**The point-by-point responses to the editorial and reviewers comments:**

**Editorial comments:**

General:

1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues.

Response: The spelling and the grammar has been checked across entire manuscript.

Protocol:

1. For each step, please ensure you answer the “how” question, i.e., how is the step performed? Alternatively, add references to published material specifying how to perform the protocol action. If revisions cause a step to have more than 2-3 actions and 4 sentences per step, please split into separate steps or substeps.

Response: The protocol has been modified based on the editorial comment.

Specific Protocol steps:

1. 4: What is a usual time scale from infestation to collection? Is there a maximum time?

Response: The following Note has been included to the step 3.4 in the protocol (Line 185-189):

*NOTE: The time from the infestation to collection of the ticks vary among different tick species and their developmental stages. For example I. scapularis and I. ricinus duration of feeding for adults, nymphs and larvae is 6-7, 3-4 and 2-3 days respectively. The list of references for the 29 different hard tick life cycle under laboratory condition can be found in Levin and Shumacher (2016).*

1. 5.2: What are usual abnormal reactions, and how are they treated? Also, Figure 3H does not exist.

Response: Although we have not experienced any abnormal reactions, in some cases the skin irritation after the capsule is removed may occur, in that case an emollient lotion can be used, we have included this in the manuscript. All the figures have been reordered and cited in the text.

Results:

1. Can you provide examples of outcomes from this procedure, in particular how its results compare to other methods? These can be from other publications, although reused Figures will need to be properly cited and copyright permission obtained (if necessary).

Response: We included/modified a paragraph in the results section giving the examples of the tick feeding success in our system. We did not provide the comparison with other methods because the feeding success does not relay on the technique but on the fitness of the ticks and suitability of the rabbit for the particular tick species as a host. The aim of this study is to offer to the community the user-friendly system which can be used for different kind of purposes when studying tick biology or maintaining the tick colonies.

The following paragraph have been included/modified in the results (Line 235-241):

*The tick feeding success in the laboratory mostly relies on the fitness of the individuals and suitability of the rabbit host for the particular tick species rather than the technique itself. This protocol has been proven for successful feeding of different live stages of several hard tick genera such as Ixodes, Dermacentor, Rhipicephalus, Hyalomma and Amblyomma. For example we observed 93.3% (n=240) engorgement rate of I. scapularis and I. ricinus adults. High success rate (data not shown) was observed also for the maintenance of colonies of these two species when feeding their developmental stages (Suppl. Fig. 1).*

2. Figure 4 has not been referenced in the text.

Response: All the figures have been reordered and cited in the text.

3. Figure 2L is not described in the Legend. ok

Response: All the figures have been reordered and cited in the text.

References:

1. Please ensure references have a consistent format.

Response: All the references have been corrected to same format.

Table of Materials:

1. Please ensure the Table of Materials has information on all materials and equipment used, especially those mentioned in the Protocol.

Response: The recommended mesh diameter of the mosquito mesh has been included (Line 124-125):

*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 Velcro loop side (Figs. 1E, F).*

**Reviewers' comments:**

**Reviewer #1:**

Manuscript Summary:

The title might not be representing the reality since infesting an animal with ticks is not indeed promoting the animal welfare. Something like "less damaging or less harmful" would be more accurate.

Response: We have changed the manuscript title to : *“A Versatile Model Of Hard Ticks Infestation On Laboratory Rabbits“*

Major Concerns:

The use of "non-irritating" materials, as far as possible and with the limitations already known, rather than the protocol in this manuscript contributes mainly to the overall idea, tick infestation and animal welfare. The use of "non-irritating" materials mostly the glue determine the damage/toxicity not only to the animal but to the ticks in every single protocol of animal tick infestation. Besides obviously the management of the animal. Using the glue once the ticks were put on the capsule is a GREAT disadvantage.

Response: We have substituted the glue with the Velcro system which allows comfortably reopen and close the capsule during the tick feeding period and does not represent any risk of the vapor or skin irritation to the ticks or rabbit during the infestation. The latex glue (solidifying in 3 minutes), for attaching the capsule to the rabbit is absolutely non-toxic (guaranteed by developers) and we did not observed any harm or abnormal reaction to this product. Base on this change the manuscript has been modified in order to emphasize the simplicity and versatility of this techniques rather than emphasizing the non-irritating materials.

Following this comment we have changed/substituted the 1.2 step in the protocol (Line 120-126):

* 1. *Glue the mosquito mesh on the capsule using fast drying cyanoacrylate glue (superglue hereafter) and leave one corner open for the later tick infestation (Fig 1 C).*

To

*1.2. Cut the 8 mm wide strips of the self-adhesive Velcro hook side and stick them to the prepared EVA foam frame (Fig. 1 C). Cut the same size strips from the Velcro loop side and bind them to the hook sides attached to the EVA-foam frame (Fig. 1D).*

*1.3. 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 Velcro loop side (Figs. 1E, F). Cut the overhangs if necessary.*

Also the steps 4.1 and 4.2 has been modified to (Line 193-203):

* 1. *Take the rabbit from the cage to the bench and unzip the jacket.*

*4.2 Gently restrain the rabbit with the hands. Open the capsule by unfastening the Velcro (Fig. 2 K, L) and collect the ticks by brushing the engorged larvae (Suppl. Fig. 1) or nymphs to a plastic dish, and using forceps for adults (Fig. 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 1.1. of the protocol. Maintain the engorged ticks at more than 95% humidity and 21 ˚C.*

* 1. *If needed, refasten the Velcro to close the capsule.*

And the following note has been removed :

*NOTE: Make sure to prevent the tick from sticking to the tape. It is also possible to reseal the capsule with another layer of the mosquito mesh. However, in this case we recommend using another glue than superglue as the vapors of the superglue may affect the ticks.*

Nothing is mentioned about the animal handling when sampling ticks. This would consider the overall stress to the animal when infesting with ticks. It assumes that the animal is going to stay still and not promoting the ticks to drop off the thin capsule (lines 268 and 269).

-The animal is restrained physically.

Response**:** Following this comment we included more detailed description of the animal handling and sampling the ticks in the protocol (Line 193-203).

***4. Collection of ticks***

* 1. *Take the rabbit from the cage to the bench and unzip the jacket.*

*4.2 Gently restrain the rabbit with the hands. Open the capsule by unfastening the Velcro (Fig. 2 K, L) and collect the ticks by brushing the engorged larvae (Suppl. Fig. 1) or nymphs to a plastic dish, and using forceps for adults (Fig. 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 1.1. of the protocol. Maintain the engorged ticks at more than 95% humidity and 21 ˚C.*

* 1. *If needed, refasten the Velcro to close the capsule.*

Minor Concerns:

Some sizes/times are mentioned like if the authors provide irrefutable evidence of the impossibility of using other sizes/values; for example in line 105, 111, 112, 127, 133.

Response: Here we understand the point of the reviewer. We use here imperative mood as JoVE requires. The sizes and values (unless specifically explained) are approximate and that is why we included the terms like: for example…, or should be…to give the users option to modified if needed.

Figure 2F, 2K, 2L are omitted in the text.

Response: All the figures have been reordered and cited in the text.

Another concern that is not mentioned is the limited use of this protocol in experiments with multiple sampling times.

Response: The capsule open-close system has been improved (Fig 1. ). This Velcro-based system allows reopen/refasten the capsule as much times as needed. The protocol has been changed appropriately as mentioned above in the major concerns of this reviewer.

Line 178. Assuming the ticks are already off the animal even though is not clearly mentioned, section 4 is collection of ticks.

Response: The section 4 of the protocol has been elaborated more in details as mentioned above.

Line 243-245. This is always the case in every single protocol of animal tick infestation.

Response: We understand the comment and have modified the paragraph (Line 299-203). One original sentence left for readers who are not familiar with the tick biology. In addition we are referring here to another possible host using our system to give the users option to change the host if needed.

Modified paragraph Line 299-203:

*Although our system has been tested for feeding of various hard tick species, the rabbit is not a natural host for all hard tick species13. This limitation may be overcome by adapting this system to other animal hosts suitable for a particular hard tick species like sheep, where the EVA foam system was adapted to feed all the developmental stages of I. ricinus (Fig. 3).*

Line 253-254. The feasibility of using EVA foam would depend of the animal size.

Response: The EVA foam system is adaptable to any animal size which is great advantage of this technique. It means the feasibility does not depends of the animal size.

Line 278. Studies regarding host-vector-pathogen interactions would require in many cases the use of the natural animal host involving the use of bigger animals. The use of the jacket or something equivalent is mandatory. For many animal host this will be an issue.

Response: Here we refer to the general EVA-foam feeding based system and not the jacket as explained in the text when EVA-foam system has been adapted to the sheep model where instead of jacket the elastic bandage has been used in instead of jacket. To void confusions and be more specific about the EVA-foam capsule system, we have changed the sentence (Line 318-320):

*This method is expected to be used for the variety of different experiments when studying tick biology, host-vector-pathogen interactions, or evaluating different control measures like acaricides or vaccines.*

To:

*The EVA-foam based tick feeding system detailed in this study is expected to be used for the variety of different experiments when studying tick biology, host-vector-pathogen interactions, or evaluating different control measures like acaricides or vaccines.*

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**Reviewer #2:**

Manuscript Summary:

As it is said, 'there are more ways than one to skin a cat," so are there more ways than one for feeding ticks on an animal. A significant number of publications had described various methods with different degree of detail, including a manual published by Levin and Schumacher in 'Experimental and Applied Acarology' 2016. Improvement of "old" techniques and development of new ones is an ongoing process and publication of these developments should be encouraged as it increases the "toolbox" available for researchers' consideration and choosing. However, advantages of one tick-feeding method over others is often just a matter of opinion and the most important/frequent consideration is which of those methods is more familiar for a particular investigator and his/her Institution. Therefore, I am quite surprised that the authors of the reviewed manuscript decided to advertise their method as something far superior that others. In my opinion, such advertisement is both UNFOUNDED and poses a risk of undermining long-established approved protocols used by other researchers.

Specifically, authors name the following as advantages of their method (lines 268-269): "Compared to other methods, we do not use anesthesia, rabbit collars, ear socks or hobbling of the rear legs. This method significantly decreases the stress of the rabbits, and they can be fully recovered after the experiment." I have to disagree with most of these assessments, especially regarding the "Emphasis on Laboratory Animal Welfare"

Response: We appreciate the comment of the reviewer. There are several ways to feed ticks on animals, and our intention with this manuscript was never to claim superiority, but to share with the researchers in this field a simplified and versatile method and compare it with existing methods as required by the JoVE policy. The tick feeding protocol by Levin and Schumacher in 'Experimental and Applied Acarology' 2016 is definitely advantageous however comparing to our method is laborious, takes a long time and its limit in the number of capsules placed on the animal. In addition we strongly believe that avoiding, E-colar, hobbling and anesthesia in our protocol significantly simplify the entire procedure and reduces the discomfort of the used animals. The rabbits in our research move freely in the cage, have easy access to the water and food and have comfortable sleep during the night or when resting, which is not the case when use the E-colar or hobbling the rear legs. However we agree with the reviewer that we do not have measurable evidence about the rabbit stress lowering. For this reason we have modified the text (original 258-262) in the part pointed out by the reviewer “The significance of the method with respect to existing/alternative methods.“

*When developing this system, we paid special attention to minimize the stress of the experimental animals. Compared to other methods6,8,9,10, we do not use anesthesia, rabbit collars, ear socks or hobbling of the rear legs. This method significantly decreases the stress of the rabbits, and they can be fully recovered after the experiment.*

To (Line311-315):

*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 legs5,6,7,8,9,10. In addition, the present protocol is not laborious, and no intensive training is required to become familiar with this technique.*

Another change has been made in “Introduction”:

*Two common strategies of tick feeding on rabbits have been frequently used: a) feeding on rabbit ears covered with cotton cloth or socks7, and b) feeding in cotton bags glued to the rabbit’s back9. 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 what is uncomfortable for the animal and makes difficult the recovery of the engorged ticks. This system is also limited only to two tick groups on ears fully covered by socks and protected by Elizabethan collars, representing a significant discomfort for the animal. The second system, where ticks are placed in cotton stockinette or neoprene cells glued to the shaved rabbit back, is more advanced but the protocols are quite laborious often involving Elizabethan collar to prevent grooming, hobbling the rear legs to avoid scratching, or even sedating or anesthetizing the animals during manipulations10.*

Has been changed to (Line: 70-75):

*Two common strategies of tick feeding on rabbits have been frequently used: a) feeding on rabbit ears covered with cotton cloth or socks6,7, and b) feeding in cotton bags9, nylon bottles10 or neoprene chambers11 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 or/and the recovery of engorged ticks difficult. This system is also limited only to two tick groups on ears fully covered by socks and protected by Elizabethan collars, representing a significant discomfort for the animal. Other systems9,10,11 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 anesthetizing or sedating the animals during manipulation, hobbling the rear legs to avoid scratching, and the use of Elizabethan collar to prevent grooming.*

Major Concerns:

1-ANESTHESIA.

In other published methodologies, sedation is used only to alleviate stress of the rabbit during the shaving procedure and to keep it calm while bags or chambers are glued to the skin. After that, no sedation is needed or recommended for the duration of tick feeding and collection. The method described here still requires shaving of the rabbit's hare but, instead of sedating the animal, authors recommend using physical restraint by a second person. This physical restraint can be quite prolonged: at least 3-5 minutes for shaving + approximately 20 minutes for gluing 4 chambers (5 min each) with additional time as needed if chamber(s) do not attach properly. That is at least 25-30 minutes of complete physical restraint of an easily spooked animal during an unusual and stressful procedure.

Laboratory Animal Welfare regulations require that "… for animal care, treatment, and practices in experimental procedures to ensure that animal pain and distress are minimized, including adequate veterinary care with the appropriate use of anesthetic, analgesic, TRANQUILIZING DRUGS, or euthanasia". As a member of Animal Use Committee, I would not approve such a stressful procedure to be conducted without appropriate sedation, especially considering that all this stress can be easily alleviated with simple and safe anesthesia.

Response: With the respect to the reviewer we do not agree with this comment. Simply we did not find a need to use anesthesia in our procedure. The reviewer is referring that the procedure may takes 30 minutes for shaving the rabbit or gluing the capsules. In reality several capsules may be glued simultaneously whish reduce the time of the process. Despite this fact the rabbits are calm. The protocol highlighted in the first comment of this reviewer Levin and Schumacher in 'Experimental and Applied Acarology' 2016 takes 3 hour just for the glue to completely solidify (in our case 3 minutes). The design of our EVA foam capsule, plus the latex glue allows it to be attached quickly that anesthetized animals is absolutely not required. In addition not every animal facility possess the anesthesia equipment’s. Also the anesthesia or tranquilizing drugs in the rabbit system may negatively affect the tick feeding. In contrary with the reviewer who would not allowed this procedure without anesthesia the ethical comity of our institution (and former ethical comity in USA , Kansas State University where this methods has been used) warmly approved this protocol.

2-USE OF EVA-FOAM CHAMBERS.

The previously published techniques use various hard-sided chambers (usually with screw top closer) or elastic fabric bags for containment of feeding ticks on animals. The reviewed manuscript suggests using suggests using elastic chambers instead. The difference in the duration of feeding within a single cohort of ticks can be 3 days for larvae and ~7 days for adults. So, it is usually recommended that ticks be removed from those containers as soon as they complete their engorgement and detach from the skin. This is necessary to both save engorged ticks from desiccation or being damaged by the animal as well as to minimize additional irritation to the animal cause by unattached ticks crawling inside the container on already irritated skin. Therefore, all previously suggested bags and chambers are designed for easy REPEATED access to ticks inside. As authors of the reviewed manuscript correctly point out (lines 259-260), "engorged-detached ticks (mainly immature stages such as larvae and nymphs) desiccate faster due to the temperature of the rabbit when left" on an animal. That is why engorged larval and nymphal ticks must be collected, removed from the animal twice daily. Elastic chambers made out of foam does not seem to allow frequent repeated reopening of the chamber for tick collection. This will result in higher tick mortality - DECREASED EFFICIENCY OF ANIMAL USE, as well as extra irritation to the animal itself.

On the other hand, I am not sure what happens to ticks that had not yet completed their engorgement by the time when feeding chambers are opened. Are those ticks left on the rabbit? Or, are they allowed to escape from an opened chamber?

Response: We agree with the comment and we have improved our system with the Velcro and as is mentioned for the reviewer 1. We have improved the capsule open-close system with the Velcro (Figure 1). This system allows comfortably reopen/refasten the capsule and collect/monitor the ticks. The text has been changed appropriately to this modification (has been already answered in the case of the reviewer no. 1).

3-USE OF RABBIT JACKETS INSTEAD OF E-COLLARS

Although it is difficult to judge the rabbit's preference for one device over another, e-collars are safe and animal-friendly devises used in veterinary practice all over the World for a wide variety of animal species from small birds to sheep and goats. In our experience, rabbits adjust to collars quickly with no changes in demeanor, behavior, food and water intake, or any other signs of discomfort. Applicability of rabbit jackets depends on their availability (they cost $100-150 each) and whether or not they can be used in combination with "traditional" (more accessible) screw top capsules or fabric bags.

Hobbles recommended in other techniques are replaced here with taping over rabbits' rear paws - equal substitution.

Response: Although E-collars may be very useful, we prefer to use jackets since in our observations that jackets are more comfortable for the animal and are better protection of the capsules containing the ticks. In addition the use of the jackets eliminate the use of the rear legs hobbling which is in our case substituted by taping the nails of the rear paws. The taping the paws (which is an optional step in our protocol) allows normal walking of the rabbit which is not the case of the hobbling. In summary in our observations the jackets are doing great job in protecting the capsules and minimizing the discomfort of the animals.

4-RECOVERY

In our experience, rabbits are quite capable of removing bags and capsules themselves within a few days after being allowed to groom themselves. Complete recovery of skin lesions or the site of tick attachment is natural process that does not depend on a specific tick-feeding technique.

Response: We are agree with your comment, however, the use of materials are non-irritant, the damage of the skin is reduced, thus the recuperation takes less time. To let rabbit to groom the capsule (as mentioned by the reviewer) is not a good idea while there is a risk of eating the EVA foam. As we mentioned in the protocol in our non-irritating glue system the capsule drop of after 3-4 weeks naturally under the rabbit jacket. So the rabbit is not exposed to the risk of eating/swallowing it.

Overall, I do not have any objections to publication of a yet another tick-feeding technique AS LONG AS CLAIMS OF SUPERIORITY ARE REMOVED.

Response: Here again, our intention has not been to claim superiority. We were trying to compare our technique with existing methods. However taking into account the reviewers points, we have change the sound of the manuscript in several places (detailed above) to avoid this impression. In addition great advantage of this technique is the use of the openable Velcro system-sealed capsule which has been included to the revised version of this manuscript.

**Reviewer #3:**

Manuscript Summary:

The manuscript describes the feeding of ticks on laboratory animals using feeding chambers made with novel materials which propose some advantages. The text is overall well written in a clear and understandable way, presenting details to enable the methods to be repeated by other researches. Specific comments about the text are written below.

Major Concerns:

The main flaw of the work is that authors need to include the methodology described by Bouchard and Wikel (2005) in the introduction and discussion. Their methodology also uses chambers made with material other than cotton bags or cloths which are more similar to the chambers made in the present work. Bouchard and Wikel suggest using 1.5 mL Eppendorf®-style tubes to feed nymphs on mice and wide-mouth Nalgene® 500 mL bottles to feed adult tick couples on rabbits, both fixed at the back of the host. The caps of the chambers are glue-free and easy to open, which is advantageous in situations when you have to daily check the ticks and an advantage in comparison to the limitations described in lines 172-176 and 258-263. (Bouchard, K.R., Wikel, S.K., 2005. Care, Maintenance, and Experimental Infestation of Ticks in the Laboratory Setting, In: Marquardt, W.C. (Ed.) Biology of Disease Vectors. Elsevier, San Diego, pp. 705-712.)

Response: The suggested references have been included**.** A advantageous open/close system has been improved in our EVA foam capsule that can be opened when required (Figs. 1, 2). This issue has been addressed in the case of the specific comments for the reviewers 1 and 2 (please see above).

Minor Concerns:

-Another work that is worth citing was published by Levin and Schumacher (2016). Although similar to others (2 and 9), this is a methodology paper giving details on blood feeding using cotton stockinettes. This work is an appropriate citation for statements in lines 64 and 192. (Levin, M.L. & Schumacher, L.B.M., 2016. Manual for maintenance of multi-host ixodid ticks in the laboratory. Exp Appl Acarol, 70: 343-367)

Response: Thank you for your comment. We have included the appropriate reference of Levin and Schumacher, 2016.

Although the welfare of the animals is improved in comparison to the methodology of using bags in the ears, I don't see significant improvements in animal welfare in comparison to methodologies that use chambers at the back of the rabbit. These are subjective comparisons (elizabethan collar x jacket and a protective tape applied to the rear paws; anesthesia x physical restraint) all with advantages and disadvantages in terms of animal welfare. Therefore, the term "welfare" should be removed from the title.

Response: We agree with the comment and we have changed the manuscript title to : *“A Versatile Model Of Hard Ticks Infestation On Laboratory Rabbits“*

Line 51 -53 - Remove reference 2; it is not appropriate for this statement. Troughton et al described tick life cycle in the laboratory and not pathogen transmission.

Response: The reference no. 2 has been removed.

Line 119 - Do you really need to completely shave the back of the rabbit? Shearing the fur completely seems to me a negative point regarding animal welfare as it can potentially hurt the animal. We have seen that just trimming the hair close to the skin is better than completely shaving the rabbit's back. Trimming instead of shaving prevents irritation of the skin and the remaining fur helps the keeping the chamber fixed and also accelerates the recovery of the animal.

Response: In our experience, shaving the rabbits does not hurt nor irritate the skin. If only trimming is done, the fur may affect the visibility of larvae or nymphs and complicates the collection of engorged ticks. In addition to properly glue the capsule shaving is necessary. By shaving the rabbit with the clippers we have not observed any irritation.

Line 155 -159 - How long after the chamber is glued to the back of the rabbit do you need to wait before introducing the tick? Usually at least a gap of 24 hours is needed for the glue to dry completely before introducing the ticks to avoid glue odors and ticks getting glued inside the chamber.

Response: The non-irritant latex glue is completely dry after 3 mins (mentioned in protocol); therefore, the animal can be infested immediately. After 3 minutes solidification the glue does not have any odor to irritate the rabbit or the ticks. I addition there is not risk to glue the tick while the glue is completely dry. This step is actually one of the important advantages of our proposed method detailed in this manuscript.

Did authors have any problem with excess humidity inside the chambers? Usually excess humidity can kill ticks and the present method seems to impair water evaporation due to the two layers formed by the mosquito mesh and the rabbit jacket.

Response: No excess of humidity is observed with this method. The rabbit jacket along the mosquito mesh has enough porosity to allow flow of evaporation. Specifically exactly opposite is the true and the replete tick have to be monitored twice daily to avoid death due desiccation caused by the temperature of the rabbit.

-Line 218- Authors should include figure "L" in the legend. Last image should be K and L.

Response: All the figures have been reordered and cited in the text.

**Reviewer #4:**

Manuscript Summary:

The authors of this paper present a method for feeding different life stages of ticks on rabbits, potentially simplifying maintenance of tick colonies and blood feeding for experiments.

Major Concerns:

-Can the authors can provide any data on tick feeding success of this method? What percentage of ticks fed? Mortality data would also be useful: we have no idea how useful this technique is compared to other methods. The authors do provide the caveat that ticks may be vulnerable to desiccation with this method, but more information would be helpful.

Response: Here we would like to mentioned that the message of the manuscript is not to highlight better engorgement results comparing to the other protocols while still we are dealing with the rabbit system which is commonly used in tick research laboratories. Here we offer simple and versatile methods to feed hard ticks on the laboratory rabbits which can be adopted by several laboratories around the world.

Following this comment, we have included the percentage of the *I. scapularis* and *I. ricinus* ticks that successfully completed the feeding using our system (Line 235-244):

*The tick feeding success in the laboratory mostly relies on the fitness of the individuals and suitability of the rabbit host for the particular tick species rather than the technique itself. This protocol has been proven for successful feeding of different live stages of several hard tick genera such as Ixodes, Dermacentor, Rhipicephalus, Hyalomma and Amblyomma. For example we observed 93.3% (n=240) engorgement rate of I. scapularis and I. ricinus adults. High success rate (data not shown) was observed also for the maintenance of colonies of these two species when feeding their developmental stages (Suppl. Fig. 1). This EVA foam capsule system may also be adapted for other type of laboratory hosts, such as sheep (Fig. 3).*

The plastic syringe method used for tick infestations could be better described. It seems the ticks would get easily damaged by the syringe plunger pushing them out or a few might remain in the barrel of the "needle" area. Perhaps a few additional sentences describing this process would clarify this part of the procedure for the reader.

Response: Tit seems that the reviewer misunderstood that the “needle area” of the plunger is completely cut from the syringe. By this way no damage of the ticks have been observed (for all life stages). A figure (Fig 1H,G) has been included to the manuscript for the clarification.

Minor Concerns:

-The "mosquito mesh" could be described in more detail. Types of cloth can vary considerably in mesh size and is a crucial detail when dealing with the minute larval stages. Is this material known by any other name (curtain material, etc.)? Perhaps the pore size of the cloth could be included in the manuscript.

Response: The recommended mesh diameter of the mosquito mesh has been included (Line 124-125):

*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 Velcro loop side (Figs. 1E, F).*

Line 186: How should one "treat appropriately"? Is there a protocol that could be referenced (perhaps the animal use protocol)?

Response: Although we did not observed any abnormal reaction to the glue or EVA-foam capsule itself. For easier understanding we have included the sentence (Line: 214-2016):

*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 to treat irritation of the skin’s rabbit.*

The quality of photos in figure 2 is not good, at least in the version I received. This may be a result of pdf conversion and not the fault of the authors, but I would suggest that high quality photos be used in the final version.

Response: We submitted high resolution images and as the reviewer assumed the quality level decreased due to the pdf conversion.

Also, there are several grammatical errors or confusing syntax in this manuscript. I have listed a few below that should be corrected, but this manuscript would benefit from having a native English speaker proofread it.

Response: The English grammar has been corrected across the entire manuscript.

-The title is grammatically incorrect. I would recommend this revision: "A Model of Laboratory Tick Infestation Emphasizing Animal Welfare"

Response: We have changed the manuscript title to: “*A Versatile Model Of Hard Ticks Infestation On Laboratory Rabbits.“*

-Line 38: "...when attached to their vertebrate hosts..."

Response: has been corrected.

-Line 60: Spelling error: should be "guarantee"

Response: has been corrected.

-Line 66: "...what is uncomfortable..." should be "...which is uncomfortable...". Also, "makes the recovery of engorged ticks difficult." makes more sense grammatically.

Response: has been corrected.

-Line 186: "abnormal reaction" should be "abnormal reactions".

Response: has been corrected.

-Line 193: "during the manipulation..." should be "during manipulation"

Response: has been corrected.

-Line 195: "...applied to a shaved rabbit's back..."

Response: has been corrected.

-Line 204: "...other types of..."

Response: has been corrected.

**Additional changes/modifications:**

1. Due to the improvement of the capsule open-close system, the Figures composition (along their panels) has been completely changed and new figures have been included.
2. One supplementary figure (Suppl. Fig. 1) has been included describing the feeding of the tick larval stages using EVA-foam capsules.
3. The Material table has been slightly modified.
4. Due inclusion of new references the citations order along the list of references have been changed.