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Rapid production of monoclonal antibodies from single mouse B cells against FMDV

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Abstract

Single B-cell antibody generation technology is an advanced method that offers several advantages, including rapid production, high efficiency, and high yield. The antibodies generated via this technique retain their natural conformation and are well suited for applications in pathogen diagnosis, disease treatment, and investigations of virus cross-species transmission mechanisms. Our study aimed to establish a platform for generating single B-cell antibodies specifically targeting the foot-and-mouth disease virus (FMDV) 146S antigen in mice. Female BALB/c mice were immunized with inactivated O-type FMDV 146S antigen, and spleen cells were collected for further analysis. Flow cytometric sorting was performed using a biotin-labeled O-type FMDV 146S antigen as a decoy to identify and select CD19 + /CD21/35 + /CD43-/IgM-/Biotin + antigen-specific individual B cells. The gene sequences encoding the variable regions of the heavy and light chains of the murine IgG antibodies were obtained via single-cell nested PCR amplification. Separate constructs were created for the heavy and light chain plasmids to ensure the proper expression of intact IgG antibodies. These plasmids were cotransfected into human embryonic kidney 293T (HEK293T) cells, leading to the successful production and purification of 15 specific monoclonal antibodies (mAbs), 10 which exhibited activity in ELISA tests, and six antibodies that displayed activity in IFA tests. These findings highlight the successful development of a method for generating mouse-derived single B-cell antibodies that target FMDV. This achievement provides a solid foundation for diagnostic techniques and the analysis of antigenic structural variations.

Keywords Single B cell, Monoclonal antibody, Antibody generation, Foot-and-mouth

Main text

Foot-and-mouth disease (FMD) is an acute and highly contagious viral disease caused by foot-and-mouth disease virus (FMDV). It affects a wide range of animals, including more than 70 cloven-hoofed animals, such as cattle, sheep, pigs and various domestic and wild animals (Jamal and Belsham 2013; Lu et al. 2022). FMD has a great impact, as it exhibits a high level of infectivity, leading to the mortality of young animals and a consequential decline in the productivity of adult animals. This disease has a great economic impact on a global scale, posing a major challenge to the development of the livestock industry.

FMDV is a nonenveloped virus that exhibits icosahedral symmetry and is classified within the *Picornaviridae*

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family (Alexandersen and Mowat 2005). Its genome consists of a single-stranded RNA molecule containing approximately 8,500 nucleotides (Avendaño et al. 2020). The three-dimensional structure of FMDV isolates and antigenic variants has been elucidated (Han et al. 2015). The viral particle (VP) has a diameter of approximately 30 nm and comprises 60 copies in total. It is composed of four distinct capsid proteins, namely, VP1, VP2, VP3 and VP4, each with 60 copies. VP1, VP2 and VP3 are located on the surface of virions, whereas VP4 is a capsid protein that interacts with viral RNA (Domingo et al. 2002). FMDV consists of three types of viral particles: 146S, 75S and 12S (Harmsen et al. 2022). Research findings have indicated that the 146S viral particle has the highest immunogenicity, offering the most effective immune protection (Doel and Chong 1982; Harmsen et al. 2011; Rao et al. 1994).

Monoclonal antibody (mAb) production has led to the development of various methods. In 1975, Köhler and Milstein pioneered hybridoma technology, a well-established but time-consuming and relatively inefficient method that yields murine-origin antibodies (KÖhler and Milstein 1975). In 1996, a groundbreaking approach for antibody production was introduced (Babcook et al. 1996). Compared with traditional hybridoma technology, this novel method offers several significant advantages. It is characterized by its simplicity, efficiency, and time-saving, allowing for the generation of multiple mAbs simultaneously within a single process. This technique combines lineage analysis of immunoglobulin (Ig) genes with Ig reactivity analysis at the single-cell level, providing a comprehensive understanding of antibody production. By harnessing the power of fluorescence-activated cell sorting (FACS) cell sorting and sequencing technologies, it becomes possible to obtain the genes responsible for antibody expression from only a small number of cells. These genes can subsequently be expressed in vitro, facilitating the rapid production of multiple mAbs with precise antigen specificity.

In this study, we immunized mice with FMDV as a basis and successfully established a platform for generating mAbs from single B cells (Fig. 1). This platform can serve as a valuable reference for establishing similar platforms aimed at producing single B-cell-derived mAbs that target other pathogens.

FMDV inactivated antigen (FMDV O/GX/09–7) was purchased from JINYU Baoling BIO-PHARMACEUTICAL Co., Ltd. The antigen underwent inactivation through binary ethyleneimine (BEI) treatment and subsequent concentration. To assess FMDV 146S quality, the

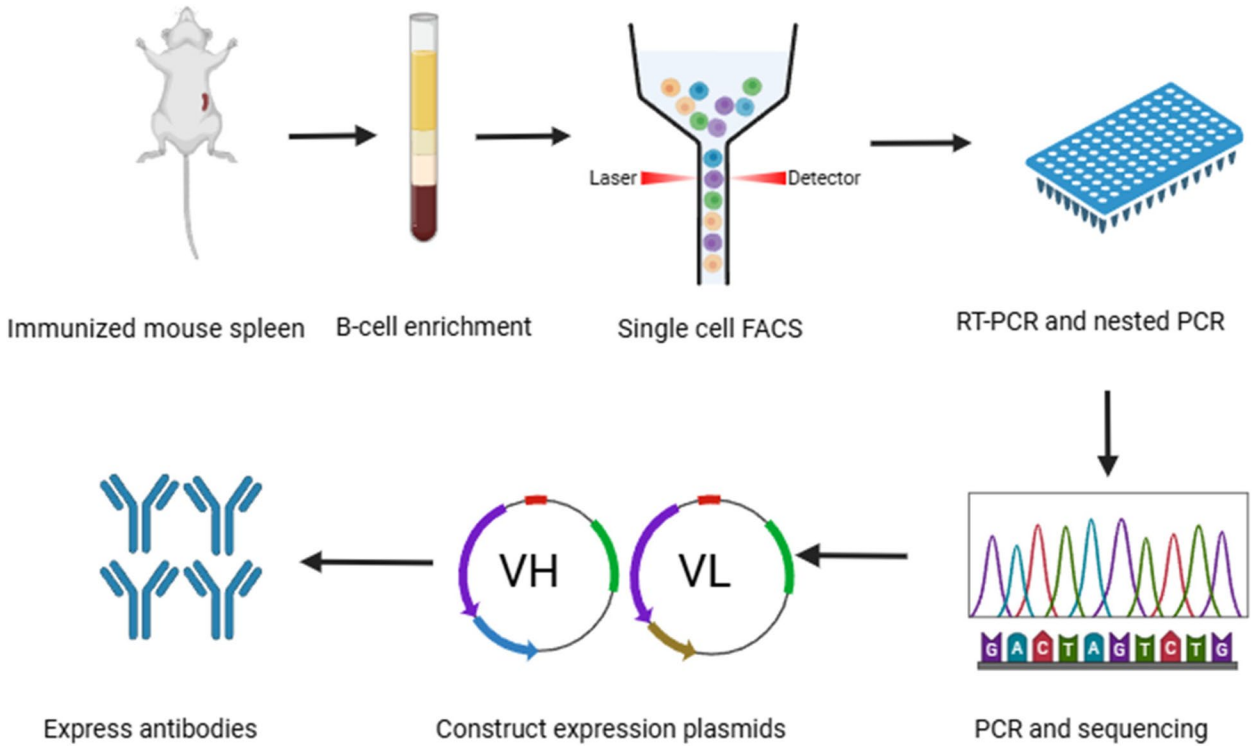

Fig. 1 Brief description of the platform for generating mAbs from single B cells in mice. B cells were enriched from immunized mice, and specific B cells were screened. The mAb sequences were amplified. Recombinant antibody expression plasmids were constructed using these sequences, and mAbs were produced (created in BioRender.com)

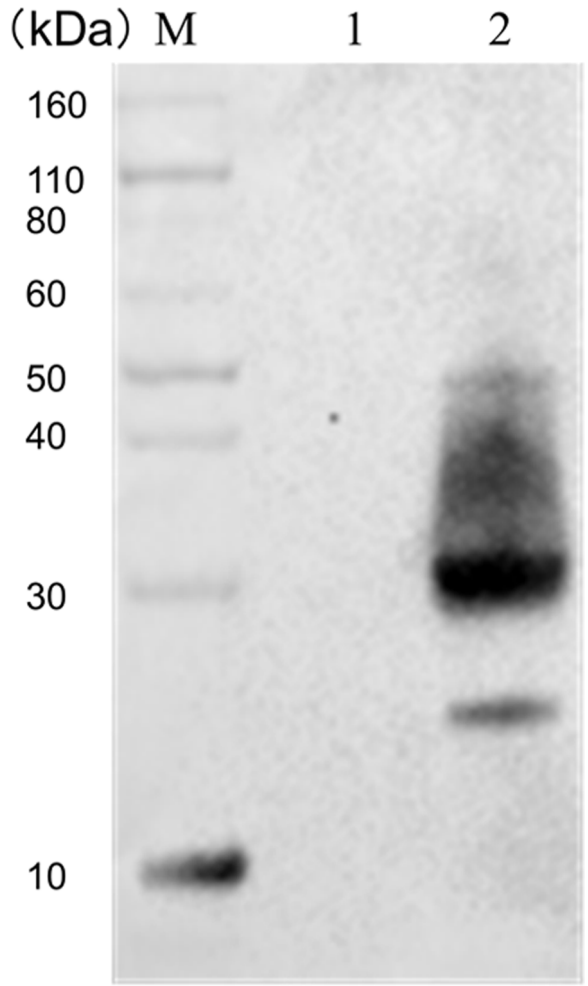

Fig. 2 Western blot analysis of Biotin-labeled FMDV 146S. Lane 1: Non-labeled FMDV 146S. Lane 2: Biotin-labeled FMDV 146S. This analysis revealed the presence of two additional bands of 10–30 kDa for the biotin-labeled FMDV 146S in comparison to the nonlabeled FMDV 146S

FMDV 146S standard and FMDV 146S were analyzed through a high-performance liquid chromatography (HPLC) system (Domingo et al. 2002), revealing characteristic chromatographic peaks.

To verify that FMDV 146S is biotinylated (Li et al. 2021), the biotin-labeled FMDV 146S and unlabeled FMDV 146S were compared via Western blotting. Two additional bands in the 10–30 kDa range were observed for the biotin-labeled FMDV 146S compared with the nonlabeled FMDV 146S (Fig. 2). These findings suggest that FMDV 146S was successfully biotinylated (Li et al. 2020).

To obtain FMDV 146S-specific B cells, mononuclear cell populations were selected from all peripheral blood mononuclear cells (PBMCs) on the basis of their size and granularity. B-cell populations were specifically identified by CD19 and CD21/35 staining, whereas T-cell populations were excluded through the use of CD43 staining for negative selection. Subsequently, CD19+/CD21+/35+/CD43-/IgM-/Biotin (FMDV)+cells were sorted via negative selection with the IgM marker and positive selection with the biotin marker. A total of approximately 1×10^8 PBMCs were sorted, and 131 FMDV 146S-specific B cells were acquired.

To obtain antibody-expressing sequences, individual B-cell samples were subjected to single-cell reverse transcription, followed by PCR amplification of variable heavy chain (VH) and variable light chain (VL) regions (Fig. 3). The resulting variable region sequences were then subjected to sequencing and analysis, yielding a total of 83 sequences for the VH gene and 39 sequences for the VL gene.

Among these, we successfully identified 20 pairs of matching heavy–light chain sequences, indicating the presence of B cells expressing antibodies with corresponding variable regions in both chains. The outcomes of the international ImMunoGeneTics information system (IMGT) analysis of these sequences are presented (Table 1), providing insights into the immunoglobulin heavy variable (IGHV) and immunoglobulin kappa variable (IGKV) gene utilization, identity percentages, and complementarity determining region 3 (CDR3) lengths. This comprehensive analysis establishes a foundational framework for understanding the genetic compositions and potential functional attributes of the identified antibodies. To verify that the antibody was expressed. We transfected twenty pairs of heavy and light chain plasmids into HEK293T cells for expression. The supernatant containing the expressed antibodies was collected and subjected to purification via a purification column. The successful expression of the antibodies was confirmed through analysis by reducing SDS–PAGE.

To validate whether the antibody can interact with FMDV, we used indirect ELISA to assess the FMDV 146S binding of 15 mAbs (500 ng/ml). Among the tested antibodies, No. 4, 31, 34, 49, 56, 57, 63, 109, 119, 121 and 123 exhibited reactivity with FMDV O/Cathay. Additionally, No. 4, 31, 34, 49, 56, 57, 63, 121 and 123 demonstrated reactivity with FMDV O/MYA98 (Fig. 4). To further verify that the antibody reacts with FMDV, we performed IFA with 1 μg/mL mAbs and observed specific fluorescence only at six mAbs with FMDV O/Cathay and four mAbs with FMDV O/Mya98, indicating the reactivity of the antibodies with their corresponding FMDV 146S. These findings suggest that the tested mAbs can effectively recognize and bind to FMDV (Fig. 5).

The rapid platform developed in this study for the efficient production of serotype O-specific antibodies

against FMDV serves as a foundation for the rapid generation of specific antibodies targeting other pathogens. Further integration of this platform with microarray technology can lead to the establishment of a comprehensive system capable of promptly responding to newly emerging pathogens and producing specific antibodies. The adoption of this platform represents a significant advancement over conventional antibody generation methods such as hybridoma technology, positioning it as the leading approach for monoclonal antibody production. This technology offers several advantages, including high-throughput capacity, preservation of natural antibody diversity, short processing time, high efficiency, and ease of operation, effectively meeting the requirements for rapid antibody production. The widespread implementation and continued development of this technology hold promising prospects for the future of antibody-based therapies.

Owing to their efficiency in protein expression, HEK293T cells are widely used in biomedical research and biopharmaceutical production. Target genes are introduced into HEK293T cells via methods such as lipid-mediated transfection, electroporation, or virus-mediated transfection. HEK293T cells are frequently used for recombinant protein expression (Jiang et al. 2022), especially for antibody production (Li et al. 2021; Ryu et al. 2022). They produce correctly folded and functional proteins, making them invaluable in creating therapeutic antibodies, vaccines, and viral vectors for gene therapy and vaccine development.

Mice were chosen as immunocompetent animals because of their well-established role in immunological research. The selection of mice as immunization hosts was based on several practical considerations, including their short immunization cycles, cost-effectiveness, and ease of handling. These factors collectively make mice a favorable choice for antibody production in our study. However, it is important to acknowledge that other animals, including rabbits, pigs, cattle (Kurosawa et al. 2012; Ramirez Valdez et al. 2023; Sok et al. 2017; Stefan et al. 2014), and even humans (Gilman et al. 2016; Iizuka et al. 2011; Liao et al. 2009; Sanam et al.

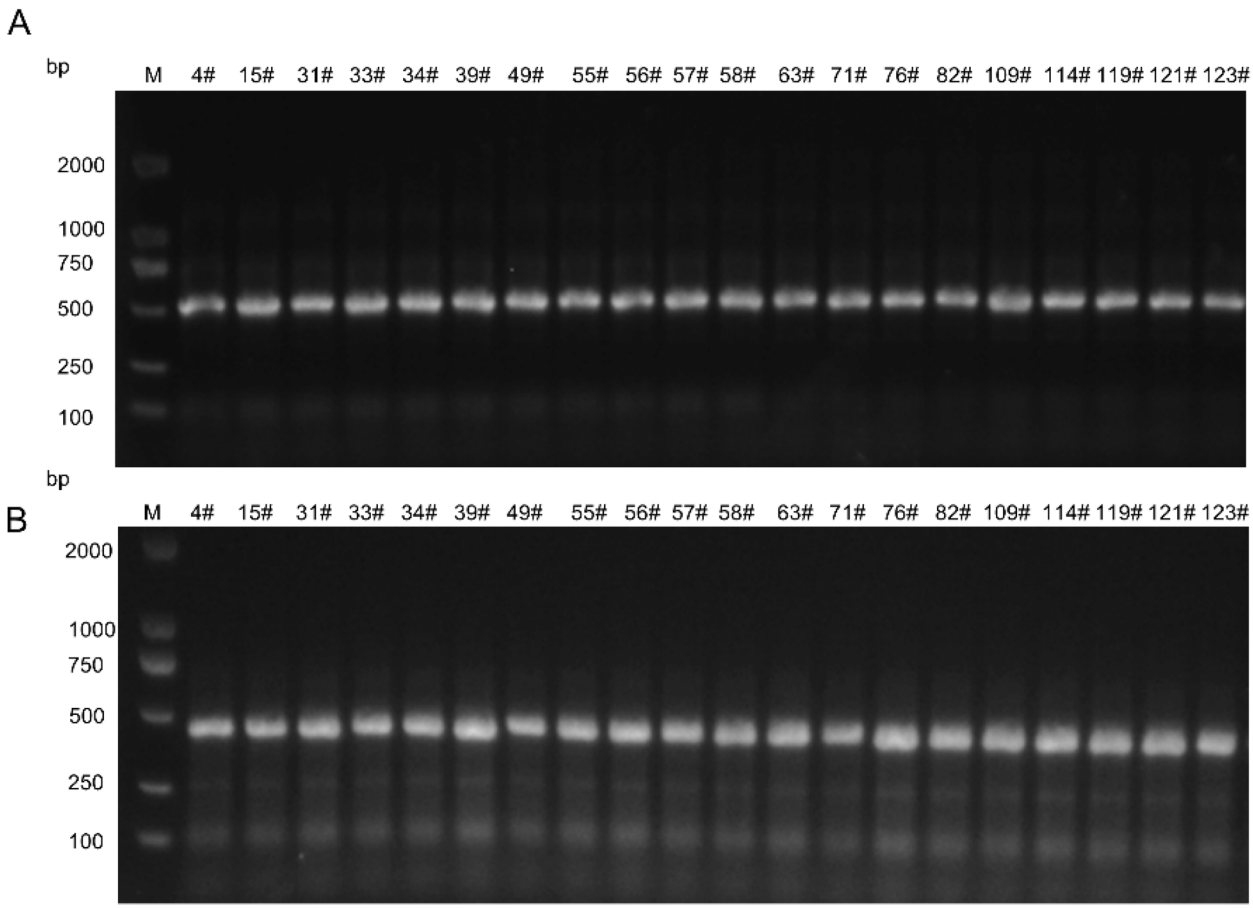

Fig. 3 PCR amplification of the VH and VL variable regions. **A** The results of antibody heavy chain amplification. **B** The results of antibody light chain amplification

Table 1 IMGT analysis of the antibody sequence

NO	Heavy chain			Light chain		
	IGHV	Identity	CDR3 length	IGKV	Identity	CDR3 length
4	1–2	76.5%	13	3–11	68.7%	9
15	1–2	76.4%	13	4–1	84.1%	9
31	1–2	74.4%	13	7–3	77.7%	9
33	1–2	74.4%	13	7–3	77.7%	9
34	1–2	74.4%	13	7–3	77.4%	9
39	1–2	74.4%	13	7–3	77.0%	9
49	1–2	74.4%	13	1–17	76.1%	9
55	1–2	74.4%	13	5–2	74.5%	9
56	1–2	74.4%	13	7–3	77.7%	9
57	1–2	74.4%	13	7–3	78.4%	9
58	1–2	74.4%	13	7–3	78.4%	9
63	1–2	74.4%	13	1–9	81.8%	9
71	1–2	75.8%	13	1–9	81.8%	9
76	1–2	74.4%	13	7–3	77.7%	9
82	1–2	74.4%	13	4–1	84.1%	9
109	1–2	74.4%	13	1–16	73.6%	9
114	1–2	74.4%	13	7–3	77.7%	9
119	1–2	74.4%	13	3–11	70.9%	9
121	1–2	74.4%	13	7–3	77.7%	9
123	1–2	73.8%	13	3–15	71.7%	9

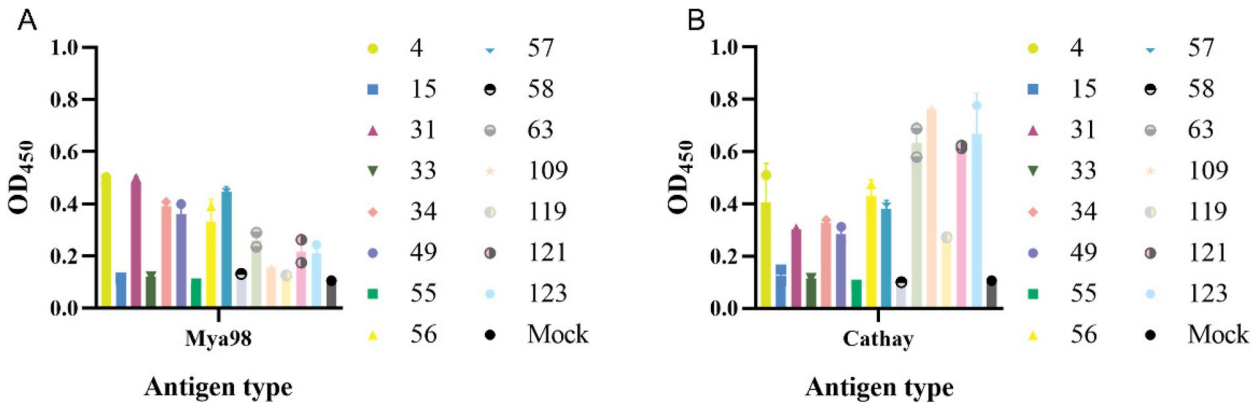

Fig. 4 Indirect ELISA results of 15 expressed antibodies. Indirect ELISA results of 15 expressed antibodies against FMDV O/Mya98 146S (**A**) and FMDV O/Cathay 146S (**B**)

2016), can also be used as suitable targets for immunization in similar studies.

Conclusion

In conclusion, this research demonstrated the utilization of a rapid platform for the generation of highly specific monoclonal antibodies against serotype O FMDV. Our findings highlight the efficiency and speed of this platform in producing monoclonal antibodies that specifically target serotype O of FMDV. Compared with conventional hybridoma methods employed for antibody production (Steinitz 2009), this platform significantly reduces the time required and allows for the acquisition of a larger repertoire of monoclonal antibodies. Compared with the existing methods for producing antibodies against bovine and porcine FMDV via this technology, our approach offers the advantages of lower costs and simpler procedures (Cao et al. 2022;

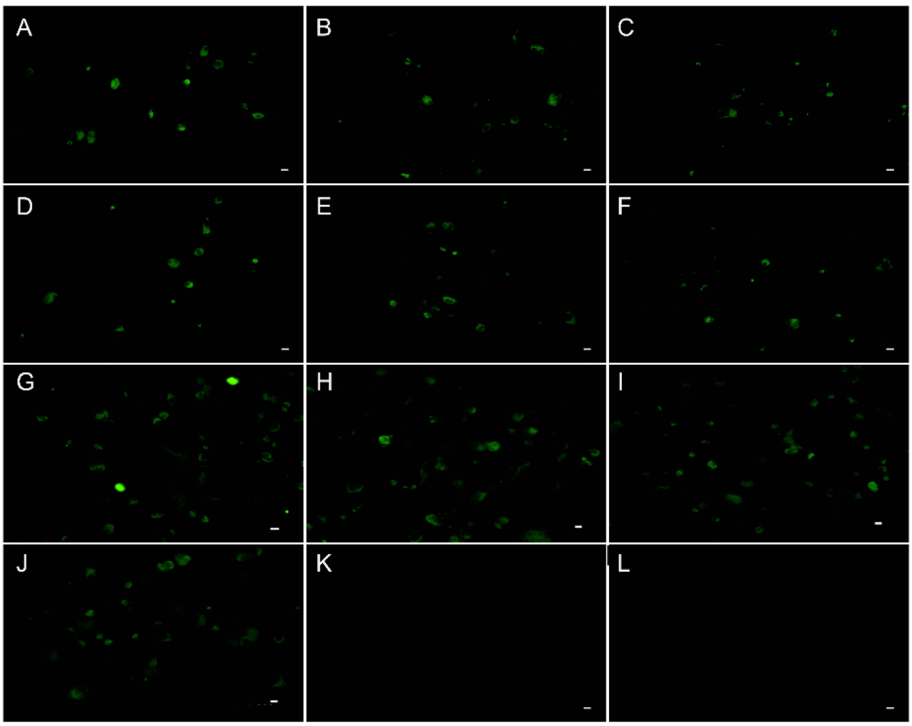

Fig. 5 IFA analysis of expressed mAbs. **A-F** No. 15,57,59,63,121,123 expressed antibodies against FMDV O/Cathay 146S. **G-J** No. 15,57,121,123 expressed antibodies against FMDV O/Cathay 146S. **K** MOCK of FMDV O/Cathay 146S. **L** The MOCK of FMDV O/Mya98 146S. (Bar = 200 nm)

Li et al. 2021). Among the more than 100 screened specific B cells, 83 heavy chain gene sequences and 39 light chain gene sequences were successfully obtained. While only 20 pairs of heavy and light chains were successfully matched, we can further increase the antibody diversity through artificial pairing (Marks et al. 1991). The platform established in this work allows for the rapid preparation of pathogen-specific antibodies. This enables the swift production of diagnostic or therapeutic antibodies against emerging pathogens, achieving a rapid response capability.

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Authors' contributions
Z.Y. carried out the original draft preparation. F.L., M.Z., and C.W. participated in the review and editing. Y.L. and L.X. were responsible for the methodology. Q.Z. and Y.L. provided supervision. W.L. and Y.Z. handled project administration and funding acquisition. All the authors have read and approved the final manuscript.

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Availability of data and materials
Not applicable.

Declarations

Ethics approval and consent to participate
All experimental procedures were approved and reviewed by the Institutional Animal Care and Use Committee of the China Institute of Veterinary Drug Control.

Consent for publication
All the authors have consented to the publication of the manuscript.

Competing interests
The authors declare that they have no competing interests. Author Wentao Li was not involved in the journal's review or decisions related to this manuscript.

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