Narrative Review Article

Applications of CRISPR-Based Gene Editing in Livestock Agriculture

Desmond Liu

Yorba Linda High, 19900 Bastanchury Rd. Yorba Linda, CA 92886, United States

ABSTRACT

This review examines genetic strategies for improving disease resistance, enhancing production efficiency, and reducing methane emissions. Livestock play an important role in global food security, providing essential sources of food and income for billions of people. However, viral outbreaks can cause the death of entire farms, resulting in food shortages and substantial economic losses. Modern challenges, such as low production efficiency and environmental impact, have made livestock cultivation increasingly difficult. Advancements in CRISPR-based gene editing offer promising solutions to these challenges. Clustered regularly interspaced short palindromic repeats (CRISPR) enable precise genetic modifications that can improve livestock health, productivity, and sustainability. Notable applications include gene knockouts of CD163 in pigs and ANP32A in chickens to confer viral resistance, as well as modifications to the MSTN gene to increase muscle mass and feed efficiency. The targeting of methane-producing microbes in ruminants has shown promising developments, hinting at a future of environmentally friendly livestock agriculture. These advancements suggest that CRISPR technology has the potential to revolutionize the livestock industry on a global scale.

Keywords: CRISPR-Cas9; Livestock; Gene Editing; Disease Resistance; Biotechnology; Agriculture

INTRODUCTION

Most people worldwide consume products produced from livestock. In the United States alone, annual consumption includes approximately 297.62 billion pounds of meat, 109.5 billion eggs, and 225.87 billion pounds of milk (1–3). These numbers underscore the importance of livestock in supporting humans. However, livestock farming has many difficulties and drawbacks. For one, livestock farming has a large carbon footprint. Greenhouse gases such as methane are released, which accelerate climate change and global warming (4). Livestock also require extensive space. A research study shows that about 98.1% of livestock agriculture is on large-scale industrialized farms, even though only 2.6% of U.S. agriculture is livestock (5). As populations continue to grow, so does the demand for livestock, resulting in an ever-increasing need for space. Additionally, antibiotic-resistant bacteria have been on an alarming rise due to the extensive use of antibiotics in farm animals. This leads to previously treatable infections becoming deadly, resulting in economic loss for farmers (4).

All these problems threaten the stability of everyday lives and can cause major problems if left unattended. A potential solution may already exist: gene editing using CRISPR. CRISPR is a gene editing technique that allows scientists to modify, add, or delete DNA. This review

Corresponding author: Desmond Liu, E-mail: desmondliu10@gmail.com.

Accepted January 27, 2026
https://doi.org/10.70251/HYJR2348.41499507

focuses on the use of CRISPR gene editing technology in livestock agriculture, examining applications and developments in disease resistance, production efficiency, and methane reduction.

THE USE OF CRISPR TO COMBAT INFECTIOUS DISEASES

Gene Knockout

One of the most common CRISPR-based methods of treating diseases in livestock is gene knock-out, which deactivates a specific gene. The process of gene knockout first uses CRISPR-Cas9 to induce a double-strand break at a specific location within the organism's genome (6). The cell responds to this break by using non-homologous end-joining (NHEJ), a form of DNA repair, to attempt to repair the break (Figure 1). NHEJ, however, is error-prone and causes insertions and deletions (indels), leading to a nonfunctional or incomplete protein and effectively knocking out the gene (7).

A notable example of the use of gene knockouts is for Porcine Reproductive and Respiratory Syndrome (PRRS) in pigs. PRRS is a highly contagious viral disease that causes severe reproductive failure in sows and respiratory illness in piglets. It is estimated that in the United States, $664 million is lost each year as a result of PRRS (8). Islam *et al.* first analyzed gene expression in peripheral blood mononuclear cells, finding that pigs do mount an immune response to PRRSV infection or vaccination (9). This led to later researchers discovering that the CD163 gene codes for a receptor on pig immune cells that the PRRS virus uses to enter the cells (10). Whitworth *et al*. used CRISPR-Cas9 to knock out CD163 and reported that the pigs were fully protected against PRRS symptoms (11).

Another example of gene knockout is the Avian Influenza Virus (AIV). AIV, also known as bird flu, is not only fatal for poultry but can also infect humans and cause death. The mortality rate for infected humans is about 50% (12). Therefore, all chickens infected with AIV are killed to prevent an outbreak. Long *et al*. discovered that a gene specific to chickens, ANP32A, is essential for the virus to replicate, and deleting a 33-amino-acid segment greatly reduced the virus's replication ability in chickens (13).

Avian leukosis virus (ALV) has caused severe

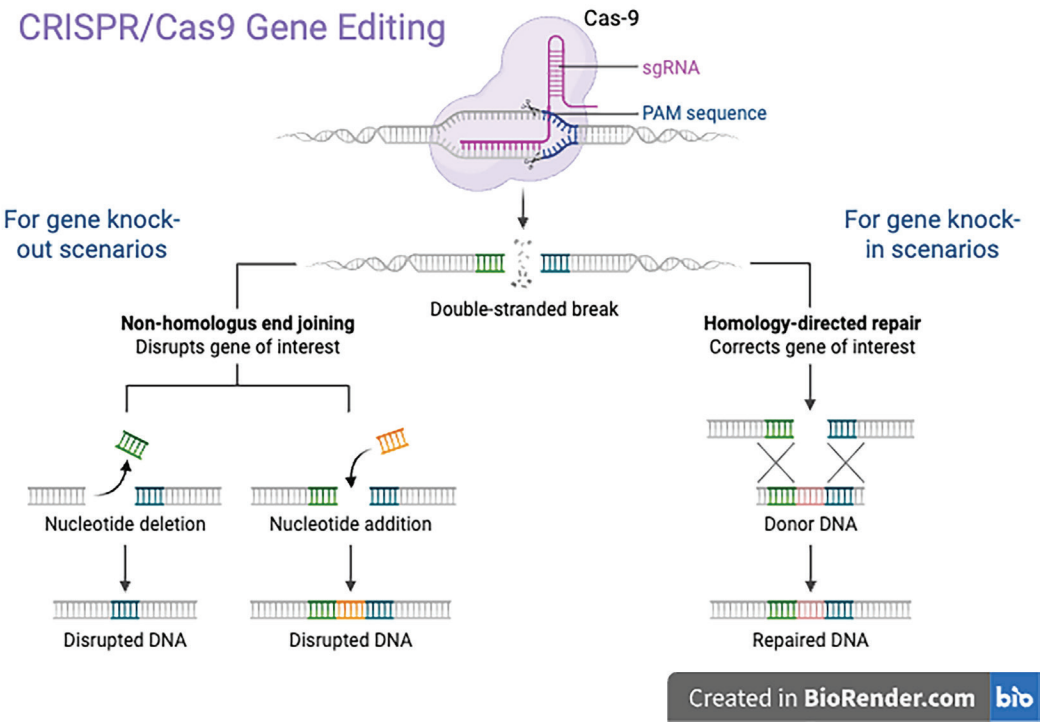

***Figure 1.* CRISPR/Cas9 Gene Editing.** *Shows how CRISPR-Cas9 induces genetic modifications via non-homologous end-joining (NHEJ) and homology-directed repair (HDR). NHEJ repair involves small insertions and deletions (indels) and is normally used for gene knockout. HDR repair requires a donor template and is often utilized for gene knockin.*

commercial losses in the poultry industry. When chickens are infected with this virus, they develop tumors, egg production decreases, and their immune system weakens (14). ALV is divided into six subgroups. Those subgroups each use either the Tva, Tvb, Tvc, or Tvj receptor (15). Some chickens naturally carry virus-resistant alleles of these receptors that disrupt their function without harming the bird (16). Based on this, Lee *et al*. used CRISPR-Cas9 to knock out these receptors, resulting in immunity to the viruses (17).

Gene Knock-In

Another popular method of using CRISPR-Cas9 to edit genomes is gene knock-in. Unlike gene knock-out, which disrupts or disables a gene, knock-in involves inserting a specific DNA sequence into the genome. The same double-strand break is generated, but instead of NHEJ, the cell uses homology-directed repair (HDR) (6). HDR repairs the break using a donor template, normally from a homologous chromosome. However, scientists can instead insert a specific DNA template of their choice (Figure 1). HDR only occurs during late synthesis and G2 phase of the cell cycle; thus, knock-ins must be timed accordingly (7). Sometimes, a single-strand break, also called a nick, can be done to trigger the HDR. This, however, requires the use of Cas9 nickase (nCas9), a more precise version of Cas9 that is created by mutating one of Cas9's two active nuclease domains, RuvC or HNH (18).

A crippling disease that appears in cattle is bovine tuberculosis (bTB), which mainly affects the lungs, causing symptoms like coughing and labored breathing. bTB is caused by the bacteria Mycobacterium bovis in cattle, resulting in over 50 million infections that create difficulties and hardship for many farmers worldwide (19). This disease can also be transmitted to humans primarily through unpasteurized milk (20). This risk thus results in mandatory testing and the slaughter of any infected cattle, causing economic loss for farmers. Holder *et al*. have found that the gene NRAMP1 is resistant to infections similar to bTB, making it a strong candidate for combating bTB. Tuggle *et al*. used nCas9 to insert the NRAMP1 gene into the genome of bovine fetal fibroblasts, a type of cell found in connective tissue from a cow fetus (21). Modified cells were then used as donor cells in somatic cell nuclear transfer (21). This process involves removing the egg cells from the mother and removing the genetic material containing nuclei from the cells (22). Then they insert the nuclei from the donor cells into the eggs and stimulate them to begin developing like normal embryos before implanting them into the recipient cows. The result is cows that are more bTB resistant as the NRAMP1 gene is correctly expressed (21).

Gene Drives

Gene drives are a relatively new method of dealing with diseases and can rapidly spread disease-resistant traits across generations. This method is based on naturally occurring site-specific homing endonuclease genes (HEGs). HEGs act by recognizing specific genomic sites that don't already have HEG and cutting that part of the chromosome. The cell responds by repairing the cut using the chromosome that does have HEG as a template, effectively copying the HEG onto the previously unmodified chromosome (23). Similarly, Cas9 and gRNA can be inserted into a targeted location in the genome and programmed to mimic HEGs, inducing double-strand breaks at locations without desired genes, resulting in the desired genes being copied over. This process allows what used to be a 50% chance of a specific trait being passed down to a 100% chance (24). However, the relatively new process has not seen much actual use due to the many risks and uncertainties. If proper safety precautions are not taken, the rapid spread of traits could have unintended consequences on the environment (25).

The ability to remove and deal with infectious diseases is critical. However, it is equally important to be able to detect diseases in the first place. This is where Cas12 and Cas13 detection systems come in. Cas12 and Cas13 enzymes were found to not only cut their DNA or RNA target sites, respectively, but also cut nearby non-target single-stranded nucleic acids (26, 27). These unique properties inspired scientists to develop systems like DETECTR and SHERLOCK, which were able to harness the collateral cleavage ability of Cas12 and Cas13. These detection systems work by first sending in a CRISPR RNA (crRNA) to guide the enzyme to a target sequence specific to a pathogen's DNA or RNA. If the target is present, the enzyme is activated and begins collateral cleavage (28). During the cleavage, a reporter molecule placed by scientists would also be cut, releasing a fluorescent signal (29). This signal is picked up by the sensitive systems, confirming the presence of the disease (30). These detection systems have been used to successfully detect diseases like PRRS and African swine fever virus (ASFV).

Despite all these advances, several limitations and technical challenges remain. Off-target edits, though reduced by methods such as nCas9 or paired gRNAs,

can still occur and may inadvertently affect animal health or development. The efficiency of germline editing also varies across species and genes, which impacts scalability and the consistent transmission of desired traits. While fast-reproducing animals like chickens may achieve edited-trait prevalence more quickly, species with long gestation periods, such as cows, require multiple generations and considerable time to effectively propagate edits (31). Addressing these constraints will be necessary for translating CRISPR innovations into scalable livestock improvements.

USING CRISPR TO IMPROVE PRODUCTION EFFICIENCY

Improving the yield efficiency of livestock agriculture is vital to meet the growing demands for food of a growing population. Several methods to increase livestock efficiency involve the MSTN gene. This protein belongs to a class of proteins called transforming growth factor beta (TGFβ), which aids in regulating the development and growth of tissues throughout the body. Found primarily in skeletal muscles, myostatin acts as a natural brake on muscle growth, preventing muscles from growing too large (32). Therefore, scientists have been developing methods involving CRISPR to modify MSTN for the pursuit of higher muscle mass and efficiency in livestock.

Gap-Don Kim *et al.* sought to knock out MSTN in chickens to increase their size. They took primordial germ cells (PGCs), which are early reproductive cells from chicken embryos, and inserted D10A Cas9 nickase with two gRNAs. This system creates two single-strand breaks instead of one double-strand break, resulting in a lower chance for off-target effects. The successfully edited cells were microinjected into early chicken embryos, creating germline chimeras that were then bred further. Male MSTN knockout (KO) chickens were observed to have, on average, higher leg and breast weight compared to the normal wild-type (WT) chicken. Specifically, the legs of MSTN KO chickens were 55.3% heavier than those of the control WT. Abdominal fat weight was also significantly lower at about 77.9% lower compared to the non-MSTN KO chickens, but no weight difference in other organs was seen. In female MSTN KO chickens, there was minimal difference in average weight between the MSTN KO and WT groups. However, similar to the male chickens, breast and leg weights were considerably heavier, and abdominal fat weight was reduced by 77.1% on average. In MSTN knockout chickens, muscle weight increased by 13.9–95.1% compared to WT chickens, with males showing greater gains than females in certain muscles (Figure 2). The M. semitendinosus and biceps femoris muscles were significantly larger in KO chickens, mainly due to muscle fiber hypertrophy (the increase in the size of individual muscle cells) or hyperplasia (the increase in the number of cells in an organ or tissue). In males, type I (slow-twitch) fibers of the biceps femoris had a 64.7% larger cross-sectional area compared to WT, while type II fibers (fast-twitch) showed little change. Females displayed modest increases in fiber size and number, but most differences were not statistically significant. The pectoralis major muscle showed no notable sex differences in fiber size or count (33).

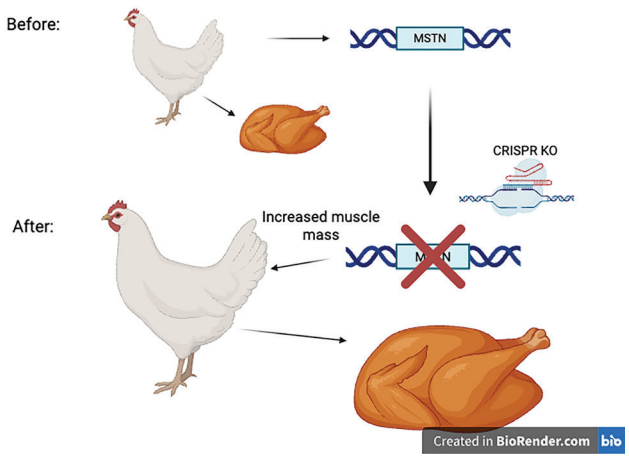

Figure 2. MSTN Knockout via CRISPR/Cas9. *The top shows a chicken with normal myostatin genes. The bottom shows the resulting chicken after the knockout of myostatin using CRISPR. Muscle mass experiences a significant increase.*

Crispo *et al.* were also able to achieve MSTN KO in sheep by targeting exon 1 using CRISPR/Cas9. Disrupting this region prevents protein from functioning. Although their birth weights had no change compared to WT, homozygous KO sheep showed a significant weight increase of 20–30% during the first two months of life, while heterozygous KO sheep showed an intermediate weight difference. The difference in body weights was primarily due to an increase in muscle mass of MSTN KO sheep (34).

Other methods that do not require MSTN KO could also help increase efficiency. For instance, Oishi *et al.* have shown that allergenicity in eggs can be reduced by disrupting the ovalbumen and ovomucoid genes.

Ovalbumen and ovomucoid are major proteins in egg whites that cause allergies. CRISPR technology can be used to disable the genes responsible for the production of these proteins, allowing eggs to be less likely to cause immune system reactions when consumed (35). Scientists have also been able to improve the meat quality and feed efficiency of pigs through Peroxisome proliferator-activated receptor gamma (PPARy), a central regulator of fat cell development and lipid metabolism. They constructed a muscle-specific expression cassette for PPARy using the muscle creatine kinase (MCK) promoter, ensuring that the gene would be active only in skeletal muscle. This was then injected into an embryo using somatic cell nuclear transfer, resulting in increased muscle mass and intramuscular fat (36). A similar approach using CRISPR could be considered.

Despite these gains, scalability remains a significant challenge. Germline editing efficiency varies across species, and the inheritance of edited traits is not guaranteed in all offspring. While fast-reproducing animals such as chickens may achieve population-level dissemination of edited traits relatively quickly, species with long gestation periods require multiple generations and substantial time investment. Furthermore, regulatory barriers are also a critical factor in CRISPR applications. Livestock genome editing overlaps with food safety regulations, animal welfare laws, and environmental protections. Approval processes vary across countries, and long-term monitoring may be required to ensure safety and compliance (31). These constraints must be addressed for translating CRISPR innovations into scalable livestock improvements.

THE USE OF CRISPR TO DECREASE METHANE LEVELS

Methane is a major contributor to climate change and is produced primarily by livestock. As a result, scientists are using CRISPR technology to reduce methane emissions. UC Berkeley researchers are actively developing tools to combat methane production (37). In ruminants, methane is produced from microbes known as methanogens, which are archaea (Figure 3). As shown in Figure 3, plant matter is broken down and utilized by archaea for the production of methane. Most genetic engineering technologies have been designed for well-studied bacteria and eukaryotes, making their application to archaea difficult due to the biological differences (38). To select successfully transformed cells in the gene editing process, scientists usually choose antibiotic resistance genes to include in the transferred material. This allows them to then introduce an antibiotic that will kill any microbe that did not take in the material. However, archaea are not susceptible to most antibiotics that work on bacteria due to the difference in their cell walls and ribosomes, ultimately making the process of finding a marker for each methanogen difficult (39). Since methanogens live in oxygen-free environments, common fluorescent proteins such as Green Fluorescent Protein (GFP) are limited in use due to their reliance on oxygen. Recently, fluorescence-activating and absorption-shifting tags (FAST and FAST2) have been developed. These proteins bind small fluorogens and cause them to fluoresce brightly, even under methanogenic conditions (40). In recent years, shuttle vectors have been developed, which are genetic elements that can replicate in multiple hosts. These tools allow genetic constructs to be developed in E. coli, where genetic methods are well established, and then transferred into methanogens, where replication will occur, and the gene will be expressed (41). *Methanococcus maripaludis* has had the most shuttle vectors developed, with puromycin, neomycin, and 6-azauracil resistance being the most common selection markers. Similar systems have been developed for other methanogens, like Methanosarcina and Methanothermobacter (42–44).

Unlike methanogens, methanotrophs are bacteria that consume methane. Although not directly found

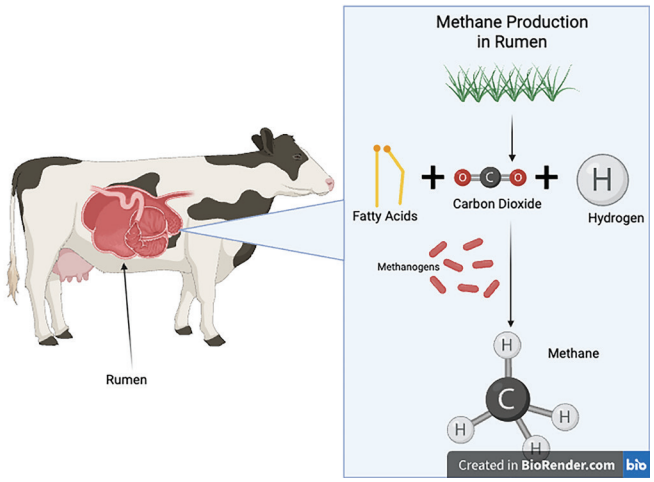

Figure 3. Methane Production in Rumen. *Illustrates how methane is produced from the rumen in cattle. Plant matter is digested in cows and broken down into fatty acids, carbon dioxide, and hydrogen. Archaea called methanogens consume carbon dioxide and hydrogen to produce methane.*

in livestock, methanotrophs can be used indirectly to reduce the methane produced by methanogens through techniques like methane capture. Promising developments and research have been conducted on methanotrophs, showing that editing methane-related microbes is possible. Rumah *et al*. constructed and validated a CRISPR/Cas9 genome editing system capable of making gene deletions and insertions in both Type I (*Methylococcus capsulatus* Bath) and Type II (*Methylocystis parvus* OBBP) methanotrophs. They built modular CRISPR/Cas9 plasmids based on pMTL9BR2 backbones that carried the Cas9 gene, sgRNA, and repair templates with homology arms on each side of the target gene, allowing scarless editing through HDR. The researchers first attempted to delete the carbon-storage gene phaC, but were unsuccessful, which they attributed to low homologous recombination efficiency in *M. parvus* OBBP, likely due to interference from the DNA repair pathway involving ligD. They then successfully deleted ligD, a DNA repair gene, which confirmed that their CRISPR system works in methanotrophs. Editing efficiencies varied but reached as high as 90%. The team also incorporated a fluorescent reporter gene with up to 60% efficiency, demonstrating that their system can do both gene deletion and insertion. To optimize outcomes, they tested different promoters, homology arm lengths, and conjugation times, showing that keeping Cas9 at moderate levels prevented it from harming the cells while still allowing efficient genome editing (45). A similar approach could be used to target methane-related genes.

Despite these technical advances, CRISPR-based methane mitigation remains at a comparatively early stage of development. Unlike disease resistance or growth-related edits, which act directly on the livestock genome and have demonstrated stable inheritance and clear phenotypic outcomes, methane-focused strategies rely on modifying complex microbial ecosystems within the rumen or through indirect biotechnological interventions. Challenges such as maintaining edited microbial populations, ensuring long-term stability, and avoiding unintended ecological consequences limit immediate translational readiness. Additionally, regulatory pathways for releasing genetically modified microbes into agricultural systems remain underdeveloped, further slowing real-world deployment.

ETHICS

Most CRISPR-based livestock editing is performed at the embryonic stage to ensure uniform genomic modification across all cells. While this approach is often considered acceptable in agricultural contexts, it raises ethical concerns related to animal welfare, unintended suffering, and irreversible genetic changes. Gene drives, in particular, pose heightened ecological risks due to their ability to spread traits rapidly and uncontrollably across populations, potentially affecting both farmed and wild species. As a result, their use in livestock remains largely theoretical. Regulatory oversight represents another major barrier. Livestock genome editing intersects with food safety regulations, animal welfare standards, and environmental protection laws, all of which vary significantly across countries. Long-term safety assessments and post-approval monitoring are likely to be required, increasing the cost and complexity of deployment. Public acceptance also plays a critical role. Consumers may view gene-edited animal products as unnatural or risky, influencing market adoption regardless of scientific evidence. Balancing economic benefits with public trust, transparency, and equity—particularly between large industrial producers and small-scale farmers—remains an ongoing ethical challenge. Comparisons to human germline editing further complicate public perception. The case of He Jiankui, who used CRISPR to edit human embryos without proper oversight, illustrates the consequences of ethical violations and has reinforced global caution around germline modification (46). Opinions on whether these ethical considerations should be extended to livestock are mixed, but ethical standards for livestock are generally more permissive.

CONCLUSION

CRISPR represents an innovative technology with the potential to redefine livestock production. New research and developments open the possibility of creating a more sustainable and resilient livestock industry that not only meets food demands but also promotes efficiency and reduces environmental impact. Additionally, the successful translation of CRISPR-based livestock technologies will depend on public acceptance and broader social considerations. Interdisciplinary studies integrating regulatory science, consumer perspectives, and ethical frameworks will be needed to ensure responsible deployment. Advancing CRISPR applications in these areas could enable a livestock industry that is simultaneously more productive, environmentally sustainable, and socially acceptable.

CONFLICT OF INTEREST

The author declares that there are no conflicts of interest related to this work.

REFERENCES

1. Industry at a Glance | Meat Institute [Internet]. Available from: https://www.meatinstitute.org/Industry_at_a_Glance (accessed on 2025-07-23).
2. Statista [Internet]. U.S. egg production: total number laid 2023. Available from: https://www.statista.com/statistics/196096/total-egg-production-in-the-us-since-2000/ (accessed on 2025-07-23).
3. Dairy Data | Economic Research Service [Internet]. Available from: https://www.ers.usda.gov/data-products/dairy-data (accessed on 2025-07-23).
4. Biggest Issues in Animal Agriculture & Ways To Overcome Them [Internet]. 2024. Available from: https://agtech.folio3.com/blogs/most-common-issues-in-animal-agriculture/ (accessed on 2025-07-23).
5. Ro C. Big Problems With Big Livestock Farms. Forbes [Internet]. Available from: https://www.forbes.com/sites/christinero/2024/09/16/big-problems-with-big-livestock-farms/ (accessed on 2025-07-23).
6. Rouet P, Smih F, Jasin M. Introduction of Double-Strand Breaks into the Genome of Mouse Cells by Expression of a Rare-Cutting Endonuclease. *MOL CELL BIOL*. 1994; 14. https://doi.org/10.1128/MCB.14.12.8096
7. Heyer WD, Ehmsen KT, Liu J. Regulation of Homologous Recombination in Eukaryotes. *Annu Rev Genet*. 2010 Dec 1; 44 (1): 113-39. https://doi.org/10.1146/annurev-genet-051710-150955
8. Holtkamp D, Kliebenstein J, Neumann E, Zimmerman J, Rotto H, Yoder T, *et al*. Assessment of the economic impact of porcine reproductive and respiratory syndrome virus on United States pork producers. *J Swine Health Prod*. 2013 Mar 1; 21 (2): 72-84. https://doi.org/10.54846/jshap/754
9. Islam MA, Große-Brinkhaus C, Pröll MJ, Uddin MJ, *et al*. Deciphering transcriptome profiles of peripheral blood mononuclear cells in response to PRRSV vaccination in pigs. *BMC Genomics*. 2016 Dec [cited 2025 July 28]; 17 (1). https://doi.org/10.1186/s12864-016-2849-1
10. Van Gorp H, Van Breedam W, Delputte PL, Nauwynck HJ. The porcine reproductive and respiratory syndrome virus requires trafficking through CD163-positive early endosomes, but not late endosomes, for productive infection. *Arch Virol*. 2009; 154 (12): 1939-43. https://doi.org/10.1007/s00705-009-0527-1
11. Whitworth KM, Rowland RRR, Ewen CL, Trible BR, *et al*. Gene-edited pigs are protected from porcine reproductive and respiratory syndrome virus. *Nat Biotechnol*. 2016 Jan 1; 34 (1): 20-2. https://doi.org/10.1038/nbt.3434
12. Madani T. Avian influenza: Reasons for concern and the challenges ahead. *J Fam Community Med*. 2005; 12 (3): 113. https://doi.org/10.4103/2230-8229.97574
13. Long JS, Giotis ES, Moncorgé O, Frise R, Mistry B, James J, *et al*. Species difference in ANP32A underlies influenza A virus polymerase host restriction. *Nature*. 2016 Jan 7; 529 (7584): 101-4. https://doi.org/10.1038/nature16474
14. Payne LN, Nair V. The long view: 40 years of avian leukosis research. *Avian Pathol*. 2012 Feb; 41 (1): 11-9. https://doi.org/10.1080/03079457.2011.646237
15. Adkins HB, Brojatsch J, Young JAT. Identification and Characterization of a Shared TNFR-Related Receptor for Subgroup B, D, and E Avian Leukosis Viruses Reveal Cysteine Residues Required Specifically for Subgroup E Viral Entry. *J Virol*. 2000 Apr 15; 74 (8): 3572-8. https://doi.org/10.1128/JVI.74.8.3572-3578.2000
16. Klucking S, Adkins HB, Young JAT. Resistance to Infection by Subgroups B, D, and E Avian Sarcoma and Leukosis Viruses Is Explained by a Premature Stop Codon within a Resistance Allele of the tvb Receptor Gene. *J Virol*. 2002 Aug; 76 (15): 7918-21. https://doi.org/10.1128/JVI.76.15.7918-7921.2002
17. Lee HJ, Lee KY, Park YH, Choi HJ, *et al*. Acquisition of resistance to avian leukosis virus subgroup B through mutations on tvb cysteine-rich domains in DF-1 chicken fibroblasts. Vet Res [Internet]. 2017 Dec [cited 2025 July 30]; 48 (1). Available from: http://veterinaryresearch.biomedcentral.com/articles/10.1186/s13567-017-0454-1. https://doi.org/10.1186/s13567-017-0454-1
18. Shen B, Zhang W, Zhang J, Zhou J, *et al*. Efficient genome modification by CRISPR-Cas9 nickase with minimal off-target effects. *Nat Methods*. 2014 Apr; 11 (4): 399-402. https://doi.org/10.1038/nmeth.2857
19. Michel AL, Müller B, van Helden PD. Mycobacterium bovis at the animal-human interface: a problem, or not? *Vet Microbiol*. 2010 Jan 27; 140 (3-4): 371-81. https://doi.org/10.1016/j.vetmic.2009.08.029
20. Lillico SG, Proudfoot C, King TJ, Tan W, *et al*. Mammalian interspecies substitution of immune

modulatory alleles by genome editing. *Sci Rep.* 2016; 6: 21645. https://doi.org/10.1038/srep21645
21. Tuggle CK, Waters WR. Tuberculosis-resistant transgenic cattle. *Proc Natl Acad Sci USA*. 2015 Mar 31; 112 (13): 3854-5. https://doi.org/10.1073/pnas.1502972112
22. Wilmut I, Bai Y, Taylor J. Somatic cell nuclear transfer: origins, the present position and future opportunities. *Philos Trans R Soc B Biol Sci*. 2015 Oct 19; 370 (1680): 20140366. https://doi.org/10.1098/rstb.2014.0366
23. Burt A. Site-specific selfish genes as tools for the control and genetic engineering of natural populations. *Proc Biol Sci*. 2003 May 7; 270 (1518): 921-8. https://doi.org/10.1098/rspb.2002.2319
24. Hammond A, Galizi R, Kyrou K, Simoni A, *et al*. A CRISPR-Cas9 gene drive system targeting female reproduction in the malaria mosquito vector Anopheles gambiae. *Nat Biotechnol.* 2016 Jan; 34 (1): 78-83. https://doi.org/10.1038/nbt.3439
25. Courtier-Orgogozo V, Danchin A, Gouyon PH, Boëte C. Evaluating the probability of CRISPR-based gene drive contaminating another species. *Evol Appl*. 2020 Sept; 13 (8): 1888-905. https://doi.org/10.1111/eva.12939
26. Abudayyeh OO, Gootenberg JS, Konermann S, Joung J, *et al*. C2c2 is a single-component programmable RNA-guided RNA-targeting CRISPR effector. *Science*. 2016 Aug 5; 353 (6299): aaf5573. https://doi.org/10.1126/science.aaf5573
27. Chen JS, Ma E, Harrington LB, Da Costa M, *et al*. CRISPR-Cas12a target binding unleashes indiscriminate single-stranded DNase activity. *Science*. 2018 Apr 27; 360 (6387): 436-9. https://doi.org/10.1126/science.aar6245
28. Abudayyeh OO, Gootenberg JS, Konermann S, Joung J, *et al*. C2c2 is a single-component programmable RNA-guided RNA-targeting CRISPR effector. *Science*. 2016 Aug 5; 353 (6299): aaf5573. https://doi.org/10.1126/science.aaf5573
29. Li SY, Cheng QX, Wang JM, Li XY, *et al*. CRISPR-Cas12a-assisted nucleic acid detection. *Cell Discov*. 2018; 4: 20. https://doi.org/10.1038/s41421-018-0028-z
30. Gootenberg JS, Abudayyeh OO, Lee JW, Essletzbichler P, *et al*. Nucleic acid detection with CRISPR-Cas13a/C2c2. *Science.* 2017 Apr 28; 356 (6336): 438-42. https://doi.org/10.1126/science.aam9321
31. Stokol T, Hasler C, Carlson SC, Behboudi S, *et al*. The genomes of precision edited cloned calves show no evidence for off-target events or increased de novo mutagenesis [Internet]. 2021. Available from: https://pubmed.ncbi.nlm.nih.gov/34139989/ (accessed on 2026-01-13).
32. MSTN gene: MedlinePlus Genetics [Internet]. Available from: https://medlineplus.gov/genetics/gene/mstn/ (accessed on 2025-08-12).
33. Kim GD, Lee JH, Song S, Kim SW, *et al*. Generation of myostatin-knockout chickens mediated by D10A-Cas9 nickase. *FASEB J.* 2020; 34 (4): 5688-96. https://doi.org/10.1096/fj.201903035R
34. Crispo M, Mulet AP, Tesson L, Barrera N, *et al*. Efficient Generation of Myostatin Knock-Out Sheep Using CRISPR/Cas9 Technology and Microinjection into Zygotes. *PLoS ONE*. 2015 Aug 25; 10 (8): e0136690. https://doi.org/10.1371/journal.pone.0136690
35. Oishi I, Yoshii K, Miyahara D, Kagami H, Tagami T. Targeted mutagenesis in chicken using CRISPR/Cas9 system. *Sci Rep.* 2016 Apr 6; 6: 23980. https://doi.org/10.1038/srep23980
36. Gu H, Zhou Y, Yang J, Li J, *et al*. Targeted overexpression of PPARγ in skeletal muscle by random insertion and CRISPR/Cas9 transgenic pig cloning enhances oxidative fiber formation and intramuscular fat deposition. *FASEB J Off Publ Fed Am Soc Exp Biol*. 2021 Feb; 35 (2): e21308. https://doi.org/10.1096/fj.202001812RR
37. Quinton AM. UC Davis. 2023 [cited 2025 Sept 7]. Can CRISPR Cut Methane Emissions From Cow Guts? Available from: https://www.ucdavis.edu/food/news/can-crispr-cut-methane-emissions-cow-guts
38. Farkas JA, Picking JW, Santangelo TJ. Genetic techniques for the archaea. *Annu Rev Genet*. 2013; 47: 539-61. https://doi.org/10.1146/annurev-genet-111212-133225
39. Lange M, Ahring BK. A comprehensive study into the molecular methodology and molecular biology of methanogenic Archaea. *FEMS Microbiol Rev.* 2001 Dec; 25 (5): 553-71. https://doi.org/10.1111/j.1574-6976.2001.tb00591.x
40. Gautier A. Fluorescence-Activating and Absorption-Shifting Tags for Advanced Imaging and Biosensing. *Acc Chem Res*. 2022 Nov 1; 55 (21): 3125-35. https://doi.org/10.1021/acs.accounts.2c00098
41. Thomsen J, Schmitz RA. Generating a Small Shuttle Vector for Effective Genetic Engineering of Methanosarcina mazei Allowed First Insights in Plasmid Replication Mechanism in the Methanoarchaeon. *Int J Mol Sci*. 2022 Oct 7; 23 (19): 11910. https://doi.org/10.3390/ijms231911910

42. Xu Q, Du Q, Gao J, Chen L, Dong X, Li J. A robust genetic toolbox for fine-tuning gene expression in the CO2-Fixing methanogenic archaeon Methanococcus maripaludis. *Metab Eng.* 2023 Sept; 79: 130-45. https://doi.org/10.1016/j.ymben.2023.07.007
43. Li J, Zhang B, Zhou L, Qi L, *et al*. The archaeal RNA chaperone TRAM0076 shapes the transcriptome and optimizes the growth of Methanococcus maripaludis. *PLoS Genet*. 2019 Aug; 15 (8): e1008328. https://doi.org/10.1371/journal.pgen.1008328
44. Fonseca DR, Halim MFA, Holten MP, Costa KC. Type IV-Like Pili Facilitate Transformation in Naturally Competent Archaea. *J Bacteriol*. 2020 Oct 8; 202 (21): e00355-20. https://doi.org/10.1128/JB.00355-20
45. Rumah BL, Claxton Stevens BH, Yeboah JE, Stead CE, *et al*. *In Vivo* Genome Editing in Type I and II Methanotrophs Using a CRISPR/Cas9 System. *ACS Synth Biol.* 2023 Jan 23; 12 (2): 544-54. https://doi.org/10.1021/acssynbio.2c00554