

# Multiple vectors and their differing ecologies: observations on two bluetongue and African horse sickness vector *Culicoides* species in South Africa

R. Meiswinkel<sup>(1)</sup>, K. Labuschagne<sup>(2)</sup>, M. Baylis<sup>(3)</sup> & P.S. Mellor<sup>(3)</sup>

(1) Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise 'G. Caporale', via Campo Boario, 64100 Teramo, Italy

(2) Onderstepoort Veterinary Institute, Private Bag X05, Onderstepoort 0110, South Africa

(3) Institute for Animal Health, Pirbright Laboratory, Pirbright, Surrey GU24 0NF, United Kingdom

## Summary

Blacklight traps were used to collect *Culicoides* biting midges weekly between September 1996 and August 1998 at 40 sites distributed equidistantly across South Africa. The seasonal and geographic prevalences of 86 species of *Culicoides* were elucidated simultaneously, and included *C. imicola* Kieffer and *C. bolitinos* Meiswinkel the principal vectors of bluetongue (BT) and African horse sickness (AHS) in the region. These two species were amongst the most prevalent *Culicoides* to be found and, together, comprised >50% of the more than three million biting midges captured. The data are presented as coloured matrices, and are transformed also into inverse distance weighting (IDW) interpolative maps. The data reveal that the prevalence of each vector is somewhat fractured and it is posited that this is (in part) due to significant differences in their respective breeding habitats. The results illustrate also that the presence of multiple vectors (in any region of the world) will complicate both the epidemiology of the orbiviral diseases they transmit and the formulation of rational livestock movement and disease control strategies. This is especially true for southern Europe where the recent devastating cycle of BT has been shown to involve at least three vectors. Finally, the influence that man has on the development of large foci of vector *Culicoides* around livestock may be less important than previously suggested but must be investigated further.

## Keywords

*Culicoides* vectors – *Culicoides imicola* – *Culicoides bolitinos* – Seasonal distribution – Geographic distribution – Interpolative maps – South Africa – Vector.

## Introduction

Pantropically some 30 species of *Culicoides* biting midges are involved in the transmission of orbiviral diseases injurious to livestock; these diseases include bluetongue (BT) and African horse sickness (AHS). Because of their negative economic impact there is a great need to model the distributions of the insect vectors involved, not only for disease control, but also for predictive purposes. To obtain data that are reliable, modellers depend heavily upon an accurate taxonomy as each vector species, in occupying a specific niche in nature, possesses a unique biology that determines its distribution in both space and time. However, accurate large-scale species-linked data sets are surprisingly few for *Culicoides*. Equally

cogent is that these diseases are, in all regions, being transmitted by more than one vector species. Not only does this complicate disease epidemiology, but also invalidates (partially) 'single vector' risk models.

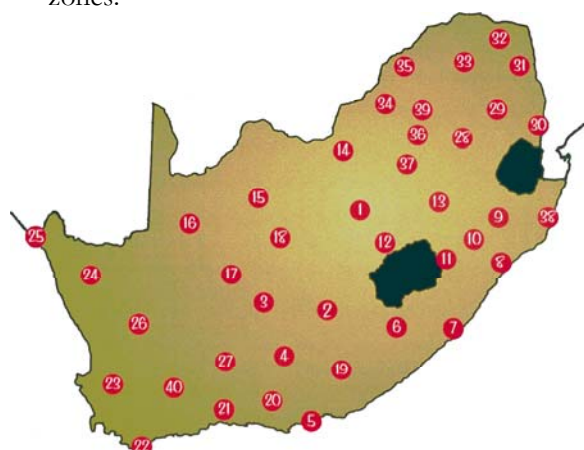
As to the involvement of multiple vectors, South Africa is a case in point: 60 years were to pass before it was realised that *C. imicola* is not the only vector of BT and AHS (5) but that *C. bolitinos* is also involved (11). To assess objectively whether the relative abundances of these two species, and their seasonal and geographic prevalences, differed significantly, a network of 40 blacklight traps was established and operated weekly across South Africa from September 1996 to August 1998. The data reveal the two species to occur widely but profound differences

in their respective ecologies impact significantly on their local prevalences and abundances. These data are used also to strengthen the argument that an unresolved taxonomy at the base of the decision-making pyramid will hobble the development of rational disease management strategies at the apex.

## Materials and methods

Blacklight traps of the type described by Venter and Meiswinkel (14) were used to capture *Culicoides*. The choice of the forty sites sampled across South Africa (Fig. 1) was determined by:

- 1) the need for equidistant monitoring
- 2) the necessary presence of livestock (of any species that included cattle, horses, sheep, goats and/or pigs)
- 3) the collection site being near (1-50 km) to an automatic weather station (AWS) maintained by the South African Weather Bureau
- 4) the need to sample a diverse range of habitats i.e. one (or two) sites in each of the 10 climatic zones of South Africa, and two (or more) sites in each of the seven broad ecological or vegetative zones.



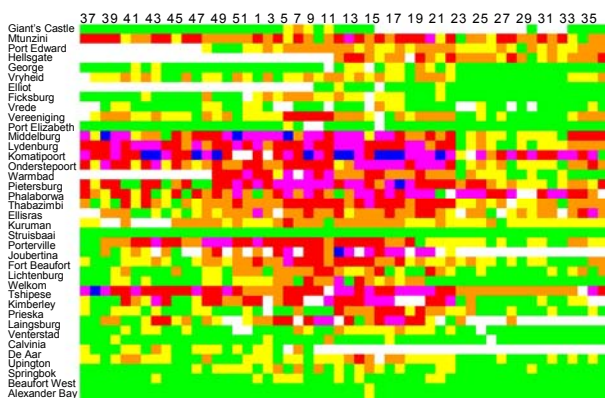
1 Welkom	21 George
2 Venterstad	22 Struisbaai
3 De Aar	23 Porterville
4 Graaff Reinet	24 Springbok
5 Port Elizabeth	25 Alexander Bay
6 Elliot	26 Calvinia
7 Port Edward	27 Beaufort West
8 Mtunzini	28 Middelburg
9 Vryheid	29 Lydenburg
10 Greytown	30 Komatipoort
11 Giant's Castle	31 Phalaborwa
12 Ficksburg	32 Tshipese
13 Vrede	33 Pietersburg
14 Lichtenburg	34 Thabazimbi
15 Kuruman	35 Ellisras
16 Upington	36 Onderstepoort
17 Prieska	37 Vereeniging
18 Kimberley	38 St Lucia
19 Fort Beaufort	39 Mabula
20 Joubertina	40 Laingsburg

Figure 1  
*Culicoides* sampling sites in South Africa, September 1996-August 1998

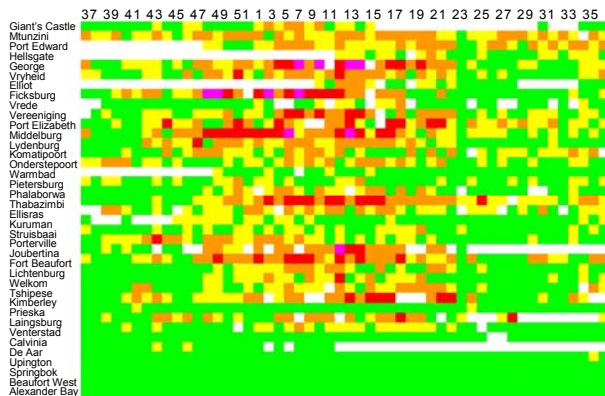
Light-trap collections were made weekly by the livestock owner whose co-operation was solicited first and, once obtained, he/she would be trained on site and provided immediately with the entire *Culicoides* collection kit. To promote sustained collaboration, owners were paid upon receipt of their light-trap collections (at six-weekly intervals) at the Onderstepoort Veterinary Institute (OVI). *Culicoides* species identifications were performed using a wing-picture atlas developed at the OVI over the last 20 years. Listed vertically in Figures 2 and 3 are 38 of the 40 sites sampled; the week number is listed horizontally (commencing 11 September 1996 [week 37] and ending in the first week of September 1997 [week 36]). In these figures the weekly abundances of the two vector species are depicted for the first year of the survey only, and are converted to  $\log(n+1)$ ; these abundances are colour-coded to aid rapid visual discernment of their geographical and seasonal prevalences: white (no collection made), green (0 *Culicoides*), yellow (1-9 *Culicoides*), orange (10-99), red (100-999), purple (1 000-9 999) and dark blue (10 000-99 999). The data were used to produce also the interpolation maps (Figs 4 and 5) with Mapinfo 7.5's inverse distance weighting (IDW) interpolator employing a distance-weighted average of data points to calculate grid cell values. Raw data were the greatest total catch of each species (males and females) made at any given site in a single night between October 1996 and September 1998. The IDW was undertaken with an exponent of 2 and a search radius of 200 km; the exponent was chosen arbitrarily, its low value increasing the influence of distant data points. The search radius was chosen to be about half of the distance between the two most distant neighbouring sites (400 km), thereby ensuring that all sites are subject to the influence of at least one other.

## Results

Of a projected 4 108 collections, 3 346 (81%) were eventually made, and included a total of >3 million *Culicoides*. These represented 86 species i.e. 70% of the >120 species of *Culicoides* known to occur in South Africa. The ten most abundant species (which together comprised 90% of the midges collected) were: *C. imicola* Kieffer (47.5%), *C. subschultzei* Cornet and Brunhes (9.8%), *C. magnus* Colaço (7.7%), *C. zuluensis* de Meillon (6.9%), *C. bolitinos* Meiswinkel (4.1%), *C. pycnostictus* Ingram and Macfie (3.9%), *C. leucostictus* Kieffer (3.6%), *C. nivosus* de Meillon (3.0%), *C. schultzei* Enderlein (2.4%) and *C. enderleini* Cornet and Brunhes (1.3%). These data show that the two principal vectors of BT and AHS, *C. imicola* and *C. bolitinos*, comprised >50% of all the *Culicoides* captured; they were also amongst the most prevalent



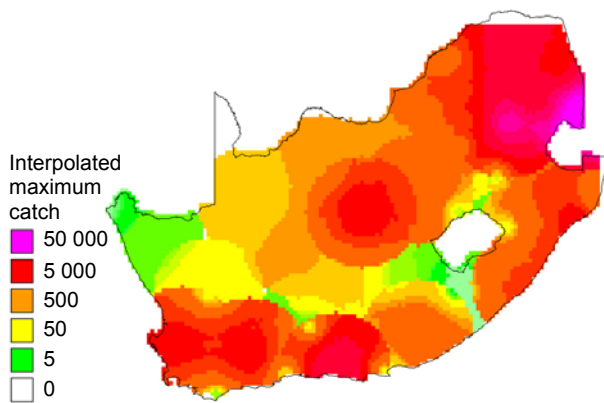
**Figure 2**  
*Culicoides imicola*: log(n+1) numbers captured weekly at 38 livestock holdings spread across South Africa between September 1996 (week 37) and September 1997 (week 36)  
 Sites are ranked from highest to lowest rainfall



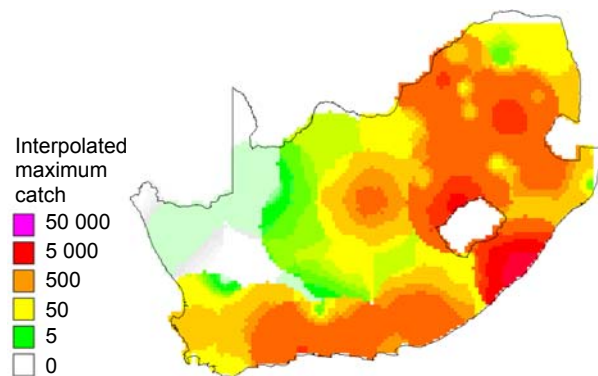
**Figure 3**  
*Culicoides bolitinos*: log(n+1) numbers captured weekly at 38 livestock holdings across South Africa between September 1996 (week 37) and September 1997 (week 36)  
 Sites are ranked from highest to lowest rainfall

species, being found at 39 and 38 of the 40 sites sampled, respectively.

In Figures 2 and 3 the collection sites are ranked according to average rainfall ranging from >1 200 mm/annum (sites 1-3: Giant's Castle to Port Edward) to <250 mm/annum (site 38; Alexander Bay). The pattern reveals *C. imicola* (Fig. 2) to occur most abundantly in those areas where the annual rainfall averages between 300-700 mm. This embraces largely the sweetveld grazing areas favoured for the raising of livestock, and previously famed for their teeming hordes of game animals (wildebeest, zebra, etc.). This pattern of prevalence suggests that the larvae of *C. imicola*, being soil-inhabiting, favour a breeding substrate that is not only moisture-retentive but one which is also rich in micro-nutrients; it is known that at annual average rainfall levels above 700 mm, such soils will become leached of nutrients, and so may help explain why *C. imicola* is virtually absent from the high rainfall



**Figure 4**  
 Inverse distance weighting interpolated maximum catches of *Culicoides imicola*, using a search radius of 200 km



**Figure 5**  
 Inverse distance weighting interpolated maximum catches of *Culicoides bolitinos*, using a search radius of 200 km

equatorial regions of Africa. Judging from the more randomised scatter of the high abundance collections of *C. bolitinos* (Fig. 3) it would appear that higher annual average precipitation levels influence less the prevalence of this cattle dung-inhabiting species.

*Culicoides imicola* was most abundant in the warmer northern and eastern areas of South Africa (sites Middelburg, Komatipoort and Tshipese); such areas have long been considered enzootic for orbiviral diseases, and so may act as source points for their southward spread. However, *C. imicola* was found abundantly also in the southern half of the country (Kimberley, Joubertina, Porterville); the latter two sites occur in the Cape Province where devastating outbreaks of AHS have occurred both in the past (1, 6) with smaller incursions more recently (8). Although *C. bolitinos* was as widespread as *C. imicola*, it was an order of magnitude less abundant. Also, and unlike *C. imicola*, it tended to predominate in cooler, higher-lying areas (site 12: Ficksburg), near where it has recently been linked unequivocally to significant outbreaks of AHS (11), and where BT can

recur almost annually. Of importance is that the latter areas mostly about those in which *C. imicola* predominates, and so this partial 'allopatry' in the geographic distributions of these two vector species effectively increases the total area at risk to orbivirus transmission in South Africa.

At a number of sites it was found that both species peaked in abundance and prevalence around week 12 (the end of March) which is mid-autumn in the southern hemisphere, and which marks (roughly) the annual commencement of the BT and the AHS 'seasons'. One prerequisite for a bloodsucking invertebrate to be classed as a vector is that its period of heightened abundance (and hence biting activity) should correlate with the appearance of the relevant disease in the vertebrate host that it may attack. Judging from the data both *C. imicola* and *C. bolitinos* fulfil this requirement. During this period, the parity rates of these two species were also at their highest seasonal levels (data not shown) i.e. the proportion of older females was found to peak also in the late summer/autumn months, and across the entire range of populations sampled. These older females are the ones to most likely harbour virus, and so transmit it to their ruminant hosts. The dual presence of two vectors with partially overlapping distribution patterns would suggest that during outbreaks the virus will (or could) be transmitted more widely.

It is a common belief that areas experiencing the coldest winters (including snow) are also those with the smallest populations of *C. imicola*. This is generally true (Giant's Castle and Elliot sites) but, unexpectedly, *C. imicola* was found to be even rarer (or entirely absent) at three sites (Port Elizabeth, Struisbaai and Alexander Bay) which possess a milder, frost-free, climate. These latter three sites occur along the southern and western coastline, and so, intuitively, one might be tempted to attribute the local absence of *C. imicola* at these sites to excessive windiness. However, the dominance of the second vector, *C. bolitinos*, at Port Elizabeth (site 5) would seem to mitigate against wind being an important inhibitor of *Culicoides* activity. Thus, it must be considered whether the sandiness of the soil (which will promote the rapid drainage of moisture from its top layer) is not the primary factor disrupting larval development in *C. imicola* locally (9, 10); if so this single edaphic 'negative' variable would cancel the effect of the more immediately obvious 'positive' variables (such as a frost-free climate, rainfall of 600 mm/annum, and abundance of livestock) and which would normally support the development of large populations of *C. imicola* at any given site. As hinted above, soil-type does not seem to act as a barrier to *C. bolitinos* due to its predilection for cattle

dung as a larval habitat. It is this predilection that enables *C. bolitinos* to penetrate into sandy areas, and also into steeply-sloped, mountainous terrain where *C. imicola* is unable to persist (in this instance presumably because of the aridification of the soils' top layer due to water runoff).

The interpolation maps (Figs 4 and 5) show (broadly) that *C. imicola* is more abundant than *C. bolitinos* almost everywhere (except around Lesotho, down to Port Edward and across to Port Elizabeth). They show also that *C. imicola* is present in two major clusters – the Cape Province and the north/north-east/east of South Africa; there is also a central cluster around Kimberley (site 18). As noted earlier this correlates well with the pattern of historical outbreaks (especially of AHS) in South Africa. Unfortunately, it means also that the AHS surveillance and protection zone, which is situated in the Western Cape Province (area south of site 23), can expect to be periodically affected by outbreaks of the disease, and is a supposition confirmed by the recent incursions of AHS into this zone in 1999 and 2004 (8). The interpolation maps show clearly that the hyper-arid north-western corner of South Africa (sites 24-26) are virtually vector free, and so would serve the country better as both export and quarantine zones.

The geographic distribution of these two vectors was found to be stable across the two seasons sampled (data not shown for the second season) and so might be harnessed as a predictive tool in risk analysis. However, there are important caveats that may profoundly influence modelling; some are natural cyclical phenomena, others are artificial (or man-induced). For example, *C. imicola* is the only species of afro-tropical *Culicoides* known to develop extraordinarily large populations during the episodic rains that affect South Africa every 10 to 15 years (10) correlating perfectly with the old adage that '...horse sickness appears as a plague following heavy rains...' (13). Not only must such climatic oscillations be factored into predictive risk maps, but, taken a step further, they imply also that the constant irrigation of grazing pastures (coupled with the maintenance of fenced livestock) not only interrupt natural wet-dry cycles but, also, may be causally linked to the establishment of extremely large (and artificial) foci of *C. imicola* locally. In the case of *C. bolitinos*, cattle husbandry may be inducing similar modifications to its 'natural' distribution across South Africa.

This study revealed that vector-free areas do exist in South Africa. One of these sites (Struisbaai) was surveyed because historical records (1) showed it to have escaped the ravages of the largest outbreak of

AHS that occurred in South Africa 150 years ago. Although Struisbaai was monitored weekly, not a single specimen of *C. imicola* was captured amongst 15 other species of *Culicoides* during 140 weeks of uninterrupted sampling. Can the absence of *C. imicola* be ascribed solely to the local soils being both sandy and calcareous? Soil type has been cited to not only explain the absence of *C. imicola* at specific points along the southern margin of its range (9, 10) but also at its northern end (4) in the region of Puglia, Italy, and where the prevailing climatic conditions would seem to favour its presence (3). These eye-catching local gaps in the distribution of *C. imicola* indicate strongly that this vector is not able to penetrate into all landscape zones (neither in the long term nor under changing climatic conditions). Although ameliorating climate change will have little foreseeable impact on local edaphic conditions, it would still facilitate the northward movement of *C. imicola* even if such a progression was to occur only patchily. Whatever the eventuality, if the *C. imicola*-free zones discovered in this survey were to be characterised precisely, and their distribution mapped across the Old World range of *C. imicola*, then vector-free enclaves could be identified. Whilst these would be of inestimable value for the quarantining and export of livestock, the absence of other competent vector *Culicoides* would still have to be assured. From Figure 3 it can be seen that the second vector, *C. bolitinos*, does occur in low numbers at Struisbaai and so introduces an element of risk. It is possible that *C. bolitinos* can be controlled (or even eliminated) locally if all cattle were to be removed. For *C. imicola*, however, its control through environmental management would appear to be well-nigh impossible to achieve. This is because it breeds in moist (and clay-type soils) which become widely available following periods of rainfall, and also under sustained irrigation, which is practised extensively on many thousands of farms throughout South Africa.

## Conclusions

With objective monitoring an immense amount of valuable data on the distribution and seasonality of *Culicoides* can be collected quite rapidly from across an extensive geographic transect. Furthermore, if the taxonomy of the local *Culicoides* fauna is resolved, then data on 50 species or more will be obtained simultaneously. Not only are such data indispensable for predictive modelling, but they arm us also with a more precise understanding of the ecological niche occupied by each species. These can help us to track changes (if any) in the dispersal pattern of any given species over time or to monitor changing prevalence amplitudes under various climatic circumstances.

Had a similar surveillance system been present in Europe, there would be a much stronger evidence base for assessing whether *C. imicola* has recently spread across the Mediterranean region.

Another hypothesis to emerge recently is that the distribution of *C. imicola* is determined not only by climate but also (and fundamentally) by soil type as it has been found to be consistently absent from sandy (and calcareous) areas which appear otherwise suitable, i.e. which possess abundant livestock and an equable climate. For *C. bolitinos*, the scenario is different: its local prevalence does not seem linked to soil but more intimately to that of cattle, buffalo and wildebeest (their dung being essential to the development of its immature stages). Thus, while some climatic variables may favour equally the adult activity preferences of both *C. imicola* and *C. bolitinos*, their geographic distributions can still differ dramatically due to their vastly different breeding habitats. These differences should prompt modellers to incorporate a separate range of 'breeding habitat' variables because, stated simply, if the larvae are not able to mature then no adult midges can emerge to fly.

The IDW maps, though broadly accurate, have shortcomings. For example, *C. imicola* from being abundant at one site can disappear at another over a fairly short distance as illustrated by the respective data (Fig. 2) collected at Struisbaai (site 22) and at Porterville (site 23). Although this heterogeneity was captured on the IDW map (Fig. 4), sampling should be performed at a finer scale (of about 50 km) to improve the resolution at a more localised level. However, this would increase the number of trapping stations (and subsequent laboratory analyses) six-fold. Clearly then, these maps are crude and show the greater value of using satellite imagery with scales down to 8 km or 1 km, and using those types of satellite data that correlate with the insect catches (using climate and soil proxies). Such a satellite map has been produced for *C. imicola* in South Africa (2) but was found also to have a limited predictive capacity probably because not all factors influencing the local distribution of *C. imicola* had been considered. Ultimately, it is necessary that the distributional data on insect vectors be highly detailed as livestock owners, especially those involved in the regular movement of animals, require information that is applicable (and reliable) at the farm level. In an AHS- and BT-endemic region such as South Africa this implies that each farmer should consider assessing the *Culicoides* vector situation on his/her holding, rather than relying on the broader 'fuzziness' of current risk maps.

Previously (9, 10), it was mooted that man, by husbanding livestock in confined spaces ('sedentary bloodbanks'), and on grazing pastures that are irrigated constantly, helps establish and maintain localised foci of both *C. imicola* and *C. bolitinos*. If so, it forces one to consider whether the currently mapped distribution of these two vectors are therefore somewhat artificial, and if this is the case, how then should man's influence be calibrated for its factoring into predictive risk models? It is not possible to provide the answer here. All that may be said is that the present data would indicate that the role of man should perhaps not be exaggerated because although both vector species were found to occur almost throughout South Africa, there was enormous geographic variability in their respective abundance levels and seasonal prevalence patterns despite the universal presence of livestock at all trapping sites. Thus we can fairly safely interpret man's impact to be but minor when compared to that exerted by climate and by soil.

Ultimately it is the presence of the insect vector that places a given area at risk to disease incursion and maintenance. As illustrated above, the ecologies of *C. imicola* and *C. bolitinos* differ markedly, and so increases the total area at risk. Furthermore, these patterns of vector prevalence will never be entirely annectant but will overlap; this applies particularly to southern Europe where the distribution of *C. imicola*, to a varying extent, overlaps that of the more northerly additional vector species of the *Obsoletus* and *Pulicaris* species complexes (7). This zone of overlap will serve to facilitate the further movement (and maintenance) of the virus and so complicates the development of rational livestock movement and disease control strategies.

Multiple vectors exist in nearly all regions of the world affected by *Culicoides*-borne orbiviral diseases. Added to this is the concern that other, and more prevalent 'non-vector' species, may possess a nascent vectorial competence that will emerge only in the future under changing climatic conditions. These possibilities warn us that vector surveillance studies should be planned with a view to mapping the distributions of all species of *Culicoides* simultaneously, and especially in those instances where the disease transmission potentials of 'suspected' vectors still remain unknown. This is especially true for the Mediterranean Basin, where the taxonomy of the 100 or more species of *Culicoides* is incompletely resolved (12), and where an enormous body of work (both in the laboratory and in the field) is still needed to understand which species are most competent at transmitting the various diseases that threaten the livestock industry there. As long as the taxonomy, and the precise

ecologies, of vector *Culicoides* in Europe remain unclear, we can expect to make only limited progress in terms of predictive risk modelling and in the development of rational control strategies.

## References

1. Bayley T.B. (1856). – Notes on the horse-sickness at the Cape of Good Hope, in 1854-'55. Saul Solomon & Co., Steam Printing Office, Cape Town.
2. Baylis M., Meiswinkel R. & Venter G.J. (1999). – A preliminary attempt to use climate data and satellite imagery to model the abundance and distribution of *Culicoides imicola* (Diptera: Ceratopogonidae) in southern Africa. *J. Sth Afr. Vet. Ass.*, **70**, 80-89.
3. Baylis M., Mellor P.S., Wittmann E.J. & Rogers D.J. (2001). – Prediction of areas around the Mediterranean at risk for bluetongue by modelling the distribution of its vector using satellite imaging. *Vet. Rec.*, **149**, 639-643.
4. Conte A., Ippoliti C., Calistri P., Pelini S., Savini L., Salini R., Goffredo M. & Meiswinkel R. (2004). – Towards the identification of potential infectious sites for bluetongue in Italy: a spatial analysis approach based on the distribution of *Culicoides imicola*. In *Bluetongue, Part I* (N.J. MacLachlan & J.E. Pearson, eds). Proc. Third International Symposium, Taormina, 26-29 October 2003. *Vet. Ital.*, **40** (3), 311-315.
5. Du Toit R.M. (1944). – The transmission of bluetongue and horse-sickness by *Culicoides*. *Onderstepoort J. Vet. Sc. Anim. Ind.*, **19**, 7-16.
6. Edington A. (1893). – Report of the Colonial bacteriological Institute for the year 1892, Part II. W.A. Richards & Sons, Government Printers, Cape Town.
7. Goffredo M., Conte A. & Meiswinkel R. (2004). – Distribution and abundance of *Culicoides imicola*, *Obsoletus* Complex and *Pulicaris* Complex (Diptera: Ceratopogonidae) in Italy. In *Bluetongue, Part I* (N.J. MacLachlan & J.E. Pearson, eds). Proc. Third International Symposium, Taormina, 26-29 October 2003. *Vet. Ital.*, **40** (3), 270-273.
8. Koekemoer J.J.O., Paweska J.T., Pretorius P.J. & van Dijk A.A. (2003). – VP2 gene phylogenetic characterization of field isolates of African horsesickness virus serotype 7 circulating in South Africa during the time of the 1999 African horsesickness outbreak in the Western Cape. *Virus Res.*, **93**, 159-167.
9. Meiswinkel R. (1997). – Discovery of a *Culicoides imicola*-free zone in South Africa: preliminary notes and potential significance. *Onderstepoort J. Vet. Res.*, **64**, 81-86.
10. Meiswinkel R. (1998). – The 1996 outbreak of African horse sickness in South Africa – the entomological perspective. *Arch. Virol.* [Suppl.], **14**, 69-83.

11. Meiswinkel R. & Paweska J.T. (2003). – Evidence for a new field *Culicoides* vector of African horse sickness in South Africa. *Prev. Vet. Med.*, **60**, 243-253.
12. Meiswinkel R., Gomulski L.M., Delécolle J.-C., Goffredo M. & Gasperi G. (2004). – The taxonomy of *Culicoides* vector complexes – unfinished business. In *Bluetongue, Part I* (N.J. MacLachlan & J.E. Pearson, eds). Proc. Third International Symposium, Taormina, 26-29 October 2003. *Vet. Ital.*, **40** (3), 151-159.
13. Theiler A. (1921). – African horse sickness (*Pestis equorum*). Department of Agriculture Science Bulletin No. 19. The Government Printing and Stationery Office, Pretoria.
14. Venter G.J. & Meiswinkel R. (1994). – The virtual absence of *Culicoides imicola* (Diptera: Ceratopogonidae) in a light-trap survey of the colder, high-lying area of the eastern Orange Free State, South Africa, and implications for the transmission of arboviruses. *Onderstepoort J. Vet. Res.*, **61**, 327-340.