Critical appraisal of the methods from the PLoS ONE paper “Climate Extremes Promote Fatal Co-Infections during Canine Distemper Epidemics in African Lions”

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Document version

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01	Initial version

1 ABBREVIATIONS

- CDV: Canine distemper virus
- FIV: Feline immunodeficiency virus

2 CONTEXT

2.1 Objectives

Review the methods and arguments from the PLoS ONE paper “Climate Extremes Promote Fatal Co-Infections during Canine Distemper Epidemics in African Lions”.

3 REVIEW

3.1 Methods

3.1.1 Statistical analyses

The Materials & Methods section does not include any description of statistical analysis, significance level or confidence level. Even so the authors did a few unspecified statistical analyses (see section 3.2).

3.1.2 Study design

The authors do not describe the study design. It can be reasonably be assumed that they have an interest in a longitudinal design, due to the scope and aims of the article. However the statistical analyses used seem to fall under the cross-sectional umbrella of methods.

3.2 Unspecified statistical analyses

In this sentence the authors refer to a result from an unspecified statistical analysis:

"We found that hemoparasite burdens were significantly higher during the high-mortality 1994 and 2001 lion die-offs (Fig. 3)"

This can be found in the figure legend:

"Levels of Babesia infection were significantly higher during the fatal outbreaks, in the Crater, and in assays performed on whole blood (all three factors significant at P<0.0001 in a multivariate analysis, n = 344)."

Several questions arise from this under-reporting:

- What type of multivariate analysis was performed and why?
- What factors were included in the model and why?
- What factors weren't included and why not?
- What were the coefficients of the model as well as their respective confidence intervals?

If the conclusion relies on these results it is odd not to have them described in the Methods of the article.

It is also noteworthy that a multivariate analysis adequate for longitudinal data would need to consider the time dimension of that data (see section 3.2). These could include either multi-level models or any time series approach, given the aims of the study.

"In support of that hypothesis, hemoparasite levels were significantly higher in lions that fed on buffalo during the 1994 Serengeti die-off than in lions from prides with low buffalo exposure (P = 0.0011, Mann-Whitney)."

Presumably the Mann-Whitney test was used as an alternative to the t-test where the data failed a normality test. Although this approach is frowned upon in the methodological literature, it is nevertheless prevalent in the applied research literature.

"Among buffalo-eating lions, hemoparasite levels were significantly higher in lions that had been infected with CDV (P = 0.0099), demonstrating the permissive effect of viral immunosuppression."

This is presumably a p-value from the t-test. From the text it must be assumed that this is a simple null hypothesis significance test, presumably the t-test for comparing averages under the assumption of a normal distribution.

3.2.1 Correlation claims

They present R^2 figures, which are related to correlation analysis indicating.

"Variations in infection and mortality across lion social groups (prides) implicate an interactive effect of CDV and Babesia: prides with higher average CDV titers had a greater proportion of individuals with high levels of Babesia ($r^2 = 0.41$, n = 11 CDV-infected prides, $P<0.05$), and prides with the highest levels of Babesia suffered the highest mortality rates with many prides suffering >67% mortality ($r^2 = 0.63$, n = 12 prides, $P<0.01$) (Fig. 4)."

Assuming R^2 is the coefficient of determination, this statistic is often used to assess the goodness of fit of a model or a hypothesis to the data. If this assumption is correct the results presented then the first figure could be interpreted as explaining 41% of the variance observed, while the second one can explain 63% of the variance.

This is found in the figure 4 legend:

"Prides with a greater proportion of individuals showing high hemoparasitemia also suffered higher mortality rates ($P<0.01$)."

One must assume a hypothesis and an adequate test for this result. Perhaps they opted to use a t-test in order to compare the average percentages in both variables.

"Quantification of hemoglobin in frozen whole blood and red blood cell pellets were shown to correlate with hemoglobin concentrations in fresh whole canine blood (data not shown)."

It is unclear why they opted to now show a single statistic. If the result is not relevant for the conclusion of the study, maybe it should have been dropped from the manuscript altogether.

3.3 Strong or causal claims

In this section a notable hypothesis was discarded:

"Similarly, feline immunodeficiency virus (FIV) infection was ruled out as a co-factor because nearly all of the adult Serengeti and Crater lions have been FIV+ each year since at least 1984, and FIV-infection in lions has never been linked with mortality[13,14]." Together these data indicate that only Babesia co-infection correlated with mortality trends."

To a non-specialist in these particular pathologies, the facts that FIV (1) was endemic in the relevant place, time and population and (2) that it is also an immunodeficiency-related disease (similar to CDV) are precisely the reasons to consider a possible interaction between CDV and FIV when mortality is concerned. It is often repeated that

correlation does not imply causation. It is worthy to add that no previous link found does not imply absence of a link.

The second cited paper (Packer, et al. 1999) does not investigate associate a possible association or interaction between FIV and CDV. The first one does not mention CDV.

In another section they observe that:

"Additionally, both lions had marked lymphocyte depletion in lymph nodes (Fig. 2B), suggesting profound immunosuppression consistent with recent CDV infection[7]."

A question from a non-specialist reader would be if those lymphocyte depletion would also be consistent with FIV infection. Was this possibility considered and discarded? If so, what tests were used and if not why not?

4 OBSERVATIONS AND LIMITATIONS

N/A

5 REFERENCES

- Munson L, Terio KA, Kock R, Mlengeya T, Roelke ME, Dubovi E, et al. (2008) Climate Extremes Promote Fatal Co-Infections during Canine Distemper Epidemics in African Lions. PLoS ONE 3(6): e2545. (https://doi.org/10.1371/journal.pone.0002545)
- Packer C, Altizer S, Appel M, Brown E, Martenson J, O'Brien SJ, Roelke-Parker M, Hofmann-Lehmann R, Lutz H. Viruses of the Serengeti: patterns of infection and mortality in African lions. J Anim Ecol. 1999 Nov;68(6):1161–78. (https://doi.org/10.1046/j.1365-2656.1999.00360.x). Epub 2001 Dec 25. PMCID: PMC7197470.

6 APPENDIX

6.1 Availability

All documents from this consultation were included in the consultant's Portfolio.

The portfolio is available at:

https://philsf-biostat.github.io/SAR-2022-039-CC/