Open Review

( ) I would not like to sign my review report

(x) I would like to sign my review report

Quality of English Language

( ) I am not qualified to assess the quality of English in this paper

( ) English very difficult to understand/incomprehensible

( ) Extensive editing of English language required

( ) Moderate editing of English language required

(x) Minor editing of English language required

( ) English language fine. No issues detected

Yes Can be improved Must be improved Not applicable

Does the introduction provide sufficient background and include all relevant references?

( ) (x) ( ) ( )

Are all the cited references relevant to the research?

(x) ( ) ( ) ( )

Is the research design appropriate?

(x) ( ) ( ) ( )

Are the methods adequately described?

( ) ( ) (x) ( )

Are the results clearly presented?

( ) (x) ( ) ( )

Are the conclusions supported by the results?

( ) ( ) (x) ( )

Comments and Suggestions for Authors

I read with interest this paper investigating Anelloviruses in domestic cats by utilising high throughput sequencing methodologies for initial detection and confirmatory PCR. Although not ground breaking research, the data presented aids in further understanding these recently discovered viruses, by including country specific data from China and attempting to situate within the context of published sequences.

In general the paper was adequately written, but found myself wanting more detailed analysis on the number of positive cats (by PCR) across the faecal sample groups and more information on how unique the recombination event is within this group of viruses. The discussion could have focussed more on the novel findings of this work and also on the other viruses identified in the metagenomics analysis. The conclusions regarding pet movement need to be validated by data or removed.

Below are a number of specific comments/questions that the authors need to address:

1. In M&M 2.2 line 83-87, please specify what the SB buffer, enzyme mix buffer and enzyme mix are.

Response: Thank you for your comments. The SB buffer, enzyme mix buffer and enzyme mix are derived from the VirOne Kit (Guangdong Magigene Biotechnology Co., Ltd. China). We have added information to the revised manuscript.

2. In M&M 2.2 line 87-90, even if you followed the manufacturer protocol, please briefly describe how the whole genome was amplified, and how genome was generated (by DNA quantification for example).

Response: Thank you for your comments. We have added information to the revised manuscript.

3. In M&M 2.3 even if you followed the manufacturer protocol, please briefly describe how the library prep was completed, and if there was any cut off applied for the quantity used.

Response: Thank you for your comments. we have provided a brief description of the steps in the revised manuscript.

4. Table 1 please add genome positions for each primer

Response: Thank you for your comments. We have added relevant information in the new table in revised manuscript.

Primer name	Sequence (5'-3')	Product size (bp)	Position(nt)[a]
FcTTV-P1	GTAAGTACACTGACGAATGGCT		8-585
FcTTV-P2	CAGTTACCACAGTTCGAGGTCGT		
FcTTV-P3	ACTGGTGACAGGACGTGCGA	314	50-363
FcTTV-P4	TGCGGAGACAAGTTGCTTCC		
FcTTV-P1Q	TCGCACGTCCTGTCACCAGT		344-69
FcTTV-P2Q	GGAAGCAACTTGTCTCCGCA		

FcTTV-P3Q	TCAGCCATTCGTCAGTGTACTTACT	1528	567-31
FcTTV-P4Q	CGACCTCGAACTGTGGTAACTG		
FcTTV-P5Q	GTTAGTGTTGCTTTACGGCAG		1969-753
FcTTV-P6Q	TGAACCAGTGGTGTCCCCAG		
FcTTV-P1	GTAAGTACACTGACGAATGGCT	579	7-585
FcTTV-P2	CAGTTACCACAGTTCGAGGTCGT		

[a] Oligonucleotide positions are referred to the FcTTV isolate SH-F1, complete genome (GenBank accession number: HM142588)

5. Results 3.1: in M&M you state that sequences under 100bp were excluded, but how long were the reads that you have included? It would be helpful to provide as a minimum the range with a mean length. Numbers of contigs obtained, and % coverage across the genome would also be interesting to the reader. The supplementary data is hard to interpret as its raw data, so a supp or additional table with this information would be helpful.

Response: Thank you for your comments. In the text, the number of bases of the assembled viral contig compared to the viral reference genome >100 bp was used as a quality control criterion to determine whether the virus was detected. The reads and contig statistics table we have been added in Supplementary DATA-S4.

6. 3.2 validation, I wonder why you didn't attempt to collect serum from the same individuals that you got the stool samples from?

Response: Thank you for your comments. Since we did not collect serum from cats at the beginning, but only feces, and the feces were performed viral metagenomics by mixing five cats into one copy and not all of the samples sequenced at that time were kept. Later we found a high rate of FcTTV infection rate in the cat population, and reviewed the literature to find that this virus can be detected from cat serum by PCR. Thus, we used this method to verify the FcTTV infection.

7. 3.3 full length sequencing. Please state in total how many positive samples you had (from faecal samples from domestic cats, faecal samples from stray cats and serum samples from domestic cats), so its clear to the reader. Is there a reason why only 5 full length genomes were obtained? Could you have used PCR to amplify ORF1 from more positive samples to enhance the phylogenetic analysis?

Response: Thank you for your comments. Since viral metagenomics experiment cannot accurately estimate the exact positive rate because of the mixed sample assay. In the validation section, we detected a total of 11 positives from 30 serum samples. These were all from domestic cats. Out of these 11, we randomly selected 5 for full-length amplification. Phylogeny shows that all 5 variants are clustered in the same branch. Indeed, as you mentioned, sequencing more ORF1 fragments would be better.

8. Discussion: You start by stating that TTV are species specific, yet go on to talk about recombination across species, if this is a risk factor please explain how and if its been observed before.

Response: Thank you for your suggestion, indeed we only detected a recombination between FcTTV variants in domestic cats. To date, no recombination between TTV strains across species has been detected, and our original descriptions only suggested a hypothesis that this might occur, with no supporting evidence, thus we have modified and removed these descriptions.

9. Discussion: You state that most of the viruses detected were likely related to the feeding and living environment of the individuals. Do you have any experimental evidence for this? If so please add this evidence in the results. For example you detect Feline leukaemia virus in 2 pools of your domestic animal faeces, why would this not be evidence of a real infection?

Response: Thank you for your comments. What we mean is that many viruses, including plant, human and many other mammalian viruses, are detected in the feces as a result of viral metagenomics, and a large proportion of these viruses may be involved by the food and environment in which of cat lives. Regarding the Feline leukaemia virus, we believe it is very likely that indeed cat infected, but this paper did not go into the validation of this virus.

10. Line 258-270: this paragraph is hard to read/interpret the relevance.

Response: Thank you for your comments. This paragraph is showed the large differences between two FcTTV variants from domestic cats reported in 2007. In our phylogenetic tree, the PRA1 variant, although isolated from cat saliva, showed that it was closer to the Lynx rufus and Puma concolor variants, whereas with the other strain, PRA4, it clustered with the FcTTV of domestic cats. We believe this is a question that deserves further study.

11. Lines 271-280: is your finding of recombination the first for TTV? If so this is a major finding, if not please introduce other recombination events that have been described and discuss how your findings fit with them.

Response: Thank you for your comments. Based on a review of the literature, clear evidence of recombination has been described in human and nonhuman TTV. We have added a description of the relevant TTV recombination and references in the revised manuscript.

12. Conclusions: line300-301: where is the evidence for pet trade involvement? Is there geographic groupings for these viruses?? If so this needs to be introduced and discussed before you make this conclusion.

Response: Thank you for your comments. We were speculating about a possible association with the pet trade based on the source of the fragments of the recombinant strain we detected showing that it

contained fragments of FcTTV from cats in the early Czech region, but there is no direct evidence that this is related to the pet trade, and we have removed the description based on your suggestion.

Comments on the Quality of English Language

The abstract and discussion could benefit from some editing to improve the English, otherwise the English is OK.

Response: Thank you for your comments. We have revised the English of the full text in the hope of achieving clarity of expression and willing to accept English revision services by the journal if further language revisions are needed.

Submission Date

15 June 2023

Date of this review

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