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Cover Image: adapted from the World Health Organization, https://www.who.int/news-room/events/detail/2025/09/28/default-calendar/world-rabies-day-2025.

This week's issue was organized by Guest Editor Wenwu Yin.

Foreword

Let's Act Now to Make Rabies History

Wenwu Yin[1,2,3,#]

In 2007, international organizations, including the World Health Organization (WHO), the World Organization for Animal Health (WOAH), and the Global Alliance for Rabies Control (GARC), jointly established World Rabies Day. This global initiative mobilizes individuals, organizations, and stakeholders worldwide in the collective effort to eliminate rabies as a public health threat. September 28, 2025, marks the 19th World Rabies Day, with the theme "ACT NOW: YOU, WE, COMMUNITIES" (*1*). This theme reinforces the global commitment to rabies prevention and control while emphasizing the critical role of individual and community engagement in achieving rabies elimination.

Over the past decade, China has demonstrated remarkable progress in rabies prevention and control through coordinated national efforts and multi-stakeholder collaboration. From a peak of 3,300 reported cases in 2007 — the inaugural year of World Rabies Day — case numbers have declined consistently for over a decade, reaching 122 cases in 2023. This achievement reflects China's steadfast implementation of a comprehensive prevention strategy that prioritizes dog management and vaccination, incorporates standardized post-exposure prophylaxis (PEP), and maintains robust surveillance, containment, and epidemic focus elimination measures (*2*). The success also demonstrates the effectiveness of prevention and control mechanisms characterized by government leadership, inter-departmental collaboration, and active public participation. However, the increase in nationally reported cases to 167 in 2024 underscores the critical need for sustained vigilance in prevention and control efforts. This epidemic resurgence correlates with inadequate implementation of prevention measures in certain areas, including suboptimal dog registration rates in specific regions, insufficient immunization coverage in rural communities, and diminished public awareness of rabies prevention strategies.

As the 2030 global target for eliminating dog-mediated human rabies approaches, China faces mounting urgency to achieve this critical milestone. Nevertheless, extensive experience both domestically and internationally has conclusively demonstrated that rabies is preventable, controllable, and ultimately eliminable. Using the core indicator of no locally transmitted dog-mediated human rabies cases reported for three consecutive years, many regions across China had already achieved regional elimination by 2024. These successes resulted from rigorous implementation of dog management regulations, enhanced canine vaccination programs, strengthened post-exposure treatment protocols, and comprehensive surveillance with rapid epidemic response measures. This progress further validates the scientific foundation of China's prevention and control strategy, with effective "implementation" serving as the decisive factor for success: regions that fully embrace their responsibilities and systematically execute prevention measures have witnessed sustained declines in rabies cases or complete elimination, while areas failing to maintain these standards remain vulnerable to epidemic resurgence. Currently, regions that have not yet reached elimination targets or have experienced recent outbreaks require immediate, precise risk assessments of local rabies transmission dynamics. These areas must leverage government-led institutional frameworks, strengthen community-based public engagement as the frontline defense, and deploy targeted, evidence-based solutions to address specific prevention and control challenges.

To address these prevention and control challenges, promote replicable experiences, and resolve critical implementation gaps, *China CDC Weekly* has specially curated this rabies-themed special issue. Drawing together research findings and practical experience from experts across multiple disciplines — including disease control and prevention institutions, clinical diagnosis and treatment, and animal epidemic prevention — this issue presents the following key research reports:

Surveillance and Analysis of Animal Rabies — China, 2004–2024: Systematically analyzes epidemic trends of rabies in dogs, cats, and other animals, identifying the primary animal hosts and transmission chains of rabies across different regions (*3*).

Efficacy Evaluation of Virus Clearance of SYN023 in A Murine Rabies Model — China, 2025: Demonstrates the efficacy of the novel anti-rabies drug SYN023 in clearing rabies virus in a murine model, establishing an experimental foundation for clinical translation (*4*).

Active Surveillance on Safety and Compliance of Freeze-dried Human Rabies Vaccine (Vero Cell) — Jiangsu Province,

China, 2023–2024: Documents the safety profile (adverse reaction rate <0.5%) and vaccination compliance (89%) of freeze-dried human rabies vaccine (Vero cell) in Jiangsu Province, providing evidence supporting vaccine deployment in primary medical institutions (*5*).

Metagenomic Next-Generation Sequencing Unmasks Atypical Rabies — Guangxi Zhuang Autonomous Region, China, 2024: Presents the diagnosis and treatment approach for atypical rabies cases (*6*).

The Clinical Advantages of Anti-Rabies Monoclonal Antibodies in Post-Exposure Prophylaxis — Worldwide, 2016–2025: Evaluates the efficacy, accessibility, and cost-effectiveness of anti-rabies monoclonal antibodies compared to traditional rabies immune globulin, emphasizing the application value of monoclonal antibodies in resource-limited settings (*7*).

Eliminating dog-mediated human rabies requires collective action — no single individual can achieve this goal alone. Success demands coordinated efforts from all stakeholders and comprehensive community-wide collaboration. As a zoonotic disease, rabies directly impacts everyone's daily life, particularly as companion animals like dogs and cats have become integral members of human communities. Moreover, rabies functions as a natural focal infectious disease: in China, foxes now represent the primary wildlife reservoir, while recent detections in raccoon dogs and badgers suggest an expanding host range (*3*). As dog-mediated human rabies comes under effective control, the risk of spillover from wild animals becomes increasingly prominent, making society-wide prevention and control efforts based on a One Health approach even more essential. Let us use this special issue and World Rabies Day as catalysts to strengthen our collective responsibility, transform scientific strategies into concrete actions, ensure effective implementation of "last-mile" rabies prevention and control efforts, and achieve the elimination of dog-mediated human rabies in China while contributing meaningfully to the global elimination goal.

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REFERENCES

1. Global Alliance for Rabies Control. World rabies day. 2025. https://rabiesalliance.org/world-rabies-day. [2025-8-25].
2. Yin WW, Fu ZF, Gao GF. Progress and prospects of dog-mediated rabies elimination in China. China CDC Wkly 2021;3(39):831 – 4. https://doi.org/10.46234/ccdcw2021.205.
3. Sun S, Ma JH, Xu Y, Zeng Z, Liu J, Hada, et al. Surveillance and Analysis of Animal Rabies — China, 2004–2024. China CDC Wkly 2025;7(39):1236 – 41. https://doi.org/10.46234/ccdcw2025.208.
4. Ma YX, Fang ZX, Tao XY, Liu SQ, Liu Q, Zhang MH, et al. Efficacy evaluation of virus clearance of SYN023 in a murine rabies model-China, 2025. China CDC Wkly 2025;7(39):1242 – 6 https://doi.org/10.46234/ccdcw2025.209.
5. Chen YJ, Wei MW, Xu XT, Shi YF, Zhang SX, Wang CH, et al. Active surveillance on safety and compliance of freeze-dried human rabies vaccine (Vero cell) —Jiangsu Province, China, 2023-2024. China CDC Wkly 2025;7(39):1247 – 52 https://doi.org/10.46234/ccdcw2025.210.
6. Li H, Wei j, Zhan ZQ, Tang JF, Wang W, Zhou SW, et al. Metagenomic next-generation sequencing unmasks atypical rabies — Guangxi Zhuang Autonomous Region, China, 2024. China CDC Wkly 2025;7(39):1253 – 8 https://doi.org/10.46234/ccdcw2025.211.
7. Chen QJ, Yin WW, Zhu ZG, Wang CL. The advantages of anti-rabies monoclonal antibodies in post-exposure prophylaxis-worldwide, 2016-2025. China CDC Wkly 2025;7(39):1259 – 63 https://doi.org/10.46234/ccdcw2025.212.

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Vital Surveillances

Surveillance and Analysis of Animal Rabies — China, 2004–2024

Sheng Sun[1]; Jihong Ma[2]; Yu Xu[3]; Zheng Zeng[4]; Jian Liu[5]; Hada [6]; Zhongzhong Tu[1]; Weidi Xu[1]; Huachao Feng[1]; Zihan Zhao[1]; Changchun Tu[1,7,#]; Ye Feng[1,8,#]

ABSTRACT

Introduction: Rabies is a zoonotic disease caused by rabies viruses(RABV). China is a high-risk country for rabies. To address China's rabies situation, the Chinese Ministry of Agriculture and Rural Affairs issued the National Animal Disease Surveillance and Epidemiological Investigation Plan. This study systematically summarized animal rabies surveillance data from the past two decades based on the Program.

Methods: Suspected rabies cases collected through the Program between 2004 and 2024 underwent confirmatory diagnosis at the National Reference Laboratory (NRL) for animal rabies using national standard protocols: direct fluorescent antibody testing (FAT) and real-time RT-PCR. Epidemiological data from confirmed cases were analyzed using Geographic Information System (GIS) mapping and statistical evaluation methods.

Results: Laboratory diagnosis confirmed 331 of 433 suspected cases (76.44%) as rabies-positive. These confirmed cases originated from 15 provincial-level administrative divisions (PLADs) and revealed two distinct transmission patterns: a) dog-mediated rabies, accounting for 47.13% of cases and predominantly endemic in southern PLADs, where it poses ongoing human exposure risks; and b) wildlife-mediated rabies in livestock, comprising 52.87% of cases and primarily transmitted by foxes in northern PLADs, with the Inner Mongolia Autonomous Region (IMAR) experiencing the highest burden.

Conclusions: This nationwide surveillance has elucidated current rabies transmission dynamics across China, revealing persistent threats from dog rabies to human health in southern PLADs and emerging threats from wildlife-mediated rabies to livestock in northern border regions. These findings underscore the critical need for enhanced surveillance systems and targeted vaccination strategies addressing both domestic dog populations and wildlife reservoirs to achieve effective rabies control.

Rabies is a zoonotic disease caused by viruses of the genus *Lyssavirus* in the family *Rhabdoviridae* of the order *Mononegavirales* (*1–2*). Rabies virus (RABV) infects nearly all warm-blooded animals (*2*). Rabies remains a significant public health concern, particularly in Asia and Africa, which account for the majority of human cases (*2*). China is still a high-risk country for rabies, with persistent transmission in both human and animal populations (*3–4*). To estimate the rabies threat and implement control measures effectively, the Chinese Ministry of Agriculture and Rural Affairs issued the National Animal Disease Surveillance and Epidemiological Investigation Plan (*5*), mandating comprehensive monitoring of rabies in animals, including dogs, cats, and other potential reservoirs. Surveillance efforts focus on animals exhibiting suspected rabies signs, particularly within key reservoir hosts such as canids, felids, and mustelids. Under this national surveillance framework, suspected animal rabies samples were collected by provincial and municipal animal disease control centers and submitted to our NRL for confirmatory diagnosis and further epidemiological analysis. This study aims to elucidate the current trends, risk factors, and spatial distribution of animal rabies, providing strong and critical evidence to support data-driven prevention and control strategies for rabies elimination in China.

METHODS

Suspected rabies cases exhibiting clinical abnormalities, aggressive behavior, or unexplained mortality were identified by local Animal Disease Control Centers and Forestry and Grassland Administrations. For post-mortem examination, brain tissue samples were collected using the straw method or by submitting entire animal heads. The majority of specimens were transported under cold chain conditions (refrigerated or frozen) to the NRL for confirmatory

diagnosis. Brain tissue specimens underwent examination using FAT with fluorescein isothiocyanate (FITC)-conjugated monoclonal antibody against rabies virus nucleocapsid protein (Fujirebio Diagnostics, Inc., Philadelphia, USA) and Taqman real-time RT-PCR according to National Standard for the Diagnosis of Rabies in Animals (*6*). Epidemiological information for these animal rabies cases from 2004 to 2024 was also collected for analysis. Map data were retrieved from the Ministry of Natural Resources of the People's Republic of China (http://bzdt.ch.mnr.gov.cn/). Figure editing was performed using ArcGIS 10.8 software (Environmental Systems Research Institute, Inc. Redlands, USA) and GIS mapping techniques. All statistical analyses were performed using Microsoft Excel 2007 (Microsoft Corp., Redmond, USA).

RESULTS

Between 2004 and 2024, brain tissues from 433 suspected animal rabies cases were collected across 20 provincial-level administrative divisions (PLADs) and submitted to the NRL for analysis. Laboratory confirmation identified 331 cases (76.44%) as RABV positive using both FAT and real-time RT-PCR methodologies (Table 1). These confirmed cases originated from 75 counties distributed across 15 PLADs. Dogs emerged as the predominant reservoir species, accounting for 47.13% of all confirmed cases (156/331) (Figure 1 and Table 1). Animal rabies cases were documented consistently throughout the entire study period from 2004 to 2024. The notable increase in case numbers primarily resulted from a severe rabies epidemic that occurred in Inner Mongolia Autonomous

TABLE 1. Animal rabies case confirmation in China, 2004–2024 (positive/total).

PLAD		Dog	Cat	Livestock						Wild Animals				Total
				Cattle	Sheep	Camel	Pig	Horse	Donkey	Fox	Badger	Racoon dog	Wolf*	
North	IMAR	10/14	–	75/83	22/27	17/18	1/2	2/6	–	25/35	3/3	3/3	–	158/191
	SX	12/13	–	1/1	3/3	–	–	–	1/1	–	–	–	–	17/18
	XUAR	3/3	–	2/3	8/11	2/2	–	–	–	1/1	–	–	–	16/20
	TJ	4/5	–	2/2	–	–	–	–	–	–	–	–	–	6/7
	SD	1/1	–	3/3	–	–	–	–	–	–	–	–	–	4/4
	HA	2/3	–	–	–	–	–	–	–	–	–	–	–	2/3
	HL	0/1	–	1/1	–	–	–	–	–	1/1	–	–	–	2/3
	GS	1/2	–	–	–	–	–	–	–	–	–	–	–	1/2
	SN	0/2	–	–	–	–	–	–	–	–	–	–	–	0/2
	HE	0/1	–	–	–	–	–	–	–	–	–	–	–	0/1
South	CQ	65/80	–	–	–	–	–	–	–	–	–	–	0/2	65/82
	SH	31/39	–	–	–	–	–	–	–	–	–	–	–	31/39
	HN	12/19	–	–	–	–	2/2	–	–	–	–	–	–	14/21
	JS	5/6	0/1	–	–	–	–	–	–	–	–	–	–	5/7
	GZAR	4/6	–	–	–	–	–	–	–	–	–	–	–	4/6
	ZJ	3/11	–	–	–	–	–	–	–	–	–	–	–	3/11
	GD	3/5	–	–	–	–	–	–	–	–	–	–	–	3/5
	HI	0/9	–	–	–	–	–	–	–	–	–	–	–	0/9
	HB	0/1	–	–	–	–	–	–	–	–	–	–	–	0/1
	JX	–	–	0/1	–	–	–	–	–	–	–	–	–	0/1
Total		156/221	0/1	84/94	33/41	19/20	3/4	2/6	1/1	27/37	3/3	3/3	0/2	331/433

Note: Ratios represent positive/total samples submitted. –: No sample submitted.
Abbreviation: IMAR=Inner Mongolia Autonomous Region; CQ=Chongqing Municipality; SH=Shanghai Municipality; SX=Shanxi Province; XUAR=Xinjiang Uyghur Autonomous Region; HN=Hunan Province; TJ=Tianjin Municipality; JS=Jiangsu Province; GZAR=Guangxi Zhuang Autonomous Region; SD=Shandong Province; ZJ=Zhejiang Province; GD=Guangdong Province; HA=Henan Province; HL=Heilongjiang Province; GS=Gansu Province; HI=Hainan Province; SN=Shaanxi Province; HE=Hebei Province; HB=Hubei Province; JX=Jiangxi Province; PLAD=provincial-level administrative division.
* Two deceased wolves were submitted by a zoological facility.

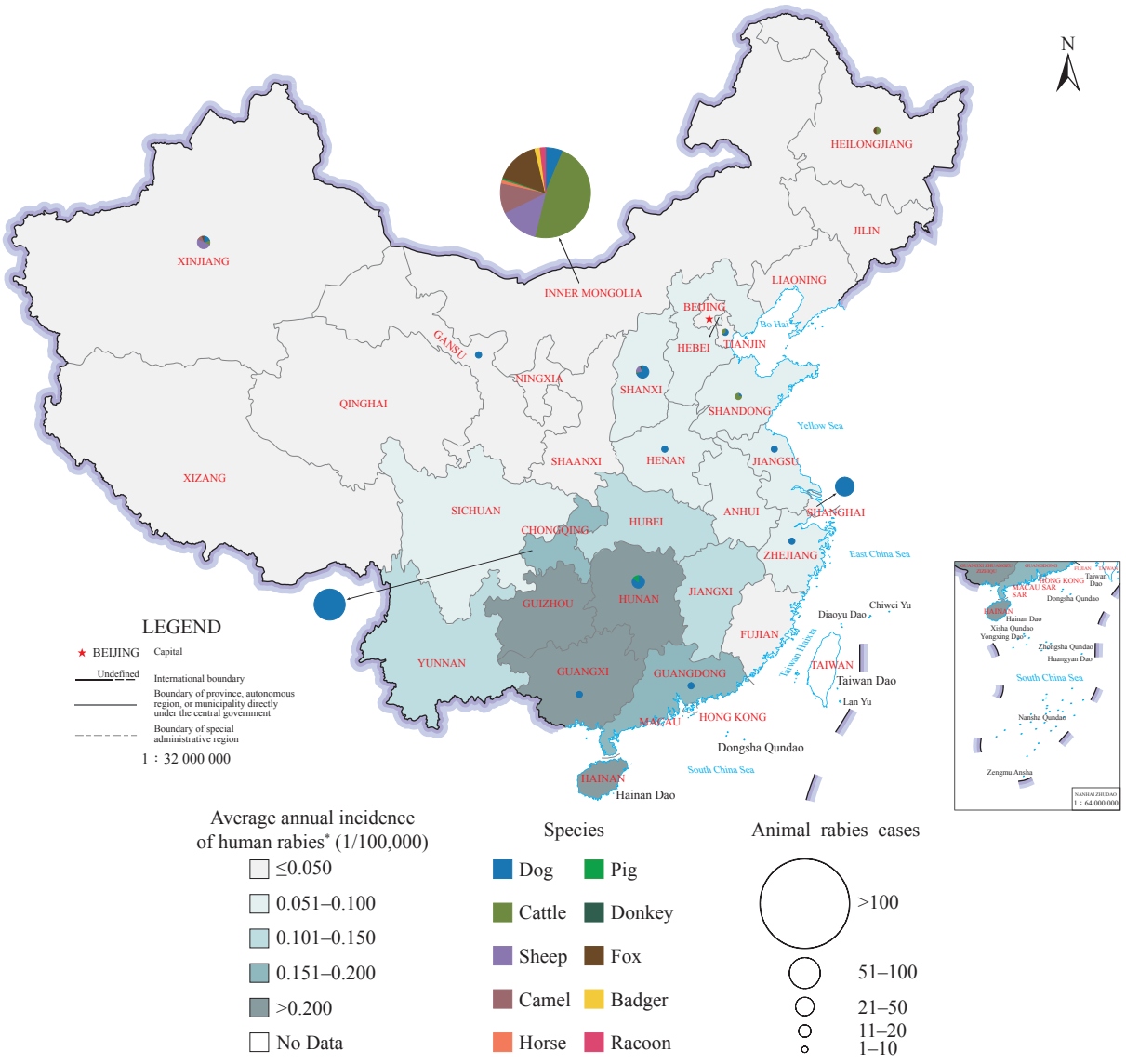

FIGURE 1. Distribution of confirmed animal rabies cases from 2004 to 2024.
Note: Figure encompassed dogs (156), livestock (142 cattle, sheep, camels, pigs, horses, and donkeys), and wildlife (33 foxes, badgers, and raccoon dogs). Map approval number: GS 京 (2025) 1687.
* Annual average incidence of human rabies in provincial-level administrative divisions (PLADs) from 2004−2020 (3).

Region (IMAR) between 2020 and 2024. Sample submission decreased in 2022 due to the impact of the coronavirus disease 2019 (COVID-19) pandemic (Figure 2). Among the 142 confirmed livestock cases, cattle demonstrated the highest infection rate (25.38%, 84/331), followed by sheep (9.97%, 33/331), camels (5.74%, 19/331), pigs (0.91%, 3/331), horses (0.60%, 2/331), and donkeys (0.30%, 1/331). Within the 33 wildlife cases, foxes (*Vulpes vulpes*) served as the predominant transmission vectors (8.16%, 27/331), followed by badgers (*Meles leucurus*) (0.91%, 3/331) and raccoon dogs (*Nyctereutes procyonoides*) (0.91%, 3/331) (Table 1). Geographically, dog rabies exhibited higher prevalence rates in southern PLADs, whereas livestock and wildlife rabies cases were predominantly concentrated in IMAR (Figure 1 and Table 1). Further epidemiological analysis revealed that infected dogs, particularly free-roaming and stray animals, constituted the major transmission sources in rural environments (94/156). The rural-to-urban distribution ratio for infected dogs was 1.52 (94∶62). Notably, 67.86% of rabid dogs (76/112) attacked multiple individuals, with one documented case involving a single dog that caused 63 separate bite incidents. All bite victims received timely post-exposure prophylaxis, preventing human rabies development. Livestock infections were

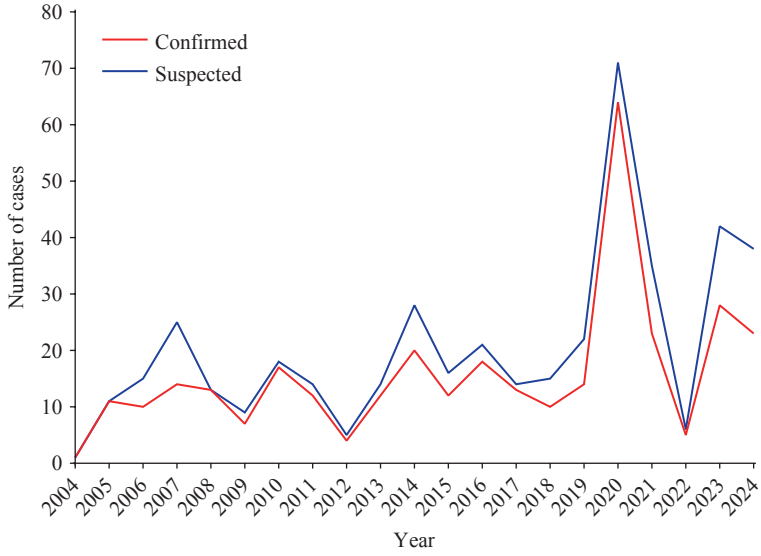

FIGURE 2. Confirmed animal rabies cases in China from 2004 to 2024.

disproportionately concentrated in IMAR (117/142, 82.39%), primarily affecting cattle (25.38%) and sheep (9.97%), with 91 cases epidemiologically linked to fox exposures. Wildlife cases totaled 33 (9.97%), with foxes dominating this category (27 cases, including 25 in IMAR). These wildlife cases demonstrated progressive inland spread from border regions, ultimately reaching the Ningxia Hui Autonomous Region by 2025. Wildlife rabies has been expanding continuously, particularly among foxes, raccoon dogs, and badgers since 2007, representing an emerging zoonotic threat. Thirty-one of the 33 wildlife cases occurred within IMAR, with spillover events documented in Xinjiang Uyghur Autonomous Region (XUAR) in 2014, and Heilongjiang Province in 2022.

DISCUSSION

China's estimated 80 to 200 million domestic dogs represent a substantial reservoir for rabies transmission, with canine-mediated infections accounting for approximately 95% of human rabies cases (*7*). Our surveillance findings confirm that dogs remain the predominant rabies vector across central, eastern, and southern China, responsible for the majority of human bite exposures requiring post-exposure prophylaxis. However, current animal rabies surveillance systems suffer from significant limitations, operating through passive reporting mechanisms that result in substantial underreporting. This surveillance gap becomes evident when comparing human and animal case detection: while human rabies cases were documented in 101 counties across 17 PLADs during 2023 (*8*), only 28 animal rabies cases received laboratory confirmation from 12 counties in 4 PLADs during the same period. This dramatic disparity — with human cases outnumbering confirmed animal cases by nearly 4∶1 — highlights critical deficiencies in animal surveillance infrastructure, as only a small fraction of aggressive or suspect animals undergo diagnostic testing. Multiple factors contribute to this underreporting, including limited regional diagnostic capacity, inconsistent compliance with sample submission protocols, inadequate reporting awareness among veterinary professionals, and substantial logistical barriers in remote and resource-constrained areas. Consequently, our confirmed case numbers likely represent a significant underestimate of the true animal rabies burden across China.

Although China harbors an estimated 80–200 million dogs, canine rabies continues to pose a substantial public health threat, accounting for approximately 95% of human rabies cases (*7*). Our surveillance data confirm that dogs remain the predominant rabies vector across central, eastern, and southern China, responsible for the majority of human bite exposures. However, wildlife rabies — particularly among foxes — has emerged as an increasingly significant concern in northern border PLADs (IMAR, XUAR, and Heilongjiang Province), creating substantial spillover risks to livestock populations. Red foxes now represent the primary wildlife reservoir, while recent rabies detections in raccoon dogs and badgers suggest an expanding host range. This epidemiological pattern aligns with global

trends, where wildlife rabies has resurged in numerous countries despite successful canine vaccination programs implemented across Europe and North America (*9*). Although rare, human rabies cases linked to wildlife exposures are increasingly documented throughout China. Since 2012, sporadic cases have been attributed to ferret badgers (Jiangxi, Anhui, and Zhejiang provinces), bats (Jilin Province), and foxes (XUAR, 2016) (*10*–*11*). Most notably, the first confirmed badger-mediated human rabies case occurred in Xilingol League (IMAR) in 2020 (*12*). These incidents underscore the critical need for enhanced wildlife rabies surveillance, particularly in northern border regions where cross-species transmission to livestock occurs frequently. Unlike canine rabies, which can be effectively controlled through mass vaccination campaigns, wildlife serve as natural RABV reservoirs with extensive movement ranges that complicate large-scale vaccination efforts. Wildlife rabies, therefore, requires alternative control strategies, including comprehensive surveillance programs and oral rabies vaccination (ORV) campaigns targeting foxes and other reservoir species. Europe's successful elimination of fox-mediated rabies through ORV programs provides a proven model for implementation in China (*13*). To contain viral circulation within these natural foci and prevent spillover into human and domestic animal populations, establishing robust immune barriers in surrounding areas remains essential. This objective can be achieved through enhanced canine vaccination campaigns in and around identified endemic regions, where high vaccination coverage will create protective buffers that interrupt transmission chains at the wildlife-domestic animal-human interface.

To effectively address animal rabies challenges in China, comprehensive strategies must be implemented within a One Health framework that combines enhanced surveillance, targeted wildlife vaccination programs, and strengthened cross-sector collaboration: 1) Improve both passive and active surveillance systems to significantly reduce underreporting of animal cases. 2) Strengthen cross-border and cross-sector collaboration while implementing real-time data sharing between animal health, forestry, and public health agencies to enhance outbreak response capabilities. 3) Establish species-specific wildlife vaccination strategies by developing oral rabies vaccines tailored for foxes, raccoon dogs, and badgers, building upon existing ORV technologies (*13*). 4) Conduct comprehensive ecological studies to map wildlife movement patterns and optimize bait distribution strategies for maximum vaccination coverage. 5) Establish a rabies-free pilot region across Hainan Province as a national demonstration model, leveraging its low dog rabies prevalence and natural island geography that provides an effective barrier against rabies transmission from China.

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REFERENCES

1. Fooks AR, Cliquet F, Finke S, Freuling C, Hemachudha T, Mani RS, et al. Rabies. Nat Rev Dis Primers 2017;3(1):17091. https://doi.org/10.1038/nrdp.2017.91.
2. Fooks AR, Jackson AC. Rabies: scientific basis of the disease and its management. 4th ed. London: Academic Press. 2020. http://dx.doi.org/10.1016/C2018-0-04330-3.
3. Meng WW, Shen TR, Ohore OE, Welburn SC, Yang GJ. Spatiotemporal distribution of human rabies and identification of predominant risk factors in China from 2004 to 2020. PLoS Negl Trop Dis 2024;18(10):e0012557. https://doi.org/10.1371/journal.pntd.0012557.
4. Feng Y, Wang YY, Xu WD, Tu ZZ, Liu TF, Huo MH, et al. Animal rabies surveillance, China, 2004-2018. Emerg Infect Dis 2020;26(12):

2825 – 34. https://doi.org/10.3201/eid2612.200303.
5. Ministry of Agriculture and Rural Affairs of China. National animal disease surveillance and epidemiological investigation plan. 2021. https://www.moa.gov.cn/nybgb/2021/202105/202110/t20211021_6380181.htm. (In Chinese).
6. State Administration for Market Regulation, National Standardization Administration. GB/T 18639-2023 Diagnostic techniques for rabies. Beijing: Standards Press of China, 2023. http://www.csres.com/detail/401165.html. (In Chinese).
7. Tu C, Feng Y, Wang Y. Animal rabies in the People's Republic of China. Rev Sci Tech 2018;37(2):519 – 28. https://doi.org/10.20506/rst.37.2.2820.
8. Qin Y, Zhang Q, Lai SJ, Chen QL, Ren Q, Yin WW, et al. Analysis of epidemic characteristics of human rabies in China in 2007-2023. Chin J Exp Clin Virol 2024;38(4):373 – 7. https://doi.org/10.3760/cma.j.cn112866-20240403-00055.
9. World Health Organization. WHO expert consultation on rabies: WHO TRS N°1012. Third report. Geneva: World Health Organization; 2018. https://www.who.int/publications/i/item/WHO-TRS-1012.
10. Miao FM, Li N, Yang JJ, Chen T, Liu Y, Zhang SF, et al. Neglected challenges in the control of animal rabies in China. One Health 2021;12:100212. https://doi.org/10.1016/j.onehlt.2021.100212.
11. Taxitiemuer A, Tuerdi G, Zhang Y, Wushouer F, Tao X, Talipu J, et al. An investigation of the first case of human rabies caused by a fox in China in May 2016. Biomed Environ Sci 2017;30(11):825 – 8. https://doi.org/10.3967/bes2017.110.
12. Feng Y, Wang YY, Hada, Deijide, Gaosuyilatu, Li X, et al. Diversity of rabies virus detected in Inner Mongolia, China, 2019-2021. Transbound Emerg Dis 2022;69(2):249 – 53. https://doi.org/10.1111/tbed.14451.
13. Müller TF, Schröder R, Wysocki P, Mettenleiter TC, Freuling CM. Spatio-temporal use of oral rabies vaccines in fox rabies elimination programmes in Europe. PLoS Negl Trop Dis 2015;9(8):e0003953. https://doi.org/10.1371/journal.pntd.0003953.

Preplanned Studies

Efficacy Evaluation of Virus Clearance of SYN023 in A Murine Rabies Model — China, 2025

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Summary

What is already known about this topic?

Rabies is a fatal, but preventable, viral disease. Post-exposure prophylaxis (PEP), which includes the administration of passive immune preparations, is critical after exposure to the rabies virus, particularly in high-risk cases. The delayed or missed application of passive immunizing agents may increase the risk of infection.

What is added by this report?

SYN023, a novel anti-rabies monoclonal antibody cocktail, can effectively reverse the course of rabies infection, even at a late stage of the disease. In a mouse model infected with the rabies virus strain SC16, multiple high-dose injections of SYN023 administered 5 days post-inoculation rescued 69% of the animals.

What are the implications for public health practice?

These findings suggest that SYN023 could serve as a promising therapeutic agent for rabies PEP, particularly in cases in which treatment initiation is delayed. This study provides a scientific basis for future clinical trials aimed at improving rabies treatment protocols.

ABSTRACT

Introduction: Rabies is a lethal yet preventable viral disease caused by lyssaviruses. It is recommended that post-exposure prophylaxis (PEP) be administered as early as possible after viral exposure. The application of passive rabies immune preparations is an important measure of rabies PEP, especially for high-risk exposures. However, owing to various factors, patients exposed to the rabies virus may not receive passive immune agents in a timely manner, thereby increasing the risk of infection.

Methods: We evaluated the clearance efficacy of SYN023, an anti-rabies cocktail monoclonal antibody (mAb), in mice infected with the rabies virus strain SC16.

Results: Our results demonstrated that SYN023 rescued 69% of the infected mice at a late stage (5 days post-inoculation).

Conclusion: Multiple daily injections of high-dose SYN023 reversed rabies infection during the late exposure stage. These findings provide a critical scientific groundwork to guide the design and implementation of future clinical trials for rabies therapy.

Rabies is a zoonotic infectious disease characterized by acute progressive encephalomyelitis caused by lyssaviruses, including the rabies virus (RABV). Despite its nearly 100% fatality rate, timely and comprehensive post-exposure prophylaxis (PEP) remains an effective preventive measure. Following rabies exposure, in addition to thorough wound cleaning and vaccination, passive immunization preparations are administered as required. The WHO Expert Advisory Panel on Rabies recommends that for individuals exposed to Category III RABV, vaccination should be accompanied by meticulous wound cleaning and administration of passive immune agents, such as human rabies immunoglobulin (HRIG), equine rabies immunoglobulin (ERIG), or rabies monoclonal antibody (mAb) to prevent viral entry into the nervous tissue and provide immediate protection (*1*).

Owing to advantages such as standardized production and higher neutralizing titers compared to polyclonal immunoglobulin preparations, mAbs represent a promising alternative to HRIG and ERIG (*2*). Notably, mAbs can easily reach a much higher neutralization titer against RABV than HRIG, allowing them to neutralize the RABV that has already been systematically exposed (*3*). In 2020, in a groundbreaking study, mice were challenged with the RABV and administered a combination of rabies mAbs (RVC20 and RVC58) via peripheral intramuscular injections at various time points along with long-term continuous

intracerebroventricular infusions using an iPRECIO microinfusion pump (*4*); this approach effectively cured rabid animals, even in advanced stages of infection.

SYN023, a cocktail of two mAbs (CTB011 and CTB012) against RABV, has been approved in China for PEP of rabies. In this study, we evaluated the clearance efficacy of SYN023 in a murine model challenged with China I strain SC16. Mice were administered SYN023 on day 5 post-inoculation, a late-stage PEP time point at which mice may begin to develop initial symptoms after SC16 injection. Notably, 69% of the treated mice recovered from rabies symptoms and survived until the end of the study with no viral presence in the brain, indicating successful reversal with SYN023.

The RABV strain used in this study, SC16 (GenBank: CSC1016D), was isolated from a dog brain in Sichuan, China, in 2010. SC16 is a representative strain of the China I group, which is the most prevalent and abundant RABV group in China (*5*). SYN023 (batch number: SYN023DP120210901) is a 1∶1 mixture of two anti-RABV mAbs, CTB011 and CTB012, produced by Synermore Biologics.

The median lethal dose (LD_{50}) of the SC16 RABV was determined by intramuscular injection in BALB/c mice. The virus, diluted 10-fold from 10^{-1} to 10^{-5}, was injected into the left hind leg muscle of each mouse (10 mice/group). The number of dead mice in each group was recorded daily for 21 days. The LD_{50} was calculated using the Reed–Muench method (*6*).

Specific pathogen-free 6-week-old female BALB/c mice were purchased from Vital River Laboratory Animal Technology Co., Ltd. (Beijing), and housed in individual ventilated cages.

For the in vivo murine rabies model, 23 BALB/c mice were intramuscularly injected with $5\times LD_{50}$ (0.1 mL) of the SC16 virus and randomly divided into a virus control group (n=10) and an SYN023 group (n=13), using a random number generator (https://www.graphpad.com/quickcalcs/randomize1/). Mice in the SYN023 group received daily injections of SYN023 (20 mg/kg) at the virus inoculation site from 5 to 30 days post-infection (dpi). The body weights and symptoms of the test animals were monitored and recorded daily. Animal care staff and administrators were blinded to the allocation groups to ensure that all animals in the experiment were handled, monitored, and treated similarly. Progressive clinical symptoms include ruffled fur, slow movement, hind limb ataxia, apathy, monoplegia, hind limb paralysis, conjunctivitis, and urine staining (*4*). Brain tissues from the mice that died or were sacrificed at the end of the study were collected for direct fluorescent antibody test (DFA). Blood from surviving mice was collected at termination to measure RABV-neutralizing antibody (RVNA) levels using the rapid fluorescence focus inhibition test (RFFIT).

The RFFIT used a standard challenge virus (CVS-11) and BSR cells. Notably, 50 μL of rabies immunoglobulin and serum (1∶3 dilution) were co-incubated with 50 μL of CVS-11 at 37°C in a 5% CO_2 incubator for 1 hour. Subsequently, 50 μL of BSR cell suspension (1×10^6 cells/m) was added and incubated for 24 hours at 37°C. Cells were fixed, stained with a fluorescent RABV antibody, and observed under a fluorescence microscope. RVNA titers were calculated using the Reed–Muench method (*6*) and expressed in IU/mL.

To evaluate the therapeutic effect of SYN023 on rabies, the SC16 challenge animal model was first established. Fifty mice were evenly divided into five groups, each comprising 10 mice. These mice were intramuscularly inoculated with serially diluted brain homogenates containing the SC16 strain. All the mice in each group received an injection volume of 0.1 mL. All mice (10/10) in the group inoculated with the highest viral concentration (10^{-1} dilution) died; all mice (10/10) inoculated with the 10^{-2} dilution also died. At 10^{-3} dilution, the mortality rate decreased to 50% (5/10). Remaining groups of mice, inoculated with lower viral concentrations (10^{-4} and 10^{-5} dilutions) showed complete survival (0/10 deaths). The DFA test results were positive in the brain tissue of all deceased mice and negative in all surviving mice. The LD_{50} of SC16 was calculated as 10^{-3}/0.1 mL using the Reed–Muench method.

The SC16 challenge mouse model was used to evaluate the effects of SYN023. Mice infected with the SC16 strain of the RABV were administered either SYN023 (20 mg/kg) or a vehicle control from 5 to 30 dpi. Mice in the control group developed obvious clinical symptoms of rabies from 5 to 7 dpi, and 100% (10/10) of these mice died within 3–6 days of symptom onset. Mice in the SYN023 group demonstrated a markedly improved survival rate of 69% (9/13) by day 30 (P<0.001, log-rank test). The remaining four mice in the SYN023 group died within 7–9 days after symptom onset, which was much longer than that in the control group (Figure 1A and B).

Both the groups progressively developed rabies symptoms. Notably, the nine surviving mice in the SYN023 group initially experienced worsening

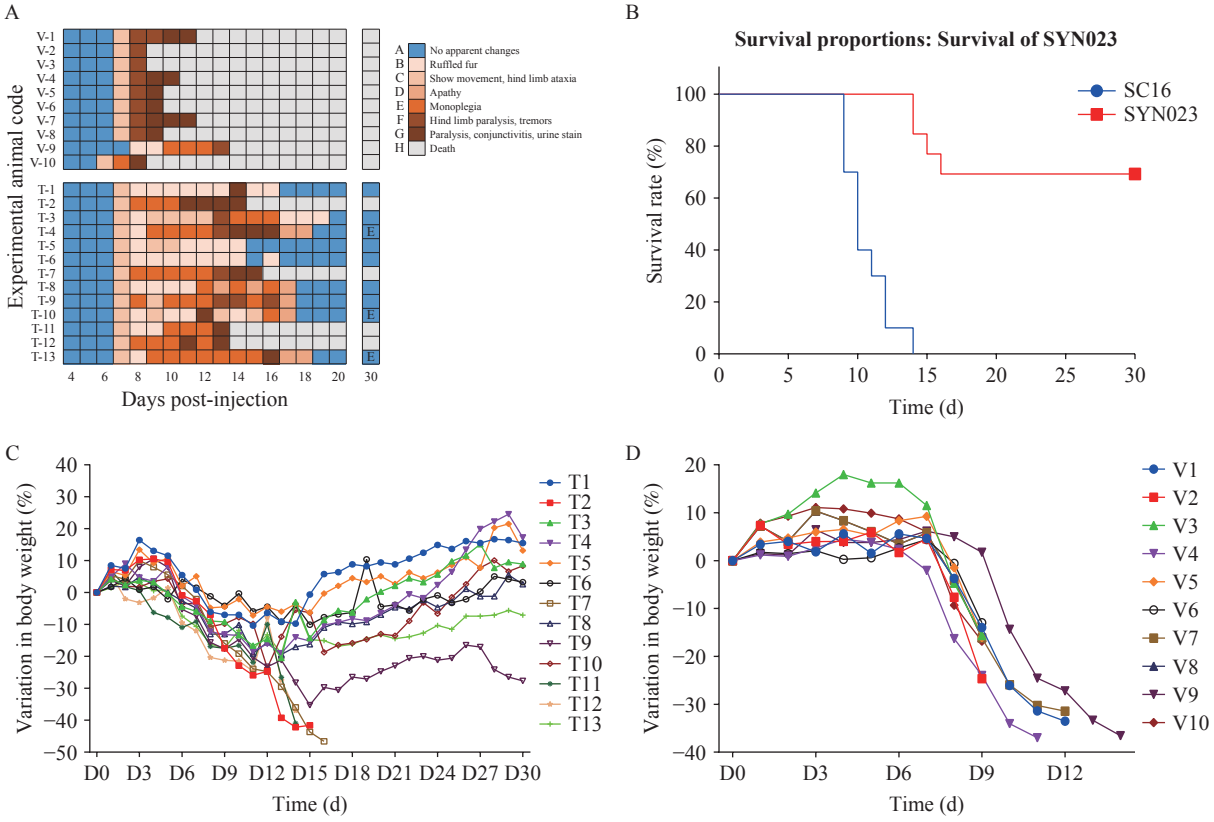

FIGURE 1. Therapeutic effect of SYN023 in the SC16 challenge model. (A) Changes in symptoms in the mice in both groups. (B) Survival curves of mice treated with vehicle control or SYN023. (C) Changes in body weight of mice treated with SYN023. (D) Changes in body weight of mice treated with vehicle control.
Note: Survival analysis revealed that SYN023 improved outcomes (69% *vs.* 0% survival); surviving mice exhibited transient weight loss during the symptomatic phase followed by recovery.

symptoms even after SYN023 administration, ultimately reversing to no apparent symptoms.

All dead mice exhibited significant weight loss before death. Notably, the nine surviving mice in the SYN023 group also lost weight during the symptomatic period, but eventually regained baseline weight after recovery (Figure 1C and 1D).

All mice brain tissues were dissected, and tested using DFA test. Fluorescein isothiocyanate-labeled mAbs against RABV nucleoprotein used for DFA were purchased from Fujirebio, catalog 311520. In the control group, 10 dead mice tested positive for DFA (Figure 2A). In the SYN023 group, four dead mice tested positive for DFA, whereas the nine surviving mice tested negative on day 30 (Figure 2B).

To further evaluate the effects of SYN023, blood samples were collected from the nine surviving mice in the SYN023 group on day 30 for RVNA detection using RFFIT. As presented in Table 1, the median RVNA titer in the nine surviving mice was 4995.12 IU/mL (range: 2698.89–12535.31 IU/mL), indicating that the sera of the animals maintained high levels of anti-RABV-neutralizing activity following the administration of a high dose of SYN023.

DISCUSSION

In 2004, Willoughby from the United States introduced the Milwaukee Protocol, a novel treatment regimen that was considered a potential breakthrough in managing symptomatic rabies cases (*7*–*9*). This innovative therapy primarily involves the induction of a medically induced coma and the administration of ketamine and amantadine. However, its efficacy remains inconclusive, and it may cause significant adverse effects; thus, the search for an effective treatment for rabies continues. The combination of RVC20 and RVC58 mAbs has been shown to effectively treat rabies-infected mice via peripheral intramuscular injections in conjunction with long-term continuous intracerebroventricular infusions (*4*). Neutralization of RABV in the brain and the Fc-mediated immune functions of RVC20 and RVC58 are potential

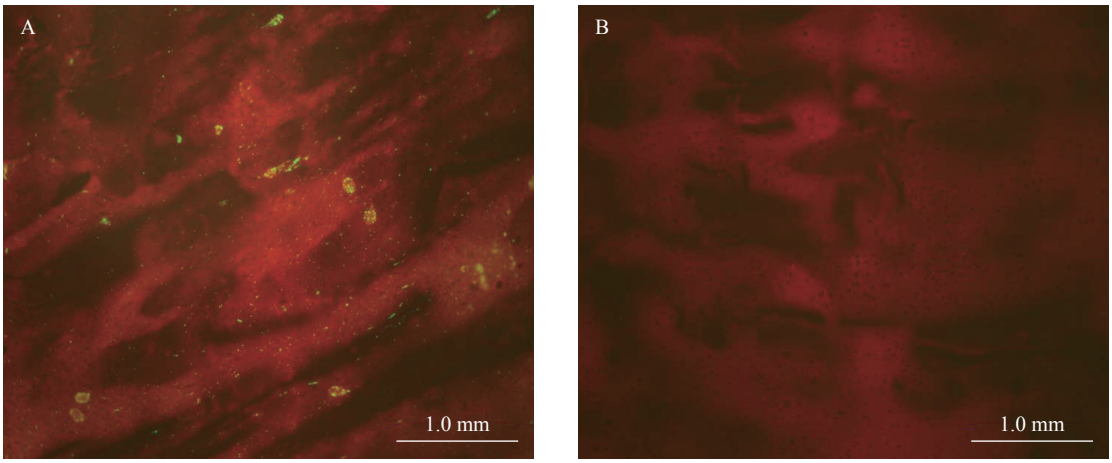

FIGURE 2. DFA results from mouse brain tissues. (A) DFA staining of brain imprints from dead mice; (B) DFA staining of brain imprints from surviving mice.
Note: The rabies virus, labeled with an FITC-conjugated monoclonal antibody against its nucleoprotein, exhibits green fluorescence.
Abbreviation: FITC = fluorescein isothiocyanate; DFA= direct fluorescent antibody test.

TABLE 1. Serum RVNA level of the surviving mice in SYN023 group.

Animal ID	RVNA (IU/mL)
T1	2,698.89
T3	4,995.12
T4	4,255.64
T5	4,145.78
T6	5,979.24
T8	4,454.98
T9	5,625.22
T10	12,535.31
T13	7,351.75

Abbreviation: RVNA= rabies virus neutralizing antibody.

mechanisms of action. In this study, the rescue rate decreased from 100% in the group treated at 6 dpi to 33% in the group treated at 8 dpi, indicating that early administration of mAbs plays a critical role in the reversal process from the onset of rabies symptoms. However, intracerebroventricular administration has a high technical threshold that may limit its clinical application.

SYN023 comprises two mAbs that target two distinct and non-overlapping epitopes, thereby ensuring a broad neutralization spectrum (*10*). Owing to its high neutralizing titer against RABV, we evaluated its therapeutic effect in a murine model challenged with the RABV strain SC16. SYN023 was administered daily at a dose of 20 mg/kg via intramuscular injection from 5 to 30 dpi with SC16. In the viral control group, all mice succumbed to the disease 3–6 days after symptom onset, whereas in the SYN023 treatment group, four mice (31%) died within 7–9 days following symptom manifestation. Notably, mAb therapy extended the survival intervals in these mice. Additionally, nine mice (69%) in the SYN023-treated group recovered to normal health 15–20 days after symptom onset, with concurrent weight gain as the rabies symptoms subsided. The DFA test confirmed RABV clearance from the brains of surviving mice treated with SYN023. This study demonstrated that even when the optimal PEP window was missed, SYN023 could effectively eliminate the RABV that had entered the central nervous system through high systemic concentrations, thereby producing a significant reversal effect in symptomatic animals. Given the similar survival rate following SYN023 administration via intramuscular injection with that of RVC20 and RVC58 mAbs administration via intramuscular injection and intracerebroventricular infusion, SYN023 could prove a more feasible clinical application. SYN023 has been successfully approved for rabies PEP in China, whereas the clinical development of the RVC20 and RVC58 mAbs was terminated in a phase 2 study. Our findings have important implications for guiding future clinical research, such as combination therapy as an add-on to the Milwaukee Protocol.

This study has several limitations. The cerebrospinal fluid of the nine surviving mice in the SYN023 group was not collected for RVNA detection; thus, the penetration capability of high-dose mAbs across the blood-brain barrier could not be evaluated. The establishment of a dose-response relationship and dosage optimization of SYN023 in a murine rabies model

requires further exploration of different dose regimens, administration routes, and durations. Furthermore, in future clinical trials, early administration of SYN023 may depend on the development of more sensitive diagnostic methods for rabies. However, the repeated administration of high-dose mAbs has shown promising efficacy in our murine rabies model. Further basic research will be conducted to explore the mechanism by which mAbs penetrate the blood-brain barrier to treat rabies. In the future, clinical trials should be designed and conducted in patients with rabies using marketed mAbs based on sufficient preclinical studies.

Conflicts of interest: No conflicts of interest.

Ethical Statement: Animals were handled in accordance with *the Guidelines for the Management and Use of Laboratory Animal Feeding*, and the study protocol was reviewed by the Animal Experimental Ethics Committee of the National Institute for Virus Control and Prevention of the Chinese Center for Disease Control and Prevention (license number: sbbdbs20250422055).

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REFERENCES

1. World Health Organization. WHO expert consultation on rabies. 3rd ed. Geneva: World Health Organization; 2018. WHO Technical Report Series, No. 1012. https://iris.who.int/bitstream/handle/10665/272364/9789241210218-eng.pdf?ua=1.
2. Liu XQ, Li JY, Zha Y, Wang ZX, Jiang Y, Zhang XY, et al. The efficacy and safety of SYN023 (Zamerovimab and Mazorelvimab injection), the recombinant humanized anti-rabies virus monoclonal antibody mixture, combined with rabies vaccine in a WHO category III rabies post-exposure population: a randomized, double-blind, positive control, phase III clinical trial. Vaccine 2025;61:127289. https://doi.org/10.1016/j.vaccine.2025.127289.
3. Ding YH, Wu M, Zhang H, Zhu XX, Hu Y, Li XJ, et al. Safety, pharmacokinetics and pharmacodynamics of SYN023 alone or in combination with a rabies vaccine: an open, parallel, single dose, phase 1 bridging study in healthy Chinese subjects. Antiviral Res 2020;184:104956. https://doi.org/10.1016/j.antiviral.2020.104956.
4. de Melo GD, Sonthonnax F, Lepousez G, Jouvion G, Minola A, Zatta F, et al. A combination of two human monoclonal antibodies cures symptomatic rabies. EMBO Mol Med 2020;12(11):e12628. https://doi.org/10.15252/emmm.202012628.
5. Tao XY, Tang Q, Rayner S, Guo ZY, Li H, Lang SL, et al. Molecular phylodynamic analysis indicates lineage displacement occurred in Chinese rabies epidemics between 1949 to 2010. PLoS Negl Trop Dis 2013;7(7):e2294. https://doi.org/10.1371/journal.pntd.0002294.
6. Reed LJ, Muench H. A simple method of estimating fifty per cent endpoints. Am J Epidemiol 1983;27(3):493 – 7. https://doi.org/10.1093/oxfordjournals.aje.a118408.
7. Zeiler FA, Jackson AC. Critical appraisal of the Milwaukee protocol for rabies: this failed approach should be abandoned. Can J Neurol Sci 2016;43(1):44 – 51. https://doi.org/10.1017/cjn.2015.331.
8. Baker HJ, Martin DR, Gross AL, Chamorro MF, Naskou MC, Johnson AK, et al. Rabies: who should care? J Am Vet Med Assoc 2022;261(4):592-6. http://dx.doi.org/10.2460/javma.22.09.0420.
9. Du Pont V, Plemper RK, Schnell MJ. Status of antiviral therapeutics against rabies virus and related emerging lyssaviruses. Curr Opin Virol 2019;35:1 – 13. https://doi.org/10.1016/j.coviro.2018.12.009.
10. Chao TY, Ren SQ, Shen EY, Moore S, Zhang SF, Chen L, et al. SYN023, a novel humanized monoclonal antibody cocktail, for post-exposure prophylaxis of rabies. PLoS Negl Trop Dis 2017;11(12):e0006133. https://doi.org/10.1371/journal.pntd.0006133.

Preplanned Studies

Active Surveillance on Safety and Compliance of Freeze-dried Human Rabies Vaccine (Vero Cell) — Jiangsu Province, China, 2023–2024

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Summary

What is already known about this topic?

Both the 4-dose (Zagreb) and 5-dose (Essen) rabies vaccination regimens demonstrate comparable immunogenicity and safety profiles in clinical trials and are approved for use in China.

What is added by this report?

This study pioneers active safety surveillance via a mobile application, identifying an adverse reaction rate of 2.10% for the Zagreb regimen and 2.70% for the Essen regimen. The Zagreb regimen had a lower out-of-window administration rate of 8.41% compared to 16.38%. Compliance was influenced by age, marital status, and exposure level, while the Essen regimen involved additional factors, including education level and perceived convenience.

What are the implications for public health practice?

This study supports broader adoption of the 4-dose regimen to reduce logistical challenges and enhance compliance. Mobile application-based active surveillance presents a novel approach to enhancing real-world monitoring quality.

ABSTRACT

Introduction: Rabies vaccination compliance and safety are critical for post-exposure prophylaxis. This study compared the freeze-dried human rabies vaccine (Vero cell) under 4-dose (Zagreb) and 5-dose (Essen) regimens in real-world settings.

Methods: In this open-label, randomized trial across Jiangsu Province, China between 2023 and 2024, 2,000 participants received Zagreb (n=999) or Essen (n=1,001) regimens. Active mobile-app surveillance monitored adverse reactions for 28 days post-vaccination. Compliance was assessed through dropout (discontinuation) and out-of-window administration (delayed dosing). Safety and compliance differences were analyzed using the chi-squared or Fisher's exact tests.

Results: Overall adverse reaction rates were comparable (Zagreb: 2.10%; Essen: 2.70%; P=0.385). Solicited local adverse reactions (pain, induration, swelling, and itching) occurred at rates of 1.50% for Zagreb and 2.10% for Essen. Solicited systemic adverse reactions (fever, diarrhea, and vomiting) were 0.60% for both. Dropout rates were statistically similar (8.51% *vs.* 8.69%; P=0.884), but Zagreb had significantly fewer out-of-window administrations (8.41% *vs.* 16.38%; P< 0.001). Compliance factors differed: Zagreb was associated with age/marital status/exposure level; Essen additionally involved education and perceived convenience.

Conclusion: Both regimens demonstrated comparable safety profiles. The Zagreb regimen showed significantly superior schedule adherence through reduced out-of-window administrations while maintaining similar dropout rates to the Essen regimen.

Rabies remains a serious public health threat, characterized by high fatality rates after clinical onset and a substantial burden on healthcare systems worldwide (*1*). Vaccination is the most effective prevention method, but compliance varies widely (*2*). Completing the recommended vaccination schedule is crucial for developing immunity (*3*). Delayed doses require adjustments to subsequent doses while maintaining proper intervals (*4*). Currently, China has approved two post-exposure prophylaxis regimens: the Essen 5-dose (1-1-1-1-1) regimen (vaccination on days 0, 3, 7, 14, 28) and the abbreviated Zagreb 4-dose (2–1–1) regimen (vaccination on days 0, 7, 21) (*5*). Reducing the number of doses decreases the number of clinic visits, which is believed to improve compliance.

This open-label, randomized trial (China Drug Trials: CTR20222797) comprised 2,000 participants with rabies exposure across five Jiangsu counties: Gaogang, Guannan, Huaiyin, Siyang, and Yixing (400 per site). Participants were randomly assigned to the Essen or Zagreb regimens. Both groups received the freeze-dried human rabies vaccine (Vero cell) manufactured by Changchun Institute of Biological Products Co., Ltd. (batch No. 20210911 and 20211016).

Safety monitoring included 30-min onsite observation after each vaccination by trained investigators, who recorded local/systemic reactions via mobile application. Participants or guardians subsequently reported solicited adverse reactions within 7 days, and unsolicited/serious adverse reactions within 28 days post-vaccination. Investigators assessed all reports for causality and severity using National Medical Products Administration criteria (2019 edition), which defined four severity grades ranging from asymptomatic (Grade 1) to life-threatening (Grade 4) (*6*). Exposure levels were categorized into three distinct classifications: Level I (animal contact or intact skin exposure), II (minor abrasions without bleeding), and III (penetrating wounds or mucosal contamination).

Compliance was evaluated through a questionnaire assessing reasons for non-compliance following vaccination, with non-compliance defined through two distinct metrics: dropout (discontinuation post-randomization) and out-of-window administration (receiving a dose later than the maximum permitted delay). As per protocol, the Zagreb out-of-window thresholds were defined as: ≥1 day (dose 1–2), ≥2 days (dose 3), or ≥3 days (dose 4); the Essen out-of-window thresholds were defined as: ≥1 day (dose 1–2), ≥2 days (dose 3–4), or ≥3 days (dose 5).

Safety comparisons and compliance comparisons between groups were conducted using the chi-squared or Fisher's exact test. All hypothesis testing was two-sided, with a P-value <0.05 considered statistically significant. All statistical analyses were performed using SAS (version 9.4, SAS Institute Inc., Cary, NC, USA).

A total of 2,000 participants were enrolled in the study between January 17, 2023 and May 21, 2024, with 999 receiving the Zagreb regimen and 1,001 receiving the Essen regimen. None received rabies immunoglobulin (RIG) or anti-rabies virus monoclonal antibody (mAb) as part of their post-exposure prophylaxis. All participants received the first injection and were included in the safety analysis set (SS) for adverse reaction monitoring. Among these, 172 participants (85 Zagreb, 87 Essen) did not complete the full vaccination schedule (Figure 1). No significant demographic differences were observed between groups at enrollment (Supplementary Table S1, available at https://weekly.chinacdc.cn/).

From the first dose to 28 days after the last dose, the overall incidence of adverse reactions was 2.10% in the Zagreb group and 2.70% in the Essen group, with no significant difference between groups. The most common solicited local adverse reactions in the Zagreb regimen group were pain (1.10%) (grade 1: 1.00%, grade 2: 0.10%), swelling (0.10%) (grade 2: 0.10%), and itching (0.30%) (grade 1: 0.30%). In the Essen regimen group, the most common solicited local adverse reactions were pain (1.80%) (grade 1: 1.30%, grade 2: 0.50%) and induration (0.30%) (grade 1: 0.20%, grade 2: 0.10%). The most common solicited systemic adverse reactions in the Zagreb group were fever (0.60%) (grade 1: 0.10%, grade 2: 0.30%, grade 3: 0.30%), diarrhea (0.10%) (grade 1: 0.10%), and vomiting (0.20%) (grade 1: 0.20%), while in the Essen group, they were fever (0.50%) (grade 1: 0.30%, grade 3: 0.20%) and vomiting (0.10%) (grade 1: 0.10%) (Figure 2). No significant differences in these symptoms were observed between groups. No serious adverse reactions or events were reported in either group.

Among the 2,000 participants, dropout rates for the second dose were 2.00% in the Zagreb regimen group compared to 0.40% in the Essen regimen group. The rates of administering the second dose outside the visit window were 4.40% and 5.79%, respectively. For the third dose, dropout rates were 6.64% in the Zagreb group and 0.80% in the Essen group, with rates of administration outside the visit window at 7.35% and 6.02%, respectively. The overall dropout rates were 8.51% for the Zagreb regimen and 8.69% for the Essen regimen, while rates of out-of-window administration were 8.41% and 16.38%, respectively. The differences in dropout rates for the second and third doses, as well as the rates of completing the vaccination series outside the visit window, were statistically significant between the two groups. However, the differences in rates of administering the second and third doses outside the visit window were not statistically significant (Table 1).

In the Zagreb group, significant differences emerged between compliant and non-compliant participants based on age (P<0.001), marital status (P=0.001), and exposure level (P<0.001). Compliant participants in the Zagreb regimen had a higher mean age (38.81±21.35 *vs.* 28.18±17.62 years), higher marriage rate (63.93% *vs.* 51.19%), and a greater proportion of individuals

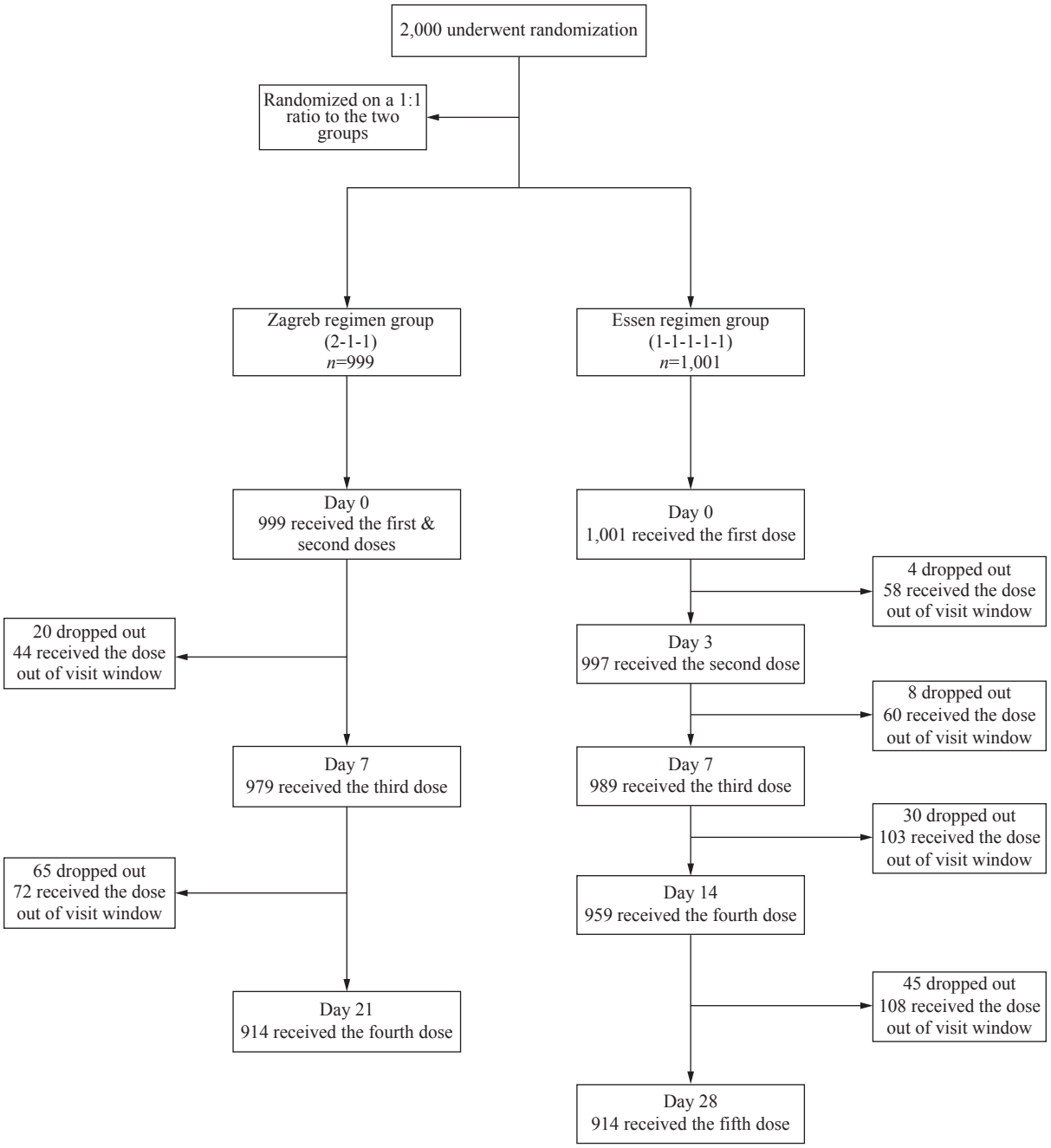

FIGURE 1. Inclusion and follow-up in analyses of safety and compliance in two groups.

with level III exposure (46.99% *vs.* 35.71%) (Supplementary Table S2, available at https://weekly.chinacdc.cn/).

In the Essen group, significant differences were observed between compliant and non-compliant participants based on age (P<0.001), marital status (P=0.014), highest education level (P=0.027), exposure level (P=0.005), and perception that the Zagreb regimen is more convenient (P=0.006). The compliant group under the Essen regimen demonstrated a higher mean age (39.54±22.47 *vs.* 30.51±20.61 years), higher marriage rate (65.23% *vs.* 53.66%), lower proportion of individuals with higher education (bachelor's degree: 12.66% *vs.* 17.68%), and a greater proportion of individuals with level III exposure (47.55% *vs.* 40.85%) (Supplementary Table S2).

DISCUSSION

Both regimens showed comparable safety, consistent with prior clinical trials (*7*–*8*). The Zagreb regimen

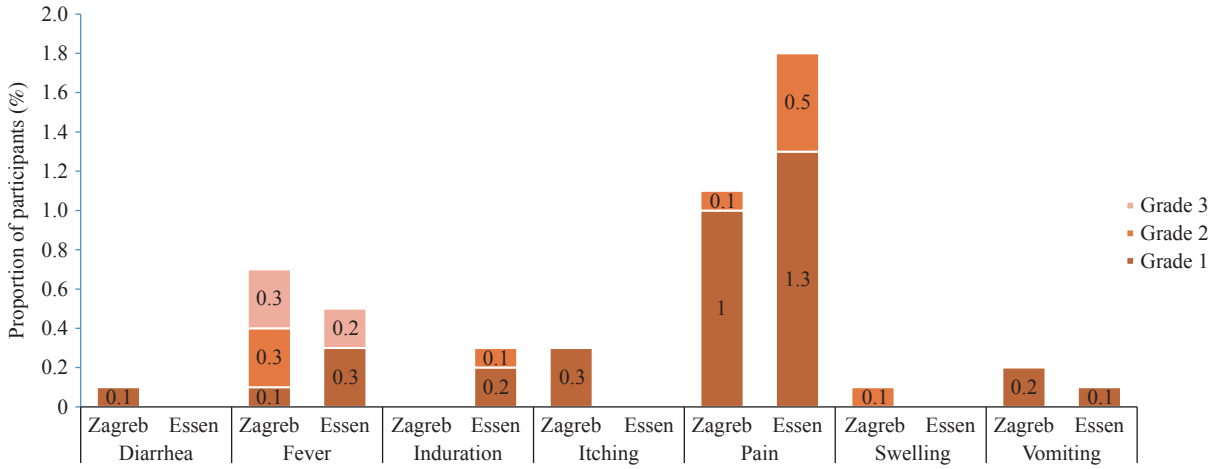

FIGURE 2. Solicited local and systemic adverse reactions between two groups.

TABLE 1. Analysis of non-compliance* in two groups.

Follow-up Status	Zagreb	Essen	Total	*P*
Eligible participants for the first visit	999	1,001	2,000	–
Dropout, *n* (%)	0 (0)	0 (0)	0 (0)	–
Out of window, *n* (%)	0 (0)	0 (0)	0 (0)	–
Eligible participants for the second visit	999	1,001	2,000	–
Dropout, *n* (%)	20 (2.00)	4 (0.40)	24 (1.20)	0.001†
Out of window, *n* (%)	44 (4.40)	58 (5.79)	102 (5.10)	0.158
Eligible participants for the third visit	979	997	1,976	
Dropout, *n* (%)	65 (6.64)	8 (0.80)	73 (3.69)	<0.001†
Out of window, *n* (%)	72 (7.35)	60 (6.02)	132 (6.68)	0.234
Eligible participants for the fourth visit		989	989	
Dropout, *n* (%)	–	30 (3.03)	30 (3.03)	–
Out of window, *n* (%)	–	103 (10.41)	103 (10.41)	–
Eligible participants for the fifth visit		959	959	
Dropout, *n* (%)	–	45 (4.69)	45 (4.69)	–
Out of window, *n* (%)	–	108 (11.26)	108 (11.26)	–
Dropout total, *n* (%)	85 (8.51)	87 (8.69)	172 (8.60)	0.884
Out-of-window total, *n* (%)	84 (8.41)	164 (16.38)	248 (12.40)	<0.001†

* The non-compliant category includes participants meeting either dropout or out-of-window administration criteria as defined in the Methods.
† Statistically significant ($P<0.05$).

demonstrated significantly lower rates of out-of-window administrations (8.41% *vs.* 16.38%, $P<0.001$), indicating better schedule adherence despite similar overall dropout rates.

Previous studies suggest that reduced dosing schedules improve compliance by lowering participant burden (*9*). Our findings confirm this benefit for schedule adherence, with fewer out-of-window doses in the Zagreb regimen (8.41% *vs.* 16.38%, $P<0.001$), but similar dropout rates between regimens (8.51% *vs.* 8.69%, $P=0.884$) indicate that factors beyond visit frequency affect completion. While fewer doses ease logistical challenges (*10*), dropout is influenced by demographics and socioeconomic status (*2*). Identifying these factors allows tailored interventions to improve adherence. Although abbreviated regimens may boost initial participation, addressing barriers after initiation is essential for full-course completion.

Active mobile surveillance detected fewer adverse reactions than phase III trials (*7*) but more than some

post-marketing studies (*8*), likely due to methodological differences. Phase III trials typically employ active and structured solicitation of a predefined list of adverse events by investigators at each visit, which may capture more minor and transient reactions. In contrast, our active surveillance relied on participant-initiated reporting via the mobile application after training, potentially leading to underreporting of milder, non-bothersome symptoms.

Several limitations warrant consideration. First, the open-label design may introduce observer and reporting biases, despite using standardized digital symptom lists. Second, operational variability arose from using two vaccine batches (both quality controlled) and non-standardized syringes across sites. Third, recruitment was limited to Jiangsu Province, restricting generalizability. Although all batches met national regulatory quality control standards, the absence of batch-tracking data prevents assessment of batch-to-batch safety variations. Despite these limitations, this study provides robust real-world evidence from prospective, app-based active surveillance of 2,000 participants across five districts. It demonstrates comparable safety between regimens while innovatively quantifying differences in schedule adherence. Participant adherence may have been enhanced by awareness of monitoring, but this approach accurately captured adverse reactions and dose-specific compliance patterns often missed by passive surveillance.

In conclusion, both the Zagreb and Essen regimens demonstrated comparable safety profiles. The Zagreb regimen showed significantly better schedule adherence through lower out-of-window administration rates while maintaining comparable dropout rates to the Essen regimen. Future studies should validate strategies addressing non-compliance drivers identified in this trial, while exploring mobile-based surveillance for broader safety monitoring.

Conflicts of interest: The freeze-dried human rabies vaccine (Vero cell) used in the trial was supplied by Changchun Institute of Biological Products Co., Ltd. XT.X., SX.Z., CH.W. and X.G. are employees of Changchun Institute of Biological Products Co. The authors have no other conflicts of interest to declare.

Ethical statement: Approved by the Ethics Committee of the Jiangsu Provincial Center for Disease Control and Prevention (JSJK2022-A038-02) and was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice. Written informed consent was obtained from all participants before enrollment.

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REFERENCES

1. Qin XA, Liu K, Fu T, Song SX, Zhao CX, Li ZZ, et al. Global burden, trends, and predictions of rabies: an analysis from the Global Burden of Disease Study 1990-2019 and projections for 2030. J Public Health (Berl) 2025;33(1):69 – 78. https://doi.org/10.1007/s10389-023-01971-9.
2. Lodha L, Manoor Ananda A, Mani RS. Rabies control in high-burden countries: role of universal pre-exposure immunization. Lancet Reg Health Southeast Asia 2023;19:100258. https://doi.org/10.1016/j.lansea.2023.100258.
3. Verdoes L, Luppino FS, Wallinga J, Visser LG. Delayed rabies post-exposure prophylaxis treatment among Dutch travellers during their stay abroad: a comprehensive analysis. J Travel Med 2021;28(3):taaa240. https://doi.org/10.1093/jtm/taaa240.
4. World Health Organization. Rabies vaccines: WHO position paper, April 2018 - recommendations. Vaccine 2018;36(37):5500 – 3. https://doi.org/10.1016/j.vaccine.2018.06.061.
5. Shi N, Zhang Y, Zheng H, Zhu Z, Wang D, Li S, et al. Immunogenicity, safety and antibody persistence of a purified vero cell cultured rabies vaccine (Speeda) administered by the Zagreb regimen or Essen regimen in post-exposure subjects. Hum Vaccin Immunother. 2017;13(6):1 – 8 https://doi.org/10.1080/21645515.2017.1279770.
6. National Medical Products Administration . Guiding principles for adverse event grading standards in clinical trials of prophylactic vaccines. https://clinregs.niaid.nih.gov/sites/default/files/documents/china/NMPA-No102-2019_Google-Translation.pdf. [2025-5-21].
7. Jin F, Zhu L, Wang YP, Qin GQ, Tian Y, Xie YH, et al. Randomized, blind, controlled phase III clinical trial: Assessing the immunogenicity and safety of freeze-dried human rabies vaccine (vero cell) with a 4-dose regimen (2-1-1) in a 10-60 year-old demographic. Vaccine 2024;42(23):126059. https://doi.org/10.1016/j.vaccine.2024.06.026.
8. Kang G, Lakhkar A, Bhamare C, Dharmadhikari A, Narwadkar J, Kanujia A, et al. Active safety surveillance of rabies monoclonal antibody and rabies vaccine in patients with category III potential rabies exposure. Lancet Reg Health Southeast Asia 202316;100207. http://dx.doi.org/10.1016/j.lansea.2023.100207.
9. Zhang W, Guo YF, She F, Liu SQ, Du X, Liu HS. Analysis of factors influencing the course of rabies vaccinations: one year study on compliance of rabies vaccination regimens in Haidian District of Beijing. Patient Prefer Adherence 2022;16:2913 – 20. https://doi.org/10.2147/PPA.S378999.
10. Kessels J, Tarantola A, Salahuddin N, Blumberg L, Knopf L. Rabies post-exposure prophylaxis: a systematic review on abridged vaccination schedules and the effect of changing administration routes during a single course. Vaccine 2019;37 Suppl 1:A107-17. http://dx.doi.org/10.1016/j.vaccine.2019.01.041.

SUPPLEMENTARY MATERIAL

SUPPLEMENTARY TABLE S1. Baseline demographic characteristics of participants who received at least one dose of the vaccine in two groups.

Characteristics	Zagreb	Essen	Total	*P*
Age (years)				
N (missing)	999 (0)	1,001 (0)	2,000 (0)	0.886
Mean±SD	37.92±21.26	38.06±22.42	37.99±21.84	
Median (IQR)	38.39 (19.29–55.21)	38.49 (17.71–55.93)	38.42 (18.63–55.52)	
Min–Max	1.79–89.01	1.68–97.73	1.68–97.73	
Sex				
N (missing)	999 (0)	1,001 (0)	2,000 (0)	0.304
Male, *n* (%)	464 (46.45)	442 (44.16)	906 (45.30)	
Female, *n* (%)	535 (53.55)	559 (55.84)	1,094 (54.70)	

Note: The data were presented as the mean±SD for continuous variables, median (IQR) or non-normally distributed variables, and number (percentage) for categorical variables.
Abbreviation: SD=standard deviation; IQR=interquartile range.

SUPPLEMENTARY TABLE S2. Factors associated with compliance between groups.

Characteristics	Zagreb				Essen			
	Compliant	Non-compliant	Total	*P*	Compliant	Non-compliant	Total	*P*
Age (years)								
N	915	84	999	<0.001*	837	164	1,001	<0.001*
Mean±SD	38.81±21.35	28.18±17.62	37.92±21.26		39.54±22.47	30.51±20.61	38.06±22.42	
Median (IQR)	40.45 (19.92–56.07)	29.53 (11.79–39.88)	38.39 (19.29–55.21)		41.91 (19.38–56.85)	28.89 (12.89–42.40)	38.49 (17.71–55.93)	
Marital status								
N	915	84	999	0.001*	837	164	1,001	0.014*
Married	585 (63.93)	43 (51.19)	628 (62.86)		546 (65.23)	88 (53.66)	634 (63.34)	
Divorced	2 (0.22)	3 (3.57)	5 (0.50)		2 (0.24)	0 (0.00)	2 (0.20)	
Other	328 (35.85)	38 (45.24)	366 (36.64)		289 (34.53)	76 (46.34)	365 (36.46)	
Highest education level								
N	915	84	999	0.903	837	164	1,001	0.027*
High school and below	641 (70.05)	59 (70.24)	700 (70.07)		605 (72.28)	109 (66.46)	714 (71.33)	
Associate degree	134 (14.64)	11 (13.10)	145 (14.51)		124 (14.81)	23 (14.02)	147 (14.69)	
Bachelor's degree	136 (14.86)	14 (16.67)	150 (15.02)		106 (12.66)	29 (17.68)	135 (13.49)	
Master's degree	4 (0.44)	0 (0)	4 (0.40)		2 (0.24)	3 (1.83)	5 (0.50)	
Exposure level								
N	915	84	999	<0.001*	837	164	1,001	0.005*
Level I	28 (3.06)	10 (11.90)	38 (3.80)		27 (3.23)	14 (8.54)	41 (4.10)	
Level II	457 (49.95)	44 (52.38)	501 (50.15)		412 (49.22)	83 (50.61)	495 (49.45)	
Level III	430 (46.99)	30 (35.71)	460 (46.05)		398 (47.55)	67 (40.85)	465 (46.45)	
Perception that the Zagreb regimen is more convenient								
N	915	84	999	0.391	837	164	1,001	0.006*
Yes, *n* (%)	720 (78.69)	69 (82.14)	789 (78.98)		521 (62.25)	114 (69.51)	635 (63.44)	
No, *n* (%)	111 (12.13)	11 (13.10)	122 (12.21)		93 (11.11)	25 (15.24)	118 (11.79)	
Not sure	84 (9.18)	4 (4.76)	88 (8.81)		223 (26.64)	25 (15.24)	248 (24.78)	

Note: The data were presented as the mean±SD for continuous variables, median (IQR) or non-normally distributed variables, and number (percentage) for categorical variables.
Abbreviation: SD=standard deviation; IQR=interquartile range.
* Statistically significant (P<0.05).

Preplanned Studies

Metagenomic Next-Generation Sequencing Unmasks Atypical Rabies — Guangxi Zhuang Autonomous Region, China, 2024

He Li[1,2,&]; Jing Wei[3,&]; Shuwu Zhou[4]; Zhiqun Zhan[5]; Jinfang Tang[6]; Wei Wang[1,#]; Huamin Tang[5,#]

Summary

What is already known about this topic?

Human rabies remains nearly universally fatal despite medical advances. Diagnosis is frequently delayed when patients present with atypical symptoms, and the failure to receive postexposure prophylaxis (PEP) continues to be a major contributor to mortality worldwide.

What is added by this report?

This represents the first confirmed human rabies case in Guangxi caused by the JSTZ190314 strain, successfully identified through metagenomic next-generation sequencing (mNGS). The patient initially presented with urinary symptoms that led to a misdiagnosis before characteristic neurological manifestations developed, ultimately progressing to brain death 28 days after neurological onset (34 days from initial urinary symptoms).

What are the implications for public health practice?

This case demonstrates the critical importance of mNGS in diagnosing atypical rabies presentations and emphasizes the urgent need for enhanced early clinical recognition, standardized PEP administration protocols, and strengthened regional viral surveillance systems.

ABSTRACT

Introduction: This study reports a confirmed case of human rabies diagnosed through metagenomic next-generation sequencing (mNGS). The patient was a 22-year-old female who developed symptoms 3 months after sustaining a scratch on the upper lip from a domesticated dog, without receiving postexposure prophylaxis (PEP). She initially presented with urinary symptoms and was misdiagnosed with a urinary tract infection. Neurological symptoms subsequently emerged, prompting intensive life support interventions including mechanical ventilation, recombinant human interferon-α 2b, ribavirin, norepinephrine, veno-arterial extracorporeal membrane oxygenation (VA-ECMO), and continuous renal replacement therapy.

Methods: Clinical data were collected from hospital records, including exposure history, symptoms, treatments, and outcomes. Saliva specimens were tested by reverse transcription polymerase chain reaction (RT-PCR) and mNGS. Sequencing data were processed by standard bioinformatics pipelines, and phylogenetic analysis was performed with MAFFT alignment and IQ-TREE maximum-likelihood reconstruction.

Results: Rabies virus infection was confirmed through reverse transcription polymerase chain reaction (RT-PCR) and mNGS analysis of saliva samples. The detected strain, JSTZ190314, represents the first documented case of this genotype in Guangxi, China. Despite initial stabilization with ECMO support, the patient's neurological condition deteriorated progressively, leading to brain death 28 days after neurological onset (34 days from initial urinary symptoms).

Conclusion: mNGS proves invaluable as a diagnostic tool for atypical rabies presentations. Enhancing early clinical recognition capabilities, ensuring timely and standardized PEP implementation, and strengthening regional viral strain surveillance represent critical components for effective rabies prevention and control strategies.

Rabies represents a fatal zoonotic disease caused by the rabies virus (RABV), resulting in over 59,000 human deaths globally each year, with the majority of cases occurring in Asia and Africa (*1–2*). Early diagnosis of rabies remains challenging due to the low detection rates of viral RNA in saliva and cerebrospinal fluid (CSF) during the initial stages of infection (*3*). Although the direct fluorescent antibody (DFA) test continues to serve as the criterion standard for rabies diagnosis, its sensitivity depends heavily on specimen quality, operator

experience, and laboratory conditions. This variable sensitivity renders the DFA test susceptible to false-negative results, particularly in patients with low viral loads or early-stage infections. Similarly, polymerase chain reaction (PCR) methods have enhanced detection capabilities but are not without limitations, particularly in resource-constrained settings where accessibility and reproducibility may be compromised (*4*–*5*).

More recently, metagenomic next-generation sequencing has emerged as a powerful, unbiased, high-throughput tool for the rapid identification of infectious pathogens, particularly in cases involving rare, atypical, or difficult-to-detect organisms (*6*–*7*). Here, we report a patient with a rabies infection who survived 28 days from neurological onset (34 days from initial urinary symptoms) caused by the JSTZ190314 strain — the first such case in Guangxi Zhuang Autonomous Region, China — as confirmed via metagenomic next-generation sequencing. This report aims to highlight the diagnostic value of metagenomic next-generation sequencing in early rabies detection, describe the clinical course under aggressive supportive care, and explore the public health implications of a newly identified RABV strain.

Clinical data were obtained from hospital medical records, including demographics, exposure history, clinical manifestations, laboratory findings, imaging, and therapeutic interventions.

Saliva samples were tested for rabies virus RNA using reverse transcription polymerase chain reaction (RT-PCR). Metagenomic next-generation sequencing (mNGS) was performed on the GeneMind FASTASeq 300 platform (single-end 75 bp, >20 million reads per sample, Q30 ≥85%). Data were processed with fastp for quality control, low-complexity reads were filtered with PRINSEQ, and host sequences were removed by alignment to the hg38 human reference genome using BWA-MEM. Non-host reads were taxonomically classified with Kraken2 and validated by re-alignment.

Complete N gene sequences were aligned using MAFFT v7.490 (--auto), and phylogenetic reconstruction was conducted with IQ-TREE v2.0.7 (GTR+G model, 1,000 ultrafast bootstrap replicates). Visualization was performed with iTOL v7.2.1.

A previously healthy 22-year-old woman developed symptoms 3 months after sustaining a scratch on her upper lip from a domesticated dog in September 2024. Although the wound bled significantly, she only rinsed it with tap water and received neither rabies vaccination nor passive immunization.

On December 22, 2024, she experienced acute urinary frequency, urgency, and vulvar burning. Three days later, she visited a local hospital, where clinicians initially diagnosed urinary tract infection and vulvitis. Despite empirical antimicrobial therapy and symptomatic treatment, her condition showed no improvement. On December 28, the patient developed fever (38.2 °C), chills, pharyngeal spasms, muscle twitching, dysphagia, and pronounced anxiety. She presented to our emergency department where rabies was suspected, prompting immediate admission to the intensive care unit due to hemodynamic instability.

Upon admission, the patient exhibited agitation, hypersalivation, and an exaggerated pharyngeal reflex. She demonstrated marked hydrophobia and aerophobia with extreme sensitivity to auditory and airflow stimuli. Her vital signs revealed instability: heart rate 145 beats/min, blood pressure 90/50 mmHg, and peripheral oxygen saturation (SpO_2) of 88% on room air. Laboratory findings showed leukocytosis (15.8×10^9/L) and elevated myocardial enzymes [creatine kinase (CK)-MB: 250.1 U/L; troponin T: 0.487 ng/mL]. Echocardiography revealed globally reduced cardiac systolic function with a left ventricular ejection fraction (LVEF) of 16%, suggesting concurrent viral myocarditis.

Due to rapid neurologic deterioration and circulatory collapse, the patient underwent emergency endotracheal intubation and mechanical ventilation on December 28. The following day, she progressed to cardiogenic shock despite high-dose norepinephrine infusion [3.5 μg/(kg·min)]. We urgently initiated veno-arterial extracorporeal membrane oxygenation (VA-ECMO) and continuous renal replacement therapy (CRRT) to stabilize vital signs. Concurrently, we administered antiviral therapy with ribavirin and recombinant interferon- α 2b, along with corticosteroids, intravenous immunoglobulin, and comprehensive supportive care.

Following standard laboratory protocols for rabies diagnosis, we initially planned to collect multiple saliva specimens for testing. However, the first specimen collected on January 3, 2025, already tested positive for rabies virus RNA by RT-PCR (Ct=26.38). Given the concordant clinical presentation, we submitted this same sample for metagenomic next-generation sequencing (mNGS) and did not pursue additional specimen collection. On January 5, mNGS confirmed the presence of RABV infection. We performed

sequencing on the GeneMind FASTASeq 300 platform using single-end 75 bp reads, generating over 20 million raw reads per sample with Q30 scores ≥ 85%. After quality control with fastp and removal of low-complexity sequences, we aligned clean reads to the human reference genome (hg38) using BWA-MEM and excluded host reads. We then taxonomically classified non-host reads with Kraken2 and validated results by re-alignment to reference genomes. To filter potential contaminants, we used negative controls and a laboratory background database, considering only microbes with reads per million (RPM) ≥3× negative template control (NTC) as positive. We annotated antimicrobial resistance and virulence genes by mapping against the CARD and VFDB databases. Sequence alignment identified the strain as JSTZ190314, marking the first reported case of this genotype in Guangxi, China. The evolutionary relationship of the patient-derived strain is shown in Figure 1.

The patient was successfully weaned off ECMO on January 7, but her neurological condition progressively deteriorated. She remained in a deep coma with no spontaneous respiration, bilaterally dilated and fixed pupils, and absent pupillary light reflexes. Neurological evaluation confirmed brain death. Following written informed consent from the patient's legal guardian, we withdrew life-sustaining treatment on January 25, and the patient was declared clinically dead. The patient progressed to brain death 28 days after neurological symptom onset (34 days from initial urinary symptoms). Figure 2 summarizes the clinical course.

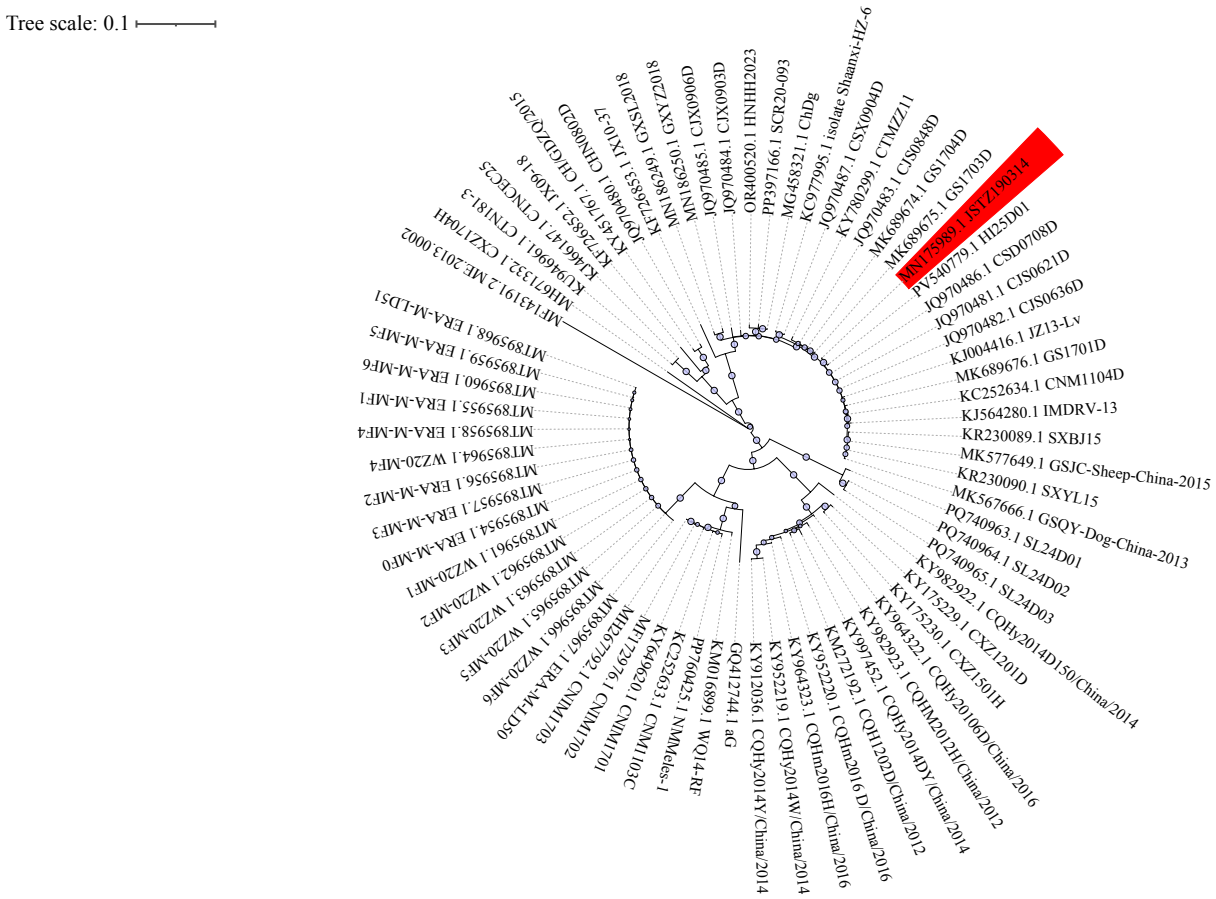

FIGURE 1. Phylogenetic analysis of Rabies lyssavirus strains in China.
Note: Circular maximum-likelihood phylogenetic tree of 70 complete N gene sequences of Rabies lyssavirus strains circulating in China. The tree was reconstructed using IQ-TREE v2.0.7with themaximum-likelihood methodunder theGTR+G substitution modeland1000 bootstrap replicates, based on multiple-sequence alignment performed by MAFFT v7.490 (–auto option). Bootstrap values (≥70) are shown at major nodes. The patient-derived strain JSTZ190314 is highlighted, with host species and geographic origins annotated from GenBank records. Visualization was performed with iTOL v7.2.1. Abbreviation: MAFFT=Multiple Alignment using Fast Fourier Transform; IQ-TREE=software for phylogenetic inference using maximum likelihood.

DISCUSSION

Rabies represents a nearly universally fatal viral infection of the central nervous system, with patients typically surviving seven to ten days following neurological symptom onset (*8*). This case involved a previously healthy 22-year-old woman who developed rabies three months after sustaining a dog scratch without receiving postexposure prophylaxis (PEP). Remarkably, her clinical course extended 28 days from neurological symptom onset (34 days from initial urinary symptoms), substantially exceeding typical survival duration and potentially reflecting temporary stabilization achieved through intensive life support interventions.

The patient's initial presentation with urinary symptoms — including dysuria, frequency, urgency, and vulvar burning — led to misdiagnosis as a genitourinary infection. Rabies was only suspected after classic neurological manifestations emerged, including aerophobia, dysphagia, and pharyngeal spasms. This clinical trajectory highlights the diagnostic challenges posed by atypical early rabies presentations and reveals critical gaps in exposure history assessment and public awareness regarding dog-related injury risks.

Traditional diagnostic approaches, including the DFA test and standard RT-PCR, demonstrate reduced sensitivity when viral loads are low or sample types are limited, frequently resulting in delayed diagnosis of rare or atypical pathogens (*9*–*10*). In this case, rabies virus RNA was initially detected in saliva samples through RT-PCR and subsequently confirmed via metagenomic next-generation sequencing, which identified the infecting strain as JSTZ190314. As an unbiased, high-throughput diagnostic method requiring no prior pathogen knowledge, mNGS demonstrates considerable promise for both public health emergencies and complex infectious disease diagnosis. However, mNGS faces significant limitations, including resource intensity, high costs, and requirements for advanced laboratory infrastructure and bioinformatics expertise. Additionally, false-positive results may occur due to sample contamination or sequencing artifacts, necessitating careful interpretation alongside clinical findings and confirmatory testing. These constraints may limit routine implementation in resource-limited or primary care settings.

To our knowledge, the JSTZ190314 strain identified in this case represents the first documented human infection with this genotype in Guangxi. Phylogenetic analysis was performed using MAFFT v7.490 (--auto) for sequence alignment, followed by reconstruction in IQ-TREE v2.0.7 under the GTR+G model with 1,000 ultrafast bootstrap replicates. This analysis confirmed that JSTZ190314 belongs to the Asian lineage and demonstrates high sequence identity with previously reported canine-origin rabies virus strains. The phylogenetic tree (Figure 1) illustrates the evolutionary placement of JSTZ190314 within the Asian lineage of Rabies lyssavirus strains circulating in China. Originally isolated in 2019 by Cheng et al. from a bovine case in eastern China, phylogenetic analysis confirmed its classification within the Asian lineage of canine origin (*11*). The detection of this strain in a human patient indicates an unrecognized transmission pathway in the region. In Guangxi, domestic dogs serve as the primary reservoir for human rabies transmission, though stray cats and bats may also contribute to viral maintenance and circulation. Given the patient's semi-rural residence and documented dog-bite exposure, local canine

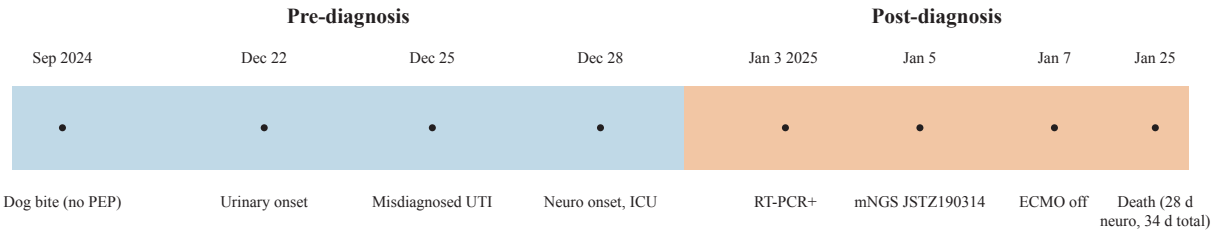

FIGURE 2. Clinical timeline of the reported rabies case.
Note: Pre-diagnosis events (blue): dog bite without PEP, urinary onset, misdiagnosis as UTI, and neurological onset with ICU admission. Post-diagnosis events (orange): saliva RT-PCR positive, mNGS confirmation of strain JSTZ190314, ECMO weaning, and death 28 days after neurological onset (34 days from initial symptoms).
Abbreviation: PEP=postexposure prophylaxis; UTI=urinary tract infection; mNGS=metagenomic next-generation sequencing; ECMO=extracorporeal membrane oxygenation.

populations represent the most probable source of infection. However, domestic dogs in such environments frequently interact with stray cats through feeding behaviors or territorial disputes, and occasionally encounter bats through predation, creating potential pathways for cross-species viral spillover. These findings highlight the critical need for enhanced molecular surveillance of dogs, stray animals, and wildlife populations within a comprehensive One Health framework. Such surveillance should include molecular tracing of circulating viral strains and potential updates to the protective spectrum of current rabies vaccines to ensure continued efficacy against emerging variants.

Following rabies confirmation, the patient received comprehensive life support interventions, including endotracheal intubation, mechanical ventilation, VA-ECMO, and CRRT. Although the patient ultimately progressed to brain death, her survival duration exceeded the typical median survival time of 7–10 days after symptom onset in rabies patients (*12*). This extended survival may reflect temporary stabilization of multiorgan dysfunction under intensive care, though causality cannot be established from a single case with multiple concurrent interventions. While ECMO likely contributed to maintaining cardiopulmonary function, other supportive measures — including CRRT, corticosteroids, and immunoglobulin therapy — may have provided synergistic benefits. The independent contribution of ECMO cannot be definitively isolated without comparative cohort data or multifactorial analysis. Kuang et al. reported a similar case diagnosed via both NGS and PCR, where the patient experienced temporary stabilization of multiorgan dysfunction under intensive support (*13*). Although no antiviral agent has demonstrated definitive efficacy against rabies, the patient received ribavirin and recombinant human interferon- α 2b based on empirical treatment strategies reported in the literature, including components of the Milwaukee protocol. These agents were administered under compassionate care principles given the absence of established alternatives. Their use required careful monitoring for potential adverse effects, including hematologic suppression and hepatic dysfunction. These cases suggest that life-sustaining measures such as ECMO and CRRT may provide temporary supportive bridges in selected patients, allowing time for virological confirmation and family communication. However, the clinical impact of such interventions on overall outcomes remains uncertain and requires further investigation. In resource-adequate settings, aggressive supportive therapy may therefore warrant additional evaluation as a component of rabies care for selected patients.

This report has several limitations, including the absence of pathological or postmortem confirmation through brain tissue immunofluorescence or virus isolation, which was not performed due to family refusal of autopsy. Additionally, sample collection was limited in both type and timing, and systematic multi-specimen testing was not conducted. Nevertheless, the diagnosis was robustly supported by characteristic clinical features, RT-PCR results, and mNGS confirmation.

Given the near-universal fatality of rabies after symptom onset (*14*), timely and standardized postexposure prophylaxis remains the cornerstone of prevention. This prophylaxis includes immediate and thorough wound cleansing, timely administration of rabies vaccines, and appropriate use of passive immunization agents (*15*–*16*). However, public awareness remains insufficient, and inconsistencies in the management of animal exposures, particularly in rural and resource-limited areas, continue to pose serious challenges to the effective implementation of PEP (*17*–*18*). Therefore, enhancing health education and capacity-building among community residents and primary healthcare providers is essential for improving prevention strategies and reducing rabies-related mortality (*19*–*21*).

Atypical early manifestations of rabies can lead to misdiagnosis and delayed diagnosis. In this case, rabies virus infection was confirmed by mNGS, with the first identification of the JSTZ190314 strain in Guangxi. The patient progressed to brain death 28 days after neurological onset (34 days from initial urinary symptoms), longer than typically reported, possibly reflecting temporary stabilization under intensive care; however, causality cannot be inferred. These findings emphasize mNGS as a valuable diagnostic tool and reinforce that timely, standardized PEP remains the cornerstone of rabies prevention.

Conflicts of interest: No conflicts of interests.

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Ethical statement: Approval from the Ethics Committee of the International Zhuang Medicine Hospital Affiliated to Guangxi University of Chinese Medicine (approval No. 202415802). Written informed consent for publication, including authorization to use clinical information and images, was obtained from the patient's legal guardian. All procedures adhered to the ethical standards established by our institutional and national research committees and complied with the 1964 Helsinki Declaration and its subsequent amendments (2013), along with comparable ethical standards.

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REFERENCES

1. Scott TP, Nel LH. Lyssaviruses and the fatal encephalitic disease rabies. Front Immunol 2021;12:786953. https://doi.org/10.3389/fimmu.2021.786953.
2. Yuson M, Bautista CT, Rees EM, Bogaardt C, Cruz VDD, Durrant R, et al. Combining genomics and epidemiology to investigate a zoonotic outbreak of rabies in Romblon Province, Philippines. Nat Commun 2024;15(1):10753. https://doi.org/10.1038/s41467-024-54255-5.
3. Damodar T, Mani RS, Prathyusha PV. Utility of rabies neutralizing antibody detection in cerebrospinal fluid and serum for ante-mortem diagnosis of human rabies. PLoS Negl Trop Dis 2019;13(1):e0007128. https://doi.org/10.1371/journal.pntd.0007128.
4. Szanto AG, Nadin-Davis SA, Rosatte RC, White BN. Re-assessment of direct fluorescent antibody negative brain tissues with a real-time PCR assay to detect the presence of raccoon rabies virus RNA. J Virol Methods 2011;174(1-2):110 – 6. https://doi.org/10.1016/j.jviromet.2011.04.009.
5. Gigante CM, Dettinger L, Powell JW, Seiders M, Condori REC, Griesser R, et al. Multi-site evaluation of the LN34 pan-lyssavirus real-time RT-PCR assay for post-mortem rabies diagnostics. PLoS One 2018;13(5):e0197074. https://doi.org/10.1371/journal.pone.0197074.
6. Haddad SF, Desimone DC, Chesdachai S, Gerberi DJ, Baddour LM. Utility of metagenomic next-generation sequencing in infective endocarditis: a systematic review. Antibiotics 2022;11(12):1798. https://doi.org/10.3390/antibiotics11121798.
7. Xi HY, Zhang LS, Xu B, Liu HY, Li SY. Metagenomic next-generation sequencing to investigate infectious endophthalmitis of brucella: a case report. Front Med 2022;9:847143. https://doi.org/10.3389/fmed.2022.847143.
8. Hemachudha T, Laothamatas J, Rupprecht CE. Human rabies: a disease of complex neuropathogenetic mechanisms and diagnostic challenges. Lancet Neurol 2002;1(2):101 – 9. https://doi.org/10.1016/S1474-4422(02)00041-8.
9. Drzewnioková P, Marciano S, Leopardi S, Panzarin V, De Benedictis P. Comparison of pan-Lyssavirus RT-PCRs and development of an improved protocol for surveillance of non-RABV Lyssaviruses. Viruses 2023;15(3):680. https://doi.org/10.3390/v15030680.
10. Ukamaka EU, Coetzer A, Scott TP, Anene BM, Ezeokonkwo RC, Nwosuh CI, et al. Economic and feasibility comparison of the dRIT and DFA for decentralized rabies diagnosis in resource-limited settings: the use of Nigerian dog meat markets as a case study. PLoS Negl Trop Dis 2020;14(2):e0008088. https://doi.org/10.1371/journal.pntd.0008088.
11. Cheng KH, Chu HM, Ren YC, Xie XL, Yu ZJ, Yang HJ. Phylogenetic analysis of the whole genome sequence of a dog lineage rabies virus detected from cattle in eastern China, 2019. Braz J Microbiol 2020;51(3):1453 – 8. https://doi.org/10.1007/s42770-020-00264-7.
12. Kuang DB, Luo M, Chen JB, Liang CM, Cai ZW, Yuan ZQ, et al. Case report: blood purification effectively relieves multiple system failure in patient with rabies. Front Public Health 2022;10:979828. https://doi.org/10.3389/fpubh.2022.979828.
13. Willoughby RE Jr, Tieves KS, Hoffman GM, Ghanayem NS, Amlie-Lefond CM, Schwabe MJ, et al. Survival after treatment of rabies with induction of coma. N Engl J Med 2005;352(24):2508 – 14. https://doi.org/10.1056/NEJMoa050382.
14. Rupprecht CE, Hanlon CA, Hemachudha T. Rabies re-examined. Lancet Infect Dis 2002;2(6):327 – 43. https://doi.org/10.1016/S1473-3099(02)00287-6.
15. Fooks AR, Cliquet F, Finke S, Freuling C, Hemachudha T, Mani RS, et al. Rabies. Nat Rev Dis Primers 2017;3:17091. https://doi.org/10.1038/nrdp.2017.91.
16. Shaughnessy HJ, Zichis J. Treatment of wounds inflicted by rabid animals. Bull World Health Organ 1954;10(5):805-13. https://pubmed.ncbi.nlm.nih.gov/13182603/.
17. Bahiru A, Molla W, Yizengaw L, Mekonnen SA, Jemberu WT. Knowledge, attitude and practice related to rabies among residents of Amhara region, Ethiopia. Heliyon 2022;8(11):e11366. https://doi.org/10.1016/j.heliyon.2022.e11366.
18. Chuchu VM, Kitala PM, Bichanga P, Ksee D, Muturi M, Mwatondo A, et al. Rabies elimination in rural Kenya: need for improved availability of human vaccines, awareness and knowledge on rabies and its management among healthcare workers. Front Public Health 2022;10:769898. https://doi.org/10.3389/fpubh.2022.769898.
19. Janeaim N, Suwanwong C, Prasittichok P, Mohan KP, Hudrudchai S. Effectiveness of educational interventions for improving rabies prevention in children: a systematic review and meta-analysis. J Educ Health Promot 2024;13:497. https://doi.org/10.4103/jehp.jehp_238_24.
20. Mabrouk Baraka SI, Abo El Nour RAE, Abdelzaher MA, Seif El-Nasr

EM. Rabies-preventive health program for mothers in maternal and child health centers: quasi experimental study. J Health Popul Nutr 2025;44(1):11. https://doi.org/10.1186/s41043-025-00740-6.
21. Tiwari HK, Vanak AT, O'Dea M, Robertson ID. Knowledge, attitudes and practices towards dog-bite related rabies in Para-medical staff at rural primary health centres in Baramati, western India. PLoS One 2018;13(11):e0207025. https://doi.org/10.1371/journal.pone.0207025.

Review

The Clinical Advantages of Anti-Rabies Monoclonal Antibodies in Post-Exposure Prophylaxis — Worldwide, 2016–2025

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ABSTRACT

Post-exposure prophylaxis (PEP) represents the critical intervention for preventing rabies and comprises three essential components: thorough wound cleansing, vaccination, and administration of rabies immunoglobulin (RIG) or monoclonal antibodies (mAbs) for category III exposures. The World Health Organization (WHO) endorses the use of mAb cocktails as an effective replacement for RIG in PEP protocols. Since 2016, four anti-rabies monoclonal antibodies (RmAbs) have received clinical approval for use in India and China. This article provides an overview of the current research status of RmAb. By reviewing clinical studies related to RmAb, it highlights the clinical advantages of RmAb over HRIG in terms of efficacy, accessibility, safety, acceptability, and clinical application in special populations. Additionally, it explores the future clinical prospects of RmAb, including their use in extremely high-risk cases, their impact on circulating antibodies, and their potential role in rabies treatment.

Rabies is an acute zoonotic disease with global prevalence and a case-fatality rate approaching 100%, causing approximately 59,000 deaths annually (*1*). China ranks second worldwide in rabies-related fatalities, and the number of reported deaths increased again in 2024 — the first increase following a continuous 17-year decline (*2*). The administration of RIG represents a critical component of PEP, providing essential early protection before vaccine-induced immunity develops. However, HRIG, the most widely utilized RIG, faces global supply shortages due to production constraints. In China, the utilization rate of passive immunization agents among post-exposure populations remains remarkably low at only approximately 15% (*3*). The *Guidelines for Prevention and Disposal of Rabies Exposure (2023)* includes content related to RmAb, mandating the use of RmAb at approved dosages (*4*). This paper examines the advantages of RmAb and explores its future clinical applications.

GLOBAL OVERVIEW OF RmAb RESEARCH AND DEVELOPMENT

The rabies virus glycoprotein contains six major antigenic sites: I, II, III, IV, G5, and 'a' (*5*). Through recombinant DNA technology, RmAb can be produced industrially to specifically target and bind these glycoprotein antigenic sites, thereby blocking viral interaction with nerve cells and providing rabies prevention (*6*). The WHO position paper recommends RmAb targeting two or more distinct antigenic sites as an alternative to RIG in PEP (*7*). Currently, 11 RmAb products worldwide have either reached market approval or remain under investigation, as detailed in Table 1. Four products have achieved market authorization: two from India — Rabishield (2016) and Twinrab (2019) — and two from China — Ormutivimab (2022) and Zamerovimab/Mazorelvimab (2024) (*8–9*).

CLINICAL ADVANTAGES OF RmAb IN PEP, COMPARED TO RIG

Superior Availability, Safety, and Patient Acceptability

The WHO initially recommended RmAb as a replacement for RIG based on availability and safety considerations in the 2018 rabies vaccine position paper (*7*).

Regarding availability, HRIG, as a blood-derived product, frequently experiences shortages in rabies-endemic countries. In contrast, RmAb demonstrates significant production and supply advantages through several key factors: 1) sustainable large-scale manufacturing ensures a stable supply and controllable

TABLE 1. RmAb Products approved and under-researched worldwide.

Name	Antigenic epitope sites	Stage	Approved Country	Active ingredient
Rabishield	Ⅲ	Approved	India (2016)	1 human-derived monoclonal antibody
Twinrab	Ⅱ, Ⅲ	Approved	India (2019)	2 murine-derived monoclonal antibodies
Ormutivimab	Ⅰ	Approved	China (2022)	1 human-derived monoclonal antibody
Zamerovimab/Mazorelvimab	Ⅲ, G5	Approved	China (2024)	2 human-derived monoclonal antibodies
GR1801	Ⅰ, Ⅲ	Under application	NA	1 human-derived bispecific antibody
NM57S /NCO8	Ⅰ, Ⅱ	Phase II	NA	2 human-derived monoclonal antibodies
CL184	Ⅰ, Ⅲ	Phase II (withdrawn)	NA	2 human-derived monoclonal antibodies
CBB1	Undisclosed	Phase I	NA	1 human-derived monoclonal antibody
RVC20 /RVC58	Ⅰ, Ⅲ	Preclinical	NA	2 human-derived monoclonal antibodies
11B6 /NP-19-9	Ⅱ, Ⅲ	Preclinical	NA	2 human-derived monoclonal antibodies
CR57/RV08 /RV3A5	Ⅰ, Ⅱ, Ⅲ	Preclinical	NA	3 human-derived monoclonal antibodies

Abbreviation: RmAb=anti-rabies monoclonal antibody, NA=not applicable.

costs, 2) industrialized quality production guarantees high batch-to-batch consistency, stable potency, and extended shelf life. For example, Rabishield (launched in India) was specifically developed to address HRIG availability challenges. This single-component, low-potency formulation (3.3 IU/kg) is priced approximately 20% higher than equine RIG (ERIG) but remains lower than HRIG, providing an affordable and accessible alternative for PEP (*9*).

In terms of safety, HRIG poses inherent risks as a blood-derived product, including potential transmission of blood-borne pathogens and the presence of multiple heterologous proteins. ERIG presents additional safety concerns as a heterologous protein product, carrying risks of anaphylactic reactions that necessitate mandatory skin testing prior to administration. In contrast, RmAb demonstrates superior safety characteristics with its precisely defined composition and complete absence of blood-borne pathogen transmission risk. The two human-derived monoclonal antibodies approved in China utilize advanced manufacturing processes, achieving purities exceeding 99.9% while remaining completely free of heterologous proteins. Clinical evidence from the Phase III trial (NCT04644484) demonstrates that Zamerovimab/Mazorelvimab produces significantly lower rates of injection site adverse reactions compared to HRIG: swelling (16.9% *vs.* 34.4%), pain (4.7% *vs.* 7.6%), and erythema (4.3% *vs.* 5.6%) (*10*). This enhanced safety profile substantially improves patient compliance and promotes better wound healing outcomes during PEP.

Regarding patient acceptability, RmAb offers considerable practical advantages over traditional immunoglobulins. The viscosity of RmAb is approximately one-fourth that of HRIG, approaching isotonic conditions that facilitate easier infiltration injection with minimal resistance. Furthermore, the required volume is significantly reduced: only 1 mL of RmAb per 10 kg body weight compared to 2 mL of HRIG for the same weight category. These characteristics enable RmAb to deliver more comprehensive neutralizing antibody coverage at anatomically restricted exposure sites, substantially reducing the tissue distension-related pain commonly experienced during clinical administration. This improved tolerability profile makes RmAb significantly more acceptable to patients undergoing PEP treatment.

Enhanced Efficacy

The WHO recommends using mAb cocktails that target at least two non-overlapping antigenic epitopes as alternatives to RIG in PEP, thereby reducing the risk of immune escape following rabies virus mutations. Both Twinrab (a murine-derived mAb cocktail) and Zamerovimab/Mazorelvimab (a humanized mAb cocktail) satisfy WHO criteria and have demonstrated dose-dependent protective effects in animal models, with significantly superior efficacy compared to HRIG (*11–12*). Compared to Twinrab, Zamerovimab/Mazorelvimab has been validated against a broader spectrum of rabies virus strains and confirmed to achieve 100% neutralization efficacy in *in vitro* neutralization experiments (*13*). Due to its potential interference with vaccines, HRIG is restricted to a clinical dose of 20 IU/kg (*14*), and live virus vaccines (e.g., measles) must be avoided within three months after HRIG administration. However, RmAb

demonstrates minimal interference with vaccines. Twinrab at 40 IU/kg and Zamerovimab/Mazorelvimab at 0.3 mg/kg have both significantly exceeded the HRIG dose (20 IU/kg); notably, Zamerovimab/Mazorelvimab at 0.3 mg/kg (*13*), when converted to potency units, is approximately 447.6±156.6 IU/kg (*14*). In the phase III clinical trial (NCT04644484), Zamerovimab/Mazorelvimab demonstrated the ability to generate higher levels of early rabies virus neutralizing antibodies (RVNA) than HRIG for the first time, providing rapid and sufficient RVNA protection during the two-week window before vaccine-induced antibodes take effect (*10*).

Enhanced Evidence Base for RmAb Use in Special Populations

In PEP protocols, elderly individuals, pregnant women, and children represent special populations requiring heightened safety considerations due to potentially compromised immune responses and stricter drug safety requirements. For these vulnerable groups, physicians have historically relied on empirical treatment approaches, as evidence-based clinical trial data remained unavailable. The two RmAb products approved in China have undergone comprehensive research in these populations, providing robust clinical evidence. Regarding elderly patients, the phase III trial of Ormutivimab enrolled 11 participants aged ≥65 years, demonstrating comparable efficacy and safety profiles to participants <65 years (*15*). Similarly, the phase III trial of Zamerovimab/Mazorelvimab enrolled 137 participants aged ≥60 years, showing equivalent efficacy and safety outcomes compared to younger participants (*10*). For pregnant population, data from the phase III clinical trial of Zamerovimab/Mazorelvimab included seven pregnant participants and revealed no adverse reactions in either the mothers or their fetuses. Ormutivimab has successfully completed pediatric clinical trials and received expanded indication approval for children aged two years and above in 2024. Zamerovimab/Mazorelvimab has initiated comprehensive pediatric clinical trials (CTR20244297), enrolling subjects across three age groups: 12–17 years, 6–11 years, and 0–5 years, with the objective of expanding application to all pediatric populations. Compared to HRIG, both RmAb products marketed in China demonstrate superior safety profiles and enhanced reliability for use in these special populations.

FUTURE PERSPECTIVES ON THE CLINICAL APPLICATION OF RmAbs

The Use of RmAb in Extremely High-risk Cases

Both the WHO and Chinese guidelines classify rabies exposure into three categories based on contact patterns and exposure severity (*4*,*7*). In 2022, researchers proposed an innovative academic framework that introduces a Grade IV exposure classification beyond the existing three-tier system. This additional category would identify exceptionally severe cases, such as extensive bites to the head, face, or neck regions. This enhanced classification aims to identify patients facing extremely high infection risks, where the incubation period may be as brief as one week. The corresponding patient management protocol emphasizes the urgent administration of sufficient or even ultra-high doses of passive immunization agents (*16*). Both the refined risk stratification system and the enhanced passive immunization protocols are designed to ensure optimal safety outcomes for high-risk populations. Regarding passive immunization strategies, the more potent RmAb agents not only demonstrate superior specific activity but also exhibit enhanced tissue diffusion properties compared to traditional alternatives.

Clinical Significance of RmAb-Generated Circulating Antibodies

Chinese guidelines recommend that after complete wound infiltration with passive immunization agents, any remaining product should be administered intramuscularly at sites distant from vaccine injection locations. In contrast, some countries advocate exclusively for local wound infiltration of passive immunizing agents. This divergence reflects not only considerations of agent availability and cost-effectiveness, but also varying clinical assessments of the protective value conferred by circulating antibodies generated through distant-site administration. In clinical scenarios, rabies virus may have limited residence time at wound sites before entering systemic circulation, or the virus may persist undetected in occult injuries that escape immediate treatment. Under these circumstances, early-onset circulating antibodies could provide critical neutralization capacity when viruses breach the initial wound barrier. However, the residual RIG volume remaining after wound

infiltration proves insufficient to generate protective levels of circulating antibodies when administered at distant sites. Only high-dose RmAb administration at remote anatomical locations can achieve clinically meaningful outcomes by producing adequate circulating antibody concentrations that meet seroconversion criteria rapidly, preceding vaccine-induced antibody development while avoiding interference with vaccine immunogenicity.

Potential Application of RmAb in Rabies Treatment

The development of effective rabies treatments has remained one of the most formidable challenges in clinical medicine (*17*), primarily due to the blood-brain barrier, which prevents large-molecule therapeutics from crossing from peripheral circulation into the central nervous system. Consequently, no effective treatments exist once clinical rabies symptoms manifest. Research demonstrates that even with ultra-high doses of macromolecular drugs, only a minimal fraction (approximately 0.02%) can penetrate the blood-brain barrier to reach the central nervous system (*18*). High-dose intravenous monoclonal antibody therapies have gained regulatory approval in both China and the United States for clearing intracranial amyloid-β plaques in Alzheimer's disease treatment, establishing a precedent for central nervous system penetration (*19*).

CONCLUSIONS

RmAbs demonstrate substantial clinical advantages over RIG in post-exposure prophylaxis, offering superior safety profiles, enhanced availability, and improved patient acceptability. These benefits are supported by robust evidence-based data from comprehensive clinical trials. The mAb cocktail formulations ensure broad-spectrum viral neutralization through complementary targeting of distinct antigenic epitopes, establishing an effective passive immunization strategy for clinical PEP protocols.

Critically, RmAbs overcome the inherent dosage constraints of traditional RIG, delivering substantially higher concentrations of neutralizing antibodies during the critical ultra-early post-exposure period without compromising vaccine efficacy. Beyond neutralizing viral particles at exposure sites, RmAbs rapidly elevate circulating antibody levels to achieve comprehensive immunological protection. The therapeutic potential of RmAbs for treating established rabies infections represents a paradigm shift that could transform clinical outcomes. Following successful completion of six global registration trials, Zamerovimab/Mazorelvimab has advanced to new drug applications across multiple rabies-endemic countries, including Turkey and Cambodia, in 2025. As clinical experience accumulates, RmAbs are positioned to become the preferred alternative to RIG in global PEP strategies, potentially enabling breakthrough advances in rabies treatment and supporting the ambitious goal of eliminating human rabies deaths worldwide by 2030.

CHALLENGES AND OPPORTUNITIES

Currently, RmAb products command relatively high prices compared to traditional alternatives. Additionally, given their recent market approval, RmAb-related products have not yet been incorporated into medical insurance reimbursement schemes across many regions. These dual factors substantially increase the financial burden on patients and limit widespread RmAb adoption. However, as additional products enter the market and industry competition intensifies, pricing is anticipated to become more accessible. Furthermore, RmAb products are expected to gain inclusion in national medical insurance reimbursement catalogs.

Despite numerous studies demonstrating the critical importance of passive immunizing agents in PEP, current utilization rates remain disappointingly low for both HRIG and RmAb products. This underutilization may stem from inadequate physician and patient education regarding rabies prevention protocols. Traditional passive immunizing agents possess inherent limitations that not only constrain their clinical utility but also perpetuate knowledge gaps concerning rabies passive immunization strategies. Moving forward, targeted educational initiatives for healthcare providers and patients should be implemented, leveraging the clinical advantages and evidence-based data supporting RmAbs. These efforts should be coupled with standardized PEP protocols that comply with national guidelines, ultimately supporting the global objective of achieving zero human rabies deaths by 2030.

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REFERENCES

1. Hampson K, Coudeville L, Lembo T, Sambo M, Kieffer A, Attlan M, et al. Estimating the global burden of endemic canine rabies. PLoS Negl Trop Dis 2015;9(4):e0003709. https://doi.org/10.1371/journal.pntd.0003709.
2. CDC. National notifiable infectious diseases surveillance. https://www.chinacdc.cn/jksj/jksj01/. [2006-1-1]. (In Chinese).
3. Chinese Center for Disease Control and Prevention. Technical guidelines for human rabies prevention and control (2016). Chin J Viral Dis 2016;6(3):161 – 88. https://doi.org/10.16505/j.2095-0136.2016.03.001.
4. General Department of the National Bureau of Disease Control and Prevention, General Office of the National Health Commission. Guidelines for the rabies postexposure prevention and disposal (2023 edition). Chin J Viral Dis 2024;14(1):22 – 4. https://doi.org/10.16505/j.2095-0136.2024.1003.
5. de Melo GD, Hellert J, Gupta R, Corti D, Bourhy H. Monoclonal antibodies against rabies: current uses in prophylaxis and in therapy. Curr Opin Virol 2022;53:101204. https://doi.org/10.1016/j.coviro.2022.101204.
6. Fan LL, Zhang L, Li JX, Zhu FC. Advances in the progress of monoclonal antibodies for rabies. Hum Vaccin Immunother 2022;18(1):2026713. https://doi.org/10.1080/21645515.2022.2026713.
7. World Health Organization. Rabies vaccines: WHO position paper, April 2018 - Recommendations. Vaccine 2018;36(37):5500 – 3. https://doi.org/10.1016/j.vaccine.2018.06.061.
8. Liu C, Lv XJ, Liu S, Chen QJ, Zhu ZG, Yu R, et al. Research trends in anti-rabies virus monoclonal antibody: a bibliometric analysis. Hum Vaccin Immunother 2025;21(1):2508559. https://doi.org/10.1080/21645515.2025.2508559.
9. Sparrow E, Torvaldsen S, Newall AT, Wood JG, Sheikh M, Kieny MP, et al. Recent advances in the development of monoclonal antibodies for rabies post exposure prophylaxis: a review of the current status of the clinical development pipeline. Vaccine 2019;37 Suppl 1:A132-9. http://dx.doi.org/10.1016/j.vaccine.2018.11.004.
10. Wang XQ, Zha YX, Wang ZX, Jiang Y, Zhang XY, Guo JS, et al. Effects of Zamerovimab/Mazorelvimab on the rabies virus neutralizing antibody level in the grade Ⅲ rabies post exposure subjects. Chin J Exp Clin Virol 2024;38(4):388 – 94. https://doi.org/10.3760/cma.j.cn112866-20240624-00097.
11. Müller T, Dietzschold B, Ertl H, Fooks AR, Freuling C, Fehlner-Gardiner C, et al. Development of a mouse monoclonal antibody cocktail for post-exposure rabies prophylaxis in humans. PLoS Negl Trop Dis 2009;3(11):e542. https://doi.org/10.1371/journal.pntd.0000542.
12. Chao TY, Ren SQ, Shen EY, Moore S, Zhang SF, Chen L, et al. SYN023, a novel humanized monoclonal antibody cocktail, for post-exposure prophylaxis of rabies. PLoS Negl Trop Dis 2017;11(12):e0006133. https://doi.org/10.1371/journal.pntd.0006133.
13. Lyu XJ, Dong GM, Liu XQ, Liu S, Chen C, Chen QJ, et al. Progress and prospect of clinical application of anti-rabies virus monoclonal antibody preparation. Natl Med J China 2023;103(32):2475 – 9. https://doi.org/10.3760/cma.j.cn112137-20230307-00341.
14. Cabasso VJ, Loofbourow JC, Roby RE, Anuskiewicz W. Rabies immune globulin of human origin: preparation and dosage determination in non-exposed volunteer subjects. Bull World Health Organ 1971;45(3):303-15. https://pubmed.ncbi.nlm.nih.gov/5316911/.
15. Liu XQ, Li YF, Li JY, Zhou JM, Guo JS, Pu Y, et al. Comparing recombinant human rabies monoclonal antibody (ormutivimab) with human rabies immunoglobulin (HRIG) for postexposure prophylaxis: a phase III, randomized, double-blind, non-inferiority trial. Int J Infect Dis 2023;134:53 – 62. https://doi.org/10.1016/j.ijid.2023.05.017.
16. Scholand SJ, Quiambao BP, Rupprecht CE. Time to revise the WHO categories for severe rabies virus exposures-category IV? Viruses 2022;14(5):1111. http://dx.doi.org/10.3390/v14051111.
17. Ledesma LA, Lemos ERS, Horta MA. Comparing clinical protocols for the treatment of human rabies: the Milwaukee protocol and the Brazilian protocol (Recife). Rev Soc Bras Med Trop 2020;53:e20200352. https://doi.org/10.1590/0037-8682-0352-2020.
18. Christensen M, Sparre-Ulrich AH, Hartmann B, Grevstad U, Rosenkilde MM, Holst JJ, et al. Transfer of liraglutide from blood to cerebrospinal fluid is minimal in patients with type 2 diabetes. Int J Obes 2015;39(11):1651 – 4. https://doi.org/10.1038/ijo.2015.136.
19. de Melo GD, Sonthonnax F, Lepousez G, Jouvion G, Minola A, Zatta F, et al. A combination of two human monoclonal antibodies cures symptomatic rabies. EMBO Mol Med 2020;12(11):e12628. https://doi.org/10.15252/emmm.202012628.

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