nature portfolio

Peer Review File

Node role of wild boars in virus circulation among wildlife and domestic animals

Corresponding Author: Dr Biao He

This file contains all reviewer reports in order by version, followed by all author rebuttals in order by version.

Version 0:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
The article titled "Node role of wild boars in virus circulation among wildlife and domestic animals" aims to conduct a national pan-viromic study of wild boars in China using a combination of DNA-specific multiple displacement amplification (MDA) and RNA-specific metatranscriptomic (MTT) methods. The article is of great importance for One Health, as it demonstrates infection by viral agents in wild boar populations of great relevance for human and animal health.

The article is well-written and detailed, and the authors have achieved the objectives. However, some revisions need to be made:

1. The Results section should be refined to focus solely on the results in an objective and clear manner, without including discussion.

2. The Discussion section needs refinement, and the statements or data presented should be supported by current references, if possible. If this is not feasible, unsupported statements or data should be removed.

3. The Methods section needs refinement. While it is well-detailed and complete, it contains unnecessary information and discussions for the body of the article.

4. Although the study is groundbreaking, the title underestimates the depth and scope of the findings. It is recommended that terms such as “pan-viromic surveillance”, “cross-species viral dynamics” or “emerging zoonoses” be included.
The other suggestions and revisions can be found below, with the page and paragraph information where they are located:

ABSTRACT:

“…across 127 locations in 26 provincial regions of China.”

- Authors: This information is not described in the Materials and Methods section. You must describe this in Materials and Methods section.

INTRODUCTION:

Extremely very long introduction, authors must reduce a lot of information, keep the focus on what is important for the manuscript:

For example, suggested removal of the text:

“Nonetheless, the breadth and circulation dynamics of wild boar viruses have rarely been investigated, which is dramatically incompatible with their population, distribution, and ecological role. Wild boars deeply interweave with humans, domestic animals, and other wildlife in ecosystems, providing ample opportunities for the cross-species transmission of viruses. Meanwhile, wild boars are ideal natural hosts for medically important mosquitoes and ticks and hence can be considered

sentinel animals to identify potential vector-borne viruses."

Page 5 – "Wild boars deeply interweave with humans, domestic animals, and other wildlife in ecosystems, providing ample opportunities for the cross-species transmission of viruses. Meanwhile, wild boars are ideal natural hosts for medically important mosquitoes and ticks and hence can be considered sentinel animals to identify potential vector-borne viruses."

Authors: To validate your information a reference must be included here.

RESULTS:

The text of the results is excessively long and dense. Clearer segmentation by topic is recommended. Suggested sub-subtitles: "ASFV variants in wild boars", "Tick-related viral circulation", "Comparison to domestic pigs", etc.

The description of the finding of co-infection with five ASFV variants is of high impact and should be highlighted with more visual emphasis (perhaps a specific figure or box).

Page 7 – "To compare the virus diversity between wild boars and domestic pigs, we prepared a VHG data set of domestic pigs by querying our previously established Pigs_Virome catalogue and GenBank and then clustered it with the counterparts of BrCN-Virome at the AAI50 level (i.e., resembling the viral family level)."

- Authors: This information should be in Methodology.

Page 9 – "These suggest that wild boars not only deeply participate in the cross-transmission of pig viruses but also are involved in the virus circulation of humans, domestic animals, vectors, and wildlife. It's interesting to note that viruses of the order Picornavirales are typically abundant and rich in mammalian viromes 18-20. However, we only discovered 19 full-length picornaviral RdRp sequences in this study, with most closely related to sapoviruses, kobuviruses, and iflaviruses and dicistroviruses that infect pigs and arthropods (Figure 3B), respectively. Furthermore, wawtorqueviruses were mainly detected in rodents 21, and gammapapillomaviruses and deltapolyomaviruses are primarily linked to human infections 22,23, but few of these viruses were ever discovered in suids 24."

- Authors: This information could be included in the Discussion, as there is no space for it in the Results.

Page 9 – "To pinpoint the risks of wild boar viruses posing to humans, domestic pigs, and wildlife, we queried BrCN-Virome sequences against Virus Pathogen Resource (ViPR) 25 to find hits with an average nucleotide identity (ANI) of ≥ 90%."

- Authors: This section should be in Methodology.

Page 10 – "These indicate that wild boars intensively participate in the virus circulation of domestic pigs and extensively interact with humans, other mammals, birds, and arthropods in the viral ecology."

- Authors: This should be part of the Conclusion or the Discussion.

Page 11 – "The H. longicornis ticks were linked to 13 virus species of BrCN-Virome, with eight being genomoviruses. The Rhipicephalus microplus ticks were associated with eight virus species of these wild boars, all being known vector-borne viruses except a Hepeviridae sp."

- Authors: This section needs to be referenced and does not appear to be part of the Results, as some points are discussed

Page 12 – "These provide adequate evidence that virus circulation occurred between wild boars and vectors."

- Authors:This should be part of the Conclusion or the Discussion.

DISCUSSION:

Page 15 – "Although wild boars are physiologically and genetically identical to domestic pigs,…"

- Authors: Authors: You cannot make this statement without including a scientific reference that validates your information. A reference must be included here.

Page 15 – "…so those viruses mainly replicating in the digestive tract, such as AstV, calicivirus, etc., …"

- Authors: Again, Authors a reference must be included in this section.

Page 16 – "their deep participation in various ecosystems. For example, wild boars freely roam in the wild and are frequently infested by arthropods, making them have a higher chance of contracting vector-borne viruses in Bunyavirales, Flaviviridae, Rhabdoviridae, etc.

-Authors: Again, a reference must be included here.

Page 16 – “In a local ecosystem, all life forms directly or indirectly interact with each other via food chain, sharing habitat, resource competition, etc., which provides ample conditions for virus circulation and cross-species transmission.”

-Authors: Again, a reference must be included here.

Page 16 – “Some viruses are known to be pathogenic to pigs, humans, sheep, cattle, and carnivores, but…”

-Authors: Again, a reference must be included here.

Page 16 – “These pig pathogens are widely distributed in wild boars across China, with some also lethal to wild boars, such as ASFV, CSFV, etc.”

-Authors: Again, a reference must be included here.

Page 17 – “Virus infection is an important cause of wild boar deaths,…”

-Authors: Again, a reference must be included here.

Page 18 – “…have already spread in China.”

-Authors: Again, a reference must be included here.

MATERIALS AND METHODS:

Methods:

-Authors: The study area needs to be described. To my best knowledge, China has almost 10 million km²; therefore, it should specify in which regions the collections were made (north, northeast, northwest, south, southeast, southwest, rural, peri-urban or forest), describing the phytocoenotic differences and abiotic and biotic factors, if possible.

Page 19 – “Wild boars have become a kind of pest animal in China 50. They frequently harass agricultural areas to trample crops and even invade urban communities to trigger human-wild boar conflicts, causing substantial ecological and economic losses to human society 13.”

- Authors: This information should not be part of the Methodology. It can be included in the Introduction or the Discussion.

Page 20 – “…series of strategies to control their population and distribution 13. The State Administration of Forestry and Grassland of China also initiated a plan to actively survey the virus background of wild boars since 2015. Forest rangers of the Local Administration of Forestry and Grassland set traps to capture wild boars or hunted them using rifles. They also routinely patrolled forests and sampled the dead wild boars they encountered. These wild boars killed by traps or shooting were all apparently and internally normal and hence were considered healthy individuals. All wild boars were dissected in the fields to collect their internal solid organ tissues using sterile tubes. Blood samples were collected if possible. All samples were immediately cryotransported to the laboratory at Changchun Veterinary Research Institute, where they were stored at -80°C.”

- Authors: This section can be summarized; as my suggestion, it is only necessary to mention that there is a population control initiative and that the study was conducted in partnership with the State Administration of Forestry and Grassland of China.

Page 20 – “Before dissection, forest rangers checked ectoparasites of wild boars and plucked them if there were any.”

- Authors: What methodology was used to assess the presence of ectoparasites?

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Page 20 – “These ectoparasites were also transported to the laboratory along with wild boar samples.”

- How was the transportation carried out? Were the ticks alive? Were they stored in any product? Have any ticks been deposited in any collection?

Page 20 – “All these arthropods were ticks, and their species were morphologically identified.

- Authors: What identification methodology was used? Which dichotomous key was applied?

- Authors, you report the species of ticks that were identified in wild boars. However, you made a very important mistake: you forgot to describe in the material and methods how the ticks were identified (what was the taxonomic identification key or were they identified by DNA sequencing technique).

Page 20 – “When necessary, the mitochondrial cytochrome c oxidase subunit I gene of tick was sequenced and subjected to a BLASTN v.2.14.0+ search against GenBank to confirm the species identification further.”

- Authors: The reference should be included in this information.

Page 31 – “Primer pairs were referred to previously published or WOAH and national recommendations, or designed based on contigs.”

- Authors: Please, reference mustbe included here. The reader needs to have access to the sequence of these primers.

The choice of minimum contig sizes (1.5 kb in MDA, 500 bp in MTT) needs to be justified. Is it possible that important small-genome RNA viruses were discarded?

Despite the inclusion of controls (BHK-21 cells), the index hopping rate observed in the blanks was not described, which is crucial in viromic studies with low viral load.

Reviewer #2

(Remarks to the Author)
I co-reviewed this manuscript with one of the reviewers who provided the listed reports. This is part of the Nature Communications initiative to facilitate training in peer review and to provide appropriate recognition for Early Career Researchers who co-review manuscripts.

Reviewer #3

(Remarks to the Author)
"Node role of wild boars in virus circulation among wildlife and domestic animals' is a very comprehensive metagenomic sequencing- and bioinformatics-based assessment of virome content in wild boars and how wild boars may contribute to viral infection of humans and other animals. Several types of sequencing are employed in this study, to maximize the recovery of DNA and RNA viruses, and multiple tissue types were examined from the animals. The results from apparently healthy boars were compared to results from dead boars, and the way the researchers proved ASFV to be the likely cause of death was interesting and methodologically sound. The researchers also demonstrated that the ASFV infection has effects on virus diversity in the tissues that were examined. They showed that healthy pigs have less virus diversity and that ASFV infection suppresses the levels of replication of other viruses (a phenomenon widely known as superinfection exclusion but rarely demonstrated in metagenomic sequencing datasets - so I appreciated this). It was also notable that the viruses identified in the ticks on the pigs were also identified in those specific pigs that the ticks fed on. Overall, the study was well-executed, with robust bioinformatics. The manuscript is very well written, and I enjoyed spending time on it. Figure 1 depicts an enormous amount of information in a very succinct manner. Below are a few specific comments, questions, and requested edits:
1. First paragraph of Results section: I think the word "proclivity" would make more sense than "priority." "Owing to the proclivity of the MDA technique to amplify single-stranded circular DNA..."
2. Same paragraph as above, I would delete "these," as in "Double-stranded DNA viruses were also efficiently recovered..."
3. The authors should delineate the meaning of each acronym at first usage. Some examples of acronyms in the manuscript that were not defined at first use are PEDV, AKAV, and WOAH.
4. In the Viromic signatures of healthy, dead, and ASFV-killed wild boars section of the Results, the authors state that the viromes did not reach saturation. This means that not all the viruses present in the animals were detected because the samples were not sequenced deeply enough. The authors should address this in the Discussion section as a caveat of their study and should explain how this might affect the conclusions.
5. There were 3 dead boars from which ASFV was not detected by sequencing and bioinformatics but was detected by PCR. Sometimes when multiplexing libraries on a sequencing run, there is variation in terms of how many reads are produced per library. Is there a statistically significant difference between the number of sequencing reads produced from dead boar samples in which ASFV was detected by sequencing and bioinformatics as compared to the number of sequencing reads produced from dead boar samples in which ASFV was not produced? If the number of reads is less in samples from which ASFV was not detected by sequencing, then the lower depth of sequencing could be the reason, and that, along with the lack of saturation the authors mentioned, would strongly suggest that all the samples should have been sequenced more deeply.
6. Among the three dead boars from which ASFV was detected by PCR but not by sequencing and bioinformatics, were there any genetic polymorphisms or was it just a case of lower titers or less reads? Were the ASFV detected in these three samples GI or GII?
7. In the first paragraph of the Discussion, the word "partially" should be inserted in the following sentence because later in the paragraph the authors talk about the effect of wild boar lifestyle contributing to virome composition: "This discrepancy is likely partially attributed to the sample type..."
8. The last sentence of that first paragraph in Discussion stating that the authors cannot rule out that some viruses

preferentially infect wild boars, I disagree with. The authors have already pointed out that wild boars and domestic pigs are genetically indistinct. I would delete this sentence.
9. In the methods, the authors describe the amount of tissue used as "a soybean-sized piece." I don't think soybean is a unit of measure. I suggest that authors replace the soybean reference with an actual length, e.g. "..a 10mm sized piece..."
10. In the first paragraph of the Methods section called Bioinformatic procedure for BrCN-Virome preparation, third sentence, the word "into" should be inserted as follows: "..can naturally integrate into host sequences."
11. In the last sentence of last paragraph in that same section, "5" should be spelled out: "five."
12. In the first sentence of the section called Viromic characterization of dead and ASFV-killed wild boars, "assess" should be past tense, "assessed."
14. In the section on Recombination analysis, I suggest more emphasis on the wording to demonstrate these are predictions. Rather than "would occur" it should say "were predicted to have occurred" and rather than "would happen to," it should say "was predicted to have occurred in."
15.In the second to last sentence of the section Molecular validation and detection, I think the word "transfect" should be replaced with "transform." Typically we use the word "transfect" to denote uptake of DNA into eukaryotic cell cultures and "transform" to denote uptake of DNA into competent bacterial cells.

Reviewer #4

(Remarks to the Author)
The authors describe a massive data set based on an extensive range of sample types from healthy and dead wild boars across many different regions in China. They present a highly detailed, comprehensive view of the wild boar virome – which has been understudies to date – and a valuable comparison to the viromes of domestic pigs. The authors also provide insights into potential cross-species jumping events of concern and clearly recognise the severe consequences this could present to human and animal health, as well as economically to the pig farming/agriculture industries.

The authors make several claims in this study. Firstly, a thorough comparison of wild boar vs domestic pig viromes with appropriate statistical analyses of alpha diversity reveals differences on virome compositions between the two data sets. The detection of viruses associated with other animals (eg., domestic pig, human, ticks) is visualised in Figure 2, as well as corroborated by placing these sequences in phylogenetic trees with known reference sequences. These results support the authors' claims that wild boars represent a node animal. This, combined with the concerning detection of ASFV in dead wild boar samples, supports the overall notion presented by the authors that the routine surveillance and of wild boar viromes and populations is warranted, given the risk they may pose to public health, agriculture, and wildlife conservation. Overall, the methods match the standard set for this kind of work in this field, and are thoroughly explained. I believe similar work could be replicated based on these methods.

Some comments and suggestions below:
• I would ask why bacteriophage were removed from the data set? They represent a significant proportion of RNA viral diversity and may provide insights into dietary or gut microbiome viruses, and may partially close the gap between domestic pigs and wild boars. I do not think this choice necessarily prevents publication, but it may be worth explaining in more detail why bacteriophage were excluded and discussing the potential limitations.
• I suggest expanding "IUCN Red List" to "International Union for Conservation of Nature Red List of Threatened Species" for clarity
• Might also be worth to explain the "least concern" category a bit further, ie, Wild boars face little to no threat of endangerment or extinction, having been assigned to the "least concern" category by the International Union for Conservation of Nature Red List of Threatened Species.
• There is a lot of background information in the beginning of the methods, namely in the "Sample collection and processing" section. I would move that to the introduction or remove what was already stated in the introduction. Only need something like "Samples were obtained as part of national and local Chinese government strategies to control wild boar populations and distribution, as well as a plan initiated in 2015 by The State Administration of Forestry and Grassland od China to actively survey the virus background of wild boars".
• Please provide more detail on the blast process – which GenBank database was used and what release date?
• Please check to make sure references to "si-libraries" and "mi-libraries" are consistently hyphenated, eg. In the "Overview of the wild boar virome", they are not hyphenated.
• References to figures should really be limited to the results section, I would recommend removing them from the discussion and methods sections (figure references can be appropriate in the methods, but usually only when referencing a figure that explains a protocol rather than results)
• Figure 1A: Genome type includes a colour (blue/green-ish colour) not present in the figure legend
• In general, the figures in this paper – while excellently delivering large amounts of data – are highly complex and busy, which can make them difficult to decipher particularly if colourblind or viewing in black&white. I encourage the authors to (where possible) match the order of categories in the figure legend as much as possible to the figure, eg. Genome type legend could be reordered to dsRNA, ssRNA(+), ssRNA(-), dsDNA, ssDNA, Mix. I also encourage the authors to minimise overly similar colours being adjacent without some kind of barrier to mark a new category, eg., Peplo/Preplasm and dsDNA/dsRNA colours are virtually indistinguishable next to each other. I acknowledge this isn't possible everywhere, but some minor changes may help improve the accessibility of these figures.
• Strange suggestion in the conclusion to abandon the free-range model of pig farming? It's important to acknowledge animal welfare concerns, where confinement can increase stress & reduce immunity, thereby increasing pigs' vulnerability to disease – as well as environmental/ethical considerations. Perhaps consider some nuance, ie better biosecurity in fencing/physical barriers, and monitoring food/water/cleanliness without worsening domestic pig living conditions.
Overall, I enjoyed reading this paper. The scale of data generated is impressive and the results were fascinating. This paper

is sure to be of interest for those working in virus discovery and ecology, as well as in wildlife conservation, public health, and agriculture.

Version 1:

Reviewer comments:

Reviewer #3

(Remarks to the Author)
I appreciate the edits the authors made in response to all the reviewers' comments. I am satisfied with the revised manuscript.

Point-by-point Responses

Reviewer #1 (Remarks to the Author):
The article titled "Node role of wild boars in virus circulation among wildlife and domestic animals" aims to conduct a national pan-viromic study of wild boars in China using a combination of DNA-specific multiple displacement amplification (MDA) and RNA-specific metatranscriptomic (MTT) methods. The article is of great importance for One Health, as it demonstrates infection by viral agents in wild boar populations of great relevance for human and animal health.

Response (R) 1: We greatly value the reviewer's constructive feedback and acknowledge his/her positive assessment of our work.

The article is well-written and detailed, and the authors have achieved the objectives. However, some revisions need to be made:
1. The Results section should be refined to focus solely on the results in an objective and clear manner, without including discussion.
R2: Agreed. We have deleted the discussion content in the Results section.

2. The Discussion section needs refinement, and the statements or data presented should be supported by current references, if possible. If this is not feasible, unsupported statements or data should be removed.
R3: Agreed. We have refined the discussion section and supplemented references to support statements.

3. The Methods section needs refinement. While it is well-detailed and complete, it contains unnecessary information and discussions for the body of the article.
R4: Agreed. We have refined the method section.

4. Although the study is groundbreaking, the title underestimates the depth and scope of the findings. It is recommended that terms such as "pan-viromic surveillance", "cross-species viral dynamics" or "emerging zoonoses" be included.
R5: We acknowledge the reviewer's valuable suggestion regarding the manuscript title. Pan-viromic surveillance constitutes a critical methodological framework employed in this study but rather than a primary study conclusion; the conceptual scope of cross-species viral dynamics and emerging zoonoses are inherently encapsulated within the original title's formulation of "virus circulation among wildlife and domestic animals." This terminology appropriately conveys both the ecological context and public health relevance of our findings. We respectfully

maintain that the current title sufficiently balances scientific precision and would therefore prefer to retain its original formulation.

The other suggestions and revisions can be found below, with the page and paragraph information where they are located:
ABSTRACT:
"…across 127 locations in 26 provincial regions of China."
- Authors: This information is not described in the Materials and Methods section. You must describe this in Materials and Methods section.
R6: This information has introduced in the Results section. See lines 90-93.

INTRODUCTION:
Extremely very long introduction, authors must reduce a lot of information, keep the focus on what is important for the manuscript:
For example, suggested removal of the text:
"Nonetheless, the breadth and circulation dynamics of wild boar viruses have rarely been investigated, which is dramatically incompatible with their population, distribution, and ecological role. Wild boars deeply interweave with humans, domestic animals, and other wildlife in ecosystems, providing ample opportunities for the cross-species transmission of viruses. Meanwhile, wild boars are ideal natural hosts for medically important mosquitoes and ticks and hence can be considered sentinel animals to identify potential vector-borne viruses."
R7: Agreed. We have incorporated the text into discussion. See lines 80-81 in Introduction and 310-312 in Discussion.

Page 5 – "Wild boars deeply interweave with humans, domestic animals, and other wildlife in ecosystems, providing ample opportunities for the cross-species transmission of viruses. Meanwhile, wild boars are ideal natural hosts for medically important mosquitoes and ticks and hence can be considered sentinel animals to identify potential vector-borne viruses."
Authors: To validate your information a reference must be included here.
R8: As responded in R7, we have removed the text and incorporated it into discussion. In addition, we have cited references to support the statements. See lines 310-312.

RESULTS:
The text of the results is excessively long and dense. Clearer segmentation by topic is recommended. Suggested sub-subtitles: "ASFV variants in wild boars", "Tick-related

viral circulation", "Comparison to domestic pigs", etc.
R9: The results section has been condensed by removing extraneous discussion texts and nonessential results. Furthermore, we have adopted the section headings recommended by the reviewer to improve structural clarity. See lines 120, 186, and 244.

The description of the finding of co-infection with five ASFV variants is of high impact and should be highlighted with more visual emphasis (perhaps a specific figure or box).
R10: Agreed. We have highlighted these variants using colored boxes. See Fig. 6F.

Page 7 – "To compare the virus diversity between wild boars and domestic pigs, we prepared a VHG data set of domestic pigs by querying our previously established Pigs_Virome catalogue and GenBank and then clustered it with the counterparts of BrCN-Virome at the AAI50 level (i.e., resembling the viral family level)."
- Authors: This information should be in Methodology.
R11: Done.

Page 9 – "These suggest that wild boars not only deeply participate in the cross-transmission of pig viruses but also are involved in the virus circulation of humans, domestic animals, vectors, and wildlife. It's interesting to note that viruses of the order Picornavirales are typically abundant and rich in mammalian viromes 18-20. However, we only discovered 19 full-length picornaviral RdRp sequences in this study, with most closely related to sapoviruses, kobuviruses, and iflaviruses and dicistroviruses that infect pigs and arthropods (Figure 3B), respectively. Furthermore, wawtorqueviruses were mainly detected in rodents 21, and gammapapillomaviruses and deltapolyomaviruses are primarily linked to human infections 22,23, but few of these viruses were ever discovered in suids 24."
- Authors: This information could be included in the Discussion, as there is no space for it in the Results.
R12: Agreed. We have removed the text from the Results and incorporated it in the Discussion. See lines 290-293 and 310-312.

Page 9 – "To pinpoint the risks of wild boar viruses posing to humans, domestic pigs, and wildlife, we queried BrCN-Virome sequences against Virus Pathogen Resource (ViPR) 25 to find hits with an average nucleotide identity (ANI) of ≥ 90%."
- Authors: This section should be in Methodology.
R13: Agreed. We have deleted it from the Results section. See lines 532-533.

Page 10 – “These indicate that wild boars intensively participate in the virus circulation of domestic pigs and extensively interact with humans, other mammals, birds, and arthropods in the viral ecology.”
- Authors: This should be part of the Conclusion or the Discussion.
R14: Agreed. We have deleted it from the Results section. See line 185.

Page 11 – “The H. longicornis ticks were linked to 13 virus species of BrCN-Virome, with eight being genomoviruses. The Rhipicephalus microplus ticks were associated with eight virus species of these wild boars, all being known vector-borne viruses except a Hepeviridae sp.”
- Authors: This section needs to be referenced and does not appear to be part of the Results, as some points are discussed
R15: We understand the reviewer’s concern, but we would like to clarify that this sentence is a part of the Results and it was based on the virus circulation between wild boars and ticks. We apologize for any confusion caused by the Results.

Page 12 – “These provide adequate evidence that virus circulation occurred between wild boars and vectors.”
- Authors: This should be part of the Conclusion or the Discussion.
R16: Agreed. We have deleted it from the Results section. See line 212.

DISCUSSION:
Page 15 – “Although wild boars are physiologically and genetically identical to domestic pigs,…”
- Authors: Authors: You cannot make this statement without including a scientific reference that validates your information. A reference must be included here.
R17: Done. We have cited two references for the statement.

Page 15 – “…so those viruses mainly replicating in the digestive tract, such as AstV, calicivirus, etc., …”
- Authors: Again, Authors a reference must be included in this section.
R18: Done. We have cited a reference for the statement.

Page 16 – “their deep participation in various ecosystems. For example, wild boars freely roam in the wild and are frequently infested by arthropods, making them have a higher chance of contracting vector-borne viruses in Bunyavirales, Flaviviridae, Rhabdoviridae, etc.

-Authors: Again, a reference must be included here.
R19: Done. We have cited a reference for the statement.

Page 16 – "In a local ecosystem, all life forms directly or indirectly interact with each other via food chain, sharing habitat, resource competition, etc., which provides ample conditions for virus circulation and cross-species transmission."
-Authors: Again, a reference must be included here.
R20: Done.

Page 16 – "Some viruses are known to be pathogenic to pigs, humans, sheep, cattle, and carnivores, but…"
-Authors: Again, a reference must be included here.
R21: Done.

Page 16 – "These pig pathogens are widely distributed in wild boars across China, with some also lethal to wild boars, such as ASFV, CSFV, etc."
-Authors: Again, a reference must be included here.
R22: Done.

Page 17 – "Virus infection is an important cause of wild boar deaths,…"
-Authors: Again, a reference must be included here.
R23: We are sorry for the confusion. This sentence summarizes the causation of wild boar deaths based on our analysis, so it's inappropriate to cite a reference here. To clarify it, we have revised it to "*Our data show that virus infection is an important cause of wild boar deaths*."

Page 18 – "…have already spread in China."
-Authors: Again, a reference must be included here.
R24: Done.

MATERIALS AND METHODS:
Methods:
-Authors: The study area needs to be described. To my best knowledge, China has almost 10 million km^2; therefore, it should specify in which regions the collections were made (north, northeast, northwest, south, southeast, southwest, rural, peri-urban or forest), describing the phytocoenotic differences and abiotic and biotic factors, if possible.
R25: Done. The sampling areas covered various landscapes including Northeastern

Forest Zone, Northwestern Arid Zone, Southeast Hilly Zone and Southwestern Mountains (Extended Data Fig. 1). See lines 379-382.

Page 19 – "Wild boars have become a kind of pest animal in China 50. They frequently harass agricultural areas to trample crops and even invade urban communities to trigger human-wild boar conflicts, causing substantial ecological and economic losses to human society 13."
- Authors: This information should not be part of the Methodology. It can be included in the Introduction or the Discussion.
R26: Done. We have incorporated the text into the Introduction and the Discussion section. See lines 65-66 and 294-299.

Page 20 – "…series of strategies to control their population and distribution 13. The State Administration of Forestry and Grassland of China also initiated a plan to actively
survey the virus background of wild boars since 2015. Forest rangers of the Local Administration of Forestry and Grassland set traps to capture wild boars or hunted them using rifles. They also routinely patrolled forests and sampled the dead wild boars they encountered. These wild boars killed by traps or shooting were all apparently and internally normal and hence were considered healthy individuals. All wild boars were dissected in the fields to collect their internal solid organ tissues using sterile tubes. Blood samples were collected if possible. All samples were immediately cryotransported to the laboratory at Changchun Veterinary Research Institute, where they were stored at -80℃."
- Authors: This section can be summarized; as my suggestion, it is only necessary to mention that there is a population control initiative and that the study was conducted in partnership with the State Administration of Forestry and Grassland of China.
R27: As suggested, we have incorporated the recommended revisions while preserving the majority of the original text, which elucidates our sampling methodology. See lines 376-382.

Page 20 – "Before dissection, forest rangers checked ectoparasites of wild boars and plucked them if there were any."
- Authors: What methodology was used to assess the presence of ectoparasites?
R28: The direct observation method was used to check the presence of ectoparasites on the wild boars' bodies, including the inner side of the ear, neck folds, groin and inner limbs.

Page 20 – “These ectoparasites were also transported to the laboratory along with wild boar samples.”
- How was the transportation carried out? Were the ticks alive? Were they stored in any product? Have any ticks been deposited in any collection?
R29: We thank the reviewer’s interests in the procedure of tick sampling. All collected ticks were euthanized through rapid freezing and subsequently stored in sterile, leak-proof tubes without preservative agents, then cryo-transported to the laboratory.

Page 20 – “All these arthropods were ticks, and their species were morphologically identified.
- Authors: What identification methodology was used? Which dichotomous key was applied?
R30: The species was morphologically identified under a stereo microscope (Nikon) based on various characters, such as the scutum, the basis capitula and palp, the anal groove and the shape and size of the spurs on the coxae. We have supplemented the methodology. See lines 386-391.

- Authors, you report the species of ticks that were identified in wild boars. However, you made a very important mistake: you forgot to describe in the material and methods how the ticks were identified (what was the taxonomic identification key or were they identified by DNA sequencing technique).
R31: Indeed, we forgot to introduce the details of identifying tick species. As we responded in R30, we have supplemented the method in the Methods section. See lines 386-391.

Page 20 – “When necessary, the mitochondrial cytochrome c oxidase subunit I gene of tick was sequenced and subjected to a BLASTN v.2.14.0+ search against GenBank to confirm the species identification further.”
- Authors: The reference should be included in this information.
R31: Done.

Page 31 – “Primer pairs were referred to previously published or WOAH and national recommendations, or designed based on contigs.”
- Authors: Please, reference must be included here. The reader needs to have access to the sequence of these primers.
R32: Done.

The choice of minimum contig sizes (1.5 kb in MDA, 500 bp in MTT) needs to be justified. Is it possible that important small-genome RNA viruses were discarded?
R33: Done. This parameterization aligns with the fact that eukaryotic DNA viruses exhibit minimum genome lengths exceeding 1500 bp (e.g., circovirus), while eukaryotic RNA viruses (including those with segmented genomes) have genomic components no shorter than 500 bp (e.g., rotavirus). Hence, important small-genome eukaryotic RNA viruses wouldn't be excluded. We have explained the reason we use such cutoffs. See lines 447-450.

Despite the inclusion of controls (BHK-21 cells), the index hopping rate observed in the blanks was not described, which is crucial in viromic studies with low viral load.
R34: While we did not perform a systematic evaluation of index hopping rates across all sequencing libraries, two quality control measures were implemented to mitigate this potential artifact. First, all libraries incorporated 10-nt unique dual indexes, which have been demonstrated to significantly reduce index misassignment in multiplexed sequencing workflows. Second, a stringent thresholding protocol was applied where relative abundance values ≤ 1 RPM were set to zero to eliminate background noise potentially arising from cross-contamination events.

Reviewer #2 (Remarks to the Author):

I co-reviewed this manuscript with one of the reviewers who provided the listed reports. This is part of the Nature Communications initiative to facilitate training in peer review and to provide appropriate recognition for Early Career Researchers who co-review manuscripts.

R35: We appreciate the reviewer for his/her efforts in evaluating our manuscript.

Reviewer #3 (Remarks to the Author):
"Node role of wild boars in virus circulation among wildlife and domestic animals' is a very comprehensive metagenomic sequencing- and bioinformatics-based assessment of virome content in wild boars and how wild boars may contribute to viral infection of humans and other animals. Several types of sequencing are employed in this study, to maximize the recovery of DNA and RNA viruses, and multiple tissue types were examined from the animals. The results from apparently healthy boars were compared to results from dead boars, and the way the researchers proved ASFV to be the likely cause of death was interesting and methodologically sound. The researchers also demonstrated that the ASFV infection has effects on virus diversity in the tissues that were examined. They showed that healthy pigs have less virus diversity and that ASFV infection suppresses the levels of replication of other viruses (a phenomenon

widely known as superinfection exclusion but rarely demonstrated in metagenomic sequencing datasets - so I appreciated this). It was also notable that the viruses identified in the ticks on the pigs were also identified in those specific pigs that the ticks fed on. Overall, the study was well-executed, with robust bioinformatics. The manuscript is very well written, and I enjoyed spending time on it. Figure 1 depicts an enormous amount of information in a very succinct manner.

R36: We extend our heartfelt gratitude to the reviewers for their thoughtful evaluation of our work, and we are particularly encouraged that our manuscript provided reviewers with an engaging and positive reading experience throughout the evaluation process.

Below are a few specific comments, questions, and requested edits:

1. First paragraph of Results section: I think the word "proclivity" would make more sense than "priority." "Owing to the proclivity of the MDA technique to amplify single-stranded circular DNA..."

R37: Agreed. We have replaced “priority” with “proclivity”.

2. Same paragraph as above, I would delete "these," as in "Double-stranded DNA viruses were also efficiently recovered..."

R38: Agreed. We have deleted the word “these”.

3. The authors should delineate the meaning of each acronym at first usage. Some examples of acronyms in the manuscript that were not defined at first use are PEDV, AKAV, and WOAH.

R39: Done.

4. In the Viromic signatures of healthy, dead, and ASFV-killed wild boars section of the Results, the authors state that the viromes did not reach saturation. This means that not all the viruses present in the animals were detected because the samples were not sequenced deeply enough. The authors should address this in the Discussion section as a caveat of their study and should explain how this might affect the conclusions.

R39: Agreed. We have supplemented the limitation of the study in the Discussion section. It reads “*This study represents the most extensive investigation conducted thus far to elucidate the virome landscape of wild boars. Nevertheless, we much acknowledge the limitations of our data set in fully capturing the complete spectrum*

of wild boar viral diversity, as both sequencing coverage and sampling scale indicate that the observed virome composition has not yet theoretically saturated." See lines 272-273 and 353-355.

5. There were 3 dead boars from which ASFV was not detected by sequencing and bioinformatics but was detected by PCR. Sometimes when multiplexing libraries on a sequencing run, there is variation in terms of how many reads are produced per library. Is there a statistically significant difference between the number of sequencing reads produced from dead boar samples in which ASFV was detected by sequencing and bioinformatics as compared to the number of sequencing reads produced from dead boar samples in which ASFV was not produced? If the number of reads is less in samples from which ASFV was not detected by sequencing, then the lower depth of sequencing could be the reason, and that, along with the lack of saturation the authors mentioned, would strongly suggest that all the samples should have been sequenced more deeply.

R40: We sincerely appreciate the reviewer's professional suggestion. A comparative analysis was performed on the quantities of high-quality unclassified reads across ASFV-positive/negative samples that were detected by viromic sequencing (viromic ±) and PCR (PCR ±). As illustrated in Figure S1 below, the read counts were comparable between groups, with no statistically significant differences observed (One-way ANOVA test). These findings suggest that the three samples likely harbor substantially lower ASFV loads compared to other ASFV-positive cases, thereby limiting the sensitivity of HTS in detecting ASFV sequences.

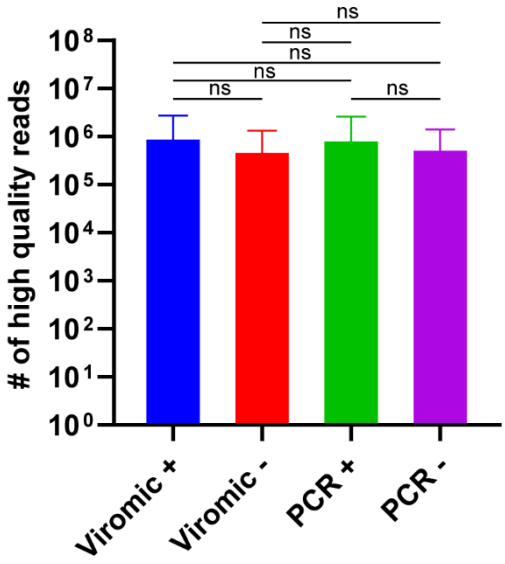

Figure S1. Comparison of the number of high-quality unclassified reads across

ASFV-positive/negative samples that were detected by viromic sequencing and PCR.

6. Among the three dead boars from which ASFV was detected by PCR but not by sequencing and bioinformatics, were there any genetic polymorphisms or was it just a case of lower titers or less reads? Were the ASFV detected in these three samples GI or GII?

R41: ASFVs in the three samples were all classified into GII without any genetic polymorphisms. The failure to detect ASFV via sequencing in the three samples was primarily attributed to the extremely low viral loads as evidenced by Ct values >35. We have supplemented these details in the Results section. See lines 252-254.

7. In the first paragraph of the Discussion, the word "partially" should be inserted in the following sentence because later in the paragraph the authors talk about the effect of wild boar lifestyle contributing to virome composition: "This discrepancy is likely partially attributed to the sample type..."

R42: Agreed. We have inserted the word “partially” in the sentence. See line 275.

8. The last sentence of that first paragraph in Discussion stating that the authors cannot rule out that some viruses preferentially infect wild boars, I disagree with. The authors have already pointed out that wild boars and domestic pigs are genetically indistinct. I would delete this sentence.

R43: Agreed. We have deleted the sentence.

9. In the methods, the authors describe the amount of tissue used as "a soybean-sized piece." I don't think soybean is a unit of measure. I suggest that authors replace the soybean reference with an actual length, e.g. "..a 10mm sized piece..."

R44: Agreed. We have changed it into “a piece approximately 6 mm in size (~0.2 g) was cut from …” See lines 392-393.

10. In the first paragraph of the Methods section called Bioinformatic procedure for BrCN-Virome preparation, third sentence, the word "into" should be inserted as follows: "..can naturally integrate into host sequences."

R45: Agreed. We have changed it into “have the capacity to integrate host-derived

genetic sequences into their own genomes." See lines 443-444.

11. In the last sentence of last paragraph in that same section, "5" should be spelled out: "five."

R46: Done.

12. In the first sentence of the section called Viromic characterization of dead and ASFV-killed wild boars, "assess" should be past tense, "assessed."

R47: Done.

14. In the section on Recombination analysis, I suggest more emphasis on the wording to demonstrate these are predictions. Rather than "would occur" it should say "were predicted to have occurred" and rather than "would happen to," it should say "was predicted to have occurred in."

R48: Agreed. We have revised these sentences.

15.In the second to last sentence of the section Molecular validation and detection, I think the word "transfect" should be replaced with "transform." Typically we use the word "transfect" to denote uptake of DNA into eukaryotic cell cultures and "transform" to denote uptake of DNA into competent bacterial cells.
R49: Agreed. We have changed the word with "transform".

Reviewer #4 (Remarks to the Author):
The authors describe a massive data set based on an extensive range of sample types from healthy and dead wild boars across many different regions in China. They present a highly detailed, comprehensive view of the wild boar virome – which has been understudies to date – and a valuable comparison to the viromes of domestic pigs. The authors also provide insights into potential cross-species jumping events of concern and clearly recognise the severe consequences this could present to human and animal health, as well as economically to the pig farming/agriculture industries.

The authors make several claims in this study. Firstly, a thorough comparison of wild boar vs domestic pig viromes with appropriate statistical analyses of alpha diversity reveals differences on virome compositions between the two data sets. The detection of viruses associated with other animals (eg., domestic pig, human, ticks) is visualised

in Figure 2, as well as corroborated by placing these sequences in phylogenetic trees with known reference sequences. These results support the authors' claims that wild boars represent a node animal. This, combined with the concerning detection of ASFV in dead wild boar samples, supports the overall notion presented by the authors that the routine surveillance and of wild boar viromes and populations is warranted, given the risk they may pose to public health, agriculture, and wildlife conservation. Overall, the methods match the standard set for this kind of work in this field, and are thoroughly explained. I believe similar work could be replicated based on these methods.

R50: We sincerely appreciate the reviewer's recognition of our work and insightful comments, which has not only encouraged us a lot but also significantly contributed to refining the manuscript.

Some comments and suggestions below:

• I would ask why bacteriophage were removed from the data set? They represent a significant proportion of RNA viral diversity and may provide insights into dietary or gut microbiome viruses, and may partially close the gap between domestic pigs and wild boars. I do not think this choice necessarily prevents publication, but it may be worth explaining in more detail why bacteriophage were excluded and discussing the potential limitations.

R51: Indeed, we did not included bacteriophages in the data set since our priority was to investigate public health-related eukaryotic viruses in these solid internal organs. We have pointed out the limitation in the revision as suggested. See lines 356-357.

• I suggest expanding "IUCN Red List" to "International Union for Conservation of Nature Red List of Threatened Species" for clarity

R52: Done as suggested.

• Might also be worth to explain the "least concern" category a bit further, ie, Wild boars face little to no threat of endangerment or extinction, having been assigned to the "least concern" category by the International Union for Conservation of Nature Red List of Threatened Species.

R52: Done as suggested.

• There is a lot of background information in the beginning of the methods, namely in

the “Sample collection and processing” section. I would move that to the introduction or remove what was already stated in the introduction. Only need something like “Samples were obtained as part of national and local Chinese government strategies to control wild boar populations and distribution, as well as a plan initiated in 2015 by The State Administration of Forestry and Grassland od China to actively survey the virus background of wild boars”.

R53: Agreed. We have condensed this section. See lines 376-379.

• Please provide more detail on the blast process – which GenBank database was used and what release date?

R54: Done. The GenBank nt database (release date: May 1 2024) was used in the blast search. We have provided the details, see lines 389-391.

• Please check to make sure references to “si-libraries” and “mi-libraries” are consistently hyphenated, eg. In the “Overview of the wild boar virome”, they are not hyphenated.

R55: We have checked them throughout the manuscript. Now they are consistently hyphenated.

• References to figures should really be limited to the results section, I would recommend removing them from the discussion and methods sections (figure references can be appropriate in the methods, but usually only when referencing a figure that explains a protocol rather than results)

R56: Agreed. We have properly removed references to figures in the Discussion section.

• Figure 1A: Genome type includes a colour (blue/green-ish colour) not present in the figure legend

R56: Sorry for the mistake. We have corrected the color scheme.

• In general, the figures in this paper – while excellently delivering large amounts of data – are highly complex and busy, which can make them difficult to decipher particularly if colourblind or viewing in black&white. I encourage the authors to (where possible) match the order of categories in the figure legend as much as

possible to the figure, eg. Genome type legend could be reordered to dsRNA, ssRNA(+), ssRNA(-), dsDNA, ssDNA, Mix. I also encourage the authors to minimise overly similar colours being adjacent without some kind of barrier to mark a new category, eg., Peplo/Preplasm and dsDNA/dsRNA colours are virtually indistinguishable next to each other. I acknowledge this isn't possible everywhere, but some minor changes may help improve the accessibility of these figures.

R57: We sincerely apologize for any inconvenience caused. The color scheme as been refined in accordance with the reviewer's suggestions, and these revisions aim to enhance the interpretability of the depicted elements.

• Strange suggestion in the conclusion to abandon the free-range model of pig farming? It's important to acknowledge animal welfare concerns, where confinement can increase stress & reduce immunity, thereby increasing pigs' vulnerability to disease – as well as environmental/ethical considerations. Perhaps consider some nuance, ie better biosecurity in fencing/physical barriers, and monitoring food/water/cleanliness without worsening domestic pig living conditions.

R58: Agreed. We have revised the conclusion as suggested. Now it reads "*Some countermeasures should be considered to mitigate the potential threats. Their chance to encounter domestic pigs can be cut off by enhancing biosecurity within free-range systems through fencing/physical barriers, and monitoring food/water/cleanliness and upgrading it to intensified industrialized production*."

Overall, I enjoyed reading this paper. The scale of data generated is impressive and the results were fascinating. This paper is sure to be of interest for those working in virus discovery and ecology, as well as in wildlife conservation, public health, and agriculture.

R59: We sincerely appreciate the reviewer's commendation, which has provided us significant encouragement. Furthermore, we are grateful for the insightful comments that have enabled us to refine the manuscript to a higher standard of academic rigor.

Point-by-point Responses

Reviewer #3 (Remarks to the Author):

I appreciate the edits the authors made in response to all the reviewers' comments. I am satisfied with the revised manuscript.

Response: We would like to thank the reviewer for his/her further assessment.