Defining the concept of ‘tick repellency’ in veterinary medicine

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SUMMARY

Although widely used, the term repellency needs to be employed with care when applied to ticks and other periodic or permanent ectoparasites. Repellency has classically been used to describe the effects of a substance that causes a flying arthropod to make oriented movements away from its source. However, for crawling arthropods such as ticks, the term commonly subsumes a range of effects that include arthropod irritation and consequent avoiding or leaving the host, failing to attach, to bite, or to feed. The objective of the present article is to highlight the need for clarity, to propose consensus descriptions and methods for the evaluation of various effects on ticks caused by chemical substances.

Key words: tick, control, companion animals, acaricides, repellency, expellency, disruption of attachment, study design.

TICK REPELLENCY: AN ILL-DEFINED CONCEPT

Ectoparasitic arthropods (insects and acarines) represent a group of organisms of major importance in veterinary medicine. Ticks and fleas are the main ectoparasites of pets, followed by sand flies and mosquitoes. Ectoparasites may impact animal health directly or indirectly through bites or their ability to transmit pathogens and associated diseases, some of which are zoonotic (Otranto et al. 2009).

Several potential approaches are available for controlling ectoparasites, including the use of chemical insecticides and acaricides, vaccination or biological control (Otranto and Wall, 2008). However, the use of different formulations of chemical insecticides and acaricides remains the most widespread approach. Depending on the nature of the compound used, the following effects may be obtained, alone or in combination: (i) disruption of contact between the arthropod parasite and the host; (ii) prevention of feeding; (iii) death of the arthropod parasite, and (iv) interference with egg fertility and subsequent development of off-host life-cycle stages. Standards

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for the evaluation of the efficacy of drugs have been
determined by pharmaceutical regulation worldwide
(for example by the European Medicine Agency
(EMA) or by the Environmental Protection Agency
in the United States) as well as by scientific societies.

Usually, the range of effects described above is
evaluated for a specific interval of time, along with
speed of kill and residual activity (Marchiondo et al.
2007; EMA, 2007). An anti-tick product for dog, for
example, should give >90% efficacy, characterized by
a reduction in tick counts 48 h after treatment and the
prevention of re-infestation (EMA, 2007). Nevertheless,
the reduction in tick counts can be caused by a
range of behaviours, not only simple mortality.
These non-lethal effects can broadly be described as
repellency, but definitions for the various behavioural
components of which it is composed are not well
established, thus resulting in the absence of specific
standards for its evaluation (Bissinger and Roe,
2010). The present article aims to explore the
definitions of various effects caused by chemical sub-
stances against ticks and to propose consensus
descriptions, as well as to suggest a standardization
of methods for evaluation of tick repellency in
veterinary medicine, which could be eventually
added to the EMA guidelines.

Arthropod repellents are defined as “chemical
substances that cause arthropods to make oriented
movements away from their source” (Dethier et al.
1960). While this definition of repellency is easily
applied to mosquitoes and other flying insects such as
sand flies, this is not the case for crawling arthropods
such as fleas and ticks. Flying insects are usually
highly mobile, transitory ectoparasites with only rapid,
short-lasting contact with their host. Repellency in
this case is characterized simply as the effect of the
chemical causing the arthropod to avoid the treated
host or substrate. For crawling arthropods and
especially for those with a long-lasting host-parasite
association, such as fleas, ticks lice or mites, where
contact with the host may last several days or even be
life long, the term repellency becomes more difficult
to define. In addition, the effect of a substance on a
pre-existing infestation of a long-lasting parasitic
infestation is not taken into account by the conven-
tional definition. Hence, there is a need to better
understand the definition of repellency. It should be
specifically defined for each type of ectoparasite and,
if not clearly applicable, other terminology should be
proposed. Here we are particularly concerned with
proposing a working definition of repellency for ticks.

THE VARIOUS EFFECTS OF CHEMICAL PRODUCTS
ON TICKS

Ticks have a specific and complex mode of para-
sitism. They wait for their host in the environment
and are able to detect through their sensorial organs
several stimuli emitted by the vertebrate host
(Sonenshine et al. 2002). Once on their host, ticks
crawl for several hours to find a suitable attachment
site. The attachment process involves skin pene-
tration, causing a feeding lesion and secretion of
cement. Ticks then begin their bloodmeal within
24 h (Sonenshine et al. 2002). Tick-borne pathogens
may be transmitted during this attachment and
bloodmeal intake phase and any feature of ticks,
from a molecular to a behavioural one, may affect
their role as vector (Randolph, 2009).

Due to the complex host location, feeding-
behaviour and vector role of ticks, the biological
activity of a chemical cannot be adequately described
by a single efficacy value (Marchiondo et al. 2007).
It seems useful, therefore, to describe the behavioural
effects as they disrupt the various steps leading to
tick infestation (Dethier et al. 1960); including tick
mobility (climbing), tick attachment and blood
feeding. A distinction must also be made between
the effect expected on pre-existing and new infesta-
tions.

The first potential influence is an irritant effect
(also called ‘hot foot’ effect), which causes the tick to
move away from the treated animal, falling off soon
after the contact with its coat. This effect could
therefore be considered as repellency sensu stricto.
The effect is short-lived and variable depending on
tick stage and species (Bissinger and Roe, 2010).
Furthermore, it is not possible to evaluate the irritant
action on a pre-existing infestation, because in such a
case, the ticks are already firmly attached. The second
effect is that of anti-attachment or disruption of
attachment (Table 1). A contact with the substance
causes disruption of the process of tick attachment
(i.e. the inhibition of attachment, the withdrawal of

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Table 1. Definition proposal for a glossary for tick control terminology

| Term                        | Definition                                                                                                                                 |
|-----------------------------|-------------------------------------------------------------------------------------------------------------------------------------------|
| Tick repellency sensu stricto| Characterized by an irritant effect, causing the tick to move away from the treated animal leading it to fall off soon after contact with its hair coat |
| Disruption of attachment    | Interference with the natural process of tick fixation including inhibition of attachment, withdrawal of mouthparts by ticks in the attaching phase, or detachment of already attached ticks |
| Tick expellency              | Causing ticks to fall off the host animal by disrupting the mechanisms of attachment: i.e. either by causing detachment of already attached ticks or by preventing attachment of new infesting ticks |
| Anti-feeding effect          | Interference with the natural process of tick feeding, avoiding any bloodmeal                                                          |
| Killing effect (lethal action)| Its ability to induce death of the arthropod and its acaricidal effect sensu stricto                                                        |
mouthparts by ticks in the attaching phase, or even detachment of already attached ticks; Dryden et al. 2006b; Ian and Bryan, 1981). Notably, it is difficult to clearly distinguish the results of an irritant effect from those of a disruption of attachment effect on the behaviour of a tick. Other effects of a chemical compound include the prevention of ingestion of the bloodmeal, or an anti-feeding effect, whose evaluation remains difficult, and finally a true acaricidal toxic effect which results in tick mortality. The ability of a product to prevent the transmission of tick-borne diseases should also be considered. The use of the term repellency in relation to tick behaviour, therefore, subsumes several effects. The use of the term 'expellency' may be more appropriate to define the combined effect of disruption of attachment and falling off the host. This term has been used previously in the pest control literature to define the effect of a product, which results in the 'expulsion' of crawling arthropods from a particular location (wood, host, hole, etc. depending on the targeted species) (Ian and Bryan, 1981; Wege et al. 2002).

Among the compounds which affect ticks, some substances have only an irritant effect, such as icaridin or DEET (Bissinger and Roe, 2010; Brown and Hebert, 1997). Other products may have both irritant and toxic effects, such as permethrin (Brown and Hebert, 1997). Substances such as amitraz present an effect which includes both an expellent effect, along with acaricidal properties (Folz et al. 1986). Finally, some substances, such as fipronil, have little impact on the tick’s behaviour but have a strong killing potential (Narahashi et al. 2010). According to the molecule or family of molecules, efficacy against tick infestation is usually a combination of 3 types of effects: repellency sensu stricto, expellency and toxicity. Unfortunately, a standardized methodology to separately assess each type of effect is still lacking.

TOWARDS ASSESSING TICK REPELLENCY AND RELATED EFFECTS IN COMPANION ANIMALS

In vitro tests (i.e. conducted in the absence of the host) to assess repellency sensu stricto are relatively easy to standardize. For example, in simple comparison tests in Petri dishes, repellency is determined by the number of ticks found on treated versus untreated surfaces (Bissinger and Roe, 2010). Climbing bioassays can also be used, where vertical supports are treated at some level above the base of the vertical climb with a chemical substance. Ticks that cross the treated region are considered not repelled whereas those that back down or fall from the treated surface are repelled (Bissinger and Roe, 2010).

The design of in vitro tests addressing separately ‘sensu stricto’ repellency, expellency and killing effect is much more difficult. According to the EMA guidelines, a repellent effect against ticks means that no ticks will attach to the animal and ticks already on the animal (attached or not) will leave the host soon after treatment. In general, no ticks should be detectable on the animal 24 h following administration of the product. However, this definition does not distinguish between ticks that are strictly repelled, or ticks that came into contact with the host and then left the host, or ticks that initially attached and then detached during the first 24 h. Distinctions between those features would particularly matter in the evaluation of the prevention for tick-borne disease transmission.

Furthermore, to date, tests used to demonstrate repellent effects lack standardized protocols. For example, the protocol used by Endris et al. (2000, 2002) exposed dogs for 2 h in individual cages to 50 unfed adult ticks (ratio male/female 1/1) at 7, 14, 21, 28 and 35 days after treatment with permethrin and other acaricidal products. After the 2-h exposure period, the numbers of live and dead ticks were counted on each dog by combing the fur and by collecting ticks from the cage. In this test, repelled ticks include those present in the cage (live and dead) and the unattached ticks on the dog 2 h after exposure. In another study (Dryden et al. 2006b), 25 adult ticks were placed on dogs for 10 min on days 3, 7, 14, 21 and 28 days post-treatment with permethrin or fipronil. During such a short time, not all ticks moved immediately into the hair coat, either on treated or untreated dogs; some dropped or crawled off the dogs. These ticks were picked up and placed back on the dogs during the 10-min infestation period. Ticks off the dog after the infestation period were considered as repelled. In a third approach (Folz et al. 1986), 50 unfed adult ticks were placed on untreated control dogs or dogs treated with amitraz on days 0, 7, 14 and 21 post-treatment. The numbers of dead and live ticks still present on the dogs 1 h after infestation were counted. In the same study, the effect of treatment on pre-existing infestation was also evaluated by counting ticks attached on dogs at 24 and 48 h after treatment. The definition of repellency was clearly different for each of these studies. Nevertheless, a few constant features can be extracted. To evaluate repellency sensu lato, or expellency, it is necessary to measure the absence of ticks on a treated animal at a given time post-infestation, compared to a non-treated control. The infestation also needs to be performed in an environment, such as a crate, where ticks falling off the host can be collected at several time-points. The selection of the time-points is of major importance and should be defined according to the tick species and life stage.

The following experimental design (Table 2, Fig. 1), based on the period of time chosen for the tick count, is suggested as an example for the evaluation of tick repellency and related effects. The use of the term repellency in relation to tick behaviour, therefore, subsumes several effects. The use of the term 'expellency' may be more appropriate to define the combined effect of disruption of attachment and falling off the host. This term has been used previously in the pest control literature to define the effect of a product, which results in the 'expulsion' of crawling arthropods from a particular location (wood, host, hole, etc. depending on the targeted species) (Ian and Bryan, 1981; Wege et al. 2002).

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The following experimental design (Table 2, Fig. 1), based on the period of time chosen for the tick count, is suggested as an example for the evaluation of tick repellency and related effects.
In addition to the classical 24 and 48 h counts recommended by the guidelines (EMA, 2007) earlier time-points are considered. From 0 to 4 h post-infestation

This first period should be dedicated to the evaluation of repellency *sensu stricto* plus expellency. Ticks will not have enough time to attach either on treated or untreated dogs and an evaluation every hour post-infestation by counting the ticks in the crate and on dogs is suggested. A total count of ticks off-host will indicate the repellency/expellency efficacy of the compound.

From 0 to 4 h post-infestation

**Early expellency and repellency *sensu stricto***

**Disruption of attachment**

**Acaricidal ‘killing’ effect**

### Table 2. Suggested methods to measure the effects of an acaricidal product on a new tick infestation

(The effect of any acaricidal product is evaluated according to the tick status and the time frame post-infestation, either in the crate or on dogs. Dogs are treated 24 h before the first infestation. In any case, the presence of a control group is required to be sure that the infestation process is normal.)

| Effect measured | Time frame post-infestation | Tick status in the crate | Tick status on the dog |
|------------------|----------------------------|--------------------------|------------------------|
| Early expellency and repellency *sensu stricto* | 0–4 hours | Alive in the crate | Alive attached on the dog |
| disrupting attachment | 4–24 hours | Dead in the crate | Dead attached on the dog |
| Acaricidal ‘killing’ effect | 0–48 hours | Alive free on the animal | Alive free on the animal |

*Tick count at 2 h is not mandatory as combing may disturb the process of infestation.*

**Fig. 1. Flow diagram of the proposed *in vivo* test for the evaluation of tick repellency and associated effects on new infestation of an acaricidal product in dogs. The diagram gives time-line, location in crate and/or on dogs and the effect being measured. *Tick count at 2 h is not mandatory as combing may disturb the process of infestation.*
Conducted because of welfare and technical reasons. Dogs cannot be maintained in small cages for such a long time, the wastes from the dog prevent reliable tick counts and ticks that are not on the dog may also be ingested. Thus, the count of attached or unattached ticks has to be performed on the dog, and compared to untreated control dogs.

between 0 and 48 h post-infestation

The lethal effect of the product can be evaluated, either on the expelled ticks collected in crates, for which survival is evaluated in an insectarium, or by counting ticks on the host, as described in classical acaricidal efficacy studies (EMA, 2007).

In addition to the evaluation of preventive efficacy described above, the measure of the effect on a pre-existing infestation is also recommended to assess the curative efficacy profile of a chemical product. In that case, infestation of dogs is performed between 2 and 1 day before treatment to let the ticks attach to their host. Tick counts are performed on dogs from 0 to 48 h post-treatment as previously described. This kind of study allows measurement of expellency, through its effect on detachment, as well as acaricidal properties of the product.

During experimental studies, evaluation of tick repellency at several time-points should be performed for an accurate evaluation of tick control products. In addition, field trials can also be conducted by comparing the number of questing ticks collected on treated and untreated dogs after walking in natural tick habitats (Dryden et al. 2006a). Tick counts additional to the 24 h or 48 h counts should be performed. The same time-points as described for the laboratory studies can be chosen as they take into account the several hours during which ticks may crawl about on their host in search of a suitable feeding site.

Concluding remarks

The impact of ticks on animal health is linked to irritation at the attachment site, to blood loss and to the risk of tick-borne pathogen transmission. Hence, the ultimate objective of tick control is to prevent both attachment and blood feeding. Precise definition of the effects of chemical compounds on these behaviours is therefore essential and, even if widely used, the term repellency does not seem to be fully appropriate for ticks without careful qualification. Distinction should be clearly made between several potential behavioural effects. Similar comments also apply to other long-term and permanent ectoparasites. The concept of expellency includes both the rate at which ectoparasites fall off the host and disruption of attachment and seems therefore more appropriate when used to assess the impact on tick control.

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