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Short communication

Efficacy of the repellent *N*,*N*-diethyl-3-methyl-benzamide (DEET) against tabanid flies on horses evaluated in a field test in Switzerland

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ABSTRACT

Female tabanid flies (Diptera: Tabanidae) can be a serious nuisance for horses because of their painful bites during blood feeding. They also play a primary role in mechanical transmission of a lentivirus causing Equine Infectious Anemia (EIA), a virus that has spread within Europe in recent years. According to the European law for products intended for use as a repellent on horses (recreational and sport horses), a field test is mandatory to demonstrate sufficient repellency of such a substance against the specific target fly species, but currently no agreed protocols are available for testing of potential repellents.

The aim of the present study was to establish a protocol for a field test to investigate the efficacy of *N*,*N*-diethyl-3-methyl-benzamide (DEET, Brum®, Huebeli-Stud Horse Care AG) in a 15–17% oil-water emulsion against tabanid flies on horses up to four hours. Between July and August 2015, four horses on three farms each were tested on two consecutive days in a cross-over design. The four horses on Farm A were used in the pre-test as well as in the main test. Two and a half hours after repellent application the horses were lunged until sweating. Tabanid fly infestations were both photographed and directly counted during five minutes 3 and 4 h after repellent application on the right side of the horses in the area from the head to the flank, belly and first third of the foreleg. Without repellent application, up to 29 tabanid flies were counted on a horse, whereas the maximum for the repellent treated horses was four. In 50% of the horses treated with DEET there were no Tabanids observed (efficacy 100%), and in all horses the tabanid fly counts were lower than in the control horses with one exemption at 4 h. The efficacy of the DEET repellent was at least 80% and 71% respectively, three or four hours after application (with a confidence level of 89%). A fly trap (Horse Pal) revealed the presence of the tabanid species *Tabanus brominus* and *Haematopota pluvialis*, but also non-specified arthropods. The design of the present study simulated practical conditions, allowed to quantify the number of tabanids flies and to demonstrate repellency of DEET in horses.

1. Introduction

Female tabanid flies (Diptera: Tabanidae) feed on blood and their painful bites can be a serious nuisance to horses (Horváth et al., 2010). The role of tabanids as nuisance pest has been reviewed recently by Baldacchino et al. (2014). In the Northern Hemisphere tabanid flies predominantly occur between June and August near cattle or horse pastures (Russell et al., 2013). Female tabanid flies detect their hosts visually or partly olfactorily; horses are more attractive as compared to cattle, sheep and goats (Baldacchino et al., 2014). Horváth et al. (2010) showed that brown and dark brown horses as well as chestnuts have a higher tabanid fly infestation than white or bright coloured horses. Tabanid flies also play a primary role in mechanical transmission of a lentivirus causing Equine Infectious Anemia (EIA) (Issel and Foil, 1984), a disease that has spread within Europe in recent years (Herholz et al., 2013,2014).

N,*N*-diethyl-3-methylbenzamide (DEET) is considered as the gold standard of insect repellent substances for the last 60 years (Frances, 2006; Katz et al., 2008). Though it is clear from several studies that DEET acts on the olfactory system of the insects, its precise mode of action remains enigmatic (Leal, 2014; Pellegrino et al., 2011). Few data are available on the efficacy of DEET when applied on horses. Blume et al. (1971) tested different concentrations of DEET aerosols on horses and also cattle. Horses sprayed with DEET at 75% concentration were protected from tabanids for as long as 3 h. However, these horses exhibited exfoliation on several body

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regions. In the study of Palmer (1969) repeated dermal application to horses produced hypersteatosis, if the solution of DEET was 15% or higher. A permethrin and DEET containing insecticide (Flymax, 6 mg/ ml permethrin and 20 mg/ml DEET, Audevard Ltd., France) is licensed for horses in Europe. Studies to demonstrate the effect of topical repellents or insecticides against flying insects on horses have been performed for other formulas than DEET, like permethrin, pyrethrin, cypermethrin, piperonyl butoxide, citronella and other etheric oils (De Raat et al., 2008; Sünder et al., 2011; Johnson, 2013). To the best of our knowledge, the efficacy of DEET against tabanid flies on horses has not been evaluated in Europe yet.

The aim of the present study was to establish a protocol for a simulated field test to investigate the efficacy of DEET against tabanid flies on horses for the period of four hours which is the duration of effect as declared by the manufacturer of the DEET-containing product.

2. Materials and methods

2.1. Farms and study design

Three farms (A–C; Farm A: 46°44′53.9″N 7°32′25.4″E; Farm B: 46°45′24.5″N 7°32′52.9″E;

Farm C: 46°45′20.5″N 7°40′18.2″E) in the canton Bern (560–1000 m above sea level) participated in the field test. The farmers and the horse owners were informed about the aim of the study and the test procedure in detail before the tests started. Farm A was used in a pre-test as well as in the main-test. Temperature and humidity were monitored via a data logger (iButton, Maxim Integrated, San Jose, CA, U.S.)

In a pre-test in the summer at the end of June 2015 the study protocol and the technical requirements for the efficacy test of the repellent were tested (Farm A). The main tests were performed during July and August.

On each farm four horses were tested on two consecutive days in a cross-over design: two randomly selected horses were not treated on day one, but were treated on day two, while the two remaining horses were treated on day one and not treated on day two. Counts of the tabanid flies were done on the horses with or without repellent application at the same time of the day.

A fly trap (HORSE PAL® fly trap, Newman Enterprises, 4552 Poygan Avenue Omro, WI 54963-9619, USA) for collection of flies was installed at a sunny and open place close to the location of the experiments at the eve (7 pm) of the experimental day. The trap was operated for twenty hours and was removed prior to the start of the horse experiments. Arthropods collected in the traps were packed in plastic bags, killed by deep-freezing, the tabanids morphologically sorted, and three specimens per morphotype genetically identified to species level at the DNA barcode locus by PCR as described by Renaud et al. (2012) and sequencing by a private company (Synergene, Switzerland).

On the eve before the experiments started, all horses were thoroughly washed with water to achieve as similar coat conditions as possible among all animals. The repellent-treated horses were again washed with water to avoid a carry-over-effect after the tests on day one. All horses were tested for a period of four hours. The tests started with the application of the repellent at 1 pm with a delay of 25 min between each horse. The first two hours after repellent application the horses stayed in their box. At 2.5 h the horses were lunged for about ten minutes until they started to sweat. Three hours after application, the horses were photographed in 30 sequences of ten seconds each during five minutes using a reflex camera (Sony 580), an automatic timer and a stative. Afterwards the horses stayed outside on the pasture and were again photographed described above at 4 h after repellent application. The horse owners lunged their horses and held them during the photographic sessions and the tabanid counts. Tabanid flies were photographed and directly counted on the right side of the horses from the head to the flank, belly and the first third of the foreleg. For each horse, photo sequences during 20 min were available (ten min with repellent treatment, ten minutes control) which corresponds to 120 photos per horse in total (six photos per minute).

2.2. Horses

A total of 12 horses were used for 16 field tests, four geldings and eight mares, with a mean age of 11 (range 3–20) years. Ten of the horses were Swiss warmbloods and two were Freiberger horses. Only dark brown (six) or brown (six) horses were selected for the tests. The four horses on Farm A were used in the pre-test as well as in the main test.

2.3. Repellent

The repellent *N*,*N*-diethyl-3-methyl-benzamide (DEET) in a 15–17% oil-water emulsion (BRUM, Huebeli-Stud Horse Care AG, Switzerland) was tested for its effect against tabanid flies. Each animal in the treated group was sprayed with the repellent on both sides on neck, hindlegs (2 spray bursts each), breast, abdomen, back to tail, forehead (1 spray burst each). In total eight spray bursts of 0.2 ml were applied. For the treatment of the forehead one spray burst was applied to the hand of the applicator and the repellent then transferred to the horses head. The treatment was always done by the same person, and the repellent was applied to the horses on a concrete surface to avoid contamination of the soil.

2.4. Statistical analysis

Several approaches are conceivable to quantify the effectiveness of the repellent. Here, we consider the relative reduction of the number of tabanid flies (cf. Table 1), i.e. after three hours

rel.3 h = (control.3 h − treated.3 h)/control.3 h,

where for each horse, control.3 h denotes the number of tabanid flies after three hours on the day without repellent treatment and treated.3 h denotes the number of tabanid flies after three hours on the day with repellent treatment. Note that each horse serves as its own control. The definition is unproblematic as the control.3 h is never zero in our sample.

Replacing 3 h by 4 h in the above equation yields the relative reduction after four hours, rel.4 h. However, horse 4–2 has control.4 h = 0 and treated.4 h = 1 (Table 1). For this single horse, the repellent is counterproductive (because of one single tabanid fly), such that the relative reduction rel.4 h must take a negative value for this horse. Due to the rank-based statistical methods under use, the precise numeric value assigned to this particular horse for rel.4 h is immaterial. We chose minus infinity (-Inf) to highlight the data point (and for consistency with the definition), but the same results are obtained if we set e.g. rel.4 h = −1 for this horse.

An exact lower confidence bound for the median of the distribution of the relative reductions was computed. As exact confidence bounds with 95% confidence level are not admissible, Table 2 contains the two confidence bounds with the nearest realizable confidence levels. Statistical analysis was performed using R (version 3.0.2.).

3. Results and discussion

Prior to the tests the following arthropods species were caught in the HORSE PAL® fly traps: Farm A: *Tabanus brominus* (n = 3); Farm B: *T. brominus* (n = 4) and *Haematopota pluvialis* (n = 4); Farm C: *T. brominus* (n = 2) and other arthropods (n = 3).

Table 1
Counts and relative reductions of the number of tabanids on repellent-treated and control horses three and four hours after application, with descriptive statistics.

Farm	Horses[a]	treated.3 h	control.3h	treated.4 h	control.4 h	rel.3 h	rel.4 h[b]
A	1–1	0	5	0	3	1	1
A	2–1	0	7	1	3	1	0.67
A	3–1	2	3	2	7	0.33	0.71
A	4–1	2	5	0	2	0.6	1
A	1-2	0	4	0	2	1	1
A	2–2	0	3	0	2	1	1
A	3–2	2	4	1	3	0.5	0.67
A	4–2	0	1	1	0	1	−Inf
B	5	0	3	0	5	1	1
B	6	0	29	1	25	1	0.96
B	7	3	9	4	6	0.67	0.33
B	8	0	15	2	18	1	0.89
C	9	4	14	2	5	0.71	0.6
C	10	2	15	0	4	0.86	1
C	11	1	5	0	5	0.8	1
C	12	1	15	0	7	0.93	1
Descriptive statistics	Minimum	0	1	0	0	0.33	−Inf (-1)
	Median	0.5	5	0.5	4.5	0.97	0.98 (0.98)
	Mean	1.06	8.56	0.88	6.06	0.84	−Inf (0.74)
	Std. Dev.	1.29	7.32	1.15	6.45	0.21	NA (0.51)
	Maximum	4	29	4	25	1	1 (1)

[a] Horses 1–4 were used in the pre-test (1–1 to 4–1) as well as in the main test (1–2 to 4–2) on farm A
[b] Due to the "−Inf" value, some descriptive statistics are not defined (NA). Values in parentheses give the descriptive statistics if the "−Inf" value is replaced by −1.

Table 2
Confidence levels and confidence bounds γ for the median relative reduction of the number of tabanids.

	Data 3 h		Data 4 h	
	confidence level	γ	confidence level	γ
Data with pretest	0.89	0.8	0.89	0.71
Data with pretest	0.96	0.71	0.96	0.67
Data without pretest	0.93	0.8	0.93	0.67
Data without pretest	0.98	0.71	0.98	0.6

The mean temperature and humidity during the study period including the pre-test was 34 °C (minimum 30.6 °C, maximum 38.6 °C) and 32% (minimum 24.0%, maximum 49.8%). On farm A the mean temperature and humidity were 34.6 °C and 29% (pre-test) respectively 33.4 °C and 32% (main test), on farm B 34.2 °C and 40% and on farm C 36 °C and 31%.

3.1. Raw data and descriptive statistics

To increase the number of observations, the pretest data (Farm A) were included in the statistical analysis, given that the data had been obtained under similar conditions. No evidence of a bias is visible in Fig. 1. The statistical analyses were also applied to the data set excluding the pretest data with similar results (Table 2).

After 3 h, the repellent treated horses have a lower median (0.5 vs. 5), mean (1.06 vs. 8.56) and maximum (4 vs. 29) number of counted tabanid flies than untreated horses, and similar differences are found after 4 h. Moreover, in 16 of the 32 measurements (combining the measurements after 3 and 4 h), no single tabanid fly was observed on repellent-treated horses (compared to only one tabanid fly free horse without repellent treatment).

3.2. Relative reductions

After 3 h, the number of observed tabanid flies was always lower in the treatment as compared to the control condition (Fig. 1). The smallest relative reduction of the number of tabanid flies was 33%, the median relative reduction was 97% and the mean relative reduction was 84% (Table 1). The situation after 4 h was similar (Fig. 2), with only one single horse showing no tabanid fly in the control and one tabanid fly after the repellent treatment. For all other horses,

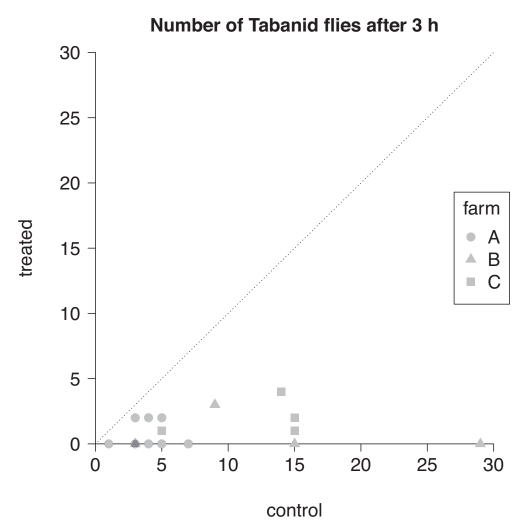

Fig. 1. Number of tabanid flies after 3 h on horses of farm A, B and C (control.3 h and treated.3 h).

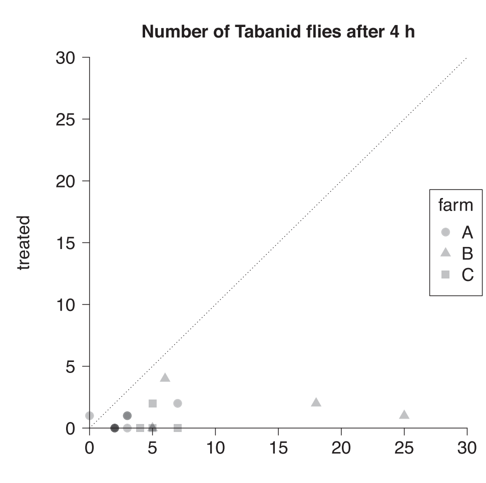

Fig. 2. Number of tabanid flies after 4 h on horses of farm A, B and C (control.4 h and treated.4 h).

after 4 h, the number of observed tabanid flies was lower with repellent treatment than without repellent treatment. The median relative reduction after 4 h was 98%. To obtain a numeric value for the mean relative reduction, the "-Inf" value has to be replaced by a number. Replacing "-Inf" by −1, a mean relative reduction of 0.51 can be calculated, but this should be interpreted with care, see Table 1.

A relative reduction of 1 (i.e. of 100%) corresponds to the reduction from any nonzero number (control) to zero (repellent treatment). This is observed in eight of 16 horses after three as well as after four hours (Table 1), leading to the high sample median relative reductions reported above.

A relative reduction of 1 was also observed for untreated horses with high tabanid counts compared to the counts with repellent treatment (i.e. 29 without treatment versus zero tabanids with repellent). As mentioned, a negative relative reduction (-Inf) was observed for one horse after four hours.

Table 2 contains the lower confidence bounds γ for the median of the relative reduction for the realizable confidence levels. They were calculated both for the data with and without pretest after 3 and 4 h. For example, it could be shown that with a confidence of 89%, the number of tabanid flies after 3 h was at least 80% lower in the median than without treatment. Whether the pretest data are used or not has little impact and only on the results after 4 h.

3.3. Discussion

These results indicate that DEET has a very good efficacy, based on the calculation of relative reduction of the number of tabanid flies three and four hours after repellent treatment of horses compared to control horses. In contrast to an earlier study done in the USA (Blume et al., 1971) in which horses were shown to be protected from certain tabanids for up to 3 h when sprayed with a 75% concentration of DEET, the horses of our study were protected from tabanids for as much as 4 h with a product containing a much lower DEET concentration of 15–17%. The different tabanid fly species that were present in these two studies on two continents with putatively different susceptibility levels to DEET might at least partly explain the different findings.

According to the European law for products intended for use as repellent on horses (recreational and sport horses), a field test is mandatory to demonstrate sufficient repellency of such a substance against the specific target fly species. In the directive 98/8/EC an efficacy test is required for repellent products containing a single active component (CA-Dec12-Doc.6.2.a, Product type 19, repellents and attractants, only concerning arthropods), but currently no agreed protocols are available for such products.

In practice, repellents are usually applied to horses during riding trips in the summer when the horses sweat and are attacked by tabanid flies. In the present study the horses were lunged until sweating before tabanid flies were counted so that the study design simulated the practical conditions very well. Tabanid infestation was directly counted as well as documented by photographic sequences. However, the differentiation of tabanid flies and other arthropods was facilitated by direct counting. Due to strong defence movements of the control horses against tabanid flies (head shaking, leg and tail movements, muscle twitches) the photographical recording and evaluation was impeded. The authors therefore strongly recommend the direct count of the tabanid flies in addition to the photographic recording.

The fly traps used for species identification were also put up with the intention to estimate the insect density before the experiments. The traps were inactivated before the start of the tests to avoid competition between the trap and the horses. However, the comparison of the numbers of tabanid flies caught in the trap during twenty hours to the numbers counted within 10 min on one horse showed that sweating horses are much more attractive to the tabanid flies than the trap. Furthermore, only dark brown or brown horses were used, as it has been demonstrated that tabanid flies are mainly attracted to black and brown horses due to positive polarotaxis (Horváth et al., 2010).

In conclusion, the use of DEET in a 15–17% oil-water emulsion applied to horses can be recommended as the efficacy was at least 80% and 71%, respectively, three or four hours after application with a confidence of 89%. In the present study, practical conditions were simulated as the repellent treatment is especially necessary to minimize tabanid attacks during riding in the summer season. Manufacturer's recommendations should be followed and attention should be paid to treat the horses on a concrete surface to avoid contamination of the environment.

Conflict of interest

No conflict of interest is declared.

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