Review of: "RNA in-situ hybridization for pathology-based diagnosis of feline infectious peritonitis (FIP): current diagnostics for FIP and comparison to the current gold standard"

Marta Vascellari

Potential competing interests: The author(s) declared that no potential competing interests exist.

This study aims to compare diagnostic performances of IHC and RNA-ISH for the diagnosis of FIP, in FFPETs.
Unfortunately, I have some concerns about this paper and the study design.
The introduction, particularly the review of diagnostic options is too long, beside adding nothing to the current knowledge. I suggest to shorten this section drastically.
Authors affirm that the major IHC limit is represented by its post-mortem application. This is partially true, since ICC (chromogenic not only fluorescent) and IHC on bioptic samples can confirm the diagnosis, also in alive cats.
RNA in situ hybridization is a well known technique that have many advantages, such as the high specificity and sensitivity, due to the probe design, that can overcome the lack of specific antibodies. On the other side, RNA ISH is more expensive than IHC and, for this reason it is primary used in research field more than in routine diagnostic.
I am not sure about the primary aim of this study. Authors affirm that they aim to compare RNA ISH diagnostic performance with the IHC gold standard. To evaluate IHC and ISH specificity a set of FIP positive and negative cases should have been selected.
Authors affirm that sensitivity of IHC can varies widely, due to the primary antibody used, as well as the IHC protocol applied. To demonstrate the superiority of ISH in terms of sensitivity, a set of FIP confirmed case (on the bases of PCR, histology or other clinical investigation) that were negative by IHC, should have been included.
In conclusion this work should aim to validate RNA-ISH with a specific designed probe. Unfortunately, this is not possible if positive and negative samples are not adequately included.
Looking at the results, no real advantages against IHC are reported, except for the better staining of tissues. In terms of diagnosis, IHC and ISH gave the same results, being ISH more expensive than IHC.
Figures are of poor quality, higher magnifications would have been appreciated.