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Antifeeding activity of *Azadirachta indica* (Neem) extract against sand fly bites on dogs

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Background

Antifeeding agents are important tools for host's protection from sand fly bites and hence prevention from sand fly-borne diseases. Chemical repellents or insecticides are the mainstay for the protection of hosts because of elevated and/or durable efficacy. However, in many communities there is a decreasing compliance with chemicals exhibiting potential adverse effects, that may represent a drawback for the control programs against sand fly-borne diseases.
Aim of this study was to evaluate the antifeeding efficacy of a natural compound, *Azadirachta indica* (Neem) extract (RP03™, 2400 ppm azadirachtin) against *Phlebotomus perniciosus* in dogs. We report herein preliminary results suggesting a potential use of this product as an additional measure for dog protection from sand fly bites and hence from *Leishmania* infections.

Fig. 1. *Azadirachta indica*: leaves and drupes; tree ; sand fly

Study design and methods

The experiments were performed on beagle dogs after owner's informed consent. They were designed to determine a minimal effective dose of RP03™ and to evaluate its anti-feeding efficacy after different treatment regimens, taking into account the low stability of the product under natural conditions (**Tab. 1**). Data from preliminary studies indicated that dog's attractiveness to colonized *P. perniciosus* may vary considerably when experiments are performed in field conditions; for example, in one experiment the same batch of flies used in parallel on 2 dogs resulted in 10.0% and 51.7% blood-feeding rate, respectively. Therefore, each dog served as own control through a sand fly feeding test performed before treatment. Topical administration of the compound was made by spraying the head. The dog was sedated and the head inserted into a cage containing about fifty 3-7 day-old unfed *P. perniciosus* females, which were recollected after 1-hour exposure (**Fig. 2)**. Protection from sand fly bites was estimated considering the rate of blood-fed flies compared with the pre-treatment rate. If less than 15% of flies took a blood meal on the dog before treatment, the test was considered invalid.

Dog	Sand fly exposure (SFE), Conditioning treatment (CT) and Full treatment (FT)														
1	SFE/CT			SFE											
2	SFE/CT	CT	CT	CT	CT	CT	CT	FT		SFE					
3	SFE/CT	CT	CT	CT	CT	CT	CT	FT		FT		SFE			
4	SFE/CT	CT	CT	CT	CT	CT	CT	FT	SFE						SFE
DAY	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15

Table 1 - Schedule of sand fly exposure and treatment for each dog

Fig. 2. Fed female sand flies recovered after one hour

Results

Five dogs have completed the study.
In dog 1, a low dose of 1.75 ml of 1000 ppm azadirachtin was given once and the sand-fly test performed at 72 h from treatment, resulting in no protection.
Dog 2 received a sub-dose (1.25 ml of 2400 ppm azadirachtin) during 7 consecutive days ("conditioning treatment") plus a full dose (2.5 ml) on day 8, the sand fly test being performed at 48 h from last treatment. A significant 74.9% protection from bites (p=0.044) was recorded.
To verify whether the treatment could be administered intermittently without loss of protection, dog 3 received the same "conditioning treatment" as above, plus full doses on day 8 and 10, and sand fly test at 48 h from last treatment. Results showed similarly high activity (67.6%, p=0.008) suggesting that intermittent treatments could maintain over time elevated protection against *P. perniciosus* bites.
To verify if protection could last longer than 48 h after last treatment, dog 4 was treated as dog 2 and sand fly tests were performed at 24 h and 7 days from last dose. Surprisingly, a significant protection detected at 7 days (89.2%, p<0.001) was even higher than at 24 h from treatment (63.0%, p=0.023). Hence, RP03™ efficacy seems to increase after a "conditioning treatment" independently from the length of the treatment gaps (**Fig. 3**).
Finally, dog 5 was excluded from the trial because only 12.2% of flies had fed in pre-treatment test.

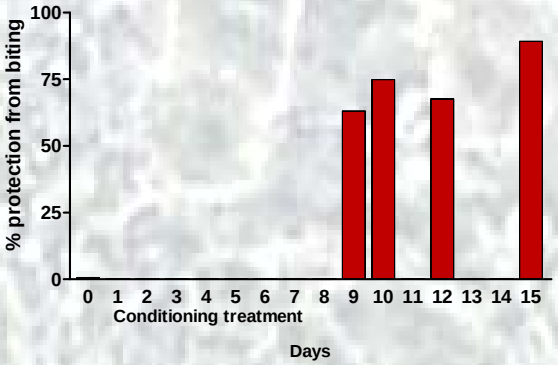

Fig. 3. Protection from sand flies bites last after "conditioning treatment", indipendently from the length of the gaps

By grouping all blood-feeding data in pre- and post-treatment exposures of dogs 2-4, the proportion of fed *P. perniciosus* before treatment was 30.7%, with a wide SE range (± 7.8%) reflecting the individual dog variability in sand fly attractiveness. After treatments, the mean percentage of fed females decreased to 8.1%, with a much narrow SE range (± 2.6%) suggesting a homogeneous response of *P. perniciosus* to the natural compound (**Fig 4**). These values gave an estimate of 73.6% protection against sand fly bites. The statistical analysis made considering each sand fly as an independent variable resulted very robust, giving an estimate of 81.3% maximum protection (p<0.0001) and 0.28 (95% CI 0.1686-0.4650) relative risk. Altogether our results suggest that the repeated use of RP03™ (2400 ppm azadirachtin) during the sand fly season can be a valid tool for protection of dogs against *P. perniciosus* bites

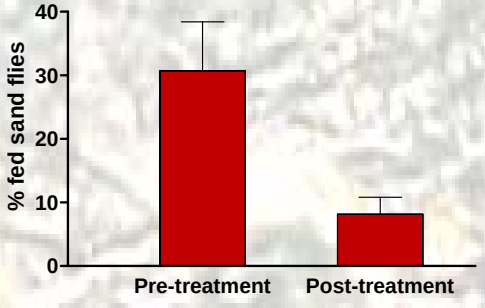

Fig. 4. Cumulative protection against *P. perniciosus* bites

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