APPLICATION OF ADVANCED BIOTECHNOLOGY TOOLS IN VETERINARY MEDICINE

AGINA, Onyinyechukwu Ada
Department of Veterinary Pathology and Microbiology, Faculty of Veterinary Medicine, University of Nigeria Nsukka, Enugu State, Nigeria.
Email: onyinye.noel@unn.edu.ng **Phone:** +234 7039010464

Received *September 13, 2022;* ***Revised*** *October 07, 2022;* ***Accepted*** *October 12, 2022*

ABSTRACT

In Veterinary Medicine, biotechnology plays a significant role in the improvements of animal health, nutrition and genetics. Advances in biotechnology have remarkably produced genetically modified animals for food production and biomedical research. Biotechnological tools applied in veterinary medicine include genetic engineering, gene editing, gene therapy, sterile insect techniques and bioinformatics. Genetic engineering led to the production of genetically modified animals (transgenics), which are tolerant to specific infectious agents, and are beneficial for human health. Gene editing technologies such as clustered regulatory interspersed short palindromic repeats/associated protein 9 (CRISPRs/Cas9) have been used to detect and eliminate possible genetic disorders in animals and biological systems. Gene therapy is utilized in cancer treatment and treatment of cystic fibrosis. Areas, where biotechnology has been applied for the advancement of animal health, include molecular diagnostics (PCR, RT-PCR, nanoPCR, biosensors, proteomics, nanotechnology), production of virus vectored vaccines and DNA vaccine technology. This review, therefore, provides an overview of recent advances and applications of biotechnology in veterinary medicine.

Keywords: Biotechnology, Bioinformatics, Gene editing, Gene therapy, Molecular biology, Transgene, Veterinary pathology

INTRODUCTION

Biotechnology is any technological approach that utilizes biological systems, animals, living organisms or their derivatives, to make or modify products for explicit use in agriculture and biomedical research (NTNU, 2022). Biotechnology has played a significant role in the improvements of three imperative areas of veterinary medicine namely: nutrition, genetics and health (Van Eenennaam, 2016). Animal biotechnology involves the use of science and engineering and started over 8000 years ago, but the enclosure of the genetic code in the mid-1950s prompted the start of the modern era of animal biotechnology. These advanced technological tools (molecular and computing technologies) have significantly modified animals for a wide range of purposes, such as scientific and medical research, and food production (Asaye *et al.*, 2014). In our current society, the advances in animal biotechnology have quickened the use of animals for agriculture, improved human health, and caused a hugely significant breakthrough in biomedical research (Schook *et al.*, 2014). A lot of pertinent traits and/or qualities such as disease-tolerant genes have been effectively introduced into various animal species using genetic engineering and gene editing approaches. These cutting-edge techniques override the traditional selection techniques but it's usually combined with the conventional breeding technique to accelerate genetic improvement in food animals (Schook *et al.*, 2014). Arising from the above, this review evaluates the recent biotechnological tools applied in veterinary medicine.

MATERIALS AND METHODS

A comprehensive internet search of the literature on biotechnological tools applied in veterinary medicine was undertaken using Google Search. The literature recovered was analyzed in pros and relevant cited table adopted.

RESULTS

Genetic Engineering: Genetic engineering is the technique of using recombinant DNA technology to introduce desirable traits or changes into an animal or biological system (Izquierdo, 2001). In other words, genes and DNA fragments are taken from one species (e.g., human, pathogen) and put into another species (e.g., animal), therefore, this technique legitimately controls, alters, modifies and transfers genetic material of cells of a host into another host. Recombinant DNA is a hybrid molecule formed when DNA fragments from at least two distinct species have been combined in vitro (Asaye *et al.*, 2014). This modern approach permits one or more genes to be isolated from large masses of DNA and produced in very huge amounts (Asaye *et al.*, 2014). The genetic code of every single living organism is comprised of similar four nucleotide building blocks; this implies that a gene can encode the same protein whether it is made in a human, animal, plant or pathogen. Recombinant DNA is constructed in such a way as to express proteins that are encoded by the genes included in the construct (Asaye *et al.*, 2014). Genetic engineering involves creating and introducing the recombinant DNA construct into a living organism so that new or changed traits or attributes can be given to that organism (Karp, 2002). Thus, a genetically engineered animal conveys a known sequence of recombinant DNA in its cells and passes that DNA onto its offspring. Several synonyms of genetically engineered animals include transgenic, genetically modified organisms (GMOs) or bioengineered animals. The technology is highly significant and recommended because it brings genetic variations that are absent in the target species, such as disease resistance. DNA from pathogens can be used to permanently immunize species against pathogens such as vector-borne parasites, viral and bacterial species (Van Eenennaam, 2016).

Use of Transgenic Animals for Advanced Animal Health in Economically Important Diseases: Incorporation of new genes into an animal's genome could be low and the varying sites where they occur may often discourage the expression of the new gene. Therefore, researchers considered cloning to be a proficient method to create transgenic animals (Wu and Bazer, 2019). In this case, once a cell line effectively incorporates and expresses a transgene that cell line becomes a donor cell for cloning. The created clones will have the transgene joined into their genome and can successfully transfer it onto their offspring through the traditional breeding methods. This, therefore, can lead to a whole group of transgenic animals expressing desirable gene alleles for biomedical and agricultural purposes. A good number of transgenic animals produced for targeting disease tolerance traits are listed in Table 1. Breeders could use these genetically or bioengineered animals for conventional breeding to facilitate genetic improvement (Schook *et al.*, 2014).

Presently, disease resistance has been reported in a variety of animals. They include avian influenza, foot and mouth disease (FMD), bovine spongiform encephalopathy (mad cow disease), and porcine reproductive and respiratory syndrome. Avian influenza affects a wide range of birds, and it is caused by the influenza virus which is zoonotic and is characterized by a high rate of mutation (WHO, 2018). The mutative nature of the virus and the pandemic associated with it, has encouraged research geared towards creating disease-resistant birds. Currently, transgenic chickens that are unable to transmit the avian influenza virus have been produced, and this is a huge achievement for the avian industry (Schook *et al.*, 2014). Foot and mouth disease caused by the FMD virus, has posed serious global economic losses for cloven-hoofed animals, and immunization as a control strategy, and has not eradicated the disease in countries that experience FMD epidemics (Naranjo and Cosivi, 2013).

Table 1: Examples of transgenic animals targeting disease resistance traits (Adapted from Asaye *et al.,* 2014; Lievens *et al.,* 2015)

Species	Gene	Origin	Effect/Goal
Cattle	Lysostaphin	Bacterial	Mastitis resistance
	Lactoferrin	Bacterial	Mastitis resistance
	Prion		BSE resistance
Chicken	Alv6 envelope glycoprotein	Viral	Disease resistance
	Short hairpin RNA	Viral	Disease resistance
	LacZ	Bacterial	Animal health
Grass Carp	Lactoferrin	Human	Grass carp haemorrhage virus resistance
Sheep	Visna virus envelope	Viral	Visna virus disease resistance
Catfish	Cercopin B	Insect	Bacterial resistance
Goat	Lysozyme	Human-Bovine	Mastitis resistance
	Monosaturated fatty acids	Rat-Bovine	Mastitis resistance
	Lactoferrin	Human	Prophylactic treatment
	Prion		BSE resistance
Pig	Mx1	Murine	Influenza resistance
Salmon	Lysozyme	Piscine	Animal Health

This was attributed to the fact that there are seven serotypes and 60 strains of the virus. Therefore, the possibility that transgenic animals can tackle FMD resistance is currently being studied. Several in-vitro and in-vivo studies have shown that the RNA interference (RNAi) is a viable antiviral strategy, either by small interfering RNAs (siRNAs) or short hairpin RNAs (shRNAs) (Haasnoot *et al*., 2003; Grubman, 2005). siRNA or shRNA genetically modified FMD-resistant animals are yet to be produced but studies are currently geared towards that. FMD is a progressive, degenerative disease of the nervous system caused by abnormal folding of the prion protein (PrP). It affects small ruminants, and because of the fatality associated with the disease, several studies have been embraced to neutralize the prion protein (PRNP) gene using knockout studies, and thus, incite protection from these prion diseases (Hirata *et al*., 2004; Richt *et al*., 2007).

Another disease of interest is the porcine reproductive and respiratory syndrome, a viral disease that affects swine and has incurred high economic losses in the swine industry because of the ability of the virus to cause reproductive failure in adult pigs and respiratory distress in piglets (Brockmeier *et al*., 2002). Pigs are vaccinated against the virus, but their efficacy varies because of the genetic diversity of the virus and its unique ability to evade the immune system. Therefore, studies are currently focused on creating transgenic pigs that are resistant to RNAi.

The mechanism involved in RNAi includes the use of RNA molecules to inhibit gene expression, and it is achieved by neutralizing the specific messenger RNA molecules (Schook *et al.,* 2014).

Antimicrobial peptides (AMPs) such as Cecropin B, has numerous antimicrobial effects (Dunham *et al*., 2002). Cecropin B was created from the giant silk moth and has antimicrobial effects against gram-negative bacteria. This was accomplished by transferring the gene encoding cecropin B into catfish and the Asian medaka fish and these transgenic fish breeds exhibited protection from numerous bacterial pathogens of fish (Dunham *et al*., 2002).

Furthermore, the production of genetically modified animals (transgenics) is also beneficial for human health. Human genes are inserted into an animal's genome so that such animals produce important human proteins, such as factor IX, which helps the blood form clot, and hence stop bleeding (Schook *et al*., 2014). Therefore, advances in biotechnology have enabled the addition of complex folding patterns and additional sugar molecules to human proteins such as factor IX and monoclonal antibodies to become biologically active. Transgenic and cloning are extraordinary, and of huge advantage for producing organs in animals for human transplants, or xenotransplantation. If animals are genetically modified to produce viable

organs for humans, cloning could drastically increase the human organ supply.

Gene Editing: Gene editing opens a new world of possibilities for transgenic animals. It is an approach that utilizes site-directed nucleases (nuclease-based genomic editing) to alter or change the genetic code at a specific location in the animal genome (Li *et al.*, 2020). This technology enables researchers to erase, add or replace letters in the genetic code (A G T C). Gene editing has been applied for the correction of diseases or disorders that have a genetic basis. It could also be used to change a less desirable gene allele to a more desirable allele without the need to bring in that allele through breeding with an animal that carries the desirable gene allele. Therefore, gene editing is more precise as it utilizes 'molecular scissors' called site-directed nucleases (SDN) to cut DNA at a specific area in the genome based on recognition of the specific, unique target DNA sequence (Jinek *et al.*, 2012). The cut site is then fixed by the DNA repair mechanisms of the cell (non-homologous end joining). These repairs can be directed to introduce, delete, or replace a series of letters in the genetic code. For example, the result of this may be a targeted single nucleotide polymorphism (SNP) edit, whereby the nucleotide A at a specific location in the genome is intentionally replaced by the nucleotide T.

Gene editing eventually leads to the production of 'custom-made animals' with improved hereditary characteristics and modifications (Schook *et al.*, 2014; Van Eenennaam, 2016). It adequately imitates the natural processes that form the basis of selective breeding programs.

Several genome editing technologies have been used to generate many edited food animals (Tan *et al.*, 2016). They include zinc finger nucleases (ZFNs) (Sood *et al.*, 2013; Watanabe et al., 2013), transcription activator-like effector nucleases (TALENs) (Tesson *et al.*, 2011; Xin *et al.*, 2013), homing endonucleases (Delacôte *et al.*, 2013) and clustered regulatory interspersed short palindromic repeats/ associated protein 9 (CRISPRs/Cas9 (Cong *et al.*, 2013; Mali *et al.*, 2013).

The CRISPR system is a family of DNA sequences found in the genomes of prokaryotic organisms such as bacteria and archaea and uses small RNAs and CRISPR-associated proteins to detect and defend against invading viruses and plasmids (Deltcheva *et al.*, 2011; Jinek *et al.*, 2012). This system has been adapted as an efficient genome editing tool in laboratory animals such as mice, rats, zebrafish and minipigs. Recently, this cutting-edge technology has been used to knock out genes in pigs and goats. Gene editing has been used to produce pigs that are genetically resistant to African swine fever (ASF) by targeting the RELA gene (Lillico *et al.*, 2016). Gene-edited pigs resistant to the porcine respiratory and reproductive syndrome (PRRS) virus have been generated and were also achieved by editing the RELA (p65) gene (Whitworth *et al.*, 2016). Ni *et al.* (2014) suppressed the prion protein gene by producing gene-edited goats resistant to spongiform encephalopathy using CRISPR/ Cas9 gene editing technology.

It is still unclear if gene editing ought to be regulated just like bioengineered animals, but from studies performed so far, there is no need to regulate gene editing. This is because gene editing does not necessarily introduce any foreign or novel genetic DNA into the genome of the edited animal. Changes produced are not discernable from naturally occurring gene alleles. For example, gene editing of a specific gene is performed using a template nucleic acid dictated by the sequence of a naturally occurring allele from the same species.

Application of Advanced Biotechnology Tools in Animal Health

Molecular diagnosis: Molecular diagnostic techniques such as polymerase chain reaction (PCR) assay is highly sensitive, specific and consistent in the detection of pathogens and diagnosis of a disease, or asymptomatic or pre-symptomatic infections (Yamamoto, 2002). Its high sensitivity is attributed to its ability to rapidly amplify the gene of interest into billions of identical DNA copies. Several forms of PCR such as real-time PCR and PCR-restriction fragment length polymorphism have been

employed for checking genetic mutation and single nucleotide polymorphism in different animal breeds. The real-time PCR or quantitative PCR has been integrated into the veterinary diagnostic laboratory (VanGuilder *et al.*, 2008), and is regarded as one of the most sensitive PCRs, because of its quantitative nature of determining the gene copy numbers. Nanoparticle-assisted PCR is a recently developed PCR technique, but it is currently used by large-scale research and/or diagnostic laboratories for the rapid detection of bacterial and viral DNA (Wang *et al.*, 2015). Another modern diagnostic technique which involves the analysis of protein expression, functions, post-translational alterations, translocation, and interactions of proteins expressed by a genome is known as proteomics (Graves and Haystead, 2002). It enhances the identification and characterization of proteins formed by pathogens and is of huge interest in veterinary medicine, as it enables the study of expression patterns of bacteria, viruses and parasites (Meyfour *et al.*, 2013; Ceciliani *et al.*, 2014). Current veterinary diagnostic tools including biosensors, fluorescent in-situ hybridisation (FISH) and nanotechnologies are currently employed in the diagnosis of diseases of veterinary importance (Schmitt and Henderson, 2005).

Virus-vectored vaccines: Virus-vectored vaccine carries a single (monocistronic) or dual (bicistronic) foreign gene. They are produced using gene editing technologies. Recently, the use of the CRISPR/Cas9-based gene editing method has enlarged the possibility of editing DNA viral genomes of several large DNA viruses, such as Herpesvirus (Tang *et al.*, 2020), Adenovirus (Bi *et al.*, 2014), Pseudorabies virus (Xu *et al.*, 2015), Vaccinia virus (Yuan *et al.*, 2015), Epstein-Barr virus (Yuen *et al.*, 2015), Marek's disease virus (Zhang *et al.*, 2019) and Infectious laryngotracheitis virus (Atasoy *et al.*, 2019). The use of this advanced gene editing technology recently generated a triple insert live avian Herpesvirus vectored vaccine (Tang *et al.*, 2020). Viral genes that were inserted in the Herpesvirus of Turkey (HVT) vector include the infectious laryngotracheitis virus, avian influenza virus and infectious bursal disease virus, resulting in a stable triple gene insert recombinant HVT vectored vaccine, which was evaluated by PCR, immunostaining and western blot (Tang *et al.*, 2020). The use of CRISPR/Cas9 technology in advancing Orthopoxvirus genome editing for vaccine and vector development has been reviewed (Okoli *et al.*, 2018). Briefly, the CRISPR/Cas9, derived from the standardized *Streptococcus* pyogenes Type II with adaptive immunity against pathogens by using CRISPR RNAs or dual RNA-guided DNA endonucleases such as Cas (Jinek *et al.*, 2012; Cong *et al.*, 2013), to target, break and cleave DNA at specific sequences, thereby silencing the invading nucleic acids (Heler *et al.*, 2015; Wei *et al.*, 2015). Orthopoxviruses are among the suitable viral vectors because of their large transgene capacity, and ability to elicit sustained cellular and humoral immune response to the vectored antigens. The Orthopoxvirus vector is heat stable as a freeze-dried compound, easy to store, transport and use, and cost-effective to produce (Gudmundsdotter *et al.*, 2009).

DNA vaccines: Biotechnological approach to vaccine development has led to the construction of DNA vaccines to combat latent or persistent infections of human and veterinary importance. One such disease is trypanosomiasis where globally, there is no effective vaccine yet. Immunization with DNA vaccines involves the delivery of DNA plasmids directly into the host cells through different delivery routes, to express the desired antigens (Weniger and Papania, 2012), to induce sustainable immune responses against a variety of infectious and non-infectious diseases (Anderson and Schneider, 2007). DNA vaccines have an advantage over the use of live vaccines because it does not replicate and is non-live, and do not revert to a virulent form (Villarreal *et al.,* 2013). The DNA vaccine construct is made of DNA plasmid, one or more antigens of interest, molecular adjuvants (cytokines), and nanoparticles (e.g., Chitosan). The cytokines facilitate the adjuvant activity of DNA-based vaccines, while the nanoparticle acts as a suitable carrier, which is biodegradable, easily absorbed and slowly releases the gene of

interest to maintain a long-lasting gene expression and elicit immunological memory. This nano-delivery system was initiated to improve DNA vaccine technologies and could also protect the digestion of DNA by DNase I or gastric enzymes if administered orally (Lim *et al.*, 2020). The molecular adjuvant is capable of selective activation of CD8+, CD4+ and increased antigen-specific antibody responses in a variety of animal models (Speiser *et al.*, 2005). DNA vaccines are less toxic, cost-effective to produce, and chemically and temperature stable under several conditions. The use of DNA vaccine stimulates strong and sustained cellular and humoral immune responses characterised by increased cytotoxicities of CD8+ T cells and higher levels of neutralizing IgG or IgA antibodies (Dong *et al.*, 2017). The route of delivery of plasmid DNA encoding a gene of interest could be subcutaneous, topical, intradermal, intranasal or intramuscular (Hu *et al.*, 2011; Villarreal *et al.*, 2013).

DNA vaccination is currently applied as a control strategy for *Haemonchus contortus* infection in lambs and goats. Its administration led to the stimulation of CD4+ T lymphocytes and elevated serum levels of antigen-specific IgG and IgA, to reduce worm burden and faecal egg count (Zhao *et al.*, 2012; Yan *et al.*, 2013; 2014). Promising work on *Leishmania*, a closely related trypanosome family carried out on mice using DNA plasmid encoding Leishmania gamma-glutaylcysteine synthetase induced specific IgG1 and IgG2a antibodies, caused a significant increase in cell-mediated immunity and reduction in liver parasite burdens (Carter *et al.*, 2007). It is believed that in the nearest future, a similar DNA vaccine strategy could be developed for the control of African trypanosomiasis. A study on the development of a DNA vaccine encoding trans-sialidase (an enzyme partly involved in the pathogenesis of anaemia in trypanosomiasis) from *Trypanosoma brucei* is being conducted (Silva *et al.*, 2009). Recently, Gupta *et al.* (2019) reported the induction of innate and T helper 1 immunity against the causative agent of Chagas disease (*Trypanosoma cruzi*) infection using DNA vaccination in mice. Tissue parasites and pathology associated with acute trypanosomiasis disease were not detected following the use of the DNA vaccine (Gupta *et al.*, 2019).

Gene therapy: Gene therapy is a therapeutic procedure in which a desirable gene is inserted into a cell to treat a metabolic disorder (Soetan and Abatan, 2008; Patil *et al.*, 2012; Ugwu *et al.*, 2019). In other words, it is the use of DNA as a therapeutic agent to treat disease. The main concept is to repair a mutated gene. A good example of gene therapy is the use of DNA that encodes a functional therapeutic gene to replace a mutated gene. It has been utilized in cancer treatment, cystic fibrosis, hepatitis, influenza and mycobacterium tuberculosis infection (Soetan and Abatan, 2008). Gene therapy involves packaging a therapeutic protein within a vector i.e. chemotherapeutics and plasmid DNA and transferring it into the host cells by electroporation (Ugwu *et al.*, 2019). It is a safe and viable strategy for the transfer of cytotoxic drugs, nucleic acids and ions into cells and tissues without hurting them (Calvet and Mir, 2016). Once the therapeutic gene is inside the host, the gene becomes expressed by the cell, resulting in the production of a therapeutic protein (Sheridan, 2011). Therefore, the whole idea in gene therapy is to remove the cells of interest, get them genetically modified in vitro, and then returned them to the body. Also, genetic modification can be done *in situ*.

Sterile insect technique: The sterile insect technique (SIT) is a control strategy that uses radiation to produce genetic mutations and generate many sterile adult male insects, which are released into the environment to suppress and eradicate wild pests or insect-borne diseases. This technique encourages mating between sterile male and female wild counterparts (Gupta and Jindal, 2013; Nayduch *et al.*, 2019). This technique has been successful in eradicating New World screwworm flies from North and Central America (Morrison *et al.,* 2010), the tsetse flies from Unguja Island in Zanzibar, Tanzania (Vreysen *et al.*, 2000), but less successful in controlling populations of

mosquito vectors of Arboviruses such as Dengue, Zika, West Nile virus, Chikungunya virus, and protozoa e.g. Plasmodium spp. since irradiated male mosquitoes have reduced performance, and hence, could not effectively compete with their wild male counterparts (Dame *et al.*, 2009). Therefore, advanced biotechnology tools deliberately manipulate and reduce insect populations to achieve insect control (Gupta and Jindal, 2013). Furthermore, advances in biotechnology involve the use of improved insect strains for mass rearing, and the transgenic introduction of fluorescent transformation molecular markers to identify the released sterile males and thus, improve the effectiveness of the sterile insect control technologies (Franz and Robinson, 2011; Gupta and Jindal, 2013). Fluorescent sperm marking systems have been introduced in mosquito species such as *Anopheles gambiae* and *Aedes aegypti* (Schetelig and Wimme, 2011). The translation of transgenic SIT strategies to insects of veterinary importance is currently in use. The robust transformation markers dependent on enhanced green fluorescent protein (EGFP) variants and the improved transformation vectors fusing with the piggyBac transposable elements are among the transgenic sterile insect techniques (Horn *et al.*, 2002; Eckermann *et al.*, 2018). These advanced techniques have transformed several species of Diptera and Coleoptera etc. (Schetelig and Wimme, 2011).

Sterile female insects are no longer released into the field because of oviposition, stinging and transmission of diseases, therefore conventional and labour-intensive hand sorting or separation of males from females based on external morphology was replaced by a transgenic sexing system. The transgenic sexing system (transgene-based embryonic lethality) does not require radiation and thus results in the death of 99.9 % of females and the mass production of sterile males. These sterile male insects are genetically modified to harbour a repressible lethal gene and the females express a conditionally lethal gene (Thomas *et al.,* 2000). The RNAi technology, a sequence-specific gene silencing approach, has now been proposed as the best control strategy over the existing transgenic approaches to insect pests that attack crops and could be employed against insect vectors of veterinary importance (Mamta and Rajam, 2017).

Bioinformatics: This is the science of collecting and analyzing complex biological data using advanced computing technology (Fernald *et al.*, 2011). Bioinformatics algorithms such as Basic Local Alignment Sequence Tool (BLAST), Fast Alignment (FASTA), and Clustal W solutions for sequence search and analysis are cost-effective, readily accessible and a time reduction strategy to acquire information on genes and proteins not easily obtainable previously (Kaikabo and Kalshingi, 2008). From a large data pool of sequenced organisms, bioinformatics has eased the discovery of vaccine targets for advancement in veterinary vaccine evolution. Current studies in developed countries have demonstrated the value of next-generation sequencing (NGS) technologies (Park and Kim, 2016). The NGS technology has evolved into a 'molecular microscope', which provides a broad examination of the bacterial whole genomes and is applied in clinical microbiology to replace conventional colony characterization of microbes in the laboratory (Deurenberg *et al.*, 2017; Tagini and Greub, 2017). The use of NGS is gradually replacing Sanger sequencing (Muir *et al.*, 2016). It requires less amount purified DNA to produce accurate, price and consistent data, and lower noise background sequence data (Kulski, 2016). Next-generation sequencing platforms include Illumina, 454 pyrosequencing, Solid, Ion Torrent PGM, Oxford Nanopore, PacBio, Helicos and Complete Genomics DNA Nanoball (Kulski, 2016).

Limitations of Biotechnology: It is worth noting that there are numerous concerns about the use of biotechnology in agriculture and biomedical research. One such concern is that food products from transgenic or cloned animals may pose risk to human health. It could have environmental, religious, cultural and ethical issues and a great impact on animal welfare (Asaye *et al.*, 2014). These concerns of significant interest have been subjected to

increased attention and discussions among the public, research scientists and regulatory bodies. One of the concerns raised was that products derived from transgenes could cause an allergenic, lethal protein to be present in food, or contain products that may contain antinutritional factors (Kochhar and Evans, 2007). Another major concern is the use of antibiotic resistance marker genes in animals and humans. These antibiotic tolerance marker genes could suppress the use and efficacy of antibiotics in the future. The presence of a huge quantity of antibiotic resistance genes in food eaten by animals, or in the environment could pass the trait of antibiotic resistance rapidly. Non-pathogenic bacteria (normal flora) can pick up genes from their environment (animal or human guts or skin) and transfer them onto pathogenic species of bacteria, thereby making diseases much more difficult to treat.

Conclusion: Biotechnology encompasses several technologies that are used to promote animal health, genetics and nutrition. The use of biotechnology in advanced vaccine technology such as in plasmid DNA vaccination proved its efficacy for use in preclinical and clinical assays. Though few veterinary plasmid DNA vaccines are available in the market, more studies are being carried out to ensure their use and availability, especially in developing countries. It is safe, easy to fabricate and stable. The use of bioengineered animals is regulated to ensure that it is safe to use animals gotten from such modern technologies to boost the livestock industry to compensate for the high demands of food from livestock farming. Application of advanced biotechnology tools in animal science has been associated with benefits such as the production of bioengineered animals that are capable of resisting diseases, animals with high meat and/or meat yield, treatment of genetic and metabolic diseases and in advanced vaccine technology.

ACKNOWLEDGEMENTS

The author would like to thank her Animal Molecular Biology Lecturer, Professor Zunita Zakaria from the Universiti Putra Malaysia, for the inspiration to write this review article.

REFERENCES

ANDERSON, R. J. and SCHNEIDER, J. (2007). Plasmid DNA and viral vector-based vaccines for the treatment of cancer. *Vaccine*, 25(Suppl. 2): B24 – B34.

ASAYE, M., BIYAZEN, H. and GIRMA, M. (2014). Genetic engineering in animal production: applications and prospects. *Biochemistry and Biotechnology Research*, 2(2): 12 – 22.

ATASOY, M. O., ROHAIM, M. A. and MUNIR, M. (2019). Simultaneous deletion of virulence factors and insertion of antigens into the infectious laryngotracheitis virus using NHEJ-CRISPR/Cas9 and Cre–Lox system for construction of a stable vaccine vector. *Vaccines*, 7(4): 207. https://doi.org/10.3390/vaccines7040207

BI, Y., SUN, L., GAO, D., DING, C., LI, Z., LI, Y., CUN, W. and LI, Q. (2014). High-efficiency targeted editing of large viral genomes by RNA-guided nucleases. *PLoS Pathogenesis*, 10(5): e1004090. https://doi.org/10.1371/journal.ppat.1004090

BROCKMEIER, S. L., HALBUR, P. G. and THACKER, E. L. (2002). Porcine respiratory disease complex. Pages 231 – 258. *In*: BROGDEN, K. A. and GUTHMILLER, J. M. (Eds.). *Polymicrobial Diseases.* ASM Press, Washington, D.C., USA.

CALVET, C. Y. and MIR, L. M. (2016). The promising alliance of anti-cancer electrochemotherapy with immunotherapy. *Cancer and Metastasis Reviews*, 35(2): 165 – 177.

CARTER, K. C., HENRIQUEZ, F. L., CAMPBELL, S. A., ROBERTS, C. W., NOK, A., MULLEN, A. B. and MCFARLANE, E. (2007). DNA vaccination against the parasite enzyme gamma-glutamylcysteine synthetase confers protection against *Leishmania donovani* infection. *Vaccine*, 25(22): 4502 – 4509.

CECILIANI, F., ECKERSALL, D., BURCHMORE, R. and LECCHI, C. (2014). Proteomics in veterinary medicine: applications and

trends in disease pathogenesis and diagnostics. *Veterinary Pathology*, 51(2): 351 – 362.

CONG, L., RAN, F. A., COX, D., LIN, S., BARRETTO, R., HABIB, N., HSU, P. D., WU, X., JIANG, W., MARRAFFINI, L. A. and ZHANG, F. (2013). Multiplex genome engineering using CRISPR/Cas systems. *Science,* 339(6121): 819 – 823.

DAME, D. A., CURTIS, C. F., BENEDICT, M. Q., ROBINSON, A. S. and KNOLS, B. G. (2009). Historical applications of induced sterilisation in field populations of mosquitoes. *Malaria Journal*, 8(Suppl. 2): S2. https://doi.org/10.1186/1475-2875-8-S2-S2

DELACÔTE, F., PEREZ, C., GUYOT, V., DUHAMEL, M., ROCHON, C., OLLIVIER, N., MACMASTER, R., SILVA, G. H., PÂQUES, F., DABOUSSI, F. and DUCHATEAU, P. (2013). High frequency targeted mutagenesis using engineered endonucleases and DNA-end processing enzymes. *Plos One*, 8(1): e0053217. https://doi.org/10.1371/journal.pone.0053217

DELTCHEVA, E., CHYLINSKI, K., SHARMA, C. M., GONZALES, K., CHAO, Y., PIRZADA, Z. A., ECKERT, M. R., VOGEL, J. and CHARPENTIER, E. (2011). CRISPR RNA maturation by trans-encoded small RNA and host factor RNase III. *Nature*, 471(7340): 602 – 607.

DEURENBERG, R. H., BATHOORN, E., CHLEBOWICZ, M. A., COUTO, N., FERDOUS, M., GARCÍA-COBOS, S., KOOISTRA-SMID, A. M. D., RAANGS, E. C., ROSEMA, S., VELOO, A. C. M., ZHOU, K., FRIEDRICH, A. W. and ROSSEN, J. W. A. (2017). Application of next generation sequencing in clinical microbiology and infection prevention. *Journal of Biotechnology*, 243: 16 – 24.

DONG, L. L., TANG, R., ZHAI, Y. J, and MALLA, T. H. K. (2017). DNA vaccine expressing herpes simplex virus 1 glycoprotein C and D protects mice against herpes simplex keratitis. *International Journal of Ophthalmology*, 10(11): 1633 – 1639.

DUNHAM, R. A., WARR, G. W., NICHOLS, A., DUNCAN, P. L., ARGUE, B., MIDDLETON, D. and KUCUKTAS, H. (2002). Enhanced bacterial disease resistance of transgenic channel catfish *Ictalurus punctatus* possessing cecropin genes. *Marine Biotechnology*, 4(3): 338 – 344.

ECKERMANN, K. N., AHMED, H. M., KARAMINEJADRANJBAR, M., DIPPEL, S., OGAUGWU, C. E., KITZMANN, P., ISAH, M. D. and WIMMER, E. A. (2018). Hyperactive piggyBac transposase improves transformation efficiency in diverse insect species. *Insect Biochemistry and Molecular Biology*, 98: 16 – 24.

FERNALD, G. H., CAPRIOTTI, E., DANESHJOU, R., KARCZEWSKI, K. J. and ALTMAN, R. B. (2011). Bioinformatics challenges for personalized medicine. *Bioinformatics,* 27(13): 1741 – 1748.

FRANZ, G. and ROBINSON, A. S. (2011). Molecular technologies to improve the effectiveness of the sterile insect technique. *Genetica*, 139(1): 1 – 5.

GRAVES, P. R. and HAYSTEAD, T. A. (2002). Molecular biologist's guide to proteomics. *Microbiology and Molecular Biology Reviews*, 66(1): 39 – 63.

GRUBMAN, M. J. (2005). Development of novel strategies to control foot-and-mouth disease: marker vaccines and antivirals. *Biologicals*, 33(4): 227 – 234.

GUDMUNDSDOTTER, L., NILSSON, C., BRAVE, A., HEJDEMAN, B., MOSS, B., ROBB, M., COX, J., MICHAEL, N., MAROVICH, M., BIBERFELD, G., WAHREN, B. and HOSPITAL, S. (2009). Recombinant Modified Vaccinia Ankara (MVA) effectively boosts DNA-primed HIV-specific immune responses in humans despite pre-existing vaccinia immunity. *Vaccine*, 27(33): 4468 – 4474.

GUPTA, S., SALGADO-JIMÉNEZ, B., LOKUGAMAGE, N., VÁZQUEZ-CHAGOYÁN, J. C. and GARG, N. J. (2019). TcG2/TcG4 DNA vaccine induces Th1 immunity against acute *Trypanosoma cruzi* infection: adjuvant and antigenic effects of heterologous *T. rangeli* booster immunization. *Frontiers in Immunology*, 10: 1456. https://doi.org/10.3389/fimm

u.2019.01456

GUPTA, V. K. and JINDAL, V. (2013). Biotechnological approaches for insect pest management. Pages 311 – 335. *In:* ABROL, D. P. (Ed.). *Integrated Pest Management: Current Concepts and Ecological Perspective*. Academic Press, Elsevier Incooporated, Cambridge, Massachusetts, United States.

HAASNOOT, J., CUPAC, D. and BERKHOUT, B. (2003). Inhibition of virus replication by RNA interference. *Journal of Biomedical Science*, 10(6): 607 – 616.

HELER, R., SAMAI, P., MODELL, J. W., WEINER, C., GREGORY, W., BIKARD, D. and MARRAFFINI, L. A. (2015). Cas9 specifies functional viral targets during CRISPR-Cas adaptation adaptation. *Nature*, 519(7542): 199 – 202.

HIRATA, R. K., XU, C., DONG, R., MILLER, D. G., FERGUSON, S. and RUSSELL, D. W. (2004). Efficient PRNP gene targeting in bovine fibroblasts by adeno-associated virus vectors. *Cloning and Stem Cells*, 6(1): 31 – 36.

HORN, C., SCHMID, B. G., POGODA, F. S. and WIMMER, E. A. (2002). Fluorescent transformation markers for insect transgenesis. *Insect Biochemistry and Molecular Biology*, 32(10): 1221 – 1235.

HU, K., HE, X., YU, F., YUAN, X., HU, W., LIU, C., ZHAO, F. and DOU, J. (2011). Immunization with DNA vaccine expressing herpes simplex virus type 1 gD and IL-21 protects against mouse herpes keratitis. *Immunological Investigations*, 40(3): 265 – 278.

IZQUIERDO, R. (2001). *Genetic Engineering.* 2nd Edition, Pyramid, Madrid, Spain.

JINEK, M., CHYLINSKI, K., FONFARA, I., HAUER, M., DOUDNA, J. A. and CHARPENTIER, E. (2012). A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. *Science*, 337(6096): 816 – 821.

KAIKABO, A. A. and KALSHINGI, H. A. (2008). Concepts of bioinformatics and its application in veterinary research and vaccines development. *Nigerian Veterinary Journal*, 28(2): 39 – 46.

KARP, G. (2002). *Cell and Molecular Biology: Concepts and Experiments*. 3rd Edition, John Wiley and Sons, Hoboken, New Jersey, USA.

KOCHHAR, H. P. S. and EVANS, B. R. (2007). Current status of regulating biotechnology-derived animals in Canada—animal health and food safety considerations. *Theriogenology*, 67(1): 188 – 197.

KULSKI, J. K. (2016). Next-generation sequencing—an overview of the history, tools, and "Omic" applications. *In:* KULSKI, J. K. (Ed.). *Next-Generation Sequencing.* IntechOpen, London. https://doi.org/10.5772/61964

LI, H., YANG, Y., HONG, W., HUANG, M., WU, M. and ZHAO, X. (2020). Applications of genome editing technology in the targeted therapy of human diseases: mechanisms, advances and prospects. *Signal Transduction and Targeted Therapy*, 5: 1. https://doi.org/10.1038/s41392-019-0089-y

LIEVENS, A., PETRILLO, M., QUERCI, M. and PATAK, A. (2015). Genetically modified animals: Options and issues for traceability and enforcement. *Trends in Food Science and Technology*, 44(2): 159 – 176.

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. and WHITELAW, C. B. A. (2016). Mammalian interspecies substitution of immune modulatory alleles by genome editing. *Scientific Reports*, 6: 21645. https://doi.org/10.1038/srep21645

LIM, M., BADRUDDOZA, A. Z. M., FIRDOUS, J., AZAD, M., MANNAN, A., AL-HILAL, T. A., CHO, C. S. and ISLAM, M. A. (2020). Engineered nanodelivery systems to improve DNA vaccine technologies. *Pharmaceutics*, 12(1): 30. https://doi.org/10.3390/pharmaceutics12010030

MALI, P., YANG, L., ESVELT, K. M., AACH, J., GUELL, M., DICARLO, J. E., NORVILLE, J. E., CHURCH, G. M. (2013). RNA-guided human genome engineering via Cas9. *Science*, 339(6121): 823 – 826.

MAMTA, B. and RAJAM, M. V. (2017). RNAi technology: a new platform for crop pest control. *Physiology and Molecular Biology of Plants*, 23(3): 487 – 501.

MEYFOUR, A., TAVIRANI, M. R. and SADEGHI, M. R. (2013). Common proteomic technologies, applications, and their limitations. *Journal of Paramedical Sciences*, 4: 115 – 125.

MORRISON, N. I., FRANZ, G., KOUKIDOU, M., MILLER, T. A., SACCONE, G., ALPHEY, L. S., BEECH, C. J., NAGARAJU, J., SIMMONS, G. S. and POLITO, L. C. (2010). Genetic improvements to the sterile insect technique for agricultural pests. *Asia-Pacific Journal of Molecular Biology and Biotechnology*, 18(2): 275 – 295.

MUIR, P., LI, S., LOU, S., WANG, D., SPAKOWICZ, D. J., SALICHOS, L., ZHANG, J., WEINSTOCK, G. M., ISAACS, F., ROZOWSKY, J. and GERSTEIN, M. (2016). The real cost of sequencing: Scaling computation to keep pace with data generation. *Genome Biology*, 17: 53. https://doi.org/10.1186/s13059-016-0917-0

NARANJO, J. and COSIVI, O. (2013). Elimination of foot-and-mouth disease in South America: lessons and challenges. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 368 (1623): 20120381. https://doi.org/10.1098%2Frstb.2012.0381

NAYDUCH, D., FRYXELL, R. T. and OLAFSON, P. U. (2019). Molecular tools used in medical and veterinary entomology. Pages 673 – 694. *In:* MULLEN, G. R. and DURDEN, L. A. (Eds.). *Medical and Veterinary Entomology*. Third Edition, Academic Press, Elsevier Incooporated, Cambridge, Massachusetts, United States.

NI, W., QIAO, J., HU, S., ZHAO, X., REGOUSKI, M., YANG, M., POLEJAEVA, I. A. and CHEN, C. (2014). Efficient gene knockout in goats using CRISPR/Cas9 system. *PloS One*, 9(9): e0106718. https://doi.org/10.1371/journal.pone.0106718

NTNU (2022). What is Biotechnology? Norwegian University of Science and Technology (NTNU). https://www.ntnu.edu/ibt/about-us/what-is-biotechnology

OKOLI, A., OKEKE, M., TRYLAND, M. and MOENS, U. (2018). CRISPR/Cas9—advancing orthopoxvirus genome editing for vaccine and vector development. *Viruses*, 10(1): 50. https://doi.org/ 10.3390/v10010050

PATIL, A., CHAN, C. T. Y., DYAVAIAH, M., ROONEY, J. P., DEDON, P. C. and BEGLEY, T. J. (2012). Translational infidelity-induced protein stress results from a deficiency in Trm9-catalyzed tRNA modifications. *RNA Biology*, 9(7): 990 – 1001.

PARK, S. T. and KIM, J. (2016). Trends in next-generation sequencing and a new era for whole genome sequencing. *International Neurourology Journal*, 20(Suppl. 2): S76 – S83.

RICHT, J. A., KASINATHAN, P., HAMIR, A. N., CASTILLA, J., SATHIYASEELAN, T., VARGAS, F., SATHIYASEELAN, J., WU, H., MATSUSHITA, H., KOSTER, J., KATO, S., ISHIDA, I., SOTO, C., ROBL, J. M. and KUROIWA, Y. (2007). Production of cattle lacking prion protein. *Nature Biotechnology*, 25(1): 132 – 138.

SCHETELIG, M. F. and WIMMER, E. A. (2011). Insect transgenesis and the sterile insect technique. Pages 169 – 194. *In:* VILCINSKAS, A. (Ed.). *Insect Biotechnology*. Biologically-Inspired Systems, Volume 2, Springer, Dordrecht.

SCHMITT, B. and HENDERSON, L. (2005). Diagnostic tools for animal diseases. *Revue Scientifique et Technique*, 24(1): 243 – 250.

SCHOOK, L. B., RUND, L. A., HU, W., DARFOUR-ODURO, K. A., KNAPP, L. A., RODRIGUES, F. M. and SCHACHTSCHNEIDER, K. M. (2014). Advances in animal biotechnology. *Encyclopedia of Agriculture and Food Systems*, 1: 1 – 16.

SHERIDAN, C. (2011). Gene therapy finds its niche. *Nature Biotechnology*, 29(2): 121 – 128.

SILVA, M. S., PRAZERES, D. M. F., LANÇA, A., ATOUGUIA, J. and MONTEIRO, G. A. (2009). Trans-sialidase from *Trypanosoma*

brucei as a potential target for DNA vaccine development against African trypanosomiasis. *Parasitology Research*, 105(5): 1223 – 1229.

SOETAN, K. O. and ABATAN, M. O. (2008). Biotechnology a key tool to breakthrough in medical and veterinary research. *Biotechnology and Molecular Biology Reviews*, 3(4): 88 – 94.

SOOD, R., CARRINGTON, B., BISHOP, K., JONES, M., RISSONE, A., CANDOTTI, F., CHANDRASEKHARAPPA, S. C. and LIU, P. (2013). Efficient methods for targeted mutagenesis in zebrafish using zinc-finger nucleases: data from targeting of nine genes using CompoZr or CoDA ZFNs. *PloS One*, 8(2): e57239. https://doi.org/10.1371/journal.pone.0057239

SPEISER, D. E., LIÉNARD, D., RUFER, N., RUBIO-GODOY, V., RIMOLDI, D., LEJEUNE, F., KRIEG, A. M., CEROTTINI, J. C. and ROMERO, P. (2005). Rapid and strong human CD8+ T cell responses to vaccination with peptide, IFA, and CpG oligodeoxynucleotide 7909. *Journal of Clinical Investigation*, 115(3): 739 – 746.

TAGINI, F. and GREUB, G. (2017). Bacterial genome sequencing in clinical microbiology: a pathogen-oriented review. *European Journal of Clinical Microbiology and Infectious Diseases*, 36(11): 2007 – 2020.

TAN, W., PROUDFOOT, C., LILLICO, S. G. and WHITELAW, C. B. A. (2016). Gene targeting, genome editing: from Dolly to editors. *Transgenic Research*, 25(3): 273 – 287.

TANG, N., ZHANG, Y., SADIGH, Y., MOFFAT, K., SHEN, Z., NAIR, V. and YAO, Y. (2020). Generation of a triple insert live avian herpesvirus vectored vaccine using CRISPR/Cas9-based gene editing. *Vaccines*, 8(1): 97. https://doi.org/10.3390/vaccines8010097

TESSON, L., USAL, C., MÉNORET, S., LEUNG, E., NILES, B. J., REMY, S., SANTIAGO, Y., VINCENT, A. I., MENG, X., ZHANG, L. and GREGORY, P. D. (2011). Knockout rats generated by embryo microinjection of TALENs. *Nature Biotechnology*, 29(8): 695 – 696.

THOMAS, D. D., DONNELLY, C. A., WOOD, R. J. and ALPHEY, L. (2000). Insect population control using a dominant, repressible, lethal genetic system. *Science*, 287 (5462): 2474 – 2476.

UGWU, G. C., EGBUJI, J. V. I., OKANYA, L. C., OMEJE, J. N. and EYO, J. E. (2019). Gene therapy, physiological applications, problems and prospects – a review. *Animal Research International*, 16(2): 3367 – 3392.

VAN EENENNAAM, A. L. (2016). The current and future uses of biotechnology in animal agriculture. *Ceiba*, 54(1): 72 – 81.

VANGUILDER, H. D., VRANA, K. E. and FREEMAN, W. M. (2008). Twenty-five years of quantitative PCR for gene expression analysis. *Biotechniques*, 44(5): 619 – 626.

VILLARREAL, D. O., TALBOTT, K. T., CHOO, D. K., SHEDLOCK, D. J. and WEINER, D. B. (2013). Synthetic DNA vaccine strategies against persistent viral infections. *Expert Review of Vaccines*, 12(5): 537 – 554.

VREYSEN, M. J., SALEH, K. M., ALI, M. Y., ABDULLA, A. M., ZHU, Z. R., JUMA, K. G., DYCK, V. A., MSANGI, A. R., MKONYI, P. A. and FELDMANN, H. U. (2000). *Glossina austeni* (Diptera: Glossinidae) eradicated on the island of Unguja, Zanzibar, using the sterile insect technique. *Journal of Economic Entomology*, 93(1): 123 – 135.

WANG, J., CHENG, Y., ZHANG, M., ZHAO, H., LIN, P., YI, L., TONG, M. and CHENG, S. (2015). Development of a nanoparticle-assisted PCR (nanoPCR) assay for detection of mink enteritis virus (MEV) and genetic characterization of the NS1 gene in four Chinese MEV strains. *BMC Veterinary Research*, 11: 1. https://doi.org/10.1186/s12917-014-0312-6

WATANABE, M., NAKANO, K., MATSUNARI, H., MATSUDA, T., MAEHARA, M., KANAI, T., KOBAYASHI, M., MATSUMURA, Y., SAKAI, R., KURAMOTO, M. and HAYASHIDA, G., (2013). Generation of

interleukin-2 receptor gamma gene knockout pigs from somatic cells genetically modified by zinc finger nuclease-encoding mRNA. *PloS One*, 8(10): e76478. https://doi.org/10.1371/journal.pone.0076478

WEI, Y., TERNS, R. M. and TERNS, M. P. (2015). Cas9 function and host genome sampling in type II – a CRISPR-Cas adaptation. *Genes and Development*, 29(4): 356 – 361.

WENIGER, B. G. and PAPANIA, M. J. (2012). Alternative vaccine delivery methods. *Vaccines,* 2013: 1200 – 1231.

WHITWORTH, K. M., ROWLAND, R. 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. and PRATHER, R. S. (2016). Gene-edited pigs are protected from porcine reproductive and respiratory syndrome virus. *Nature Biotechnology*, 34(1): 20 – 22.

WHO (2018). Influenza (Avian and other zoonotic): Key facts. World Health Organization (WHO), Geneva, Switzerland. https://www.who.int/news-room/fact-sheets/detail/influenza-(avian-and-other-zoonotic)

WU, G. and BAZER, F. W. (2019). Application of new biotechnologies for improvements in swine nutrition and pork production. *Journal of Animal Science and Biotechnology*, 10: 28. https://doi.org/10.1186/s40104-019-0337-6

XIN, J., YANG, H., FAN, N., ZHAO, B., OUYANG, Z., LIU, Z., ZHAO, Y., LI, X., SONG, J., YANG, Y., ZOU, Q., YAN, Q., ZENG, Y. and LAI, L. (2013). Highly efficient generation of GGTA1 biallelic knockout inbred mini-pigs with TALENs. *PLoS One*, 8(12): e0084250. https://doi.org/10.1371/journal.pone.0084250

XU, A., QIN, C., LANG, Y., WANG, M., LIN, M., LI, C., ZHANG, R. and TANG, J. (2015). A simple and rapid approach to manipulate pseudorabies virus genome by CRISPR/Cas9 system. *Biotechnology Letters*, 37(6): 1265 – 1272.

YAMAMOTO, Y. (2002). PCR in diagnosis of infection: detection of bacteria in cerebrospinal fluids. *Clinical and Vaccine Immunology*, 9(3): 508 – 514.

YAN, R., SUN, W., SONG, X., XU, L. and LI, X. (2013). Vaccination of goats with DNA vaccine encoding Dim-1 induced partial protection against *Haemonchus contortus*: A preliminary experimental study. *Research in Veterinary Science*, 95(1): 189 – 199.

YAN, R., WANG, J., XU, L., SONG, X. and LI, X. (2014). DNA vaccine encoding *Haemonchus contortus* actin induces partial protection in goats. *Acta Parasitologica*, 59(4): 698 – 709.

YUAN, M., ZHANG, W., WANG, J., AL YAGHCHI, C., AHMED, J., CHARD, L., LEMOINE, N. R. and WANG, Y. (2015). Efficiently editing the vaccinia virus genome by using the CRISPR-Cas9 system. *Journal of Virology*, 89(9): 5176 – 5179.

YUEN, K., CHAN, C., MICKEY WONG, N., HO, C., HO, T., LEI, T., DENG, W., TSAO, S., CHEN, H., KOK, K. and DONG-YAN, J. (2015). CRISPR/Cas9-mediated genome editing of Epstein-Barr virus in human cells. *Journal of Gerneral Virology*, 96(3): 626 – 636.

ZHANG, Y., TANG, N., LUO, J., TENG, M., MOFFAT, K., SHEN, Z., WATSON, M., NAIR, V. and YAO, Y. (2019). Marek's disease virus-encoded MicroRNA 155 ortholog critical for the induction of lymphomas is not essential for the proliferation of transformed cell lines. *Journal of Virology*, 93(17): e00713-19. https://doi.org/10.1128/JVI.00713-19

ZHAO, G. W., YAN, R. F., MULEKE, C. I., SUN, Y. M., XU, L. X. and LI, X. R. (2012). Vaccination of goats with DNA vaccines encoding H11 and IL-2 induces partial protection against *Haemonchus contortus* infection. *Veterinary Journal*, 191(1): 94 – 100.