Culicoides species abundance and potential over-wintering of African horse sickness virus in the Onderstepoort area, Gauteng, South Africa

In South Africa, outbreaks of African horse sickness (AHS) occur in summer; no cases are reported in winter, from July to September. The AHS virus (AHSV) is transmitted almost exclusively by Culicoides midges (Diptera: Ceratopogonidae), of which Culicoides imicola is considered to be the most important vector. The over-wintering mechanism of AHSV is unknown. In this study, more than 500 000 Culicoides midges belonging to at least 26 species were collected in 88 light traps at weekly intervals between July 2010 and September 2011 near horses in the Onderstepoort area of South Africa. The dominant species was C. imicola. Despite relatively low temperatures and frost, at least 17 species, including C. imicola, were collected throughout winter (June–August). Although the mean number of midges per night fell from > 50 000 (March) to < 100 (July and August), no midge-free periods were found. This study, using virus isolation on cell cultures and a reverse transcriptase polymerase chain reaction (RT-PCR) assay, confirmed low infection prevalence in field midges and that the detection of virus correlated to high numbers. Although no virus was detected during this winter period, continuous adult activity indicated that transmission can potentially occur. The absence of AHSV in the midges during winter can be ascribed to the relatively low numbers collected coupled to low infection prevalence, low virus replication rates and low virus titres in the potentially infected midges. Cases of AHS in susceptible animals are likely to start as soon as Culicoides populations reach a critical level.

Introduction

African horse sickness (AHS), an arthropod-borne viral disease affecting all equines, is considered to be the most lethal viral disease of horses. The agent, AHS virus (AHSV), a double-stranded ribonucleic acid (RNA) virus in the genus Orbivirus (Reoviridae), is enzootic in sub-Saharan Africa. Outbreaks can occur, however, well beyond this region (Coetzer & Guthrie 2004; MacLachlan & Guthrie 2010). All nine serotypes (Howell 1962; McIntosh 1958) are transmitted almost exclusively by certain species of blood feeding flies, 1 mm – 3 mm in size, of the genus Culicoides (Diptera: Ceratopogonidae) (Wilson et al. 2009). The geographic distribution and seasonal incidence of AHSV depend not only on the presence of the virus and susceptible equines but also on the presence and abundance of competent vectors (Mellor, Boorman & Baylis 2000). As a result of its host preference for larger mammals, wide geographic distribution, abundance in light trap collections and isolation of AHSV and other viruses from field-collected specimens, Culicoides imicola is considered to be the principal vector of orbiviruses of livestock in southern Africa (Meiswinkel, Venter & Nevill 2004; Nevill, Venter & Edwardes 1992).

In South Africa, AHS is endemic in the frost-free north-eastern parts of the country, where it is maintained in zebra (Equus burchelli) populations that foal throughout the year (Barnard 1993). Although the first cases of AHSV for a particular season are recently often reported from Gauteng, KwaZulu-Natal or even the Eastern Cape (AHS Trust 2012), it is still generally accepted that seasonal epidemics spread from the Kruger National Park and adjacent parks moving south and westwards (Coetzer & Guthrie 2004). The extent of these annual AHS epidemics is dependent on favourable climatic conditions that will increase breeding and spread of the vector (Coetzer & Guthrie 2004). Epidemiological data from 1955 to 1995 showed that outbreaks occurred nearly every summer in the subtropical northern parts of South Africa, once every 5–10 years in the cooler centre of the country, and only rarely in the Western Cape in the south (Bosman, Brückner & Faul 1995). Outbreaks are usually associated with large C. imicola populations (Venter, Koekemoer & Pawska 2006), with a peak in the latter half of summer and no cases reported between July and


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September. Despite the interruptions in normal transmission, AHSV is able to persist for long periods during adverse conditions without new cases. These absences of up to eleven and a half months can create the deceptive impression that an outbreak has ended (Wilson et al. 2009).

Based on diagnostic results, outbreaks of AHS have increased over the last decade and tend to occur earlier than usual in most parts of South Africa. For example, during 2012/2013 cases of AHS were detected in Gauteng in November 2012, at least 2 months before cases were reported from the more northerly areas of South Africa (AHS Trust 2012).

The objective of this study was to monitor the seasonal abundance of Culicoides midges in the Onderstepoort area, Gauteng, South Africa and to determine if AHSV can potentially over-winter in adult populations. The presence of adult midges and over-wintering of AHSV will imply that the virus circulates all year long in the area and does not need to be introduced annually from more subtropical regions.

Materials and methods

Collection site

The Agricultural Research Council – Onderstepoort Veterinary Institute (ARC-OVI) (25°39’S, 28°11’E, 1219 m.a.s.l.) is situated in the Gauteng province of South Africa. There is a mean of 32.8 days when the minimum temperature is below freezing point, and a further 70.8 days when it is below 5 °C. It is a summer rainfall area with an annual mean rainfall of 706 mm (Venter et al. 2004). The predominantly black cotton soils, characteristic of the Onderstepoort area, are an ideal breeding habitat for C. imicola (Meiswinkel et al. 2004).

A single light trap was placed underneath the eaves of a stable building between 20 and 30 horses at night. The stable was surrounded by open camps, characterised by some trees and patches of grass, where an additional 10–15 horses were kept. There were other horses in open camps 100 m – 200 m away. Daily maximum and minimum outside temperatures at the light trap were recorded using a Tinytag Explorer (Gemini Data Loggers UK Ltd.).

Insect collection

An 8-watt 220-volt down-draught Onderstepoort black light trap was used to collect adult Culicoides midges (Venter et al. 2009). Trapping was conducted one night a week from July 2010 to September 2011. Frequency of collection was increased (up to 5 nights per week) during the colder winter months (July and August 2011) (Table 1). The maximum collected in a single night was 71.3 midges through winter (Figure 1; Table 1). Although Culicoides midges were absent from the traps on 04–06 July and 26–27 July 2011, the mean for 20 collections in July was 71.3 midges per night (Table 1). The mean for 20 collections in July was 71.3 midges per night (Table 1). The maximum collected in a single night during July was 240.

Culicoides midges were collected in phosphate buffered saline (PBS) to which 0.5% Savlon® antiseptic (Johnson & Johnson, South Africa), containing chlorhexidine gluconate 0.3 g/100 mL and cetrimide 3.0 g/100 mL, had been added (Walker & Boreham 1976). Collections were retrieved the following morning and the insects washed with PBS and stored in PBS at 4 °C until analysed. Large collections were subsampled (Van Ark & Meiswinkel 1992). Based on abdominal pigmentation (Dyce 1969), the females of all species were age-graded into unpigmented (nulliparous), pigmented (parous), gravid or freshly blood-fed. Males were also counted. After the Culicoides midges were analysed down to species level and age-graded, they were removed from the PBS and stored in 1.5 mL vials at -80 °C until assayed for the presence of AHSV.

Virus detection

Virus detection was done on the weekly Culicoides collections using baby hamster kidney cell cultures (BHK-21) as well as by molecular methods. Virus isolation from pools of field-collected midges was done as described previously (Venter et al. 2006). Insects were homogenised (Precellys Lysing Kit CK 14, Anatech, South Africa) and tested for the presence of AHSV RNA by reverse transcriptase polymerase chain reaction (RT-PCR) according to a published protocol (Quan et al. 2010).

Ethical considerations

Materials used in the experiment posed no health risk to researchers and no vertebrate animals were harmed. The project was approved and financially supported by the Gauteng Department of Agriculture and Rural Development (project number: OV07/23/C231).

Results

Midges were collected weekly for 15 months (from end of July 2010 to beginning of September 2011) and more than 500 000 were collected in 88 collections (Table 1). The monthly mean per night ranged from 67.0 in July 2010 to 52 434.6 in March 2011 and the overall mean for the period was 5747.8 midges per night (Table 1). Relatively high numbers were found throughout winter (Figure 1; Table 1). Although Culicoides midges were absent from the traps on 04–06 July and 26–27 July 2011, the mean for 20 collections in July was 71.3 midges per night (Table 1). The maximum collected in a single night during July was 240.
The minimum monthly temperature recorded at the trap ranged from -3.7 °C in June to 13.5 °C in December (Figure 2). The maximum monthly temperature recorded ranged from 25.8 °C in June to 42.0 °C in December (Figure 2). In June and July 2011, there were at least 17 nights when the minimum temperature dropped to below 0 °C (-4.5 °C).

Culicoides midges belonging to 26 different species were collected (Table 1). Culicoides imicola was dominant throughout the period (Table 1). The mean numbers of C. imicola collected per night ranged from 58.5 in July 2011 to 48 650.4 in March 2011 (Table 1). Its representation ranged from 74.9% in January 2011 to 96.2% in August 2010 whilst its mean representation was 90.8% (Table 1). The second most abundant species, Culicoides enderienei, accounted for 2.3% of the total number (Table 1) and its highest mean number (1598.6) was collected in March 2011 (Table 1). This species was absent from the collections made in July (Table 1). Culicoides nevilli, at 1.4%, was the third most abundant (Table 1). In the table, where Culicoides species numbers appear instead of a species name, the numbering system of Meiswinkel (1995) was followed to refer to as yet undescribed species.

Parous females (females that have had at least one blood meal and completed one gonotrophic cycle and therefore potentially infected with AHSV) were present throughout the year (Figure 1). Their mean number ranged from 13 361.4 collected in March 2011 (Table 1) and its mean representation was 90.8% (Table 1). The second most abundant species, Culicoides enderienei, accounted for 2.3% of the total number (Table 1) and its highest mean number (1598.6) was collected in March 2011 (Table 1). This species was absent from the collections made in July (Table 1). Culicoides nevilli, at 1.4%, was the third most abundant (Table 1). In the table, where Culicoides species numbers appear instead of a species name, the numbering system of Meiswinkel (1995) was followed to refer to as yet undescribed species.

During December and January, Culicoides midges belonging to at least 23 species were collected (Table 1) whilst during June and July 2011 the number of species present dropped to 10 (Table 1). Despite the lower species richness, midges belonging to at least 17 species were present throughout winter (from June to August) (Table 1). Even if only parous females are taken into account, a minimum of 13 species remained throughout winter.

Virus detection

Virus isolation, using cell culture, was attempted on all 60 weekly pools (Figure 1). After three passages on BHK-21 cells, viable virus was only recovered from four pools. These viruses were not typed or further identified. These midges were collected in summer during the nights of 08-09 December 2010 (pool size n = 1770), 10–11 January 2011 (n = 1514), 01–02 February 2011 (n = 10 728) and 22–23 March 2011 (n = 13 519). These positive isolations correlate well with relatively high numbers of midges (> 1000) (Figure 1) and the seasonal occurrence of AHSV in the Onderstepoort area.

Representative samples covering the 15-month collection period were subjected to RT-PCR. Viral nucleic acid was detected in 12 of 35 pools assayed (Figure 1) with cycle threshold (CT) values ranging between 26.20 and 39.02. The first RT-PCR positive pools were found early in October 2010 (Figure 1). Virus was detected in one of three pools assayed in November and all 11 pools tested between December 2010 and May 2011, except for one pool in January, were positive (Figure 1). No positive pools were found after 24 May and this related to a drop in the mean catch size and proportion of parous individuals (Figure 1). As no viral nucleic acid was detected in any pool of < 3500 midges, positive RT-PCR results, as for virus isolation, seemed to correlate with high numbers of midges (Figure 1).

Discussion

Despite the fact that there were nights when the minimum temperature at the collection site was well below freezing point (-4.5 °C) with frost occurring, nulliparous, parous, freshly blood-fed and gravid females as well as males were present throughout the sampling period. Culicoides imicola, considered a proven vector of AHSV, was found throughout the year and no midge-free periods were recorded. Although the mean number collected per night dropped from > 50 000 in March to < 100 in July and August, midges were present each week. The maximum number of C. imicola collected in July was 189 individuals, of which 188 were females, including 23 parous ones. The presence of males indicates that breeding continued throughout winter. The presence of freshly blood-fed females of most species, including C. imicola, in winter implies that blood feeding and the transmission of AHSV can potentially continue throughout winter. The results of this study, done near horses, is supported by previous work conducted near livestock at Onderstepoort (Nevill 1971; Venter, Nevill & Van der Linde 1997) and also near a variety of caged wild animals at the nearby National Zoological Gardens of South Africa in Pretoria (Labuschagne 2007). These studies also showed that adult midges could be present throughout winter.
In the absence of transovarial transmission (Jones & Foster 1971; Nunamaker et al. 1990), competent Culicoides females only become infected after feeding on a viraemic host. Once infected systemically, a competent female will remain persistently infected, and be able to transmit virus with each subsequent blood meal, until she dies (Mellor et al. 2000). Adult Culicoides females have an average life span of < 20 days, but depending on the environmental conditions can survive up to 90 days (Mellor et al. 2000). Culicoides imicola adults can survive for more than 15 days and some species, for example Culicoides psenonstictus, up to 54 days at temperatures as low as -1.5 °C (Nevill 1971). Adult longevity increases with a decrease in ambient temperature (Gerry & Mullens 2000; Hunt, Tabachnick & McKinnon 1989; Lysyk & Danyk 2007; Wellby et al. 1996). In C. imicola, the number of days from blood feeding to ovipositing and subsequent blood feeding can increase from between 2 and 13 days at 28 °C to between 19 and 27 days at 20 °C (Veronesi & Danyk 2007). Comparably with the results found for AHSV and Culicoides sonorensis (Mellor et al. 1998; Wellby et al. 1996), C. imicola and C. bovinus can harbour bluetongue virus at low levels for more than 20 days at 10 °C and replicate the virus to transmittable levels when the ambient temperatures increase (Carpenter et al. 2011; Paweska, Venter & Mellor 2002). It is possible that Culicoides females infected with AHSV can survive throughout winter.

The relatively low virus isolation rates in field-collected Culicoides midges in the present study correspond with previous work done in South Africa (Venter et al. 2006). Even during AHSV outbreaks, the infection prevalence in field-collected C. imicola was calculated to be as low as 0.003% (Venter et al. 2006). In most outbreak situations, however, the superabundance of C. imicola will compensate for the relatively low infection prevalence (Venter et al. 2006). Even during outbreak situations the number of viraemic equines with a sufficiently high virus titre in the blood to infect midges in an area will be much less abundant compared with the number of non-viraemic mammals from which midges can take blood. Relatively low infection prevalence in vector species collected in the field is therefore typical of arbovirus infections (Walter, Hildreth & Beaty 1980). Considering that no virus was detected in any pool < 1000 midges, it is possible that the numbers of parous females assayed during winter may have been too low for detection with the techniques used. The minimum temperature required for the replication of orbiviruses, including AHSV, in the Culicoides vector was determined to be between 11 °C and 13 °C (Carpenter et al. 1996). In most outbreak situations, however, the superabundance of C. imicola will compensate for the relatively low infection prevalence (Venter et al. 2006).
Because of the low winter temperatures, the rate of virus replication and consequently the titre in these potentially infected midges would also have been low (Mellor et al. 1998; Paweska et al. 2002; Wellby et al. 1996).

The number of species found, 27 in summer and 17 in winter, each with a largely unique and mostly unstudied biology and largely unknown vector capacity and competence, will complicate the epidemiology of AHS in the area. Most of the species collected in winter were shown to either blood-feed on horses (C. imicola, Culicoides magnus, C. nevilli, C. pygostictus, Culicoides zuluensis, C. bolitinos, Culicoides gulbenkiani and C. enderleini) or to breed in horse dung (C. sp.# 54 d.f. [dark form] and C. sp.# 107) (Meiswinkel et al. 2004; Nevill, Nevill & Venter 2009). Based on the relative abundance countrywide, geographic distribution, virus isolation from field specimens, host preference and larval habitats, Nevill et al. (1992) classified all of these species, except for C. sp.# 54 d.f. and C. sp.# 107, as having a high vector potential for the transmission of orbiviruses.

The most abundant Culicoides species in an area will not inevitably be the most competent for a specific virus (Standfast, Dyce & Muller 1985). Less abundant species should therefore not be excluded from risk assessments. Cumulative laboratory oral susceptibility studies over the last decade indicate at least six of the these, namely C. imicola, C. magnus, C. nevilli, C. zuluensis, C. bolitinos and C. enderleini, to be orally susceptible to infection with AHSV (Paweska, Prinsloo & Venter 2003; Venter, Graham & Hamblin 2000; Venter et al. 2006; Venter & Paweska 2007). Laboratory susceptibility is supported by field isolations of AHSV from C. imicola and C. bolitinos (Meiswinkel & Paweska 2003; Nevill et al. 1992; Venter et al. 2006). For some of these species, oral susceptibility in the laboratory was significantly higher than that of C. imicola. Culicoides bolitinos, also present throughout the year, is considered to be a potential vector of AHSV (Meiswinkel & Paweska 2003; Mellor et al. 2000; Paweska et al. 2003; Venter et al. 2000, 2006; Venter & Paweska 2007). The highest numbers of this species were found from April to June and based on the number of parous females it was the third most abundant species.

In the evaluation of composition and numbers, it should be remembered that light traps do not sample diurnal Culicoides species. These, together with some Culicoides midges with a temporal shift in summer and winter activity (Nathan 1981; Viennet et al. 2012), including C. imicola (Braverman et al. 2003), may play a role in the transmission of AHSV. Mechanical aspiration of horses at Onderstepoort has shown that Culicoides females of different species can be collected from horses before sunset (Scheffer et al. 2012). This phenomenon can become even more pronounced in winter (Braverman et al. 2003; Viennet et al. 2012). Importantly, the attraction range of the light trap may be rather limited and only a relatively small portion of the active, blood-seeking females are intercepted (Venter et al. 2012). The exact size of this portion is not known but it could be < 0.0001% and is influenced by a great variety of environmental factors. Flight activity and therefore the efficiency of the light traps are suppressed at temperatures below 10 °C (Mellor et al. 2000).

Whilst the first cases of AHSV in South Africa during the study period were only confirmed in November 2010 (AHS Trust 2012), AHSV viral nucleic acid in field collections was detected as early as October 2010 by RTPCR. Although this may indicate over-wintering in adults, these detections may also represent the vaccine strains of AHSV. It has been shown that midges become infected and replicate the attenuated vaccine strains of AHSV (Venter & Paweska 2007). This early detection of AHSV, which may be field or vaccine, indicates that populations were large enough to become infected and transmit the virus.

The significantly higher number of positive polymerase chain reactions (PCRs) confirms this method to be more sensitive than cell cultures for the detection of AHSV. It should be remembered that whilst virus isolation will detect only viable virus, nucleic acid is detected by PCR. The detection of viral residues by PCR may indicate that at least a primary viraemia had occurred at some point (Quan et al. 2010). The preferred virus detection method used in a study will largely be dictated by the specific objectives of the study.

Conclusion

The results of this study indicate that, despite a sharp drop in numbers during winter, adult Culicoides midges of several species, including C. imicola, remain present and that blood-feeding and breeding continues throughout the winter in the Ondersteepoort area. The apparent absence of AHSV in the field-collected midges during winter can be ascribed to the relatively low numbers collected, low infection prevalence and low virus titres, all resulting from lower development rates, in the infected midges. The absence of vector-free periods implies that the virus does not need to be introduced from the warmer, frost-free north of the country. It can potentially be present in adult Culicoides midges all year long and outbreaks of AHS can therefore commence as soon as populations reach a critical level.

Acknowledgements

Competing interests

The authors declare that they have no financial or personal relationship(s) which may have inappropriately influenced them in writing this article.

Authors’ contributions

G.J.V. (Agricultural Research Council–Ondersteepoort Veterinary Institute) was responsible for the project design. S.N.B.B. (Agricultural Research Council–Ondersteepoort Veterinary Institute) was responsible for the collection of the insects. G.J.V., D.M. (Agricultural Research Council–Ondersteepoort Veterinary Institute) & K.L. (Agricultural Research Council–Ondersteepoort Veterinary Institute) did the Culicoides species analyses and age grading of the