A Capsule-Based Model for Immature Hard Tick Stages Infestation on Laboratory Mice

Lourdes Mateos Hernandez, Sabine Rakotobe, Baptiste Defaye, Alejandro Cabezas Cruz, Ladislav Simo

jove

A Capsule-Based Model for Immature Hard Tick Stages Infestation on Laboratory Mice

Lourdes Mateos-Hernández[1], **Sabine Rakotobe**[1], **Baptiste Defaye**[1,2,3], **Alejandro Cabezas-Cruz**[1], **Ladislav Šimo**[1]

[1] UMR BIPAR, INRAE, Ecole Nationale Vétérinaire d'Alfort, ANSES, Université Paris-Est [2] Faculté de Pharmacie, Université de Limoges [3] UMR SPE 6134 CNRS, Université de Corte Pascal Paoli

Corresponding Author

Ladislav Šimo
ladislav.simo@vet-alfort.fr

Citation

Mateos-Hernández, L., Rakotobe, S., Defaye, B., Cabezas-Cruz, A., Šimo, L. A Capsule-Based Model for Immature Hard Tick Stages Infestation on Laboratory Mice. *J. Vis. Exp.* (), e61430, doi:10.3791/61430 (2020).

Date Published

June 16, 2020

DOI

10.3791/61430

URL

jove.com/video/61430

Abstract

Ticks are obligatory blood feeding parasites at all stages of development (except eggs) and are recognized as vectors of various pathogens. The use of mouse models in tick research is critical for understanding their biology and tick-host-pathogen interactions. Here we demonstrate a non-laborious technique for the feeding of immature stages of hard ticks on laboratory mice. The benefit of the method is its simplicity, short duration, and the ability to monitor or collect ticks at different time points of an experiment. In addition, the technique allows attachment of two individual capsules on the same mouse, which is beneficial for a variety of experiments where two different groups of ticks are required to feed on the same animal. The non-irritating and flexible capsule is made from easily accessible materials and minimizes the discomfort of the experimental animals. Furthermore, euthanasia is not necessary, mice recover completely after the experiment and are available for re-use.

Introduction

Ticks are important vectors of several pathogens and represent a serious risk to animal and human health[1]. Setting up an effective feeding system is crucial when studying their biology, tick-host-pathogen interactions, or establishing effective control measures. Currently, several artificial feeding systems, which avoid the use of live animals are available for ticks[2,3,4] and these should be utilized whenever experimental conditions allow. However, in various experimental settings these systems fail to appropriately mimic the specific physiologic features and the use of live animals is necessary to achieve relevant results.

Laboratory mice are commonly used for the study of many biological systems and are routinely utilized as hosts for feeding ticks[5,6,7,8,9]. The two most common methods of feeding immature ticks on mice include free infestations and the use of confinement chambers attached to the mouse. Free infestations are primarily used for larval stages and engorged ticks can drop to an area where they can be recovered.

Confinement chambers are usually composed of acrylic or polypropylene caps which are glued to the mouse's back. The first technique is an effective natural system for tick feeding but does not allow close monitoring during the experiment because the individual ticks are dispersed in different parts of the host body. Additionally, engorged ticks that drop to a recovery area can become contaminated with feces and urine[10, 11, 12, 13, 14] that may severely affect the tick fitness or they can be damaged or eaten by the mouse if there is no separation between the animal and the recovery area[15]. Chamber-based systems allow the confinement of ticks to a defined area, however, the gluing process is laborious and the caps are often weakly adherent to the glue and thus they often detach during the experiment[16, 17, 18, 19]. The caps are also stiff, uncomfortable, and lead to skin reactions, which prevent the re-use of the mice and necessitates their euthanasia after the experiment.

In our previous study, we successfully developed an effective system using chambers made of ethylene-vinyl acetate (EVA) foam for feeding ticks on laboratory rabbits[20]. Herein, we adapted this system to a mouse model and propose a simple and clean method to feed immature hard tick stages in closed capsules made from EVA-foam. Specifically, our system uses elastic EVA-foam capsules glued to the shaved mice back with fast drying (3 min), non-irritating latex glue. This technique allows firm and long-lasting attachment of capsules to the experimental mouse, as well as effective tick infestation/collection during the entire course of the experiment. The flat capsule is made from flexible materials and does not impede manipulation of the mouse for blood collection or other purposes. The system is suitable mainly for the nymphal tick stages, but with slight modification it can be used for feeding larvae as well. The method can be completed by one single experienced person and extensive training is not required.

Protocol

Please note that this protocol can be only applied when all welfare and safety measures are met in the laboratory. This protocol received permission to use mice for tick feeding by the Ethics Committee for Animal ExperimentsComEth Anses/ ENVA/UPEC, Permit Numbers E 94 046 08. For the endpoint, the animals were exposed to CO_2 for 9 min in two phases of 4 and 5 min each one.

1. Preparation of the capsule

1. Stick 2 mm thick EVA-foam and the adhesive double sticky foam together (**Figure 1A**).
2. Using a 20 mm diameter leather hole punch, cut a circle from the sticked foam pieces. Then, using a 12 mm diameter hole punch, cut the interior to create the double foam circle (**Figure 1B**).
   NOTE: The frame thickness of the capsule should be greater than 3 mm in size to guarantee sufficient surface for the gluing process to the host skin (see below).
3. Peel the protective paper strip from the adhesive double sticky foam (**Figure 1C**) and attach a transparent circular plastic of 20 mm diameter (**Figure 1D**).
   NOTE: If feeding larvae, do not remove the protective paper strip from the adhesive foam and directly move to the step 2 in the protocol. Glue the double foam ring, including protective paper strip to the mouse.
4. Make a ~ 1 cm slit in the transparent plastic (**Figure 1E**).
5. Create at least 10 small holes with an entomological pin (**Figure 1F**) to allow excessive moisture evaporation during the experiment.

NOTE: The capsule (**Figure 1G**) has a total height of 4 mm (2 mm EVA-foam together with 2 mm adhesive foam) and can be used to feed nymphs and larvae of all the hard tick species. The capsule size (**Figure 1H**) of 20 mm outer diameter is suitable for most of the mouse strains but can be modified if necessary.

2. Preparation of the mice before tick infestation

NOTE: In this study, 10 - 12 weeks old female experimental mice (strain C57BL/6 and BALB/cByJ) were maintained in standard cages with food and water offered *ad libitum* (Green line ventilated racks at -20 Pa) 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 experienced technicians for any abnormal skin reactions, health problems or complications.

1. Anesthetize mouse with isoflurane in the induction chamber. Once anesthetized, place mouse to the manipulation pad and attach to a nose cone for the continuous isoflurane supply (**Figure 2A**). Monitor the breathing rate and reduce isoflurane level to ensure it is less than 80 breaths per minute.
   NOTE: Prior to the manipulation, label the individual mouse by tattooing or radio-frequency identification chip if necessary. It is recommended to keep the individual mice in separate cages to avoid capsule damage by biting.
2. Shave the anterior part of the mouse from behind the shoulder blades up to the area just behind the ears (**Figure 2A**).
   NOTE: The shaved area should be greater than the capsule surface.
3. Apply non-irritating latex glue to the entire EVA-foam site of the prepared capsule and wait for 1 min (**Figure 2B**).
4. Glue the capsule to the mouse back by slight 3 min constant pressure with the finger(s) (**Figure 2C**), especially on the left and right side of the capsule. Slightly lift the capsule to visually check its attachment to the skin. If non-attached regions are found, apply more glue using a spatula and press for another 3 minutes.

3. Tick Infestation

1. For nymph infestation, introduce the individual nymphs into the capsule via the cut made in step 1.4) (**Figure 2D**).
   NOTE: For *Ixodes* tick species a maximum of 20 nymphs is recommended per one capsule.
2. Slightly squeeze the capsule from two sides to allow the transparent plastic to bend for easier introduction of individual nymphs using fine dissection forceps (**Figure 2D**). Push the individual nymphs via the cut inside the capsule. Once inside, turn the forceps in 90˚ and pull out the forceps to deposit ticks inside the capsule.
3. For larvae infestation remove the paper slip from the attached capsule (**Figure 2E**). Place the syringe, containing larvae (**Figure 2F**), directly inside the capsule and deposit ticks by pushing the syringe plunger. Gently turn the plunger towards the skin to remove the remaining larvae attached.
   NOTE: Place larvae into a 1 mL syringe with cut end plugged by piece of cotton prior to the experiment.
4. Once the larvae are deposited onto the skin, close the capsule by attaching the transparent plastic (**Figure 2G**).
5. Apply the protective plastic band around the capsule (**Figure 2H**).
   NOTE: The protective plastic band greatly improved the durability of the capsule for the entire duration of the experiment (**Figure 2I,J**). It is possible to attach two

capsules to one individual mouse (**Figure 2K**). In this case, a minimum of 3 mm space between the capsules is required and the shaved area should be increased appropriately.

6. Return the mice to the cage.

4. Collection of Ticks

1. Anesthetize the mouse as in step 2.1 above.
2. Make a cross shaped cut (**Figure 3A**) to the plastic with a scalpel.
   NOTE: This cross shaped cut enables easy collection of engorged ticks or detachment of the feeding ticks if necessary.
3. If needed, reclose the capsule by sticking an adhesive plastic patch to the transparent plastic (20 mm diameter, **Figure 3B**).
   NOTE: If collection of ticks at multiple time points is desired, the same sticky plastic patch can be used. If the protocol requires, one may also euthanize the mouse, remove the capsule, and collect/detach the ticks (**Figure 3C**).

5. Recovery of the mice

1. Keep the mice in cage for one additional week.
2. Let the capsule detach naturally.
   NOTE: In this case, it takes about 8-9 days for capsules to fall off. When the capsule is removed, it is important to check for abnormal reactions on the skin of the mice. In case of irritation apply an emollient lotion, although normally no treatment is required. If the ethical protocol allows, the recovered mice (**Figure 3D**) can be reused for another tick infestation or different experiment(s).

Representative Results

We propose the detailed step-by step method for feeding immature hard tick stages in EVA-foam capsules applied to a mouse's back (**Figure 2**). This non-laborious protocol is suitable for various types of experiments when precise tick monitoring and collection is required. The main advantages of this method are its simplicity, easily accessible cost-effective materials, and short duration. In addition, we succeeded in attaching two capsules to one mouse individual (**Figure 2K**) allowing to us to feed two different groups of ticks on the same animal. The use of the highly effective, fast-drying, and non-irritating latex glue ensures that the capsule is firmly attached within 3 min. Also, the capsule remained attached for at least one week (**Figure 2J**) which was enough time for engorgement of most of the immature hard tick species[21, 22, 23, 24]. Due to the capsule elasticity, further manipulation of the mouse for blood collection or other purposes was very convenient. This procedure also allows complete recovery of the mice after the experiments (**Figure 3D**) giving the opportunity to reuse the animals and avoid euthanasia. Our system has been successfully used to feed *Ixodes ricinus* nymphs (**Figure 4**). A moderate to high engorgement success rate was achieved in C57BL/6 and BALB/cByJ mouse strains, respectively. In both cases all nymphs finished the feeding within 4 – 5 days, while the majority (~75%) dropped off on the fourth day.

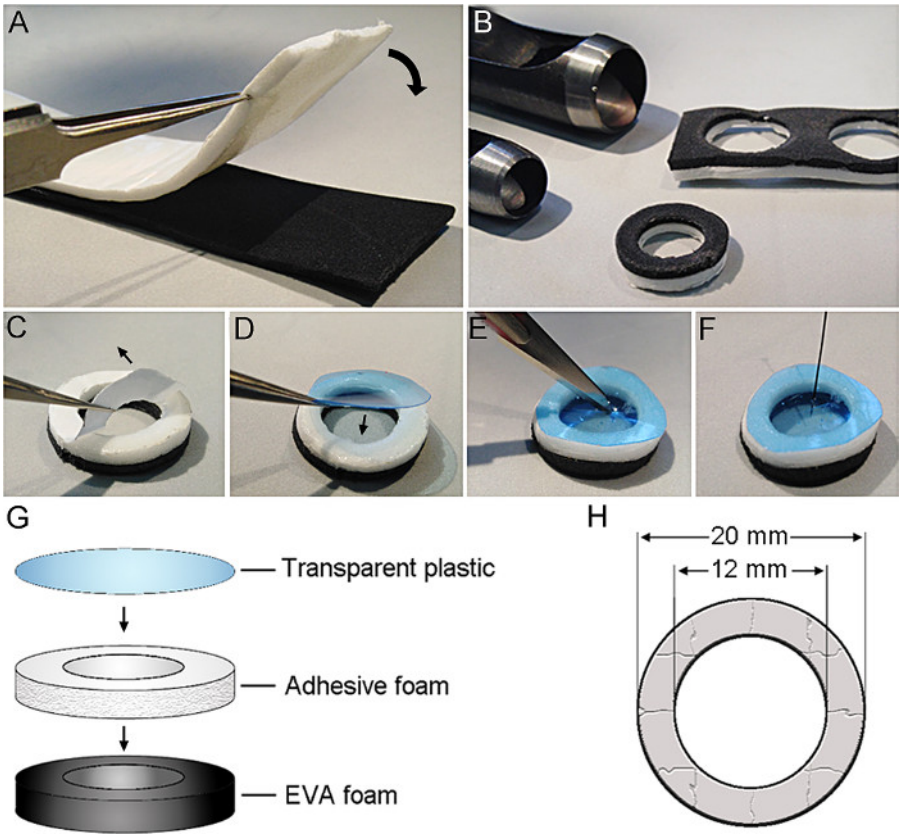

Figure 1: EVA-foam capsule preparation. (**A**) Attachment of EVA-foam (black) and adhesive double sticky foam (white). (**B**) Cutting 20 mm diameter outer and 12 mm inner circle using leather hole punches. (**C**) Removal of the paper protection tape from the adhesive double sticky foam. (**D**) Attachment of the transparent plastic to the capsule. (**E**) Cutting the slit in the transparent plastic with a scalpel. (**F**) Creation of holes using an entomological pin in the plastic. (**G-H**) Schematic drawing of the different parts of the capsule and dimensions. Please click here to view a larger version of this figure.

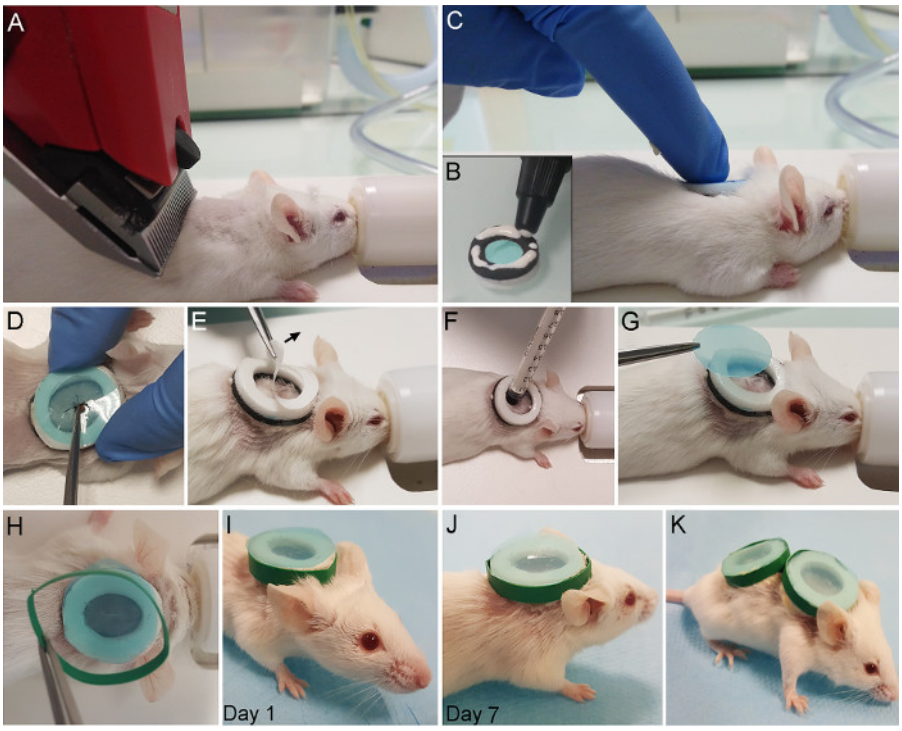

Figure 2: Gluing the capsule to the mice and tick infestation. (**A)** Shaving mouse's back anterior part. (**B**) Application of the latex glue to the EVA-foam side of the capsule. (**C**) Attachment of capsule to the mouse. (**D**) Placing the nymph in the capsule via the cut in the transparent plastic. (**E**) Peeling the paper protection tape from the adhesive double sticky foam before larvae infestation. (**F**) Injections of larvae inside the capsule using a cut syringe. (**G**) Closing the capsule with the transparent plastic. (**H**) Placing a protective plastic band around the capsule. (**I**) Mouse with the attached capsule - 1st day. (**J**) Mouse with the attached capsule - 7th day. (**K**) Mouse with two capsules attached. Please click here to view a larger version of this figure.

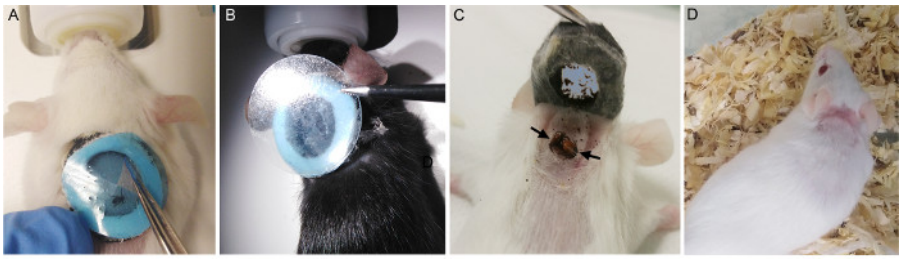

Figure 3: Tick collection and mouse recovery. (**A**) Cutting cross-shape opening for tick collection. (**B**) Resealing the capsule with adhesive plastic patch. (**C**) Capsule removal from a euthanized mouse. Arrows show the attached ticks. (**D**) Recovered mouse after dropped off capsule. Please click here to view a larger version of this figure.

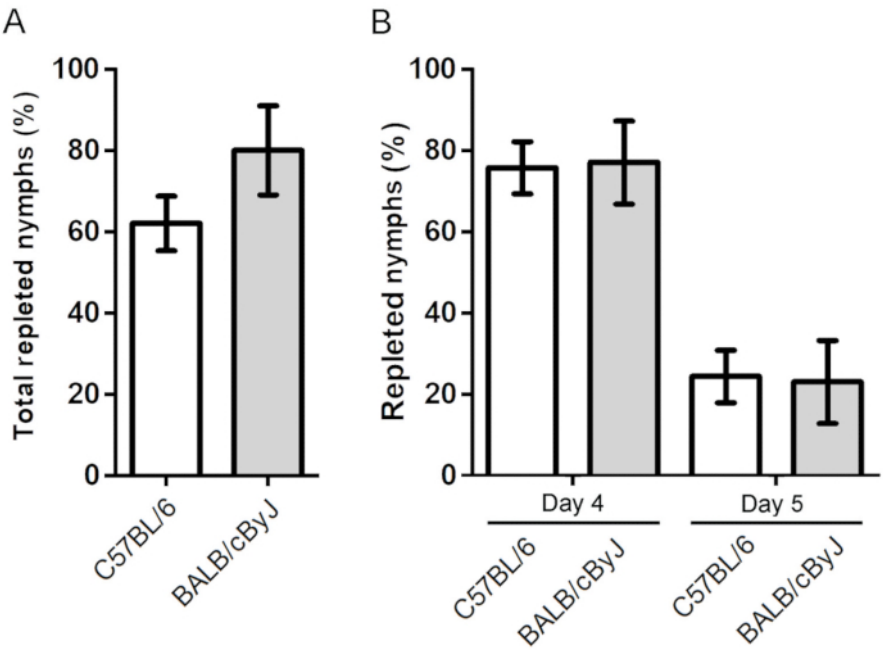

Figure 4: Engorgement success and feeding duration of *Ixodes ricinus* nymphs feeding on mice. (**A**) Total percentage of engorged nymphs in C57BL/6 and BALB/cByJ mice. (**B**) Duration of nymph engorgement in C57BL/6 and BALB/cByJ mice. The (n) numbers for infested nymphs are 130 and 25 for 15 individual C57BL/6 and 5 individual BALB/cByJ mice, respectively. Please click here to view a larger version of this figure.

Discussion

The most critical step in the protocol is firm gluing of the capsule to the mouse skin. Therefore, the latex glue should be homogenously applied to the entire EVA-foam surface of the capsule and constant pressure for 3 minutes should be applied, especially to the left and right side of the capsule.

We also recommend placement of the capsule as far forward on the back as possible to avoid its removal by the mouse using its rear paws. In our experiments, only the adhesion of the EVA-foam and latex glue to the mouse skin has been validated and we cannot guarantee the achievement of same results using different materials.

During our experiments, detachment of the capsule from the skin within first seven days was not observed. We strongly recommend protecting the outer surface of the capsule using the plastic band (**Figure 2H**). If the protective band is damaged over the course of tick feeding, it can be replaced with a new one. The diameter of the capsule can be modified for different mouse strain sizes. We suggest monitoring the feeding ticks at least twice daily and to collect engorged ticks immediately after detachment to avoid their desiccation.

The number of infested ticks is limited by the capsule diameter, as well as the host size. In our experiments we used maximum of 20 nymphs or 100 larvae of *I. ricinus* for one mouse. For the larger size ticks such *Amblyomma* or *Hyalomma* sp., etc the number of infested ticks should be reduced to avoid harm to the host from blood loss[19, 26, 27]. Therefore, this technique is not suitable for the maintenance of tick rearing colonies, where large numbers of ticks are required to feed. For this purpose, larger hosts like rabbits or sheep are recommended[20, 27] to reduce overall animal requirement.

Our technique is suitable for various types of experiments where a mouse model is required, and it is necessary to keep ticks in enclosed area for easy collection and/or monitoring of their biological parameters. Compared to other techniques[10, 11, 12, 13, 14, 15, 16, 17, 18], this simple protocol greatly reduces the overall anesthesia time (approximately 5 minutes) per mouse and the fast drying, non-irritating latex glue does not cause harm to the animal. The highly adhesive EVA-foam capsule protects the tick feeding area and minimizes the risk of lost, damaged, or eaten ticks as reported in free infestation systems[10, 11, 12, 13, 15]. The great advantage of the proposed technique is the flat-shape capsule and its firm long-lasting attachment to the skin allowing easy manipulation with the mouse if required. Special attention has been paid on usage of elastic and non-irritating materials to reduce the discomfort to the experimental animals allowing complete recovery of the mouse host after experiment (**Figure 3D**).

The method is expected to be used for a variety of the experiments when studying tick-host-pathogen interactions, tick manipulation of host immune systems, evaluating different tick control measures or tick biology.

Disclosures

The authors have nothing to disclose.

Acknowledgments

We acknowledge the technical assistance of Alain Bernier French National Institute of Agricultural Research (INRAE), and Océane Le Bidel (ANSES). The study was supported by the DIM One Health - *Région Île-de-France (Acronym of the project: NeuroPaTick)*. The mice were purchased by ANSES. Dr. Jeffrey L. Blair is acknowledged for reviewing the earlier version of the manuscript.

References

1. Sonenshine, D. E., Roe, M. *Biology of Ticks.* Oxford University Press. (2014).
2. Kröber, T., Guerin, P.M. *In vitro* feeding assays for hard ticks. *Trends in Parasitology.* **23** (9), 445-449 (2007).

3. Bonnet, S. et al. Transstadial and transovarial persistence of Babesia divergens DNA in Ixodes ricinus ticks fed on infected blood in a new skin-feeding technique. *Parasitology.* **134** (2), 197-207 (2007).
4. Bonnet, S., Liu, X. Laboratory artificial infection of hard ticks: A tool for the analysis of tick-borne pathogen transmission. *Acarologia.* **52** (4), 453-464 (2012).
5. Kohls, G. M. Tick rearing methods with special reference to the Rocky Mountain wood tick, *Dermacentor andersoni.* In: *Culture methods for invertebrate animals.* Edited by Galtsoff, P. S., Lutz, F. E., Welch, P. S. and Needham, J. G., 246–256, Comstock Publishing Co., Ithaca, N.Y. (1937).
6. Faccini, J. L. H., Chacon, S. C., Labruna, M. B. Rabbits (Oryctolagus cuniculus) as experimental hosts for Amblyomma dubitatum. Neumann (Acari: Ixodidae). *Arquivo Brasileiro de Medicina Veterinária e Zootecnia.* **58** (6), 1236-1239 (2006).
7. Chacon, S. C., Freitas, L. H. T., Barbieri, F. S. Relationship between weight and number of engorged Amblyomma cooperi. Nuttal (sic.) and Warburton, 1908 (Acari: Ixodidae) larvae and nymphs and eggs from experimental infestations on domestic rabbit. *Brazilian Journal of Veterinary Parasitology.* **13**, 6-12 (2004).
8. Sonenshine, D. E. Maintenance of ticks in the laboratory. In: *Maintenance of Human, Animal, and Plant Pathogen Vectors.* Edited by Maramorsch, K. and Mahmood, F., 57-82, *Science Publishers Inc.* Enfield NH (1999).
9. Levin, M. L., Schumacher, L. B. M. Manual for maintenance of multi-host ixodid ticks in the laboratory. *Experimental and Applied Acarology.* **70** (3), 343-367 (2016).
10. Almazán, C. et al. Identification of protective antigens for the control of Ixodes scapularis infestations using cDNA expression library immunization. *Vaccine.* 21 (13-14), 1492-1501 (2003).
11. Banks, C. W., Oliver, J. H., Hopla, C. E., Dotson, E.M. Laboratory life cycle of *Ixodes woodi.* (Acari:Ixodidae). *Journal of Medical Entomology.* **35**, 177-179 (1998).
12. Almazán, C. et al. Characterization of three Ixodes scapularis cDNAs protective against tick infestations. *Vaccine.* **23** (35), 4403-4416 (2005).
13. Levin, M. L., Ross, D. E. Acquisition of different isolates of Anaplasma phagocytophilum by Ixodes scapularis from a model animal. *Vector Borne Zoonotic Diseases.* 4 (1), 53-59 (2004).
14. Heinze, D. M., Wikel, S. K., Thangamani, S., Alarcon-Chaidez, F. J. Transcriptional profiling of the murine cutaneous response during initial and subsequent infestations with Ixodes scapularis nymphs. *Parasites & Vectors.* **6** (5), 26 (2012).
15. Nuss, A. B, Mathew, M. G, Gulia-Nuss, M. Rearing, Ixodes scapularis, the Black-legged Tick: Feeding Immature Stages on Mice. *Journal of Visualized Experiments.* (123) e55286 (2017).
16. Wada, T. et al. Selective ablation of basophils in mice reveals their nonredundant role in acquired immunity against ticks. *Journal of Clinical Investigation.* **120** (8), 2867-2875 (2010).
17. Saito, T. B., Walker, D. H. A Tick Vector Transmission Model of Monocytotropic Ehrlichiosis. *The Journal of Infectious Diseases.* **212** (6), 968–977 (2015).
18. Boppana, V. D., Thangamani, S., Alarcon-Chaidez, F. J., Adler, A. J., Wikel, S. K. Blood feeding by the Rocky

Mountain spotted fever vector, Dermacentor andersoni, induces interleukin-4 expression by cognate antigen responding CD4+ T cells. *Parasites & Vectors.* **2** (1), 47 (2009).

19. Gargili, A., Thangamani, S., Bente, D. Influence of laboratory animal hosts on the life cycle of Hyalomma marginatum and implications for an in vivo transmission model for Crimean-Congo hemorrhagic fever virus. *Frontiers in Cell and Infection Microbiology.* **20** (3), 39 (2013).
20. Almazán, C. et al. A Versatile Model of Hard Tick Infestation on Laboratory Rabbits. *Journal of Visualized Experiments.* (140) e57994 (2018).
21. Zhijun, Y., et al. The life cycle and biological characteristics of *Dermacentor silvarum* Olenev (Acari: Ixodidae) under field conditions. *Veterinary Parasitology.* **168** (3–4), 323-328 (2010).
22. Ahmed, B. M., Taha, K. M., El Hussein, A. M. Life cycle of Hyalomma anatolicum Koch, 1844 (Acari: Ixodidae) fed on rabbits, sheep and goats. *Veterinary Parasitology.* 177 (3-4), 353-358 (2011).
23. Široký, P., Erhart, J., Petrželková, K. J., Kamler, M. Life cycle of tortoise tick Hyalomma aegyptium under laboratory conditions. *Experimental and Applied Acarology.* **54**, 277-284, (2011).
24. Chen, X., et al. Life cycle of Haemaphysalis doenitzi (Acari: Ixodidae) under laboratory conditions and its phylogeny based on mitochondrial 16S rDNA. *Experimental and Applied Acarology.* **56**, 143-150 (2012).
25. Jin, S. W., et al. Life Cycle of Dermacentor everestianus Hirst, 1926 (Acari: Ixodidae) under Laboratory Conditions. *Korean Journal of Parasitology.* **55** (2), 193-196 (2017).
26. Labruna, M. B., Fugisaki, E. Y., Pinter, A., Duarte, J. M., Szabó, M. J. Life cycle and host specificity of Amblyomma triste (Acari: Ixodidae) under laboratory conditions. *Experimental and Applied Acarology.* **30** (4), 305-16 (2003).
27. Breuner, N. E. et al. Failure of the Asian longhorned tick, Haemaphysalis longicornis, to serve as an experimental vector of the Lyme disease spirochete, Borrelia burgdorferi sensu stricto. *Ticks Tick Borne Diseases.* **11** (1), 101311 (2020).