Culicoides *spp. (Diptera: Ceratopogonidae) as vectors of Bluetongue virus in South Africa – a review*

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Summary
The aim of this paper is to consolidate vector competence studies on *Culicoides* midges (Diptera: Ceratopogonidae) as vectors of Bluetongue virus (BTV) done over a period 25 years at the Agriculture Research Council - Onderstepoort Veterinary Institute in South Africa. In 1944, it was demonstrated for the first time in South Africa that *Culicoides* midges transmit BTV. In 1991, field-collected *Culicoides imicola* were fed on blood containing BTV-3 or BTV-6 and the infection rates were established as being 31% and 24%, respectively. In 1998, *Culicoides bolitinos* was shown to have a higher infection prevalence and virus titre/midge than *C. imicola*. This species was then shown to have a higher transmission potential for BTV-1 over a range of incubation temperatures wider than the one showed by *C. imicola*. Attenuation of BTV also does not reduce its ability to infect competent *Culicoides* species. Oral susceptibility studies, involving 29 BTV isolates of various serotypes, indicated differences between various geographic virus isolates and *Culicoides* populations evaluated. While low recovery rates of European BTV strains from South African *Culicoides* species suggest co-adaptation between orbiviruses and vectors in a given locality, co-adaption was shown not to be essential for virus transmission. Cumulative results since 1991 provide evidence that at least 13 livestock-associated *Culicoides* species are susceptible to BTV. Susceptibility results are supported by field isolations from 5 of these species. This implies that multi-vector potential for the transmission of BTV will complicate the epidemiology of BT. It must be emphasised that neither oral susceptibility nor virus isolation/detection from field-collected specimens is proof that a species is a confirmed field vector.

Specie di Culicoides *(Diptera: Ceratopogonidae) vettori del virus della Bluetongue in Sud Africa*

Riassunto
Il presente lavoro ha l'obiettivo di descrivere i risultati degli ultimi 25 anni di ricerca condotta all'Agriculture Research Council - Onderstepoort Veterinary Institute in Sud Africa, sul ruolo dei *Culicoides* (Ditteri: Ceratopogonidae) come vettori del virus della Bluetongue (BTV). Nel 1944, in Sud Africa, è stato dimostrato per la prima volta il loro ruolo di vettori di BTV. Nel 1991, *Culicoides imicola* catturati in campo e nutriti con sangue contenente i sierotipi 3 o 6 del BTV hanno evidenziato percentuali di infezione, rispettivamente, del 31% e 24%. Nel 1998 è stato dimostrato che *Culicoides bolitinos* ha una prevalenza di infezione e un titolo virale per insetto superiori a quelli di *C. imicola*. È stato quindi dimostrato che *C. bolitinos* ha un maggior potenziale di trasmissione di BTV-1 in un intervallo di temperatura di incubazione più esteso di quello riportato per *C. imicola*. L'attenuazione del BTV non riduce la sua capacità di infettare specie competenti di *Culicoides*. Studi di competenza vettoriale attraverso infezione orale con 29 ceppi diversi appartenenti a vari sierotipi di BTV hanno evidenziato differenze a seconda dell'origine geografica dei ceppi e delle popolazioni di *Culicoides* esaminate. I bassi livelli di infezione dei ceppi europei di BTV in specie sudafricane di *Culicoides* suggeriscono l'esistenza di un possibile co-adattamento tra *Orbivirus* e vettori in una data zona, co-adattamento che tuttavia non è essenziale per la trasmissione del virus. In tutti questi anni è stato possibile

dimostrare che almeno 13 specie di *Culicoides* associate al bestiame sono suscettibili al BTV. Tali risultati sono supportati da isolamenti di campo su 5 di queste specie. Ciò implica che il potenziale multi-vettoriale per la trasmissione di BTV complicherà l'epidemiologia di BT. Va sottolineato che né la suscettibilità orale né l'isolamento/rilevamento del virus da insetti catturati in campo provano che una data specie sia vettore certo del BTV.

Introduction

Bluetongue (BT), an infectious viral disease of wild and domestic ruminants, was reported for the first time more than 125 years ago with the introduction of European breeds of sheep into the Cape Colony of Southern Africa (Howell and Verwoerd 1971). The disease was subsequently recognized in other parts of Africa, Europe, the Middle East, the Indian subcontinent, the Americas, and Asia. The causative agent, Bluetongue virus (BTV), is a double stranded RNA virus, within the genus *Orbivirus* of the family *Reoviridae* (Borden *et al.* 1971), which is transmitted almost exclusively by certain species of *Culicoides* biting midges (Diptera: Ceratopogonidae) (Mellor *et al.* 2000, Purse *et al.* 2015). Although BTV infects all known species of ruminants (Barnard 1997), severe disease is usually restricted to certain breeds of sheep (*e.g.,* Merino) and some species of deer (Taylor 1986, MacLachlan 1994).

Outbreaks of BT and its geographic distribution are determined both by the presence of susceptible host animals and by the abundance of specific vector-competent *Culicoides* species. Bluetongue was historically considered to occur more commonly in the tropical and sub-tropical regions of the world, between the latitudes of 40°N and 35°S (Gibbs and Greiner 1994). However, since 1998 there has been a dramatic expansion in the worldwide distribution in its occurrence (Purse *et al.* 2005, MacLachlan 2010), with several serotypes of BTV causing outbreaks in Southern and Central Europe (Mellor and Wittmann 2002, Mellor *et al.* 2008). In September 2006, outbreaks of BTV-8 were confirmed as far North as the Netherlands, Belgium, Germany, and Northern France (Thiry *et al.* 2006), and overwintering subsequently occurred in the area (Hoffmann *et al.* 2008).

Only a small portion of the more than 1,300 *Culicoides* species described worldwide is deemed a proven vector of BTV (Purse *et al.* 2015). The apparent absence of BTV in the North-Eastern United States was ascribed *e.g.* to low the apparent absence of BTV in the North-Eastern United States was ascribed to low oral susceptibility rates of *Culicoides variipennis* (*i.e., Culicoides variipennis variipennis*), the dominant *Culicoides* species in the area (Tabachnick and Holbrook 1992). Similarly, in Australia *Culicoides*

brevitarsis, a relative inefficient but abundant vector of BTV, is considered to play a more important role than the more efficient vector, yet less abundant, *Culicoides fulvus* (Standfast *et al.* 1985). For effective risk management it will be essential to know the species composition and abundance of the *Culicoides* populations in an area. At the same time, to determine the role they might play in the epidemiology and occurrence of BT, the vector capacity must be known. Vector capacity refers to the ability of a vector population to transmit a pathogen and it is determined by vector abundance, biting rates (the number of feedings on specific host as well as the period between subsequent feedings), host preferences, survival rates, vector competence, and duration of the extrinsic incubation period (Garrett-Jones 1964, Mullens *et al.* 2004).

Vector competence refers to the ability of a vector to support infection, replication, dissemination, and subsequent transmission of the virus to susceptible hosts. It is a measure of the number of midges that actually become infective after feeding on a viraemic host and is dependent upon the genetic makeup (Tabachnick 1991) of the vector midge alongside external environmental factors (Wellby *et al.* 1996, Mellor *et al.* 1998, Wittmann *et al.* 2001). Oral susceptibility (infection and replication), as an indication of vector competence, of *Culicoides* species can be experimentally assessed by allowing females to feed on a viraemic animal or a blood virus suspension. Engorged midges are kept alive for the extrinsic incubation period (time from when the vector ingested an infected blood meal until excretion of the virus in the saliva) and then assayed for the presence of virus.

Culicoides species as vectors of BTV in South Africa

In South Africa the climate ranges from tropical to sub-tropical high Summer rainfall areas in the North-Eastern parts of the country to semi-desert and relatively arid conditions in the North Western parts. Despite these variations, the whole of South Africa can be considered endemic for BTV (Gerdes 2004). Over the last 45 years, more than 112 *Culicoides* species (a third of which are still undescribed) (Meiswinkel *et al.* 2004), of the 1,357 described

worldwide (Borkent 2014, Purse *et al.* 2015) were collected in South Africa. Light trap surveys have shown that more than 20 of these species are collected regularly near livestock (Nevill *et al.* 1991b, Venter *et al.* 1996). Of these 20 species *Culicoides imicola* is the most abundant livestock-associated *Culicoides* species to be collected, especially in the warm, frost-free Summer rainfall areas of the country (Meiswinkel 1989, Venter *et al.* 1996). Based on host preference, wide geographical distribution, oral susceptibility, field isolation of the virus, and high abundance at livestock, *C. imicola* is considered the most important vector of BTV and other livestock viruses in South Africa (Nevill *et al.* 1991 a, b, Meiswinkel *et al.* 2004). The vast numbers (> 1 million) that can be collected with the Onderstepoort black light trap under suitable conditions near livestock may be indicative of high attack rates on livestock (Meiswinkel *et al.* 2004). In evaluating these results, it must be taken into account that, as for other studies conducted in Europe (Gerry *et al.* 2009, Viennet *et al.* 2013), light traps results in South Africa are not always directly related to the attack rate on the host (Scheffer *et al.* 2012).

Since *C. imicola* is rare or absent from some of the cooler mountainous or more arid areas (Jupp *et al.* 1980, Venter and Meiswinkel 1994, Venter *et al.* 1996), it cannot be regarded as the only vector of BTV in South Africa. Abundant species in the latter areas include *Culicoides zuluensis* and members of the Culicoides schultzei group (Venter *et al.* 1996). *Culicoides imicola* was also shown to be

absent in collections made in the sandy dune fields adjoining Port Elizabeth in the Eastern Cape Province (Meiswinkel 1997) and at some sites on the Southern and Western coastlines (Meiswinkel *et al.* 2004). The absence of *C. imicola* in these areas was attributed to the sandiness of the area (Meiswinkel 1997, Meiswinkel *et al.* 2004).

To determine the role that the various livestock associated *Culicoides* species might play in the epidemiology of BTV in South Africa efforts were made over the last 2 decades to assess the vector competence of these species. The results of these vector competence and oral susceptibility studies done on South African livestock associated *Culicoides* species are summarised and evaluated in the rest of this article.

Materials and methods used to test vector competence in South Africa

A membrane feeding method developed in 1991 (Figure 1) proved suitable for the artificial feeding of field-collected midges (Venter *et al.* 1991) and has been used ever since to determine and compare South African livestock associated *Culicoides* species for a variety of viruses including BTV. Field-collected *Culicoides* were fed in batches of 300-400 for 30-45 minutes on defibrinated horse or sheep blood containing viruses through a one-day-old chicken-skin membrane (Figure 1). After feeding blood engorged specimens were separated on
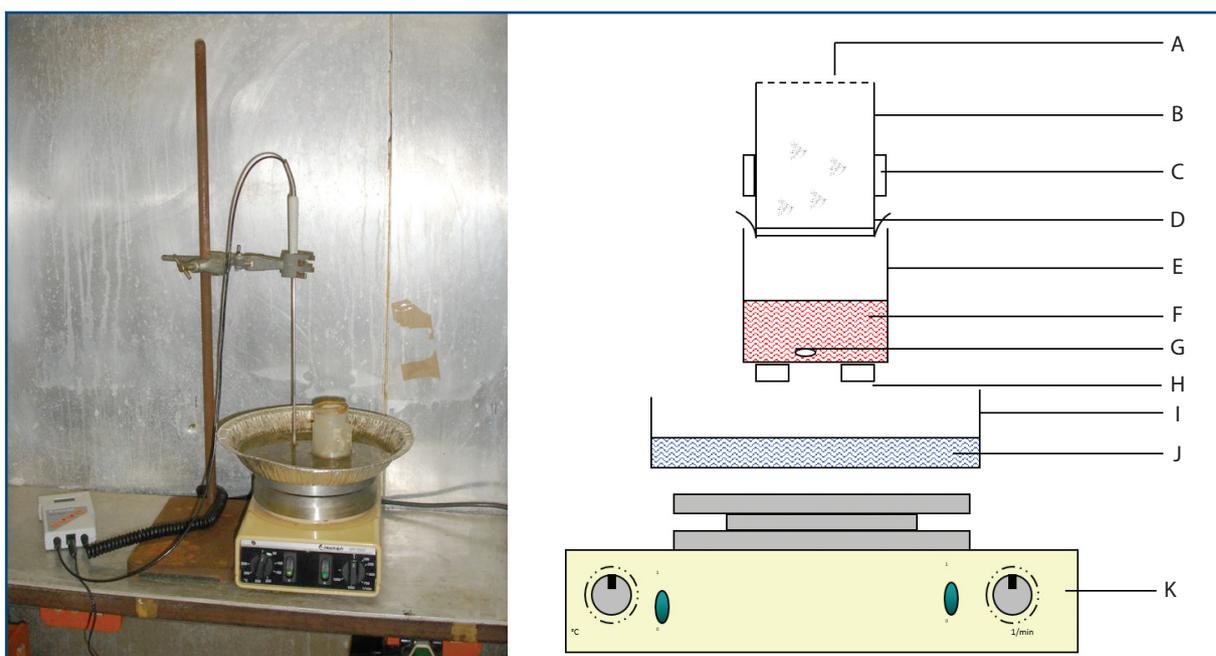
Figure 1. *Blood-feeding device for field-collected* Culicoides *species.* A = gauze top; B = feeding chamber (40 mm diameter plastic 'pill-bottle'); C = foamrubber; D = one day old chicken skin membrane; E = blood container (45 mm diameter plastic 'pill-bottle'); F = blood/virus mixture kept at ± 37℃; G = magnetic stirrer bar; H = rubber stopper support; I = water-bath; J = water; K = magnetic heater/stirrer (redrawn from Venter *et al.* 1991).

a chill table and incubated for 10 days at 23.5°C before being assayed for virus. A comparative study indicated that midges take a bigger blood meal, resulting in higher infection prevalence, when feeding through a membrane compared to feeding on cotton wool pledgets drenched in virus-infected blood (Venter *et al.* 2005). Due to logistical problems minimal feeding on live hosts were attempted. The feeding rate of *Culicoides* midges during these attempted feedings on live hosts (sheep) were low, in contradiction to earlier work at the Agriculture Research Council - Onderstepoort Veterinary Institute (ARC-OVI) (Du Toit 1944, Carpenter *et al.* 2015).

Viruses used for susceptibility studies were maintained in culture on laboratory cell lines. Virus detection was done using BHK-21 cell cultures (infectious virus); and virus neutralization tests were used for serotyping and identification of isolates (Venter *et al.* 2006b). Virus detection using cell cultures are considered less sensitive and specific than presently available polymerase chain reaction (PCR) methods for the detection of orbiviruses (Quan *et al.* 2010). While PCR will detect relatively small amounts of viral RNA, which may not always be indicative of replicating virus, only live replicating virus will be detected in cell cultures.

A potential shortcoming of the present studies may be that whole midge isolates were used for virus detection and it was therefore not possible to determine whether the infection was restricted to the cells of mid-gut or to a full dissemination involving the salivary glands (Mellor *et al.* 2000). Relatively high virus titres found in some individual infected midges after incubation, however, suggested full dissemination and that onwards transmission might indeed be possible (Jennings and Mellor 1987).

In evaluating these results it must be taken into account that field collected specimens were used, when laboratory colonies were absent. These studies focused on 2 areas, the ARC-OVI, Onderstepoort (25°3′S, 28°11′E; 1,219 m a.s.l.) and Koeberg Farm near Clarens (28°32′S, 28°25′E; 1,631 m a.s.l.), where *C. imicola* and *Culicoides bolitinos* are respectively abundant.

Historical time line of vector competence results in South Africa

Based on preliminary field surveys, mosquitoes were initially suspected to be the most probable vector group for BTV (Nieschulz *et al.* 1934, Carpenter *et al.* 2015). Following several failed attempts involving mosquitoes, in 1944 it was shown for the first time that *Culicoides* midges, after having been fed for 10 days on experimentally infected sheep, could transmit BTV successfully to susceptible sheep

(Du Toit 1944). These field studies were subsequently confirmed in the US in the early 1960's in insect-proof accommodation (Foster *et al.* 1963).

Virus isolations from light trap collections made from 1979 to 1985 in 25 sites in South Africa confirmed the dominance of *C. imicola* and showed BTV to be abundant and widespread (Nevill *et al.* 1991 a, b). At least 14 serotypes of BTV were isolated from 526 (11.7%) of 4,506 pools tested over a period of 6 years (Nevill *et al.* 1991a). Midges were mainly tested in mixed pools (pool size ranging from <50 to a few thousand), and yet BTV was also isolated from single pools of *Culicoides expectator* and *Culicoides pycnostictus* (Nevill *et al.* 1991a).

In 1991 artificial membrane feeding proved successful for the infection of field-collected midges and *C. imicola* (Northern parts of the country) were subsequently fed on sheep blood containing BTV-3 or BTV-6 (Venter *et al.* 1991). After an extrinsic incubation period of 10 days at 25-27°C, the recovery rates were 31% (BTV-3) and 24% (BTV-6) (Venter *et al.* 1991). No African horse sickness virus (AHSV) was recovered in this study and no virus was detected in the relatively low numbers of an additional 5 species tested (Venter *et al.* 1991).

Culicoides bolitinos was recognised as a separate species from *C. imicola* in 1989 (Meiswinkel 1989). The Imicola group currently consists of at least 9 species, of which 2 do not occur in South Africa (Meiswinkel 1995). Of this group, *C. imicola* and *C. bolitinos* are the most abundant and wide spread livestock associated species in South Africa; of them *C. imicola* is more widely distributed and is found from the Southern tip of Africa Northwards into Southern Europe and Eastwards as far as Laos, Vietnam, and Southern China (Meiswinkel 1989). Although *C. bolitinos* is restricted to Africa, it is equally widely distributed in South Africa, although it is on average 10 times less abundantly than *C. imicola*. It can, however, become abundant in the cooler mountainous areas in the East of the country. *Culicoides bolitinos* was also shown to be abundant in the Winter rainfall region of the Western Cape Province (Venter *et al.* 1996); it is the dominant *Culicoides* species, in the absence of *C. imicola*, in the sandy dunefields adjoining Port Elizabeth in the Eastern Cape Province (Meiswinkel 1997). Outside of South Africa, *C. bolitinos* has been collected in Lesotho, Zimbabwe, Malawi, Kenya, Nigeria, Mauritius, Ivory Coast (Meiswinkel 1989), Botswana (Mushi *et al.* 1999), Namibia (Becker *et al.* 2013), and Senegal (Fall *et al.* 2015).

Oral susceptibility studies during 1998 showed BTV-1, BTV-3 and BTV-4 to have a higher infection prevalence and virus titre/midge in *C. bolitinos* (22.7% to 82.0%) than *C. imicola* (1.9% to 9.8%). These same isolates displayed an apparently lower infection prevalence in US colonies of *Culicoides*

sonorensis (Venter *et al.* 1998). Follow up studies (Paweska *et al.* 2002) showed that *C. bolitinos* also had a significantly higher transmission potential for BTV-1 than *C. imicola* over a range of incubation temperatures (10°C to 30°C). In addition to *C. imicola* and *C. bolitinos*, BTV was also isolated from *Culicoides magnus*, *Culicoides bedfordi*, *Culicoides leucostictus*, *Culicoides pycnostictus*, *Culicoides gulbenkiani* and *Culicoides milnei* (Paweska *et al.* 2002). In the same study no virus was isolated from an additional 14 species tested after incubation (Paweska *et al.* 2002).

In South Africa attenuated live-virus vaccines have been used to control BT for many years. In 2004, the oral susceptibility of South African *Culicoides* species for the attenuated vaccine strains (BTV-1, BTV-4, BTV-9 and BTV-16) was compared to that of a field strain (BTV-1) (Venter *et al.* 2004). While the attenuated strains were recovered from 65 of 5,611, the field one was recovered from 65 of 393 of individuals tested after incubation (Venter *et al.* 2004). In addition, 1 or more of the strains were also recovered from *C. magnus* and *Culicoides huambensis* (Venter *et al.* 2004). This study was followed in 2007 by 16 attenuated strains of BTV fed to field collected midges (Venter *et al.* 2007). As in previous studies, BTV recovery rates and mean titres were higher in *C. bolitinos* than in *C. imicola* and BTV was also isolated from *Culicoides enderleini*. No virus was isolated from low numbers of an additional 14 species that survived incubation (Venter *et al.* 2007).

With the unpredicted outbreaks of BTV in Europe, it became relevant to determine and compare the vector competence of European *Culicoides* species with that of the proven BTV vector, *C. imicola*. European *Culicoides* species, however, were found to be reluctant to feed through membranes in the laboratory. In an effort to overcome this limitation, constraint cotton pads saturated with blood/virus mixtures were compared with membrane feeding for the assessment of susceptibility in *C. imicola* and *C. bolitinos* (Venter *et al.* 2005). Although infection rates were significantly lower, the cotton pad method was shown to be useful in situations where membrane feeding is not viable (Venter *et al.* 2005). Reduced infection rates were partly ascribed to a 30% smaller volume of blood taken up with cotton pads (Venter *et al.* 2005). The average blood meal for *C. imicola*, as determined by membrane feeding, is 0.045 µl (Venter *et al.* 2005). Cotton pad feeding subsequently showed recovery rates of BTV-9 from *Culicoides obsoletus* and *Culicoides pulicaris* comparable to (or higher than) that found in the proven BTV vector *C. imicola* (Carpenter *et al.* 2006).

Oral susceptibility studies were continued in 2006 with 13 low passage BTV reference strains fed to field collected midges. The adaption of attenuated virus

to cell culture may have an impact on their detection rate in the *Culicoides* midge (Coetzee *et al.* 2012). To minimise the impact of the *in vitro* passaging of BTV on susceptibility results, low passage virus, referring to the number of passage on cell cultures, was used in these experiments. After incubation, 7 of these strains were recovered from *C. imicola* and 11 from *C. bolitinos* (Venter *et al.* 2006b). Recovery rates and virus titres were higher in *C. bolitinos* (Venter *et al.* 2006b). Significant differences were found between the used isolates. However, since only 1 isolate of each serotype was used, it was not possible to link these differences to any one serotype. In addition 1 or more of the isolates used came from *C. enderleini*, *C. milnei* or *C. zuluensis* (Venter *et al.* 2006b).

Following the outbreak of BTV-8 in Europe in 2006, 4 isolates of BTV-8 (3 European and 1 South African) were fed to South African midges. The recovery rates of all these isolates, including the South African one, was relatively low (< 1%) from *C. imicola*. The recovery rates were somewhat higher, though, for *C. bolitinos* (up to 6.4%) (Venter *et al.* 2011). While no virus could be isolated from low numbers of an additional 13 species assayed after incubation, 1 or more of the isolates used was recovered from *C. leucostictus, C. gulbekiani*, and *Culicoides* nr *angolensis* (Venter *et al.* 2011). These studies were followed by the feeding of *C. imicola* on Spanish isolates of BTV-1, BTV-2, BTV-4 and BTV-8 (Del Rio *et al.* 2011). As for the previous study, virus recovery rates in *C. imicola* were below 1%. Relatively high virus concentrations, >2.5 $\log_{10}$ 50% tissue culture infectious dose ($TCID_{50}$)/midge, in individual infected midges, including *C. imicola*, suggests virus replication. Although these results do not preclude *C. imicola* as a vector, its low to near refractory status indicates that other less abundant species may have the potential to play decisive roles in the epidemiology of these viruses (Del Rio *et al.* 2011).

In the UK differences were shown in vector competence for various orbiviruses in the same vector for a range of *Culicoides* species (including *C. imicola* and *C. bolitinos*) and for the same orbivirus in different vectors (Carpenter *et al.* 2011). In this study the virus replication rate and minimum temperature required for replication (11°C to 13°C) were found to be consistent for different orbiviruses across different *Culicoides* species (Carpenter *et al.* 2011).

Discussion

Cumulative oral susceptibility studies indicate that competence for BTV may be widespread in the genus *Culicoides*. Bluetongue virus replicates in *Culicoides* species of 5 subgenera implicating a multi-vector potential for the transmission of

this virus. The subgenera and species involved were *Avaritia (C. imicola, C. bolitinos, C. gulbenkiani, C. huambensis), Synhelea (Culicoides exspectator, C. bedfordi), Meijerehelea (C. leucostictus, C. pycnostictus), Culicoides (C. magnus), Remmia (C. enderleini)*, and also 3 species, *C. zuluensis, C. milnei,* and *C.* nr *angolensis*, not allocated to a subgenus. Susceptibility results are supported by field isolation of BTV from at least 5 of these species (Nevill *et al.* 1991a, Meiswinkel and Paweska 2003, Meiswinkel *et al.* 2004, Venter *et al.* 2006a). Although these 13 species are collected regularly at livestock, some (*e.g., C. leucostictus* and *C. pycnostictus*) are considered to be mainly ornithophilic and their role (vector capacity) is still unclear. The involvement of a variety of *Culicoides* species, each with a unique and mostly unstudied biology, increases the complexity of the epidemiology. Despite the fact that half of the 10 species involved in the transmission of orbiviral disease that affect livestock (Meiswinkel *et al.* 2004) belong to the subgenus *Avaritia*, the involvement of a variety of species belonging to different sub-genera make it difficult to substantiate a clear relationship between phylogenetics and vector competence. This multi-vector potential for the transmission of BTV is supported by its detection from various field collected *Culicoides* species in Europe (Caracappa *et al.* 2003, De Liberato *et al.* 2003, Torina *et al.* 2004, Meiswinkel *et al.* 2007). Limited comparable data seem to indicate that the susceptibility of some Palaearctic species/populations to BTV may be equal to or higher than that of the proven vector *C. imicola*.

Despite the variation in susceptibility results among different studies, recovery rates of BTV from *C. bolitinos* were consistently higher than that of *C. imicola*. This indicates that *C. bolitinos*, which utilizes bovine dung as a breeding medium (Nevill 1968), may indeed have a higher vector competence for BTV. Although *C. bolitinos* is widespread in South Africa, it is less abundant than *C. imicola*. Studies in Australia have shown that the most abundant species, often determined by light trap collection, is not by default the most competent (Standfast *et al.* 1985). A competent vector may have a low vectorial capacity due to low biting rates or survival capacity, while a vector with low competence may be more efficient in virus transmission (Standfast *et al.* 1985). *Culicoides bolitinos* are more abundant in the cooler areas of South Africa (Meiswinkel 1989). Interestingly, *C. bolitinos* did not show a consistently higher infection prevalence than *C. imicola* for African horse sickness (Paweska *et al.* 2003) or equine encephalosis virus (Paweska and Venter 2004).

Cumulative studies also seem to indicate that attenuation of BTV does not reduce its ability to infect competent *Culicoides* species (Venter *et al.* 2004,

Venter *et al.* 2007). Adaption of attenuated virus to cell culture may increase their detection rate in the *Culicoides* midge. The transmission of attenuated vaccine viruses by *Culicoides* to susceptible animals was confirmed in study describing active circulation of BT vaccine virus serotype-2 among unvaccinated cattle in Italy (Ferrari *et al.* 2005). It was also shown that *C. sonorensis* infected through feeding on vaccinated sheep was able to transmit attenuated virus to susceptible animals (Foster *et al.* 1968). Similarly, the results obtained for AHSV show that at least 7 South African livestock-associated *Culicoides* species, belonging to more than 6 subgenera, are susceptible to infection with attenuated strains of the virus (Paweska *et al.* 2003).

Although low recovery rates of the European BTV strains from *C. imicola* and *C. bolitinos*, as well as the differences found in the oral susceptibility for isolates of the same serotypes, suggest co-adaptation between orbiviruses and vectors in a given locality, co-adaption was shown not to be an essential prerequisite for virus transmission.

Neither oral susceptibility, as demonstrated in the laboratory, nor virus isolation/detection from field-collected specimens is proof that a species is a confirmed field vector of BTV. Although virus detection from field specimens indicates that the species involved may indeed have fed on a viraemic animal, it does not prove virus replication or potential transmission. Despite being genetically controlled, vector competence and oral susceptibly are influenced by a number of intrinsic and extrinsic factors, *e.g.* environmental temperature, co-infections with other viruses and parasites, and level of viraemia in the host. Vector competence is not constant and can change over time and between populations. In evaluating these results, it must be taken into consideration that studies were restricted to 2 areas in South Africa. Given the wide geographic distribution of *C. imicola* and the number of extrinsic factors influencing competence, the extent to which these results will be applicable to other populations still needs to be determined. Real-time monitoring of vector competence might be difficult, as it would require assessing local *Culicoides* populations using variants of orbiviruses currently in circulation.

A consistently higher infection in *C. bolitinos*, compared to that of *C. imicola*, linked to a close relationship with bovines and other livestock, leaves little doubt that this species will play a role in the transmission of BTV (Mellor *et al.* 2000). However, it still needs to be demonstrated that this species will transmit virus to susceptible hosts. All infection prevalence work in South Africa was done with field-collected material. As different field studies focus on specific geo-temporal conditions, it is difficult to compare results among different studies.

The availability of a colony will enable a more reliable determination of infection prevalence and a more precise assessment of the factors that may influence it. The high numbers of *C. imicola* found in light traps and the inclination of this species to feed under laboratory conditions will make it an ideal candidate for colonization. Taking into account the apparent discrepancies between light trap results and attack rate on animal hosts (Gerry *et al.* 2009, Scheffer *et al.* 2012, Viennet *et al.* 2013), it will be essential to determine the attack rate of *C. imicola* and other South African *Culicoides* species on livestock. It is, however, clear that there is a crucial need for standardization (terminology/definitions, feeding, virus detection methods and interpretation of infection and light trap results).

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