28 November 2025

Report manuscript "Efficacy of a Modified-Live Virus Combination Vaccine (CDV, CAV, CPV, CPiV), CanigenTM DHPPi, in Puppies Vaccinated at Six Weeks of Age"

Journal Viruses - MDPI

The study addresses an important topic in veterinary vaccinology (early-life immunization in the presence of MDA) and the authors present two well-structured experiments encompassing both challenge and serological components. The work is generally well-organized, clearly written, and supported by detailed experimental procedures. Nevertheless, several issues require clarification or additional detail before the manuscript can be considered suitable for publication. Overall, I recommend minor to moderate revision:

1. Both experiments use small sample sizes, which is common in regulatory safety/efficacy trials, but some justification is needed. For example: experiment A uses n=12 per group, which is acceptable but should include a brief statement on how this number aligns with EP monographs or prior power expectations. Experiment B uses n=6, making between-animal variability more impactful. Add one or two sentences acknowledging limitations related to sample size and how the design still fulfills the regulatory efficacy criteria.
2. The serology study is explicitly non-blinded, which introduces potential bias when interpreting clinical and laboratory endpoints. Explicitly discuss this limitation in the Methods or Discussion and justify why the risk of bias is minimal (e.g., lab-based endpoints, objective neutralization assay).
3. The text states positivity thresholds (e.g., 10^0.7, 10^1.17), but how these relate to clinically protective levels is not entirely clear for all pathogens. Add a brief justification or citation explaining how these thresholds correspond to protection (e.g., WSAVA guidelines, previous efficacy studies).
4. Figure 1 conveys trends but lacks confidence intervals or individual animal traces, which would provide useful granularity. If possible, include error bars or shaded confidence regions. Clarify in figure legend whether data points represent means of log10 values ± SEM or ± SD.
5. The manuscript repeatedly emphasizes that respiratory signs were mild in both groups. Given the nature of CPiV challenges, this is expected. Add one sentence explaining that mild clinical disease is typical for CPiV infection, thereby contextualizing the findings.
6. The use of mixed models is appropriate, but please state the covariance structure chosen (e.g., AR(1), unstructured).
7. Ensure consistent use of: CPV-2 vs CPV-2C; Log10 formatting (sometimes 10^1.17 is written differently); "Seroneutralizing antibody titers" vs "neutralizing titers".

8. A few sentences are duplicated in the results (e.g., clinical description around D21–D28). Removing redundancy will improve flow.
9. Consider consolidating ethics statements into a single paragraph for clarity.

This is a relevant and valuable study that contributes meaningful data on early-age vaccination in puppies. The findings support the safety and immunogenicity of Canigen™ DHPPi when administered at six weeks of age. With minor clarifications and additional detail, the manuscript will be suitable for publication.