Review Article

A REVIEW ON NANO FORMULATION FOR VETERINARY APPLICATION

K. Chitra[1], M. Rasheeda Shameem [2] and C. Soundararajan[3*]

Centre for Animal Health Studies, Tamil Nadu Veterinary and Animal Science University Chennai – 600 051, India

ABSTRACT

Nanotechnology has been used in a variety of medical applications, including diagnostics, immunisation, medication and gene delivery. Nanotechnology has opened up new ways for molecular biology and biotechnology applications, and it has revolutionised practically every area of veterinary and animal sciences by introducing new, small-scale instruments and materials that are advantageous to living species. Metallic nanoparticles, quantum dots, carbon nanotubes, magnetic nanoparticles, fullerenes, liposomes, and dendrimers are among the several forms of nanomaterials employed in detection of various diseases, therapy, drug administration, animal nutrition and breeding. It is concluded that, before making nano-products available for animal use, extensive risk evaluations should be carried out to ensure their safety. So, this article provides an overview of existing and promising nano applications in veterinary research that may lead to increased efficiency and production.

Key words: Animal health, Nanoformulations, Nanomedicines, Nanotechnology, Veterinary Science

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INTRODUCTION

Nanotechnology is a rapidly growing science with the goal of building innovative materials with a diameter of 1 to 100 nano meters. Nano is derived from the Latin word nanus, which is synonymous with tiny and denotes small size (1nm equals 10^{-9}m). Nanotechnology is an emerging technology with applications in a variety of disciplines, including research, agriculture, and infection therapy. Nanomaterials also have the potential to have an impact on *in-vivo* and *in-vitro* biomedical applications and research (Shaalan *et al.,* 2016; Youssef *et al.,* 2019; Tatli Seven *et al.,* 2018). There are numerous classifications of nanoparticles based on, (1) the origin of their nano-materials, which can be divided into organic nanoparticles (proteins, peptides, or

[1]Scientist, Translational Research Platform for Veterinary Biologicals, Centre for Animal Health Studies, TANUVAS, Chennai – 600 051

[2] Project Executive, Jeshron Biotech Solution Pvt Ltd, VIF @ TANUVAS, Chennai – 600 051

[3]Director, Centre for Animal Health Studies, Tamil Nadu Veterinary and Animal Science University, Chennai – 600 051, Corresponding author Email id: dcahs@tanuvas.org.in

lipids); inorganic nanoparticles (gold, silver, silica, iron, magnesium, or grapheme); and hybrid nanoparticles, (2) shape (spheres, tubes, or liquid drops), (3) application and type of payload (therapeutics, vaccines, diagnostics) (El-Sayed and Kamel, 2020). The potential applications of nanotechnology in veterinary medicine and animal health are unlimited. Every area of veterinary medicine can be benefitted from nanotechnology. The nanoparticles can be used to eradicate a variety of animal diseases, including blood parasites, intracellular pathogens, and those that cause severe chronic illnesses (Prasad *et al.*, 2021). Nanotechnology also offers enormous potential in terms of improving drug delivery. The fascination with newly synthesised molecules may lead to the development of a new useful therapeutic products to treat diseases, protect animals from viral or bacterial infections, and promote wound healing. Furthermore, these novel mixtures could transport pharmaceutical drugs into cells for therapeutic purposes. The use of nanotechnology devices for animal disease diagnosis or as animal models for human disease diagnosis is a significant achievement in the field of nano science. Advances in nano science have the potential to solve substantial challenges related to animal health, reproduction, production, hygienic procedures, and food supplements. However, nanotechnology is not widely used in these fields (Tatli Seven *et al.,* 2018; Youssef *et al.,* 2019). Nanotechnology-based solutions have the potential to aid in the early detection, prevention and monitoring of a variety of diseases. Many developed nano systems are currently undergoing clinical trials for major diseases such as cancer, infections, cardiovascular, neurodegenerative, musculoskeletal, and inflammatory diseases, and there are currently approved nanotechnology products for therapeutic and diagnostic purposes in human health. Veterinary medicine has likewise entered a new era with remarkable changes. Although nanotechnology applications in veterinary medicine is not same as in humans, they do provide increased safety and efficacy in therapy, as well as early/rapid diagnosis and effective monitoring in a wide range of species (Şenel, 2020). So, this review aims to give a clear idea about the types of nanocarriers used in various veterinary applications.

Nano carriers and Types

Nano carriers are colloidal nanoparticles that are commonly employed to deliver medicinal agents or other substances to a specific target spot. Nano carriers have a diameter ranging between 1 to 1000 nano meters (nm) and for therapeutic applications nano carriers with a diameter of less than 200 nm is required (Chamundeeswari *et al.,* 2019). Nano carrier technolohy is a good technique for reformulating existing dosage forms of compounds having low-solubility, thereby enhancing the bioavailability and lowering their toxicity. Organic nano carriers, inorganic nano carriers and hybrid nano carriers are the three types of nano carriers (Muktar *et al.,* 2015; Carvalho *et al.,* 2020; Chariou *et al.,* 2020). The principal nano carriers (Fig 1) utilised to manufacture nanomedicines for veterinary applications like, liposomes (Fig

1a), dendrimers (Fig 1b), nano emulsion (Fig 1c), micelles (Fig 1d), solid lipid nanoparticles (Fig 1e), quantum dot (Fig 1f), carbon nanotube (Fig 1g), mesoporous silica nanoparticles (Fig 1h) and magnetic nanoparticles (Fig 1i) are discussed in this study.

Liposomes

Liposome is a phospholipid bilayer vesicle having a nonpolar tail and a polar head. Phospholipids are divided into two categories: phosphoglycerides and sphingolipids. Phospholipids of natural and synthetic origin are commonly employed in the preparation of liposomes. Based on the number of lamellae and size, liposomes are classified as small unilamellar vesicles, large unilamellar vesicles, large multilamellar vesicle and multivesicular vesicle. It has several benefits like its high efficacy, therapeutic index, increased stability of encapsulated drug, reduced toxicity, flexibility with site-specific ligands, and selective passive targeting. When employed as liposomal medications, cancer chemotherapeutic agents and other hazardous pharmaceuticals like amphotericin and hamycin have substantially greater efficacy and safety than conventional preparations (Prasad *et al.,* 2021;Rode *et al.,* 2018).

Dendrimers

Dendrimers are nano molecules with a branching structure. The size of the dendrimer that can be adjusted is determined by the number of branches. They are used for gene therapy and can replace conventional viral vectors. Their major stress in the treatment of cancer is due to the fact that these nano metric particles passively accumulate at the site of tumours, enter the cells via endocytosis and transport the DNA into the nucleus for transcription of the applied gene. According to current studies, dendrimers could be used as drug carriers for the treatment of diseases since they have the ability to deliver a prolonged release with less adverse effects (Muktar *et al.,* 2015; Shaalan *et al.,* 2016).

Nano emulsions

Nano emulsions, are also called as submicron emulsions, ultrafine emulsions and mini emulsions. They are submicron-sized colloidal particulate systems that are thermodynamically and kinetically stable isotropic dispersions made up of two immiscible liquids, such as water and oil, stabilised by an interfacial film made up of a suitable surfactant and co-surfactant to form a single phase (Gurpreet and Singh, 2018). Each droplet in a nano emulsion has a protective coating of emulsifier molecules with a diameter ranging from 10 to 200 nanometres. A water-based, or oil-in-water emulsion has water as the continuous phase and oil as the dispersed phase, whereas an oil-based, or water-in-oil emulsion has the opposite condition. Nano emulsions' small droplet size enables homogeneous deposition and penetration of active substances via the skin (Marzuki *et al.*, 2019).

Micelles

Micelles are amphiphilic polymer-based drug carrier systems that are divided into

two parts: a hydrophobic nucleus (inner part) and a hydrophilic crown (outer part) with a size of 10 to 100 nm. Micelles can be made from a variety of polymers which divides them into four classes based on the hydrophilic portion's components. Phospholipid micelles (with phospholipids in the crown) are classified as class number one, while Pluronic® micelles are classified as class number two, poly (L-amino acid) micelles are classified as class number three and polyester micelles obtained from biocompatible polymers are classified as class number four (Carvalho *et al.*, 2020).

Solid Lipid Nanoparticles (SLN)

Solid lipid nanoparticles (SLN) are made up of lipids that are solid at normal temperature, stabilised with a surfactant and suspended in water. SLNs have significant advantages for example, they have a better drug entrapment efficiency, great physical stability, more flexibility in controlling the release of the loaded ingredient, can be delivered orally, topically and intravenously and gives chemical protection of incorporated active compounds. They also have some drawbacks like inadequate loading capacity, drug ejection after polymorphic transition during storage and relatively high water content (Underwood and van Eps, 2012; Irache *et al.,* 2011).

Quantum Dots

Quantum dots are semi-conductor nanoparticles with a diameter of 2–10 nm. They are made up of an inorganic core, an inorganic shell and an aqueous coating that can be coated with biomolecules. They fluoresce when stimulated by light and the colour of light emitted is determined by the size of the core (Underwood and van Eps, 2012). The colour will be brighter as the quantum dots are smaller. They stay illuminated for considerably longer than dyes, typically for hours or days. They allow us to brand distinct biological components, such as proteins or DNA strands, with specific colours, similar to fluorescence. Quantum dots could be injected into the bodies of animals. They can detect cells that are not functioning correctly once injected into the body. Quantum dots can be used to screen proteins in a blood sample that signal a higher predisposition for various diseases. Quantum dots, on the other hand, have a restricted therapeutic utility due to their probable cytotoxicity and slow elimination (Mohanty *et al.*, 2014).

Carbon nanotubes

Carbon nanotubes made up of a graphite sheet folded into a nanoscale tube are called as single-wall carbon nanotubes or additional graphene tubes wrapped around the centre of an object are called as multi-wall carbon nanotubes. The diameters of a carbon nanotube range a few nanometres, and the lengths range from a few centimetres to several centimetres, with both ends generally capped by fullerene-like structures (Norizan *et al.*, 2020; Giri *et al.,* 2021). These carbon nanotubes have a big surface area, so that they have high ability to penetrate cell membranes, they also act as an effective micro-needles which makes them excellent for medication delivery, including anti-tumour medicines

(Shaalan *et al.*, 2016). The electrical, magnetic, and chemical properties of these carbon allotropic tubes are all unique. Carbon nanotubes have the potential in drug delivery mechanisms due to their unique structure and various efforts have been made to make them more effective for medical conditions (Mohanta *et al.*, 2019). Several researchers have demonstrated that carbon nanotube bundles, carbon nanotube membranes, polymer - carbon nanotube composites and carbon nanotubes - modified electrodes can be used as effective electrochemical biosensors, for the high electron transfer rate and high electrocatalytic activity (Gupta *et al.*, 2019).

Mesoporous silica nanoparticles

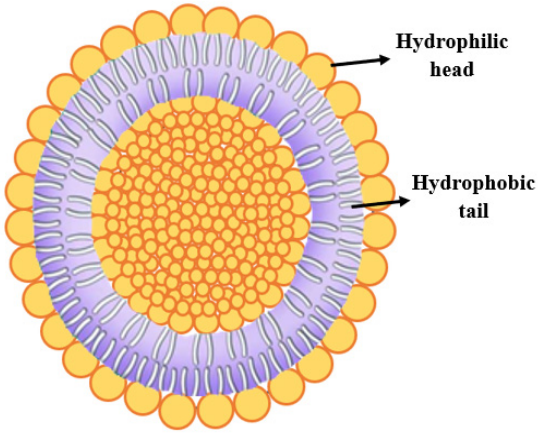
Mesoporous silica nanoparticles are made up of an amorphous silicon dioxide (silica) matrix with porosity in mesoporous range. Textural qualities, physical chemical stability and ease of chemical modification are some of the primary attributes that make mesoporous silica materials an appealing candidate for drug delivery systems (Paris and Vallet-Regí, 2020). Mesoporous silica nanoparticles have unique features such as meso-nanoscale pores, variable pore size and flexible morphology. They can act as an excellent drug delivery vehicle as the pores can be filled with therapeutic medicines and then capped with appropriate gatekeeper molecules (Bakhshian *et al.*, 2020).

Magnetic nanoparticle

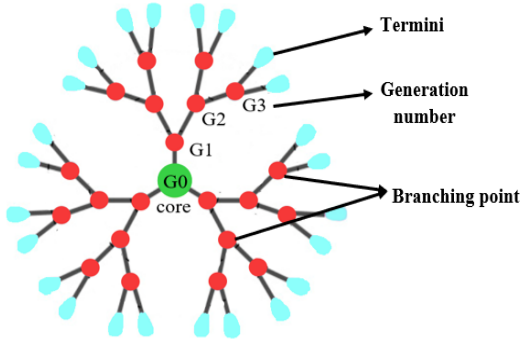
Magnetic nanoparticles are a type of imaging that uses super para magnetic iron oxide particles as tracer particles to provide very sensitive and specific images in a variety of applications, such as cardiovascular imaging, neuroimaging, tumour imaging, magnetic hyperthermia, and cellular tracking. Furthermore, antibacterial surface functionalization of metal oxides is relatively simple (Billings *et al.*, 2021). Magnetic nanoparticles can be made using variety of methods with highly saturated magnetization materials such as transition metals (Iron, Cobalt, Nickel) and metal oxides (Fe_3O_4 and Fe_2O_3). Pure metals, such as iron nanoparticles, have the maximum magnetism, but they are usually poisonous and prone to oxidation. As a result, pure metal nanoparticles are not recommended for biomedical purposes (Quan *et al.*, 2021).

Application of nanotechnology in veterinary science

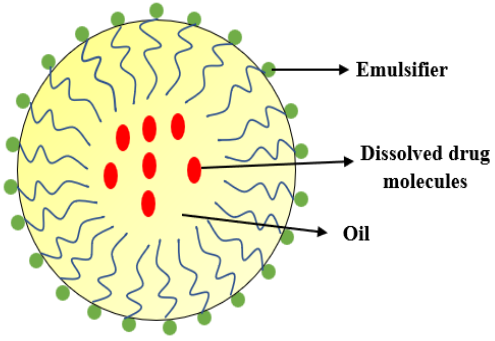
In future nanotechnology will have a significant impact in the field of treating of animals. Nanotechnology has the potential in providing solutions for formulating domesticated animal diet, veterinary care, prescription medicines and vaccinations. Figure 2 shows various applications of nanotechnology in veterinary science. The nano carrier materials used in these veterinary formulations are still under pre-clinical and clinical trials. Imrestor (Elanco, US) made with protein and poly ethylene glycol (PEG) for treating inflammation in the breast tissues of cattle and Paccal vet – Cal (Osmania Pharmaceuticals) made with micelle nanocarrier to treat canine mast cell tumours



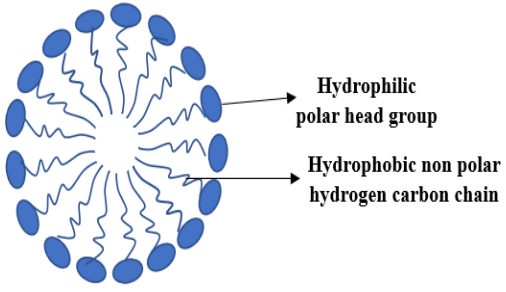
(a) Liposomes



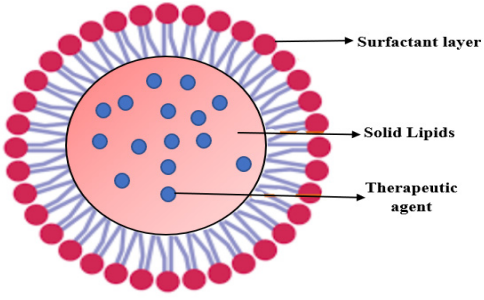
(b) Dendrimers



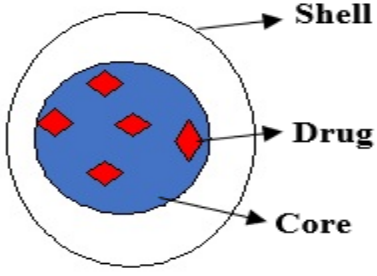
(c) Nano emulsion



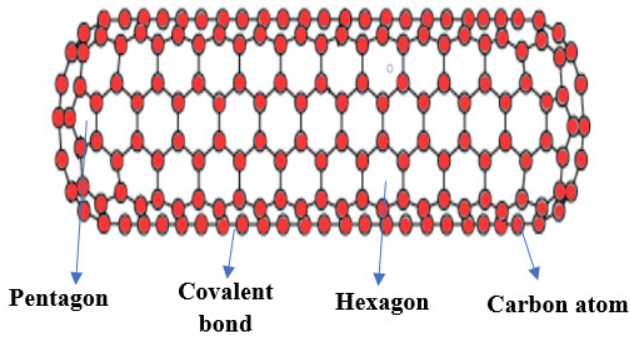
(d) Micelle



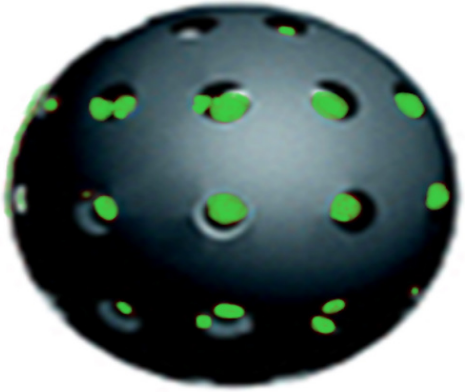
(e) Solid lipid nanoparticles (SLN)



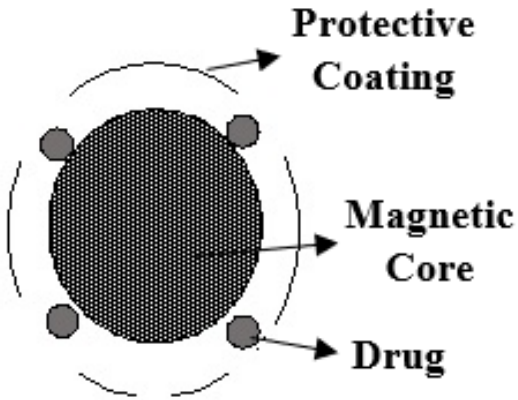
(f) Quantum dot



(g) Carbon nanotube



(h) Mesopourous Silica Nanoparticles



(i) Magnetic Nanoparticles

Fig 1: Different types of nano carriers used in veterinary formulations: (a) Liposomes, (b) Dendrimers, (c) Nano emulsion, (d) Micelle, (e) Solid lipid nanoparticle (SLN), (f) Quantum dot, (g) Carbon nanotube, (h) Mesoporous silica nanoparticle and (i) Magnetic nanoparticle

are some of the approved veterinary nano carrier drugs. Most of the nano carriers will not survive the clinical trials due to their poor efficacy (Chariou *et al.,* 2020). So, in order to boost production and effectiveness, the use of nano capsules to cap and protect some specific enzymes and proteins might be helpful. Certain drugs, like antibiotics, vaccines and probiotics, would be more successful when used at the nanoscale level in treating infections, nutrition and metabolic diseases. Medicinal application at the nanoscale level has multimodal qualities that allow it to break down biological barriers and improve medicine's efficiency. The key advantages of using nanotechnology in drug therapy are the precise timing of drug release, self-regulatory capabilities and capacity planning. Furthermore, the benefits of nanoparticles in drug delivery systems include reduced toxicity and site-specific targeting, which can be accomplished by attaching targeted ligands to the surface of the particles and delivering them by oral, nasal, parenteral and intraocular routes (Banumathi *et al.*, 2017).

Drug Delivery

Different nano carriers have been employed for drug delivery systems, in which liposomes, micelles, nanoparticles and nanogels are being highlighted as key nano carriers in drug delivery. Nanotechnology-based drug delivery targets effective drug encapsulation, effective drug delivery to the specified area of the body and effective drug release. Liposomes are one of the most widely used nano carriers in transdermal drug delivery systems for both systemic (intravenous) and topical drug administration. Liposomes provide a number of benefits over traditional drug delivery techniques and they are typically employed for a system's long-term or regulated drug delivery. As a result, liposomes are prospective drug carriers that have been intensively investigated due to their ease of production and regulated size distribution (Prasad *et al.*, 2021). Nanotechnology also aids in the treatment of various veterinary diseases including infections of intracellular or blood pathogens, tuberculosis, brucellosis, methicillin-resistant *Staphylococcus aureus* (MRSA), foot and mouth disease (FMD) and so on (El-Sayed and Kamel, 2020). Knottenbelt *et al.* (2020) prepared a novel topical liposome-encapsulated preparation of bleomycin and studied its efficacy in 118 equine sarcoid cases. The prepared liposome encapsulated with bleomycin was compared with tazarotene, 5-fluorouracil (5-FU) and the bleosome in combination with each of these two treatments. The obtained results showed that after 12 months, 44% of the sarcoid cases had recovered. According to these findings it is stated that, bleomycin in a liposomal carrier could be an effective treatment for superficial, diffuse and verrucose sarcoids. When compared to radiation and other treatments, liposome-encapsulated bleomycin is less expensive and can be applied efficiently by the owner. Various polymeric and macromolecular materials (protein, polysaccharides) and nanoparticles have been extensively investigated for targeted drug delivery for tumour regression; nonetheless, premature drug release into the

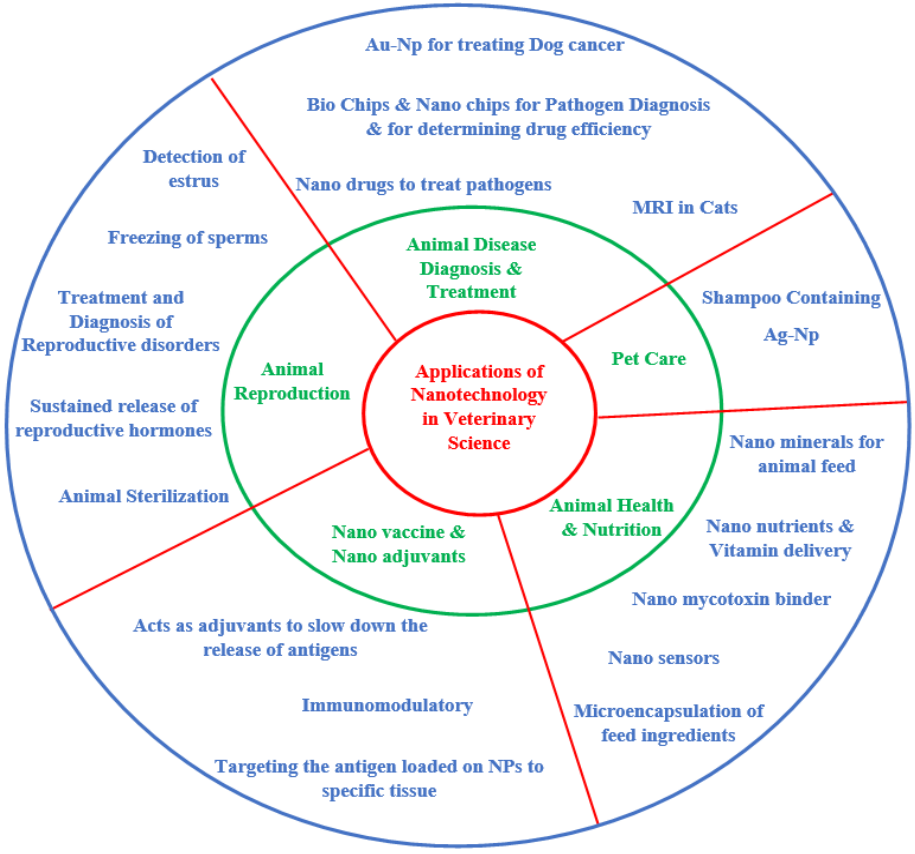


Fig 2: Applications of nanotechnology in veterinary science (El-Sayed and Kamel, 2020)

bloodstream is a primary cause of their failure. Guo *et al.,* (2012) developed a solid lipid nanoparticles (SLNs) carrier as a vehicle for transdermal drug delivery of ivermectin. The SLNs were nearly spherical in shape and had good stability with excellent encapsulation efficiency. It was found that ivermectin encapsulated in amorphous form within SLNs demonstrated delayed release from SLNs without bursting and these findings suggest that SLNs can be used as an effective carrier for transdermal delivery of ivermectin in the treatment of scabies. Hydrogels are three-dimensional cross-linked polymeric networks with a hydrophilic character that absorb water. Hydrogels are always regarded as promising drug delivery strategies due to their biocompatibility, softness, and porous nature (Raza *et al.*, 2018).

Animal breeding and reproduction

Nanotechnology has begun to develop in the field of animal reproduction. The goals of these nanotechnology-based animal reproduction studies are to characterise nanoscale features of gamete cells using atomic force microscopy and other scanning microscopic techniques, to develop nano-bio sensors for physiological or altered detection of reproductive status, chemical tactics for the production of metal nanoparticles for fertility control applications and nano devices for secure cryopreservation of gametes and embryos. Furthermore, nanotechnology devices such as microfluidics, nanoparticles and bioanalytic nano-sensors can help answer even more issues in animal health, reproduction, disease prevention and treatment. Microfluidics and nanofluidics are cutting-edge techniques for improving *in vitro* fertilisation and the growth of *in vitro* embryos. Microfluidics have been demonstrated to be effective in insulating motile sperm without centrifugation in recent studies (Kalkidan *et al.,* 2021). Wang *et al.,* (2017) generated transgenic mice using magnetic nanoparticles as nano carriers, as they have various properties like high carrying efficiency, non-immunogenicity, and strong targeting activity which is induced by magnetic fields. Transgenic mice were generated using magnetic nano carriers with sperm-mediated gene transfer possessing enhanced green fluorescent protein (EGFP) gene. The authors conclude that this method is extremely simple, quick, and cost-effective, with greater transfection rates.

Veterinary Vaccine delivery

The nano vaccine is gaining popularity because of the new techniques of vaccination. Nano vaccines are more potent than regular vaccines at stimulating both humoral and cell-mediated immune responses. They hold the potential of directing the immune system to attack pathogens and prevent infection and spread of the disease. The current vaccination process has shifted away from live and killed organisms, toward synthetics and recombinants, which are far safer. Such novel vaccination candidates are frequently immunogenic and susceptible to degradation, necessitating the use of a tailored adjuvant to improve immunogenicity. As conventional adjuvants are inflexible, the development of nanotechnology has brought about a series of new antigen-carrying techniques. Such nanoparticle-based adjuvants can be tailored for lower dose frequency and a more comfortable mode of administration to produce a specific target immunological response, such as improving target mucosal immunity. This makes them ideal for veterinary practise, where huge numbers of animals must be handled at once, or where traditional vaccination methods are unfeasible due to complex management systems or a lack of accessibility (Kalkidan *et al.,* 2021). Nano vaccines can be classified based on a variety of factors including form, source, size, characteristics, and structural restriction. As a result, it provides scientists and researchers with the possibility to develop new creative methodologies as well as nanovaccinology applications. Nano vaccination with nano-

beads is one of the most successful approaches for vaccine delivery. The investigation into the identification of nano-beads has taken a lot of time and effort. Antigen is coated on the surface of these nanoparticles. Because of their appealing characteristics like high storage stability and the need of a minimal dosage of antigen to function, nano-beads vaccination has proven to be effective. Liposomes have been used as a drug carrier system in nano vaccines to improve therapeutic index and immune response. Liposomes have the unique ability to solubilize the phospholipid nature of the cell membrane, with their biocompatibility and biodegradability properties, they become attractive drug delivery vehicles (Yadav *et al.*, 2018). Watarai *et al.* (2014) studied the efficacy of mucosal vaccine based on pH-sensitive fusogenic polymer [(succinylated poly glycidol (SucPG) and 3-methylglutarylated poly glycidol (MGluPG) - modified liposomes as carrier material. To evaluate the efficiency of the prepared vaccines the mice were inoculated intravenously with SucPG-modified liposomes carrying ovalbumin. Significant antigen-specific antibodies were found in the serum and gut of mice after immunisation. Both IFN and IL-4 mRNA were found in spleen cells from mice inoculated with ovalbumin (OVA)-containing SucPG-modified liposomes. Similar results were observed in mice immunized with ovalbumin (OVA)-containing MGluPG modified liposomes. The authors also studied the immunological responses in chickens after intraocular immunisation with *Salmonella enteritidis* antigen containing MGluPG-modified liposomes, as well as the protective impact against *S. enteritidis* challenge. Immunization with MGluPG-modified liposomes carrying *S. enteritidis* antigen resulted in significant antibody responses against *S. enteritidis* in the serum and gut. The chicken immunized with modified liposomes showed less microorganisms in the faecal discharge and in caecum than the unimmunized controls.

Delivery of Animal Feed Supplements

Feeding has a direct impact on the animals growth rate, producing capacity and health. Certain nutrients like arginine, glutamine, zinc and conjugated linoleic acid can increase fertility, pregnancy success, immunological function, neonatal survival and growth, feed efficiency and meat quality through regulating gene expression and metabolic processes. Nanotechnology is also employed in animal nutrition to manufacture nano-minerals, particularly trace minerals, which help livestock and poultry to improve digestion, immunity and performance. Nano zinc, with particle sizes ranging from 1 to 100 nm, has been shown to improve immunity in pigs, milk production in cattle and poultry growth. Many studies have found indications that nanoparticles could be effective antimicrobials and growth promoters for animals. Encapsulating the important or functional feed ingredients also protects them from being lost during the preparation of the feed (Şenel, 2020). Patyra and Kwiatek, (2021) quantified sulfonamides in medicated feeds using micellar liquid chromatography.

The proposed method was simple, sensitive, rapid and was able to determine sulfadiazine, sulfamethazine, sulfaguanidine, sulphamerazine and sulfamethoxazole in the medicated feeds given to poultry and pigs.

Diagnosis of veterinary diseases

In veterinary medicine, when an animal is infected with any disease, it might take days, weeks or even months for clinical indications to appear. By that time, the infection may have spread, necessitating to destroy the entire herd. Nanotechnology works on the same scale as a virus or a disease-infecting particle, and hence has the ability to detect and eradicate diseases very early. The use of nanotechnology devices for animal disease diagnosis or as animal models for human disease diagnosis is a significant achievement in one health effort (Tatli Seven *et al.*, 2018). Nanotechnology also aids in the creation of novel diagnostic instruments and therapies for veterinary animals. Metal nanoparticles have been employed in veterinary applications such as animal disease detection and treatment, as well as biological sensing. Several metal nanoparticles, such as zinc nanoparticles have been extensively used in animal lymphomas, cutaneous malignancies, transmissible venereal tumours and equine sarcomas for diagnostic and therapeutic purposes (Hassan *et al.*, 2020). Biosensors as an application for animal health management have emerged as a recent trend in global market (Şenel, 2020). As a result, nanotechnology has the potential to be an useful tool in delicate clinical diagnosis (Mohanty *et al.*, 2014).

CONCLUSION

Nanotechnology has provided progressive revolutionary advancements in animal health and productivity throughout the previous decades. Currently, several nanoparticles are being employed as nano antifungals, for disease diagnosis and therapy and as additives in animal feed. The number of studies employing nanotechnology in veterinary medicine is increasing and the results are promising. Several chemotherapy medications that were previously too toxic, unstable, or difficult to synthesise have been reformulated using nanotechnology and have entered clinical trials. Veterinary medicine is well positioned to benefit from these developments. However, the toxicity of nanomaterials must be assessed carefully prior to their use in any veterinary applications.

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Conflict of Interest

Authors have declared that no conflict of interest exist.

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