A publication of the 3R Research Foundation Switzerland

3R-Info-Bulletin

No. 27, September 2004

Editor: Peter Maier, Scientific Adviser of the 3R Research Foundation

The tick blood meal: From living animals or from a silicone membrane?

27

ISSN 1421-6590

The development of pesticides against hard ticks requires large numbers of animals of different species for host feeding during tests with potential acaricides. The aim of the present successful project Nr. 79-01, supported by the 3R Research Foundation, Switzerland, has been to develop in vitro screening methods for products with which to control ticks. The project was guided by Patrick Guerin and*

realized by Thomas Kröber. The present development of a feeding unit suggests that in the future a large portion of the animals used as hosts can be replaced in screenings of acaricides.

Dr. Patrick Guerin is Director of Research in Animal Physiology, Institute of Zoology, University of Neuchâtel. He is the head of a research group on sensory physiology and ecophysiology of blood-sucking arthropod vectors of diseases. His research covers chemical stimuli from hosts that play a key role in host selection by these ectoparasites.

Dr. Thomas Kröber is a Post-Doc in this research group and focuses his investigations on factors guiding host attachment and feeding in ticks. His primary goal is the development of experimental methods to investigate these vital processes in ticks.

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Animals as hosts for ticks

Animal husbandry could not be practised over large areas of the planet without acaricides. This persistent reliance on pesticides has led to the development of resistance in ticks against the major classes of acaricide treatments. There is a continual requirement for new types of molecules to target physiological processes that are crucial to tick survival. The development of animal health products against ticks* requires hundreds of cattle, dogs, rabbits and gerbils for *in vivo* trials with acaricides, placing the annual worldwide use of animals in acaricide research in the tens of thousands. Small mammals used in such trials may suffer from skin inflammation and anaemia, and may be submitted to restrictions by the Elizabethan collar inhibiting cleaning behaviour. For controlled studies, dogs have to be kept in small cages, cattle are kept in isolation, in climatic boxes, where their movement is confined. Apart from the ethical aspects of using experimental animals, the costs of maintaining suitable hosts for ticks are high.

In vitro feeding: outwitting the ticks

Tick control on animals is achieved either through contact with a product applied either topically or orally. Ideally, an *in vitro* assay should permit both the assessment of products that either affect a tick's capacity to attach for a blood meal, or that restrict feeding once the tick has started to take

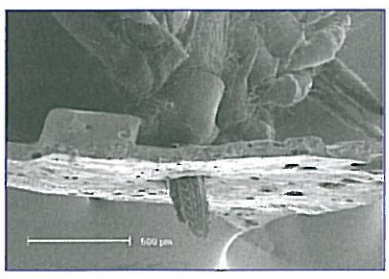

Figure 1: *Ixodes ricinus* female attached to the silicone membrane. Half of the 500 µm food canal has pierced the membrane. (Photo: M. Vlimant)

blood. When ticks start to attach to the skin, they penetrate the uppermost keratin rich stratum corneum with outward lacerating movements of their cutting mouthparts. Strong retrograde food canal denticles anchor the tick in the skin (Fig. 1) allowing the cutting mouthparts to move deeper until the corium containing blood vessels is reached. The artificial feeding unit, that we have developed, has a silicone membrane that replaces host skin and provides the tick with a similar perch over blood (Fig. 2a+b).

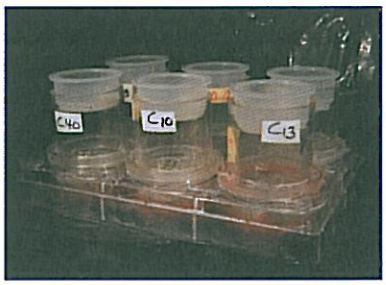

Figure 2a: Six-well plate with feeding units over blood.

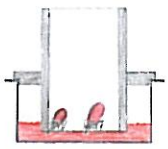

Figure 2b: Schema of a section through one feeding unit depicting the ticks feeding on blood through the membrane.

The feeding unit consists of a silicone membrane reinforced with cellulose rayon glued across one end of a piece of acrylic glass tubing (44 mm high and 26 mm i.d.). The membrane is a modified version of one developed in a previous 3R project (No. 10-88)[1,2]. We rendered the membrane softer to facilitate feeding by the European tick, *Ixodes ricinus*. The new membrane allows ticks with shorter mouthparts (500 µm in the case of female *I. ricinus*; Fig. 1) to be accommodated and the mouthparts of ticks to be withdrawn and reattach elsewhere. The previous penetration site closes by elastic retraction forces in the membrane preventing leaking of blood into the unit. The feeding units are set up in six well plates (Fig. 2a) where each is equipped with an outer ring that limits the depth to which it sinks into the 3.5 ml of blood applied per well. Tick feeding proceeds at body temperature on a support in a water bath. Bovine blood, collected

weekly from an abattoir, is manually defibrinated and supplemented with glucose, an antibiotic and ATP (as a feeding stimulus). Blood is exchanged twice daily.

Attachment by ticks at predilection sites on hosts is preceded by a behavioural sequence that depends on the presence of an appropriate array of mechanical, olfactory and contact chemostimuli[3]. We achieved a 75-95% attachment rate by *I. ricinus* in these feeding units by applying a combination of chemical and mechanical stimuli. A cow hair extract was applied to the membrane in addition to mosquito netting and a layer of cow hair (Figs. 2 and 3).

Testing an acaricide *in vitro*

Fipronil, an odourless phenyl pyrazole, is toxic to a broad range of arthropods via contact or ingestion. This acaricide disrupts ion flow by interacting with GABA-gated chloride channels of the CNS causing hyperexcitation. The effect of different doses of fipronil dissolved in the bovine blood on *I. ricinus* mortality was assessed over 9 days in our feeding units. Fipronil killed all females within two days at 10 µg/ml of blood, at 1 µg/ml no females survived longer than four days, and at 0.1 µg/ml all females were killed by day 7 (Fig 4). These are similar to the doses of fipronil required to protect companion animals against ticks. At the lowest doses of 0.01 and 0.001 µg fipronil/ml female ticks survive but their feeding activity is reduced by 35% and reproduction is inhibited. Females feeding on 0.001 µg fipronil/ml laid eggs but none of these hatched.

Figure 3: Feeding unit with partially engorged female *Ixodes ricinus* sucking bovine blood for over a week. Less engorged females (see arrow) can readily reattach to complete the blood meal.

Since fipronil, like other acaricides, is also applied to animals as a spot-on in the fur, we tested the contact effects of this acaricide in our feeding units by applying it to the surface of the silicone membrane before the ticks were introduced to the feeding units. Fipronil applied in this manner inhibited feeding and strongly affected tick survival: mortality reached 69 % at 10 ng fipronil/cm^2 and 100 % at 1 µg fipronil/cm^2 within 30 h (controls 19 %, $P \leq 0.001$ and ≤ 0.0001, respectively, Fisher's exact test).

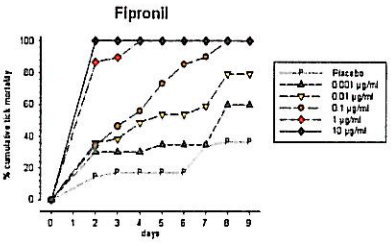

Fig. 4: Cumulative mortality of *Ixodes ricinus* females feeding on bovine blood through a silicone membrane with dimethylsulfoxide (DMSO) added (placebo) and with increasing doses of fipronil in DMSO.

Advantages of the *in vitro* feeding assay

In vivo trials with animals as hosts require repetitions with 10 to 20 animals due to the inherent variation between them. Furthermore, large amounts of products and ticks are needed for such tests. By contrast, our *in vitro* assay requires approximately one hundred fold less of the test product and only about 40 ticks are required per dose. Survival curves calculated over the different doses of fipronil in different feeding experiments showed that the observed effects were significant, were obtained within 5-6 days and highly reproducible. In addition, this *in vitro* assay permits setting up more standardised conditions since the placebo, a reference acaricide and test products can be tested in blood from the same donor animal. Together these reasons suggest that the in vitro feeding assay for hard ticks is preferable to *in vivo* screening trials on animals.

Reference:

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3. *Guerin PM, Kröber T, McMahon C, Guerenstein P, Grenacher S, Vlimant M, Diehl PA, Steullet P and Syed Z, Chemosensory and behavioural adaptations of ecto-parasitic arthropods. Novo Acta Leopoldina* ***83*** *213-229 (2000).*

✻ *The tick blood meal*

The prevention of tick bites in animals reduces tick-transmitted diseases such as anaplasmosis, babesiosis, theileriosis and heartwater disease. The duration of the tick's blood meal takes 2 to 14 days depending on whether it is a larva, nymph or adult. During the first days of feeding, only small amounts of blood are imbibed. However, this a period during which the tick undergoes a variety of physiological changes to accommodate the blood meal in females. Numerous physiologically active agents are injected by the parasite into the feeding lesion inducing strong inflammatory, vasodilatory and immunological responses by the host. The tick's engorgement occurs during the last 24 hours of feeding during which the females imbibe 2 to 8 times as much blood as they finally gain in weight and multiply their body mass by as much as 100 fold with protein and lipid rich nutrient for the production of thousands of eggs. Males do not engorge themselves with blood. In some ticks (e.g. the European tick, *Ixodes ricinus*), every life stage feeds on a different host: larvae and nymphs mainly on small vertebrates and females on sheep, deer and other larger mammals. As man is occasionally parasitized, this contributes to this tick's role as a vector of spring-summer meningo-encephalitis and bacterial (Lyme borelliosis) diseases.

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