TRADITION AND MODERNITY IN VETERINARY MEDICINE, 2026, vol. 11, No 2(21): 30–43

UNDERSTANDING THE GENESIS AND SIGNIFICANCE OF RABIES VIRUS STRAIN TC-80

Vitalii Nedosekov

National University of Life and Environmental Sciences of Ukraine, 15 Heroiv Oborony Street, Kyiv 03041, Ukraine
Corresponding e-mail: nedosekov06@gmail.com
ORCID: 0000-0001-7581-7478 V.N.
(Submitted: 5 January 2026; Accepted: 30 March 2026; Published: 30 June 2026)

ABSTRACT

Aim: This study aimed to analyse the biological, molecular and immunobiological characteristics of rabies virus strain TC-80 and to evaluate its significance as a veterinary vaccine strain. The novelty lies in the integrated presentation of long-term data on TC-80, combining biological productivity, molecular differentiation and stability, and immunobiological response in several animal species.

Materials and methods: TC-80 was propagated in continuous cell cultures, with assessment of virus and antigen accumulation. Genome-region stability was evaluated during serial passages in BHK-21 and saiga kidney (SK) cells. PCR-restriction analysis of an 878 bp fragment of the G-L intergenic/pseudogene region, partial sequencing of nucleoprotein and pseudogene fragments, and FAVN testing of sera from vaccinated cattle, horses, dogs and foxes were performed.

Results: TC-80 propagated efficiently in several cell systems, with the highest virus titres in BHK-21 and SK cells, reaching approximately 7.2-7.6 log10 MLD_{50}/cm^3. The analysed restriction profile remained stable during passage. PCR-restriction analysis and sequence comparison placed TC-80 within the SAD/ERA-related group and showed 99% nucleotide homology with SAD B19, while retaining distinguishable molecular markers. TC-80-derived vaccines induced virus-neutralizing antibody levels equal to or above 0.5 IU/ml in 88.5% of cattle and 81% of horses nine months after parenteral vaccination, and in 87.0% of dogs and 65% of foxes six months after oral vaccination.

Conclusions: These findings support the characterization of TC-80 as a biologically productive, genetically distinguishable and immunogenic fixed rabies virus strain with relevance for veterinary rabies research, vaccine-strain characterization and diagnostic applications..

Key words: fixed vaccine strain; virus-neutralizing antibodies; FAVN test; PCR-restriction analysis; saiga kidney cells; oral vaccination.

Introduction

Rabies remains one of the most important fatal zoonotic diseases worldwide and continues to circulate in domestic animals and wildlife, causing fatal infections in humans (WOAH, 2024). Despite the availability of effective vaccines, rabies control still depends on reliable vaccine strains, sustained vaccination coverage, laboratory surveillance and post-vaccination monitoring of immunity (WOAH, 2023; Moore, 2021). Current international strategies place particular emphasis on mass dog vaccination as the central measure for interrupting dog-mediated rabies transmission and reducing the risk of human exposure (WHO, FAO, WOAH and GARC, 2018; WOAH, 2024).

Fixed rabies virus strains remain the biological foundation of many veterinary rabies vaccines and diagnostic systems. Historically, vaccine production has used a range of fixed strains, including PV, PM, Flury, SAD, ERA, Vnukovo-32 and other cell-culture-adapted rabies virus strains (Lépine, 1938; Sakamoto *et al.*, 1994; Yang *et al.*, 2013; Natesan *et al.*, 2023). However, contemporary

rabies vaccinology increasingly requires not only evidence of immunogenicity, but also clear information on strain origin, cell-culture adaptation, genetic identity, stability during passage and relationship to other vaccine strains. These characteristics are important for vaccine quality control, seed-lot management and the scientific interpretation of vaccine performance (Horiya *et al.*, 2022; Borutzki *et al.*, 2022).

The need for detailed characterization of rabies vaccine strains has become especially relevant in the context of modern oral rabies vaccination and next-generation rabies vaccine platforms. Oral rabies vaccination has been successfully used in wildlife and is now considered an important complementary tool for reaching free-roaming dogs that are difficult to vaccinate by injection (Wallace *et al.*, 2020; United Against Rabies Forum, 2023). Recent studies of the oral rabies vaccine strain SPBN GASGAS have shown that modern rabies vaccine candidates are assessed according to strict international requirements, including safety, efficacy and genetic stability (Borutzki *et al.*, 2022; Bobe *et al.*, 2023). In parallel, new approaches such as recombinant, viral-vectored and mRNA rabies vaccines show that rabies vaccinology is moving towards better-defined and more controllable vaccine platforms (Natesan *et al.*, 2023; Li *et al.*, 2024). In this context, historical and regionally used fixed strains should be re-evaluated using biological, molecular and immunobiological criteria.

The rabies virus strain TC-80 is one such fixed strain. It has been used in biotechnology and veterinary rabies prevention for more than 40 years (Nedosekov *et al.*, 2001). Vaccines and diagnostic approaches based on TC-80 have been developed for veterinary use, including inactivated parenteral vaccines, recombinant vaccine constructs for oral vaccination of wildlife and methods for evaluating rabies vaccine activity (Nedosekov *et al.*, 2001; Nedosekov *et al.*, 2004; Shmarov *et al.*, 2006). The strain TC-80, originally designated **T**issue **C**ulture-19**80**, was initially passaged in suckling mouse brain, then adapted to bat embryo fibroblast culture and subsequently to BHK-21/clone 13 cells. After cloning and serial passage in BHK-21 cells, an avirulent and highly immunogenic rabies virus clone was obtained (Safonov *et al.*, 1991).

Previous studies indicated that TC-80 has low reactogenicity after intramuscular and intracerebral inoculation. In comparative experiments with TC-80 and Vnukovo-32, all inoculated calves and sheep remained alive during the 90-day observation period after intracerebral inoculation with TC-80, whereas sheep inoculated with Vnukovo-32 developed clinical signs of paralytic rabies (Safonov *et al.*, 1991). Later comparative studies of the invasiveness of rabies virus strains RB-71 and TC-80 in mice and guinea pigs also classified TC-80 among avirulent rabies virus strains (Slivko, 2003).

However, despite its long history of practical use, the current scientific status of TC-80 remains insufficiently defined. Existing information on this strain is dispersed across earlier experimental reports, diagnostic studies and vaccine-related observations, rather than being presented as a single integrated vaccine-strain profile (Safonov *et al.*, 1991; Nedosekov, 2003). This gap is important because contemporary rabies vaccinology requires vaccine strains to be characterized not only by their ability to induce immunity, but also by their origin, cell-culture behaviour, genetic relationship to established fixed strains, stability during passage and measurable immune response in target animal species (Horiya *et al.*, 2022; Borutzki *et al.*, 2022).

The novelty of this study lies not in the initial description of TC-80, but in its integrated re-evaluation as a fixed rabies virus vaccine strain in the context of contemporary strain-characterization requirements. By bringing together long-term biological, molecular and immunobiological

data, this study converts previously fragmented information on TC-80 into a coherent vaccine-strain profile. The article integrates original data on virus propagation in continuous cell cultures, accumulation of infectious virus and protective antigen, stability of PCR-restriction profiles during passage, molecular differentiation from related fixed vaccine strains and induction of virus-neutralizing antibodies in vaccinated cattle, horses, dogs and foxes. This integrated approach distinguishes the present work from earlier descriptive or technical reports and provides a more robust basis for interpreting the origin, identity, biological properties and veterinary significance of TC-80.

The aim of this study was to analyse the biological, molecular and immunobiological characteristics of rabies virus strain TC-80 and to evaluate its significance as a veterinary vaccine strain for rabies vaccine research, vaccine-strain characterization and diagnostic applications.

Materials and Methods

Virus strain and cell cultures

The rabies virus strain TC-80 was used in this study. The virus material had an infectious activity of 7.0-7.6 log10 MLD_{50}/cm^3 and had been passaged in BHK-21 and saiga kidney (SK) cell cultures (Nedosekov *et al.*, 2004). The infectious activity of virus-containing material in vivo and in vitro was determined according to standard rabies laboratory methods (Meslin *et al.*, 1996).

The propagation, titration and accumulation of rabies virus (RV) were studied in continuous cell cultures, including saiga kidney cells (SK), baby hamster kidney cells (BHK-21), African green monkey kidney cells (Vero), African goat embryo kidney cells (PEAK) and pig embryo kidney cells (PPK-66b) (Nedosekov, 2003).

Virus propagation and antigen accumulation

To study the reproduction cycle of TC-80, SK cells were infected with virus at a multiplicity of infection of 0.1 MLD_{50}/cell. Virus accumulation was assessed separately in the cellular fraction and in the culture supernatant at defined time points after infection (Nedosekov *et al.*, 2004). The dynamics of rabies virus antigen accumulation were also evaluated during cultivation of TC-80 in SK cells using the roller culture method in a circular monolayer of SK cells. Virus titres and antigen accumulation were recorded descriptively during the cultivation period.

PCR-restriction analysis

PCR-restriction analysis was used to evaluate the molecular characteristics of TC-80 and to compare its restriction profile with those of other fixed rabies virus vaccine strains. Primers flanking an 878 bp fragment of the variable G-L intergenic/pseudogene region of the rabies virus genome were used for amplification. This region was selected because it is informative for differentiating rabies virus strains (Naumkina, 1999; Tsybanov, 2001).

The amplified PCR products were cleaved with restriction endonucleases BamHI, HindIII, RsaI and TaqI, which had previously been selected for differentiation of rabies virus vaccine strains. Additional restriction analysis, including DraI, was used where necessary for further differentiation of TC-80 from closely related vaccine strains.

Analysis of PCR products

Aliquots of PCR products, usually 5-10 µl, were separated by electrophoresis in 1.5-2.0% agarose gel. Fragment sizes were estimated by comparison with a molecular weight marker based on HindIII-cleaved lambda phage DNA. Restriction patterns were interpreted according to the

presence or absence of characteristic DNA fragments and their estimated sizes in base pairs (bp) (Naumkina, 1999; Tsybanov, 2001).

Sequencing and nucleotide sequence comparison

Partial nucleotide sequencing was performed for selected genome fragments of rabies virus strain TC-80 and closely related vaccine strains. The analysed regions included fragments of the nucleoprotein gene and the G-L intergenic/pseudogene region. Sequence comparison was used to assess nucleotide homology between TC-80 and related fixed rabies virus vaccine strains, particularly SAD B19. The analysis focused on nucleotide substitutions, sequence homology and the molecular relationship of TC-80 to established rabies vaccine strains (Naumkina, 1999; Tsybanov, 2001).

Immunobiological assessment

Modified rabies vaccines derived from the TC-80 strain were used for assessment of immunobiological activity. Virus-neutralizing antibodies (VNA) were measured using the fluorescent antibody virus neutralization (FAVN) test.

The study included 120 serum samples from cattle immunized with inactivated rabies vaccines derived from TC-80, 37 serum samples from horses, 124 serum samples from dogs and 21 serum samples from foxes vaccinated with TC-80-derived rabies vaccines. Blood samples were collected at defined intervals after vaccination, including 1, 3, 6 and/or 9 months, depending on the animal group and vaccination scheme. Serum samples were tested for the presence of rabies virus-neutralizing antibodies, and antibody levels equal to or above 0.5 IU/ml were interpreted as meeting the commonly used serological threshold for rabies vaccination monitoring (Nedosekov, 2003).

Ethical approval

Ethical approval is described in the Ethical approval section.

Statistical analysis

No inferential statistical analysis was performed. Data are presented descriptively as virus titres, restriction profiles, nucleotide sequence comparisons, antibody titres, percentages or proportions, according to the design of the historical experimental studies. The numbers of tested serum samples were 120 for cattle, 37 for horses, 124 for dogs and 21 for foxes.

Results

Cell-culture properties and virus accumulation of rabies virus strain TC-80

Rabies virus strain TC-80 propagated in all tested continuous cell cultures. The highest infectious titres were observed in BHK-21 and saiga kidney (SK) cells, where the virus reached 7.3-7.6 log10 MLD_{50}/cm^3 after 10 passages.

During serial cultivation, TC-80 retained a stable level of infectious activity over the observation period. To characterize the reproduction cycle of the strain in SK cells, virus accumulation was assessed separately in the cellular fraction and in the culture supernatant. The latent period of virus reproduction was approximately 6 hours. Maximum accumulation of intracellular and extracellular virus was observed at 72-96 hours after infection and reached 5.8±0.3 and 7.3±0.2 log10 MLD_{50}/cm^3, respectively.

Immunofluorescence analysis showed progressive accumulation of rabies virus antigen in infected SK cells. At 8-10 hours after infection, viral antigen was detected on the cell membrane

and in the cytoplasm as fluorescent granules. At 12 hours, antigen expression was mainly cytoplasmic and appeared as granules of different sizes. At 24 hours, antigen clusters of irregular shape were observed. At 36 hours, the number of cells containing viral inclusions increased, and fluorescence foci became more evident (Figure 1). At later stages, diffuse cytoplasmic fluorescence, rounded inclusions and larger amorphous antigen aggregates were observed, followed by involvement of the whole cell monolayer.

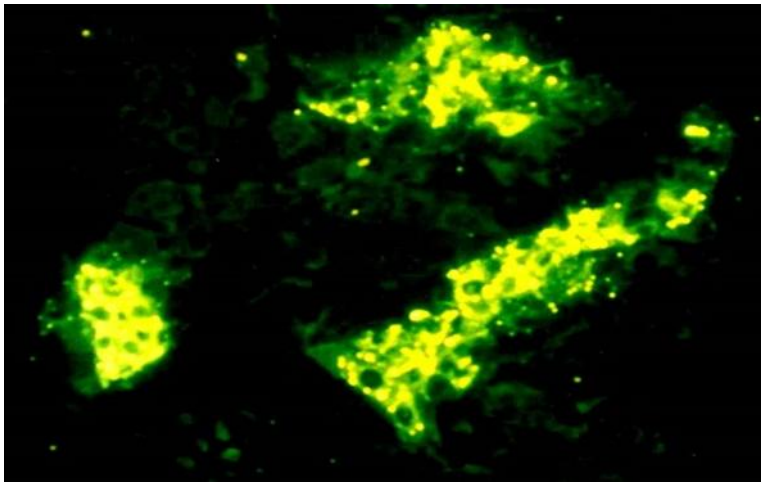

Figure 1: Fluorescence of rabies virus strain TC-80 in SK cells 36 hours after infection. Magnification ×280.

The dynamics of infectious virus and protective antigen accumulation were also assessed during cultivation of TC-80 in SK cells using the roller culture method. The infectious virus titre reached its maximum level on day 4 of cultivation, at 7.2 ± 0.2 log10 MLD_{50}/cm^3. In contrast, the maximum accumulation of protective rabies virus antigen was observed on day 6. Addition of DEAE-dextran at concentrations of 0.01, 0.1 and 1.0 mg/cm^3 did not increase virus titre or antigen accumulation under the tested conditions. These results indicate that TC-80 propagated efficiently in SK cells and achieved high infectious virus titres under laboratory cultivation conditions.

Stability of the analysed genome region during passage in BHK-21 and SK cells

PCR-restriction analysis was used to evaluate whether the analysed G–L intergenic/pseudogene region of TC-80 remained stable during serial passage in BHK-21 and SK cells. The amplified 878 bp fragment was cleaved with BamHI, HindIII, RsaI and TaqI. The restriction profile was identical in TC-80 samples obtained after different passage histories in BHK-21 and SK cells (Table 1).

Table 1: Restriction profile of the amplified G–L intergenic/pseudogene fragment of rabies virus strain TC-80 during passage in BHK-21 and SK cells.

№	cell system	number of passages	Rabies virus titre, log10 MLD_{50}/cm^3	*BamHI*	HindIII	RsaI	TaqI
1	BHK-21	10	6.5	-	+	+	+
2	SK	23	6.7	-	+	+	+
3	SK	71	6.6	-	+	+	+
4	SK	74	6.7	-	+	+	+

Note: "+" indicates cleavage of the amplified fragment by the corresponding restriction endonuclease; "–" indicates no detectable cleavage.

The absence of changes in the restriction profile indicates stability of the analysed G-L intergenic/pseudogene region of TC-80 during the tested passages. This result should be interpreted as evidence of stability of the investigated genome region, rather than as complete evidence of whole-genome stability.

Molecular differentiation of TC-80 from other fixed rabies virus vaccine strains

To determine the molecular relationship between TC-80 and other fixed rabies virus vaccine strains, PCR-restriction analysis of the 878 bp G-L intergenic/pseudogene fragment was performed for SAD, TC-80, ERA, Vnukovo-32, PV, Shchelkovo-51, RB-71 and CVS. Cleavage with BamHI, HindIII, RsaI and TaqI separated the analysed vaccine strains into distinct restriction groups (Table 2).

Table 2: Restriction patterns of amplified G-L intergenic/pseudogene fragments of rabies virus vaccine strains.

Restriction endonuclease	Rabies virus vaccine strains			
	SAD	ERA, Vnukovo-32, TC-80	PV, Shchelkovo-51, RB-71	CVS
BamHI	-	-	+(672 and 206 bp)	+(308 and 570 bp)
HindIII	+(672 and 206 bp)	+(672 and 206 bp)	-	-
RsaI	+(436 and 442 bp)	+(436 and 442 bp)	-	-
TaqI	+(290 bp)	+(580 bp)	-	-

Note: "+" indicates cleavage of the amplified fragment by the corresponding restriction endonuclease; "–" indicates no detectable cleavage. Fragment sizes are given in base pairs (bp).

The restriction profile of TC-80 corresponded to the ERA/Vnukovo-32-related group, indicating a close molecular relationship between these strains. The BamHI restriction pattern also allowed separation of Pasteur-type strains from SAD/ERA-related strains. Additional analysis showed that DraI cleavage was useful for differentiating TC-80 from closely related vaccine strains.

These results indicate that TC-80 can be molecularly distinguished from other fixed rabies virus vaccine strains using the selected PCR-restriction approach.

Partial sequencing and sequence comparison of TC-80 and SAD B19

Partial sequencing was performed to further assess the relationship between TC-80 and the closely related SAD B19 vaccine strain. A 398 nt fragment of the nucleoprotein gene and fragments of the G-L intergenic/pseudogene region were analysed. The pseudogene fragments included a 310 nt sequence obtained using the Gfor primer and a 329 nt sequence obtained using the Lrev primer (Figures 2 and 3).

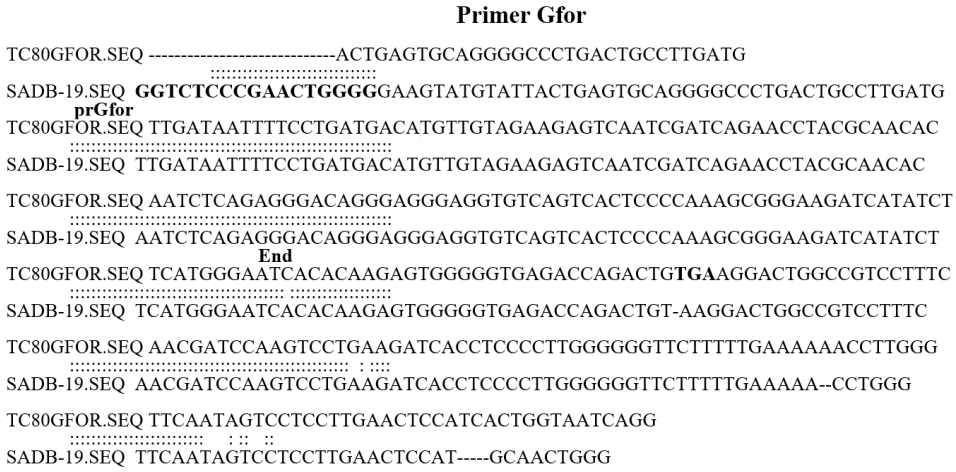

Figure 2: Comparative nucleotide sequence of the G-L intergenic/pseudogene fragment of rabies virus vaccine strains SAD B19 and TC-80 using the Gfor primer.

Figure 3: Comparative nucleotide sequence of the G-L intergenic/pseudogene fragment of rabies virus vaccine strains SAD B19 and TC-80 using the Lrev primer.

Sequence comparison showed a high degree of nucleotide homology between TC-80 and SAD B19. In the analysed fragment, four nucleotide substitutions distinguished TC-80 from SAD B19. Despite these substitutions, the overall nucleotide homology between the compared fragments was 99%. Comparison of the analysed translated region did not reveal amino acid differences between the compared TC-80 and SAD B19 sequences. These findings support a close genetic relationship between TC-80 and SAD B19, while also indicating the presence of molecular markers that distinguish TC-80 from SAD B19.

The combined PCR-restriction and partial sequencing data placed TC-80 within the SAD/ERA-related group of fixed rabies virus vaccine strains. At the same time, the observed restriction and nucleotide-sequence differences indicate that TC-80 retains distinguishable molecular features.

Immunobiological properties of TC-80-derived rabies vaccines

The immunobiological activity of TC-80-derived rabies vaccines was evaluated by measuring virus-neutralizing antibodies in vaccinated cattle, horses, dogs and foxes using the FAVN test. The numbers of tested serum samples were 120 for cattle, 37 for horses, 124 for dogs and 21 for foxes. Antibody levels equal to or above 0.5 IU/ml were interpreted as meeting the commonly used serological threshold for rabies vaccination monitoring (Figure 4).

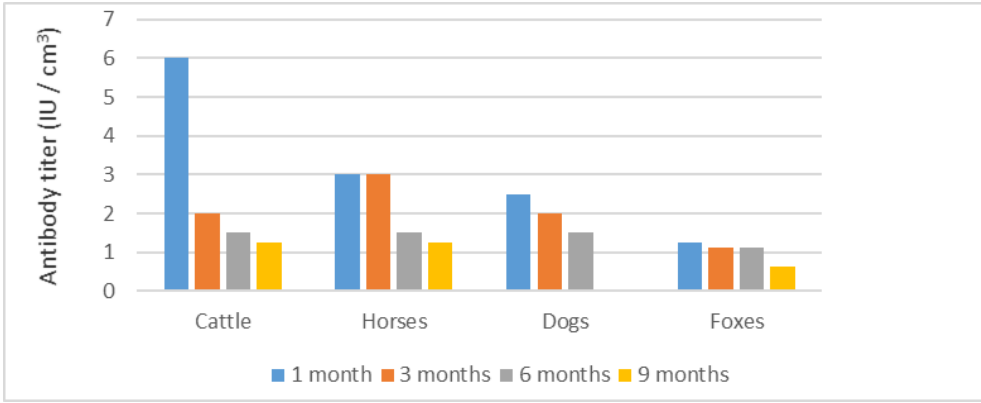

Figure 4: Virus-neutralizing antibody levels in cattle, horses, dogs and foxes after vaccination with TC-80-derived rabies vaccines.

All tested cattle had antibody levels equal to or above 0.5 IU/ml at one and three months after vaccination. At six and nine months after vaccination, 88.5% of cattle remained above the 0.5 IU/ml threshold.

In horses, all vaccinated animals had virus-neutralizing antibody levels equal to or above 0.5 IU/ml at one and three months after vaccination. The proportion of horses with antibody levels above this threshold was 89.2% at six months and 81% at nine months after vaccination.

In dogs, the proportion of animals with antibody levels equal to or above 0.5 IU/ml was 96.8% one month after vaccination, 93.6% after three months and 87.0% after six months.

In foxes, antibody levels equal to or above 0.5 IU/ml were detected in 75% of vaccinated animals one month after vaccination, 65% after six months and 43% after nine months.

These results show that TC-80-derived vaccines induced virus-neutralizing antibodies in all tested animal species. The highest and most persistent serological responses were observed in cattle

and horses after parenteral vaccination. Dogs and foxes also developed measurable virus-neutralizing antibody responses after oral vaccination, although the proportion of animals remaining above the 0.5 IU/ml threshold declined over time, particularly in foxes. These data should be interpreted as evidence of serological response and immunogenicity during the observation period, rather than as definitive proof of protection in all vaccinated animals.

Discussion

The present study provides an integrated biological, molecular and immunobiological characterization of rabies virus strain TC-80. This is important because TC-80 has been used for many years as a veterinary vaccine strain, but its properties have not previously been interpreted as a single strain profile in relation to contemporary requirements for rabies vaccine-strain evaluation. Modern rabies vaccinology requires vaccine strains to be described not only by their immunogenicity, but also by their cell-culture productivity, molecular identity, stability during passage, relationship to other fixed strains and relevance for vaccine quality control (Finke *et al.*, 2012; Horiya *et al.*, 2022; Natesan *et al.*, 2023). In this context, the value of the present study lies in bringing together several lines of evidence that allow TC-80 to be considered as a defined biological and immunobiological entity rather than only as a historically used vaccine strain.

The cell-culture data show that TC-80 propagated efficiently in several continuous cell systems, with the highest virus titres observed in BHK-21 and saiga kidney (SK) cells. This is relevant because reproducible propagation in cell culture remains one of the practical requirements for vaccine-strain use, seed-lot preparation and laboratory standardisation. Classical rabies vaccine strains, including PV, Flury, SAD and ERA-related strains, became useful for vaccine production only after adaptation to suitable laboratory or cell-culture systems (Yang *et al.*, 2013; Natesan *et al.*, 2023). More recently, Harada *et al.* (2024) showed that adaptation of HEP-Flury to Vero cells improved viral growth while maintaining antigenic characteristics similar to the parental strain. Against this background, the ability of TC-80 to reach high virus titres in BHK-21 and SK cells supports its technological relevance. However, these findings should be interpreted as evidence of efficient laboratory propagation, not as complete proof of suitability for contemporary vaccine manufacture, which would require standardised seed-lot characterization, antigen quantification, vaccine potency testing and cell-substrate safety assessment.

The molecular data place TC-80 within the SAD/ERA-related group of fixed rabies virus vaccine strains. PCR-restriction analysis of the G-L intergenic/pseudogene region allowed differentiation of vaccine strains into several groups and indicated a close relationship between TC-80 and ERA/Vnukovo-32-type strains. Partial sequencing of nucleoprotein and pseudogene fragments also showed high nucleotide homology between TC-80 and SAD B19, while several nucleotide substitutions distinguished TC-80 from SAD B19. This pattern is consistent with the general concept that fixed rabies virus vaccine strains may share common ancestry but acquire strain-specific molecular markers during adaptation, cloning and serial passage (Ajorloo *et al.*, 2018; Horiya *et al.*, 2022). The restriction profile of the analysed genome region remained unchanged during the tested passages in BHK-21 and SK cells, supporting stability of this region under the conditions of the study. However, these findings do not demonstrate whole-genome stability. Contemporary studies increasingly use whole-genome sequencing to reconstruct vaccine-strain origin, passage history and genetic relationships, as shown for historical rabies vaccine strains by Horiya *et al.* (2022) and for the oral vaccine strain SPBN GASGAS by Borutzki *et al.* (2022).

Therefore, the present molecular data support the identity of TC-80 as a SAD/ERA-related fixed strain, while also indicating the need for whole-genome confirmation.

This molecular interpretation provides an important bridge between strain identity and immunobiological performance. Earlier comparative analyses of rabies vaccine strains showed that fixed rabies virus strains may form distinct genetic groups despite their shared use as vaccine strains. Metlin *et al.* (2008) reported that the Russian vaccine strains "Ovechiy" and RV-97 formed a separate group and differed from several internationally used vaccine strains, including SAD B19, Ni-Ce and Flury-HEP, by more than 7%. Krasnov *et al.* (2020) later confirmed the close relationship between the "Ovechiy" lineage and Moscow 3253. These observations illustrate that rabies vaccine strains cannot be interpreted only by their historical use or immunogenicity; their molecular position also matters for strain identification, quality control and comparison with other vaccine lineages. In this context, the high nucleotide homology between TC-80 and SAD B19 supports the placement of TC-80 within the SAD/ERA-related group, while the detected nucleotide substitutions and restriction-profile features indicate that TC-80 retains distinguishable molecular markers. This provides a more precise basis for interpreting the immunobiological data generated with TC-80-derived vaccines.

The immunobiological results show that TC-80-derived vaccines induced virus-neutralizing antibodies in cattle, horses, dogs and foxes. In cattle and horses, antibody levels equal to or above 0.5 IU/ml were maintained in a high proportion of animals after parenteral vaccination. In dogs and foxes, virus-neutralizing antibodies were also detected after oral vaccination, although the proportion of animals remaining above the 0.5 IU/ml serological threshold declined over time, particularly in foxes. The 0.5 IU/ml threshold is widely used as an indicator of an adequate rabies virus-neutralizing antibody response, especially for vaccination monitoring and international movement of animals (WOAH, 2023). However, rabies serology must be interpreted according to the context, assay type, timing of sampling, host species and purpose of testing (Moore, 2021). Therefore, the TC-80 antibody data should be interpreted as evidence of serological response and immunogenicity, rather than as definitive proof of protection in all vaccinated animals.

The oral vaccination data are particularly relevant when considered in relation to modern studies of rabies vaccination in difficult-to-reach animal populations. Bobe *et al.* (2023) showed that dogs receiving oral vaccine baits containing SPBN GASGAS were protected against challenge infection according to international standards, although not all vaccinated dogs reached the ≥0.5 IU/ml threshold before challenge. Field studies with SPBN GASGAS in Namibia and Indonesia also demonstrated that oral vaccination can be a useful complementary strategy for free-roaming dogs that are difficult to reach by parenteral vaccination (Molini *et al.*, 2021; Freuling *et al.*, 2022; Saputra *et al.*, 2023). These studies support the relevance of oral rabies vaccination as a control tool, but they also show that modern evaluation requires standardized assessment of safety, efficacy, bait delivery, field feasibility and challenge protection. In this context, the TC-80 oral vaccination data remain scientifically relevant, but should be regarded as serological evidence of immunogenicity rather than as a complete contemporary assessment of oral vaccine efficacy.

When TC-80 is considered against contemporary vaccine-strain requirements, its strengths and limitations become clear. The present data document efficient propagation in BHK-21 and SK cell cultures, retention of a stable restriction profile in the analysed genome region during passage, distinguishable molecular markers within the SAD/ERA-related group, and induction of virus-neutralizing antibodies in several animal species. These features support the view that TC-80 is a

biologically productive, genetically distinguishable and immunogenic fixed rabies virus strain. At the same time, modern vaccine-strain evaluation would normally require whole-genome sequencing, standardized seed-lot characterization, comparative potency testing, expanded safety assessment and challenge studies in relevant target species. Field observations on the practical use of TC-80-derived vaccines may be considered supportive historical information, but they should not be treated as definitive evidence of field efficacy unless supported by standardized epidemiological and experimental data.

In summary, the present study positions TC-80 as a historically important and biologically relevant fixed rabies virus strain that merits further modern characterization. Its efficient propagation in continuous cell cultures, stable PCR-restriction profile in the analysed genome region, close molecular relationship with SAD/ERA-related strains and ability to induce virus-neutralizing antibodies in several animal species provide a coherent basis for its vaccine-strain profile. However, TC-80 should not be presented as a fully validated contemporary vaccine seed without additional evidence. Stronger conclusions about its position among modern rabies vaccine strains or its direct suitability for contemporary vaccine manufacture would require whole-genome sequencing, standardized seed-lot analysis, comparative potency testing, expanded safety evaluation and challenge experiments.

Conclusion

The present study provides an integrated biological, molecular and immunobiological characterization of the rabies virus strain TC-80, a fixed vaccine strain with a long history of veterinary use. The results show that TC-80 can be efficiently propagated under laboratory conditions in continuous cell cultures, with the highest levels of virus accumulation observed in BHK-21 and saiga kidney (SK) cells. These findings support the value of these cell systems for further laboratory and technological evaluation of TC-80.

PCR-restriction analysis of the amplified G–L intergenic/pseudogene region showed that the restriction profile of TC-80 remained unchanged during the tested passages in BHK-21 and SK cells. This finding indicates stability of the analysed genome region under the conditions of the study. However, it should not be interpreted as complete evidence of whole-genome stability, which would require full-genome sequencing of early and late passage materials.

Restriction analysis and partial sequencing placed TC-80 within the SAD/ERA-related group of fixed rabies virus vaccine strains and showed a high degree of nucleotide homology with SAD B19. At the same time, the detected nucleotide substitutions indicate that TC-80 retains distinguishable molecular features. These results clarify the genetic relationship of TC-80 to established rabies vaccine strains and contribute to a more precise understanding of its origin and identity.

Vaccines derived from TC-80 induced virus-neutralizing antibodies in cattle, horses, dogs and foxes. Antibody levels equal to or above 0.5 IU/ml were detected in 88.5% of cattle and 81% of horses nine months after parenteral vaccination, and in 87% of dogs and 65% of foxes six months after oral vaccination. These data support the immunogenic potential of TC-80-derived vaccines. Nevertheless, the results should be interpreted as evidence of serological response rather than definitive proof of protection in all vaccinated animals.

Overall, the data presented in this study support the characterization of TC-80 as a biologically productive, genetically distinguishable and immunogenic fixed rabies virus strain. Its efficient propagation in BHK-21 and SK cells, stable restriction profile in the analysed genome region, close relationship with SAD/ERA-related vaccine strains and ability to induce virus-neutralizing antibodies in several animal species indicate its continued relevance for veterinary rabies research, vaccine-strain characterization and diagnostic applications.

Acknowledgments

The author is grateful to Prof. S. Tsybanov, V. Balyshev, S. Yurkov, Dr I. Slivko, M. Naumkina and Ya. Tsybanov for their valuable scientific discussions and technical assistance during the original experimental work on the TC-80 rabies virus strain. This acknowledgement refers solely to their scientific and technical contribution to the historical research described in this manuscript.

Funding

This research received no external funding.

Conflict of interest

The author declares no conflict of interest.

Ethical approval

The animal procedures and collection of biological material described in this study were reviewed and approved by the Bioethics and Animal Welfare Committee of VNIIVViM, protocol No. 23/2001. The study was conducted in accordance with the institutional animal welfare requirements applicable at the time of the original experimental work.

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