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

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Author Questionnaire

1. **Microscopy**: Does your protocol involve video microscopy, such as filming a complex dissection or microinjection technique? **No**

2. **Software:** Does the part of your protocol being filmed include step-by-step descriptions of software usage? **No**

3. **Filming location:** Will the filming need to take place in multiple locations? **No**

Introduction

1. Introductory Interview Statements

REQUIRED:

1.1. **Ladislav Šimo:** Our technique is suitable for various types of experiments where a mouse model is required for feeding ticks and it is necessary to keep the ticks in an enclosed area for easy collection and monitoring.

1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. Videographer NOTE: last one

1.2. **Ladislav Šimo:** The benefit of this simple method is its efficiency, short duration, and the ability to monitor or collect ticks at different time points of an experiment.

1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. Videographer NOTE: last one

OPTIONAL:

1.3. **Lourdes Mateos-Hernández:** Our system is suitable for studying tick biology, host-vector-pathogen interactions, or evaluating different control measures of these medically important arthropods.

1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. Videographer NOTE: last one

1.4. **Sabine Rakotobe:** This method requires accuracy and attention to detail as immature tick stages are small and feeding them on mice necessitates the enclosed capsule to be firmly attached to the mouse for several days.

1.4.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Videographer: This statement is optional. If you don't have time, don't film this.* Videographer NOTE: last one

Ethics Title Card

1.5. Procedures involving animal subjects have been approved by the Ethics Committee for Animal Experiments ComEth Anses/ENVA/UPEC.

Protocol

2. Preparation of the Capsule

2.1. Begin by sticking together a 2-millimeter EVA foam and adhesive double sticky foam **[1-TXT]**. Use a 20-millimeter diameter leather hole punch to cut a circle from the foam pieces **[2]**. Then, use a 12-millimeter hole punch to cut the interior **[3]**.

2.1.1. Talent sticking together the 2 foam pieces. **TEXT: EVA: ethylene vinyl acetate** Videographer NOTE: take 2

2.1.2. Talent punching the circle out of the foam. Videographer NOTE: cu + med

2.1.3. Talent punching the inner circle.

2.2. Peel the protective paper strip from the adhesive double sticky foam **[1]** and attach a 20-millimeter transparent plastic circle to the foam **[2]**. If feeding larvae, do not remove the protective paper strip from the adhesive foam and proceed directly with capsule attaching. **[3-added]**.

2.2.1. Talent peeling the paper strip off.

2.2.2. Talent attaching the transparent plastic circle.

2.2.3. Extra Shot: Comparing the 2 versions of the capsule

2.3. Make a 1-centimeter slit in the transparent plastic **[1]**, then use an entomological pin to create at least 10 small holes for moisture evaporation during the experiment **[2]**.

2.3.1. Talent making the slit in the plastic.

2.3.2. Talent making holes in the plastic.

3. Capsule Attachment to the Mouse

3.1. After the mouse has been anesthetized, place it on the manipulation pad and attach a nose cone for continuous isoflurane supply **[1]**. Ensure that the breathing rate is less than 80 breaths per minute by adjusting the isoflurane level **[2]**.

3.1.1. Talent positioning the mouse and attaching the cone. Videographer NOTE: take 2

3.1.2. Talent adjusting the isoflurane level.

3.2. Shave the anterior part of the mouse from behind the shoulder blades up to the area just behind the ears, creating a shaved area that is greater than the capsule surface **[1]**. Apply non-irritating latex glue to the entire EVA-foam site of the capsule and wait for 1 minute **[2]**. *Videographer: This step is important!*

3.2.1. Talent shaving the mouse.

3.2.2. Talent applying glue to the capsule.

3.3. Place the capsule on the mouse's back and apply constant pressure for 3 minutes, especially on the left and right side of the capsule **[1]**. Slightly lift the capsule to visually check its attachment to the skin **[2]**. *Videographer: This step is difficult and important!*

3.3.1. Talent placing the capsule on the mouse's skin and holding it there.

3.3.2. Talent lifting the capsule.

3.4. **Lourdes Mateos-Hernández**: To ensure the firm attachment of the capsule, apply a slight, 3-minute constant pressure with the fingers, especially on the left and right side of the capsule.

3.4.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Videographer: This statement is optional. If you don't have time, don't film this.* Videographer NOTE: last one

3.5. If non-attached regions are found, apply more glue with a spatula and press for another 3 minutes. If a non-attachment region forms during the experiment, fix it in the same way **[1]**. *Videographer: This step is important!*

3.5.1. Talent adding glue and continuing to hold down the capsule.

4. Tick Infestation

4.1. Introduce individual nymphs into the capsule via the 1-centimeter slit **[1]**. Slightly squeeze the capsule from two sides to allow the transparent plastic to bend, then push the nymph inside the capsule. Turn the forceps 90 degrees and pull them out **[2]**.

4.1.1. Nymphs ready for infestation.

4.1.2. Talent inserting a nymph into the capsule.

4.2. For larvae infestation remove the paper slip from the attached capsule **[1]** and place the syringe with the larvae directly inside **[2]**. Deposit the ticks by pushing the syringe plunger, then gently turn the plunger towards the skin to remove the remaining attached larvae **[3]**.

4.2.1. Talent removing the paper slip.

4.2.2. LAB MEDIA: Larvae Syringe.mp4.

4.2.3. Talent depositing the syringe and turning the plunger.

4.3. Once the larvae are deposited onto the skin, close the capsule by attaching the transparent plastic circle **[1]**.

4.3.1. Talent attaching the transparent plastic circle.

4.4. Cut a 3.5-millimeter strip of the protective plastic band **[1]** and apply the protective plastic band around the capsule **[2]**, then return the mouse to the cage **[3]**.
Videographer: This step is important!

4.4.1. Talent cutting the protective band.

4.4.2. Talent applying the protective band.

4.4.3. Talent retuning the mouse to the cage.

5. Collection of Ticks

5.1. To collect the ticks, create a cross shaped cut in the plastic with a scalpel **[1]** and use forceps to collect the ticks **[2].** If necessary, reclose the capsule by sticking an adhesive plastic patch on the transparent plastic. The same plastic patch can be used if collection of ticks at multiple time points is required **[3]**.

5.1.1. Talent making a cross shaped cut. Videographer NOTE: take 2

5.1.2. Collect the ticks by forceps.

5.1.3. Talent reclosing the capsule with adhesive patch.

5.2. Alternatively, the mouse can be euthanized and the ticks collected or manually detached after capsule removal **[1].**

5.2.1. LAB MEDIA: Figure 3C. Euthanized mouse with removed capsule and attached ticks.

5.3. For successful recovery of the mice, keep them in a cage for one additional week and let the capsules detach naturally **[1-TXT]**. Once the capsule is off, check for abnormal reactions on the skin. Apply an emollient lotion if there is any irritation **[2]**.

5.3.1. Mouse in the cage. **TEXT: 8 – 9 days**

5.3.2. Talent examining the mouse after capsule detachment.

Videographer: Film a fully recovered mouse for 6.2.2. in the results section.

Results

6. Results: Engorgement Success and Feeding Duration of *Ixodes ricinus* Nymphs on Mice

6.1. This protocol makes it possible to feed immature hard ticks in EVA-foam capsules applied to a mouse's back. The use of the fast-drying and non-irritating latex glue ensures that the capsule is firmly attached within 3 minutes **[1]** and remains attached for at least 7 days **[2]**.

6.1.1. LAB MEDIA: Figure 2 I.

6.1.2. LAB MEDIA: Figure 2 I and J.

6.2. Attaching two capsules makes it possible to feed two different groups of ticks on the same animal **[1]**. Furthermore, complete recovery of the mice after the experiments makes it possible to reuse the animals and avoid euthanasia **[2]**.

6.2.1. LAB MEDIA: Figure 2 K.

6.2.2. Fully recovered mouse. *Videographer: Please film this.*

6.3. This system has been successfully used to feed *Ixodes ricinus* nymphs. A moderate to high engorgement success rate was achieved in two different mouse strains **[1]**. In both strains, all nymphs finished the feeding within 4 to 5 days **[2]**, while the majority dropped off on the fourth day **[3]**.

6.3.1. LAB MEDIA: Figure 4_updated A.

6.3.2. LAB MEDIA: Figure 4_updated B.

6.3.3. LAB MEDIA: Figure 4_updated B. *Video Editor: Emphasize the Day 4 bars.*

Conclusion

7. Conclusion Interview Statements

7.1. **Lourdes Mateos-Hernández:** When attempting this protocol, it is important to shave a sufficient area of mouse skin and effectively attach the capsule to avoid its accidental detachment during the experiment.

7.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 3.2.1 and 3.3.1.* Videographer NOTE: the one before last

7.2. **Ladislav Šimo:** Besides the study of basic tick biology, this method can be used to explore pathogen transmission from tick to host or host to tick.

7.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. Videographer NOTE: last one

7.3. **Ladislav Šimo:** Additionally, the option to reuse the mouse offers a possibility for multiple reinfestations that can be beneficial for immunological studies or investigating different tick-borne pathogen transmissions.

7.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Videographer: This statement is optional. If you don't have time, don't film this.* Videographer NOTE: last one