CHAPTER 34

RECOMBINANT THERAPEUTICS EXPRESSED IN TRANSGENIC PLANTS WITH POTENTIAL APPLICATIONS IN VETERINARY DISEASES

Atif Ahmed[1], Shafique Ahmed[2], Sohail Ahmad[3], Aiman Zahra[1,4], Muhammad Azmat Ullah Khan[5], Muhammad Azam Ali[1,3], Adnan Iqbal[6] and Sidra Hasnain[2]

[1]Centre of Excellence in Molecular Biology, University of the Punjab, Lahore – Pakistan
[2]The Superior University, Lahore – Pakistan
[3]Clinic of Nephrology and Hypertension, Otto Von Guerick University Magdeburg – Germany
[4]Virtual University of Pakistan – Pakistan
[5]University of Gujrat, Gujrat – Pakistan
[6]The University of Lahore, Lahore – Pakistan
*Corresponding author: shafique.ahmed@superior.edu.pk

INTRODUCTION

The livestock industry has grown up swiftly from the last decade and earned $1.4 trillion annually (Sohaib and Jamil 2017). This industry is continuously evolving due to rapidly increasing demand of livestock products. Therefore, use of veterinary drugs is essential for therapeutic and prophylactic purposes in livestock production to improve growth, productivity, and food safety (Falowo and Akimoladun 2019). Only one segment of this sector i.e., meat production valued at 838.3 billion U.S. dollars in 2020 and forecasted to increase to 1157.6 billion U.S. dollars by 2025. These drugs are globally utilized to enhance the profitability and productivity of modern food animal production by facilitating higher animal densities, earlier weaning, meat quality, cheaper feeds, and carcass yield (Moreno and Lanusse 2017). These life-saving agents include a broad range of natural, synthetic, and semi-synthetic compounds such as, antibiotics, antiparasitics, β-agonist and vaccines (Moreno and Lanusse 2017). Among the antibiotics used in livestock production, commonly consumed are amprolium, penicillin, tetracyclines, streptomycin, tylosin, sulphonamides, aminoglycosides, β-lactams, quinolones macrolides and lincosamides (Landoni and Albarellos 2015; Alhaji et al. 2018) while antiparasitic drugs include anthelmintics or coccidiostats such as stilbenes, nitrofurans, amphenicols, nitroimidazoles, pyrethroids, carbamates and sedatives (Falowo and Akimoladun 2019).

The extensive use of antimicrobial agents leads to continuously increasing antimicrobial resistance. Mostly, scientists believe that improper and immense administration of antimicrobials is a single most significant factor that is responsible for emergence of resistance (Hoelzer et al. 2017). The veterinary researchers have identified that intestinal microbiome of food producing animals can act as a reservoir of resistant bacteria in the society (Graveland 2011; Patchanee 2014; Moradigaravand et al. 2017). However, there is high risk of multi-drug resistant bacterial zoonosis and pose a serious threat to the public health (Zhu et al. 2013; Jans et al. 2017; Lugsomya et al. 2018). At present, the average annual utilization of antimicrobial compounds per-kilogram of animal produced is approximately at >100mg/kg worldwide (Vishnuraj et al. 2016). It has been estimated that almost 80% of the antibiotics consumed in veterinary field are growth promoters, which mostly exceed the amount of total antibiotic consumption in human medical care (Vishnuraj et al. 2016). The antibiotic residues in edible animal products have increased beyond the acceptable level in most of developing countries (Use, 2017). Moreover, many scientific reports revealed that consistently use of antimicrobial agents in enormous amount result in deposition of drug residues in different organs and muscles of animals (Sanz et al., 2015). These residues in edible animal products can cause severe health risks to humans when ingested (Use, 2017). The development of antimicrobial resistance and hypersensitivity reactions are most common outcome in humans (Use, 2017).

Dawn of Recombinant Therapeutics

The recombinant proteins are gaining much attention worldwide due to its variety of applications. Efficient strategies are utilized to produce high quality proteins in enormous amount with low cost (Palomares et al. 2004). The potential of engineered recombinant proteins are widely explored for the development of therapeutic and prophylactic use (Gifre et al. 2017). These include antibodies, enzymes, cytokines, growth factors and vaccines (Schillberg et al. 2019). These proteins are synthesized in various expression systems depending upon the type of protein. Commonly used expression systems are bacteria, yeast, filamentous fungi, and unicellular algae (Legastelois et al. 2017; Owczarek et al. 2019). All expression systems have their own merits and demerits, and its selection depends upon the protein of interest to be expressed, such as, eukaryotic protein modifications are only possible in eukaryotic expression system because prokaryotic system does not support these modifications (Rai and Padh 2001). Moreover, cell free expression systems are now attracting the attention of scientific community to be utilized for the fast synthesis of recombinant proteins with eliminating the processes of purification (Swiech et al. 2012).

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These proteins are widely utilized due to its dynamic properties. The gene encoding the particular protein is isolated from the respective organism and synthesized in various expression systems. Thereafter, same protein is again injected in the living body e.g., insulin. Therapeutic proteins minimize the issues related to the synthetic and semi-synthetic drugs e.g., antibiotic associated diarrhea, unpleasant taste and reduce absorption from the gut. Appropriate modifications are required to increase specificity, to prolong half-life, and to improve functionality (Gupta et al. 2017). The continuous impressive work by the scientists in the recombinant protein technology have brought multiple therapeutic proteins into clinical applications (Kim et al. 2017). Due to these advancements, demand of recombinant proteins is increasing in the livestock sector as well.

Different Ranks of the Recombinant Therapeutics

Vaccines are very successful method for disease prophylaxis in humans and animals. Most deadly diseases are cured today through the conventional and modern vaccines which significantly decrease the graph of diseases in livestock (Jorge and Dellagostin 2017). Majority of conventional vaccines today in market include live attenuated vaccines, killed vaccines, inactivated vaccines, toxoids and cell membrane compounds (McVey and Shi 2010; Unnikrishnan et al. 2012). Attenuated vaccines are very effective in stimulation of immune response both humoral and cell mediated (Rizzi et al. 2012; da Costa et al. 2015). However, killed and inactivated vaccines are preferred over the attenuated vaccines due to its safety profile in the animal body, but they are less effective to elicit the immune response in the host along with its adverse effects (Cho et al. 2002; Moreira et al. 2016). The main problem associated with the attenuated vaccines is its reversion back to its virulent form after inoculation into the body (Shimoji et al. 2002; Unnikrishnan et al. 2012). Toxoid vaccines are raised against lethal and fatal bacterial and mycotoxins after inactivation through chemical agents (Arimitsu et al. 2004). Toxoids are effective in a sense that these induce reliable humoral immune response but negligible cell-mediated immune response (Jorge and Dellagostin 2017). The widespread use of these prophylactic agents prominently improves animal health. Although, multiple bacterial and viral diseases in animals are efficiently treated with conventional vaccines but they are still expensive to produce and require administration of multiple doses to achieve optimal immune response (Meeusen et al. 2007; Delany et al. 2014). Therefore, it is the necessity of the time to introduce the more immunogenic, safer and economical vaccines, which are more capable to efficiently control and eradicate the animal diseases.

The advancements in next generation sequence technologies and understanding the molecular mechanisms of pathogenesis of various pathogens resulted in the introduction of recombinant veterinary vaccines in the market (Jorge and Dellagostin 2017). It enables the genome and proteome screening with the aid of next generation technologies, which prominently enhance the chances of more appropriate antigen discovery. The vaccines based on the relevant epitopes could successfully evoke the optimal protective immunity in the host (Jorge and Dellagostin 2017). However, impressive progress in the field of genomics initiated the era of 'third generation' of vaccines using the applied technologies such as, reverse vaccinology that open it up in the broad spectrum (Dellagostin 2011; Rappuoli et al. 2014). This approach led to the identification of less abundant and non-identified proteins as vaccines. Furthermore, we can recognize the potential targets for vaccines by eliminating the process of passaging. The next generation vaccines could be multivalent, better safety profile and optimal immune stimulation (Oliveira et al. 2015).

Different types of recombinant vaccines have been designed for livestock animals for preventive purpose. Subunit vaccines are based on small, specific and non-infectious proteins. Therefore, these vaccines are safe and non-replicative immune-modulatory agents (Jorge and Dellagostin 2017). Moreover, we can evoke the immune response against multiple pathogens or multiple serotypes of same pathogen by inoculating the cluster of multiple proteins in one subunit vaccine (Dellagostin 2011). However, major drawbacks of subunit vaccines are moderate immune response and requirement of an adjuvant to produce robust immune response (Jorge and Dellagostin 2017). On the other hand, vector-based vaccines contain the core of pathogen and display multiple membrane bounded antigens (Jorge and Dellagostin 2017). The core is also utilized as a carrier to deliver genes for other pathogens; these antigens will express to stimulate immunogenic response. In contrast, DNA vaccines contain only the template that codes for the antigenic proteins. It not only overcomes the safety issues but also the production of cytotoxic T cells (Meeusen et al. 2007).

Recombinant Therapeutics Producing Plants – Need and Rationale

Plants have been historically used as medicine and lately the trend is shifting back to further explore their potential against bacteria, fungus, and other pathogens (Hamayun et al. 2021; Tariq et al. 2020; Khan and Javaid 2020; Rehman et al. 2020). Scientists are extensively studying the applications of plants related to the production of biopharmaceuticals. The plants are the ultimate source of food and various nutrients for living organisms on earth especially animals and humans. Now, scientists want to merge the biopharmaceutical objectives with their contribution in food manufacturing (Walmsley and Arntzen 2000). Therefore, the potential of plants is utilized for the production of growth factors, enzymes, vaccines, hormones, antibodies and peptide based antibiotic drugs along with the synthesis of essential proteins, primary sugars and vital amines. For this purpose, transgenic plants are developing using various applied techniques. The transgenic plants are preferred to achieve the therapeutic needs due to its enormous production and strengthening antigenicity (Rybicki 2009).

How does it work?

The manufacturing process of plant-based vaccines begin with the selection of gene of interest expressing the particular antigenic determinant. The candidate gene for particular vaccine is cloned in the plant expression cassette that have ability of promoting and terminating expression (Rybicki 2009). Subsequently, the expression cassette is delivered to the plant for synthesis of recombinant protein (Walmsley and Arntzen 2000). The stable or transient transformation occur after successful delivery of expression cassette to the plant carrying the particular gene. The transient gene expression is quick and convenient method but yield of protein is low in amount and production of foreign protein for temporary period (Liew and Hair-Bejo 2015). Contrastingly, the candidate gene is

permanently incorporated in the plant genome which is the principal benefit of stable transgenic expression. The antigenic trait is inherited in the genome, which allows the transfer of desired character over multiple generations (Santi 2009). Thus, mass stocks of transgenic seeds are available for the cultivation of next generation (Joensuu et al. 2008).

The plants for vaccine production are grown in plant factory systems instead of conventional soil-based cultivation. Artificial environment is created in plant factory systems to control the CO2 concentration, temperature, humidity, light quality and quantity, and defined hydroponic media (Shim et al. 2019). In contrast to egg-based vaccine production, which requires at least 180 days, the plant-based systems take only 21 days for vaccine production (D'Aoust et al. 2010). However, cultivation of transgenic plants in natural environment demands basic requirements such as, sunlight, water and nutrients for simple and economical propagation. Additionally, harvest and further processing do not need complicated procedures to achieve the final product (Mason et al. 2002). These systems are 10 to 40 times more economical than vaccine production by *E. coli* fermentation (Giddings 2001; Mett 2008) and 140 times cheaper than baculovirus insect-based system (Rosales-Mendoza et al. 2017). Moreover, we can manipulate the glycosylation pathway to produce diversity of similar antigens instead of specific glycosylation (Rosales-Mendoza et al., 2017). These systems also help in the synthesis of cheap and natural vaccines. Thus, plant factory systems are considered as alternative methods for the production of biopharmaceuticals worldwide. Despite its advantages, it requires the more attention of scientists to work out in this domain because only few vaccines have passed the pre-clinical trials and now passing through the clinical trials. The plant-based systems got success to develop the vaccine against most common disease in cloven-hoofed animals i.e., the foot and mouth disease. The VP1 whole coat protein or the antigenic peptides of FMDV are successfully expressed in different transgenic plants like, alfalfa, arabidopsis and potato. In addition, VP1 is also expressed using the plant viral vector such as, tobacco mosaic virus and tobacco leaf curl virus. The leaf extracts were prepared and delivered by ingestion and injection into the intraperitoneal cavity of mice. The mice developed protective immunity and upon the live FMDV challenge, it showed protection against it. Afterwards, experiments were conducted in the swine, the natural host of FMDV. The VP1 immunogenic peptide was inserted in the modified coat of bamboo mosaic virus followed by its infection to *Chenopodium quinoa,* the host for Bamboo mosaic virus. Two doses of 5mg of leaf extract were prepared and inoculated intramuscularly in the pigs. This resulted in the synthesis of anti-FMDV antibodies. Then the pigs were challenged with the live FMDV and after four weeks of booster dose showed complete protection (Liew and Hair-Bejo 2015). Similarly, the same approach was used to produce vaccine against mink enteritis virus and rabbit hemorrhagic disease. In MEV vaccine, the viral VP2 capsid was expressed in black-eyed bean. The short epitope was incorporated in the cotton mosaic virus followed by infection to the plant. Two doses of 1mg leaf extract were injected subcutaneously that developed optimal immunity in the mink (Dalsgaard et al. 1997). Likewise, Vp60 of rabbit hemorrhagic disease was inserted in the potatoes to produce immunity in the rabbits (Castanon et al. 1999).

Apart from livestock vaccines, plant based transgenic poultry vaccines are also under process against major poultry diseases. The infectious bursal disease is highly contagious and deadly disease of young chickens. The VP2 capsid contain two segments, segment A and B (Nick et al. 1976). The strain E gene of segment B contain neutralizing antigenic determinants, which is incorporated in the *Arabidopsis thaliana* (Wu et al. 2004). In another study, gene of attenuated segment A of VP2 was expressed in rice seeds. In oral immunization trial with rice seeds, four consecutive doses of 5g transgenic rice seeds were fed with the interval of one week each (Wu et al., 2007). One dose contained 10mg of VP2 protein and stimulate optimal immunity in the chickens. The chickens remained healthy when infectious IBDV was challenged to it (Mason and Herbst-Kralovetz 2011).

The transgenic plants seem to be excellent alternative source for the production of biopharmaceuticals. The transient expression systems produce rapid synthesis of therapeutic proteins while stable expression requires permanent insertion of genetic element in the plant genome. The transgenic plants grown in the natural systems require only basic plant needs, which are able to produce recombinant proteins. The glycosylation pathway can be manipulated to produce diverse post-translational modification (Shim et al. 2019). Moreover, cheap and enhanced productivity in the plant factory are the prominent advantages in the current economic situation worldwide.

Challenges of Recombinant Therapeutics Production in Plants

Plant based recombinant therapeutics are in high demand due to their low cost, high efficacy, edible property and ease of administration. Host plant system act as a bioreactor for the intended transgenic protein and express it alongside other host proteins. Although plant derived recombinant proteins are holding a promising future yet this system is not completely ideal and risk free. There are certain limitations which hinder the full utilization of plant derived recombinant protein production i.e. (1) selection of plant host (2) limited product yield (3) Safety and health concern (4) Environmental risks.

Selection of Protein and Plant Expression Host

First and most critical step in recombinant protein production is the selection of suitable plant expression system as well as desired protein. This stage is crucial as all the plant expression hosts are not compatible with desired protein or antigen and hence will compromise the expression. Careful selection and use of modern approaches (such as genomics and proteomics analysis) help in development of vaccines for poorly characterize pathogens (Rigano and Walmsely 2005; Sharma and Sood 2011).

Limited Product Yield

In plant based recombinant therapeutics, product yield is of prime importance and defined as grams of product obtained per unit of plant biomass. Although biomass production is scalable in molecular pharming but product expression is low in magnitude and require more attention and efforts to achieve the desired targets. There are various factors behind the yield limitation i.e. genetic elements choice, epigenetic, environmental and biochemical factors alongside downstream processing techniques (Twyman 2013).

Yield Limiting Genetic Factors

Product yield is largely depending upon the transcriptional and translational efficiencies of transgene and require special attention while designing the construct. Choice of upstream and downstream regulatory elements is critical in this regard. Strong promoter alongside other genetic elements is necessary for higher, stable and organ specific transgene expression. A strong constitutive promoter is often required to enhance the transcriptional yield which will further enhance the product (protein) yield. Organ specific promoter is often desirable where transgene product is hindering vegetative growth of host plant or a specific plant organ is intended for harvesting and post-harvest applications. Seed based edible vaccines are an example of it as they are easy to administer and store. Apart from promoter, certain other genetic elements can also be introduced into expression constructs to enhance mRNA stability or to enhance the translation efficiency. These elements are either endogenous elements such as 5' or 3' UTRs (Untranslated regions) or exogenous such as introns, Kozak's consensus sequence etc. (Mitsuhara et al. 1996; Sharma et al. 2008; Lu et al. 2008; Peremarti et al. 2010).

Epigenetic Factors Affecting Yield

As compared to genetic factors, epigenetic effects are independent from DNA sequence. They mainly influence the expression cassette through their position, structure or complexity of the locus. These factors are hard to control as transgene integration is random instead of sequence specific. Due to the above-mentioned limitations, transgene integration into host genome is of prime importance. Surrounding genetic elements as well as integration into a silencing locus (positional silencing) both can influence transgene stability and expression. Sometime multi copy transgene are prone to instability and silencing. But many other instances shows that high copy number is proportional to greater gene expression, hence proving that copy number is not the reason behind silencing but some other factors influence it, such as hairpin loop formation etc. In order to avoid epigenetic silencing, intervening genetic elements (MARs matrix attachment regions) can be introduced in between transgene and surrounding host genetic elements. Transient gene expression (which involves the expression of transgene into non-transgenic plants without integrating into host genome) is often preferred to avoid epigenetic silencing. Although transient expression is short lived due to transgene degradation and environmental stresses but still its ability to give high yield in a short period of time is promising and utilized in large scale vaccine manufacturing to cope the high demand (Topping et al. 1991; Kohli et al. 2003; Datta et al. 2003; Halweg et al. 2005; Kohli et al. 2006; Paul et al. 2013).

Yield Limiting Biochemical and Environmental Factors

Expression and accumulation of recombinant protein in plants is depending upon both intracellular as well as external factors. Biochemical species within cells (include proteases, free radicals, pH and salt etc.) often interfere with recombinant protein, limiting its accumulation and stability. Through experimental investigations, it is learnt that secretory pathway of protein synthesis is more feasible in terms of protein folding and post translational modification as compare to cytosolic protein synthesis. A common approach in this regard is the attachment of a signal peptide to target recombinant protein into ER and Golgi bodies from where they will be either stored into vacuole or apoplast (Schillberg et al. 1999; Vitale and Denecke 1999).

External factors (such as nutrients, heat, pH, light etc.) can also interfere in plant growth ultimately affecting expression of recombinant protein. Nitrogen availability is critical in plant metabolism as it pivotal in amino acid biosynthesis. In order to ensure uniform growth, plants are grown under controlled condition and all growth requirements are monitored regularly (Fischer et al. 2012; Twyman 2013).

Downstream Processing or Harvesting Issues

Downstream processing or harvest intended for the extraction of recombinant protein is critical in both plants derived as well as conventional expression systems. Innovative separation strategies are being utilized to overcome this issue such as co-extraction of proteins with lipid fraction (Oleosin platform) followed by endo-proteolytic cleavage. Other strategies include expression of recombinant proteins into edible parts of plant such as seed or fruits and consume directly. Despite of their promise, edible recombinant proteins havecertain limitations such as dosage determination, antigen selection, efficacy, quality control and regulatory issues (Paul and Ma 2011).

Health and Safety Concern

Plant derived recombinant proteins pose some health and safety concerns which need to be considered while utilizing the plant-based systems. One of the safety concerns is the development of hypersensitivity (allergy) especially against orally administrated vaccines and therapeutics. Certain post translational modification such as N glycosylation and administration of vaccines with adjuvants may cause hypersensitivity issues. In order to produce plant derived protein of biopharmaceutical use, manufacturing facility should be well equipped and follow the guidelines of regulatory authorities. Stringent quality control management which includes Good Agriculture Practices (GAP) and Good Manufacturing Practices (GMP) is the mandatory and should be primary responsibility of manufacturer. Implementation of GMP standards is a huge challenge which can be addressed through in process monitoring, skilled workers and by the proper design of the production facility (Cox et al. 2012; Guan et al. 2013; Sato et al. 2014).

Environmental Risk

Escape of the transgene into environment is the biggest concern in genetic engineering. Many GMO varieties utilize the toxic or resistance genes which if escape can cause some serious problems. Molecular pharming of transgenic plant alongside the non-transgenic varieties can contaminate the non-GMOs and confer them toxic or resistance properties. During the production of recombinant proteins, transgene might escape and contaminate the normal food chain; this will result in safety and health issues (allergic reactions). Majority of recombinant protein production utilizes the antibiotic resistance gene markers; hence imparting the resistance issues in bacteria and other microorganisms (WHO 1992).

REFERENCES

Alhaji NB et al., 2018. Antimicrobials usage assessments in commercial poultry and local birds in North-central Nigeria: Associated pathways and factors for resistance emergence and spread. Preventive Veterinary Medicine 154: 139-147.

Arimitsu H et al., 2004. Vaccination with recombinant whole heavy chain fragments of Clostridium botulinum Type C and D neurotoxins. Clinical and Vaccine Immunology 11: 496-502.

Castanon S et al., 1999. Immunization with potato plants expressing VP60 protein protects against rabbit hemorrhagic disease virus. Journal of Virology 73: 4452-4455.

Cho HW et al., 2002. Review of an inactivated vaccine against Hantaviruses. Intervirology 45: 328-333.

Cox LS et al., 2012. Clinical efficacy of 300IR 5-grass pollen sublingual tablet in a US study: the importance of allergen-specific serum IgE. Journal of Allergy and Clinical Immunology 130: 1327-1334.

D'Aoust MA et al., 2010. The production of hemagglutinin-based virus-like particles in plants: a rapid, efficient and safe response to pandemic influenza. Plant Biotechnology Journal 8: 607-619.

da Costa C et al., 2015. Tuberculosis Vaccines–state of the art, and novel approaches to vaccine development. International Journal of Infectious Diseases 32: 5-12.

Dalsgaard K et al., 1997. Plant–derived vaccine protects target animals against a viral disease. Nature Biotechnology 15: 248-252.

Datta K et al., 2003. Bioengineered 'golden'indica rice cultivars with β-carotene metabolism in the endosperm with hygromycin and mannose selection systems. Plant Biotechnology Journal 1: 81-90.

Delany I et al., 2014. Vaccines for the 21st century. EMBO Molecular Medicine 6: 708-720.

Dellagostin OA, 2011. Recombinant vaccines against leptospirosis. Human Vaccines 7: 1215-1224.

Falowo AB and Akimoladun OF, 2019. Veterinary drug residues in meat and meat products: occurrence, detection and implications. Veterinary Medicine and Pharmaceuticals 1: 53-69.

Fischer R et al., 2012. GMP issues for recombinant plant-derived pharmaceutical proteins. Biotechnology Advances 30: 434-439.

Giddings G, 2001. Transgenic plants as protein factories. Current Opinion in Biotechnology 12: 450-454.

Gifre L et al., 2017. Trends in recombinant protein use in animal production. Microbial Cell Factories 16: 1-17.

Graveland H, 2011. Persistence of livestock associated MRSA CC398 in humans is dependent on intensity of animal contact. PloS One 6: e16830.

Guan ZJ et al., 2013. Recent advances and safety issues of transgenic plant-derived vaccines. Applied Microbiology and Biotechnology 97: 2817-2840.

Gupta V et al., 2017. Production of Recombinant Pharmaceutical Proteins. In Basic and Applied Aspects of Biotechnology 1: 77-101.

Halweg C et al., 2005. The Rb7 matrix attachment region increases the likelihood and magnitude of transgene expression in tobacco cells: a flow cytometric study. The Plant Cell 17: 418-429.

Hamayun M et al., 2021. Efficiency of Virgin's Mantle (Fagonia cretica L.) as an Antibacterial and Antifungal Agent. Advancements in Life Sciences 8: 233-237.

Hoelzer K et al., 2017. Antimicrobial drug use in food-producing animals and associated human health risks: what, and how strong, is the evidence. BMC Veterinary Research 13: 1-38.

Paul MJ et al., 2013. Target product selection-where can Molecular Pharming make the difference. Current Pharmaceutical Design 19: 5478-5485.

Paul M and Ma JKC 2011. Plant-made pharmaceuticals: Leading products and production platforms. Biotechnology and Applied Biochemistry 58: 58-67.

Jans C et al., 2017. East and West African milk products are reservoirs for human and livestock-associated Staphylococcus aureus. Food Microbiology 65: 64-73.

Joensuu JJ et al., 2008. Transgenic plants for animal health: plant-made vaccine antigens for animal infectious disease control. Phytochemistry Reviews 7: 553-577.

Jorge S and Dellagostin OA, 2017. The development of veterinary vaccines: a review of traditional methods and modern biotechnology approaches. Biotechnology Research and Innovation 1: 6-13.

Khan IH and Javaid A, 2020. Anticancer, antimicrobial and antioxidant compounds of quinoa inflorescence. Advancements in Life Sciences 8: 68-72.

Kohli A et al., 2006. The quest to understand the basis and mechanisms that control expression of introduced transgenes in crop plants. Plant Signaling and Behavior 1: 185-195.

Kohli A et al., 2003. Transgene integration, organization and interaction in plants. Plant Molecular Biology 52: 247-258.

Landoni MF and Albarellos G, 2015. The use of antimicrobial agents in broiler chickens. The Veterinary Journal 205: 21-27.

Legastelois I et al., 2017. Non-conventional expression systems for the production of vaccine proteins and immunotherapeutic molecules. Human Vaccines and Immunotherapeutics 13: 947-961.

Liew PS and Hair-Bejo M, 2015. Farming of plant-based veterinary vaccines and their applications for disease prevention in animals. Advances in Virology. 2015: 1-12.

Lu J et al., 2008. Gene expression enhancement mediated by the 5′ UTR intron of the rice rubi3 gene varied remarkably among tissues in transgenic rice plants. Molecular Genetics and Genomics 279: 563-572.

Lugsomya K et al., 2018. Routine prophylactic antimicrobial use is associated with increased phenotypic and genotypic resistance in commensal Escherichia coli isolates recovered from healthy fattening pigs on farms in Thailand. Microbial Drug Resistance 24: 213-223.

Twyman MR, 2013. Optimizing the yield of recombinant pharmaceutical proteins in plants. Current Pharmaceutical Design 19: 5486-5494.

Mason HS and Herbst-Kralovetz MM, 2011. Plant-derived antigens as mucosal vaccines. Mucosal Vaccines 101-120.

Mason HS et al., 2002. Edible plant vaccines: applications for prophylactic and therapeutic molecular medicine. Trends in Molecular Medicine 8: 324-329.

McVey S and Shi J, 2010. Vaccines in veterinary medicine: a brief review of history and technology. The Veterinary clinics of North America. Small animal practice 40: 381.

Meeusen EN et al., 2007. Current status of veterinary vaccines.

Clinical Microbiology Reviews 20: 489-510.
Mett V, 2008. Plants as biofactories. Biologicals 36: 354-358.
Mitsuhara I et al., 1996. Efficient promoter cassettes for enhanced expression of foreign genes in dicotyledonous and monocotyledonous plants. Plant and Cell Physiology 37: 49-59.
Moradigaravand D et al., 2017. Evolution of the Staphylococcus argenteus ST2250 clone in Northeastern Thailand is linked with the acquisition of livestock-associated staphylococcal genes. MBio 8: e00802-17.
Moreira JC et al., 2016. Protective potential of recombinant non-purified botulinum neurotoxin serotypes C and D. Anaerobe 40: 58-62.
Moreno L and Lanusse C, 2017. Veterinary drug residues in meat-related edible tissues. In New Aspects of Meat Quality 581-603.
Nick H et al., 1976. Structural and growth characteristics of infectious bursal disease virus. Journal of Virology 18: 227-234.
Oliveira TL et al., 2015. Evaluation of the Leptospira interrogans outer membrane protein OmpL37 as a vaccine candidate. PloS One 10: e0142821.
Owczarek B et al., 2019. A brief reminder of systems of production and chromatography-based recovery of recombinant protein biopharmaceuticals. BioMed Research International 1-13
Palomares LA et al., 2004. Production of recombinant proteins. Recombinant Gene Expression 15-51.
Patchanee P, 2014. Occurrence and characterization of livestock-associated methicillin-resistant Staphylococcus aureus in pig industries of northern Thailand. Journal of Veterinary Science 15: 529-536.
Peremarti A et al., 2010. Promoter diversity in multigene transformation. Plant Molecular Biology 73: 363-378.
Rai M and Padh H, 2001. Expression systems for production of heterologous proteins. Current Science. 80 1121-1128.
Rappuoli R et al., 2014. Vaccines, new opportunities for a new society. Proceedings of the National Academy of Sciences 111: 12288-12293.
Rehman KU et al., 2020. Competence of benzoil tree (moringa oleifera l.) as antibacterial and antifungal agent. Advancements in Life Sciences 7: 135-139.
Rigano MM and Walmsley AM, 2005. Expression systems and developments in plant-made vaccines. Immunology and Cell Biology 83: 271-277.
Rizzi C et al., 2012. Vaccination with a BCG strain overexpressing Ag85B protects cattle against Mycobacterium bovis challenge. PLoS One 7: e51396.
Rosales-Mendoza S et al., 2017. A perspective on the development of plant-made vaccines in the fight against Ebola virus. Frontiers in Immunology 8: 1-13.
Rybicki EP, 2009. Plant-produced vaccines: promise and reality. Drug Discovery Today 14: 16-24.
Santi L, 2009. Plant derived veterinary vaccines. Veterinary Research Communications 33: 61-66.
Sanz D et al., 2015. Incidence of antimicrobial residues in meat using a broad-spectrum screening strategy. European Journal of Nutrition & Food Safety 5: 156-165.
Sato S et al., 2014. Immunotherapy in food allergy: towards new strategies. Asian Pacific Journal of Allergy and Immunology 32: 195-202.
Schillberg S et al., 1999. Apoplastic and cytosolic expression of full-size antibodies and antibody fragments in Nicotiana tabacum. Transgenic Research 8: 255-263.
Schillberg S et al., 2019. Critical analysis of the commercial potential of plants for the production of recombinant proteins. Frontiers in Plant Science 10: 720-730.
Sharma MK et al., 2008. Expression of toxin co-regulated pilus subunit A (TCPA) of Vibrio cholerae and its immunogenic epitopes fused to cholera toxin B subunit in transgenic tomato (Solanum lycopersicum). Plant Cell Reports 27: 307-318.
Sharma M and Sood B, 2011. A banana or a syringe: journey to edible vaccines. World Journal of Microbiology and Biotechnology 27: 471-477.
Shim BS et al., 2019. Plant factory: new resource for the productivity and diversity of human and veterinary vaccines. Clinical and Experimental Vaccine Research 8: 136-139.
Shimoji Y et al., 2002. Erysipelothrix rhusiopathiae YS-1 as a live vaccine vehicle for heterologous protein expression and intranasal immunization of pigs. Infection and Immunity 70: 226-232.
Sohaib M and Jamil F, 2017. An insight of meat industry in Pakistan with special reference to halal meat: a comprehensive review. Korean journal for food science of animal resources, 37: 329.
Swiech K et al., 2012. Human cells: new platform for recombinant therapeutic protein production. Protein Expression and Purification 84: 147-153.
Tariq A et al., 2020. In vitro Micropropagation of Citrullus colocynthis (L.) Schrad: an endangered medicinal plant. Advancements in Life Sciences 8: 52-56.
Topping JF et al., 1991. Functional tagging of regulatory elements in the plant genome. Development 112: 1009-1019.
Unnikrishnan M et al., 2012. Recombinant bacterial vaccines. Current Opinion in Immunology 24: 337-342.
Use FA, 2017. Resistance in Nigeria: Situation Analysis and Recommendations. Federal Ministries of Agriculture. Environment and Health.
Vishnuraj MR et al., 2016. Occurrence, public health hazards and detection methods of antibiotic residues in foods of animal origin: A comprehensive review. Cogent Food & Agriculture 2: 1235458.
Vitale A and Denecke J, 1999. The endoplasmic reticulum—Gateway of the secretory pathway. The Plant Cell 11: 615-628.
Walmsley AM and Arntzen CJ, 2000. Plants for delivery of edible vaccines. Current Opinion in Biotechnology 11: 126-129.
World Health Organization, 1992. Good manufacturing practices for biological products. WHO Technical Report Series 822: 20-29.
Wu H et al., 2004. Expression of immunogenic VP2 protein of infectious bursal disease virus in Arabidopsis thaliana. Biotechnology Letters 26: 787-792.
Wu J et al., 2007. Oral immunization with transgenic rice seeds expressing VP2 protein of infectious bursal disease virus induces protective immune responses in chickens. Plant Biotechnology Journal 5: 570-578.
Zhu YG et al., 2013. Diverse and abundant antibiotic resistance genes in Chinese swine farms. Proceedings of the National Academy of Sciences 110: 3435-3440.