively).

However, neither the DEET nor the DPM treatments completely prevent vector-host contact (Schreck and Kline 1983). The Coefficient of Protection from Intrusion (CPI) calculated as {[(A - B) x 100] / A}, where A is the number of *Culicoides* collected inside the untreated area and B is the number collected in the treated area, can be utilised as a measure of the effectiveness of an area protection method incorporating a correction for the local population density of the target vector (Schreck and Kline 1983). A CPI of 100% indicates that the method completely excludes the target vector, while a CPI of ≥ 80% was considered by Schreck and Kline (Schreck and Kline 1983) to indicate an effective control measure, a CPI of < 80% indicated a failure in protection. Schreck and Kline (Schreck and Kline 1983) found that netting treated with either DEET or DPM at 1.25 mg/cm^2 provide a CPI of 99.7 and 100.0% at 1 hour post-application. The CPI of the DEET treatment was ≥ 89% for 24 to 96 hours post-application, when no rain fell on the netting, rainfall resulted in a rapid loss of the protection conferred. While the CPI of the DPM treatment was ≥ 86% for 24 to 120 hours post-application, with the DPM treatment appearing to be more rain-resistant than the DEET treatment (Schreck and Kline 1983). A lack of consistency in the sampling periods utilised by Schreck and Kline (Schreck and Kline 1983) prevents a full assessment of the duration of activity of either of the treatments tested. In addition, this study highlights the importance of having sufficient biological and technical replicates to enable appropriate statistical analysis of the resulting experimental data to be able to account for the highly variable local population densities observed in *Culicoides*.

Following on from the efforts of Schreck and Kline (Schreck and Kline 1983) to use treated netting for area protection to reduce human biting nuisance, the veterinary community focused on other *Culicoides* species of veterinary importance. When applied to polyester mesh at 13.0 mg/m^2 (1.36 mm aperture mesh) (Braverman *et al.* 1999) at 11.0 g/m^2 (3-4 mm aperture mesh), DEET has been shown to repel *C. imicola*, the Afro-Asiatic vector of BTV and AHSV (Page *et al.* 2009). At the same time, meshes treated at 183.0 mg/m^2 with a formulation of PMD, (+)-Citronellol and isopulegol as the active ingredients (1.36 mm aperture mesh) were found to attract, in the first 2 hours post-application, up to 4x the number of *C. imicola* in comparison to those untreated used for control (Braverman *et al.* 1999). Despite this formulation previously being shown to be an effective repellent when used as a topical repellent against *C. impunctatus* in laboratory-studies (Triggs and Hill 1996). Citronella oil (0.6%) applied at 40 mg/m^2 to polyester mesh (3-4 mm aperture mesh) also had no significant effect ($P \geq 0.05$) on the number of *C. imicola* collected by a UV light trap during a 14 hour period (Page *et al.* 2009). The lack of a significant effect in the latter study is likely to be due to the volatile nature of the formulation leading to little or no active ingredients being present on the mesh after the first few hours of the study.

Previous research into the use of insecticide treated nets (ITNs) has focused on the equine industry; however, the Northern European BTV-8 outbreak has driven research into the potential for ITNs to be used to protect livestock. Currently the only commercially available ITNs are treated with the pyrethroid insecticide deltamethrin to which *Culicoides* have previously been shown to be highly susceptible (Del Río *et al.* 2014b, Venail *et al.* 2015). The use of pre-treated deltamethrin-treated mosquito fences (100 mg/m^2 deltamethrin; 200 µm aperture) surrounding cattle paddocks, did not reduce the abundance of *Culicoides* within the treated paddocks in comparison to untreated paddocks, as measured by UV light trap collections as a proxy for vector-host contact (Bauer *et al.* 2009). Similarly, 2 studies conducted in Spain found no significant difference between the number of *Culicoides* collected by a UV light trap within untreated net in comparison to those collected by a UV light trap within the commercially-available deltamethrin ITN ZeroVector® Durable Lining (Dart Association, Lausanne, Switzerland; 0.4% deltamethrin; 200 µm aperture) and ZeroFly® Livestock (Vestergaard, Lausanne, Switzerland; 0.4% deltamethrin; 200 µm aperture) (Del Río *et al.* 2014b, Miranda *et al.* 2014). This was despite the fact that deltamethrin was lethal to field-collected *Culicoides* in laboratory contact bioassays (Del Río *et al.* 2014b, Venail *et al.* 2015).

Manual treatment of ITNs has focused on the use of formulations containing cypermethrin as the primary active ingredient. Cypermethrin-treated canvas barriers (2.6 m height; 0.5% *w/v* cypermethrin) used to surround a pen containing cattle provided only partial to no protection against *C. imicola*, as it becomes evident when comparing the number of *C. imicola* collected within the ITN using a UV light-suction trap to the number of specimens collected within a pen surrounded by untreated net (Calvete *et al.* 2010). According to the authors, *C. imicola* seemed able to fly above the barrier avoiding contact with the insecticide-impregnated canvas. In another study based in Spain, blue-shading nets, made from inert polyethylene fibres (200 µm aperture) were manually sprayed with a 1% *w/v* cypermethrin solution and placed in a cylinder formation (1.5 m high; 1 m wide) with a UV light trap inside (Del Río *et al.* 2014a). Cypermethrin-treated ITNs were found to be ineffective with no significant difference in the number of *C. imicola* collected by the UV light-suction trap surrounded by the cypermethrin-treated ITN and the UV light-suction trap surround by the untreated control net.

In the Republic of South Africa, high density polyethylene (HDPE) nets (300 µm aperture) manually treated with α-cypermethrin (Fendona®6, BASF Agro BV Arnhem, Zürich, Switzerland; 20-40 mg/m^2) did not significantly reduce the entry of *Culicoides* to UV light traps directly covered by the treated net in comparison to an untreated HDPE net (Page *et al.* 2014). However, the presence of either untreated or treated HDPE net did significantly reduce the number of *Culicoides* collected in comparison to a trap screened only to exclude large insects with a wide-aperture polyester mesh (2,000 µm aperture). Following this study, a field-based study found α-cypermethrin (Fendona®6, BASF Agro BV Arnhem, Zürich, Switzerland; 20-40 mg/m^2) treated HDPE net (300 µm aperture) did not reduce the number of *Culicoides* collected by a UV light trap in a jet stall designed for the transport of horses in airplanes (KLM HMA, European Horse Services, Meetkerke, Belgium) (Page *et al.* 2015). The netting did reduce the number of *Culicoides* directly aspirated from horses in a jet stall whose entrances had been covered with treated net in comparison to a no-net negative control, indicating that ITNs may be successful in reducing vector-host contact in this scenario (Page *et al.* 2015).

The latest research focusing on ITNs developed by Baker and colleagues (Baker *et al.* 2015) found during a series of highly standardised WHO cone bioassay studies significant variation in mortality and subsequent blood-feeding rates of colony-reared *C. nubeculosus* which had been exposed to commercially-available SP insecticides applied to black polyvinyl-coated polyester insect screen (1.6 mm aperture; 1.6 mm thickness). With some SPs being no more effective at inducing mortality or reducing subsequent blood-feeding than untreated controls. However, Baker and colleagues (Baker *et al.* 2015) did find that a formulation of cypermethrin (0.15% *w/w*) and pyrethrins (0.2% *w/w*) (Tri-Tec 14®, LS Sales (Farnham) Ltd, Bloxham, UK) when applied to black polyvinyl-coated polyester insect screen (1.6 mm aperture; 1.6 mm thickness) inflicted 100% mortality on batches of *C. nubeculosus* following a 3 minute exposure in the WHO cone bioassays at 1, 7, and 14 days post-treatment. In subsequent field trials, the authors (Baker *et al.* 2015) found that the mean CPI for untreated mesh and Tri-Tec 14®-treated mesh in comparison to a no mesh control was 71% and 96% respectively, when used to screen stables containing a horse. Indicating that insecticide-treated mesh, when used to screen stable entrances, can provide increased but not complete protection of stabled horses from exposure to *Culicoides*, in comparison to either untreated screens or no screens.

Larvicides

Only 1 study in the last decade has investigated the potential to control *Culicoides* using larvicides, reflecting the difficulties of targeting these treatments effectively and the increasing environmental concerns over indiscriminate use (Carpenter *et al.* 2008a). The study, which was carried out in Maharashtra, India, found that the herb-based fly repellent AV/FRC/18 (M/S Myurvet Ltd., Mathura, Uttar Pradesh, India) [active ingredients: oil of *Eucalypta globus* Labill (Tasmanian blue gum tree), *Cedrus deodara* (Roxb.) G.Don (Himalayan cedar tree) and *Pinus roxburghii* Sargent, 1897 (= *Pinus longifolia*) (Chir pine tree)] exhibited a dose-dependent oviposition deterrent and ovicidal effect in field-collected *Culicoides peregrinus* Kieffer, 1910 and *Culicoides oxystoma* Kieffer, 1910 (= *Culicoides schultzei*) (Naraladker *et al.* 2011). This product was also demonstrated to provide a larvicidal effect in *C. peregrinus* during laboratory trials and resulted in a reduction in larval abundance post-treatment in a field trial.

Host systemic treatments

There has been little advance in the potential to use ivermectin or other avermectins for *Culicoides* vector control, either through toxicity to blood-feeding adults or via a residual effect on larvae developing in dung from treated livestock. Adult mortality and other sub-lethal effects including reduced ovarian development, decreased fecundity, and reduced larval survival of subsequent generations have previously been reported in *C. brevitarsis*, which had fed upon cattle treated with ivermectin (Standfast *et al.* 1984, Standfast *et al.* 1985). In a study conducted in the USA, however, no significant difference was found in the mortality rate of *C. sonorensis* fed bloodmeals from treated and untreated horses, sheep, and elk (Reeves *et al.* 2009). This supports previous findings that *C. sonorensis* is more resistant to ivermectin than *C. brevitarsis*, with regards to adult mortality (Holbrook and Mullens 1994). In this respect it is worth mentioning the study conducted by Reeves and colleagues (Reeves *et al.* 2009), which reports that that blood from sheep treated with Ivomec® Plus (Merial Inc., Duluth, GA, USA: 200 µg/kg ivermectin and 2,000 µg/kg clorsulon) when mixed 1:1 with BTV-17 in cell culture media at 7 $\log_{10}$ $TCID_{50}$ results in a significantly ($P < 0.05$) reduced susceptibility to BTV infection, as determined by the presence of virus in the head detected by infrared reverse transcription polymerase chain reaction (RT-PCR) (Kato and Mayer 2007), in colony-reared *C. sonorensis* incubated at 24°C for 12 days. No significant difference ($P > 0.05$) was found by the authors in the corresponding study using EHDV-2 at 7 $\log_{10}$ $TCID_{50}$ with blood from Ivomec® Plus treated Elk. No significant difference ($P > 0.05$) in mortality was observed between treated and untreated controls in either the BTV or EHDV studies, the mechanism by which the reduction in susceptibility to BTV-17 infection was achieved has not yet been explained and is worthy of further investigation.

Sollai and colleagues (Sollai *et al.* 2007) proposed that avermectins may have the potential to disrupt host location due to the impact of avermectin treatment on the treated host's kairomone profile. In a laboratory-based study, the authors found that the response of female field-collected *C. imicola* to the host kairmones L-(+)-lactic acid and butanone were significantly ($P \leq 0.05$) reduced when they were co-exposed to the odour of commercially available avermectin treatments, Dectomax® (Zoetis, Rome, Italy: 1% *w/v* doramectin) and Ivomec® (Merial Animal Health, Rome, Italy: 1% *w/v* ivermectin) during electroantennography (EAG) experiments. In addition, the EAG response of female field-collected *C. imicola* exposed to clipping of fleece from sheep 1 day post-treatment with Dectomax® or Ivomec® decreased by 80% compared with the one of the fleece of the same animal before treatment, Nonetheless, there was no significant effect ($P > 0.05$) on EAG responses following exposure to fleece clippings obtained 7 days post-treatment (Sollai *et al.* 2007). No further studies have been conducted to investigate other *Culicoides* species, the impact of avermectin treatment on biting rates in the field or the level of inter- and/or intra-breed variation in the impact of avermectin treatment on host kairomone profiles. It is likely that the limited duration of the effect of avermectins on vector EAG responses reported by Sollai and and colleagues (Sollai *et al.* 2007) would limit the impact of this potential method for reducing host-vector contact in the field. The use of avermectins treatments to reduce vector-host contact, however, may have a place within an integrated control strategy during high-risk, but short duration exposure periods, like, for example, in the case of animal transport through a high-risk area from a low-risk to low-risk area. Concerns remain, however, over the impact of widespread use of avermectins on dung beetle communities (Bishop *et al.* 2005) and in promoting resistance to helminths, which is the primary role of these products (Carpenter *et al.* 2008a).

Semiochemical-based systems

The use of semiochemicals in research of *Culicoides* has been reviewed in detail (Logan *et al.* 2010). Results with host kairomone-baited traps remain varied and studies in this area remain targeted towards reducing populations of nuisance biting species around human habitations. Cilek and Hallmon (Cilek and Hallmon 2005) found no significant reduction in local *Culicoides* abundance, species composition or perception of biting nuisance by owners in Florida gardens between gardens with and without a Mosquito Magnet® trap (American Biophysics, East Greenwich, Rhode Island, USA) operating [Carbon dioxide (CO_2), racemic 1-octen-3-ol bait]. This was consistent with previous research, which found kairomone-baited ABC Pro insect suction traps (Clarke Mosquito Products Inc., Roselle, Illinois, USA) (CO_2 and a 4:1:8 octenol/phenol mixture of 1-octen-3-ol: 3-n-propylphenol:4-methylphenol bait) were ineffective at reducing local *Culicoides* abundance in Florida gardens (Cilek *et al.* 2003). Research on the host location in livestock-associated *Culicoides* remain in their infancy, although recent studies in the UK have examined combinations of host kairomone cues based on enantiomers of 1-octen-3-ol in combination with carbon dioxide (Harrup *et al.* 2012). Suction traps using a semiochemical lure of predominantly the (R) enantiomer of 1-octen-3-ol with CO_2 have been found to attract consistently a greater abundance of female *Culicoides* than traps using either CO_2 alone ($P \leq 0.01$) or a racemic mix of (R) and (S) enantiomers of 1-octen-3-ol ($P \leq 0.05$) (Harrup *et al.* 2012). Harrup and colleagues (Harrup *et al.* 2012) found that the species complement attracted to suction traps with a lure of (*R*)-(-)-1-octen-3-ol at ~4.7 mg/h with CO_2 at 500 ml/min were comparable to those collected in a sheep-baited drop trap. Due to the study design, the abundance of female *Culicoides* collected between the sheep-baited and semiochemical trap were not directly comparable. However, the semiochemical trap is unlikely to be sufficiently attractive to lure *Culicoides* away from a host in order to reduce biting rates. Semiochemical baited traps may, however, present a method to monitor the effectiveness of control measures in a more host-appropriate manner than light-suction traps.

Biological control methods

Biological control refers to methods where entomophagous and entomogenous organisms are used by human beings, either in manipulated or natural forms, to suppress a pest species (Van Den Bosch and Stern 1962). Research on *Culicoides* in this subject area during the last 10 years has been confined to a handful of laboratory-studies showing the larvicidal effects of insect pathogenic fungi. In a series of laboratory and semi-field based experiments in the UK, insect-pathogenic fungi from 4 genera [*Metarhizium anisopliae* (Metchnikoff) Sorokin, *Beauveria bassiana* (Balsamo) Vuillemin, *Paecilomyces fumosoroseus* (Wize), and *Verticillium longisporum* (Starke) Karapapa, Bainbr and Heale] were found to kill colony-reared *C. nubeculosus* larvae (Ansari *et al.* 2010) and reduce adult survival (Ansari *et al.* 2011) in laboratory-studies. Similarly, Nicholas and McCorkell (Nicholas and McCorkell 2014) also found *M. anisopliae* to be pathogenic to adult field-collected *C. brevitarsis* and to reduce the emergence of *C. brevitarsis* from field-collected substrate samples in laboratory-studies. While, Narladkar and colleagues (Narladkar *et al.* 2015) found *M. anisopliae* and *Beauveria bassiana* to be pathogenic to field-collected *C. peregrinus* larvae and blood-fed adults. While, Stephen and Kurtböke (Stephen and Kurtböke 2011) found no significant larvicidal effects of *Oomycete fungi* on *Culicoides* species associated with the inter-tidal mangrove areas of Queensland, Australia. To date these studies have not been transferred to the field.

Use of the gram-positive bacterium *Bacillus thuringiensis israelensis* (*Bti*) as a low environmental impact alternative to chemical pesticides has been successfully developed and deployed in the field for the control of mosquitos (Boyce *et al.* 2013). Initial laboratory trials have, however, showed *Bti* to be ineffective against field-collected larvae of *C. impunctatus* (Blackwell and King 1997), *C. mississippiensis*, *Culicoides guttipennis* (Coquillett), 1901 (Kelson *et al.* 1980), and colony-reared *C. sonorensis*, and *Culicoides occidentalis*, Wirth and Jones, 1957 (Kelson *et al.* 1980). The use of *Bti* for *Culicoides* control has therefore not been taken further either commercially or by researchers. It is likely that the organically enriched semi-aquatic nature of the majority of *Culicoides* breeding habitats would make it challenging to develop a *Bti* formulation that would penetrate the substrate and be sufficiently and persistently effective.

Insect Growth Regulators, Mermithid parasites (Stichosomidae: Mermithidae), iridescent viruses, pansporoblastic microsporidia, and aquatic cnidarians *Hydra* spp. (Anthomedusae, Hydridae) have all previously been investigated as potential biological control agents and are reviewed in detail by Carpenter and colleagues (Carpenter *et al.* 2008a) and by Mullens and colleagues (Mullens *et al.* 2008). For a variety of reasons none of these agents have been taken forward to field trials, with the exception of Mermithids (*Hereidomermis* spp.), which were subject to a small semi-field trial in Southern California (Mullens *et al.* 2008). In this study, the authors seeded 8 sections of a cattle slurry pond (40 cm wide by 55 cm long by 10 cm deep) with ~1,500 *C. sonorensis* eggs per section on day 0 and on day 7 (the experimental sections had been screened to prevent oviposition by wild *Culicoides*). Four of the 8 enclosures were also inoculated with ~40,000 pre-parasites of *Heleidomermis magnapapula* Poinar and Mullens 1987 per section on day 7. The numbers of *C. sonorensis* emerging from the treated mermithid sections were found to be significantly lower ($P \leq 0.01$; 84% reduction) than that of the untreated areas at day 21 (Mullens *et al.* 2008). However, none of the newly emerged adult *C. sonorensis* collected from the mermithid treated areas was infected with mermithids (Mullens *et al.* 2008), raising doubt over the potential persistence of the control method and the ability of the mermithids to be dispersed to other non-treated habitat areas. Significant logistical obstacles for the utilisation of *Heleidomermis* spp. of mermithids for biological control of *Culicoides* also exist. The most logistically efficient method of distributing *Heleidomermis* to target field-sites would be eggs. However, *Heleidomermis* lack an environmentally-resistant egg stage (Mullens *et al.* 2008). In addition there are currently no in vitro rearing systems for *Heleidomermis*, meaning that a *Culicoides* colony would also be required to rear the *Heleidomermis* (Mullens and Velten 1994).

The use of endosymbionts to reduce pathogen transmission and adult longevity has gained increasing attention in recent years, and a strain of the endosymbiont bacterium *Wolbachia* is successfully being utilised in the field to reduce dengue virus transmission by *Aedes aegypti* (L. in Hasselquist, 1762) (Ritchie 2014). Prelimiary surveys of the microbiome of Culicodies have identified *Cardinium* and *Wolbachia* (Lewis *et al.* 2014, Mee *et al.* 2015, Morag *et al.* 2012, Nakamura *et al.* 2009) together with a range of other microbiota (Campbell *et al.* 2004) inhabiting *Culicoides*.

It is unclear whether *Wolbachia* or other endosymbiont infection(s) in *Culicoides* have any influence on viral blocking, fecundity, parthenogenesis, sex ratios and/or, mate descrimination, traits which have been observed in other *Wolbachia*-infected arthropods, and are the basis of *Wolbachia*'s utility in vector control (Hoffmann *et al.* 2015, Werren *et al.* 2008). While vector-microbiome interactions present a fascinating area of research, considerable work is required to investiagate the influence of endosymbionts on *Culicoides* biology and, in turn, their influence on vector capacity prior to the use of endosymbionts, such as *Wolbachia*, for *Culicoides* population control and/or a tool to limit *Culicoides*-borne pathogen transmission.

Genetic control

The last decade has seen significant developments in the application of genetics and genomics to the study of *Culicoides*. There has been a proliferation in the use of PCR-based assays and Sanger sequencing for the identification of vector species in surveillance datasets and vector incrimination studies (for review see Harrup *et al.* 2015), in publications and analysis of reference transcriptomes (Campbell *et al.*, 2005, Nayduch *et al.* 2014 b, c), and proteomes (Russell *et al.* 2009), culminating in the imminent release of the first annotated full genome build for *C. sonorensis* (Nayduch *et al.* 2014a). These new genetic and genomic resources have not been used yet to either optimise currently available vector control strategies via, for example, screening for genetic markers of insecticide resistance in *Culicoides* or for the development of a genetic modification-based vector control system.

Vector control strategies, which are based on genetic modification, can be utilised to either suppress the abundance of the target vector population or promote a refractory phenotype within the target vector population (for review see Alphey 2014). In addition, there is also the possibility to develop control strategies through gene manipulation via RNA interference (RNAi) (for review see Bartel 2004). While RNAi is not considered a genetic modification, it relies on a detailed knowledge of gene regulation within the target vector. Recent proof-of-principal studies have demonstrated the potential to induce RNA interference (RNAi) in *C. sonorensis* both *in vivo*, using larval cell lines (Schnettler *et al.* 2013), and *in vitro* (Mills *et al.* 2015). This technique is an exciting tool to be exploited for gene function analysis and in particular investigate the contribution of the small interfering (siRNA) pathway to *Culicoides* vector competence (Mills *et al.* 2015).

Many parallels can be drawn between the publication of the first *Culicoides* genome and the publication of the first mosquito genome, *Anopheles gambiae* Giles 1902 (Holt *et al.* 2002). In response to the publication of the *An. gambiae* genome, Tabachnick (Tabachnick 2003) outlined 3 goals that had to be addressed prior to the new genomic resources being capable of advancing vector control. The same goals can now equally be reiterated and applied to *Culicoides*, they are (i) developing genetic engineering tools that can be used with *Culicoides* vectors, (ii) identifying anti-pathogen effector genes, (iii) developing gene-driven systems capable of introgressing the gene(s) identified in (ii) throughout the wild vector populations. The *Culicoides* community will hopefully be able to leverage the considerable technological advances that have been made in areas (i) and (iii) by the mosquito community (Gantz *et al.* 2015). However, the global heterogeneity and complexity of transmission dynamics of *Culicoides*-borne pathogens may lead to the identification of anti-pathogen effector genes being highly complex. For example, BTV circulates globally as at least 26 serotypes, with at least 14 major vector species associated with transmission (Purse *et al.* 2015). Many areas have multiple vector species present in addition to multiple serotypes of BTV circulating. Can a suitable effector gene(s) be identified with anti-pathogen effect sufficient to significantly reduce transmission across virus species, serotypes, and topotypes? The alternative strategy of developing a genetic modification strategy that aims to suppress the vector population, e.g. sterile males, would also be subject to the same limitation of potentially needing to be effective across multiple vector species.

The availability of genomic resources or the techniques to implement for example gene-drive systems are, however, not the greatest limitation to the application of genetic control strategies to *Culicoides*. Instead, it is limitations in the availability of basic biological resources for the vectors of interest, principally colonies of vector species of *Culicoides*. *C. sonorensis* is currently the only major vector of arboviruses in colony worldwide (Nayduch *et al.* 2014). In addition, transmission of *Culicoides*-borne arboviruses in the regions where this species exists is not generally considered a research priority due to a lack of clinical disease. While progress has been made in colonising other major vector species, such as *C. imicola* (Veronesi *et al.* 2009), the paradox of the most tractable system for experimental research on *Culicoides* being in one of the least affected regions for *Culicoides*-borne disease poses a challenge for the next decade.

Conclusions and future directions

The reduction of *Culicoides* populations or the protection of livestock as a means of interrupting arbovirus transmission will always be a secondary technique to vaccination in the control of disease. However, this review has explored the many scenarios in which vector techniques remain an important aspect of combatting arboviruses of livestock (Carpenter *et al.* 2008a, MacLachlan and Mayo 2013). While future research in this area would be best aimed at the areas where these techniques have greatest impact (subsistence farmers in resource-poor regions), the vast majority of research presented in this review is from epidemic areas where BTV impacts on large-scale farming. This is unlikely to change dramatically in the next 10 years. Nonetheless, a greater focus on producing quantitative data regarding the use of traditional means of *Culicoides* control would be of significant interest and improve communication of techniques worldwide.

A key flaw in current studies of control has been the inability to infer the probability of transmission of arboviruses from *Culicoides* abundance data (the measure used most commonly by researchers to define control success). To date very few studies have examined this impact by monitoring infection in ruminant hosts before and after treatment, not least due to the logistically challenging nature of these trials. Studies of this type have been conducted in the USA for use of a permethrin insecticide treatment (Mullens *et al.* 2001) and for the use of habitat manipulation (Mayo *et al.* 2014), and in Australia for the use of OP-impregnated ear tags, stabling, and OP and SP dipwashes (Melville *et al.* 2004, Melville *et al.* 2005). The fact that all these studies were preceded by preliminary experimentation showing that the techniques used in the field trials would reduce *Culicoides* populations is significant when examining recent research in Europe.

European research has been dominated by field trials to assess the effectiveness of products already commercially available against *Culicoides* and which had originally been developed to target non-*Culicoides* species. The impact of fully integrated control systems using combinations of chemical, mechanical, and/or biological control systems targeting multiple life-stages *Culicoides* have never been assessed via a systematic screening of potential control measures in standardised laboratory and semi-field conditions in order to select appropriate and complementary control techniques for use in field trials. With respect to this, the development of semi-field experimental systems for *Culicoides* similar to the one developed for mosquitos (Ng'habi *et al.* 2015) would greatly aid the transition of vector control methods from laboratory-studies to full field-studies.

Within Europe, significant progress has been made towards the development of standardised techniques for testing the efficacy of insecticides and levels of insecticide resistance in the laboratory through the adoption of the World Health Organisation Pesticide Evaluation Scheme (WHOPES) techniques (World Health Organisation 2002) with only minor modifications required for their use with *Culicoides* (Baker *et al.* 2015, Del Río *et al.* 2014b, Venail *et al.* 2011, Venail *et al.* 2014). The use of insecticide screening in stabling and transport will remain a major focus of research, as these areas are of interest both in the initial stages of arbovirus incursion and during vaccine deployment (Carpenter *et al.* 2008a). Further work is, however, required to develop a standardised framework for the testing and evaluation of these control measures in the field and the development of minimum requirements for publication of research into vector control measures similar to the Minimum Information for Publication of Quantitative Real-Time PCR Experiments (MIQE) guidelines recently published (Bustin *et al.* 2009). The adoption of standard reference insecticide and repellent compounds, such as deltamethrin and DEET respectively, as positive controls for chemical control experiments would allow for cross-comparison of mortality rates between the studies to be performed. This is, at the moment, a common flaw across previous research in this area. In addition, a move towards systematic testing of repellents and insecticides based on initial efficacy in laboratory trials through to large-scale field studies would be advantageous.

Conflict of interest statement

The authors know of no financial or personal conflicts of interest with any person or organisation that could inappropriately influence this work. Funders had no role in study design or the collection, analysis and interpretation of data. Mention of proprietary products does not constitute an endorsement or a recommendation by the authors for their use.

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