July 8, 2016

Avital Braiman, PhD

Director of Editorial of *JoVE*

One Alewife Center, Suite 200

Cambridge, MA 02140

Dear Dr. Braiman,

Enclosed, please find our revised manuscript (JoVE54850) entitled “Cefoperazone treated mouse model of clinically relevant *Clostridium difficile* strain R20291” that we are submitting for consideration for publication in *JoVE.* We have addressed all the reviewers’ comments in the attached “Rebuttal Comments” section.

*Clostridium difficile* is an anaerobic, gram positive, spore forming enteric pathogen that is associated with increasing morbidity and mortality consequently posing an urgent threat to public health. Recurrence with *C. difficil*e infection (CDI) after successful treatment with antibiotics is high, occurring in 20-30% of patients, thus necessitating discovery of novel therapeutics against this pathogen. Current animal models of CDI result in high mortality rates and thus do not approximate the chronic insidious disease manifestations seen in humans with CDI. To evaluate therapeutics against *C. difficile* a mouse model approximating human disease utilizing a clinically relevant strain is needed. This protocol outlines the cefoperazone mouse model of CDI using a clinically relevant and genetically tractable strain, R20291. Techniques for clinical disease monitoring, *C. difficile* bacterial enumeration, toxin cytotoxicity, and histopathologic changes throughout CDI in a mouse model are detailed in the protocol.

Successful execution of a murine model of CDI is challenging. Researchers struggle with certain aspects of CDI the mouse model and JoVE provides an excellent format to clarify some of the challenges researchers can encounter. In particular, we are interested in highlighting the most challenging and critical steps of the mouse model using JoVE’s video production including: calculation of the inoculum of *C. difficile* spores administered to mice, sterile collection of mouse feces, feces and cecal content bacterial enumeration of *C. difficile*, and completion of the Vero cell cytotoxicity assay.

This manuscript would be the first that fully details all steps of the mouse model of CDI using a clinically relevant strain. Compared to other mouse models of CDI, this model is not uniformly lethal at the dose administered, allowing for observation of a prolonged clinical course of infection concordant with human disease. Therefore, this cefoperazone mouse model of CDI proves a valuable experimental platform to assess effects of novel therapeutics on amelioration of clinical disease and restoration of colonization resistance against CDI. We anticipate that researchers interested in *C. difficile* pathogenesis and the development of novel therapeutics against *C. difficile* would greatly benefit from the protocol outlined in this manuscript.

This manuscript is the product of the team and all authors made substantial contributions to the conceptualization, acquisition, and interpretation of the data presented in this protocol. In addition, all authors contributed to the preparation of this manuscript for submission.

Thank you again for your consideration and I look forward to your response.

Sincerely,

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