Video Article

A Versatile Model of Hard Tick Infestation on Laboratory Rabbits

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Abstract

The use of live animals in tick research is crucial for a variety of experimental purposes including the maintenance of hard tick colonies in the laboratory. In ticks, all developmental stages (except egg) are hematophagous, and acquiring a blood-meal when attached to their vertebrate hosts is essential for the successful completion of their life cycle. Here we demonstrate a simple method that uses easily openable capsules for feeding of hard ticks on rabbits. The advantages of the proposed method include its simplicity, short duration and most importantly versatile adjustment to the needs of specific experimental requirements. The method makes possible the use of multiple chambers (of various sizes) on the same animal, which permits feeding of multiple stages or different experimental groups while reducing the overall animal requirement. The non-irritating and easily accessible materials used minimizes discomfort to the animals, which can be easily recovered from an experiment and offered for adoption or reused if the ethical protocol allows it.

Video Link

The video component of this article can be found at https://www.jove.com/video/57994/

Introduction

The hard ticks (Ixodidae) are well known as slow feeding arthropods and can be attached on a host for several days, or weeks, depending on the species and developmental stage[1]. These obligatory hematophagous arthropods are vectors of a wide variety of infectious agents, such as bacteria, protozoa, and viruses, and thus present a significant risk to humans and animal health[1]. When studying tick biology or evaluating new control methods, the establishment of an effective tick feeding system is crucial in order to effectively design the experiments and accomplish the goal(s) of the study. Recently, several artificial tick feeding systems (avoiding the use of live animals) have been developed[2,3,4] and they should be used whenever possible. However, these systems have not been able to completely replace tick feeding on live animals, and they are not suitable substitutes for many physiologic conditions required for scientific studies. Therefore, in some cases, the use of experimental animal hosts is crucial to guarantee the relevance of experimental results.

Laboratory New Zealand rabbits have shown to be the most suitable and accessible hosts for several ixodid tick species[5,6,7,8,9]. Two common strategies of tick feeding on rabbits have been frequently used: a) feeding on rabbit ears covered with cotton cloth or socks[6,7], and b) feeding in cotton bags[9], nylon bottles[10] or neoprene chambers[11] glued to the rabbit's back. The feeding on rabbit's ears is not an elegant system, because ticks (especially early stages, larvae or nymphs) may crawl and attach deep in the ear canal, which is uncomfortable for the animal and makes the monitoring of tick feeding and/or the recovery of engorged ticks difficult. This system is also limited to only two tick groups on ears fully covered by socks protected by Elizabethan collars,representing a significant discomfort for the animal. Other systems[9,10,11] are definitely more advanced and well suited for hard tick colony maintenance. However, they are limited in the number of experimental groups placed on the rabbit as well as in the modifiable sizes/shapes of the feeding chambers. In addition, these protocols often require hobbling the rear rabbit legs to avoid scratching and the use of Elizabethan collars to prevent grooming.

Herein we propose a simple, non-laborious and very effective method to feed multiple groups of hard ticks in closed chambers glued to the rabbit back covered by a jacket, eliminating the need for Elizabethan collars or hobbling during the experiment. Specifically, our system uses elastic capsules made from an ethylene-vinyl acetate (EVA) foam sheet covered by mosquito mesh and glued to the shaved rabbit back with fast solidifying (3 min) non-irritating latex glue. This technique allows the attachment of multiple capsules of desired size or shape, and few weeks after the experiment the rabbits are fully recovered. The system is suitable mainly for the nymphal and adult hard tick stages, but with a little modification it can be used for larvae feeding as well. The EVA foam-based methods for hard tick feeding may be adapted to other types of vertebrate hosts, for example sheep (which is shown as one of the alternatives in this paper).

Protocol

NOTE: In this study, rabbits were maintained in standard cages with food and water offered *ad libitum* at the French Agency for Food, Environmental and Occupational Health & Safety (ANSES) accredited animal facilities in Maisons-Alfort, France. Animals were monitored twice daily by two experienced technicians for any abnormal skin reactions, health problems or complications. The experimental room was secured by framing the interior of the doorway with double-sided tape to avoid accidental escape of ticks. The method works best if two people work as a team, but it is possible to complete single handedly by one experienced person. Although most rabbits are docile and calm, signs of stress may occur during the manipulation. To ensure that a rabbit does not injure itself by struggling, manual restraint may be accomplished by gently holding of the scruff of the neck in one hand while the other hand supports it hind quarters. The six month old, Rambouillet female sheep was kept at the Centre for Biomedical Research (CRBM) facilities at the National Veterinary School of Alfort (ENVA), where water and food were supplied *ad libitum,* and it was checked twice daily.

NOTE: Our laboratory has received permission to use rabbits and sheep for tick feeding by the Ethics Committee for Animal Experiments ComEth Anses/ENVA/UPEC, Permit Numbers 01741.01 and 11/10/16-5B, respectively. Since we used only pathogen-free ticks in our experiments, all the rabbits used in this study were offered for adoption via the White Rabbit Association, Paris, France.

1. Preparation of the Capsules

1. Cut the desired size of the capsule from the EVA foam sheet (**Figure 1A**). Round the outer corners (**Figure 1B**) of the capsule to minimize accidental detachment when gluing it to the rabbit skin.
   NOTE: The frame thickness of the capsule should be around 8 mm. Use a 5 mm thick foam sheet for the larvae, nymphs, and small tick adult species such as *Ixodes*. A 1 cm thickness foam sheet is suitable for large size adult ticks such as *Amblyomma* sp., *Hyalomma* sp., *etc.* The size of the capsule varies based on the experimental requirements. For example, for 20 *Ixodes* adult couples, 200 nymphs, or 1,000 larvae, we use an inner capsule size of 5 x 5 cm^2, 6 x 7 cm^2 or 7 x 9 cm^2, respectively.
2. Cut the 8 mm wide strips of self-adhesive hook tape (see **Table of Materials**) and stick them to the prepared EVA foam frame (**Figure 1C**).
3. Cut the same size strips from self-adhesive loop tape (see **Table of Materials**) and bind them to the hook sides attached to the EVA-foam frame (**Figure 1D**).
4. Cut the appropriate size of the fine mosquito mesh (mesh size less than 50 µm) to the size of the EVA foam frame and stick it to the self-adhesive loop (**Figure 1E** and **1F**). Cut the overhangs if necessary.
   NOTE: This type of capsule can be used to feed nymphs and adults of hard tick species, while a different sealing system of the capsules is needed for larval feeding (**Supplemental Figure 1**) to prevent accidental escape of the larvae via the fastened hook-and-loop side.

2. Preparation of the Rabbit before Tick Infestation

1. Shave the area of the rabbit back and sides to be used with clippers (**Figure 2A**).
2. Apply non-irritating latex glue to the entire surface of the prepared capsule and wait for 1 min (**Figure 2B**).
3. Glue the capsule by pressing to the skin (especially at the corners) with the fingers for about 3 minutes (**Figure 2C** and **2D**).
   NOTE: When gluing more than one capsule, make sure to keep at least 5 mm space between them (**Figure 2E** and **2F**). We usually avoid the region of the spine, but it may be used if needed.
4. Slightly lift the capsules to visually check their attachment to the skin. If non-attached regions are found, apply the glue using a spatula and press for another 3 minutes.
5. Apply protective tape to the rear paws of the rabbit to prevent jacket damage (**Figure 2G**).
   NOTE: This step is optional and is mainly to prevent jacket damage, not damage to the tick capsule.
6. Put on the rabbit jacket by placing the front legs through the openings and tightening the neck, making sure rabbit breathing remains comfortable. Do not place the rear legs through the elastic enclosures at this step and leave the zipper open (**Figure 2H**).

3. Tick Infestation

1. Place the ticks into a plastic syringe (1 or 5 mL depending of the number of the individuals) with the needle-end cut and plugged with cotton (**Figure 1G**). If a small amount of ticks are to be infested, use forceps.
   NOTE: For tick colony maintenance allow the engorged tick female(s) to lay eggs with subsequent hatching inside the syringe (5 or 10 mL) covered by the mosquito mesh wrapped by a rubber band[12] to avoid laborious manipulation of the larvae at the time they are applied to the host (**Figure 1H**). Also, fully engorged larvae may be allowed to molt in the syringe (**Supplemental Figure 1I**; 5 or 10 mL) for direct infestation of the rabbit with nymphs.
2. Place the syringe deep into the capsule via the open corner and inoculate the ticks by pushing the syringe plunger. Slowly twist the plunger toward the rabbit skin to remove the remaining ticks attached to the plunger and simultaneously pull it out from the capsule (**Figure 2I**).
   NOTE: If some of the individuals crawl out of the capsule, return them using forceps.
3. Close the capsule by refastening the hook-and-loop tape.
4. Place the rear legs of the rabbit into the rear elastic enclosures of the jacket and zip closed.
   NOTE: Make that an index finger can fit between the neck of the jacket and the rabbit to ensure comfort and also to avoid chewing on the jacket.
5. Return the rabbit to the cage (**Figure 2J**).
   NOTE: The time from the infestation to collection of replete ticks vary among different tick species and the developmental stages. For example, for *Ixodes scapularis* and *Ixodes ricinus*, the durations of feeding for adults, nymphs, and larvae are 6–9, 3–4, and 2–3 days,

respectively. A list of references for 29 different hard tick life cycles under laboratory conditions can be found in Levin and Shumacher (2016)[9].

4. Collection and Monitoring of Ticks

1. Take the rabbit from the cage to the bench and unzip the jacket.
2. Gently restrain the rabbit with your hands. Open the capsule by unfastening the hook-and-loop tape (**Figure 2K** and **2L**) and collect the ticks by brushing the engorged larvae (**Supplemental Figure 1**) or nymphs to a plastic dish or using forceps for adults (**Figure 2L**). If partially fed (not replete) ticks are required, use a tick twister or forceps to detach them.
   NOTE: If maintaining the tick colonies please see the note in step 3.1. Maintain the engorged ticks in appropriate humid and temperature conditions according to the particular tick species.
3. If needed, refasten the hook-and-loop tape to close the capsule.

5. Recovery of the Rabbit

1. Remove the mosquito mesh completely from the capsule and leave the jacket on the rabbit (**Figure 2M**).
2. Wait 3-4 weeks and try to remove the capsule by gently trimming one of the corners (**Figure 2N**). If the capsule is still firmly attached, repeat this step one week later.
3. Remove the jacket and let the rabbit recover in the cage.
   NOTE: Once the capsule is off, check the skin of the rabbit for abnormal reactions. Although normally no treatment is required, an emollient lotion can be used in case of irritation.
4. If the protocol and experiments allow, the recovered rabbit (**Figure 2O**) can be reused or offered for adoption.
   NOTE: Rabbits have been shown to acquire tick resistance once exposed to repeated tick infestations[13]; therefore, reinfestations are not recommended unless the experiment requires.

Representative Results

Here we propose for the first time a detailed step-by step method of hard tick feeding in EVA foam capsules applied to a shaved rabbit's back, covered by a jacket (**Figure 1** and **Figure 2**). This protocol is suitable for various types of experiments when different tick groups on the same host are required and can be also used for mass rearing of hard ticks. The tick feeding success in the laboratory mostly relies on the fitness of the tick individuals and suitability of the rabbit host for the particular tick species, rather than the technique itself. Our system using EVA-foam capsules glued to the rabbit back have been proven to be highly successful when feeding different developmental stages of various hard tick species (**Table 1**) and may also be adapted for other type of laboratory hosts, such as sheep (**Figure 3**).

The main advantages of this method are the simplicity, easily accessible materials (**Table of Materials**) and, most importantly, a comfortable open-close system allowing easy monitoring of the ticks during the feeding. In addition, this versatile method offers the possibility of a variety of different experimental settings based on the modifiable number, shape, and composition of the capsules on the host (**Figure 2D-F**), meeting the challenges of the particular study. The use of the highly effective, fast-drying, and non-irritating latex glue ensures that the capsule is firmly glued in three minutes and remains attached for at least three weeks. This procedure also allows complete recovery of the rabbit hosts after the experiments (**Figure 2O**).

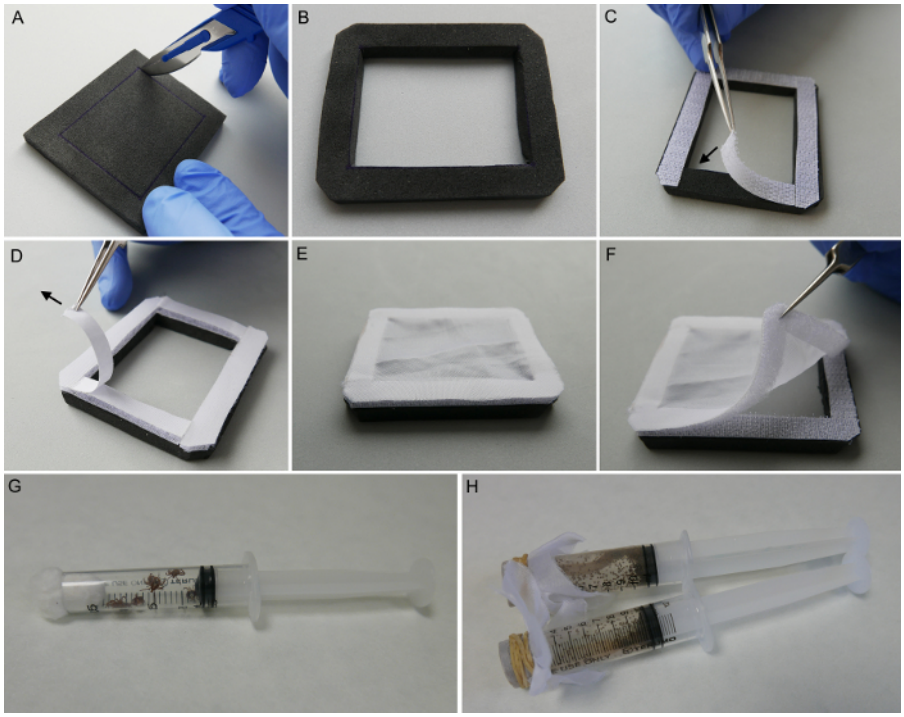

Figure 1: Preparation of the EVA foam capsule and ticks. (**A, B**) Cutting the capsule from the EVA foam. (**C**) Placing the strips of self-adhesive hook tape to the capsule. (**D**) Binding the loop side strips and peeling the tape from the fasteners. (**E, F**) Sticking the mosquito mesh to the adhesive strips. (**G**) Example of adult hard ticks inside the syringe (5 mL) with the cut needle-end covered by cotton. (**H**) Example of freshly hatching larvae inside the syringe with the cut needle-end covered by the mosquito mesh held in place by a rubber band. Please click here to view a larger version of this figure.

Figure 2: Gluing the capsule to the rabbit, tick infestation/recovering and capsule removal. (**A**) Shaved rabbit's back. (**B**) Application of the glue to the prepared EVA foam capsule. (**C, D**) Attachment of different size capsules to the rabbit. (**E, F**) Dorsal view of a rabbit showing two or several chambers attached to the back, respectively. (**G**) The tape is positioned around the rear feet. (**H**) The jacket is applied starting from the front legs and neck, and the rear part is left open. Opening the corner of the capsule is also shown. (**I**) Placing the ticks to the capsule via the open corner using the syringe. (**J**) The jacket is zipped completely and the rabbit is relocated to the cage. (**K**) Monitoring the ticks during their feeding by opening the capsule. (**L**) Collecting replete ticks using the forceps. (**M**) Empty capsule after tick removal. (**N**) Detachment of the capsule (after 3–4 weeks) from the rabbit. (**O**) Fully recovered rabbit Please click here to view a larger version of this figure.

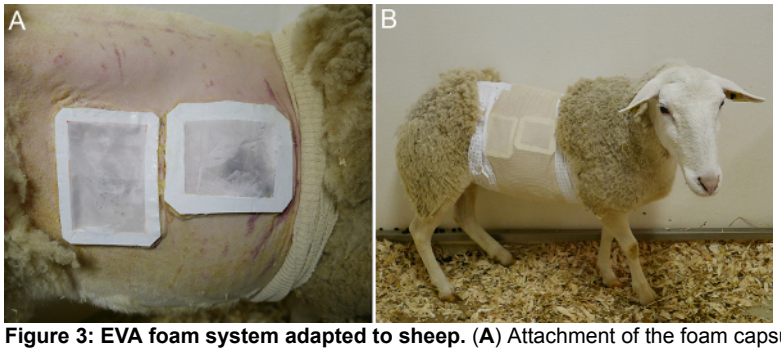

Figure 3: EVA foam system adapted to sheep. (**A**) Attachment of the foam capsules to a shaved and cleaned area in the lateral side of the sheep back. (**B**) After infestation, the attached capsules are covered with a bandage (orthopedic stockinette), instead of a jacket. Please click here to view a larger version of this figure.

Tick species	Number of infested ticks/Number of engorged ticks (%)		
	Larvae	Nymphs	Females
Ixodes ricinus	-	692/557 (80.49%)	670/592 (88.35%)
Ixodes scapularis	-	-	40/34 (85%)
Dermacentor reticulatus	3,550/3255 (91.7%)	900/803 (89.22%)	323/305 (94.42%)
Rhipicephalus appendiculatus	3,300/2822 (85.52%)	490/421 (85.91%)	370/362 (97.83%)
Rhipicephalus pulchelus	-	1,920/1831 (95.36%)	282/257 (91.13%)
Amblyomma variegatum	332/225 (67.77%)	404/308 (76.24%)	207/146 (70.53%)
Amblyomma americanum	-	140/134 (95.71%)	31/27 (87.1%)
Hyalomma excavatum	1,000*	510* (58.8%)	380/313 (82.36%)

Table 1: Engorgement rate of different developmental stages of hard tick species feeding on rabbits inside EVA-foam capsules. *Due to the fast molting process of *H. excavatum* immature stages, the engorged larvae (data not shown) were left to molt into nymphs that subsequently engorged in the same capsule.

Discussion

The most important step in this entire protocol is to glue the capsule firmly to the shaved skin. For this reason, constant pressure for at least 3 minutes is critical, especially at the corners. When inoculating the ticks into the capsule, it is important to apply them deep into the opposite corner from the open one to avoid tick escape during the sealing. When planning the experiments, make sure that all the capsules are covered by the jacket to avoid damage by chewing or scratching. Make sure that the neck region of the jacket is tight enough to prevent chewing but sufficiently loose that the rabbit remains comfortable.

One of the main advantages of the described technique is its simplicity and that modifications in terms of size and number of the capsules can be used. During our experiments, detachment of capsules from skin were not observed. However, occasional damage of the jacket (but not the capsule) by the rabbit may occur.

In rabbits, we have observed that, in the capsule, fully engorged-detached ticks (mainly immature stages such as larvae and nymphs) desiccate faster due to the temperature of the rabbit. For this reason, we suggest to estimate the duration of the feeding period of particular tick species and stage, to plan engorged tick collection immediately after their detachment. Although our system has been tested for feeding of various hard tick species (**Table 1**), the rabbit is not a natural host for all hard tick species[14]. This limitation may be overcome by adapting this system to other animal hosts suitable for a particular hard tick species. Here we reported the use of the EVA foam system adapted to sheep to feed all the developmental stages of *I. ricinus* (**Figure 3**). In this particular case, the sheep needs to be shaved and washed with cotton impregnated with 70% ethanol to eliminate the oil present at the surface of the skin. Afterwards the same procedure as the one described for rabbits was followed, but instead of a jacket, a cotton bandage was used around the back as shown in **Figure 3**.

When developing this system, we paid special attention to minimize the amount of the materials used and steps of the procedure. Compared to other methods, we do not use anesthesia, rabbit collars, ear socks or hobbling of the rear legs[5,6,7,8,9,10]. In addition, the present protocol is not laborious, and no intensive training is required to become familiar with this technique. The EVA-foam based tick feeding system detailed in this study is expected to be used for a variety of different experiments when studying tick biology, host-vector-pathogen interactions, or evaluating different control measures like acaricides or vaccines. Our future direction will be adapting the EVA foam capsule feeding system to the mouse model to feed immature stages of hard ticks.

Disclosures

The authors have nothing to disclose.

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