Journal of Integrative Agriculture 2026, 25(8): 3508–3510

Available online at ScienceDirect

Journal of Integrative Agriculture



LETTER

Coronavirus-like particles package the negative-strand genome of coronavirus

Xinyu Yang*, Wenzhen Qin*, Ning Kong*, Yuchang Liu, Guangzhi Tong, Tongling Shan#

Shanghai Veterinary Research Institute, Chinese Academy of Agricultural Sciences, Shanghai 200241, China

Highlights

- The particles of coronavirus package the negative-sense genome of coronavirus into the coronavirus-like particles.
- In PEDV particles purified by sucrose density gradient centrifugation and treated with antibodies and nuclease, approximately 20 to 46% contain negative-sense genomes.
- Coronavirus-like particles are capable of adsorption and internalization.

Classifying viruses based on their genomic characteristics is an important principle for virus classification (Walker *et al.* 2022). It is known that the coronaviruses belong to single-stranded positive-sense RNA viruses, and which particles package the single-stranded positive-sense genomes (Maier and Bickerton 2020; Woo *et al.* 2023). The replication of the coronavirus genome begins with the synthesis of negative-strand genomic and subgenomic RNAs, which serve as templates for producing new positive-strand genomes (Sola *et al.* 2015; Malone *et al.* 2022). Detecting the negative-strand sequence of coronavirus is the starting point for determining virus RNA synthesis after virus adsorption and internalization. We simultaneously detected the positive and negative-strand sequences of porcine epidemic diarrhea virus (PEDV) after the adsorption and internalization before the synthesis of negative sense RNA. The results are confusing. To reveal the discovery, we found that the particles of coronavirus belonging to *Alpha*, *Beta*, and *Deltacoronavirus* package the negative-sense genome of coronavirus into the coronavirus-like particles. In PEDV particles purified by sucrose density gradient centrifugation and treated with antibodies and nuclease, approximately 20% to 46% contained negative-sense genomes. These particles were capable of adsorption and internalization. The study explains why the negative-sense sequences of coronaviruses can be detected after virus adsorption and internalization and showed that coronavirus particles not only package the positive stranded genomes, but also package the negative-stranded genomes during the assembly process of coronavirus.

The synthesis of negative strands is the first step in coronavirus RNA synthesis. To confirm the starting point of genome synthesis after the adsorption and internalization of PEDV belonging to *Alphacoronavirus*, we simultaneously detected the positive and negative-sense sequences of virus with the ratio of positive and negative chains about 23–654 using the primers (Appendix A) targeting nsp2, nsp15 and N (Fig. 1-A and B). However, we could not determine the starting point of genome synthesis. We firstly speculated that the negative-sense sequences of PEDV originate from the free negative-sense viral RNA which is synthesized in the virus-infected cells. Then, PEDV supernatant was treated with nuclease to remove the free RNA outside the viral particles (Shan *et al.* 2022), but the positive and negative-sense sequences of PEDV were also simultaneously detected with the ratio about 8–29 (Fig. 1-C), although the viral RNA of the nuclease-treated samples was less than that of untreated samples. The negative-sense viral RNA detected in the positive-sense RNA viruses is confusing. To examine whether viral or host proteins protect RNA from nuclease degradation, virus particles were immunopurified using magnetic beads conjugated with monoclonal antibodies against M or S proteins, followed by nuclease treatment. The positive and negative-sense sequences of PEDV in the purified and treated viral samples were also simultaneously detected with ratio about 12–75 (Fig. 1-D). Similarly, viral particles were purified by sucrose density gradient centrifugation, followed by nuclease treatment to remove free RNA. Subsequently, immunopurification was performed using magnetic beads conjugated with monoclonal antibodies against the M or S protein. The positive and negative-sense genomes of PEDV in

Received 28 September 2025; Received in revised form 5 November 2025; Accepted 25 November 2025; Available online 22 December 2025
#Correspondence Tongling Shan, E-mail: shantongling@shvri.ac.cn
*These authors contributed equally to this study.

© 2026 CAAS. Publishing services by Elsevier B.V. on behalf of KeAi Communications Co. Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/). Peer review under responsibility of Editorial Board of *Journal of Integrative Agriculture*.
doi: 10.1016/j.jia.2025.12.042

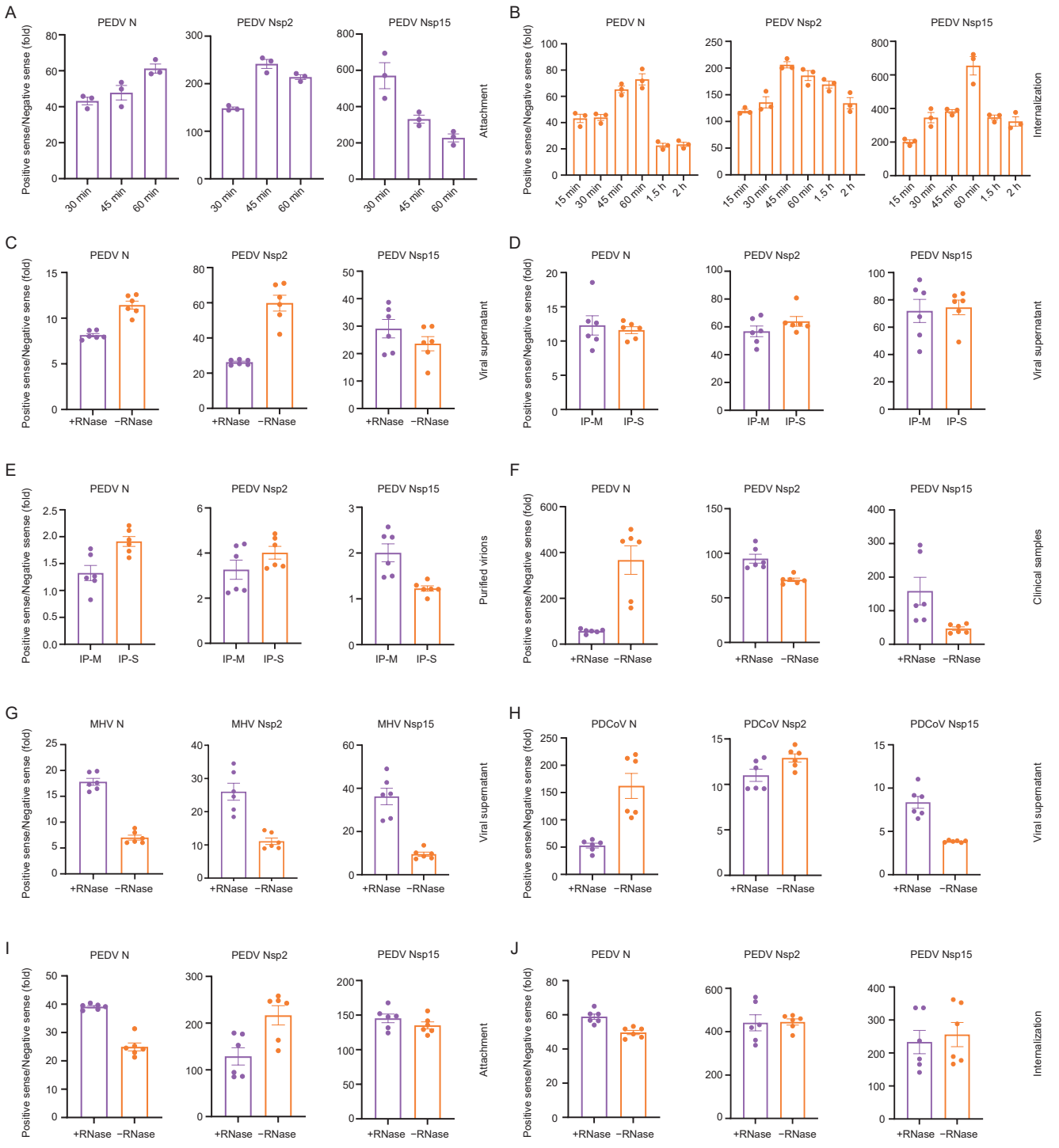


Fig. 1 Coronavirus-like particles package the negative-sense genome of coronavirus. A and B, the ratio of positive-sense to negative-sense RNA of PEDV during the PEDV attachment (A) and internalization (B). Quantification of the positive-sense to negative-sense RNA ratio of coronavirus by qRT-PCR targeting PEDV N, nsp2, and nsp15 during viral adsorption (A) and internalization (B) at the indicated times. C, nuclease-treated PEDV contains the negative-sense of virus. qRT-PCR detection with specific primers following nuclease treatment of viral supernatant. D, PEDV particles by Co-IP contains the negative-sense of virus. Nuclease-treated PEDV particles were immunoprecipitated using beads conjugated with anti-M or anti-S antibody. RNA was analyzed by strand-specific qRT-PCR for the indicated genes. E, PEDV purified by the sucrose density gradient centrifugation and Co-IP contains the negative-sense of virus. PEDV particles were purified by the sucrose density gradient centrifugation and Co-IP, and which were treated with nuclease for qRT-PCR. F, PEDV particles from PEDV-positive clinical sample contain the negative-sense of virus. Clinical samples from swine infected with PEDV were treated with nuclease and then analyzed by strand-specific qRT-PCR to quantify the positive-sense and negative-sense RNA. G and H, MHV (G) and PDCoV (H) particles contain the negative-sense of virus. PDCoV and MHV were treated in the presence or absence of nuclease to digest nucleic acids for strand-specific qRT-PCR to quantify the relative levels of positive-sense and negative-sense ratio. I and J, PEDV-like particles entry cells. Ratio of positive-to-negative strand RNA by qRT-PCR in cells infected with nuclease-treated or untreated PEDV following adsorption (I) and internalization (J). Results are represented to be mean±SD from three or six independent samples.

the purified and treated viral samples were also simultaneously detected with ratio about 1.2–4 (Fig. 1-E). The results showed that the negative-sense viral RNA is packaged in PEDV-like particles (named PEDV particles packaging the negative-sense viral genome as PEDV-like particles). To identify whether PEDV-like particles package the complete viral genomes, we performed RT-PCR using the primers (Appendix B) targeting overlap sequences as well as the 5′ and 3′ ends (poly19T or poly19A as the upstream and downstream primers). The results showed that PEDV-like particles package the complete negative-sense genomes that lack poly(A) or poly(T) tails at either terminus. To verify whether PEDV-like particles package negative-sense genomes of viruses in infecting pigs, clinical small intestine samples from PEDV-infected pigs were treated with nuclease to remove the free RNA outside the viral particles for detecting the positive and negative-sense sequences of PEDV. The results showed that the positive and negative-sense sequences of PEDV were also detected with the ratio about 57–158 (Fig. 1-F).

To investigate whether coronavirus-like particles of other genera in the *Coronaviridae* family package the negative-sense sequences of coronaviruses, mouse hepatitis coronavirus (MHV) belonging to *Betacoronavirus* and porcine deltacoronavirus (PDCoV) belonging to *Deltacoronavirus* were chosen to reveal that the negative-sense sequences of virus are packaged into viral particles. The samples of MHV and PDCoV were also treated with nuclease to remove the free RNA outside the viral particles for detecting the negative-sense sequences of the viruses *via* the primers targeting N, nsp2 and nsp15. Similar to PEDV, both MHV and PDCoV also package the negative-sense RNA, with the ratio of positive and negative strands about 18–36 and 8–53, respectively (Fig. 1-G and H). The results suggest that *Alpha*, *Beta and Deltacoronavirus*-like particles package the negative-sense genomes of the viruses into coronavirus-like particles.

To test the infectivity of PEDV-like particles, nuclease-treated PEDV was used to infect cells. Following adsorption and internalization, the ratios of positive to negative strand RNA were approximately 39–145 and 59–441 in two independent experiments (Fig. 1-I and J) in cells after the adsorption and internalization of PEDV treated with nuclease. The results showed that PEDV-like particles could enter cells. To investigate whether PEDV-like particles can replicate, we constructed infectious clones containing the negative-sense and positive-sense genomes of PEDV using yeast recombination (Thi Nhu Thao *et al.* 2020). The infectious clones of the positive-sense genomes of PEDV are successfully rescued, but the negative-sense genomes are not rescued.

In summary, the data explain why the negative-sense sequences of coronaviruses can be detected after virus adsorption and internalization before the synthesis of negative-sense RNA, and reveal that coronavirus-like particles including *Alpha*, *Beta*, and *Deltacoronavirus*, package the negative-sense genome of coronavirus into the viral particles in the assembly process of coronavirus, which are about 1.3% to 46% of coronaviruses, and which have the activity of adsorption and internalization. The negative-sense genome of coronavirus might be incorrectly packaged into coronavirus-like particles in the assembly process of coronavirus. Further study is needed to uncover the mechanisms of incorrect packaging and the biological functions of coronavirus-like particles for developing novel antiviral strategies, and to confirm whether this phenomenon exists in other single stranded viruses.

Acknowledgements

This work was supported by the National Key Research and Development Programs of China (2023YFD1801300) and the National Natural Science Foundation of China (32272999).

Declaration of competing interest

The authors declare that they have no conflict of interest.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this manuscript, the authors used DeepSeek to assist in improving the clarity and readability of the language. All content generated was carefully reviewed and revised by the authors as necessary, and the authors take full responsibility for the final content of the article.

Ethical statements

This study did not involve animal subjects, and thus, no ethical approval was required.

Appendices associated with this paper are available at https://doi.org/10.1016/j.jia.2025.12.042

References

Maier H, Bickerton E. 2020. *Coronaviruses Methods and Protocols*: *Methods and Protocols*. Humana Press, New York, NY.

Malone B, Urakova N, Snijder E J, Campbell E A. 2022. Structures and functions of coronavirus replication-transcription complexes and their relevance for SARS-CoV-2 drug design. *Nature Reviews Molecular Cell Biology*, **23**, 21–39.

Shan T, Yang S, Wang H, Wang H, Zhang J, Gong G, Xiao Y, Yang J, Wang X, Lu J, Zhao M, Yang Z, Lu X, Dai Z, He Y, Chen X, Zhou R, Yao Y, Kong N, Zeng J, *et al.* 2022. Virome in the cloaca of wild and breeding birds revealed a diversity of significant viruses. *Microbiome*, **10**, 60.

Sola I, Almazán F, Zúñiga S, Enjuanes L. 2015. Continuous and discontinuous RNA synthesis in coronaviruses. *Annual Review of Virology*, **2**, 265–288.

Thi Nhu Thao T, Labroussaa F, Ebert N, V'Kovski P, Stalder H, Portmann J, Kelly J, Steiner S, Holwerda M, Kratzel A, Gultom M, Schmied K, Laloli L, Hüsser L, Wider M, Pfaender S, Hirt D, Cippà V, Crespo-Pomar S, Schröder S, *et al.* 2020. Rapid reconstruction of SARS-CoV-2 using a synthetic genomics platform. *Nature*, **582**, 561–565.

Walker P J, Siddell S G, Lefkowitz E J, Mushegian A R, Adriaenssens E M, Alfenas-Zerbini P, Dempsey D M, Dutilh B E, García M L, Curtis Hendrickson R, Junglen S, Krupovic M, Kuhn J H, Lambert A J, Łobocka M, Oksanen H M, Orton R J, Robertson D L, Rubino L, Sabanadzovic S, *et al.* 2022. Recent changes to virus taxonomy ratified by the International Committee on Taxonomy of Viruses. *Archives of Virology*, **167**, 2429–2440.

Woo P C Y, de Groot R J, Haagmans B, Lau S K P, Neuman B W, Perlman S, Sola I, van der Hoek L, Wong A C P, Yeh S H. 2023. ICTV virus taxonomy profile: Coronaviridae. *Journal of General Virology*, **104**, 001843.

Executive Editor-in-Chief Dongming Zhao
Managing Editor Juan Zhang