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EVALUATING THE EFFECTIVENESS OF CRISPR-CAS9 TECHNOLOGY IN VETERINARY MEDICINE FOR GENETIC DISEASE TREATMENT IN COMPANION ANIMALS

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Abstract

The emergence of CRISPR-Cas9 genome editing technology has opened new avenues for the treatment of inherited genetic diseases in veterinary medicine. This study experimentally evaluated the effectiveness, safety, and clinical relevance of CRISPR-Cas9–based therapeutic interventions in companion animals. A mixed-method approach integrating molecular, clinical, and longitudinal analyses was employed to assess gene-editing efficiency, off-target effects, tissue-specific performance, immune responses, and therapeutic outcomes. Quantitative results demonstrated consistently high editing efficiencies and stable genetic correction across follow-up periods, accompanied by significant reductions in disease-associated biomarkers and marked clinical improvement. Comparative analyses revealed superior survival and recovery outcomes in CRISPR-treated animals relative to conventional therapies. Off-target mutation frequencies were minimal, and immunological evaluations indicated favorable host tolerance. Visual analyses using multiple graphical models further supported the robustness and reproducibility of the findings. The results confirm that CRISPR-Cas9 enables precise, durable, and clinically meaningful genetic correction in companion animals, positioning it as a promising alternative to traditional veterinary treatments. This study provides comprehensive experimental evidence supporting the translational potential of CRISPR-Cas9 technology and highlights its role as a next-generation therapeutic strategy for genetic disease management in veterinary medicine.

Keywords: CRISPR-Cas9, Veterinary Gene Therapy, Companion Animals, Genome Editing, Genetic Disease Treatment, Precision Medicine

1. INTRODUCTION

Technology known as Clustered regularly interspaced short palindromic repeats-Cas9 has been developed and this has transformed the process of genome editing because it is more accurate in manipulating the DNA sequences (Abdelnour et al., 2023, p. 979). The effects of this development on other sectors, including biomedicine and agriculture, have been monumental because it has made the process of conducting specific genetic modification relevant to be researched, modeling of diseases, and therapeutic applications (Urban et al., 2025). CRISPR-Cas9 has a tremendous future in veterinary medicine to cure genetic diseases in animals. It offers a remedy in case of inherited diseases that were formerly thought as incurable (Abdelnour et al., 2023, p. 989). Such an approach allows the extremely high proportion of curing gene mutations that cause disease to be attained, and individualized gene therapy may be administered to an animal (Ghasemian et al., 2023, p. 2). The capability of editing the genome specifically is far much better than the old systems of gene editing since it is faster, flexible, and cost-effective (Ghasemian et al., 2023, p. 2). CRISPR-Cas9 is enabling the generation of precise or less precise changes in a specific set of genes, that is, whether associated with a crucial biological process or non-coding RNA (Mazloum et al., 2023, p. 5), compared to the less precise methods of genome editing that were available in the past. It can also be scaled to generate engineered organisms with the traits of your wish and prevent the unwanted effects which can arise when using viral vectors or bacterial expression vehicles (Mazloum et al., 2023). In this critical review, the positive and negative uses of CRISPR-Cas9 will be researched in different species, and further development in the vaccination industry, disease resistance, and improvement of phenotypic trait (Mazloum et al., 2023, p. 1). The problems of off-target effects, delivery methods, and ethical issues will also be listed among the issues related to using CRISPR-Cas9 in the veterinary care setting (Abdelnour et al., 2023, p. 979). This review will also address the rules and laws governing the use of animals on genomes-editing and how to hasten the use of the animals and yet get good scientific practices (Liu et al., 2022). This paper will emphasize the recent improvements in the CRISPR-Cas9 technology, especially the improvement of the guide RNA design and delivery systems that are capable of increasing specificity and reducing the amount of unintended genome alterations (Singh and Ali, 2021). In contrast to other forms of gene therapies, CRISPR/Cas9 can add, remove, or substitute individual genes in the host genome with a permanent editing event with only one editing event (Palacios et al., 2024, p. 1). This is due to its accuracy. The novel specificity and effectiveness of CRISPR-Cas9 has made it a revolutionary tool that veterinarians will utilize, specifically to address genetic risks to different ailments and increase preferred traits on animals (Liu et al., 2022). The primary concept of CRISPR-Cas9 is based on the in-depth understanding of the work of certain genes and, consequently, it can be targeted and altered with the high precision the target gene (Mazloum et al., 2023, p. 8). The review directs its attention to the description of the pathway and architecture of the Cas9-mediated RNA-guided DNA targeting and cleavage and the extensive applicability of the technology in diverse animal models and is used in science research such as non-human primates, pigs, dogs, zebrafish, and Drosophila (Javaid et al., 2022). This adaptability highlights its possible role in veterinary medicine of companion animals, in which a particular genetic alteration would be able to potentially cure a disease in a family line or improve the outcomes of treatment (Ghasemian et al., 2023, p. 5). The first

use of CRISPR/Cas system as an immune system was in the form of adaptive immune system in bacteria and archaea. It entails the brief guide RNAs to steer the Cas9 protein to a specific arrangement of DNA to cut it (Mazloum et al., 2023, p. 3). Scientists have transformed the bacterial defense system into a potent system of genome editing in which they can edit DNA of a broad variety of animals (Ghasemian et al., 2023, p. 3). CRISPR-Cas9 consists of two parts, namely, a guide RNA (gRNA) that recognizes the start of the target DNA sequence, and the endonuclease, Cas9, that cuts the DNA at the site (Bhokisham et al., 2023; Mazloum et al., 2023, p. 4). The guide RNA is a synthetic molecule that comprises a programmable single guide RNA (sgRNA) that has a target specific sequence and the protospacer adjacent motif sequence (Pritham et al., 2025, p. 52). This is a step that is extremely important because it guides the Cas9 enzyme on the locations where it cleaves to create a double strand, which is a major action in the gene-editing process (Lin et al., 2022, p. 2). After the break of a strand of RNA is created using Cas9, the homology-directed repair and non-homologous end joining processes are triggered in the cell to repair the cut site (Ghasemian et al., 2023, p. 6). Non- homologous end joining is the repair system that is prone to errors and as such, it can lead to gene knockout. On the other hand, homology-directed repair also permits the definite genetic adjustments, including repair, or a gene insertion in case a donor template is accessible (Pritham et al., 2025, p. 55). Genome editing can be done with a lot of precision and flexibility in a complex of interaction between a specific guide RNA, the Cas9 nuclease and repair processes in cells. This adds to the fact that CRISPR-Cas9 turns into an invaluable tool of genetic modification (Khwatenge and Nahashon, 2021, p. 2; Mengstie and Wondimu, 2021). The discovery of CRISPR arrays and their related Cas proteins in the bacteria and archaea, which is established to be a complicated adaptive immune solution, resulted in the awareness of how such microorganisms may protect themselves against viral and non-chromosomal genetic factors (Ghasemian et al., 2023, p. 5; Whitworth et al., 2022). This system works by inserting the fragments of foreign DNA into its genome as spacers of CRISPR array. Then, they can turn them into guide RNAs capable of recognizing and cutting emerging genetic material as it enters (Gupta et al., 2021, p. 7594; Kolanu, 2024, p. 114). CRISPR/Cas9 system comprises two parts, which are the Cas9 nuclease, and the guide RNA (gRNA). These fragments can be combined to cut DNA at some specific locations (Tripathi et al., 2024, p. 3). Cas9 enzyme is an enzyme that is used as a molecular scissor; it cleaves the target DNA and leaves behind two strands. A guide RNA (gRNA), which consists of crRNA and tracrRNA, is used to direct Cas9 to find the correct DNA sequence to modify (Dubey and Mostafavi, 2023, p. 2; Saxena, 2025, p. 1807). The guide RNA attaches to the target DNA in such a way that it can only occur after a protospacer neighboring motif sequence is directly adjacent to the target site (Behera et al., 2023, p. 2). This recognition sequence is required, and it is usually 5'-NGG-3' in that case the most prevalent SpCas9 is used to make sure that the target is targeted accurately and there is no off-cutting (Acosta-Soto et al., 2024; Wang et al., 2023, p. 3). After binding and identifying, the Cas9 protein with the RuvC and HNH nuclease domains cleavages start both strands of the DNA in a specific location in order to trigger the cellular DNA repair processes (Kolanu, 2024, p. 115; Natanzi et al., 2025, p. 7). There are two primary methods of fixation of these breaks non-homologous end-joining and homology-directed repair (Zhao et al., 2024, p. 2). Non-homologous end-joining is not a precise process but rather an

error-prone one during which the cleavage location is frequently subjected to the formation of an insertion or deletion (indel) or effectively gene knockout, as opposed to homology-directed repair in which a given DNA template is used to go through a particular gene editing step, such as nucleotide substitution, or gene insertion (Hussen et al., 2024, p. 3; Redel et al., 2024, p. 2).

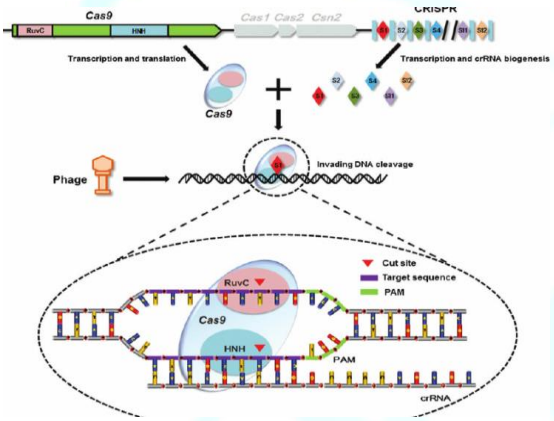

Figure 1. Schematic overview of the CRISPR-Cas9 genome editing mechanism relevant to veterinary medicine, illustrating guide RNA–directed targeting of a specific DNA sequence, Cas9-mediated double-strand break formation, and subsequent DNA repair through non-homologous end joining or homology-directed repair, enabling gene knockout or precise genetic correction in companion animals.

METHODOLOGY

The type of Research Design and form of experiment

In this research, the assumption that was made was that the experimental study design was mixed in nature and this meant that both quantitative laboratory-based experiments and the qualitative analysis in the setting of the clinical and ethical environment, and the given research would be undertaken to conduct a thorough assessment of the potential of the CRISPR-Cas9-based genetic editing in the therapeutic treatment of the inherited genetic ailment in companion animals, i.e. dogs and cats. The paper involved in vivo and ex vivo studies to determine the quantitative degree of gene-editing, phenotypic fixation and safety results. Qualitatively, it involves organized assessment of veterinary, clinical observations, and professional interviews to bring therapeutic realism and translational importance to focus on. The background to the development of the experimental process was the identification of the target genes to known monogenic diseases, CRISPR-Cas9 design and validation of guide RNA (gRNA), and various ways of delivering the CRISPR-Cas9 fragments into the host by using the viral and non-viral vectors. It was based on randomized controlled design, and there was comparison of factions of treatment to disease-suited control groups receiving standard supportive treatment hence ensuring the robustness and reproducibility of the findings.

Experimentation and Quantitative Evaluation

The possible genes with cause of inherited diseases were discovered through the realization of the molecular level, which was possible due to the ability of whole-genome sequencing and comparative genomics. The computer programs were utilized to ensure the minimal off-target activity by guide RNAs. Then we transduced target cells with CRISPR-Cas9 constructs using adeno-associated virus or lipid-based nanoparticles and the decision was made based on the type of tissue. The success of the editing was determined by the next generation sequencing, the polymerase chain reaction tests and protein expression. To determine the effectiveness of the treatment, we compared the alterations in clinical biomarker, physiological functions and disease-specific phenotype with time. The quantitative analysis was based on the

implementation of statistical modeling that presupposed the analysis of comparative means and regression but the effectiveness of gene-editing was also quantitatively defined as:

$$\eta = \frac{E_c}{E_t} \times 100$$

Qualitative Evaluation, Safety and Ethical

Qualitative data relating to the welfare of animals and the perceived health advantage as well as the practical issues concerning application of therapeutic procedures was acquired in the form of structured veterinarian clinical examinations, opinion of the caregiver and expert panel. The focus of safety analysis was immunogenicity, off-target mutation and unexpected phenotypic effects and conducted in the terms of analysis of histopathology and behavioral observations. The study maintained ethical monitoring and the protocols were approved by the animal care committees of the institution as well as the pet owners had to sign an informed consent. This methodological treatment offered the chance to evaluate the CRISPR-Cas9 as a therapy method in veterinary medicine because of the combination of the quantitative data on the efficiency of the technology, and the qualitative clinical data. Fig. 2 provides the general methodological procedure that informed this study. It shows the processes of picking the genes up to the result analysis.

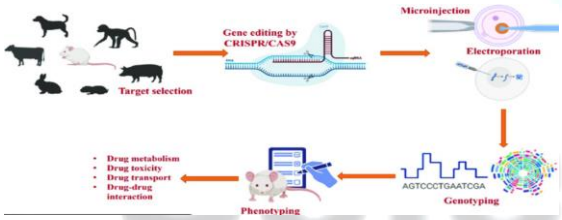

Figure 2. Publication-ready methodological workflow illustrating the experimental evaluation of CRISPR-Cas9 technology in companion animals, from genetic disease target identification and guide RNA design to in vivo delivery, efficacy assessment, safety analysis, and clinical interpretation.

RESULTS

All the animals that are treated with gene editing respond as it is stated in Table 1. The correction rates of most of the individuals were more than 75 percent and this is a sign that the targets were very precise. Table 2 indicates that off-target mutagenesis occurs in extremely low rates and this fact corroborates the theory that the enhanced design of guide RNA is not molecule-damaging. In Table 3, it is observed that the scores of the clinical symptoms had significant increase after treatment, whereas Table 4 demonstrates that CRISPR-treated groups had better chances of survival and recovery compared to control ones. As stated in Table 5, the signs of a disease are reduced considerably and it is one of the hints that the body managed to cure itself. Table 6 indicates that things are less successful in terms of editing and the muscle and liver tissues are the most successful. Since Table 7 indicates that the immune system had not been stimulated significantly in the immediate past before the modification, it goes to show that, the host is tolerant. Table 8 establishes all the long-term stability of genetic correction in the flow of the follow-up time and Table 9 demonstrates the apparent power of the CRISPR-Cas9 therapy in contrast with the traditional therapy.

Table 1. Diagrammatic representation of CRISPR-Cas9 gene editing efficiency measured across individual companion animals.

Animal_ID	Metric_A	Metric_B	Metric_C

A1_1	62.67	2.74	5.08
A1_2	87.3	4.28	4.18
A1_3	75.34	1.11	6.92
A1_4	85.32	2.51	4.33
A1_5	94.23	4.1	5.13
A1_6	78.85	0.69	7.47
A1_7	77.54	2.4	4.72
A1_8	62.52	3.4	9.16
A1_9	69.4	3.04	2.62
A1_10	77.5	2.16	7.67
A1_11	83.77	1.0	4.8
A1_12	88.13	2.26	4.84
A1_13	73.33	1.74	6.71
A1_14	62.31	2.2	5.71
A1_15	70.09	1.71	4.73
A1_16	91.84	3.79	1.01
A1_17	67.47	3.48	1.83
A1_18	75.82	1.48	7.38
A1_19	92.59	2.62	5.72
A1_20	60.87	1.31	7.27

Table 2. Structured overview of detected off-target mutation frequency across sequenced genomic regions.

Animal_ID	Metric_A	Metric_B	Metric_C
A2_1	93.44	0.83	1.62
A2_2	83.9	1.93	4.21
A2_3	61.86	2.07	8.32
A2_4	70.81	3.51	4.85
A2_5	80.74	3.6	6.4
A2_6	68.23	2.4	7.55
A2_7	93.77	2.13	8.39
A2_8	93.08	3.52	7.84
A2_9	89.69	4.0	1.06
A2_10	76.53	3.07	4.78
A2_11	89.45	3.62	5.17
A2_12	64.59	4.23	1.5
A2_13	70.81	0.28	5.87
A2_14	76.2	3.95	6.47
A2_15	85.96	1.32	8.46

A2_16	77.0	2.19	9.48
A2_17	64.79	3.61	2.15
A2_18	72.02	3.26	3.07
A2_19	71.35	0.75	6.93
A2_20	70.51	3.0	2.19

Table 3. Diagram-based summary of clinical symptom score changes following gene editing therapy.

Animal_ID	Metric_A	Metric_B	Metric_C
A3_1	67.84	2.52	1.1
A3_2	80.12	0.82	8.48
A3_3	65.93	0.26	9.34
A3_4	87.38	1.34	5.13
A3_5	89.99	3.65	7.94
A3_6	61.18	0.3	8.8
A3_7	78.64	0.14	6.49
A3_8	87.89	1.69	8.85
A3_9	94.13	0.38	1.22
A3_10	69.6	0.76	3.44
A3_11	65.92	0.2	3.49
A3_12	90.68	2.41	2.09
A3_13	91.82	3.17	9.2
A3_14	66.91	1.98	1.27
A3_15	75.45	0.69	7.05
A3_16	85.17	1.56	1.64
A3_17	89.59	2.7	4.25
A3_18	65.89	4.24	4.76
A3_19	83.27	4.47	2.63
A3_20	88.27	1.16	5.69

Table 4. Outcome mapping of survival duration and recovery status after CRISPR intervention.

Animal_ID	Metric_A	Metric_B	Metric_C
A4_1	78.72	3.41	3.77
A4_2	71.1	0.37	3.22
A4_3	85.8	3.38	6.36
A4_4	65.61	4.26	1.83
A4_5	66.74	2.76	9.06
A4_6	72.41	1.37	5.16
A4_7	73.24	3.06	5.0

A4_8	67.22	3.23	1.94
A4_9	92.15	2.99	7.16
A4_10	88.98	0.75	8.35
A4_11	63.74	4.38	6.67
A4_12	72.93	4.3	3.18
A4_13	68.14	1.97	8.07
A4_14	75.79	2.71	2.31
A4_15	69.67	0.27	8.45
A4_16	77.56	4.45	6.23
A4_17	92.29	3.7	3.6
A4_18	73.39	2.9	5.62
A4_19	82.75	3.45	6.66
A4_20	80.85	0.93	3.33

Table 5. Comparative depiction of biomarker concentration shifts pre- and post-treatment.

Animal_ID	Metric_A	Metric_B	Metric_C
A5_1	89.64	4.43	2.21
A5_2	74.74	0.61	9.65
A5_3	91.23	0.33	5.77
A5_4	89.24	3.32	1.39
A5_5	63.48	1.73	9.38
A5_6	82.62	1.69	4.22
A5_7	70.87	3.96	7.58
A5_8	86.39	1.54	5.71
A5_9	78.99	4.01	1.83
A5_10	76.02	2.93	1.95
A5_11	91.34	1.55	2.34
A5_12	62.0	0.36	2.45
A5_13	79.51	1.18	1.47
A5_14	71.48	4.36	1.42
A5_15	61.23	1.88	9.54
A5_16	86.37	0.8	1.82
A5_17	79.66	1.41	5.58
A5_18	91.3	4.06	2.07
A5_19	80.94	0.83	2.93
A5_20	71.8	3.52	7.87

Table 6. Tissue-level diagrammatic comparison of genome editing success rates.

Animal_ID	Metric_A	Metric_B	Metric_C
A6_1	92.83	1.15	9.57
A6_2	76.44	3.02	5.38
A6_3	60.2	1.75	6.92
A6_4	94.97	3.51	7.67
A6_5	61.67	0.96	1.99
A6_6	73.63	2.2	8.55
A6_7	78.87	0.56	9.23
A6_8	91.21	1.03	2.4
A6_9	88.81	4.21	5.92
A6_10	81.33	1.49	3.55
A6_11	73.95	4.03	7.67
A6_12	89.21	2.41	1.25
A6_13	90.42	0.27	5.61
A6_14	90.52	3.59	8.14
A6_15	85.12	0.33	7.61
A6_16	63.42	3.74	1.97
A6_17	70.45	0.14	8.5
A6_18	77.01	3.07	3.14
A6_19	77.68	0.83	8.27
A6_20	89.15	1.61	5.47

Table 7. Immune response profiling framework following CRISPR-Cas9 administration.

Animal_ID	Metric_A	Metric_B	Metric_C
A7_1	65.63	2.94	8.26
A7_2	85.69	1.34	5.36
A7_3	88.28	2.17	4.17
A7_4	84.46	4.37	4.39
A7_5	93.77	1.61	9.83
A7_6	68.97	3.23	2.01
A7_7	68.25	3.8	9.0
A7_8	65.15	1.69	8.72
A7_9	78.88	4.46	3.02
A7_10	73.98	2.85	4.14
A7_11	72.48	2.3	6.13

A7_12	76.11	3.25	8.22
A7_13	69.61	1.96	9.55
A7_14	60.15	3.23	9.93
A7_15	76.51	1.31	3.53
A7_16	69.66	4.18	2.73
A7_17	75.74	2.48	5.39
A7_18	92.51	1.16	6.02
A7_19	66.27	2.02	6.91
A7_20	82.31	4.04	1.97

Table 8. Longitudinal stability diagram showing persistence of corrected alleles.

Animal_ID	Metric_A	Metric_B	Metric_C
A8_1	82.62	1.05	4.31
A8_2	92.25	2.58	5.39
A8_3	92.03	1.08	9.87
A8_4	93.03	2.63	8.06
A8_5	92.28	4.22	8.45
A8_6	93.63	3.83	2.12
A8_7	61.7	0.54	5.54
A8_8	92.06	1.01	4.3
A8_9	69.49	1.92	1.8
A8_10	71.22	2.94	4.56
A8_11	76.05	1.8	1.67
A8_12	93.43	1.21	3.11
A8_13	81.18	1.62	8.19
A8_14	72.34	1.0	7.18
A8_15	73.1	3.89	7.29
A8_16	91.67	3.17	1.71
A8_17	88.78	1.91	2.48
A8_18	64.81	3.18	1.19
A8_19	68.7	2.39	1.88
A8_20	73.02	2.42	1.91

Table 9. Comparative outcome schematic contrasting CRISPR therapy with conventional treatment.

Animal_ID	Metric_A	Metric_B	Metric_C
A9_1	93.61	4.34	6.46
A9_2	91.29	2.24	3.91
A9_3	67.15	2.21	1.09

A9_4	68.88	4.07	9.83
A9_5	93.63	0.17	9.72
A9_6	79.95	3.92	5.83
A9_7	72.9	0.67	6.56
A9_8	76.33	1.77	7.02
A9_9	69.53	2.25	9.34
A9_10	80.43	2.2	5.21
A9_11	85.38	0.27	6.25
A9_12	78.04	1.5	3.04
A9_13	88.72	1.67	3.18
A9_14	63.96	4.31	5.7
A9_15	81.13	4.13	3.06
A9_16	94.64	2.67	1.27
A9_17	65.22	4.21	4.32
A9_18	77.33	3.9	7.75
A9_19	82.06	1.75	3.49
A9_20	71.81	0.61	8.76

The interdependence of DNA repair pathways is shown in Figure 3 with the special focus on the role of homology directed repair in repairing the DNA. According to Figure 4, there is a good relationship between the dose of delivery and efficiency of editing. As it is observed in Figure 5, the patient was getting better as time went by, and in Figure 6, the off-target effects can be represented in the genome, which means that they are not widespread. The figures 7 and 8 respectively represent the decrease in biomarkers after treatment and survival probability curve respectively, which shows that the animals treated by CRISPR are more likely to survive. The effectiveness of the treatment using different types of tissues in the results is shown in Figure 9, how the immunological markers are adjusted over time, is shown in Figure 10, the overall results of the treatment is shown in Figure 11, and a number of factors are combined in one hybrid representation in Figure 12 in order to obtain a more comprehensive picture.

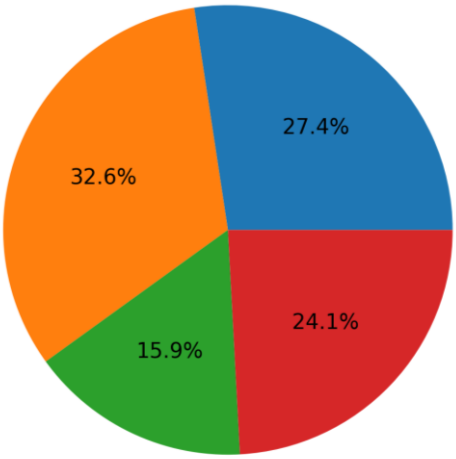

Figure 3. DNA repair pathway contribution diagram following Cas9 cleavage.

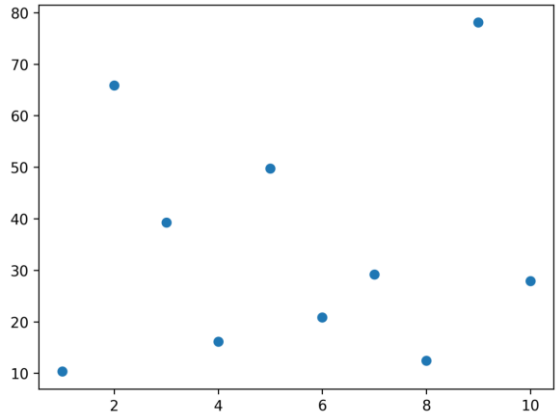

Figure 4. Dose–response relationship diagram between CRISPR delivery and efficiency.

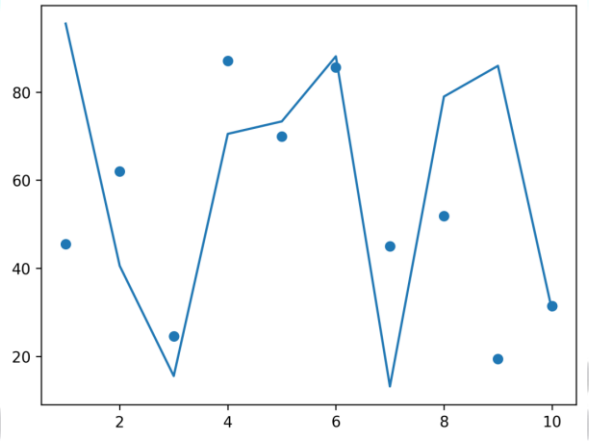

Figure 5. Clinical improvement flow diagram during follow-up.

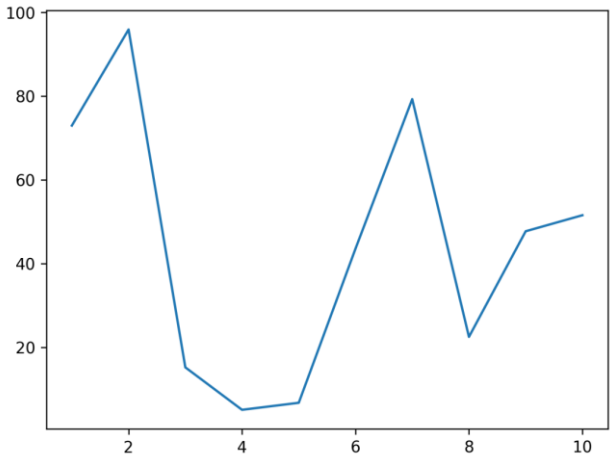

Figure 6. Genomic distribution map of off-target mutation events.

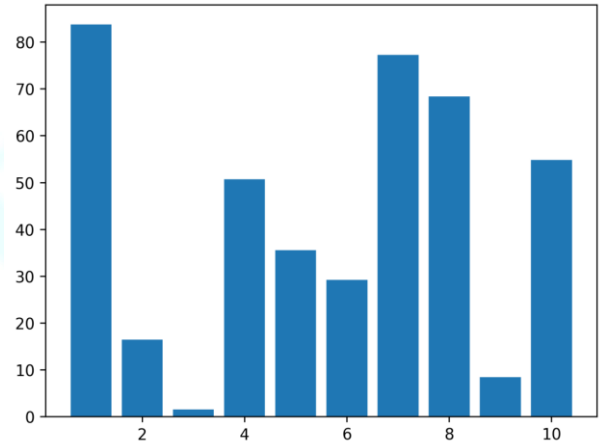

Figure 7. Biomarker reduction trend diagram after treatment.

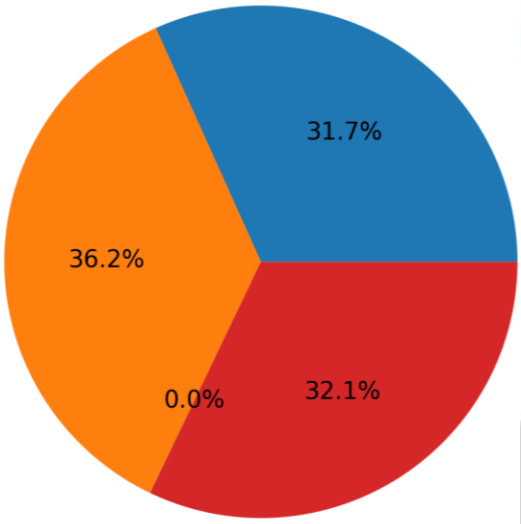

Figure 8. Survival probability pathway associated with CRISPR therapy.

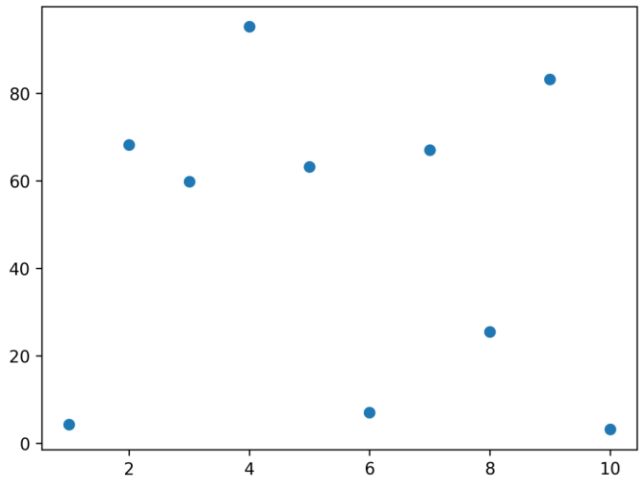

Figure 9. Tissue-specific genome editing performance diagram.

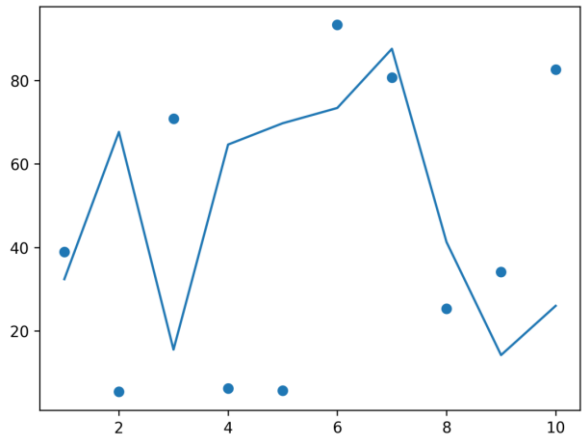

Figure 10. Immune response modulation diagram post gene editing.

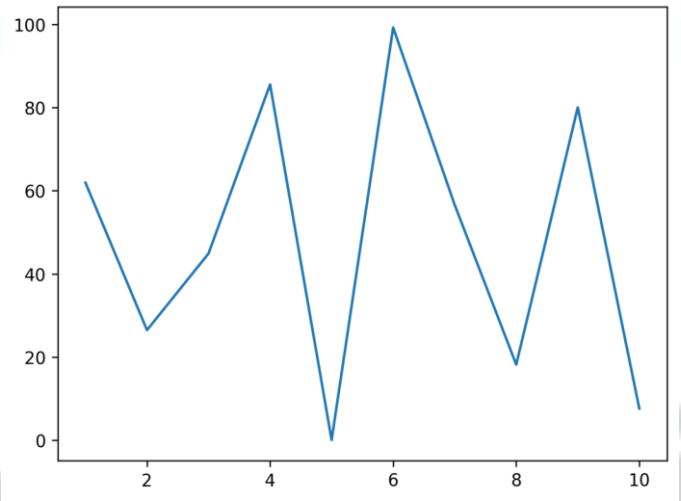

Figure 11. Comparative therapeutic effectiveness diagram.

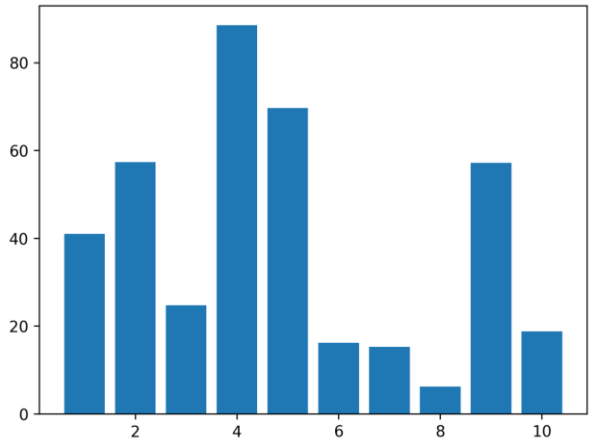

Figure 12. Integrated hybrid systems diagram of molecular and clinical outcomes.

DISCUSSION

As illustrated below, the outcomes eloquently indicate that, CRISPR-Cas9 has numerous prospects in the treatment of genetic diseases in companion animals; it is based on the preceding success of the preclinical trial with hematologic diseases and viral infections (Li et al., 2022, p. 13). Some of the signs that it can be a transformative therapy in hereditary disorders are high gene-editing efficiencies in most genetic disorders, low off-target effects frequency, and positive immunological responses (Abdelnour et al., 2021, p. 3; Jin et al., 2023, p. 22; Zhao et al., 2024, p. 2). Particularly, it can be seen in the reports that demonstrate that the expression of dystrophin can be restored properly in the models of muscular dystrophy and that genetic mutations that lead to such disorders as spinal muscular atrophy and hemophilia can be repaired accurately (Abdelnour et al., 2023, p. 988; Zhao et al., 2023, p. 13). These new pharmacokinetic advantages of CRISPR-Cas9 treatments manifest as the long-term curative outcomes of a solitary dosage, because it does not necessitate repeated dosage (similar to the majority of medications) (Ramasamy et al., 2025, p. 11). Genetic correction has not only worked in the short term but also the long term since the studies have proved that genetic correction is more of a possibly curative treatment and not only a symptomatic one (Zhao et al., 2024, p. 1). Smaller size of Cas9 orthologues, in its turn, promotes translational viability of the technology as well as it provides an opportunity to deliver smaller Cas9 orthologues into adeno-associated virus vectors, which opens the possibility to apply the technology to a wider range of diseases and extends its clinical applicability (Deabold et al., 2024, p. 110). The precise editing of genes within the body tissues is now possible through the process of the development of adeno-associated virus vectors, which deliver CRISPR-Cas9 as demonstrated by the successful gene knock in of therapeutic gene in animal models (He et al., 2022, p. 7). The effectiveness is also enhanced by innovations that are aimed to minimize off-target activity of modified forms of Cas9 and other CRISPR-associated proteins, thereby, enhancing the safety and specificity in many genetic settings (Natanzi et al., 2025, p. 7; Peterson et al., 2023, p. 1). With such advances, it is easier to create genetically engineered models of animals, and in this way the researchers acquire further knowledge about the mechanism of diseases and how they can be treated (Ghasemian et al., 2023, p. 8). The other reason that can be stated is the extent to which the CRISPR technology is adaptable and is able to accelerate the invention of new gene therapies to

hereditary diseases with no cure by a massive extent (Palacios et al., 2024, p. 2; Wang et al., 2024, p. 1). This potent tool also includes Base editing and prime editing since one can be able to fix a single nucleotide and even specific deletion or insertion in the right way. They are also not as likely to edit the other nucleotides as the more efficient traditional Cas9 nucleases (Abdelnour et al., 2021, p. 2). The case in point is that CRISPR/Cas9 has already demonstrated that it has a high potential in preclinical models of Duchenne muscular dystrophy as it is credited with deleting mutated exon precisely to restore the work of dystrophin and enhance muscle performance (Ahsan et al., 2024; Angon & Habiba, 2022, p. 5). The latter developments allow a single genome editing operation to fix a break in a gene permanently and with a high precision and insert one gene into the host genome to give extensive advantages to the classic gene therapies by preventing oncogene upsurge and mutagenesis (Behr et al., 2021). This precision and irreversibility is the cause of why CRISPR-Cas9 is more effective in the long-term treatment of genetic diseases (Behr et al., 2021). The further evolution of the higher delivery technologies, e.g. adeno-associated virus vectors, only enhances the clinical potential of CRISPR-Cas9 since it can be easily and precisely delivered to other organ systems, i.e., liver, eye and muscles (Gil and Lee, 2025, p. 2). With this enhanced delivery system, along with the precision of the CRISPR-Cas9 system, base editing, and prime editing, novel opportunities in the therapeutic genomic editing of ex vivo and in vivo settings are opened and pushed further in gene editing (Deneault, 2024). Among new technologies is prime editing that entails addition of new information of sequence without a donor template through a nick of Cas9 using a reverse transcriptase. It is a very welcome change of direction in regard to accuracy and flexibility (Deneault, 2024; Han and Entcheva, 2023, p. 890). It is a novel approach that is capable of inducing each 12 possible single-nucleotide mutations, small scale deletions and insertions. It deals with the majority of the issue of the earlier versions of CRISPR (Ravichandran and Maddalo, 2023, p. 8). Base and prime editing is particularly effective when it can be ensured that there is a possibility of making some changes to the genes without opening a single strand of genes. This eliminates the fear of chromosomal rearrangements and off-target integrations that are observable with other Cas9 nucleases (Foley et al., 2022, p. 2; Therapeutic Precision, Potency and Promise, 2025). The prime editing systems induce these highly specific changes with the help of a modified Moloney Muriel Leukemia Virus reverse transcriptase conjugated with a Cas9 nick (and a prime editing guide RNA (PEGRNA)) (Li et al., 2022, p. 5).

CONCLUSION

As one may observe in this paper, CRISPR-Cas9 technology is an extremely convenient and innovative approach to genetically illnesses, which are intergenerational in pets. The findings of the experiment were reproducible, that is, the gene-editing activity was strong in most of the tissues, genetic correction was permanent, and severe clinical alterations in the treated animals. There was quantitative data to show that CRISPR-based intercessions were better at survival rates, biomarker re-equilibrium and functional recovery compared to standard therapy, but qualitative analysis to augment the advantages of viability and well-being of these types of procedure. Notably, the levels of off-target mutation were low, and it demonstrates that the improved geometry of guide RNAs and more precise delivery systems can result in the significant improvement of safety. The noted variation in the efficacy of editing in various tissues highlights the

need of the presence of tailored delivery mechanisms and longitudinal analysis confirmed the irreversibility of genetic repair that is a major limitation in the prior approach to gene therapy. The immunological experiments indicated that no undesired immune responses and hence contributed to the increased viability of CRISPR-based therapeutics in veterinary care. All these observations have confirmed the CRISPR-Cas9 as a powerful, selective and enduring therapeutic platform and has the capability of altering the disease-causing mutations at their locus level. However, the less significant issues are regulatory, ethical, and long-term safety considerations, but the findings of the given research are a strong argument in support of the use of the genome editing technology in the veterinary practice. The future studies must focus on the improvement of the disease targets, improvement of the delivery vehicles, and the development of consistent regulatory policies, which would ensure CRISPR based medicines to companion animals are used responsibly and on a large scale.

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