Reviewer’s report

Title: Establishment of a leptospirosis model in guinea pigs using an epicutaneous inoculations route

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Reviewer: Mathieu Picardeau

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In the manuscript by Zhang et al., the authors have conducted a very basic set of experiments in which guinea pigs were infected with L. interrogans by epicutaneous inoculations. This is in contrast with most of the published challenge experiments where animals were infected by intraperitoneal inoculation of leptospires. As mentioned by the authors, epicutaneous inoculations may reflect conditions encountered during natural infection.

Major Compulsory Revisions

1) The authors should make sure that their study is placed in its proper context. For example, they did not cite landmark studies such as the one from Faine in 1957 on the dissemination of leptospires in infected guinea-pigs (“Virulence in Leptospira. II. The growth in vivo of virulent Leptospira icterohaemorrhagiae” British Journal of Experimental Pathology, Vol. 38, Pages 8-14). Some more recent studies (not cited in the text) have also used qPCR to follow bacterial load in animal models, including guinea pigs (ex. Lourdault et al. Use of quantitative real-time PCR for studying the dissemination of Leptospira interrogans in the guinea pig infection model of leptospirosis. 2009).

2) The authors should compare the dissemination (kinetics, bacterial load in target organs), histopathology, LD50 (not determined in this study), ... in guinea pigs infected by epicutaneous and the classical intraperitoneal inoculations.

3) subcutaneous infections: how long do the the filter discs with the bacterial suspension are put in contact with the skin? any idea of the true inoculum dose? this could be evaluated by qPCR of filter discs before and after contact with the skin.

Minor Essential Revisions

4) references for the EMJH medium should include Ellinghausen et al. (Nutrition of Leptospira pomona and growth of 13 other serotypes: fractionation of oleic albumin complex and a medium of bovine albumin and polysorbate 80. Am J Vet Res. 1965;26:45-51) and Johnson et al. (Differentiation of pathogenic and saprophytic leptospires. J Bacteriol. 1967;94:27-31).

5) Discussion: again, authors should compare their findings with previous papers on the use of PCR (or other methods) to detect Leptospira in a range of tissues
in a range of animal species by different routes of inoculations.

**Level of interest:** An article of importance in its field

**Quality of written English:** Needs some language corrections before being published

**Statistical review:** No, the manuscript does not need to be seen by a statistician.