Transmission potential of South African *Culicoides* species for live-attenuated bluetongue virus

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Summary

Field-collected *Culicoides* were fed on sheep blood-virus mixtures, each containing one of four live-attenuated vaccine strains of bluetongue virus (BTV), namely: BTV-1, BTV-4, BTV-9, and BTV-16. A South African field isolate of BTV-1 was used as the non-attenuated control virus. Titres of vaccine strains in blood meals ranged from 5.1 to 6.1 log<sub>10</sub>TCD<sub>50</sub>/ml; the titre of the field isolate of BTV-1 was 7.1 log<sub>10</sub>TCD<sub>50</sub>/ml. Recovery rates of vaccine viruses from *Culicoides* assayed immediately after feeding varied from 0% to 10.6%. This indicates that virus concentrations in blood meals were too low to ensure that all individuals ingested detectable amounts of virus. Thus, the oral susceptibility of *Culicoides* to infection with BTV vaccine strains determined in this study might be an underestimation. Of a total of 6 540 *Culicoides* that survived a 10-day extrinsic incubation period at 23.5°C, 124 tested positive for BTV; 65 individuals yielded vaccine strains, and the remaining 59, the field isolate of BTV-1.

Infection prevalences with the vaccine viruses ranged from 11.0% in *C. bolitinos* fed on blood containing 6.1 log<sub>10</sub>TCD<sub>50</sub>/ml of BTV-1 down to 0.3% in *C. imicola* fed on a blood containing 5.3 log<sub>10</sub>TCD<sub>50</sub>/ml of BTV-4. The infection rate for *C. imicola* and *C. bolitinos* fed on the field isolate of BTV-1 was 9.5% and 36.0%, respectively. In most infected midges the replication levels of vaccine strains were below the postulated threshold for a systemic infection with an orbivirus as previously calculated in the larger American vector, *C. sonorensis* (>2.5 log<sub>10</sub>TCD<sub>50</sub>/midge) but some individuals replicated BTV vaccine strains to high titres. This carries an implication that if ruminants become viraemic after vaccination with live-attenuated BTV vaccines, they might act as a source for the infection of *Culicoides* vectors.

Keywords


Introduction

A potential problem with current live-attenuated bluetongue virus (BTV) vaccines is that they can induce a low level of viraemia in vaccinated animals. Thus, there is the fear that vaccine viruses may reverse to virulence through passage in vectors, and then be transmitted in the field (16). *Culicoides sonorensis* infected through feeding on vaccinated sheep have been shown to transmit vaccine virus to susceptible animals (3). Another concern is that reassortment between vaccine and wild-type viruses may occur in the field and generate strains of different virulences (12). The fact that Théiler’s original BTV strain 4 has been used as a vaccine strain for 80 years without detectable antigenic drift and the absence of any evidence indicating that new serotypes have recently arisen suggests, however, that such events, should they occur at all, are likely to be very rare (15).

*Culicoides imicola* has been implicated as the major field vector of BTV in southern Africa (6, 7, 10) and southern Europe (2, 8). However, evidence is growing that other *Culicoides* species may also act as competent field vectors (7, 9, 14). In South Africa, it has been found that *C. bolitinos* supported replication of BTV-1,
BTV-3 and BTV-4 to higher levels (14) and that it had significantly higher vector competence for BTV-1 over a range of different incubation periods and temperatures than *C. imicola* (11). In addition, non-*Avaritia* South African *Culicoides* species such as *C. bedfordi*, *C. leucostictus*, *C. pycnostictus* and *C. milnei* were shown to be susceptible to BTV infection (11). In addition, at least three European *Culicoides* species namely: *C. melanocephalus*, *C. pulicaris* and *C. obsoletus* can become infected with BTV after feeding on viraemic sheep (7). Thus, data on vector competence of *Culicoides* midges for BTV live-attenuated vaccine strains are of importance for risk assessment analyses and particularly when intervention with live-attenuated vaccines is being considered in regions vulnerable to virus incursions.

The oral susceptibility and vector competence of South African livestock-associated *Culicoides* species for vaccine viruses that are included in the polyvalent BTV vaccine in current use has not yet been evaluated. The aim of this study was to determine the oral susceptibility of *C. imicola*, *C. bolitinos* and other livestock-associated *Culicoides* species to cell culture-attenuated BTV vaccine strains that are currently used for the annual vaccination of sheep in southern Africa.

### Materials and methods

#### Viruses

The viruses used and the titres/ml of blood meal are listed in Table I. Stocks of vaccine viruses for oral infection studies were grown in BHK-21 cells, titrated and stored in 10% foetal bovine serum in 1 ml aliquots at −70°C. Aliquots of virus stocks for oral infection were titrated during the experiments using a previously described procedure (14).

#### Insects

*Culicoides* were collected near farm animals at the Onderstepoort Veterinary Institute (25°39’S, 28°11’E; 1 219 m above sea level) and on Koeberg Farm near Clarens (28°32’S, 28°25’E, 1 631 m above sea level) in the eastern Free State from January to April 2002 as described previously (14).

#### Feeding technique

Before feeding, the field-collected *Culicoides* were held without access to nutrients or water for 24 h at 23.5°C and a relative humidity of 50-70%. Lighting in the room was dimmed to ~1% daylight (~65 lux). Surviving flies were subsequently fed in batches of 250-300 for 30-45 min on defibrinated sheep blood.

### Table I

<table>
<thead>
<tr>
<th>Culicoides species</th>
<th>Field strain (virus titre)</th>
<th>Bluetongue virus strain and mean virus titre (log10TCID50/ml) of blood meal</th>
<th>Vaccine strain (virus titre)</th>
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<tr>
<td></td>
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<tr>
<td></td>
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<td>1 (5.1)</td>
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<td>1/23</td>
<td>0/6</td>
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<tr>
<td></td>
<td>1.9</td>
<td>1.7</td>
<td></td>
</tr>
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<td></td>
<td>1/3</td>
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<td>1.7</td>
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<td>0/8</td>
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<td>C. gallenkiani</td>
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</table>

*a) log10TCID50/midge  
b) number positive/number tested*
containing one of the vaccine viruses through a one-day-old chicken-skin membrane using techniques described previously (14). A blood-virus mixture was freshly prepared immediately before feeding as described previously (11). The blood-virus mixture was maintained at 35.5°C and stirred constantly during feeding. Immediately after feeding, midges were immobilised by holding them at 4°C until movement ceased. Blood-engorged females were separated out on a chill-table and handled as described previously (14). To improve the survival of the Culicoides after blood feeding, midges were maintained on a 5% (w/v) sucrose solution containing 500 IU penicillin, 500 µg streptomycin and 1.25 µg per ml of sucrose solution (1). All female Culicoides surviving the incubation period were sorted into species on a chill-table and stored individually in 1.5 ml microfuge tubes at –70°C until assayed.

Processing of Culicoides and virological assays

Flies were assayed for virus immediately after feeding on blood-virus mixtures (the day 0 value) and after 10 days extrinsic incubation at 23.5°C. Processing of samples and virus microtitration assays in BHK-21 cells were performed as described previously (11). The identity of virus isolates was determined by a micro-titre virus-neutralisation procedure (4) using type-specific antisera produced in guinea-pigs.

Results

The feeding rate in the laboratory for field-collected Culicoides varied between 10% and 70% regardless of site and time of collection. Of a total of 10 506 Culicoides fed, 6 540 (66.3%) survived the 10-day incubation period at 23.5°C. Due to high bacterial and fungal infections in the field-collected Culicoides, results could not be obtained from all the Culicoides that survived incubation. The numbers of midges examined, from which results could be obtained, are shown in Tables I and II.

None of 57 Culicoides that fed on blood containing less than $6 \log_{10} \text{TCID}_{50}/\text{ml}$ tested positive for virus immediately after feeding. Of a total of 141 midges fed on blood containing more than $6 \log_{10} \text{TCID}_{50}/\text{ml}$ of virus, 15 (10.6%) were positive immediately after feeding. In positive midges, virus titres ranged between 1.7 and $1.9 \log_{10} \text{TCID}_{50}/\text{midge}$ (Table I).

Of the 124 Culicoides that assayed positive for virus after incubation, 59 were infected with the field isolate of BTV-1. Although there were significant differences between the different serotypes of the virus used, all of the BTV vaccine strains were recovered from at least four of the 18 Culicoides species assayed, namely: C. bolitinos, C. imicola, C. huambensis and C. magnus (Table II). In infected C. bolitinos and C. imicola, the maximum virus titres of BTV vaccine strains, after incubation, ranged from 0.7 to $4.4 \log_{10} \text{TCID}_{50}/\text{midge}$ and of the field isolates of BTV-1, from 3.9 to $4.4 \log_{10} \text{TCID}_{50}/\text{midge}$ (Table II).

Discussion

The level of viraemia that is necessary to infect Old World Culicoides vectors with BTV is not known. Based on the blood-meal size of C. imicola and C. bolitinos (see below) it can be calculated that, theoretically, 5 $\log_{10}$ infectious doses per ml of blood are needed to expose vectors to approximately one TCID$_{50}$ of virus. Consequently, none of the Culicoides which fed on blood/BTV mixtures with concentrations lower than $6 \log_{10} \text{TCID}_{50}/\text{ml}$ were found infected and only 10.6% of midges, which fed on blood/BTV mixtures with concentrations higher than $6 \log_{10} \text{TCID}_{50}/\text{ml}$ assayed positive for virus immediately after feeding (Table I). The low recovery rate in this study was not due to the inactivation of BTV during the 3-4 hour feeding period as blood samples assayed before and after each feed showed no drop in virus titres. This low rate of virus detection, in day 0 midges is not surprising as the average blood-meal size of C. imicola and C. bolitinos is between 0.01 µl and 0.06 µl, and in this study only 75 µl of the 200 µl of original midge homogenate was tested (minimum detection level of the infectivity assay = 0.63 TCID$_{50}$/midge). Thus, these results indicate that the virus titre in the infecting blood-meal in relation to the volume of the blood-meal ingested by the midges was too low to allow all engorging midges to take up virus. Prevalence of infection, as determined in this study, could therefore be much lower than the true susceptibility rate of the Culicoides species tested.

Despite low virus titres in the blood-meals, this study also shows that the two major BTV vectors in South Africa, C. imicola and C. bolitinos, are susceptible to oral infection with the current BTV vaccine strains. Moreover, BTV has been recovered after the 10 days extrinsic incubation period from non-Avaritia livestock-associated species (C. magnus and C. huambensis) (Table II). The possible involvement of non-Avaritia species in vectoring orbiviruses in South Africa is supported by the successful isolation of BTV after 10-day incubation period from orally infected C. bedfordi, C. magnus and C. fycnostictus (11).

Concentration of live virus in the head or body of infected midges, higher than $2.5 \log_{10} \text{TCID}_{50}$/insect has been postulated as an indicator of fully disseminated infection (5). Since this threshold titre distinguishes between potentially transmissive and non-transmissive
individuals, the epidemiological significance of laboratory oral infection studies can be assessed. At present however, it is unknown if this precise threshold value, derived from results with the much larger C. sonorensis-African horse sickness virus (AHSV)/BTV model applies directly to other, differently sized Culicoides species, without extensive experimental validation. In contrast to the highly standardised AHSV/BTV model, vector competence studies on field-collected Culicoides cannot be fully controlled. Culicoides are a biologically highly diverse genus and thus extrapolation of vector competence data from one species to others is not advisable. C. sonorensis is at least four times bigger than either C. imicola or C. bolitinos. In the absence of experimental data this suggests that the infectivity threshold for transmission potential in smaller Culicoides species may be significantly lower than in C. sonorensis.

As has been found previously (11, 14), C. bolitinos was shown to be more susceptible to infection with the field strain of BTV-1 than C. imicola (Table II). Furthermore, the prevalence of infection in both species, as well as the maximum virus titre in midges
Epidemiology and vectors

assayed after 10 days incubation, was 2 to 3 times higher in midges fed on the field strain of BTV than in those fed on the vaccine strains (Table II). However, the virus titres of the blood-meals containing the field strain of BTV were 10 times higher than those containing the vaccine strains (Table II) so direct comparisons on susceptibility to field and vaccine strains of BTV are not possible at present.

In view of the very high abundance of the two highly competent Orbivirus vectors, C. imicola and C. bolitinos, in South Africa (13), the level of viraemia in vaccinated animals, transmission of BTV vaccine strains from vaccinated to unvaccinated animals may well occur. However, such an event has not yet been demonstrated in South Africa and neither has vaccine virus reversion to virulence been demonstrated on vector insect passage so the epidemiological significance of these results remains uncertain.

Further studies, e.g. including sequencing analysis of vaccine strains and field isolates of BTV and reversion studies on insect-passaged vaccine virus, are urgently required. It is also important to develop experimental data to estimate the threshold level of viraemia in the vertebrate host above which experimental data to estimate the threshold level of susceptibility to oral infection with bluetongue virus.

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References


