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Application of Biotechnology towards Diagnosis and Treatment in Veterinary Medicine in Africa: Potentials and Future Developments

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Abstract

Biotechnology is an already established technique in several areas of medicine, but its application in the field of veterinary medicine has only started to emerge with the potential to revolutionize veterinary practice. This paper therefore reviews the potential applications of biotechnology in veterinary medicine towards diagnosis and treatment in Africa which includes; molecular gene cloning, production of recombinant biotechnology derived vaccines, application of polymerase chain reaction (PCR), Real time polymerase chain reaction (RT-PCR), Polymerase chain reaction - restriction fragment length polymorphism (PCR-RFLP) and Bioinformatics to diagnosis of infectious and parasitic diseases , gene therapy, disease diagnosis, treatment delivery systems and many more. These components occur in other parts of the world and are therefore presumed to be consolidated into the African delivery framework as a private enterprise in a foreseeable future. Whilst it is sensible to postulate that biotechnology application and its peculiar evolution will imminently transform veterinary medicine, there is immense treat, amidst stakeholders in the industry, about food health and safety and other civil and ethical concerns which can hinder this novel scientific breakthrough. The ethical concerns which include; theory of the Three Rs (Reduction of animal population, Refinement of enactments and farm managements to curtail affliction and despair, Replacement of animals with non-animal surrogate wherever necessary. Limitations regarding the application to veterinary practices are extensively discussed. This review has implication on the future of revolutionalisation of veterinary practice and increase in animal protein source for human consumption.

Keywords: Bioinformatics; Biotechnology; Gene cloning; Polymerase chain reaction (PCR); Vaccines; Veterinary medicine

Introduction

The term Biotechnology is broadly defined as the ability to use living organisms or substances to improve or reconstruct a product, to ameliorate animals or plants or to evolve micro-organisms for peculiar purposes [1,2]. Conventional animal breeding involving the collection and breeding of phenotypically desired individuals is an ideal illustration of a well-established application of biotechnology [3]. However, the latest biotechnology comes from the recent breakthroughs such as recombinant Deoxyribonucleic Acid (DNA), the hereditary substances in all living organisms from bacteria to an elephant, restricts and regulates all the functions of living organisms [4,5]. DNA technology and its corresponding techniques, monoclonal antibody techniques, embryo manipulation technology, Polymerase chain reaction (PCR) have underlined feasibilities for manipulating biological systems for the well-being of humanity through genetic manipulations using microorganisms and vector hosts [6]. Although, human medicine has apparently profited the bulk from biotechnology, successful application of veterinary biotechnology has predominantly been restricted to developed nations. Explicitly, there are rarely any success stories of the application of biotechnology in the advancement of animal health and husbandry in the developing nations. The aim of this paper therefore is to review accessible biotechnologies with prospective application in disease diagnosis and treatment and to ascertain those which have been or may be administered in Africa in particular and other developing nations including Asian continent. Taking into consideration the scope of the subject matter, not much magnitude is given to the description of each section. Comparatively, an effort is made to accentuate the technologies adjudged to have current or potential application in the field of veterinary medical practice. This review paper concludes with a cursory reportage of the impediments concerning the prospective environmental threats of genetic engineering and other biotechnologies, necessitating their ethical appraisal for a global regulatory mechanism.

Applications of Biotechnology to Veterinary Medicine

Modern biotechnology is often employed in the diagnosis (e.g. to differentiate closely related disease agents), the production of commercial veterinary drugs and vaccines which have the potential to significantly affect the way veterinarians will practice veterinary medicine [7]. The application of biotechnology to animal health, predominantly prophylactic (prevention-related) concerns include; molecular gene cloning, the evolution and production of therapeutic (medicinal) products and biotechnologically derived vaccines; advanced veterinary diagnostic procedures; immunocastration and other biotechnological applications [8].

Molecular gene cloning

Molecular gene cloning is the technique whereby multiple copies of a plasmid or other cloning vehicles are produced by inserting the plasmid into a suitable host capable of producing multiple copies and growing in a bulk culture. The bacterium *Escherichia coli* are often used as the host organism for this purpose [9]. The word gene cloning is the duplication of definite cell types from a "parent" cell, or the duplication of a definite part of the cell or DNA to duplicate a precise preferable

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genetic trait [10,11]. There are three distinct forms of cloning: DNA cloning, therapeutic cloning and reproductive cloning [12]. On the basis of this review, the term "cloning" refers to reproductive cloning, as this is the most probable to culminate to animal well-being concerns. Reproductive cloning is used if the objective is to generate an animal that has similar nuclear DNA as another currently, or previously prevailing animal. The technique used to generate this category of cloned animal is called somatic cell nuclear transfer (SCNT). However, the application of this technique is still at experimental level [10,13]. Recent studies in China, Southeast Asia on veterinary diagnostics revealed the cloning of a partial exon 3 sequence of *LEP* (*qLEP*) and four different leptin receptor splicing variants, including a long receptor (*qLEPRl*) and three soluble receptors (*qLEPR-a*, *qLEPR-b* and *qLEPR-c*) in Japanese quail (*Coturnix japonica*) [14].

Production of biotechnology-derived veterinary recombinant vaccines

The evolution of recombinant vaccines for the prophylactic effect of a plethora of bacterial and viral diseases is one of the monumental improvements in human and veterinary medical practices [15,16]. These biotechnology-derived veterinary vaccines are often used not only to control infectious diseases, but also to improve yield by modulating hormones or immune system functions, coupled with the control of ectoparasites and immunocastration [8,17]. Since Edward Jenner's establishment of a therapeutic approach using a smallpox virus acquired from cow (Latin vacca) in 1796, the use of vaccines has largely accorded to the eradication of infectious diseases [18]. This cattle-derived 'drug' was called vaccine, and the therapy was labelled vaccination, which still has a crucial effect on the prophylaxis of infectious diseases. These vaccines including DNA vaccines, subunit vaccines and vectored vaccines, proffer elevated protection above orthodox inactivated or killed vaccines. Nonetheless, a few other different vaccines developed employing biotechnology is hitherto in use and many more are at various phases of production. Significantly, amidst variant therapeutic procedures like gene therapy, interferons, monoclonal antibodies, phage therapy, RNA interference and stem cells have been emerging in the human medicines and are destined to ameliorate the accessible therapeutic alternatives in the veterinary medicines too [16]. Their benefits include; reduction in the prospect of restoration to virulence of live vaccines and of intercession with antibodies induced by passive immunisation, evade contamination with other viruses, reduce degeneration throughout storage [8]. Recent studies in Malaysia, Southeast Asia and Pakistan, South-western Asia demonstrated the use of transgenic vaccines from plants as attractive alternatives for the immunization of cattle against Foot and Mouth Disease (FMD) conventional FMD vaccines [19,20].

Immunocastration

Immunocastration is an alternative method used to replace surgical castration commonly performed in valued animals [21]. It has been revealed that immunocastration is been used as an advantageous alternative in commercially viable livestock (cattle, goats, horses, pigs) and pets (cats and dogs) to enhance the aggressive performance of males, the odour and flavour of meat and feed conversion, to yield underweight carcasses and to mitigate the utilization of livestock feed [21,22]. Recent studies in Romania, Southeast Europe revealed that due to the previous setbacks (irreversibility, infections, hernia and immunosuppression leading to death) experienced from the conventional methods of castration such as surgery and administration of steroids, animal immunocastration has been adopted. The test was carried out in pigs by successfully utilizing peptides corresponding to gonadotropin releasing hormone (GnRH), incorporated with proteins, to activate antibodies that nullify the activity of GnRH [23].

Veterinary Diagnostic Systems

The major prerequisite to the control of any newly occurring diseases is detection and proper identification of the causal organism also known as diagnosis. 'Diagnosis' is generally termed as an art of identification of the cause of a particular disease (Dia=through, gnosis=knowledge) [24]. Conventional techniques of veterinary diagnosis including serological and microscopic examinations do not differentiate between species and subspecies of disease causing organisms [25]. Molecular diagnostic techniques including single DNA sequences proffer an elevated level of sensitivity, specificity and reliability in the diagnosis and regulation of pathogenic microorganisms, and Polymerase chain reaction (PCR) technique allows significantly elevated specificity levels [24]. Furthermore, these molecular diagnostic methods have also markedly minimized the subjectivity characteristic in the elucidation of morphological and biological data [8]. In the other hand, proteomics, a technique used in the analysis of expression, localization, functions, post-translational modifications, and interactions of proteins expressed by a genome at a specific condition and at a specific time [26] facilitate the identification and characterization of proteins formed by pathogens and are of huge interest to veterinary diagnosis, facilitating the protein expression pattern of viruses, bacteria and more pathogens under consideration [27]. Similarly, proteomics permits the investigation of proteins that are expressed or repressed exponentially as a consequence of being invaded by pathogens, which is exceedingly critical for the determination of new methods for employing vaccines, medicinal products or alternative means to regulate pathogens. Recent study in Germany, Central-western Europe revealed that Acute Phase Proteins (APPs); highly conserved plasma proteins increasingly secreted by the liver in response to a varieties of injuries, independently of their location and cause have been applied as general diagnostic parameters in both human and veterinary medicine [28]. Other forms of technologies including biosensors, Fluorescent In situ Hybridisation (FISH) and nanotechnologies are being integrated as current veterinary diagnostic tools [29].

Polymerase Chain Reaction (PCR)

PCR is an efficient, simple and inexpensive tool used to amplify the desired sequence or sequences of DNA into billions of identical copies. It essentially employed for checking STRs, SNP in different breeds, parentage identification; genetic mutation, etc. The principal constituent of the PCR are the DNA template, primers (forward and reverse), Taq polymerase enzyme, and the PCR machine (Thermocycler) which sustains the ideal temperature for each step in every cycle. PCR involves three major steps in each cycle which include; Denaturation, Annealing and Extension or Elongation steps [30]. The PCR is often employed for applications which entail an elevated level of reliability and specificity including blood screening, biodefense, clinical diagnostics, forensics and genetic testing [31]. In order to ensure whether the PCR produced the anticipated DNA fragment, agarose gel electrophoresis is often employed for the separation of the sizes of PCR amplicons (products). The size (s) of PCR amplicon is resolved by juxtaposition with a DNA ladder (a molecular weight marker), which contains DNA fragments of known size, run on the gel apace with the PCR amplicon [32].

Applications of polymerase chain reaction (PCR)

The PCR is the most sensitive of the existing rapid methods to detect microbial pathogens in clinical specimens [33]. In Brazil, South America the first report of the successful use of PCR for detection of epizootic hemorrhagic disease virus (EHDV) serogroup-4 and the development of a PCR-based assay for specific identification of EHDV-2 provided the basis for future diagnostic techniques [34,35]. Further studies in the United States of America (USA), North America also revealed that Bacilli were recognized as *Mycobacterium genavense* by the use PCR, sequencing of the 16S-23S ribosomal RNA intergenic spacer (ITS) gene and mycolic acid evaluation by high-performance liquid chromatography. *Mycobacterium genavense* is established to be the usual cause of mycobacteriosis in birds (free-ranging and captive) [36].

Applications of real-time polymerase chain reaction (RT-PCR)

Real Time Polymerase Chain Reaction (RT-PCR): Real-time PCR (RT-PCR) also known as Real Time Quantitative PCR (RTQ-PCR) is the current technology being employed in scientific research [37]. High specificity, high sensitivity, low cost, rapid time-to-result, scalability, and quantitative nature are some of the benefits of RT-PCR [38]. RT-PCR is one of the latest advancements in PCR techniques to have been integrated by veterinary diagnostic laboratories [39]. Furthermore, RT-PCR allows the use of quantitative techniques. The sequencing of whole genomes of pathogens generates crucial elements for biological research and for enhancing parasite control and diagnosis. The current microarray techniques permit investigation for the genotypes of specific parasites and proffer crucial support for epidemiological studies of parasites of veterinary importance [40]. The discovery of Real Time PCR was prompted by some critical impediments of the standard PCR technique. For instance, by first amplifying the DNA sequence and then interpreting the product, evaluation was extremely demanding considering that the PCR gave rise to substantially the same amount of amplicon independently of the initial amount of contemporary DNA template molecules [41]. This impediment was unravelled in 1992 by the evolution of Real-Time PCR. By employing the real time PCR, it is attainable to determine the number of DNA molecules amplified in a complex sample by monitoring the cycles (checking the fluorescence of dyes) [42,43]. The quantities of DNA or RNA are then resolved by juxtaposing the results to a standard curve generated by Real-Time PCR of serial dilutions of a known quantity of DNA or RNA [44]. Conventional application of realtime PCR includes; analysis of chromosome aberrations, gene expression analysis, pathogen detection, single nucleotide polymorphism (SNP) analysis, and most lately protein detection by Real-Time immuno PCR [43]. Recent studies in Egypt, North Africa revealed that RT-PCR was applied using specific primers for genomic region encoding the capsid protein VP60 to detect Rabbit haemorrhagic disease virus (RHDV), a world-wide infectious disease of rabbits [45].

Application of polymerase chain reaction-restriction fragment length polymorphism (PCR- RFLP) in molecular identification of pathogens

The Polymerase Chain Reaction (PCR) approach was developed as an alternative to existing diagnostic procedures such as direct detection of parasites by microscopic examination of clinical specimens or by cultivation [46]. Recent studies in Egypt, North Africa demonstrated that RFLP assay was used as a diagnostic tool for the differentiation of ovine theileriosis thereby enabling direct, concurrent, highly specific and sensitive identification of *Theileria* spp. [47].

Application of bioinformatics to diseases diagnosis

Bioinformatics is simply defined as the science of organizing and interpreting biological data employing improved computing techniques [48]. In view of these accelerated sequence biological information, bioinformatics tools and algorithms have now been evolved. Employing bioinformatics algorithms such as BLAST (Basic Local Alignment Sequence Tool), Fast Alignment (FASTA) and Clustal W solutions for sequence search and analysis, in conjunction with other techniques comprise a time reduction and cost-effective strategy to acquire an essential data on gene and protein levels information not easily attainable through other techniques [49]. Bioinformatics has strengthened the regimen of scientific investigation and future veterinary vaccines evolution because it has provided new tools for discovery of vaccine targets from sequenced biological data of organisms. Nevertheless, the prospect of employing this technology relies heavily on the accessibility of bioinformatics knowledge in the public domain. Recent studies in Belgium, Western Europe have demonstrated the value of Next Generation Sequencing (NGS) technologies to veterinary medicine, with particular focus on applications on diagnosis of diseases and treatment [50].

Application of gene therapy in veterinary medicine

Gene therapy is a therapeutic technique in which a functioning gene is inserted into a cell in order to ameliorate a metabolic abnormality or to introduce a new function is one of the outcomes of breakthroughs in molecular biology [51,52]. Gene therapy is a promising approach to the treatment of cancer and other genetic diseases in human and veterinary medicine [51]. The Combination of cytotoxicity with chemotherapy along with the anti-tumour immune responses of immunomodulatory therapy hinder tumour growth in multiple types of cancer and electroporation (EP) appears as an attainable approach for cautiously and adequately combining this therapeutics [53]. Electroporation is therefore a real technique for transfecting agents, such as chemotherapeutics and plasmid DNA (pDNA), into host cells. EP is increasingly being used among the scientific and the medical communities, as it is a safe and efficient technique to transfer a variety of material (e.g. nucleic acids, cytotoxic drugs and ions) into target cells and tissues without harming them [54]. In EP, brief electric pulses activate transient pores in the cell membrane and convey the agents into the cytosol. EP often does not induce any critical harmful end results, and therefore various veterinary clinical trials have demonstrated the safety and efficacy of electrochemotherapy (ECT), chemotherapy delivered *via* EP [53]. The efficacy of gene therapy has also been demonstrated in large animal models of X-linked retinitis pigmentosa which accords a course for translation to human therapeutics [55]. Previous study in Spain, South west Europe demonstrated that it is possible to generate a "glucose sensor" in skeletal muscle through co-expression of glucokinase (GCK) and insulin (Ins), increasing glucose uptake and correcting hyperglycemia in diabetic mice [56]. Further study in the USA revealed the success of liver gene therapy with Adeno-associated virus -functional coagulation factor VIII (AAV-FVIII). This case involves two outbred, privately owned dogs with severe haemophilia A (HA) that resulted in sustained expression of 1–2% of normal FVIII levels and prevented 90% of expected bleeding episodes [57].

Cancer immunotherapy

The area of tumor immunotherapy is altering swiftly and has galvanised progressive enthusiasm in human and veterinary medicine. In addition to biological response modifiers, veterinary oncologists will soon have access to tumor depleting antibodies, as well as modified T-cells and checkpoint molecule blocking antibodies [58]. Spectacular achievements are also being accomplished with current tumor vaccine stratagem, integrating new antigen targets and delivery technologies [59]. Recent studies in Japan, Eastern Asia demonstrated that oncolytic virotherapy with rMV-SLAM blind can be a novel method for treating canine mammary tumors (CMTs), the most frequent canine tumors which account for more than 40% of all tumors in female dogs [60].

Limitations

To control animal diseases in many parts of Africa however, the problem is not how to develop new, more effective diagnostic methods, drugs or vaccines. Rather, it is their accessibility to the local societies. For this, more effective organisational structures of veterinary services are more important than further refinement of diagnostic methods or better vaccines. More effective services also include community-based animal health workers, who live in the livestock-keeping communities and provide the local animals with inexpensive “first aid” for the effective management of diseases.

Ethical Issues

Biotechnology is sure to be a part of the future of veterinary medicine; animal management and health. However, any new technology carries an ethical responsibility for a successful application and the recognition that there are potential unforeseen hazards that may come with the tremendous positive potential. Therefore ethical concerns, including animal welfare issues, can emerge at various phases in the propagation and life span of a respective genetically engineered animal. The succeeding segment enumerate some of the concerns that have emanated during the peer-driven requirement evolution procedure and related impact investigation consultations conducted by the Canadian Council of Animal Care (CCAC) [10]. The CCAC accomplishes an adequate ethic of animal use in science, which include; theory of the Three Rs (Reduction of animal population, Refinement of enactments and farm managements to curtail affliction and despair, Replacement of animals with non-animal surrogate wherever necessary [61]. Simultaneously, the Three R’s aspire to curtail any despair and affliction experienced by the animals used and as such, they are pondered the principles of humane experimental approach [62]. Although, numerous measures have been employed to curtail despair and affliction, there is corroboration of public issues that go over the Three Rs and animal welfare concerning the formation and utilization of genetically engineered animals in veterinary medical practice [10].

Conclusion and Future Perspectives

In conclusion, we are persuaded that biotechnology will suffice as a revolution in every discipline of life sciences including veterinary medicine in many parts of Africa. Egypt has hitherto demonstrated the precedence in the African continent in the application of Biotechnology in Veterinary Medicine. However, revolution alone devoid of practical enactment never had an impression on our community. It is therefore of greatest significance that the breakthrough accomplished in basic and clinical science becomes elucidated in to public endeavour and in practical applications. Finally, it is hoped that biotechnology will in the nearest future vigorously accord to the advancement in the field of diagnosis in veterinary medicine in the entire continent of Africa. This will therefore provide tools and biomarkers that will allow an exceptional understanding of the mechanisms in the propagation of livestock epidemics.

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