Gene Editing for Disease Resistance: Practice, Perception and Policy

Alison Van Eenennaam, PhD
Distinguished Professor of Extension
Animal Genomics and Biotechnology
Department of Animal Science
One Shields Avenue, Davis, CA 95616
University of California, Davis
Davis, CA 95616
Email: alvaneenennaam@ucdavis.edu

Abstract

Gene editing (GnEd) refers to the introduction of targeted changes into the genome of animals to result in a desired phenotype. There are 39 papers documenting edits intended to produce a disease resistance phenotype in animals for agricultural applications, two of which aimed to improve resilience to the bovine respiratory disease complex. The first of these was an edit to affect an amino acid substitution in the signal peptide of CD18, the β subunit of β2 integrins, to prevent *Mannheimia haemolytica* leukotoxin from binding to leukocytes and causing leukotoxin-induced cytolysis. The second was a 6 amino acid substitution in the bovine viral diarrhea virus (BVDV)-binding domain of the bovine CD46 gene which reduced susceptibility to BVDV infection. A UK-based company, Genus llc, has announced it plans to obtain regulatory approval and commercialize the first disease-resistant GnEd food animal, a porcine respiratory and reproductive syndrome (PRRS) virus-resistant pig GnEd at CD163. Three species of fast-growing GnEd fish have been commercialized in Japan. Despite expected pushback from the Japanese public and activist groups given the global experience with food from genetically modified organisms (GMOs), there was no sustained opposition to these fish in the marketplace, and media coverage was mostly positive. It was suggested that this was due to government-led innovation policy and improved regulatory governance, the fact that these products were

commercialized by a local Japanese-based university startup, and a new interest in both the sustainable development goals and environmental, social, and governance investing. Regulations regarding GnEd in animals are currently under development in many countries. Some countries are regulating GnEd animals that could have been achieved using conventional breeding (i.e. contain no foreign DNA) no differently to those produced by conventional breeding. Ultimately, the fate of GnEd in livestock will be reliant upon the development of risk-proportional, science-based regulatory frameworks.

Keywords: Gene editing, food-producing animal, livestock, disease resistance**,** regulations

Practice

Gene editing (GnEd) offers a powerful approach to introduce targeted alterations into the genome of livestock to achieve a desired outcome. Several groups are working on using GnEd to introduce disease resistance traits. The peer-reviewed literature of GnEd in livestock for agricultural applications (i.e. specifically excluding biomedical applications), ultimately resulting in the production of fetuses or live animals includes 39 papers targeting disease resistance; 8 in cattle, 20 in pigs, 5 in aquatic species, 5 in poultry, and one in an insect (**Table 1**). The editing system refers to the nuclease that was used to introduce the alteration. Site directed nuclease (SDN) applications have been categorized as one of three types; **SDN-1**: produces a double-stranded break in the genome without the addition of foreign DNA. The spontaneous repair of this break can lead to a modification or deletion, causing gene silencing, gene knockout (KO) or a change in the activity of a gene, **SDN-2**[1]: Produces a double-stranded break, and while the break is repaired by the cell, a small nucleotide template that is complementary to the target region is supplied, which is used by the cell to repair the break. The template contains one or several small sequence changes in the genomic code, that are copied into the animal's genetic material resulting in a modification of the target gene. and **SDN-3**: Also induces a double-stranded break in the DNA, but is accompanied by a template containing a gene or other sequence of genetic material. The cell's natural repair process then utilizes this template to repair

[1]This category is often associated with applications involving the insertion of genetic material into a recipient organism from a donor that is sexually compatible (crossable). Cisgenesis and intragenesis are genetic modifications involving genetic material obtained from outside the host organism and transferred to the host using various delivery strategies; the incorporated sequences contain an exact copy (cisgenesis) or a re-arranged copy (intragenesis) of sequences already present in the species or in a sexually compatible species. The meaning of bp size when referring to a “small” nucleotide template is ill-defined.

the break; resulting in the introduction of the genetic material. SDN-1 and SDN-2 animals do not contain inserted "foreign" DNA, while SDN-3 animals may contain "foreign" DNA - meaning DNA introduced from non-sexually compatible species (i.e. transgenic DNA) (Broothaerts et al., 2021).

Of particular interest to this audience is GnEd applications associated with the Bovine Respiratory Disease (BRD) complex. There are two specifically targeting this disease complex. The first was a paper from 2016 suggesting that an amino acid substitution introduced into the gene CD18, the β subunit of β2 integrins, by CRISPR-Cas9 editing in a cell line resulted in the cleavage of the signal peptide. The intact signal peptide binding site is normally where *Mannheimia haemolytica* leukotoxin binds uniquely to ruminant leukocytes resulting in acute inflammation and lung tissue damage.

A bovine GnEd fetus homozygous for the Q(–5)G at amino acid position 5 upstream of the signal peptide cleavage site was harvested and leukocytes were shown to be resistant to leukotoxin-induced cytolysis. The authors suggested that this could be used to produce lines of cattle genetically resistant to *M. haemolytica*-caused pneumonia (Shanthalingam et al., 2016). There are no peer-reviewed reports of the generation of live cattle with this genomic alteration.

The second paper targeting the bovine respiratory disease complex was the production of a calf GnEd to be resistant to bovine viral diarrhea virus (BVDV). This work, a collaboration between the USDA ARS Meat Animal Research Center, the University of Nebraska, and livestock genome-editing company Acceligen (Minnesota), produced a single Gir calf following cloning of an edited fibroblast cell line (**Figure 1**). The edit that was introduced involved substituting six amino acid A82LPTFS87 in the BVDV binding domain of bovine CD46. The calf with

demonstrated reduced susceptibility to infection following natural challenge by cohabitation with the BVDV-PI calf for 7 days as measured by reduced clinical signs and the lack of viral infection in white blood cells. The calf had no obvious adverse effects from the on-target edit in the first 20 months after birth.

There are other reports of cattle that have been edited in an attempt to introduce resilience to Trypanosomiasis (African Sleeping Sickness), a vector-borne parasitic disease caused by protozoans of the genus Trypanosoma, and transmitted to humans by bites of tsetse flies (glossina) which have acquired the parasites from infected humans or animals. These cattle will be edited to be both thermal-tolerant SLICK and also trypanosome resilient by editing the ferredoxin 2 (fdx2) and dehydrogenase/ reductase 4 (dhrs4) candidate genes (Hallerman et al., 2024) based on learning regarding trypanotolerance derived from the West African N'Dama breed. Additionally, Oxitec is developing a platform for producing Asian blue ticks, a major parasite and disease vector for cattle, that carry a self-limiting gene. This company has previously developed reproductively confined mosquitos, including *Aedes aegypti* and *A. albopictus* (the vectors of dengue and zika) and *Anopheles stephensi* (malaria).

Perception

A study examined the views of 3,698 participants in five developed countries (Canada, the US, Austria, Germany and Italy) on genome editing. Five applications of genome editing were assessed: 1) Resistance to AIDs in humans, 2) Resistance to mildew in Wheat, 3) Resistance to PRRS virus in pigs, 4) Allergen-free cow's milk, and 5) Increased muscle yield in cows. In all countries, participants evaluated the application of disease resistance in humans as most right to do, followed by disease resistance in plants, and then in animals, and considered changes in

product quality and quantity in cattle as least right to do (Busch et al., 2022). Interestingly, the third example, resistance to PRRS virus in pigs, is likely to be the first gene edited livestock application approved as a New Animal Drug (NAD) by the United States FDA (Burger et al., 2024). This application is being sponsored by the Pig Improvement Company (PIC), a subsidiary of UK-based Genus plc as outlined in detail in Cigan and Knap (2022).

There are, however, already three approved gene edited fish, commercially available and being sold to consumers in Japan. These include a myostatin KO Sea Bream (Kishimoto et al., 2018) , and leptin receptor KO Tiger pufferfish and flounder. These actual products provide an interesting test case of Japanese consumers' willingness to accept products from genome edited animals. A paper entitled the "Implications & Lessons From the Introduction of Genome-Edited Food Products in Japan" (Matsuo and Tachikawa, 2022) anticipated that "*Given the low public acceptance of GM [genetic modification] in Japan, it was anticipated that the societal introduction of genome editing technologies would face a degree of public controversy. A previous consumer perception survey found more support for tight regulations of genome-editing-derived foods which were designed to reduce the risk to as close to zero as possible rather than scientifically proven regulations and technically reasonable*." However, the Japanese government decided that fish with no foreign DNA, i.e. SDN-1, were not going to be made to go through the same regulatory requirements as traditional genetically modified organisms (GMOs) harboring a transgenic ("foreign DNA") construct. Rather, the Japanese regulators asked only for molecular characterization of the products prior to making a decision that they were not GM and could enter the market without additional GMO regulatory authorizations.. There was a disconnect between the anticipated and actual public response, described as follows, "*even though there were indeed some social actions, for instance, some groups were against the use of*

genome-editing; petitions were made by some consumer groups; they did not develop into a mass mobilization, and media coverage was mostly positive. After filing the notifications, there were no considerable public reactions, nor did they receive any sustained attention."

Matsuo and Tachikawa (2022) concluded that 3 factors influenced this outcome including: 1) improved R&D environments as a result of government-led innovation policy and regulations which have sought a balance between science and social demand; 2) changes in the players (i.e. university startups), that engage in R&D and the strategies used for social introduction; and 3) social value changes—the recent rise in momentum for sustainable development goals (SDGs) and environmental, social, and governance (ESG) investing. This example highlights the importance of regulatory policy on commercialization timelines, costs, and public acceptance. Although it should be noted that these fish were domestically developed niche products not intended for export, and their commercialization did not pose potential trade issues for Japan.

Policy

The regulation of genetically engineered animals has typically required an approval before a product could come to market. Despite an almost 30-year history of genetically engineered livestock (**Figure 2**), only one biotech animal in the world that was developed for food production, the fast growing AquAdvantage Atlantic salmon, has reached the market under a "GMO" or recombinant DNA (rDNA) approval process (Hallerman et al., 2024). A second genetically engineered animal, the GalSafe pig with an inactivated α1,3-galactosyltransferase (GGTA1) gene that was originally developed for biomedical xenotransplantation purposes (Lai et al., 2002), was also given a limited food use approval in the United States in December, 2020. The approval applies to a single swine farm that can produce up to 1,000 pigs yearly.

Following the lead of Argentina (Whelan and Lema, 2015), another eight countries in Latin America, and countries from Africa, Asia and Oceania are treating SDN-1 edits, and those that could have been achieved using conventional breeding, no differently to conventional breeding (Whelan et al., 2020). This was the approach that allowed the gene edited fish to come to market in Japan. Argentina reports that their gene edited oversight approach has enabled a faster development rate of GnEd plants, animals, and microorganisms for agricultural use, originating from a more diverse group of developers, and led mostly by small and medium enterprises (SMEs) and public research institutions. In addition, they report that product profiles are also more diversified in terms of traits and organisms.

Figure 3 shows the global situation for development of policies for oversight of gene edited animals for agricultural purposes. For most but not all countries, the same process applies to modified plants, microorganisms and animals. Currently, the United States FDA Center for Veterinary Medicine (CVM) regulates gene edited animals, and the USDA Animal and Plant Health Inspection Service (APHIS) regulates gene edited plants. CVM regulates any "intentional genomic alteration" (IGA) in the genome of an animal as a regulated article using the same legal framework it uses to regulate new veterinary drugs under the Federal Food, Drug, and Cosmetic Act They are proposing a tiered approach (FDA 2024a) for the evaluation of animals with IGAs with three categories requiring differing levels of data review. These are Category 1: no review of data, Category 2: review of data to determine low risk prior to an enforcement discretion decision, or Category 3: full approval application (equivalent to a GMO approval).

The first categorical enforcement discretion decision only applies to non-food species laboratory animals, such as rats and mice, that are raised in contained and controlled laboratory conditions for research (Category 1 IGAs). The second has previously been used for research models of

food species (pigs) and for aquarium pet fish, but was expanded to allow enforcement discretion decisions for food animals, such as those that have DNA edits that can be demonstrated to already exist in conventionally-bred animals. This was the approach that was used to evaluate two SLICK (SDN-1 edits of the prolactin receptor) beef cattle that were produced by Acceligen and that were determined to be low risk based on the data provided by the company to the FDA (https://www.fda.gov/media/155706/download?attachment; Accessed 3/29/2024), and were given enforcement discretion in March 2022. According to the FDA's website "*This is not a determination of "safety" under the Federal Food, Drug, and Cosmetic Act but is instead a determination that we understand the product's risks for the specified intended use and have concluded we have no safety concerns. If FDA becomes aware of new information about risk, it may revisit these decisions*."

In 2024, the FDA clarified that for "Category 2" IGAs, defined as, "*IGAs in food-producing animals that are equivalent to genomic sequences that are found in animals of the same species with a history of safe use in animal agriculture food production, or those where (1) the alteration is equivalent to what could be theoretically achieved through conventional breeding; (2) based on the genomic sequence, the alteration is not expected to result in changes to food composition; (3) the intended use of the alteration does not include any effect on animal disease, human disease, or other health outcome; and (4) the alteration has no identified risks of concern to humans, animals, or the environment for the intended use*", no submission of an investigational new animal drug (INAD) application is required to market these types of IGAs in animals. Instead, prior review of risk factor data submitted by the developer through a Veterinary Master File (VMF) is required "*to support the evaluation of potential risk factors, developers generally submit data and information based on an appropriate comparator for the intended use (e.g., an*

unmodified comparator of the same species). This includes information about the methodology used to generate the IGA, characterization of the genomic sequence, and information addressing animal safety, food safety, and risk of impacts on the environment, as appropriate for the intended use of the product, as the types of risks we are concerned with will vary for particular products depending upon the nature of the IGA, the species of animal, and other factors specific to each product". The FDA's determination that the IGA meets the Category 2 description above and is low risk such that it qualifies for enforcement discretion, is required ***prior*** to introduction of food derived from such animals with IGAs into the food supply (FDA 2024a).

To obtain a new animal drug (NAD) approval for a Category 3 IGA (e.g. genetically engineered transgenic animals), developers must open an INAD file, and perform studies to document the safety and effectiveness of the new animal drug (FDA 2024b). It was further clarified that when the FDA states that it "may not expect developers to seek an approval" prior to marketing certain IGAs, it is meant that on a case-by-case basis, the FDA does not intend to take action against a developer for the introduction or delivery for introduction into interstate commerce of an unapproved IGA in an animal and the marketed item(s) containing the IGA (e.g., eggs, semen, embryos, live animals, etc.) **if** that IGA in an animal has been determined by the FDA to be a low-risk Category 2 IGA (FDA 2024a). A list of IGAs in animals that have been "Risk-Reviewed" and given a low risk determination and enforcement discretion is maintained on the FDA website (https://www.fda.gov/animal-veterinary/intentional-genomic-alterations-igas-animals/intentional-genomic-alterations-igas-animals-low-risk-igas; Accessed 5/18/2024).

Summary

Currently there are only a dozen or so GnEd cattle applications that attempt to introduce disease resilience/resistance traits into the bovine genome. Not all pathogen receptors will be amenable to a GnEd approach to control infections in livestock. In some cases, receptor genes may play other essential roles in addition to providing an entry site for disease-causing pathogens, such that altering their sequence is lethal or has other undesired effects on production or performance. The two examples of GnEd cattle produced to address some aspect of the bovine respiratory disease complex provide examples of minimal and precise genomic alterations based on biological understanding of the target gene to reduce the disease phenotype associated with exposure to *Mannheimia haemolytica* and BVDV, respectively, while preserving normal cellular functions of the target gene. However, some caution may be warranted as to date no live animals with the Q(–5)G amino acid substitution in the signal peptide of CD18 that results in *in vitro* protection against leukotoxin-induced cytolysis have yet been reported. Introducing edited alleles into the larger population, especially if they need to be the homozygous state, will require considerable resources even prior to regulatory considerations, as documented by the efforts of Genus plc to produce PRRS virus resistant pigs in their four grandparent lines. Global regulations regarding gene editing in animals are currently in development, and differ markedly among countries. Ultimately, the fate of genome editing in livestock will be highly dependent upon the development of risk-proportional, science-based regulatory frameworks that are sufficiently aligned and cross-compatible to allow for the international trade of GnEd animal products, including eggs, semen, embryos, and live animals, among global trading partners.

Figure 1. Schematic representation of reproductive cloning. Primary skin fibroblasts were edited to replace 6 amino acids in the bovine CD46 gene with "ALPTFS" and subsequently fused to enucleated oocytes (somatic cell nuclear transfer) and the resultant embryos implanted into synchronized recipient cows. Image from Workman et al. (2023). This article is a work of the United States government. Such works are not entitled to domestic copyright protection under U.S. law and are therefore in the public domain.

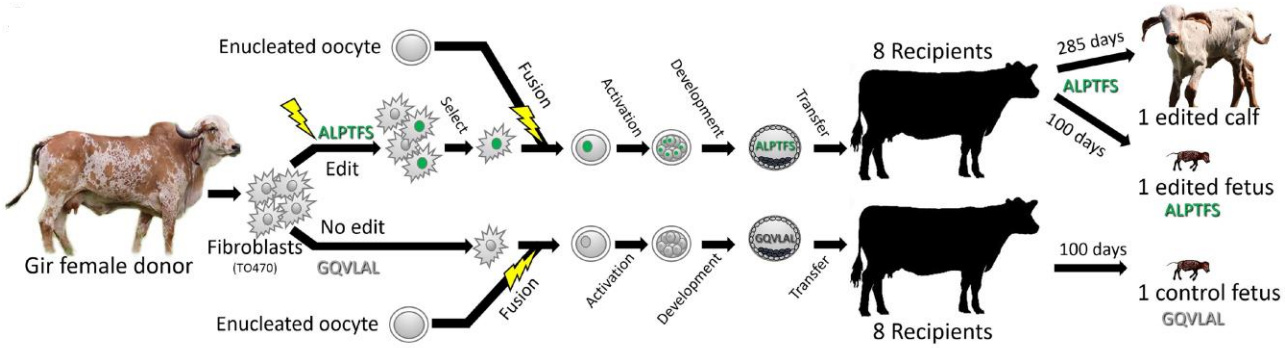


Figure 2. An abbreviated schematic history of 35 years of genetically engineered livestock featuring some of the well-known celebrities of the field. Abbreviations: CRISPR/Cas9, clustered regularly interspaced short palindromic repeat targeted by Cas 9 nuclease; SCNT, somatic cell nuclear transfer; TALEN, transcription activator-like effector nuclease; ZFN, zinc-finger nuclease. Image from Van Eenennaam et al. (2021).

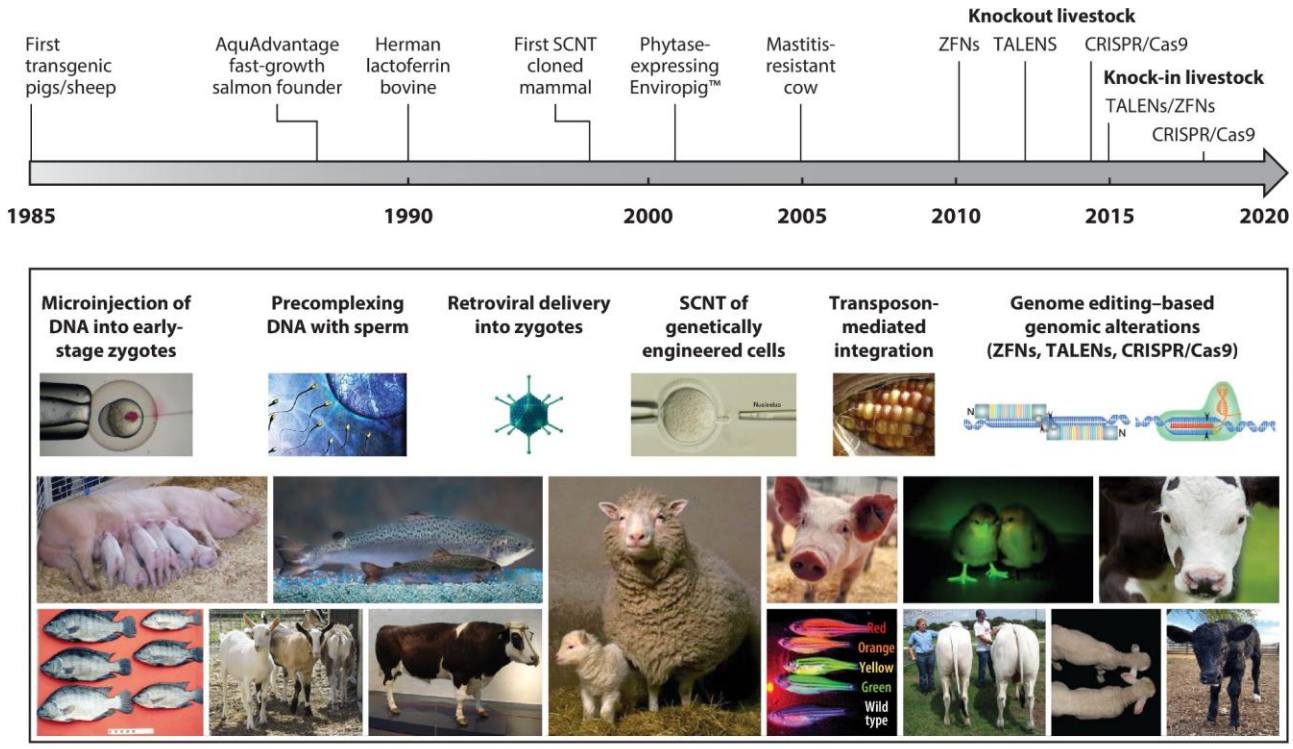
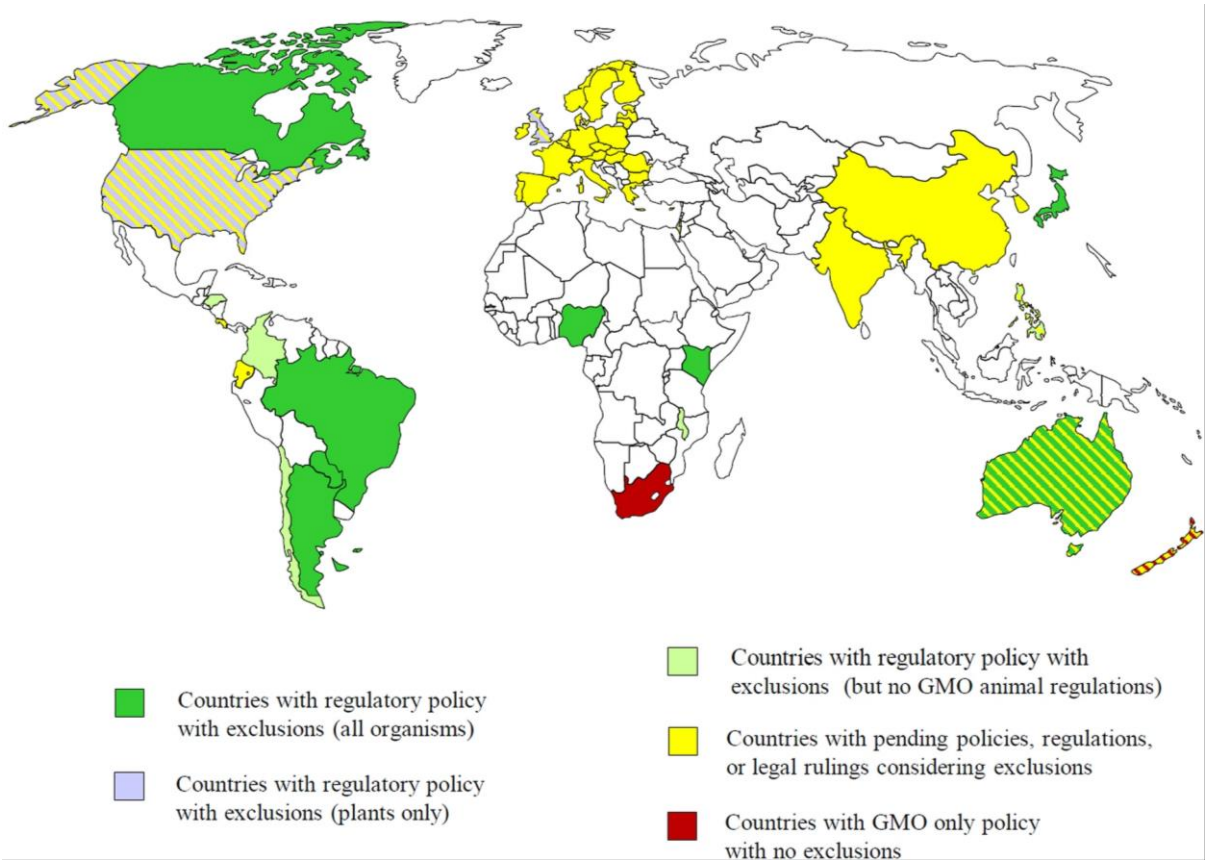


Figure 3. Countries that show progress in development of policies for oversight of GnEd agricultural animal. Figure from Hallerman et al. (2024) and current as of 16 June, 2023.



Targeted animal	Editing System	SDN	Clone?	Methodology	Gene targeted	Targeted Disease	Reference
Cattle	TALEN	3	Yes	Knockin	SP110	Tuberculosis resilience	(Wu et al., 2015)
	CRISPR/ Cas9	3	Yes	Knockin	NRAMP1	Tuberculosis resilience	(Yuan et al., 2021)
	CRISPR/ Cas9	3	Yes	Knockin	NRAMP1	Tuberculosis resilience	(Gao et al., 2017)
	ZFN	3	Yes	Amino acid substitution	CD18	*Mannheimia haemolytica* leukotoxin resilience	(Shanthalingam et al., 2016)
	CRISPR/ Cas9	3	Yes	Amino acid substitution	CD46	Bovine viral diarrhea virus (BVDV) resilience	(Workman et al., 2023)
	CRISPR/ Cas9	1	No	Knockin	PRNP	Prion diseases resilience	(Park et al., 2020)
	CRISPR/ Cas9	3	Yes	Nucleotide substitution	Isoleucyl-tRNA synthetase	Prevention of Isoleucyl-tRNA synthetase syndrome	(Ikeda et al., 2017)
	ZFN nickase	3	Yes	Knockin	Lysozyme	Mastitis resilience	(Liu et al., 2013)
Pig	CRISPR/ Cas9	1	No	Knockout	ANPEP	Transmissible gastroenteritis virus (TGEV), & Porcine epidemic diarrhea virus (PEDV) resilience	(Whitworth et al., 2019)
	CRISPR/ Cas9	1	No	Knockout	CD163	PRRS virus resilience	(Whitworth et al., 2016)
	CRISPR/ Cas9	1,3	Yes	Replacement of Cysteine-Rich Domain 5	CD163	PRRS virus resilience	(Wells et al., 2017)
	CRISPR/ Cas9	3	Yes	Replacement of exon 7 with hCD163L1	CD163	PRRS virus resilience	(Chen et al., 2019)
	CRISPR/ Cas9	1	Yes	Knockout	CD163	PRRS virus resilience	(Yang et al., 2018)
	CRISPR/ Cas9	1	Yes	Double knockout	CD163 and ANPEP	PRRS virus and TGEV resilience	(Xu et al., 2020)

	CRISPR/Cas9	1	No	Knockout	CD163	PRRS virus resilience	(Tanihara et al., 2021)
	CRISPR/ Cas9	1	No	Knockout	CD163	PRRS virus resilience	(Hung et al., 2022)
	CRISPR/ Cas9	1	No	Knockout	CD163/CD1D	PRRS virus resilience	(Whitworth et al., 2014)
	CRISPR/ Cas9	1	No	Knockout	CD163	PRRS virus resilience	(Burkard et al., 2017)
	CRISPR/ Cas9	1	Yes	Knockout	CD163	PRRS virus resilience	(Wang et al., 2019)
	CRISPR/ Cas9	3	Yes	Knockin	shRNAs	Classical swine fever virus (CSFV) resilience	(Xie et al., 2018)
	CRISPR/ Cas9	1	Yes	Knockout	PCBP1	CSFV resilience	(Qi et al., 2022)
	CRISPR/ Cas9	2	Yes	Knockin	RSAD2	African swine fever (ASFV) and pseudorabies virus (PRV) resilience	(Xie et al., 2020)
	ZFN	2	No	Interspecies allele substitution	RELA	ASFV resilience	(Lillico et al., 2016)
	CRISPR/ Cas9	1	Yes	Knockout	PCBP1	CSFV resilience	(Qi et al., 2022)
	CRISPR/ Cas9	2	Yes	Knockin	APN	Enteric coronaviruses resilience	(Liu et al., 2023)
	CRISPR/ Cas9	1	No	Knockout	glycolylneuraminic acid hydroxylase (CMAH) gene	Reduced severity and delayed appearance of porcine epidemic diarrhoea virus (PEDV)	(Tu et al., 2019)
	CRISPR/ Cas9	1	Yes	Double gene modification	CD163 & MSTN	PRRS virus resilience and increased muscle growth	(Zhang et al., 2022)
	Cytidine base editors	1	Yes	SNP replacement at multiple sites	MSTN, IGF2 & CD163	Improved growth performance and PRRS virus resilience	(Song et al., 2022)

Chicken	CRISPR/ Cas9	1	No	Knockout	Tva cell surface receptor	Avian leukosis virus resilience	(Koslová et al., 2021)
	CRISPR/ Cas9	2	No	Amino acid deletion	chNHE1	Avian leukosis virus resilience	(Hellmich et al., 2020)
	CRISPR/ Cas9	1	No	Knockout	chNHE1	Avian leukosis virus resilience	(Koslová et al., 2020)
	CRISPR/ Cas9	3	No	Expression of CRISPR/Cas9 to target a virus	ICP4 of Marek's disease virus	Marek's disease resilience	(Challagulla et al., 2021)
	CRISPR/ Cas9	3	No	two ANP32A amino acid substitutions	ANP32A	Avian influenza	(Idoko-Akoh et al., 2023)
Blue catfish	CRISPR/ Cas9	3	No	Knockin	Alligator CATH	Increased bacterial resistance	(Wang et al., 2023a)
Channel catfish	CRISPR/ Cas9	3	No	Knockin	Alligator CATH	Antimicrobial activity	(Simora et al., 2020)
	CRISPR/ Cas9	1	No	Knockout	TICAM 1 and RBL	Innate immune related genes	(Elaswad et al., 2018)
	CRISPR/ Cas9	3	No	Knockin	CEC and CATH	Increased bacterial resistance	(Wang et al., 2023b)
Labeo rohita carp (Rohu)	CRISPR/ Cas9	3	No	Knockin	TLR22	Immunity model	(Chakrapani et al., 2016)
Silkworm	CRISPR/ Cas9 CRISPR/ Cas12a	3	No	Knockin	Lines expressing Cas & guide sequences	Nucleopolyhedro virus resilience	(Dong et al., 2020)

Table 1. A list of gene-edited food and agricultural animals edited for the trait of disease-resilience/resistance. Updated from Van Eenennaam (2023).

References

Broothaerts, W., Jacchia, S., Angers, A., Petrillo, M., Querci, M., Savini, C., Van Den Eede, G. & Emons, H. (2021). New genomic techniques – State-of-the-art review. doi/10.2760/710056: European, Commission Joint Research Centre Publications Office. doi/10.2760/710056.

Burger, B. T., Beaton, B. P., Campbell, M. A., Brett, B. T., Rohrer, M. S., Plummer, S., Barnes, D., Jiang, K., Naswa, S., Lange, J., Ott, A., Alger, E., Rincon, G., Rounsley, S., Betthauser, J., Mtango, N. R., Benne, J. A., Hammerand, J., Durfee, C. J., Rotolo, M. L., Cameron, P., Lied, A. M., Irby, M. J., Nyer, D. B., Fuller, C. K., Gradia, S., Kanner, S. B., Park, K. E., Waters, J., Simpson, S., Telugu, B. P., Salgado, B. C., Brandariz-Nuñez, A., Rowland, R. R. R., Culbertson, M., Rice, E. & Cigan, A. M. (2024). Generation of a Commercial-Scale Founder Population of Porcine Reproductive and Respiratory Syndrome Virus Resistant Pigs Using CRISPR-Cas. *Crispr j,* 7**,** 12-28.

Burkard, C., Lillico, S. G., Reid, E., Jackson, B., Mileham, A. J., Ait-Ali, T., Whitelaw, C. B. A. & Archibald, A. L. (2017). Precision engineering for PRRSV resistance in pigs: Macrophages from genome edited pigs lacking CD163 SRCR5 domain are fully resistant to both PRRSV genotypes while maintaining biological function. *PLoS pathogens,* 13**,** e1006206.

Busch, G., Ryan, E., Von Keyserlingk, M. a. G. & Weary, D. M. (2022). Citizen views on genome editing: effects of species and purpose. *Agriculture and Human Values,* 39**,** 151-164.

Chakrapani, V., Patra, S. K., Panda, R. P., Rasal, K. D., Jayasankar, P. & Barman, H. K. (2016). Establishing targeted carp TLR22 gene disruption via homologous recombination using CRISPR/Cas9. *Developmental & Comparative Immunology,* 61**,** 242-247.

Challagulla, A., Jenkins, K. A., O'neil, T. E., Shi, S., Morris, K. R., Wise, T. G., Paradkar, P. N., Tizard, M. L., Doran, T. J. & Schat, K. A. (2021). In Vivo Inhibition of Marek's Disease Virus in Transgenic Chickens Expressing Cas9 and gRNA against ICP4. *Microorganisms,* 9.

Chen, J., Wang, H., Bai, J., Liu, W., Liu, X., Yu, D., Feng, T., Sun, Z., Zhang, L., Ma, L., Hu, Y., Zou, Y., Tan, T., Zhong, J., Hu, M., Bai, X., Pan, D., Xing, Y., Zhao, Y., Tian, K., Hu, X. & Li, N. (2019). Generation of Pigs Resistant to Highly Pathogenic-Porcine Reproductive and Respiratory Syndrome Virus through Gene Editing of CD163. *Int J Biol Sci,* 15**,** 481-492.

Cigan, M. A. & Knap, P. W. (2022). Technical considerations towards commercialization of porcine respiratory and reproductive syndrome (PRRS) virus resistant pigs. *CABI Agriculture and Bioscience,* 3**,** 34.

Dong, Z., Qin, Q., Hu, Z., Zhang, X., Miao, J., Huang, L., Chen, P., Lu, C. & Pan, M. (2020). CRISPR/Cas12a Mediated Genome Editing Enhances Bombyx mori Resistance to BmNPV. *Frontiers in Bioengineering and Biotechnology,* 8.

Elaswad, A., Khalil, K., Cline, D., Page-Mccaw, P., Chen, W., Michel, M., Cone, R. & Dunham, R. (2018). Microinjection of CRISPR/Cas9 Protein into Channel Catfish, Ictalurus punctatus, Embryos for Gene Editing. *J Vis Exp*.

FDA (2024a). US Food and Drug Administration. Draft Guidance for Industry #187A. Heritable Intentional Genomic Alterations in Animals: Risk-Based Approach. *Federal Register* [Online], 89. Available: https://www.fda.gov/media/150658/download.

FDA (2024b). US Food and Drug Administration.. Draft Guidance for Industry #187B. Heritable Intentional Genomic Alterations in Animals: The Approval Process *Federal Register* [Online], 89. Available: https://www.fda.gov/media/150658/download.

Gao, Y., Wu, H., Wang, Y., Liu, X., Chen, L., Li, Q., Cui, C., Liu, X., Zhang, J. & Zhang, Y. (2017). Single Cas9 nickase induced generation of NRAMP1 knockin cattle with reduced off-target effects. *Genome Biol,* 18**,** 13.

Hallerman, E., Bredlau, J., Camargo, L. S. A., Dagli, M. L. Z., Karembu, M., Kovich, D., Muia, A. N., Murrone, M. L., Rocha-Salavarrieta, P. J., Romero-Aldemita, R., Tizard, M., Walton, M. & Wray-Cahen, D. (2024). Enabling regulatory policy globally will promote realization of the potential of animal biotechnology. *CABI Agriculture and Bioscience,* 5**,** 25.

Hellmich, R., Sid, H., Lengyel, K., Flisikowski, K., Schlickenrieder, A., Bartsch, D., Thoma, T., Bertzbach, L. D., Kaufer, B. B., Nair, V., Preisinger, R. & Schusser, B. (2020). Acquiring Resistance Against a Retroviral Infection via CRISPR/Cas9 Targeted Genome Editing in a Commercial Chicken Line. *Front Genome Ed,* 2**,** 3.

Hung, S. W., Chuang, C. K., Wong, C. H., Yen, C. H., Peng, S. H., Yang, C., Chen, M. C., Yang, T. S. & Tu, C. F. (2022). Activated macrophages of CD 163 gene edited pigs generated by direct cytoplasmic microinjection with CRISPR gRNA/Cas9 mRNA are resistant to PRRS virus assault. *Anim Biotechnol***,** 1-14.

Idoko-Akoh, A., Goldhill, D. H., Sheppard, C. M., Bialy, D., Quantrill, J. L., Sukhova, K., Brown, J. C., Richardson, S., Campbell, C., Taylor, L., Sherman, A., Nazki, S., Long, J. S., Skinner, M. A., Shelton, H., Sang, H. M., Barclay, W. S. & Mcgrew, M. J. (2023). Creating resistance to avian influenza infection through genome editing of the ANP32 gene family. *Nature Communications,* 14**,** 6136.

Ikeda, M., Matsuyama, S., Akagi, S., Ohkoshi, K., Nakamura, S., Minabe, S., Kimura, K. & Hosoe, M. (2017). Correction of a Disease Mutation using CRISPR/Cas9-assisted Genome Editing in Japanese Black Cattle. *Sci Rep,* 7**,** 17827.

Kishimoto, K., Washio, Y., Yoshiura, Y., Toyoda, A., Ueno, T., Fukuyama, H., Kato, K. & Kinoshita, M. (2018). Production of a breed of red sea bream Pagrus major with an increase of skeletal muscle mass and reduced body length by genome editing with CRISPR/Cas9. *Aquaculture,* 495**,** 415-427.

Koslová, A., Trefil, P., Mucksová, J., Krchlíková, V., Plachý, J., Krijt, J., Reinišová, M., Kučerová, D., Geryk, J., Kalina, J., Šenigl, F., Elleder, D., Kožich, V. & Hejnar, J. (2021). Knock-Out of Retrovirus Receptor Gene Tva in the Chicken Confers Resistance to Avian Leukosis Virus Subgroups A and K and Affects Cobalamin (Vitamin B(12))-Dependent Level of Methylmalonic Acid. *Viruses,* 13.

Koslová, A., Trefil, P., Mucksová, J., Reinišová, M., Plachý, J., Kalina, J., Kučerová, D., Geryk, J., Krchlíková, V., Lejčková, B. & Hejnar, J. (2020). Precise CRISPR/Cas9 editing of the NHE1 gene renders chickens resistant to the J subgroup of avian leukosis virus. *Proc Natl Acad Sci U S A,* 117**,** 2108-2112.

Lai, L., Kolber-Simonds, D., Park, K. W., Cheong, H. T., Greenstein, J. L., Im, G. S., Samuel, M., Bonk, A., Rieke, A., Day, B. N., Murphy, C. N., Carter, D. B., Hawley, R. J. & Prather, R. S. (2002). Production of alpha-1,3-galactosyltransferase knockout pigs by nuclear transfer cloning. *Science,* 295**,** 1089-92.

Lillico, S. G., Proudfoot, C., King, T. J., Tan, W., Zhang, L., Mardjuki, R., Paschon, D. E., Rebar, E. J., Urnov, F. D., Mileham, A. J., Mclaren, D. G. & Whitelaw, C. B. (2016). Mammalian interspecies substitution of immune modulatory alleles by genome editing. *Sci Rep,* 6**,** 21645.

Liu, X., Wang, Y., Guo, W., Chang, B., Liu, J., Guo, Z., Quan, F. & Zhang, Y. (2013). Zinc-finger nickase-mediated insertion of the lysostaphin gene into the beta-casein locus in cloned cows. *Nat Commun,* 4**,** 2565.

Liu, Z., Zhang, M., Huang, P., Ji, Z., Qi, C., Jiao, S., Zhao, D., Jiang, Y., Chen, X., Lv, D., Pang, D., Zhang, X., Feng, L., Xie, Z. & Ouyang, H. (2023). Generation of APN-chimeric gene-edited pigs by CRISPR/Cas9-mediated knock-in strategy. *Gene,* 851**,** 147007.

Matsuo, M. & Tachikawa, M. (2022). Implications and Lessons From the Introduction of Genome-Edited Food Products in Japan. *Frontiers in Genome Editing,* 4.

Park, K. E., Frey, J. F., Waters, J., Simpson, S. G., Coutu, C., Plummer, S., Campbell, M., Donovan, D. M. & Telugu, B. P. (2020). One-Step Homology Mediated CRISPR-Cas Editing in Zygotes for Generating Genome Edited Cattle. *Crispr j,* 3**,** 523-534.

Qi, C., Pang, D., Yang, K., Jiao, S., Wu, H., Zhao, C., Hu, L., Li, F., Zhou, J., Yang, L., Lv, D., Tang, X., Ouyang, H. & Xie, Z. (2022). Generation of PCBP1-deficient pigs using CRISPR/Cas9-mediated gene editing. *iScience,* 25**,** 105268.

Shanthalingam, S., Tibary, A., Beever, J. E., Kasinathan, P., Brown, W. C. & Srikumaran, S. (2016). Precise gene editing paves the way for derivation of Mannheimia haemolytica leukotoxin-resistant cattle. *Proc Natl Acad Sci U S A,* 113**,** 13186-13190.

Simora, R. M. C., Xing, D., Bangs, M. R., Wang, W., Ma, X., Su, B., Khan, M. G. Q., Qin, Z., Lu, C., Alston, V., Hettiarachchi, D., Johnson, A., Li, S., Coogan, M., Gurbatow, J., Terhune, J. S., Wang, X. & Dunham, R. A. (2020). CRISPR/Cas9-mediated knock-in of alligator cathelicidin gene in a non-coding region of channel catfish genome. *Sci Rep,* 10**,** 22271.

Tanihara, F., Hirata, M., Nguyen, N. T., Le, Q. A., Wittayarat, M., Fahrudin, M., Hirano, T. & Otoi, T. (2021). Generation of CD163-edited pig via electroporation of the CRISPR/Cas9 system into porcine in vitro-fertilized zygotes. *Anim Biotechnol,* 32**,** 147-154.

Tu, C. F., Chuang, C. K., Hsiao, K. H., Chen, C. H., Chen, C. M., Peng, S. H., Su, Y. H., Chiou, M. T., Yen, C. H., Hung, S. W., Yang, T. S. & Chen, C. M. (2019). Lessening of porcine epidemic diarrhoea virus susceptibility in piglets after editing of the CMP-N-glycolylneuraminic acid hydroxylase gene with CRISPR/Cas9 to nullify N-glycolylneuraminic acid expression. *PLoS One,* 14**,** e0217236.

Van Eenennaam, A. L. (2023). New Genomic Techniques (NGT) in animals and their agri/food/feed products. *EFSA Supporting Publications,* 20**,** 8311E.

Van Eenennaam, A. L., De Figueiredo Silva, F., Trott, J. F. & Zilberman, D. (2021). Genetic Engineering of Livestock: The Opportunity Cost of Regulatory Delay. *Annu Rev Anim Biosci,* 9**,** 453-478.

Wang, H., Shen, L., Chen, J., Liu, X., Tan, T., Hu, Y., Bai, X., Li, Y., Tian, K., Li, N. & Hu, X. (2019). Deletion of CD163 Exon 7 Confers Resistance to Highly Pathogenic Porcine Reproductive and Respiratory Viruses on Pigs. *Int J Biol Sci,* 15**,** 1993-2005.

Wang, J., Su, B., Al-Armanazi, J., Wise, A. L., Shang, M., Bern, L., Li, S., Xing, D., Johnson, A., Wang, W., Hettiarachchi, D. U., Coogan, M., Bruce, T. J. & Dunham, R. A. (2023a). Integration of alligator cathelicidin gene via two CRISPR/Cas9-assisted systems enhances bacterial resistance in blue catfish, Ictalurus furcatus. *Aquaculture,* 576**,** 739860.

Wang, J., Su, B., Bruce, T. J., Wise, A. L., Zeng, P., Cao, G., Simora, R. M. C., Bern, L., Shang, M., Li, S., Xing, D., Wang, W., Johnson, A., Coogan, M., Hettiarachchi, D. U., Al-Armanazi, J., Farias, R. S. & Dunham, R. A. (2023b). CRISPR/Cas9 microinjection of transgenic embryos enhances the dual-gene integration efficiency of antimicrobial peptide genes for bacterial resistance in channel catfish, Ictalurus punctatus. *Aquaculture,* 575**,** 739725.

Wells, K. D., Bardot, R., Whitworth, K. M., Trible, B. R., Fang, Y., Mileham, A., Kerrigan, M. A., Samuel, M. S., Prather, R. S. & Rowland, R. R. R. (2017). Replacement of Porcine CD163 Scavenger Receptor Cysteine-Rich Domain 5 with a CD163-Like Homolog Confers Resistance of Pigs to Genotype 1 but Not Genotype 2 Porcine Reproductive and Respiratory Syndrome Virus. *J Virol,* 91.

Whelan, A. I., Gutti, P. & Lema, M. A. (2020). Gene Editing Regulation and Innovation Economics. *Frontiers in Bioengineering and Biotechnology,* 8.

Whelan, A. I. & Lema, M. A. (2015). Regulatory framework for gene editing and other new breeding techniques (NBTs) in Argentina. *GM Crops & Food,* 6**,** 253-265.

Whitworth, K. M., Lee, K., Benne, J. A., Beaton, B. P., Spate, L. D., Murphy, S. L., Samuel, M. S., Mao, J., O'gorman, C., Walters, E. M., Murphy, C. N., Driver, J., Mileham, A., Mclaren, D., Wells, K. D. & Prather, R. S. (2014). Use of the CRISPR/Cas9 system to produce genetically engineered pigs from in vitro-derived oocytes and embryos. *Biol Reprod,* 91**,** 78.

Whitworth, K. M., Rowland, R. R., Ewen, C. L., Trible, B. R., Kerrigan, M. A., Cino-Ozuna, A. G., Samuel, M. S., Lightner, J. E., Mclaren, D. G., Mileham, A. J., Wells, K. D. & Prather, R. S. (2016). Gene-edited pigs are protected from porcine reproductive and respiratory syndrome virus. *Nat Biotechnol,* 34**,** 20-2.

Whitworth, K. M., Rowland, R. R. R., Petrovan, V., Sheahan, M., Cino-Ozuna, A. G., Fang, Y., Hesse, R., Mileham, A., Samuel, M. S., Wells, K. D. & Prather, R. S. (2019). Resistance to coronavirus infection in amino peptidase N-deficient pigs. *Transgenic Res,* 28**,** 21-32.

Workman, A. M., Heaton, M. P., Vander Ley, B. L., Webster, D. A., Sherry, L., Bostrom, J. R., Larson, S., Kalbfleisch, T. S., Harhay, G. P., Jobman, E. E., Carlson, D. F. & Sonstegard, T. S. (2023). First gene-edited calf with reduced susceptibility to a major viral pathogen. *PNAS Nexus,* 2**,** pgad125.

Wu, H., Wang, Y., Zhang, Y., Yang, M., Lv, J., Liu, J. & Zhang, Y. (2015). TALE nickase-mediated *SP110* knockin endows cattle with increased resistance to tuberculosis. *Proceedings of the National Academy of Sciences,* 112**,** E1530-E1539.

Xie, Z., Jiao, H., Xiao, H., Jiang, Y., Liu, Z., Qi, C., Zhao, D., Jiao, S., Yu, T., Tang, X., Pang, D. & Ouyang, H. (2020). Generation of pRSAD2 gene knock-in pig via CRISPR/Cas9 technology. *Antiviral Res,* 174**,** 104696.

Xie, Z., Pang, D., Yuan, H., Jiao, H., Lu, C., Wang, K., Yang, Q., Li, M., Chen, X., Yu, T., Chen, X., Dai, Z., Peng, Y., Tang, X., Li, Z., Wang, T., Guo, H., Li, L., Tu, C., Lai, L. & Ouyang, H. (2018). Genetically modified pigs are protected from classical swine fever virus. *PLoS Pathog,* 14**,** e1007193.

Xu, K., Zhou, Y., Mu, Y., Liu, Z., Hou, S., Xiong, Y., Fang, L., Ge, C., Wei, Y., Zhang, X., Xu, C., Che, J., Fan, Z., Xiang, G., Guo, J., Shang, H., Li, H., Xiao, S., Li, J. & Li, K. (2020). CD163 and pAPN double-knockout pigs are resistant to PRRSV and TGEV and exhibit decreased susceptibility to PDCoV while maintaining normal production performance. *Elife,* 9.

Yang, H., Zhang, J., Zhang, X., Shi, J., Pan, Y., Zhou, R., Li, G., Li, Z., Cai, G. & Wu, Z. (2018). CD163 knockout pigs are fully resistant to highly pathogenic porcine reproductive and respiratory syndrome virus. *Antiviral Res,* 151**,** 63-70.

Yuan, M., Zhang, J., Gao, Y., Yuan, Z., Zhu, Z., Wei, Y., Wu, T., Han, J. & Zhang, Y. (2021). HMEJ-based safe-harbor genome editing enables efficient generation of cattle with increased resistance to tuberculosis. *J Biol Chem,* 296**,** 100497.