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Laboratory survival and blood feeding response of wild-caught *Culicoides obsoletus* Complex (Diptera: Ceratopogonidae) through natural and artificial membranes

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Summary

In late summer 2002, live wild-caught midges of the Obsoletus Complex were collected using blacklight traps placed at a horse stable in Teramo (Abruzzo, Italy). For the survival study under laboratory conditions, 1 500 Obsoletus Complex midges were kept at 17°C-25°C and provided only with a sucrose solution. Of these, 150 (10%) survived for at least 40 days and 3 midges were still alive after 92 days. In addition, 10 midges survived 10 days at 4°C. For the feeding trials, 40 blood-meals (9 440 midges) were administered, 27 of which were successful (67.5%); the feeding rate ranged from 0.3% to 16.7%, with a total of 592 engorged midges. Similar feeding rates (U Mann-Whitney test=129.5 $p>0.05$) were obtained when natural (day-old chicken skin) and artificial (stretched parafilm) membranes were used. To infect the insects, a field strain of bluetongue (BT) virus (BTV) serotype 2 isolated from the spleen of a sheep during the 2000 Italian outbreak was added to the blood-meal. Two different viral solutions, with titres of 10^6TCID$_{50}$/ml and 10^7TCID$_{50}$/ml, were prepared. Uninfected blood was significantly more appetising (U Mann-Whitney test=88.5 $p<0.05$) than the infected meal and the midges preferred (U Mann-Whitney test=48 $p<0.05$) to feed on blood containing BTV-2 at a lower titre. A total of 251 midges were fed on BTV-2 infected blood and were then incubated at 23°C-25°C and fed with a sucrose solution for 10 days. During the incubation period, the dead insects were collected daily and analysed for evidence of virus infection. Of the 251 engorged midges, 54 (21.5%) died in the feeding chambers or during sorting on the chill table, 136 died within the first 10 days and 61 survived longer. BTV was isolated only from those which died just after feeding (52.6%; 10/19) or 24 h later (47.8%; 11/23). Considering the small number of midges tested after 10 days of incubation, the prevalence of infection detected in this study (95% probability) would have been higher than 4.74%. These preliminary results appear very promising as this is the first time that midges of the Obsoletus Complex have been successfully fed under laboratory conditions.

Keywords

Bluetongue – Obsoletus Complex – *Culicoides obsoletus* – Blood feeding – Laboratory survival.

Introduction

The Obsoletus Complex includes a group of *Culicoides* species belonging to the subgenus *Avaritia* (Diptera: Ceratopogonidae). There is strong evidence that the species of the Obsoletus Complex are vectors of bluetongue (BT) virus (BTV). They have been found associated with BT outbreaks in areas where the main vector, *C. imicola*, is rare or absent (1). They have also been capable of sustaining BTV replication when inoculated intrathoracically (2); perhaps more importantly, BTV serotypes 4, 2 and 9 have been isolated from parous individuals caught during clinical outbreaks (4, 5). Being abundant and widely distributed, they might play an important role in the spread of BTV in Europe. A crucial step to assess the vector competency of a *Culicoides* species is to isolate the virus 10 days after artificial feeding (incubation period). According to the literature, all attempts to feed *Culicoides obsoletus* under laboratory

conditions have been unsuccessful (2, 3). The aim of this study was to develop methods to improve both insect survival rates and feeding under laboratory conditions.

Materials and methods

In the late summer of 2002, live wild-caught midges of the Obsoletus Complex were collected using blacklight traps placed at a horse stable in Teramo (Abruzzo, Italy). Two years of daily trapping at this site demonstrated that the Obsoletus Complex represented 90%-95% of the total *Culicoides* (Fig. 1) and that the males present in the catches belonged to at least two species of the group, *C. obsoletus* (Meigen), 1818 and *C. scoticus* Downes and Kettle, 1952. For the survival study under laboratory conditions, 1 500 Obsoletus Complex midges were kept at 17°C-25°C and were provided only with a sucrose solution. The feeding trials were performed in accordance to the method described by Venter *et al.* (6). Forty afternoon or evening blood-meals were given, involving 9 440 midges (200-300 midges for each meal).

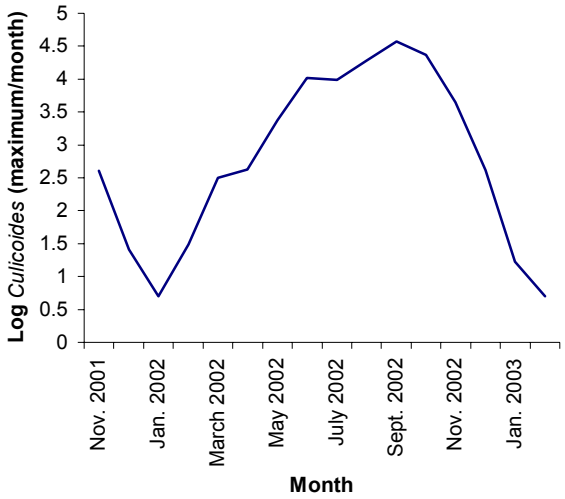

Figure 1
Seasonal dynamics of the Obsoletus Complex population in the collection site, 2002

The midges were fed at least four days after collection and did not receive a sucrose solution in the 24 h that preceded meals. Sheep defibrinated blood was used for feeding. During feeding, the blood-meal was kept at 37°C and agitated using a magnetic stirrer (Fig. 2). For the infection, a BTV serotype 2 field strain isolated from the spleen of an infected sheep during the 2000 BT outbreak in Italy, was added to the blood-meal. Two different viral solutions with titres of 10^6TCID$_{50}$/ml and 10^7TCID$_{50}$/ml were prepared.

Figure 2
Midges of the Obsoletus Complex feeding through an artificial membrane

Day-old chicken skin and stretched parafilm were used as natural and artificial feeding membranes, respectively. After the infected meal, the engorged midges were incubated at 23°C-25°C for 10 days and fed with a sucrose solution (Fig. 3). Dead insects were collected daily and analysed for evidence of virus infection.

Figure 3
Infected midges of the Obsoletus Complex fed with a sucrose solution during the incubation period

Results

Of the 1 500 midges fed with a sucrose solution alone, 150 (10%) survived for at least 40 days and 3 midges were still alive after 92 days (Fig. 4). This is the longest survival period recorded for *Culicoides*. In addition, 10 midges survived 10 days at 4°C. Engorged midges of the Obsoletus Complex were obtained in 27/40 (67.5%) blood-meals.

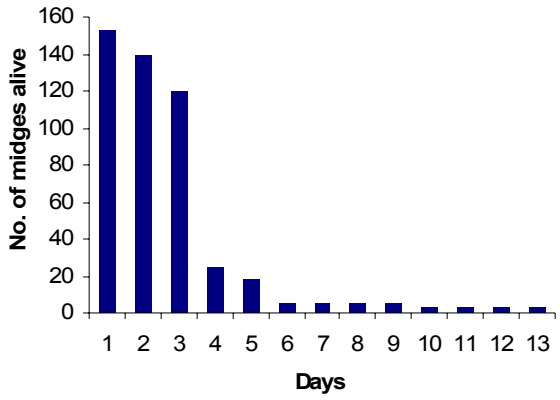

Figure 4
Number of midges of the Obsoletus Complex that survived at least 40 days under laboratory conditions

Similar feeding rates (U Mann-Whitney test =129.5 $p>0.05$) were obtained when natural and artificial membranes were employed (Table I). In the 27 successful blood-feeding trials, the feeding rate ranged from 0.3% to 16.7%, with a total of 592 engorged midges of the Obsoletus Complex of a total of 7 140.

Table I
Blood feeding response of midges of the Obsoletus Complex, fed through natural and artificial membranes

Membrane	No. successful/ No. total meals (%)	Engorged/total midges fed (%)
Natural (day-old chicken skin)	7/10 (70)	130/2 000 (6.5)
Artificial (stretched parafilm)	20/30 (66.67)	462/7 440 (6.21)
Total	27/40 (67.5)	592/9 440 (6.27)

Uninfected blood was significantly more appetising (U Mann-Whitney test = 88.5 $p<0.05$) than the infected blood and midges of the Obsoletus Complex preferred (U Mann-Whitney test = 48 $p<0.05$) to feed on blood containing BTV-2 at a lower titre (Table II).

Table II
Blood feeding response of midges of the Obsoletus Complex fed with uninfected and infected meals

Sheep blood	No. successful/ total meals (%)	Engorged/total midges fed (%)
No virus	11/13 (84.62)	341/3 030 (11.25)
With BTV-2 10^6TCID$_{50}$/ml	13 17 (76.47)	204/4 750 (4.29)
With BTV-2 10^7TCID$_{50}$/ml	3/10 (30)	47/1 660 (2.83)
Total	27/40 (67.5)	592/9 440 (6.27)

Of the 251 midges engorged with infected blood, 54 (21.5%) died in the feeding chambers or during sorting on the chill table. Of the remaining 197 midges, only 61 survived for 10 days (Fig. 5). All the midges that survived were analysed for evidence of virus and BTV was isolated only from those which died within 24 h after feeding (Table III).

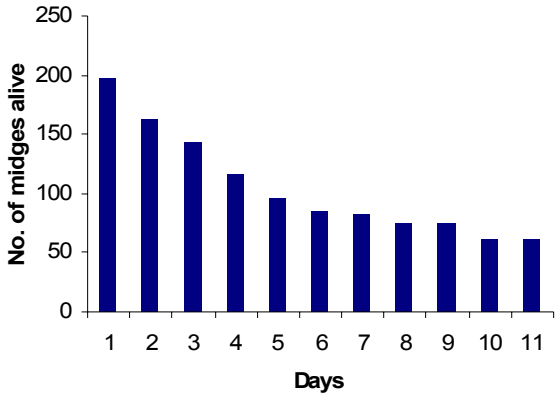

Figure 5
Number of midges of the Obsoletus Complex that survived the incubation period after oral infection with BTV-2

Table III
Bluetongue virus serotype 2 isolation from artificially infected midges of the Obsoletus Complex during a 10-day incubation period

Incubation day	Positive/analysed midges (%)
0	10/19 (52.6)
1	11/23 (47.8)
2	0/5 (0)
3	0/27 (0)
4	0/39 (0)
5	0/0 (0)
6	0/1 (0)
7	0/7 (0)
8	0/2 (0)
9	0/13 (0)
10	0/61 (0)
Total	21/197 (10.66)

Discussion and conclusions

These results clearly show that females of the Obsoletus Complex, at least those belonging to the species *C. obsoletus sensu stricto* and *C. scoticus*, were able to survive for up to 92 days at 17°C-25°C without a blood-meal (Fig. 4). This study also demonstrated that midges of the above species

recovered with ease after being kept for 10 days at 4°C. This lengthy life-span and resistance to low temperature could play an important role in BTV persistence if their vector competence for BTV is confirmed. After infection, the survival rate could probably be improved if antibiotics, never used in this study, were added to the sugar solution. The blood-feeding response of the wild-caught midges through natural and artificial membranes appeared very promising and this is the first time that midges of Obsoletus Complex have been successfully fed under laboratory conditions. It also appears evident that the presence of BTV and the titre thereof in some way influenced the feeding rate (Table II). The fact that BTV was isolated from engorged midges 24 h after the infected meal would suggest that the virus was still alive in the gut; no virus was isolated from midges which survived 10 days after feeding on an infected meal (Table III). According to the number of midges tested 10 days after feeding, it is predicted that a prevalence of infection of up to 4.74% could be detected (95% probability). Consequently, to better assess the vector competence (if any) of the Obsoletus Complex, the study should now be performed with a larger sample of midges incubated for a period of 10 days.

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