Author's response to reviews

Title: Cyclical changes in seroprevalence of leptospirosis in California sea lions: endemic and epidemic disease in one host species?

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Author's response to reviews: see over
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Editor
BMC Infectious Diseases

Dear Editor,

Attached please find the revised version of the manuscript entitled “Cyclical changes in seroprevalence of leptospirosis in California sea lions: endemic and epidemic disease in one host species?” by James O. Lloyd-Smith and co-authors (MS # 81587961333932). We have addressed all points raised by the reviewers, and feel that the manuscript is improved as a result. Below we include a point-by-point description of how we addressed the reviewer comments.

We have also formatted the manuscript in keeping with your formatting checklist, and references have been formatting using the biomedcentral.ens style file for Endnote. We attach a Word document with all changes highlighted, as well as separate image files for all figures (in PDF format created in Adobe Illustrator CS2). Please let us know if other files are needed.

This manuscript is our original unpublished research and is not being considered elsewhere. It has been seen and approved by all authors. Thank you for your consideration.

Sincerely,

James O. Lloyd-Smith
Responses to reviews

Referee 1

Major Compulsory Revisions

Other investigations of California sea lion populations have incorporated serovar Cynopteri in their MAT panel with significant numbers of animals showing seropositivity for this particular serovar. The authors should explain reasons for exclusion of this serovar from their MAT panel.

Response: As we have now specified in the manuscript, the MAT panel used in this study was a convenience panel incorporating the six serovars of greatest diagnostic interest in California over the period of the study. Other serovars were not deliberately excluded, indeed Cynopteri was not included in the panel available to the researchers at the time when serological analysis was started. With this said, we believe it is unlikely that inclusion of Cynopteri would substantively change the results of the present study. The previous study that reported high seroprevalence of Cynopteri in California sea lions reported a maximum titer of 1:50 (below our threshold for seropositivity of 1:100, and far below the maximum titer of ≥1:204,800 to Pomona that was observed frequently in our study (Fig. 3a)). Also all Leptospira isolates from wild California sea lions have been Pomona, and we are not aware of any isolates of Cynopteri from any species in the western hemisphere. Finally, the sea lions that tested seropositive for Cynopteri was drawn from the Gulf of California population, which has been shown to be genetically isolated from the coastal population we are studying [1]. We have added text in the discussion addressing the possibility that unmeasured serovars are circulating, with particular reference to Cynopteri and Autumnalis.

A reference should be provided for the MAT method used. The description of the end point reading used should be provided.

Response: done

The source of Leptospira cultures used for the MAT should be provided.

Response: done

The authors need to review their nomenclature. The correct formatting of species and serovar in the paper is important, the International Leptospirosis Society website should be consulted for the correct details. Genus and species must be italicized, with the serovar name not italicized and with an upper case first letter.

Response: done

Provision of further detail on the breakdown of the specific MAT results (ie. by serovar) or by including a table(s) which summarises the MAT results versus serovars.

Response: In-depth analysis of the full data set for all six serovars is a large-scale undertaking, and will be the subject of a future publication in collaboration with the developers of the new methodology of antigenic cartography [2]. Meanwhile we have added Table 3, which compares titer scores for all six serovars and shows that serovar Pomona had the highest of all measured titers for the great majority of samples.
Discretionary Revisions (which the author can choose to ignore)

Reason for why males are 3.2 fold more likely to have high titres than females.

Response: This is a very interesting question which has been raised in previous studies of this system [3-6]. We have added text outlining one possible explanation for the pattern, associated with the different migratory behavior of male and female sea lions.

Some animals are serological (MAT) negative but can be shedders/carriers - has this been considered fully in the study.

Response: This is an excellent point, and is evolving into a focus of our on-going research on this system. In the current ms, we have addressed the serological impacts of chronic shedders only in the context of titer decay times by saying: “The possible role of chronic shedders, in maintaining their own low-titer seropositivity and in boosting the antibody responses of others, requires investigation.”

References

Referee 2

General: The authors utilized a unique and difficult to obtain set of data to address important questions regarding the epidemiology of leptospirosis in California sea lions. They conclude from their study that the findings suggest that sea lions may serve as both a reservoir and incidental host for leptospirosis. This analysis was based however on results using only the serovar pomona, without mention of what titers were obtained for the five other serovars tested. They offer as an explanation that leptospirosis in sea lions is only caused by serovar pomona (Refs. 15, 18, 25). If titers >100 were obtained in their study to any of the five other serovars tested, then these other serovars must be evaluated in their study as well. Therefore, it is imperative that the authors report all titers for all six of the serovars they tested and then proceed accordingly based on the titers.

Response: In-depth analysis of the full data set for all six serovars is a large-scale undertaking, and will be the subject of a future publication in collaboration with the developers of the new methodology of antigenic cartography [2]. Meanwhile we have added Table 3, which compares titer scores for all six serovars and shows that serovar Pomona had the highest of all measured titers for the great majority of samples.

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Major Compulsory Revisions

See comment above under General as well as the following points:
1. In the logistic model each outbreak year was compared with all other years. Does this mean the reference group changes depending on the outbreak year or does it remain constant for each epidemic year.


2. The authors state that "Blood chemistry (but not serology, gross necropsy and histopathology were used to classify sea lions that stranded because of leptospirosis". Rather than just giving a reference, the authors should explain the specific criteria used for a diagnosis of leptospirosis and whether they consider this to be indicative of a definite or probable diagnosis. It is unclear why serology would not be used to make a definitive diagnosis of leptospirosis if it was available, since this is the standard diagnostic procedure in veterinary and human medicine. Even using serology, a single titer >100 should not be considered to be diagnostic. Only a fourfold rise in paired titers, PCR, or isolation of the organism is definitive.

Response: We have included the specific criteria in the revised ms. Serology was not used as a diagnostic criterion because we wanted an independent measure of symptomatic leptospirosis for the purposes of this study. Also, as noted by the referee, there are significant complications in diagnosing leptospiral disease from a single titer, and paired samples are often not obtainable from these animals.

3. I am not aware that any MAT test is 100% sensitive and 100% specific for leptospirosis in any species of animal. Please explain how this was established in addition to citing a reference. Also, what criteria were used to define a single MAT titer of >1:100 as diagnostic? A significantly higher titer is usually used for the diagnosis of leptospirosis in people and dogs.
Response: We have added text to clarify the reported finding on sensitivity and specificity. Regarding the titer cut-off, again we are specifically not using serology as a diagnostic measure in this study. We have followed Levett and Colagross-Schouten in using 1:100 as the minimum cut-off for seropositivity (indicative of past exposure), but most of our analyses are based on the higher cut-off of 1:800 that we consider to be a more reliable indicator of recent exposure.

4. What were the causes of of stranding for the the 200 sea lions in the study with positive titers and how do you know that the stranding was not due to leptospirosis in these seropositive animals?

Response: Of those 200 individuals, 30 stranded due to domoic acid toxicity, 40 due to malnutrition, and the remainder due to a variety of causes including trauma, cancer, behavioral problems and unrecorded causes (Grieg et al 2005). Of course we cannot know that stranding was not due to leptospirosis, only that those animals did not exhibit the symptoms (now described in greater detail) that we considered diagnostic for leptospirosis. We have added a statement to clarify this fact.

5. The Discussion starts with a sentence indicating that sea lions "do not fit into a clear dichotomous classification of maintenance and accidental hosts for particular serovarS of L. interrogans". This indicates to me that more than one serovar affects sea lions. Yet, the rational given for only evaluating pomona in the study is that it is the ONLY serovar that causes leptospirosis in seal lions. Which is it?

Response: The comment regarding specific serovars was intended as a broader statement regarding the classical host-serovar associations for Leptospira. We have altered the text so that hopefully this is more clear.

6. Serovar autumnalis is now one of the predominant serovars diagnosed in dogs in the U.S. Why therefore wasn't autumnalis included in the battery of serovars tested by MAT, especially since cross reactivity between pomona and autumnalis is common?

Response: The panel of serovars used was a convenience panel, based on the Leptospira serovars of diagnostic interest in California during the study period. Autumnalis was not specifically excluded, but was not included in the panel available to the researchers at the time the serological tests were initiated. The fact that all isolates from California sea lions have been Pomona, and Autumnalis to our knowledge has not been isolated from US dogs, would seem to argue that this was not a significant omission for the current study. All the same further investigation, including isolation and identification of Leptospira species both during sea lion outbreaks and in intervening years, is warranted to confirm the current observations. We have added text in the discussion to clarify this point.

Reference

Referee 3

Major Essential Revisions

1. My main comment concerns the use of the logistic regression model – the dataset has as its outcome a three factor variable (uninfected, low titre, high titre). Therefore the most appropriate model to fit would be a multinomial logistic regression model rather than 2 individual models. This would then allow the authors to present tests of whether or not the effect of age, sex etc are different between high and low titres.

Response: We thank the referee for bringing this point to our attention. We have re-analyzed the data using a multinomial logistic regression model, and we report the results as relative risk ratios and alter our language accordingly.

2. My other main comment concerns the analysis presented in Fig 2(b) and how stable the analyses are. One of the assumptions in the regression line is that the residual errors are normally distributed. However there does appear, as the authors note, to be an outlier for the year 2000 and possibly 2004. I wonder how much influence these points have on the estimate of the intercept and whether there are any suggestions of departure from normality. Also as the authors propose a reasonable explanation for the year 2000 being anomalous there could be a strong case for omitting this data point – what impact would this have on the results?

Response: We now present a second set of regression results with the year 2000 point excluded as an outlier. This fit explains a considerably greater proportion of the variance ($R^2=0.98$ vs $R^2=0.80$ for the full dataset), and yields a slightly higher and more precise estimate of the probability of antibody decay. We have also included a simple test for non-linearity in the data (by fitting a quadratic model) and for non-normality of the residuals (by Kolmogorov-Smirnov test), neither of which shows any significant effect.

Minor Essential Revisions

1. It would be helpful to the reader if, in Table 3, the numbers of infected/disease free sea lions are included in each of the groups.

Response: done

2. In the labels for Fig 1 it would be helpful to describe the differences between panels a b and c.

Response: done

3. Could the authors check Fig 3 as I think they have panel b and c the wrong way round.

Response: this has been corrected – thanks for catching it!

4. There may be a good reason for this (I'm not an expert in sea lions!) but why use different age classifications in Table 1 for males and females – and more importantly to me, how does this translate into the analysis.
Response: These are the standard age classifications based on observable size and morphological characteristics of seal lions. We agree that it is inconvenient that the age classes do not align for males and females. We can see two possible impacts on the analysis:

1. The juvenile age class is strictly male, while all other age classes include both males and females. Because of the higher risk of seropositivity in males compared to females, this could account for some of the elevated RRR for the juvenile age class. We have added a note to clarify this point.
2. Any analysis of time trends for particular age classes would be complicated by the non-matching age ranges for male and female age classes (since, e.g., adult males have a different time period of past exposure than adult females). However we are not examining time series for particular age classes in this study, so this is not a concern.

5. I think that 2 paragraph in the analysis section would benefit from a little more explanatory text to explain some of the terms/equations in English as I found that I had to read it a few times as well as look at the results to understand what the authors were doing.

Response: We have made changes to the text in the Methods and Results sections to clarify this material.