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Letter

Feline calicivirus VP1-based mRNA vaccine protects cats against FCV challenge

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Dear Editor,

The proportion of families in China owning pets has been rising annually. In 2024, the number of pets reached 124.11 million, a 2.1% increase from 2023, with 71.53 million being pet cats, up 2.5% from 2023. Cats have overtaken dogs to become the most commonly kept type of pet. Feline calicivirus (FCV) is one of the major causes of respiratory disease in cats. Many cats recovered from the disease shed the virus for a long time and become persistent carriers of the virus, this gives the virus a high degree of genetic diversity, which contributes to its extreme infectiousness and aids its spread. In recent years, there have been successive outbreaks of feline malignant systemic disease caused by highly virulent FCV strains (virulent systemic disease, VSD) in multiple locations worldwide (Pedersen et al., 2000; Duclos et al., 2024), and hosts of VSD strains tend to be adult cats, which experience higher disease severity and a greater risk of death. The comprehensive prevention and control system of FCV takes vaccination as the core. Due to the high genetic variability and pathogenicity of FCV (Tan et al., 2025), current vaccines could only alleviate symptoms, but not completely prevent infection. The development of a new vaccine against FCV has clinical significance. The mRNA vaccine strategy has demonstrated promising outcomes in clinical trials for the treatment or prevention of various diseases, garnering significant attention (Fitz-Patrick et al., 2025; Oda et al., 2024; Schnyder et al., 2025). This platform holds considerable potential in calicivirus family, with significant progress notably seen in human norovirus vaccine development (Wei et al., 2025). These vaccines offer advantages, including robust immune activation, high safety profile, rapid research and development, ease of mass production, thereby offering extensive application potential (Pardi et al., 2019). In this study, we engineered mRNA vaccines encoding the full-length viral protein 1 (VP1), encapsulated within lipid nanoparticles (LNPs), and evaluated their protective efficacy in cats. Our findings indicate that the mRNA vaccine we developed successfully induced a protective immune response, conferred resistance against challenges with highly pathogenic strains, and effectively prevented FCV infection in cats. This approach offers a novel and promising strategy for the prevention of FCV infection.

Here, we selected FCV VP1 the main immune protective antigen, as the mRNA vaccine antigen, which based on the sequence of a highly pathogenic strain HBDL2 (GenBank No. PX458762). The FCV HBDL2 strain was isolated in 2021 from a clinical sample of a cat presenting with severe systemic symptoms. It is a highly virulent VS-FCV strain that causes systemic signs, including oral ulcers, ocular and nasal discharge, depression, anorexia, transient fever, sneezing, dyspnea, and in some cases, facial or paw edema. Individual severe cases result in death (Heng et al., 2025a). The strain was isolated and identified, which could induce robust immune responses against highly pathogenic viral strains (Heng et al., 2025b). The mRNA vaccine used in this study contains 5′ and 3′ UTRs from the *α-globin* gene, the feline-derived codon-optimized HBDL2 *VP1* gene with CD5 signaling peptide, and 110 poly(A) tail. The mRNA was encapsulated in a lipid mixture containing lipids, ethanol, ionizable lipids, DSPC and cholesterol and was synthesized and prepared by Zhongqi Pharmaceutical Technology Co., Ltd. To validate the antigen expression of FCV-VP1-based mRNA vaccine *in vitro*, The CRFK cells were treated with mRNA vaccine for 24 h. The results of indirect immunofluorescence assay (IFA) and Western blotting analysis indicated that the vaccine could effectively express the full-length VP1

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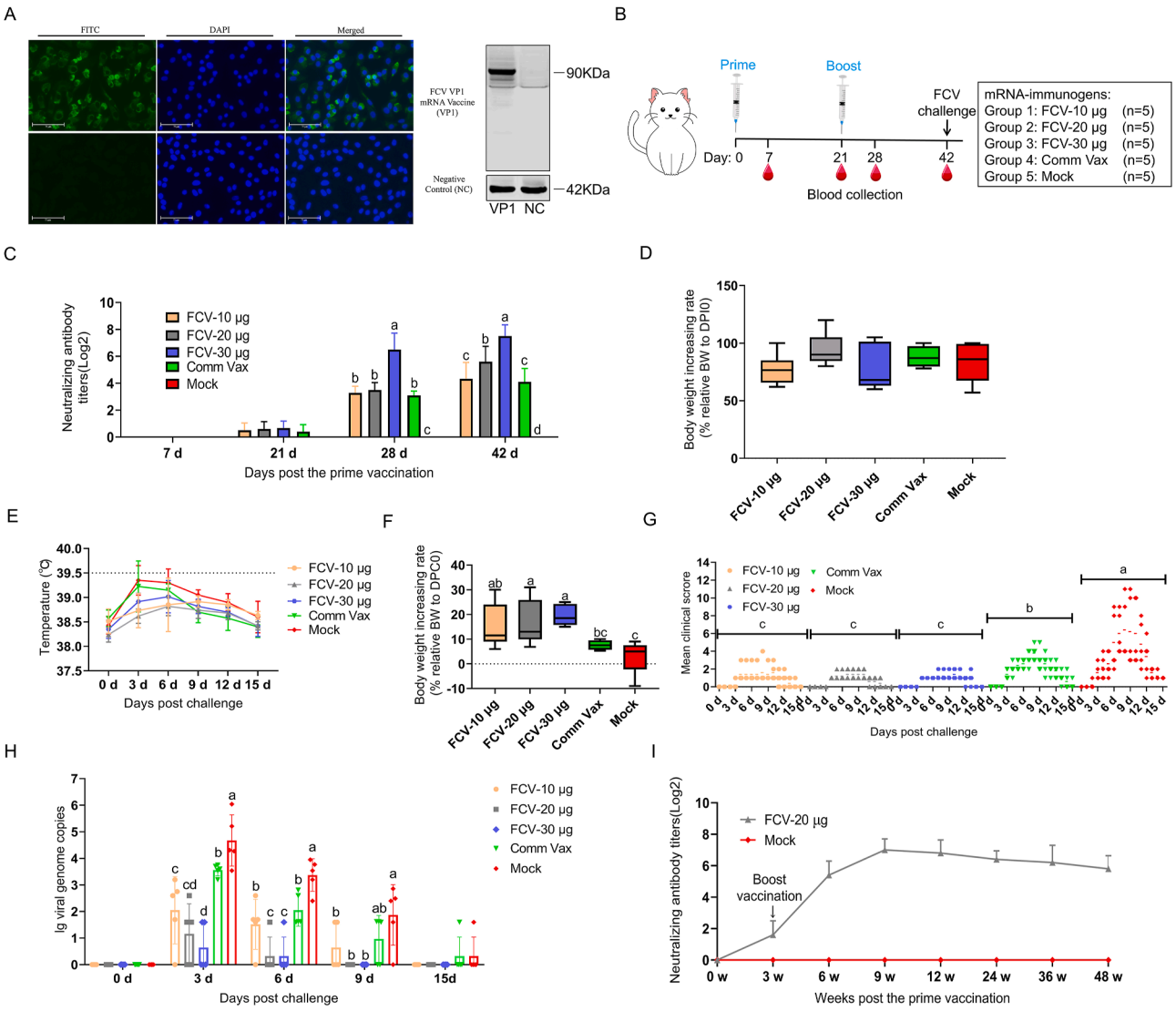

Fig. 1. Feline calicivirus VP1-based mRNA vaccine protects cats against FCV challenge.
A Identification of mRNA vaccines encoding FCV-VP1 by IFA and WB in vitro. **B** Experimental design for evaluation of the immune efficacy in cats. **C** Neutralizing antibody levels after immunization. **D** The body weight gain rate of cats during the immunization period. **E**, **F**: Change Variations in body temperature and weight after challenge. **G** The clinical signs were monitored daily and scored of cats following challenge. **H**: The blood viral load was measured via RT–qPCR (a 135 bp conserved region spanning the P76-VP1 junction). **I**: Kinetics of FCV Neutralizing antibody titers from week 0 to week 48. Statistical analyses were conducted using one-way ANOVA (**F**) or two-way ANOVA (**C**, **G** and **F**) followed by Tukey's multiple comparisons test. Different lowercase letters (a, b, c, d) above the bars indicate statistically significant differences between groups ($P < 0.05$), while groups sharing the same letter are not significantly different.

precursor (90 kDa) in feline-derived cells (Fig. 1A). To evaluate the efficacy of the mRNA vaccine in eliciting anti-FCV antibody production, the healthy British Shorthair cats (2-months) confirmed seronegative by neutralization prior to vaccination were subjected to five groups (5 cats per group, within a dedicated animal room 3 m × 1.5 m), including mRNA vaccine low-dose group (FCV–10 μg), medium-dose group (FCV–20 μg), high-dose group (FCV–30 μg), commercial inactivated vaccine (Comm Vax), and the control group. The immunized groups were immunized twice with an interval of 21 days between each immunization. Blood samples were collected on 7 and 21 days after each immunization to assess neutralizing antibody titers (Fig. 1B). The results showed that no seroconversion was observed in any of the immunized cats after the initial immunization, and showed a significant increase in FCV neutralizing antibodies after booster immunization (Fig. 1C). On 21 days after each immunization, the mean serum neutralizing (SN) antibody titers in Comm Vax group ($1:2^{4.1}$) were significantly lower than the titers in mRNA vaccine medium-dose ($1:2^{5.6}$) and high-dose group ($1:2^{7.5}$), titers were close to those of the low-dose group ($1:2^{4.3}$). Moreover, to evaluate safety, vaccinated and control cats were monitored for 7 days after each immunization. No adverse reactions (injection site reactivity, lethargy, anorexia, and fever) were observed in the vaccinated cats, and there was no significant difference in the body weight gain rate among the groups (Fig. 1D). These findings demonstrate that FCV-VP1-based mRNA vaccine immunization effectively induces anti-FCV neutralizing antibody responses in cats and has a favorable safety profile.

Challenge experiments were performed after verifying the induction effect of neutralizing antibodies. All the cats were challenged with the homologous FCV HBDL2 strain 0.5 mL (10^8 $TCID_{50}$) via intranasal and ocular administration, the clinical symptoms were recorded daily (over a 15-day period) using a quantitative scoring system derived from our previous studies (Tian et al., 2020; Heng et al., 2025b). This system evaluated four main categories: depression and anorexia, respiratory signs, ulcer lesions, and ocular or nasal discharges, with each category assigned a score based on severity. In addition to daily observations of clinical symptoms, body temperature and weight were measure, and blood viral load was

measured via RT–qPCR to determine viremia. Despite the 100% survival rate across all groups, marked differences in clinical manifestations were observed. In terms of body temperature and weight change after challenge, the results showed that the body temperature of the Comm Vax group and the blank control group would briefly exceed 39.5 °C and then return to within the normal range, while the body temperature of the FCV mRNA vaccine immunized groups remained at the level of the normal physiological range (Fig. 1E). The body weight gain rate of mRNA immunized group was higher than that of Comm Vax group and control group, while the rate of weight gain in the blank control group was consistently less than 10% (Fig. 1F). The clinical symptoms of cats were analyzed by the clinical scores. The results showed that compared with the cats in mock group, the incidence of FCV infection was significantly reduced in all vaccinated groups, only a few cats in the low-dose group of FCV mRNA vaccine and Comm Vax groups presented mild clinical symptoms (Fig. 1G). In the blood samples, compared with mRNA vaccine groups, the mock group and Comm Vax group exhibited more severe viremia. Viral RNA was detected in all cats in these groups on day 3 post-challenge and persisted until the end of the experiment. Instead, only some cats in mRNA vaccine groups showed detectable viral copy numbers on day 3 post-challenge, and viral RNA rapidly declined below the detection limit (Fig. 1H). In summary, the FCV-VP1-based mRNA vaccine provides robust immune protection against FCV infection.

To further evaluate the immunogenicity of the FCV mRNA vaccine, we monitored the duration of antibody. Ten cats aged between two and three months were assigned to an immunization group (N = 5) and a control group (N = 5). Based on the above results, cats in the immunization group were vaccinated twice intramuscularly at 21-day interval (Day 0 and Day 21) with medium-dose of mRNA vaccine (FCV–20 μg). All cats were periodically tested for FCV antibodies during the immunization phase. Similar to the previous results, all vaccinated cats seroconverted against FCV after boost vaccination. The booster vaccination induced a strong anamnestic response in all vaccinated cats. So far, the serum antibody titer of cats vaccinated with the mRNA vaccine maintain high level for nearly 1-year post-immunization shown in Fig. 1I. Further research revealed that the immune response induced by this mRNA vaccine is not only rapid but also long-lasting, enabling them to continuously and effectively resist the invasion of FCV.

In the current study, we designed an mRNA-LNP vaccine encoding the full-length VP1 protein, with features to optimize immunogenicity. Although the leader capsid (LC) region is cleaved during natural viral maturation, we retained it based on preliminary data suggesting its role in supporting proper VP1 folding and overall immunogenicity. The construct also incorporates a CD5 signaling peptide to promote antigen secretion, enhancing availability for B cell recognition, while intracellular antigen enables MHC-I presentation—a dual mechanism contributing to the potent humoral and potential cellular immunity induced. This design yielded a higher molecular weight product, as evidenced by the expression of the full-length VP1 precursor. The vaccine successfully induced robust and durable neutralizing antibodies, which correlated with complete clinical protection against challenge. Compared to our previous replication-deficient FCV vector platform that prioritizes rapid antibody induction, this mRNA-LNP strategy offers a complementary, fully synthetic alternative with an enhanced safety profile and design flexibility. This platform also holds considerable potential for protection against heterologous FCV strains. This potential is due to the VP1 antigen itself, derived from the HBDL2 strain, which has been shown in our prior studies to elicit broadly cross-neutralizing antibodies against diverse FCV strains, including VS-FCV variants (Heng et al., 2025a, 2025b). Moreover, the ease of co-encapsulating multiple antigen-encoding mRNAs, significantly simplifies the development of multivalent vaccines to address FCV diversity. The choice between platforms may thus depend on weighing the rapidity of immune onset against factors such as safety and antigen design flexibility. Nevertheless, we acknowledge that a comprehensive evaluation of vaccine-induced cellular immune responses was not included. This limitation stems primarily from the current lack of well-validated, available reagents and standardized protocols in feline models. Future studies designed to characterize the cellular immune profile will provide a more complete understanding of the immune mechanisms. In the future, we plan to conduct clinical trials to further verify the vaccine efficacy through larger-scale clinical studies, while simultaneously validating the safety and effectiveness of the mRNA vaccine in practical applications. It is expected that this work will open up a new and efficient immunization approach for the health protection of cats.

FOOTNOTES

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APPENDIX A. SUPPLEMENTARY DATA

Supplementary data to this article can be found online at https://doi.org/10.1016/j.virs.2026.03.010.

REFERENCES

Duclos, A.A., Guzmán Ramos, P.J., Mooney, C.T., et al., 2024. Virulent systemic feline calicivirus infection: a case report and first description in Ireland. Ir. Vet. J. 77, 1.

Fitz-Patrick, D., McVinnie, D.S., Jackson, L.A., Crowther, G., Geevarughese, A., Cannon, K.D., Garcia, L.M., Pineiro Puebla, Y., Yi, Z., Cunliffe, L., Maniar, A., Zareba, A.M., Ianos, C.A., Gomme, E., Koury, K., Suphaphiphat Allen, P., Anderson, A.S., Gurtman, A., Lindert, K., Pfizer C4781004 Trial Investigators, et al., 2025. Efficacy, immunogenicity, and safety of modified mRNA influenza vaccine. N. Engl. J. Med. 20, 2001–2011.

Heng, W., Zang, D., Li, R., Qi, R., Jiang, Q., Liu, J., Jia, H., Kang, H., et al., 2025a. A virulent systemic Feline calicivirus strain isolated in China could produce broadly neutralizing antibodies against multiple strains. Transbound. Emerg. Dis. 7, 6853477.

Heng, W., Zang, D., Li, R., Jiang, Q., Liu, J., Jia, H., Kang, H., et al., 2025b. A novel replication-deficient FCV vaccine provides strong immune protection in cats. J. Virol. 19, e0009325, 99.

Oda, Y., Kumagai, Y., Kanai, M., Iwama, Y., Okura, I., Minamida, T., Yagi, Y., Kurosawa, T., Greener, B., Zhang, Y., Walson, J.L., et al., 2024. Immunogenicity and safety of a booster dose of a self-amplifying RNA COVID-19 vaccine (ARCT-154) versus BNT162b2 mRNA COVID-19 vaccine: a double-blind, multicentre, randomised, controlled, phase 3, non-inferiority trial. Lancet Infect. Dis. 24, 351–360.

Pedersen, N.C., Elliott, J.B., Glasgow, A., Poland, A., Keel, K., et al., 2000. An isolated epizootic of hemorrhagic-like fever in cats caused by a novel and highly virulent strain of feline calicivirus. Vet. Microbiol. 73, 281–300.

Pardi, N., Hogan, M.J., Porter, F.W., Weissman, D., et al., 2019. mrna vaccines -a new era in vaccinology hhs public access author manuscript strategies for optimizing mrna pharmacology. Nat. Rev. Drug Discov. 17, 261–279.

Schnyder Ghamloush, S., Fanning, S., Essink, B., Eder, F., Snape, M.D., Stoszek, S.K., Guo, R., Dhar, R., Righi, V., Morsy, L., Kapoor, A., El Asmar, L., Shaw, C.A., et al., 2025. Safety and immunogenicity of an mRNA-based RSV vaccine in seropositive children aged 12-59 months. Hum. Vaccines Immunother. 21, 2557676.

Tan, X., Lin, L., Zhang, Q., Li, N., Dong, N., Wang, S., Zhu, W., Luo, M., Pang, S., Xu, Y., Liu, G., Meng, C., et al., 2025. First isolation and genomic characterization of a novel inter-genotype recombinant feline calicivirus from domestic cats: implications for viral evolution. Virol. Sin. 40, 672–675.

Tian, J., Kang, H., Huang, J., Li, Z., Qu, L., et al., 2020. Feline calicivirus strain 2280 p30 antagonizes type I interferon-mediated antiviral innate immunity through directly degrading IFNAR1 mRNA. PLoS Pathog. 16, e1008944.

Wei, J., Bystol, K., Alabanza, L., Lim, S., Greig, J., Gopal, P., Reyes Ballista, J.M., Galli, J. D., Warren, C., Swartz, A., David, C., Jeong, U., Gonzalez-Fernandez, E., DeWitt, L. A., Go, G., Lago, A., Radcliffe, S., May Fulton, C.D., Shen, P., Fridman, A., Gaspar, J., Pirrone, A., Wen, Z., O'Donnell, G., Austin, L., McKenney, D., Krosky, P., Liu, Y., Bett, A.J., Zhang, L., et al., 2025. Characterization of humoral and cellular immunity induced by mRNA vaccines expressing norovirus VP1 proteins in mice and NHPs. Mol. Ther. 12. S1525-0016(25)00751-8.