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Pathogen Detection and Eradication in Animals Using Nanotechnology Advancement

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Abstract: Today, nanotechnology has made great strides, important in biotechnology and biomedicine related to human and animal sciences, including increasing health safety, productivity, and rising national revenue. There are various categories of nanomaterial applications in veterinary medicine such as effective diagnostic and therapeutic tools, drug delivery, animal nutrition, reproduction, and essential additives. Additional benefits include detection of bacteria, proteins, biological molecules, antimicrobial agents, dietary supplements, nutrient delivery, and reproductive resources. There are many nanomaterials and nanocomposites that can be used in nanomedicine such as metal nanoparticles, liposomes, carbon nanotubes, and quantum dots. In the near future, nanotechnology research will have the potential to develop new tools to improve animal health and production. Therefore, this chapter is designed to highlight the novel techniques created by nanotechnology for use in improving animal health and production.

Keywords: Nanotechnology, Nanoparticles, Microbial diseases, Drug delivery

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Introduction

Infectious diseases are very important in veterinary medicine as they are responsible for multiple appointments in veterinary hospitals and clinics. Significant economic losses occur in animal production, and the potential zoonotic risk of many of these etiological agents. Each disease has different sources of infection, transmission paths, entry points and potentially vulnerable animals,

which require effective diagnosis and treatment (Mignani *et al.*, 2022). It is also important to reinforce the concept of "One Life", which aims at the diversity of human, animal, and environmental health. Many epidemics and animal-borne pathogens, including influenza and coronaviruses, emphasize the importance of early diagnosis, continuous monitoring, and effective prevention of

emerging or regenerating animal diseases to reduce their impact on animal health, diet, food safety and security, as well as public health (Kobayashi *et al.*, 2021).

Nanotechnology has revolutionized the field of infectious diseases, as well as the development of treatments and prevention. Their importance in biomedical use is due to their small size and unique physicochemical properties that allow for controlled drug release, targeted drug delivery, and *in vivo* immunomodulation (Fig. 1). Nanotechnology has been used in various aspects of veterinary medicine including diagnostics, treatment, development of adjuvants and vaccines, drug delivery, and problem-solving in animal and reproductive problems (McElwain and Thumbi, 2017). Nanotechnology provides the most effective diagnostic tools and treatments (Table 1), whether depending on sensitivity, specificity, speed, or cost. Various nanomaterials have been used in animal diagnostics and treatments including metal nanoparticles, polymeric nanoparticles, nanoemulsions, liposomes, and nanocrystals (McElwain and Thumbi, 2017). The use of nanotechnology in veterinary medicine will continue in the future leading to the development of diagnostic and therapeutic diseases in animals that protect the health of animals and humans (Youssef *et al.*, 2019).

The purpose of this research study was to integrate the application of nanotechnology to the detection of animal pathogens (viruses, protozoa, and fungi) and to the control of animal diseases (treatments and vaccines). This study describes diagnostic methods, control measures and highlights the use of nanoparticles as an antibacterial agent in animal diseases. These studies have shown the development of nanotechnology in the field of animals, especially in the diagnosis of emerging diseases. There is a great potential for these nanoparticles in the development of new products and processes for detecting, preventing and eliminating germs from animal sources (Scott, 2007).

Pathogen detection in animals using nanosensors

Nanotechnology has an incredible potential to bring revolution in the livestock, poultry and agriculture sector. The basic tools of nanotechnology like nanomaterials, miniaturized microfluidic systems, and nanosensors provide versatile applications in human and animal health sector by offering early diagnosis and treatment of diseases. The rapidly growing human population has drastically put burden on the healthcare systems. These applications of nanotechnology will have profound effect in the human and animal health sector. For animal health, the detection and diagnosis can help to overcome challenges posed to a large population which is at risk and can give a boost to livestock production, while reducing the overall costs for treatment (Takei *et al.*, 2015). There is a variety of nanomaterials having multiple applications in the health, energy and environment. Among them includes carbonaceous nanomaterials comprising of carbon nanotubes, graphene nanosheets, and carbon quantum dots and their composites with metallic nanostructures can be used for the qualitative and quantitative evaluation of disease causing agents. As nanotechnology continues to develop and garner more attention, its applications in the animal production industry will become more expensive. The regular inclusion of nano-supplements to fortify livestock feed is likely possible in the near future; however, it will take longer for nanoparticles to fully replace antibiotics in feed as many biocidal candidates must still be tested *in vivo* before undergoing clinical trials and food safety tests in accordance with government regulations. External uses for nanoparticles have already been integrated into some aspects of animal production, i.e. antiseptic wound dressings, and more are to follow (Younis *et al.*, 2022). For studies interested in nanoparticles with anti-cancer properties, it is important to investigate nanoparticle cytotoxicity in both cancer cell lines and normal healthy cell lines. Only using cancer cells and claiming the nanoparticle under investigation has anti-cancer properties may be misleading, as the nanoparticle may be cytotoxic to all cell types. *In vivo* studies are needed for

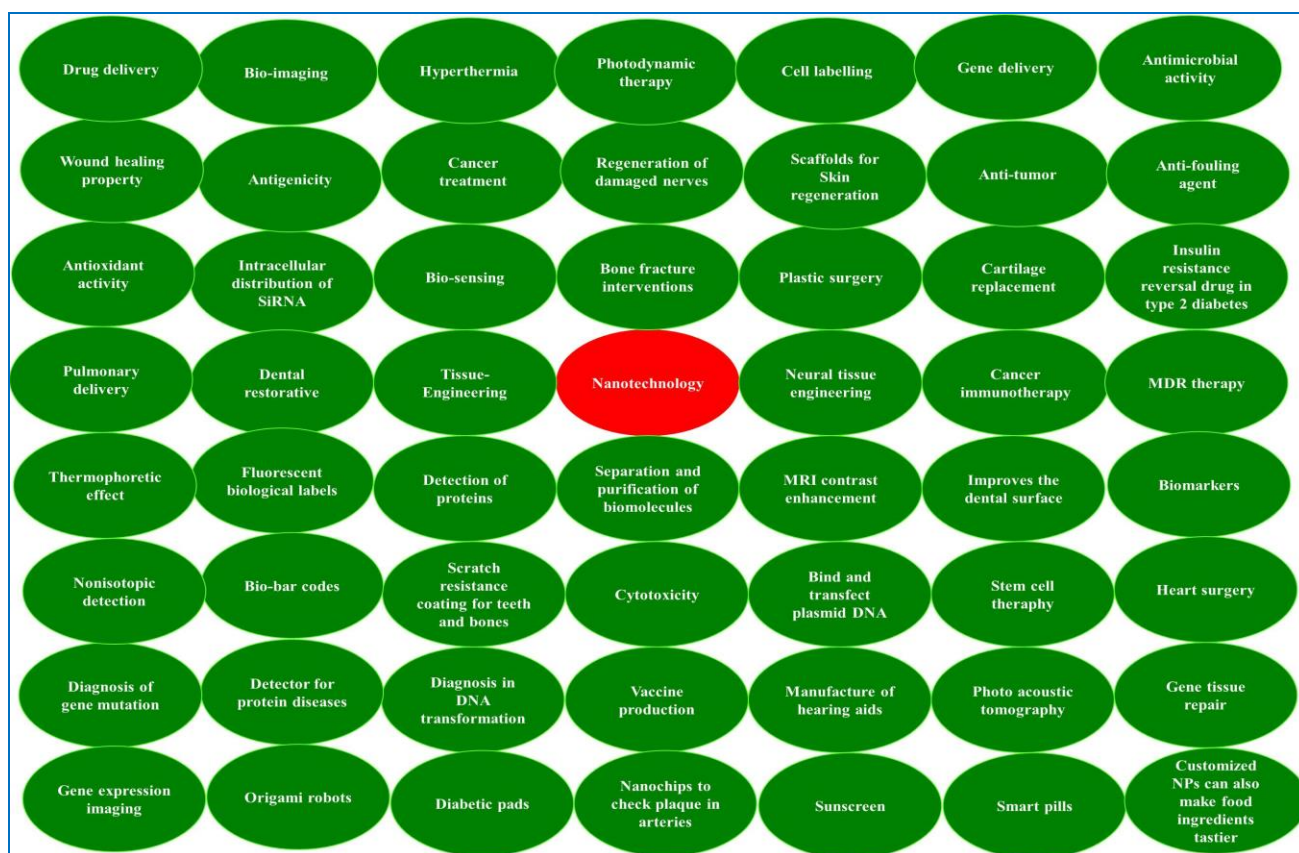


Fig. 1: Nanotechnology's biomedical uses.

Table 1: Nanodiagnostic tools/medicines uses for the detection of microbial infections in animals

Name of the diseases	Disease causing agent	Mechanism	Diagnostic tools used	References
Anthrax	<i>Bacillus anthracis</i>	Anthrax vaccine nanoemulsions appear to be an efficient mucosal adjuvant for a recombinant protective antigen. These formulations elicit long-lasting, powerful, and specific humoral and cellular responses, appear to be free of side effects, and can stabilise antigen	Nanoemulsion	Bielinska <i>et al.</i> (2007)
Food and mouth disease virus (FMDV)	Not mentioned	The extent of the immunological response elicited by FMDV-AuNPs conjugated peptides is associated with the accumulation of gold nanoparticles (AuNPs) in the spleen	AuNPs	Chen <i>et al.</i> (2010)
Pertussis	<i>Bordetella pertussis</i>	Pertussis antigens, pertussis toxin and filamentous haemagglutinin, encapsulated in PLG particles, generate powerful T cell and	Poly-lactide-co-glycolide (PLG)	Conway <i>et al.</i> (2001)

		antibody responses and provide excellent protection against a <i>Bordetella pertussis</i> challenge		
FMDV	Not mentioned	Multiple peptides can be conjugated to individual nanobeads or combined to generate large cell-mediated and humoral immune responses	Inert nanobeads	Greenwood <i>et al.</i> (2008)
Herpes simplex virus-2 (HSV-2)	HSV-2 and Epstein-Barr virus	High titers of IgG2a antibody and neutralising antibody were generated by calcium phosphate nanoparticles (CAPNPs) injected intraperitoneally with HSV-2 and Epstein-Barr virus proteins, allowing for a high level of protection against viral infection.	CAPNPs	He <i>et al.</i> (2002)
Rabies	Not mentioned	Inflammatory cytokines release mechanism	AgNPs	Asgary <i>et al.</i> (2016)
Mastitis	<i>Staphylococcus aureus</i> , <i>Streptococcus agalactiae</i> and <i>E. coli</i>	Nanomaterials may disrupt signalling molecules, preventing the development and modification of biofilm structures by inhibiting gene expression pathways. This may cause the biofilm's resistance to decrease	AgNPs and CuNPs	Lange <i>et al.</i> (2021)
Mastitis	<i>Staphylococcus aureus</i>	Some reduction in cell viability may be deemed a reasonable compromise in the interest of achieving effective control of <i>S. aureus</i> 's intracellular survival capacity. Controlled host cell damage may be an acceptable cost of eradicating the virus on some levels	Propolis NPs (PNPs)	Pinheiro <i>et al.</i> (2019)
Mastitis	<i>Staphylococcus aureus</i>	AgNPs may provide for a two-fold reduction in the length of serous mastitis therapy. Elevation in serum lysozyme activity and serum bactericidal activity after therapy with this drug, which might imply a stimulating impact of this medication on nonspecific immunity via phagocytosis activation	AgNPs	Nefedova and Shkil (2021)
Mastitis in sheep	<i>Staphylococcus aureus</i> and <i>E. coli</i>	The concentration and size of ZnONPs have a significant impact on their inhibitory activity	ZnONPs	Alekish <i>et al.</i> (2018)
Anthrax	<i>Bacillus anthracis</i>	When combined with an antigen, CpG boosts the synthesis of IFN-. The quantities of IL-4 generated by the splenocytes were modest in all of the groups, but the TMC-PA group secreted the most IL-4, regardless of the vaccination method	CHNPs	Malik <i>et al.</i> (2018)

verification of nanoparticle functions seen in *in vitro* research.

The current socio-economic situation and the growing consumer demand for healthy food have led to the development of new research methods for nanotechnology and its use in livestock and poultry. Livestock and poultry provide us with the most nutritious nutrients as protein and are essential for sustainable agriculture. The use of poultry and livestock production systems is related to human diet in many ways and as a result may affect food quality (Hassan *et al.*, 2020). Diseases in poultry, livestock and other animals are divided into different groups according to their causes. Other causative agents of pathogens and viruses appear in each case and lead to the spread of the global infection among animals (El Sabry *et al.*, 2018).

Research into the development and use of diagnostic nanosensors to detect pathogens in animals, livestock, and poultry has brought about changes in the field of animal health. The development of sensitivity sensors has led to timely diagnostic detection leading to increased management in the animal health sector and reduced economic losses. The incorporation of nanotechnology into the development of animal diagnostic nanosensors has provided practical solutions for various applications in livestock and poultry production systems (Ikonomopoulos). These are responsible for improving animal health and productivity due to the early detection of pathogens. Pre-acquisition strategies required a laborious process of sample preparation, metal availability and time-consuming (Younis *et al.*, 2022).

Nanosensors are easy to use because of their simplicity, sensitivity and low cost (Gattani *et al.*, 2019). The rapid detection of bacterial and animal diseases in livestock, and poultry has improved animal infrastructure and reduced global threats to emerging infectious diseases (Parthiban *et al.*, 2015). Diagnosis is an important factor in fighting, reducing and eliminating the spread of viral or viral infections. Early detection systems have been

shown to be more alert in the event of an outbreak of disease. The invention of nanosensors has helped to control the outbreak of infectious diseases (Pejcic *et al.*, 2006).

Viral infection detection in animals

Livestock viruses mainly include avian influenza virus, cattle respiratory virus, foot-and mouth virus, rift valley fever virus, bluetongue virus, all of which have become a major threat to livestock and poultry (Pejcic *et al.*, 2006). Livestock farms are often at risk of cattle diarrhea, cattle herpes virus and oral and viral infections. Many types of sensors have been developed to control the presence of the virus in animal sera. Biosensor technology offers the advantage of replication, suitable for large livestock and poultry applications and has the potential to detect outbreaks of viral diseases (Neethirajan, 2020). The avian influenza virus has caused epidemics in humans and animals around the world for many years (Neethirajan, 2020). Neurosurgery has led to the early detection of suspected cases. Following the re-emergence of bird flu virus in the Asian climate each year, Lin *et al.* (2015) developed a low-cost nano-sensor impedance to detect Avian influenza (H5N1) virus spots in chickens. The nerves are detected in the wild spots of the virus from chickens in just one hour (Lin *et al.*, 2015).

Infectious bronchitis belongs to the family of avian coronaviruses which has greatly affected chickens and other pets for decades causing significant economic losses to the poultry industry. A novel and sensitive molybdenum disulphide based fluorescent immunosensor was developed to diagnose Infectious bronchitis (IB). This two-dimensional nanosensor works best when applied to an antibody labeled dye using the resonance force between MoS₂ and fluorescence dye. The sensor showed remarkable sensitivity and successfully detected serum-infected chicken serum due to the different extinguishing properties of molybdenum disulphide (Weng and Neethirajan, 2018). In a similar research study, an electrochemical immunosensor based on carbon

electrode was developed to detect H5N1 flu virus in hen serum. The sensor was also able to distinguish between vaccinated and vaccinated chicken pox against the flu virus and was found to be effective in vaccinating chickens in large quantities (Jarocka *et al.*, 2014).

Foot-and-mouth disease (FMD) is a highly contagious disease that affects cattle, sheep, goats and many other species of mammals. In older animals, they do not kill but in younger animals can cause serious ulcers (Longjam *et al.*, 2011). The galactosidase allosteric Biosensor was used to study the conditions of animals with foot-and-mouth disease in general. Studies have been performed on infected pigs, cattle, and sheep. The sensor provided another effective way of differentiating serological differences between infected and vaccinated animals. An antigen-coated potentiometric immunosensor was studied to detect Bovine Herpes Virus-1 in chickens. To test sensory abilities as a diagnostic tool, the BHV-1 viral protein antigen, could be moved to the sensory surface to capture a specific antibody produced in cattle as a response to a viral infection. The sensor was found to be highly selective of the anti-gE present in the anti-BHV-1 antiserum present by commercialization in serum samples of real cattle (Tarasov *et al.*, 2016).

In the livestock and poultry industry, *Salmonella*, *Escherichia coli* and the most debilitating campylobacter bacteria are occasionally found on the skin and intestines of birds, sheep and goats. These germs are the leading cause of diseases in poultry that cause foodborne illness worldwide. The increase in poultry consumption worldwide is due to the accessibility and affordability of a low-cost protein source. Increased use of poultry has forced researchers to use the possible use of nanotechnology to synthesize nanosensors in order to meet the challenges posed by these pathogens in livestock, poultry and other animals (King *et al.*, 2018).

Anthrax, a very common disease that affects pets, is caused by the *Bacillus anthracis*. A multi-

walled fluorescent apta-sensor sensor made of carbon nanotube was used to detect the reconstituted antigen base of *Bacillus anthracis* which is a major cause of anthrax. Fluorescent aptamer sensor that works with adsorption on multi walled carbon nanotubes. Results have shown that the nanosensor is as potent as a sensitive, rapid and inexpensive diagnostic system for anthrax in cattle (Karimi and Dabbagh, 2019). A highly sensitive immunosensor made of quartz crystals using nanobead magnets was used to detect the bacterium *Campylobacter jejuni* in chickens, mice and rabbits. Quartz crystal nanosensor, prepared in a flow cell, was coated with a magnetic nanobead to detect C spots. A nanosensor was shown to detect bacterial color in less than 30 min and atomic force microscopy was used to measure C spots.. This highly flexible and flexible approach can be in the poultry industry with effective means of detecting pathogens in the poultry industry (Wang *et al.*, 2018).

Animal health is very important for human survival. Major food products e.g. milk, cheese, butter, meat and eggs are procured from animals. Pets and livestock are also important for many other purposes especially in the leather industry and agriculture. Any outbreak of infectious disease in animals will directly affect human health in many ways. There is a need to design such new, unique, very clear and sensitive visual systems that can detect unwanted or disease-causing substances even at nano molar levels in a complex biological system. Such technology is available on laboratory and clinical scales, but generally for humans. Nowadays, researchers are making significant efforts to market nanosensors in the global human and animal market for livestock and aquaculture. Nanomaterials based nanosensors are designed for the detection of bacteria, viruses, other bacteria and antibiotics.

Conclusion

Over the past few decades, nanotechnology has provided continuous novel development to improve animal health and production. Today, several nanomaterials are used as

nanoantifungals, in addition to having other benefits such as diagnostics, diagnosis and treatment, use of animal feed additives and their products, and ultimately food safety. The important therapeutic and protective properties of nanoantimicrobials, especially zinc nanomaterials, have been tested against a variety of fungal infections and mycotoxicosis in animals. Also, super paramagnetic iron, semiconductor quantum dots and gold nanoparticles receive early and sensitive detection applications followed by detailed predictions and cancer treatment. Both inorganic polymeric nanomaterials and organic polymeric materials have also been used for the target delivery of various vaccines, rapid and local detection of signed bacteria or proteins and other biological molecules. The mechanisms of nanoantimicrobial activity are related to their ability to penetrate the cell membrane, damaging the cytoplasmic content, leading to loss of function and cell death. Therefore, further studies showing cellular toxicity pathways that lead to oxidative stress and lead to genotoxicity and cancer require detailed testing to determine the nanomaterial role in animal health. In addition, the toxicity of nanomaterials should be determined prior to the use of nanomaterials in animal medicines to protect animal health and their role in animal production.

Ethical Statement

This is a review article hence no Ethical Approval needed.

Author Contributions

Each author has contributed equally to the study's planning, analysis, writing, and editing. All authors have read and agreed to the published version of the manuscript.

Conflict of Interest

The authors declare no conflicts of interest.

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