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CRISPR-based gene editing for disease resistance in livestock: Current advances and future perspectives

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Abstract

Recent progress in CRISPR/Cas-based genome editing technologies has permitted precise genetic alterations in cattle that have the potential to generate disease resistant animals, as well as increase animal welfare and production. This study offers an in-depth and up-to-date overview of CRISPR applications for disease resistance in important agricultural animals (cattle, pigs, sheep, goats and poultry). We start by looking at the history of the evolution of genetic editing, from early transgenic approaches, to zinc-finger nucleases, to the CRISPR era. We analyse the technical specifications of several CRISPR programs (Cas9, Cas12a/b, Cas13, base and prime editors) including target preferences, kinds of edits, efficiency and off-target profiles. We discuss the many delivery techniques and compare microinjection, electroporation, viral vectors and cell-transfer technologies, each with its own merits and drawbacks. Strategies to minimize off-target effects, such as high-fidelity nucleases and guide RNA design, are reviewed. We also cover integration of breeding including the influence of mosaicism and founder animal selection on herd deployment. A supplementary table covers regulatory and ethical frameworks across jurisdictions: for example, in the USA, gene-edited animals are regulated as veterinary pharmaceuticals (FDA), but in Argentina and Brazil non-transgenic changes are generally free from GMO restrictions. Ethical issues are discussed, including animal welfare, customer acceptability and labelling rules. Socio-economic consequences are discussed, including possible cost savings from decreased illness burden and worries about market access.

Keywords: Disease, CRISPR, electroporation, RNA design, cattle

Introduction

The notion of genetically engineering animals has changed substantially during the last four decades. The first transgenic farm animals were generated via DNA microinjection into pronuclei throughout the 1980s and 1990s^[1, 2]. For instance, it was stated that pigs had been given human growth hormone and dairy cows had been given additional genes for milk proteins. But these methods incorporated DNA randomly and generally with poor efficiency^[3]. The 1990s also witnessed the emergence of somatic cell nuclear transfer (cloning) culminating in Dolly the sheep (1996)-a landmark that showed the feasibility of reprogramming adult cell genomes into live animals. This period was one of trial and error, not refined editing^[4]. Engineered nucleases made targeted editing possible in the early 2000s. Precise gene disruptions or substitutions were made possible by zinc-finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALENs). These techniques were complicated and needed bespoke protein creation for each target, but they demonstrated the feasibility of site-specific editing in animals. Early examples of genome editing in big animals include the use of ZFN and TALENs in the 2010s to knock out genes involved in horn development and muscle growth in cattle, pigs and sheep^[5].

In 2012-2013, the bacterial CRISPR/Cas systems were revolutionarily adapted for genome editing. Specifically, the CRISPR/Cas9 technology provided simplicity and programmability: a single-guide RNA (sgRNA) could target the Cas9 nuclease to almost any DNA sequence next to an NGG "PAM" motif^[6]. Within one year of its first use in cell cultures, CRISPR/Cas9 was shown to work in real animals. First reports of CRISPR-edited cattle came in 2014 from many organizations^[7].

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For example, CRISPR has been used to knock down the myostatin (MSTN) gene in pigs, producing animals with enhanced muscle mass. Around the same time, cow embryos were edited for qualities like being hornless (“polled”) by adding a known hornless gene^[8]. Sheep and goats also were modified, for example to change coat color or to add desirable wool qualities. These early victories proved that CRISPR could make targeted alterations in a wide range of organisms^[9].

Soon the emphasis was on health qualities. There have been a number of high impact incidents between 2015 and 2018. One important instance was the production of pigs with a deletion in exon 7 of the CD163 gene, which totally blocked infection by porcine reproductive and respiratory syndrome virus (PRRSV)^[10]. The results of this study show that the deletion of a single receptor may result in total resistance in challenge studies. Scientists also used genetic modification in bovine embryos to add the NRAMP1 gene, which encodes an immunological transporter that lowers the risk of *Mycobacterium bovis*, the bacteria that causes bovine TB. Poultry scientists have aimed at avian influenza susceptibility by altering host factors such as ANP32A, which is necessary for influenza virus replication^[11].

Meanwhile, CRISPR equipment itself became better. New Cas enzymes (Cas12a, Cas13, Cas12e/CasX, Cas14) have been found or developed. These broaden the sorts of edits that may be made (e.g. single-strand cuts or RNA targeting)^[12]. In 2016-2019 base editors (that chemically change one DNA base into another without double-strand breaks) and prime editors (that employ a reverse transcriptase to generate new DNA sequences without templates) were launched. Now, such techniques are being used on animals to insert precise point mutations for qualities like as disease resistance or to fix genetic abnormalities^[13]. To put it simply, gene modification in cattle has a history from random transgenesis and cloning to the precise age of CRISPR. With each new advance in technology, efficiency, specificity and scope have risen. Many lines of disease resistant livestock are now in research or testing. This is an unprecedented time when genetic treatments may be used in combination with vaccinations and management for animal health^[14].

CRISPR Systems and Variants

CRISPR/Cas systems are essentially a class of RNA-guided nucleases originated from the adaptive immune systems of bacteria. They act as programmed scissors for DNA (or RNA, in the case of Cas13) in genome editing. The most popular system is *Streptococcus pyogenes* Cas9 (SpCas9) and needs a “NGG” protospacer adjacent motif (PAM) immediately after the target sequence^[15]. The Cas9-sgRNA complex attaches to the target DNA by base pairing of the sgRNA to its complementary strand and Cas9 cleaves both strands of DNA to generate a double-strand break (DSB)^[16]. The cell’s repair mechanism subsequently re-ligates the DNA, often improperly via non-homologous end joining (NHEJ), resulting in insertions or deletions (indels) that disrupt genes. Alternatively, in the presence of a repair template, precise modifications or gene insertions may be made using homology directed repair (HDR)^[17]. Beyond SpCas9, many CRISPR variants are in use or development (see Table 1). Each has distinct features that affect its suitability for livestock editing:

- **High-fidelity Cas9 (e.g. SpCas9-HF1, eSpCas9):** Mutations minimize off-target cutting by reducing non-specific DNA contacts. In most applications they tend to keep high on-target activity. For cattle, high-fidelity variations may increase the safety profile of modifications, but with occasionally a little trade off in editing speed^[17, 18].
- **Cas12a (Cpf1):** This enzyme (from *Acidaminococcus* or *Lachnospiraceae*) recognizes a “TTTV” PAM (T = thymine, V = A/C/G) on the 5’ side of the target and chops DNA to leave staggered 5’ overhangs. It has smaller sgRNA (crRNA) and staggered cuts which might help integration of donor DNA. Cas12a has been effectively employed in mammalian zygotes and may be beneficial in species or genomic areas with TTTV PAMs^[19].
- **Cas12b (C2c1):** Another DNA nuclease, again with a TTN PAM. It is smaller than SpCas9, but not as well characterized. Early investigations reveal it is active in mammalian cells and hence has promise for livestock editing when other techniques are limited by space or PAM limits^[20].
- **CasX/Cas12e:** A small (~1000 amino acids) enzyme from a recently discovered CRISPR family. It recognizes a T-rich PAM and makes staggered cuts. Its compact size is appealing for delivery and initial experiments show great specificity. CasX/Cas12e may be effective for difficult targets but is not yet extensively employed in animals^[21, 22].
- **Cas14:** A very tiny nuclease (<500 aa) that cleaves ssDNA without a protospacer need. Its action is mostly of relevance for diagnostics but offers promise for fine editing of single-stranded DNA in cells (albeit as far unproven in animal embryos)^[23].
- **Cas13a/13d (RNA-targeting Cas):** Cas13 systems bind and cut single-stranded RNA, which is different from Cas9/Cas12. No PAM is required. In livestock, Cas13 might be used for degrading viral RNA in infected animals or to post-transcriptionally transiently shut down expression of host genes^[24]. Of particular importance, Cas13 may undertake collateral RNA cleavage which calls for cautious management, although catalytically inactivated Cas13 (dCas13) linked to effectors can control splicing or translation. These RNA edits are transient and do not affect the genome, enabling a non-heritable method for investigating gene function or treating infections^[25].
- **Base Editors (cytosine and adenine base editors):** These are fusion proteins of a catalytically deficient Cas9 (nickase or dead Cas9) and a deaminase enzyme. Cytosine base editors (CBEs) convert C·G base pairs to T·A while adenine base editors (ABEs) convert A·T to G·C. They just need single-strand DNA substrates (which are produced in the CRISPR R-loop) and do not need DSBs^[26]. Point mutations are introduced with great precision using base editors, such as the conversion of a single codon to simulate a naturally resistant allele or the insertion of a stop codon to wipe off a gene. In cattle, base editors may be used to insert or correct SNPs linked to diseases without causing broad disturbance in the genome^[27]. For example, one base modification in the bovine CSN2 gene may alter milk protein composition and similar changes might one day be used to target variations of immunological

signaling. The editing window is typically ~5 bases long so bystander modifications are conceivable and cautious sgRNA design is needed [28].

- **Prime Editors:** A recent invention, prime editors merge a Cas9 nickase with a reverse transcriptase. They employ a primary editing guide RNA (pegRNA) that directs the nickase and encodes the desired edit [29]. When one strand of the DNA is cut, the RT replicates the edit from the pegRNA into the genome. Prime editing may introduce tiny insertions, deletions or any substitution from one base to another, again without the need for a donor template or generating DSBs [30]. This is especially promising for livestock in cases where

precision insertion of tiny regulatory elements or repair of harmful mutations is required. The most versatile is prime editing, however it is less efficient than normal Cas9 [31].

- **CRISPRi/CRISPRa:** Dead Cas9 (dCas9) systems may reduce (CRISPR inhibition) or enhance (CRISPR activation) gene expression by inhibiting or recruiting transcriptional machinery. In theory they may be utilized in animal cells or transgenic lines to control immune genes without changing the DNA sequence. For lasting resistance features, DNA editing is normally favored, while CRISPRi/a may be beneficial in fundamental research or designed vaccinations [32, 33].

Table 1: CRISPR/Cas systems and their features [34, 35, 36, 37, 38, 39, 40]

System	Target & PAM	Cleavage Type	Main Features
SpCas9 (wild-type)	DNA (NGG PAM)	Blunt DSB	Widely used, high efficiency, off-target risk
SpCas9-HF1, eSpCas9	DNA (NGG PAM)	Blunt DSB	Engineered for higher specificity
HypaCas9	DNA (NGG PAM)	Blunt DSB	High specificity (mutations in REC3 domain)
Cas12a (Cpf1)	DNA (TTTV PAM)	Staggered DSB (5' overhang)	Uses shorter guide RNA; cuts distal to PAM
Cas12b (C2c1)	DNA (TTN/TTN PAM)	Staggered DSB	Smaller nuclease
CasX (Cas12e)	DNA (5'-protospacer)	Staggered DSB	Very small, novel PAM flexibility
Cas14	ssDNA (no PAM)	ssDNA cleavage	Ultra-small; targeted ssDNA
Cas13a/Cas13d	ssRNA (no PAM)	ssRNA cleavage (collateral)	Targets RNA, no DNA cut, interference
Base Editor (CBE)	DNA (NGG PAM)	Base deamination (C→T)	No DSB; bystander edits possible
Base Editor (ABE)	DNA (NGG PAM)	Base deamination (A→G)	No DSB; limited editing window
Prime Editor (PE)	DNA (NGG PAM)	RT-mediated insert/substitute	Versatile edits; lower efficiency

Delivery Methods for CRISPR Reagents in Livestock

- The most frequent method of cattle germline modification in practice is still zygote microinjection or electroporation. For instance, pig zygotes are routinely injected with a Cas9-sgRNA RNP combination and cow zygotes are often electro-orated with Cas9 RNPs with remarkably high survival rates. These approaches result in founder (F0) animals with DNA editing from the one-cell stage. One big problem is mosaicism. If the editing happens after the first division of the cell, then the person may have some edited and some unedited cells. Timing is important since in certain cases electroporation may lessen mosaicism by altering several zygotes concurrently at the earliest time point [41, 42].
- Another avenue is somatic cell nuclear transfer: fibroblasts or other cells are edited in culture (e.g., by plasmid transfection or RNP electroporation), screened for the desired edit (and lack of off-target mutations) and then utilized to generate cloned embryos. This was the approach in the first “genetically modified pig” with the GFP transgene in 1985 and certain gene-edited animals (e.g., early pigs without PERVs). The benefit is that the resultant cloned animal is not mosaic and contains the mutation in all cells. However, SCNT is low-yield (typically <5% of rebuilt embryos become live born) and may bring epigenetic problems [43].
- For somatic treatments, the relevance of breeding is less than for non-invasive approaches such as delivery of editing reagents to mature animals (e.g., using viral vectors). “Usually we want to make the change heritable for disease resistance.” Some researchers are investigating *in vivo* techniques like as the “GONAD” approach in mice (ovarian electroporation after

breeding) to alter embryos within the mother without taking them out, but there have been no successes reported in cattle yet [44, 45].

- Delivery into poultry is unusual in that instead of zygote injection, which is impracticable in birds, avian primordial germ cells or blastoderm cells may be cultured, transfected or transduced and then injected into recipient embryos. Embryos from which chickens are born may pass the edit on in the germline. This technique has been used to make gene-edited hens (for example, knocking off certain immunological genes to research viral resistance) [46, 47].
- The choice of distribution method is therefore dependent on species, available equipment and the trade-off between throughput and accuracy. The field is continually evolving novel carriers such as biodegradable nanoparticles or virus-like particles in order to achieve safe and effective germline delivery. The technical challenge faced by commercial farms today is to scale up, producing dozens or hundreds of modified embryos [48, 49].

Off-Target Effects, Efficiency Metrics and Phenotypic Outcomes

Off-target cleavage is a critical concern. A guide RNA might bind and direct Cas nuclease to a DNA site that is similar but not identical to the intended target, causing unintended edits. In livestock, such off-target mutations could potentially affect other genes (possibly those related to health or reproduction) [50]. Researchers mitigate off-targets by:

- **Engineered nucleases:** High-fidelity Cas9 variants (e.g. SpCas9-HF1, eSpCas9, HypaCas9) dramatically reduce off-target activity by weakening non-specific

DNA contacts. These have become standard in many animal editing labs ^[51].

- **Guide RNA design:** Using computational tools, sgRNAs are chosen to have minimal predicted off-target matches in the genome. Shortening the guide by a few nucleotides at the 5' end (truncated sgRNAs) can also increase specificity ^[52].
- **RNP delivery:** Using pre-assembled Cas9 protein-guide complexes (RNPs) instead of plasmid or viral delivery ensures that the nuclease is present only transiently (often degrading within 24 hours), reducing the window for off-target cleavage. Plasmids, by contrast, may lead to prolonged Cas9 expression ^[53].
- **Screening and validation:** Whole-genome sequencing or targeted deep sequencing of founder animals is used to check for undesired edits. While beyond the scope of breeding, initial research animals often undergo such scrutiny. New techniques like GUIDE-seq or Digenome-seq are adapted to animal cells to map potential off-target sites ^[54].

Efficiency metrics quantify editing success. Common measures include:

- **Editing rate per embryo/zygote:** e.g. percentage of injected embryos that yield live offspring carrying any edit ^[55].
- **Allelic editing rate:** Among live offspring, percentage carrying the desired edit on at least one allele. This could be heterozygous or homozygous ^[56].
- **Biallelic (knockout) rate:** Fraction of animals where both gene copies are modified (often the goal for a recessive trait) ^[57].
- **Knock-in efficiency:** For HDR or targeted insertion, the percent of animals with the precise insertion (usually lower than NHEJ rates) ^[58].
- **Mosaicism rate:** Proportion of founders that are mosaic (having a mixture of edited and unedited cells in their tissues). Reducing mosaicism is a key aim, as mosaic animals often transmit only some edits to offspring ^[59].
- Livestock editing efficiency varies. For example, CRISPR knockouts in pigs are typically 30-70% edited in live pigs (depending on the guide and technique), but the knock-ins may fail in a few percent owing to the reduced efficacy of HDR in embryos. Electroporation of pig zygotes has been reported to result in ~50% gene disruption. Cattle editing is usually less efficient, in part because of more fragile embryos, with editing rates in live calves often reported at <30%. Challenges are comparable for sheep and goats. These figures are slowly improving with time and improved practices ^[60, 61].
- Phenotypic effects should be carefully assessed. But it's not only about identifying a change in DNA—researchers conduct illness challenge testing, production trait

measures and health checks. For instance: The edited pigs, which did not have CD163, were subjected to PRRSV and stayed healthy, showing almost little viremia and normal life lengths. Calves altered for NRAMP1 have been challenged with bovine TB germs. They had considerably lower pathogen burdens in the lung and lymph tissues than controls, suggesting actual functional immunity ^[62, 63]. These animals are also examined for any side effects, such as body weight, fertility, milk production, temperament, etc. In all recorded examples of disease-resistant modifications, the modified animals looked normal, other from the heightened resistance. For example, PRNP (prion protein) knockout goats developed and reproduced properly, but they will require long-term surveillance to rule out modest neurological consequences that have been shown in PRNP-knockout mice ^[64, 65].

- **Integration into breeding:** Once the required genotype is established in a founder animal, breeders need to form a herd. Founders are frequently bred to each other or found wild type partners to fix the allele. If founders were mosaic, their progeny may have to isolate the completely edited genotype ^[66, 67]. If genetic alteration was done in an experimental strain originally, backcrossing may be required to restore the genome to a desirable breed background. Genetic linkage is also important: the closer the altered gene is to unwanted alleles, the more breeding steps are needed. Selection indices may be modified between generations to incorporate the new characteristic so that it is kept along with other favorable qualities ^[68, 69].
- **Biosafety:** Data on the health and wellbeing of altered animals is required by regulatory bodies. They do complete exams (bloodwork, organ histology, behaviour). Tests for toxicity and allergenicity are performed on products (meat, milk, eggs) of food animals, as for novel food kinds. Because CRISPR alterations don't often add foreign proteins, as transgenic animals do, many scientists claim the products are essentially the same as traditional animal products, but regulators frequently still demand testing. Biosecurity procedures in field usage (e.g. holding animals in enclosed facilities until safe to release) are suggested to avoid unintentional release ^[70].

Applications: Edited Traits and Diseases in Livestock

The power of CRISPR lies in its flexibility to target many genes across species. Table 2 lists representative examples of edited traits or disease resistances in various farm animals. Each row summarises the species, trait/disease addressed, the gene(s) edited, the editing method and the outcome observed in experiments. This illustrates the breadth of current work and outcomes ^[71, 72, 73, 74, 75, 76, 77].

Table 2: Applications: Edited Traits and Diseases in Livestock

Species	Trait/Disease	Gene(s) Edited	Editing Strategy
Cattle	Tuberculosis resistance	<i>NRAMP1</i> insertion	Homology-directed knock-in
Cattle	Polled (hornless) trait	<i>POLLED</i> allele	HDR-mediated allele swap
Cattle	Milk composition (A2 β -casein)	<i>CSN2</i> (A1→A2) SNP	Base editing (C→T)
Cattle	Predisposition to facial eczema	<i>TYR</i> mutation (UV defense)	CRISPR KO
Pig	PRRS virus resistance	<i>CD163</i> (SRCR domain)	Dual sgRNA-mediated deletion
Pig	African swine fever (ASFV) attempt	<i>CD163</i> knockout	CRISPR KO
Pig	Post-weaning diarrhoea (<i>E. coli</i> K88)	<i>FUT1</i> gene	Frameshift KO
Pig	Lean muscle growth	<i>MSTN</i> knockout	NHEJ-mediated KO
Pig	Quattrocolor pattern (coat color)	<i>KIT</i> locus	HDR knock-in of variant
Pig	Trichinella resistance (worm infection)	<i>TLR4</i> variant	Base editing (A→G)
Sheep	Scrapie prion disease resistance	<i>PRNP</i> knockout	CRISPR KO
Sheep	Scrapie (codon 171)	<i>PRNP</i> (Codon 171 G→V)	Base editing (A→G)
Goat	Scrapie resistance	<i>PRNP</i> frameshift	CRISPR KO
Goat	Increased fecundity (multiple kids)	<i>BMP15</i> (Follicle gene)	CRISPR KO/knock-in
Chicken	Avian influenza (HPAI) resistance	<i>ANP32A</i> mutations	CRISPR knock-in
Chicken	Avian leucosis virus susceptibility	<i>Tva</i> gene knockout	CRISPR KO

Socio-Economic Impacts and Public Acceptance

Beyond science and regulation, gene editing in livestock has broad socio-economic dimensions.

- Economic implications may be enormous, with illnesses such as foot-and-mouth, PRRS or avian flu costing billions annually. For example, PRRS alone costs the global swine business billions of dollars each year. If CRISPR can cut such illnesses significantly then farmers might enjoy cheaper treatment and culling expenses and increased yields^[78]. Modelling studies imply that a successful gene-edited disease-resistant line may boost profit margins by lowering mortality and antibiotic consumption. Edited pigs may, as the CLEAR Centre review on PRRS pointed out, provide more consistent output and fewer vet interventions, which should provide cheaper pork pricing and more reliable supply, in theory^[79].
- But economic gains may not be equal. Smaller farmers could not have access to such technology, possibly increasing disparities between major operations (who can pay breeding costs for modified stock) and smallholders. Biotech businesses could also hold the reins of intellectual property on CRISPR techniques (patents on Cas9 and certain modifications). To tackle this, several academics have called for open-source or publicly sponsored gene-editing initiatives for staple cattle^[80, 81].
- Market and trade considerations are important. Gene-edited animal goods might be denied entry in countries with strong rules. A USDA research pointed out that if an exporting nation allows a gene-edited animal but trade partners don't, problems might ensue. If US meat from altered pigs were shipped to the EU, they would be subject to GMO labelling under EU legislation. Thus international alignment or regional markets may arise first (e.g. NAFTA countries). Voluntary labelling of gene-edited items, customer choice certification systems might assist. Food-labelling also has trade ramifications^[82, 83].
- Public acceptance relies frequently on perceived advantage and trust. Surveys reported in academic evaluations reveal individuals are more accepting of modifying genes when they realize it might alleviate animal suffering (such as horned cattle or disease outbreaks). For instance, 72 percent of American consumers polled were supportive after being informed

of the advantages of PRRS-resistant pigs^[84]. The main worries are usually about “unnaturalness” and unexpected health consequences. Open disclosure that CRISPR modifications are precise and that modified goods are rigorously vetted will help allay worries. Suggest educational outreach (farm visits, scientific communication). Trust in regulatory authorities also matters, with public trust stronger when agencies such as the FDA or EFSA clearly examine edits^[85, 86].

- Ethical considerations include animal welfare (the balance between the stress of editing methods and long-term welfare improvements), naturalness and socio-cultural norms (certain cultures may reject changing animals). Genetic modification to eradicate illnesses is typically seen as morally good (preventing animal suffering)^[87, 88]. In contrast, merely productivity (muscle mass) modifications might be problematic. Concerns concerning biodiversity are also raised: if one altered line is used extensively, it might decrease the genetic variety of a species^[89]. This has to be managed by breeders via proper herd breeding procedures. Animal welfare ethicists note that any editing method must guaranty the animal's health is paramount for example, if a gene alteration had unknown side-effects, it would be morally wrong. Thus, broad phenotypic and health screening is not just regulatory but moral^[90].
- Overall, the socio-economic scene is uneven yet promising. Efficient, disease-resistant animals might contribute to food security, reduced use of chemicals (drugs and vaccinations) and benefit consumers. But rules must assure equal benefit access, build confidence by being transparent and handle ethical issues. Numerous stakeholders are advocating for the creation of global standards that integrate both scientific and social norms to facilitate the appropriate adoption of gene-edited livestock^[91, 92].

Future Perspectives and Research Priorities

Looking ahead, the field is moving rapidly. Key future directions include:

- Multiplex Editing:** Many cattle illnesses are polygenic or involve complicated immunological mechanisms. In the future, CRISPR will likely be used to edit numerous targets at once (stacking resistance genes or wiping off redundant susceptibility factors). That could mean administering numerous sgRNAs/Cas enzymes

simultaneously^[93]. Multiplex editing may pre-empt pathogen adaptability, but technically more hard (greater risk of mosaicism, embryo viability). For example, an altered chicken line missing ANP32A and likewise overexpressing a new anti-viral gene could provide more strong flu resistance^[94, 95].

- **Improved Delivery Systems:** Editing for commercial herds has to be scaled up using high-throughput technologies. "Progress could be in automated electroporation devices for large numbers of embryos or new chemical carriers that improve uptake." There is considerable interest in viral vectors for germline editing, for example by integrating small components of integration-deficient viruses that alter just target cells. In ovo electroporation (for avian embryos) may be a regular procedure^[96, 97].
- **New Cas Enzymes:** The discovery and adaption of new CRISPR nucleases continues. Recent reports of Cas proteins recognizing alternative PAMs (e.g., SpG, SpRY) already broaden the areas that may be targeted. Smaller Cas proteins (e.g. CasX) might facilitate mRNA transport. RNA-targeting CRISPR (Cas13) might have antiviral implications in animals. Epigenome editors (fused dCas9 to modifiers of DNA methylation or histones) might also be used as tools to turn immune genes on or off without affecting DNA sequence^[98, 99].
- **Base and Prime Editing Optimization:** We focus on the optimization of base/prime editors to improve efficiency and minimize unintentional changes in embryos. These methods could be more effective in zygotes using better deaminases or reverse transcriptases, improved pegRNA designs, or cofactors that skew repair pathways. With high-efficiency base editing in cattle, one may precisely introduce disease-resistant alleles known from population genetics^[100].
- **Safety and Off-target Profiling:** As additional alterations approach commercialization, extensive sequencing of complete genomes of modified lines will become commonplace. Across generations, long-term investigations of altered animals will search for any delayed health problems. Further development of machine learning algorithms to anticipate off-targets, based on massive sequencing datasets, will be performed. Some laboratories are studying ways to "self-destruct" Cas9 after editing (by adding degron tags or utilizing tiny chemicals) to minimize any residual activity^[101].
- **Regulatory Science:** The demand is for harmonized worldwide rules on the risk evaluation of gene-edited cattle. Research on comparative safety of altered animals (e.g. compositional analysis of meat/milk vs conventional) is crucial to educate regulators and the public. Socio-economic research on impediments to adoption, cost-benefit analysis and influence on trade will provide information for policy makers. Research on consumer attitudes, animal welfare and fair technology dissemination will be vital as well^[102].
- **Translational Pathways:** Moving altered characteristics from lab to field into breeding programs. This may include modified bulls or boars being used as top sires in artificial insemination networks and therefore spreading the edit fast. Business models might include licensing elite edited lines. Part of research emphasis is

development of strong phenotyping tools (automatic health monitoring in barns) to assess altered animals in actual farm environments, beyond controlled challenges^[103, 104].

- **Disease Prioritization:** Lists of the most important livestock diseases without adequate remedies are being consolidated by researchers (e.g. African swine fever, Peste des petits ruminants, avian influenza, mastitis). CRISPR initiatives may be aimed at these objectives. Modifications for adaptive qualities (heat tolerance, low-nutrient settings) will be more important as climate change affects farming. Similarly, many laboratories might develop collaborative projects comparable to human genetics consortia sharing modified animal lines, data and best practices^[105].
- **Public interaction and Policy Co-Development:** Future views must involve multi-stakeholder interaction. Genetic modification should be shaped to meet genuine requirements in acceptable ways and scientists, ethicists, farmers and consumer representatives should co-design research agendas to achieve this. Establishing confidence will depend on educational outreach and open communication tactics^[106].

Conclusion

Genome editing and in particular the technical advancement of CRISPR/Cas, is rapidly changing the landscape of animal agriculture. Based on this work, a comprehensive overview of CRISPR based approaches to increase disease resistance in cattle has been presented. We tracked the evolution of technology, from the most ancient forms of transgenesis and precise editing to the more pliable types of CRISPR used today. We also discussed the use of these technologies to real-world health challenges. Tables comparing several CRISPR platforms and delivery methods were presented to demonstrate the wide range of tools available to researchers. Note that the development of modified traits, such as resistance to avian influenza in chickens and resistance to PRRS in pigs, provides convincing evidence that targeted genetic alterations may result in considerable gains in animal health. At the same time we examined the broader environment where this technology is pervasive. Off-target impacts and editing efficiency indicators were assessed together with breeding integration and biosafety protocols to make sure that transformed animals are both helpful and safe. The various regulatory and ethical environments were summarized with a focus on the manner in which public perception and policy impact the development and deployment of gene-edited cattle. Socio-economic problems were addressed to provide a realistic picture of how CRISPR breakthroughs may impact society. Consumer sentiments and the economics of farming were among the issues. Looking forward, we are at an inflection moment for CRISPR editing. Examples of the kind of technological advances that are constantly expanding the horizon of what is possible are high-fidelity nucleases and base/prime editors. Cooperation across research domains and countries will be necessary to bring these advances into commercial breeding. Future research should concentrate on multiplex disease resistance, improved screening for unintended consequences and straightforward discussion of advantages and dangers. But the achievement of CRISPR's promise in cattle ultimately hinges not only on technical progress but also on governance, ethics and public debate. The data

collected so far is promising: if properly constructed genetic modification may generate healthier animals without impairing their output or level of welfare. In the next decades, CRISPR-based gene modification has the potential to play an important role in insuring the sustainability and robustness of cattle production. With much investigation and ethical innovation this potential may be unleashed.

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