A comparison of the susceptibility of the biting midge *Culicoides imicola* to infection with recent and historical isolates of African horse sickness virus

G. J. VENTER¹, I. M. WRIGHT² and J. T. P A WESKA³

¹Vectors and Vector-Borne Diseases Programme, Agricultural Research Council-Onderstepoort Veterinary Institute (ARC-OVI), Onderstepoort, South Africa, ²Molecular Epidemiology and Diagnostics Programme, ARC-OVI, Onderstepoort, South Africa and ³Department of Virology, Special Pathogens Unit, National Institute for Communicable Diseases, Sandringham, South Africa

Abstract. The susceptibility of *Culicoides* (Avaritia) *imicola* Kiefer (Diptera: Ceratopogonidae) to 21 isolates representing all nine known serotypes of African horse sickness virus (AHSV), recovered from clinical cases of the disease in South Africa during 1998–2004, was compared with its susceptibility to approximately 40-year-old isolates stored at the Agricultural Research Council-Onderstepoort Veterinary Institute. Field-collected *C. imicola* were fed through a chicken skin membrane on sheep blood spiked with one of the virus isolates to a concentration in the range of 5.6–7.5 log₁₀ TCID₅₀/mL. After 10 days incubation at 23.5 °C, five of the nine historical serotypes (AHSV-1, -2, -3, -7 and -9) could not be isolated from *C. imicola*. All nine serotypes were recovered for the 21 recent isolates, for 16 of which the virus recovery rates were higher than for the corresponding historical isolates. These results emphasize the need to assess the oral susceptibility of local *Culicoides* populations to viruses in circulation during outbreaks in order to estimate their vector potential.

Key words. *Culicoides imicola*, African horse sickness virus, oral susceptibility, South Africa.

Introduction

African horse sickness (AHS) is an infectious, non-contagious, arthropod-borne viral disease that can cause up to 90% mortality in susceptible horses. The disease has been present in Africa for many centuries. In South Africa, where no indigenous horses existed, it was first noticed in 1652 after the introduction of horses from Europe and the Far East to the Cape of Good Hope (Henning, 1956). The worst outbreak on record in South Africa is that of 1854–1855, when an estimated 40% (64 850) of the horse population succumbed to the disease (Bayley, 1856). Traditionally, AHS has been most prevalent in the northern parts of South Africa and major epidemics occur every 10–15 years. A strong link between the timing of these epidemics and the warm El Niño/Southern Oscillation (ENSO) has been demonstrated (Baylis et al., 1999). African horse sickness is listed by the Office Internationale des Épizooties (OIE) as a notifiable disease, which greatly impedes the movement of horses from South Africa to rest of the world.

All nine distinct serotypes of AHS virus (AHSV) are endemic in South Africa (McIntosh, 1958; Howell, 1962), but it appears that they are not equally abundant. Of the 280 diagnostic submissions to the AHS OIE Reference Centre at the Agricultural Research Council-Onderstepoort Veterinary Institute (ARC-OVI) during 1981–2005, 32.9% and 22.9% were diagnosed as serotypes 7 and 2, respectively, and only 3.2% as serotype 8. During outbreaks different serotypes circulate simultaneously within an area, but usually only one is found to dominate during a particular season (Coetzer & Erasmus, 1994). The epidemiological parameters determining
the prevalence and incidence of serotypes and serotype succession are unknown.

The virus is transmitted when infected *Culicoides* species (Diptera: Ceratopogonidae) feed on a susceptible host, but only a limited number of species are classified as competent vectors (Mellor & Hamblin, 2004). Based on its abundance near livestock and its wide geographical distribution, the most important vector is the Afro-Asiatic species *Culicoides (Avaritia) imicola* Kieffer. *Culicoides imicola*, considered a proven vector, is probably one of the world’s most widely distributed *Culicoides* species (Mellor et al., 2009). It is the most abundant livestock-associated *Culicoides* species in the summer and winter rainfall areas of South Africa and usually represents more than 90% of all *Culicoides* species collected (Venter et al., 1996, 2006). It can become superabundant in the warm, frost-free summer rainfall AHS endemic areas of the country (Meiswinkel, 1989; Venter et al., 1996). During outbreaks of the virus more than 1 000 000 midges can be collected in a single light trap collection (Meiswinkel et al., 2004).

Paweska et al. (2003) showed significant differences in the susceptibility of *C. imicola* to different serotypes of the attenuated strains of AHSV, but no correlation could be found between susceptibility and serotype abundance in the country. The purpose of the present study was to determine and compare the oral susceptibility of *C. imicola* to various isolates of all nine serotypes of the virus. As part of this comparison, the earliest typed isolates of AHSV were compared with more recent isolates recovered from clinical cases of the disease in South Africa during 1998–2004.

**Materials and methods**

**Viruses and insects**

Adult *Culicoides* midges were collected using 220-V ultraviolet light traps as described by Venter et al. (1998). Between December 2004 and April 2005, 36 different AHSV isolates were fed to *C. imicola* collected in the vicinity of about 30 stabled horses at the ARC-OVI, Onderstepoort (25° 29’ S, 28° 11’ E; 1219 m a.s.l.). All virus isolates were obtained from the OIE Reference Centre for AHS at the ARC-OVI. These isolates included the earliest typed isolates from the 1960s, as well as more recent isolates obtained from diagnostic field samples obtained during 1998–2004 (Table 1). Stocks of viruses for oral infection studies were grown in BHK-21 (baby hamster kidney-21) cells, titrated and stored as described previously (Venter et al., 1998).

**Insect feeding technique**

Field-collected flies were allowed an acclimatizing period of 2–3 days at 23.5 °C and relative humidity of 50–70%, after which they were fed on virus-infected blood through a chicken skin membrane (Venter et al., 1998). After a feeding period of 30–40 min, engorged females were separated out on a refrigerated table and incubated for 10 days at 23.5 °C. In addition, 67 and 177 midges fed on historical and recent isolates, respectively, were stored at −70 °C immediately after feeding. The engorged females were maintained on 5% (w/v) sucrose solution containing antibiotics (Venter & Paweska, 2007). After incubation, surviving *Culicoides* females were immobilized at −4 °C and *C. imicola* females were sorted out on a refrigerated table and stored individually in 1.5-mL microfuge tubes at −70 °C until assayed.

**Processing of Culicoides, virological assays and statistical analysis**

Individual midges were processed for virus microtitration assays on BHK-21 cells as described by Paweska et al. (2002) and the identity of all virus isolates determined by a micro-titre virus-neutralization procedure (House et al., 1990), using type-specific antisera produced in guinea pigs. Virus concentrations were calculated using the method described by Kärber (1931). Statistical differences between experimental groups were analysed using Fisher’s exact test. A *P*-value of <0.05 was used as the cut-off for statistical significance.

**Results**

**Historical isolates**

Between January and March 2005, 4737 field-collected midges were fed in 14 feeding attempts on the 40-year-old historical isolates of the virus stored at the OIE Reference Centre for AHS at the ARC-OVI. Virus concentrations in the bloodmeal were in the range of 6.1–7.5 log_{10} TCID_{50}/mL.
(Table 2). Feeding rates varied between 10% and 70%; 56.2% of engorged females survived incubation. Virus could be isolated from 48 of 67 (71.6%) *C. imicola* assayed immediately after feeding. The average virus concentration in midges from which virus could be isolated was 2.0 log_{10}TCID_{50}/midge.

Despite relatively high viral concentrations in the bloodmeal, five (serotypes 1, 2, 3, 7 and 9) of the nine historical isolates could not be recovered from *C. imicola* after incubation (Table 2). The highest recovery rate (48.4%) was that for serotype 6 in midges that had fed on a bloodmeal with a virus concentration of 7.5 log_{10}TCID_{50}/mL (Table 2). This recovery rate was significantly higher (*P* < 0.001) than that for serotype 5 (virus concentration 7.1 log_{10}TCID_{50}/mL of blood). When the virus concentration of serotype 6 in the bloodmeal was decreased 10-fold, the virus recovery rate dropped significantly (*P* < 0.001) to 1% (Table 2). No similar drop in virus recovery rate was found for serotype 5. The virus recovery rate obtained at 7.1 log_{10}TCID_{50}/mL of bloodmeal was not significantly different (*P* = 0.280) from that obtained at a virus concentration of 6.1 log_{10}TCID_{50}/mL of blood (Table 2).

If we consider only virus isolates which were fed at comparable concentrations (6.1–6.5 log_{10}TCID_{50}/mL) (Table 2), the recovery rate of serotype 4 appears to have been significantly higher than that of any of the other serotypes (*P* < 0.001 in all cases).

Average virus concentrations in midges from which virus could be isolated after incubation were in the range of 1.4–3.1 log_{10}TCID_{50}/midge (Table 2).

### Recent isolates

From December 2004 to April 2005, 9866 field-collected midges were fed in 36 separate feeding attempts on field isolates of the virus obtained from diagnostic field samples during 1998–2004 (Table 1). Feeding rates varied between 10% and 70%; 58.0% of engorged midges survived incubation. Virus concentrations in the bloodmeal were in the range of 5.6–6.3 log_{10}TCID_{50}/mL of blood (Table 2) and virus could be isolated from 87 of 177 (49.2%) midges assayed immediately after feeding. The average virus concentration in these 87 midges was 2.4 log_{10}TCID_{50}/midge.

Unlike the historical isolates, only one (isolate 29/03 of serotype 8) of the 21 field isolates could not be recovered from *C. imicola* after incubation (Table 2). Statistically significant differences in virus recovery rates were found for different serotypes of the virus. The highest virus recovery rate of 8.1%, obtained for isolate 10/04 of serotype 1 (Table 2), was not significantly higher than those for AHSV-3 76/04 (*P* = 0.086), AHSV-4 60/04 (*P* = 0.158), AHSV-5 88/99 (*P* = 0.005) or AHSV-8 88/99 (*P* = 0.865).

Similarly, just as differences were found between serotypes, significant differences in virus recovery rates were also seen between different isolates of the same serotype (Table 2). For serotype 1 the recovery rate of isolate 10/04 was significantly higher than that of isolate 33/04 (*P* < 0.001); for serotype 3 the recovery rate of isolate 32/03 was significantly higher than that of isolate 76/04 (*P* = 0.005), and for serotype 7 the recovery rate of isolate 18/99 was significantly higher than those

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**Table 2.** Virus recovery rates in field-collected *Culicoides* midges from Onderstepoort after 10 days incubation at 23.5 °C following feeding on blood containing African horse sickness virus (AHSV), from December 2004 to April 2005.

<table>
<thead>
<tr>
<th>AHSV serotype</th>
<th>Positive/tested, n (%)</th>
<th>Range in virus titre in infected midges, log_{10}TCID_{50}/midge (mean)</th>
<th>Virus titre of bloodmeal, log_{10}TCID_{50}/mL</th>
<th>Range in virus titre in infected midges, log_{10}TCID_{50}/midge (mean)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recent isolates</td>
<td></td>
<td>96.1</td>
<td>0/264</td>
<td>5.7</td>
</tr>
<tr>
<td>1</td>
<td>6.1</td>
<td>0/280</td>
<td>10/04</td>
<td>6.3</td>
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<tr>
<td>2</td>
<td>6.5</td>
<td>0/188</td>
<td>19/03</td>
<td>5.9</td>
</tr>
<tr>
<td>3</td>
<td>6.5</td>
<td>0/203</td>
<td>32/03</td>
<td>5.7</td>
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<tr>
<td>4</td>
<td>6.5</td>
<td>32/199 (16.1)</td>
<td>76/04</td>
<td>5.7</td>
</tr>
<tr>
<td>5</td>
<td>7.1</td>
<td>2/187 (1.1)</td>
<td>70/04</td>
<td>6.0</td>
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<tr>
<td>6</td>
<td>7.5</td>
<td>119/246 (48.4)</td>
<td>60/04</td>
<td>6.3</td>
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<tr>
<td>7</td>
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<td>2/200 (1.0)</td>
<td>70/04</td>
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<tr>
<td>8</td>
<td>6.5</td>
<td>1/225 (4.9)</td>
<td>26/03</td>
<td>6.2</td>
</tr>
<tr>
<td>9</td>
<td>6.5</td>
<td>0/164</td>
<td>0/199</td>
<td>5.8</td>
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<tr>
<td><strong>Historic isolates</strong></td>
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<td>0/262</td>
<td>5.7</td>
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<tr>
<td>1</td>
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of isolates 23/98 \((P = 0.048)\), 45/98 \((P = 0.006)\) and 93/99 \((P = 0.007)\).

Virus recovery rates in \(C.\ imicola\) for 16 of the 21 recent isolates were higher than for the corresponding historical isolates (Table 2). For five of these \(\text{AHSV-1} 10/04\ (P < 0.001)\), \(\text{AHSV-2} 19/03\ (P = 0.035)\), \(\text{AHSV-3} 76/04\ (P = 0.002)\), \(\text{AHSV-6} 26/03\ (P < 0.001)\), \(\text{AHSV-7} 18/99\ (P = 0.013)\) and \(\text{AHSV-9} 36/04\ (P = 0.013)\), virus recovery was significantly higher. For serotype 4, however, virus recovery of isolates 60/04 and 70/04 \((P < 0.001\) in both cases) was significantly lower than for its historical isolates. In addition, for serotype 8 the recovery rate of isolate 29/00 was significantly lower \((P < 0.001)\) than that of its historical isolate.

Average virus concentrations in midges from which virus could be isolated after incubation were in the range of \(0.9–2.7 \log_{10}\text{TCID}_{50}/\text{midge}\) (Table 2).

**Discussion**

The surprisingly low or negative virus recovery rates demonstrated in this study, especially for the historical isolates, indicate extremely low vector competence or even refractory status for the Onderstepoort \(C.\ imicola\) population for AHSV. In general very low concentrations of virus were found in infected midges, despite their oral challenge with relatively high doses of the virus. Virus concentrations in infected midges were mostly below levels that indicate a possible salivary gland infection, a prerequisite for potential onward transmission (Jennings & Mellor, 1987). In this context it is worth mentioning that \(C.\ imicola\) is also considered a proven vector of bluetongue virus (BTV), for which its low oral susceptibility (Venter et al., 2000; Paweska et al., 2003), significant differences were found in the oral susceptibility of \(C.\ imicola\) to the nine different serotypes of the virus (Table 2). Significant differences were, however, also found between geographically distinct isolates of the same serotype of the virus (Table 2). The relatively low virus recovery rates for the majority of the isolates used make it difficult to draw direct comparisons between isolates and to correlate these with field occurrences of these serotypes.

The lower susceptibility of \(C.\ imicola\) to the historical isolates, as determined in the present study, may be the result of co-adaptation between virus and vector over relatively long periods (Venter & Paweska, 2007). Vector competence is dependent upon the genetic makeup of the vector midge and is greatly influenced by a vast number of external environmental factors (Mellor et al., 2009). The present results indicate complex interactions between the virus and its potential host and stress the need to use orbiviruses currently in circulation in the assessment of vector competence in local \(Culicoides\) populations. Low susceptibility in the vector may play a key role in the evolution of the viruses it transmits. Future studies should therefore be directed towards investigating the biochemical and molecular mechanisms in both the virus and the vector that regulate oral susceptibility.

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**References**


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