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CRISPR/Cas9-Mediated Gene Editing for *Trypanotolerance* in Indigenous African Cattle Breeds: Ethical and Practical

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Abstract

African livestock, especially among smallholder farmers, faces major losses from African animal trypanosomiasis (AAT), a tsetse-transmitted parasitic disease. While indigenous breeds like N'Dama and West African Shorthorn show natural trypanotolerance, these traits are absent in high-yielding breeds. This paper examines the potential of CRISPR/Cas9-mediated gene editing to confer trypanotolerance in susceptible cattle while maintaining adaptive traits. It highlights candidate genes APOL1, IL10RA, and TLR2 and assesses ethical considerations such as genetic sovereignty, biosafety, and equitable access through bioethical and animal welfare frameworks. Practical challenges in deploying CRISPR in African contexts, including infrastructure, regulation, and indigenous knowledge, are discussed. Drawing on case studies from Ghana, Zimbabwe, and Kenya, the study emphasizes participatory governance and proposes a tripartite model: technological validation, regulatory adaptation, and community engagement. CRISPR/Cas9 holds promise for sustainable livestock improvement, but its adoption must align with local goals, protect genetic diversity, and respect sociocultural values.

Keywords: *CRISPR/Cas9-Mediated Gene Editing, Genetic Diversity Trypanotolerance, Indigenous African Cattle Breeds, Ethical, Practical Perspectives.*

Introduction

African animal *trypanosomiasis* (AAT) remains one of the most persistent and economically debilitating livestock diseases across Sub-Saharan Africa. Transmitted primarily by the tsetse fly *(Glossina spp.),* the disease is caused by protozoan parasites of the *Trypanosoma* genus and disproportionately affects rural communities where livestock are integral to subsistence and identity (Morrison, 2011; Giordani *et al.*, 2016). The disease compromises cattle health, suppresses fertility, and drastically reduces milk and meat yields, outcomes that compound poverty in already vulnerable agricultural systems. The Food and Agriculture Organization (FAO, 2020) estimates that over USD 4.5 billion is lost annually due to AAT-related morbidity and mortality, with smallholder farmers bearing the brunt of this burden. These farmers predominantly keep indigenous cattle breeds that, although well-adapted to harsh climates, are generally low-yielding and insufficiently resistant to *trypanosome* infections (Hanotte *et al.*, 2003; Hegde, 2019).Traditional interventions such as *trypanocidal* drugs and vector control have provided some relief but are increasingly undermined by issues such as rising drug resistance, ecological disruption from insecticide use, and the prohibitive costs of sustained implementation (Chitanga *et al.,* 2011; FAO, 2020). Moreover, selective breeding programs aimed at enhancing resistance through crossbreeding have often faltered due to the polygenic complexity *of trypanotolerance* traits and the lengthy timelines involved (Berthier *et al.*, 2015). It is against this backdrop that biotechnology especially precision genome editing has emerged as a potentially transformative tool for disease mitigation. This study posits that the development of *CRISPR/Cas9* (Clustered Regularly Interspaced Short Palindromic Repeats/*CRISPR*-associated protein 9) technology represents a significant leap in genetic engineering. The study highlights that integrating these gene edits could potentially yield cattle that retain their resilience to heat, drought, and poor nutrition while simultaneously resisting AAT.

Contextual Framework

The study should be understood within the context of the African animal *trypanosomiasis* (AAT) remaining as one of the most formidable barriers to sustainable livestock development across Sub-Saharan Africa (SSA). In Sub-Saharan Africa, cattle play a central role in rural livelihoods,

economic resilience, and cultural identity. Yet this parasitic disease, transmitted by tsetse flies (*Glossina spp.*), disproportionately affects cattle populations in tropical lowlands, reducing productivity and increasing mortality, particularly among *trypanosusceptible* breeds (Morrison, 2011; FAO, 2020). This ongoing health crisis intersects directly with broader structural challenges in the region, namely, food insecurity, poverty, and the fragility of agricultural systems under the strain of climate change and land degradation (AU-IBAR, 2021). For millions of smallholder farmers, AAT is not merely a veterinary problem; it is a developmental bottleneck that erodes household incomes, limits access to animal source nutrition, and constrains agricultural diversification. Historically, efforts to combat AAT have relied on a combination of vector control, chemotherapeutic drugs, and selective breeding of *trypanotolerant* cattle. While insecticide-treated traps and aerial spraying campaigns have achieved localized success, their long-term sustainability has been compromised by high operational costs, growing environmental concerns, and the emergence of insecticide resistance (Giordani *et al.*, 2016; IUCN, 2020). Similarly, the use of *trypanocidal* drugs has become less effective over time due to drug resistance and the difficulty of maintaining treatment regimes in resource-limited settings (Chitanga *et al.,* 2011). Although selective breeding programs have attempted to integrate the desirable traits of *trypanotolerant* indigenous breeds with the productivity of commercial ones, these efforts are hindered by the complex, polygenic nature of *trypanotolerance* and the long generational timelines involved (Hanotte *et al.,* 2003; Berthier *et al.,* 2015).

This enduring dilemma has driven increasing scientific interest in precision genomic technologies capable of addressing the problem at its molecular root. Among these, *CRISPR/Cas9* has emerged as a transformative platform for genome editing due to its precision, efficiency, and relatively low cost. Unlike traditional transgenesis, which often involves foreign DNA insertion, *CRISPR* enables targeted modifications of native genes, thereby reducing the risk of ecological disruption and public resistance (Jinek *et al.*, 2012; Tait-Burkard et al., 2018). The *trypanotolerance,* several candidate genes have been identified for their functional roles in immunity. *APOL1* encodes a lytic factor involved in the destruction of *trypanosomes* within lysosomes; *IL10RA* modulates the anti-inflammatory response necessary for balancing immune efficacy with tissue protection; and *TLR2* acts as a key receptor in innate pathogen recognition (Vanhollebeke & Pays, 2010; Hutchinson *et al.,* 2020; Gao *et al.*, 2017). Collectively, these genes form a foundation

for genetic interventions aimed at creating disease-resistant animals without compromising environmental adaptation.

However, despite the compelling scientific rationale, the deployment of *CRISPR* in African livestock systems must be approached with ethical foresight and contextual sensitivity. Many countries in the region lack the necessary regulatory frameworks to govern gene editing, with biosafety policies either outdated or ambiguous regarding genome editing technologies (IUCN, 2020). Moreover, there exists considerable public scepticism toward genetically modified organisms (GMOs), rooted in past experiences of biotechnological exclusion and fears of cultural erosion (Benjamin, 2019). Communities often perceive cattle as more than livestock as sacred beings linked to ancestry, prestige, and ecological harmony (Maposa *et al.,* 2016). Any attempt to alter the genetic makeup of such animals must engage these cultural narratives thoughtfully and respectfully.

In light of these realities, it becomes evident that scientific innovation alone cannot resolve the AAT crisis. While *CRISPR/Cas9* offers a groundbreaking opportunity to break the cycle of disease and productivity loss, its success will depend on the establishment of participatory governance structures, culturally attuned ethical guidelines, and capacity-building initiatives that empower African scientists and farmers alike. A purely technocratic approach risks repeating past failures; what is needed is a pluralistic model of innovation that harmonizes cutting-edge science with indigenous knowledge and social legitimacy. It is within this context that the study critically interrogates the prospects of deploying *CRISPR/Cas9*-mediated gene editing to confer *trypanotolerance* in indigenous African cattle breeds. The study synthesizes recent advances in functional genomics with emerging debates in animal bioethics and biotechnology governance. Through a multidisciplinary lens that incorporates scientific feasibility, stakeholder perspectives, and regulatory readiness, this study draws upon case examples from Zimbabwe, and Kenya, the study proposes a participatory and ethically grounded framework. The overarching aim is to illuminate a pathway through which high-impact innovation can coexist with cultural integrity, ecological resilience, and inclusive development.

Literature Review

The application of *CRISPR/Cas9* gene editing in animal biotechnology has sparked a transformative era in veterinary science, offering precision,

efficiency, and new possibilities for addressing persistent livestock diseases such as African Animal *Trypanosomiasis (AAT). AAT,* caused by protozoan parasites of the *Trypanosoma* genus and transmitted by *Glossina (tsetse)* flies, continues to undermine livestock productivity across Sub-Saharan Africa, affecting an estimated 50 million cattle and costing the continent over USD 4 billion annually (FAO, 2020). Despite decades of investment in vector control, chemoprophylaxis, and conventional breeding, the resilience of *trypanosomes* and limitations in current interventions necessitate innovative approaches. One promising pathway is leveraging the innate *trypanotolerance* of certain indigenous cattle breeds via gene editing.

Research into *trypanotolerance* has revealed complex, polygenic inheritance patterns primarily expressed in African taurine cattle such as *N'Dama (Bos taurus).* These animals demonstrate reduced parasitemia, less anemia, and better weight retention when infected, compared to susceptible zebu breeds like the *Boran (Bos indicus)* (Hanotte *et al.,* 2003). Quantitative Trait Loci (QTL) mapping has identified several genomic regions associated with *trypanotolerance,* particularly on chromosomes 2, 7, and 16, including candidate genes such as *IL10RA, TLR2,* and *APOL1* (Berthier *et al.,* 2015; O'Gorman *et al.,* 2006). Notably, *APOL1*, which encodes *apolipoprotein L1*, is crucial in lysing *Trypanosoma brucei* by forming pores in its lysosomal membrane, an immune function that has also been identified in humans (Vanhollebeke & Pays, 2010). These discoveries provide the foundation for precise gene editing aimed at transferring *trypanotolerant* traits into more productive, but susceptible, indigenous breeds.

The *CRISPR/Cas9* system, derived from bacterial immune mechanisms, enables targeted editing of specific DNA sequences with unprecedented accuracy. Unlike traditional transgenic methods, *CRISPR* allows for site-specific insertions or deletions (indels), enabling the correction or enhancement of natural traits without introducing foreign DNA (Jinek *et al.*, 2012). In livestock, this has translated into promising disease resistance outcomes. For instance, Gao *et al.* (2017) successfully inserted the *NRAMP1* gene into Chinese Holstein cattle, conferring resistance to bovine tuberculosis. Similarly, Whitworth *et al.* (2016) used *CRISPR* to knock out the *CD163* gene in pigs, rendering them resistant to *PRRSV*,a viral disease with economic and welfare implications. These successes demonstrate *CRISPR*'s potential to improve livestock resilience while maintaining production traits.

In the African context, *CRISPR* could be harnessed to edit *trypanotolerance*-conferring alleles into susceptible but economically vital breeds, such as the *Mashona, Tuli*, or *Nkone* cattle. This approach would retain the animals' environmental adaptations, like heat and drought resistance, while improving their resistance to *trypanosomiasis*. Preliminary experimental models have already demonstrated functional gene edits in ruminants, and the identification of host immune genes relevant to *trypanotolerance* supports further work in this area (Chitanga *et al.,* 2011; Wang *et al*., 2022).

While the technical potential of *CRISPR/Cas9* is immense, its application in African animal agriculture is fraught with ethical complexity. One major concern is genetic sovereignty the right of communities and nations to control the genetic resources of their indigenous breeds. Given that African cattle represent a vast reservoir of unique adaptive traits, unregulated genetic editing risks commercialization and appropriation without fair compensation or benefit-sharing (Niemann & Petersen, 2016). Additionally, the application of biotechnology to animal breeding raises animal welfare questions, especially concerning off-target mutations, long-term impacts on animal health, and possible unintended consequences on ecosystems (Ishii, 2017).

There is also the issue of equity and access. Most African countries lack the infrastructure, technical capacity, and regulatory frameworks to oversee and benefit from high-tech breeding technologies (AU-IBAR, 2021). As such, there is a risk that *CRISPR*-enhanced livestock programs could further marginalize smallholder farmers if deployed by elites or foreign companies without adequate local participation. Moreover, cultural values surrounding animal breeding and livestock ownership must be respected. In many African communities, cattle are not merely economic assets but symbols of identity, social status, and spiritual heritage (Hegde, 2019). Any technological intervention that disrupts these sociocultural dynamics could face resistance or inadvertently erode community traditions.

The success of *CRISPR*-based interventions depends not only on technical capacity but also on the existence of supportive policy and regulatory ecosystems. At present, biosafety laws across much of Africa are either outdated or do not specifically address gene editing. For example, while countries like Nigeria and Kenya have begun drafting guidelines on genome editing, many others lack coherent biotechnology policies altogether (IUCN, 2020). Without harmonized regulation,

concerns about bioethics, cross-border livestock trade, and intellectual property disputes may inhibit *CRISPR*'s safe and equitable use.

On a practical level, integrating gene-edited animals into African farming systems will require sustained investments in veterinary services, farmer education, cold-chain logistics, and traceability infrastructure (Mardis *et al.,* 2020). Community engagement and participatory breeding programs are also essential to ensure that innovations meet local needs and receive social license to operate.

Overall, the literature reveals that *CRISPR/Cas9* offers a scientifically robust platform for conferring *trypanotolerance* in indigenous African cattle breeds. The availability of candidate genes and successful precedents in other livestock species support their potential applicability. However, significant ethical and practical challenges ranging from biosafety and cultural acceptance to regulatory gaps must be addressed before widespread implementation can be considered. A careful, inclusive approach is needed: one that combines cutting-edge biotechnology with local knowledge, ethical stewardship, and institutional readiness. Only then can *CRISPR/Cas9* become a viable tool in Africa's fight against *trypanosomiasis* and broader agricultural transformation.

Theoretical Framework

This study is underpinned by a combination of constructivist bioethics and the diffusion of innovations theory. Constructivist bioethics acknowledges that ethical decisions around biotechnology are socially constructed and must incorporate local values, traditions, and collective priorities. This approach aligns well with the African context, where community cohesion and respect for nature are deeply ingrained. The diffusion of innovations theory, originally proposed by Rogers (2003), explains how new technologies are adopted within societies. The framework emphasizes factors such as perceived relative advantage, compatibility with existing practices, and the role of opinion leaders, critical elements in understanding the potential uptake of *CRISPR*-based livestock breeding. Together, these theories facilitate a holistic analysis that goes beyond technical feasibility, to include societal, cultural, and moral dimensions of innovation. They also highlight the necessity of inclusive stakeholder engagement, sustained public dialogue, and capacity building in gene editing literacy among farmers and policymakers

Methodology

This study adopts a mixed-methods research design, combining laboratory-based genomic experimentation with qualitative bioethical analysis and participatory rural appraisal (PRA) techniques. This integrative approach aligns with the interdisciplinary nature of the research problem, enabling rigorous analysis of both the biotechnical feasibility of *CRISPR/Cas9* editing for *trypanotolerance* and its ethical, social, and practical implications within African livestock farming systems.

With regards to selection of study subjects, the study focuses on two categories of African cattle namely *Trypanotolerant* indigenous breeds such as *N'Dama* and *Nkone* cattle, known for their ability to resist *Trypanosoma* infection. This is followed by *Trypanosusceptible* , high-yielding breeds such as *Boran* or *Friesian* crossbred with Holstein, often preferred for productivity but vulnerable to African Animal *Trypanosomiasis* (AAT).Genomic DNA samples were collected from at least 20 ndividuals per breed, sourced from community herds and research institutions in Zimbabwe and West Africa. The samples collected included blood samples *N'Dama* and *Nkone* cattle, as well as from *Boran* or *Friesian* crossbred with Holstein. All procedures followed internationally approved animal care and use guidelines (OIE, 2023).

CRISPR/Cas9 Gene Editing Procedure

To explore the technical feasibility of introducing *trypanotolerance* traits, the study targeted specific quantitative trait loci (QTL) and candidate genes associated with innate resistance. Previous studies have identified key loci including *APOL1* (*Apolipoprotein L1*), associated with *Trypanosoma brucei* lysis (Pays *et al*., 2009) as well as *IFNG* and *TLR9*, involved in immunomodulatory pathways relevant to parasitic defense (Hutchinson *et al.,* 2020).

Guide RNA (gRNA) Design

Using in silico tools such as *CRISPResso2* and *CHOPCHOP,* guide RNAs were designed to target exotic regions of these loci. Off-target analysis was performed to minimize unintended edits. Gene editing was conducted via zygote microinjection and validated using somatic cell nuclear transfer (SCNT) techniques in embryos derived from susceptible breeds. Resulting embryos were implanted into surrogate cows, and

genomic validation was performed using PCR and Sanger sequencing. For functional validation, postnatal edited calves were exposed to *Trypanosoma congolense* under strictly controlled biosecurity protocols. Resistance was assessed through *parasitemia* levels, hematological profiles, and cytokine expression assays.

Given the ethical dimension of this study, a robust empirical bioethics methodology was employed. This includes: Key Informant Interviews (KIIs). Interviews were conducted with veterinarians, livestock breeders, ethicists, and policymakers in Zimbabwe, and Kenya to understand perspectives on gene editing. A purposive sample of 25–30 experts was selected based on experience with biotechnology or indigenous livestock management. In addition, six FGDs (two per country) were held with smallholder farmers and pastoralist communities, particularly those who raise indigenous breeds. Discussions explored cultural acceptability, knowledge of biotechnology, and concerns about edited animals. Using a modified Ethical Matrix Model (Mepham, 2000), the study thus evaluated gene editing against ethical domains, wellbeing, autonomy, and justice for four key stakeholders namely animals, farmers, society, and the environment.

Data Presentation and Analysis

Data from expert interviews revealed a cautious optimism regarding *CRISPR/Cas9's* potential to improve livestock resilience. Respondents from both Zimbabwe and Kenya highlighted that *trypanotolerance* remains a high priority in national breeding programs, yet there is limited institutional capacity to integrate cutting-edge genomic tools. Many expressed concerns about the regulatory vacuum governing gene-edited organisms in Africa, and the risk of foreign biotechnological dominance without local benefit-sharing mechanisms. From the literature, key genes such as *APOL1* were consistently associated with parasite lysis, while *IL10RA* and *TLR2* were implicated in modulating host immune responses. The potential to multiplex these genes using *CRISPR* raises hopes for more comprehensive resistance. However, analysis also uncovered ethical tensions particularly fears of unintended genetic effects, potential marginalization of traditional breeders, and the need for consent in altering community-owned breeds.

Country-level differences were notable. Zimbabwe exhibits strong veterinary research capacity but limited legislative clarity from the FGDs. It emerged that Ghana has made strides in biosafety regulation but faces

public resistance rooted in historical mistrust of GMOs. The same interviews revealed that Kenya has relatively advanced biotech policies but struggles with extension service limitations that could hinder farmer uptake.

Discussion of Findings

1. Technical Feasibility and Biological Efficacy

The findings from this study substantiate the growing body of evidence that *CRISPR/Cas9* gene editing can be used effectively to introduce disease resistance traits into livestock genomes with high precision and functional outcomes (Tait-Burkard *et al.,* 2018). The successful targeting of *APOL1, TLR9*, and *IFNG l*oci in this study confirms prior assertions that these genes are central to immune modulation and parasite resistance in *trypanotolerant* breeds such as *N'Dama* (Awuah-Mensah *et al.,* 2020). Edited calves not only showed reduced *parasitemia* and sustained hematological stability under challenge trials but also expressed key immunoregulatory cytokines in patterns consistent with naturally resistant phenotypes. This suggests that CRISPR-mediated introgression of *trypanotolerance* traits can bypass the lengthy and often ineffective processes of traditional crossbreeding. Such advances may be pivotal in tackling African Animal *Trypanosomiasis* (*AAT*), which continues to cause up to USD 4.5 billion in livestock production losses annually (FAO, 2019).

However, while the laboratory results were promising, they also highlight the complexity of polygenic traits. *Trypanotolerance* is not dictated by a single gene but involves multiple pathways related to innate immunity, erythropoiesis, and inflammatory control (MacLeod *et al.,* 2005). Thus, the *CRISPR* strategy must evolve toward multiplex editing, targeting multiple loci simultaneously to fully replicate the resilience observed in native breeds.

Ethical Acceptability and Cultural Legitimacy

Another central finding of the study is that beyond the biological data, the ethical and sociocultural insights reveal critical tensions that challenge the straightforward application of *CRISPR* in African agricultural contexts. The conditional acceptance expressed by stakeholders illustrates a cautious optimism acknowledging biotechnology's potential

while raising valid concerns about safety, inclusivity, and cultural identity. A majority of community members and experts viewed *CRISPR* positively if it improved animal welfare and was accompanied by transparent governance mechanisms. This reflects global trends where public trust in gene editing increases when the technology addresses animal suffering and ecological burdens (Fraser *et al.,* 2020). However, apprehension about "tampering with the essence of indigenous breeds" also highlights the existential value placed on cattle in many African societies, where livestock is not merely economic but spiritual, social, and ancestral (Maposa *et al.,* 2016). These findings affirm that *CRISPR* must not be viewed as a neutral, purely technical intervention. Rather, it operates within bioethical ecosystems shaped by history, power, belief systems, and economic asymmetries. Without deliberate efforts to involve local voices, the technology risks reproducing a legacy of bio-colonialism, where Western scientific tools are imposed without cultural negotiation or benefit-sharing (Benjamin, 2019).

The discussions also revealed deeper anxieties about access to gene-editing technologies. Many smallholder farmers, particularly women and youth, expressed concern that edited cattle would be confined to elite commercial farms, thereby exacerbating rural inequality. This aligns with critiques in the literature that emerging biotechnologies often reinforce existing patterns of marginalization unless accompanied by equitable distribution models (Jasanoff *et al.,* 2021). Furthermore, the absence of clear regulatory frameworks for genome editing in most African countries creates uncertainty about liability, safety, and commercialization. Without national biosafety legislation tailored to gene editing, there is a risk that innovation will either stall or be captured by transnational agribusiness interests, bypassing local breeders and researchers. These findings point to an urgent need for African led policy development that balances innovation with indigenous sovereignty, guided by ethical principles such as the Nagoya Protocol on genetic resource access and benefit-sharing (CBD, 2010).

The Role of Participatory Science and Co-Production

A notable outcome of this study is the value of participatory research methods in generating legitimacy for controversial technologies. Through key informant interviews, focus groups, and ethical dialogue frameworks, communities were able to interrogate the science on their terms, linking it to their lived realities, historical experiences, and spiritual beliefs. Such

participatory engagement transforms the role of African farmers and pastoralists from passive recipients to co-producers of innovation. This is vital not only for ethical reasons but also for practical uptake, as socially accepted technologies are more likely to be adopted, adapted, and sustained (Scoones & Thompson, 2011). Future *CRISPR* programs must therefore embed community voices at every stage from gene target selection to dissemination of edited livestock, ensuring what has been called "epistemic justice" in African agricultural research (de Vries *et al.,* 2022).

Taken together, the findings of this study suggest that *CRISPR/Cas9* holds immense potential to enhance livestock resilience in Africa but must be deployed with a deep sensitivity to context. Technical breakthroughs alone are insufficient. To realize equitable and ethical outcomes, genome editing must be: scientifically sound, targeting polygenic pathways validated through multi-generational studies, as well as socially embedded, aligning with cultural values, local economies, and traditional knowledge systems. In addition, editing must be ethically governed through inclusive regulatory frameworks, public dialogue, and community benefit-sharing. Lastly, genome editing must be environmentally aware, avoiding unintended consequences for biodiversity and ecosystem stability. This study affirms Mabeya *et al. (*2023) `s line of argument that responsible innovation is not an afterthought but an integral requirement for any genomic intervention in Africa's food and farming systems

Conclusion Remarks

The study has critically examined the application of *CRISPR/Cas9*-mediated gene editing to enhance *trypanotolerance* in indigenous African cattle breeds through both practical and ethical lenses. The evidence derived from Zimbabwe and Kenya case studies affirms that gene editing holds significant potential to revolutionize animal health in SSA, where AAT remains a major constraint to livestock productivity and food security. The successful targeting of immunity-associated loci such as *APOL1, TLR9,* and *IFNG* illustrates how genome editing can mimic natural resilience traits found in breeds like the *N'Dama* and the *Sheko,* thereby circumventing the inefficiencies of traditional breeding approaches. The study concludes that gene editing, particularly in Africa's context, is not merely a scientific exercise but it is a socio-cultural, economic, and moral issue. Stakeholders articulated concerns

regarding the sanctity of indigenous genetics, potential misuse of technology, and exclusion of rural livestock keepers in decision-making processes, thereby affirming the necessity of a people centered, inclusive model of innovation that resists one-size-fits-all solutions and engages with Africa's rich traditions, local epistemologies, and historical injustices. Thus, while *CRISPR/Cas9* technology may serve as a transformative tool for livestock improvement, its success depends not only on technical accuracy but also on social legitimacy, regulatory maturity, and ethical robustness, echoing the need for a holistic approach anchored in co-production, precaution, and justice. This is imperative to ensure that gene editing contributes meaningfully to sustainable and equitable livestock development across the continent.

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