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LIPOSOME-BASED DRUG AND VACCINE DELIVERY SYSTEM IN VETERINARY APPLICATION: RECENT ADVANCEMENT AND FUTURE TRENDS – A REVIEW

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Abstract

Liposomal technology has become a significant advancement in the field of veterinary therapeutics, offering increased performance and safety in the delivery of treatments such as antibiotics, anti-inflammatories, and anti-cancer medications. With their superior stability, improved encapsulation of drugs, and more efficient transport mechanisms, nanoparticles are becoming a key tool for future uses in veterinary applications. This review article explores the latest developments and potential future applications of liposomes for delivering drugs and vaccines in veterinary medicine. Furthermore, we delve into the potential of liposomes for ground-breaking applications. These include delivering drugs precisely to specific tissues and organs, alongside the incorporation of immunomodulatory agents to boost vaccine effectiveness in veterinary medicine. It will emphasize the critical role of liposomes in the administration of vaccines and pharmaceuticals, as well as their potential as nanoscale carriers in veterinary applications. It will highlight the expanding significance of liposomes in veterinary medicine and delve into their potential as a foundational technology for advancing animal health management strategies.

Key words: liposomes, nanoparticles, drug delivery system, vaccine delivery system, veterinary applications

In the dynamic field of modern healthcare, the utilization of technology has emerged as a fundamental aspect of both human and veterinary medical practices. The veterinary pharmaceutical industry supplies a broad range of pharmacological agents for diverse types of farms, companions, and laboratory animals (Sadozai and Saeidi, 2013). The past several decades have witnessed a surge in the veterinary pharmaceutical industry. A desire to improve the well-being of companion and wild animals, coupled with the need for safer, more productive, and high-quality food sources are several factors that are contributing to the increase in demand in the industry (da Silva et al., 2020).

Veterinary medicine plays a crucial role in safeguarding the health of billions of livestock and poultry worldwide, with estimates suggesting over 3 billion livestock and 50 billion poultry rely on it (Anon, 2019). This industry's economic significance is substantial, with the global animal medicine market exceeding US$33.8 billion in 2018. Reflecting this trend, in the United States alone, antibiotic use in animals reached nearly 12 million kilograms (kg) in the same year. Further highlighting its growth trajectory, the market has experienced a significant increase from $33.8 billion in 2018 to $44.2 billion by 2020 (Scott et al., 2020). Veterinary medicines play a crucial role in animal healthcare, but they can also carry risks. Therefore, a thorough and responsible evaluation is essential before their market introduction. Additionally, a safe, controlled distribution system minimizes potential side effects and ensures animal well-being (Lúcia et al., 2020).

Recent years have seen significant progress in the implementation of nanotechnology in both human and veterinary medicine (Meena et al., 2018) and it also influences numerous therapeutic applications (Filipczak et al., 2020). Its application in medical contexts revolves around the utilization of nanoparticles as targeted delivery vehicles for drugs, heat, light, or other therapeutic agents, enabling precise interventions at the cellular level, particularly for cancer cells (Meena et al., 2018). It also holds significant promise for advancements in animal health, veterinary medicine, and various aspects of animal production (Youssef et al., 2019). Nanotechnology stands at the forefront of science and technology, focusing on the design and study of substances and materials at a nanometre scale, specifically within the range of 1 to 100 nm (Baig et al., 2021). At such a minuscule scale, materials exhibit novel properties and behaviors, allowing for the precise tailoring and application of these substances across various fields (Mahendra Kumar et al., 2023). By leveraging their unique properties and functionalities, diverse nanocarrier systems like liposomes, metallic nanoparticles, polymeric nanospheres, functionalized fullerenes, carbon nanotubes, dendrimers, polymer-coated nanocrystals, and nanoshells offer tailored approaches to address various challenges in animal health and production. These systems can act as targeted delivery vehicles, diagnostic tools, or biocompatible materials for improved animal health outcomes (Youssef et al., 2019).

A surge of research in recent years highlights the exciting potential of nanoparticles in combating various microorganism-induced diseases, including fungi (Alghuthaymi et al., 2021), bacteria (Soliman et al., 2019; Hu et al., 2020), and viruses (Ross et al., 2019). Nanoparticle's unique properties hold promise for targeted drug delivery, advanced diagnostic imaging, and novel therapeutic strategies. Notably, nanoscience overcomes biological limitations, paving the way for improved disease detection and diagnosis (Tatli Seven et al., 2018). Veterinary medicine has witnessed the exploration and development of diverse nanoparticles for treating both infectious and non-infectious diseases (Bai et al., 2018; Youssef et al., 2019; Chariou et al., 2020; da Silva et al., 2020). Their ability to modulate surface properties and co-deliver adjuvants effectively makes them compelling candidates for vaccine development (Zaheer et al., 2021).

Liposome-based drug delivery systems have received considerable attention for veterinary use over the past decade due to their versatility, ease of modification, and formulation. Their unique structure, featuring self-assembling lipid bilayers and an aqueous core, allows for diverse therapeutic encapsulation (Sadozai and Saeidi, 2013). The continued refinement of liposome technology has contributed to the growing utilization of liposome-based formulations, with a significant number of products currently advancing through various stages of clinical trials (Filipczak et al., 2020).

This review will highlight the most recent developments in liposomal drug and vaccine delivery for veterinary uses. It will emphasize the importance of liposomes in vaccine and drug application, challenges and opportunities for the industrial market, and future promise as nanoscale delivery tools in veterinary medicine. Additionally, we will explore how liposome research in veterinary species could potentially translate to advancements in human medicine. A review exploring recent progress in this area is essential given the multifaceted applications of liposomes in relation to veterinary applications.

Liposomes: an overview

Liposomes, discovered by Alec D. Bangham in 1965, have emerged as a prominent delivery system for therapeutic molecules in diverse disease treatments. Their biocompatibility and biodegradability stem from their primary composition: lipids and fatty acids, mirroring the natural constituents of cell membranes (Large et al., 2021). This liposome is spherical phospholipid vesicles ranging from 5 to 200 nm in diameter and with a membrane thickness of approximately 4–5 nm (Andra et al., 2022), which can encapsulate both hydrophilic and lipophilic drugs. This encapsulation occurs within the aqueous phase or bilayer membrane due to the affinity between the drug and specific vesicle components (Li et al., 2019). This versatility has made liposomes a valuable tool in pharmaceutical industries (Ahmadi Ashtiani et al., 2016).

Liposomes are constructed from amphiphilic molecules, each possessing a hydrophilic head and two hydrophobic tails (Li et al., 2019; Andra et al., 2022), derived from phospholipids as the major components, which are stabilized by cholesterol (Sriwidodo et al., 2022). Cholesterol is commonly used to stabilize the liposome structure by integrating into the phospholipid bilayer and filling in any gaps (van der Koog et al., 2022), which is necessary for bilayer consolidation, phospholipid molecules packaging enhancement, controlling drug retention and reducing the bilayer permeability (Handali et al., 2018). Due to their amphiphilic nature, consisting of both hydrophilic and hydrophobic regions, and their extended aliphatic chains that promote self-assembly, liposomes readily form membranes in aqueous environments (Hu et al., 2021). These interactions, influenced by factors like concentration, temperature, and geometry, lead to the formation of various structures, including spherical, polyhedral, and tubular vesicles, primarily through van der Waals forces and hydrogen bonds (Frolov et al., 2011).

A diverse range of phospholipids can be employed in liposome construction, with the specific choice significantly impacting the desired therapeutic outcome (Moghimipour et al., 2018). Liposome shape and stability are critically influenced by the selected phospholipids, as different combinations adopt distinct conformations. Liposomes can be tailored for various functions by designing them with specific sizes, shapes, and even multilayered structures (Tseu and Kamaruzaman, 2023), in-

fluencing their stability both in living organisms (*in vivo*) and in laboratory settings (*in vitro*) (Yan et al., 2020).

Liposomes emerge as compelling drug carriers due to their biomimetic membrane structure, excellent biocompatibility, minimal immune response, and capability to shield drugs from degradation. These features extend the drug's half-life by prolonging its circulation and enhancing efficacy with reduced toxicity (Abbasi et al., 2023). Furthermore, traditional lipid components can be structurally and superficially modified to create novel liposomes with targeted biological effects, expanding their applications in biomedicine and fostering the development of improved delivery systems (Li et al., 2019). Due to their bilayer morphology, close resemblance to cell membrane and their versatility in encapsulating various substances, liposomes have emerged as a highly attractive drug delivery system. This has spurred extensive research over the past six decades with liposomes continuing to be a subject of intense investigation. They are widely recognized as the most effective drug carrier system to date (Selim et al., 2017; Mathiyazhakan et al., 2018), valued for their unique biological and technological advantages in both *in vitro* and *in vivo* delivery of therapeutic agents. Remarkably, the past two decades have witnessed significant advancements, with several liposome-based biomedical applications on the verge of clinical use or market entry (Bozzuto and Molinari, 2015).

These aforementioned characteristics highlight the potential of liposomes in various veterinary applications, as further explored throughout this review article. They emerge as effective delivery vehicles for various therapeutic agents, including drugs, vaccines, pain medications, and also cancer treatments.

Recent advancement of liposomes in veterinary application

Recent advancements in liposome technology have led to significant improvements in veterinary applications. Their unique ability to encapsulate and deliver drugs offers exciting possibilities for targeted therapy in animals.

A key advantage of liposomes over other colloidal delivery systems lies in their tunability. Liposomal structure and physicochemical properties can be actively modified, enabling targeted delivery to specific sites within an organism (Guimarães et al., 2021). This level of control offers significant advantages in therapeutic applications. The main four generations of liposomal systems are classified by their functions and compositions; conventional, stimulus-responsive or stealth, ligand-targeted, and multifunctional liposomes (Sogut et al., 2021), which are shown in Figure 1. The key characteristics, advantages, disadvantages, and specific veterinary applications for conventional, stimulus-responsive, stealth, ligand-targeted, and multifunctional liposomal delivery systems were highlighted in Table 1.

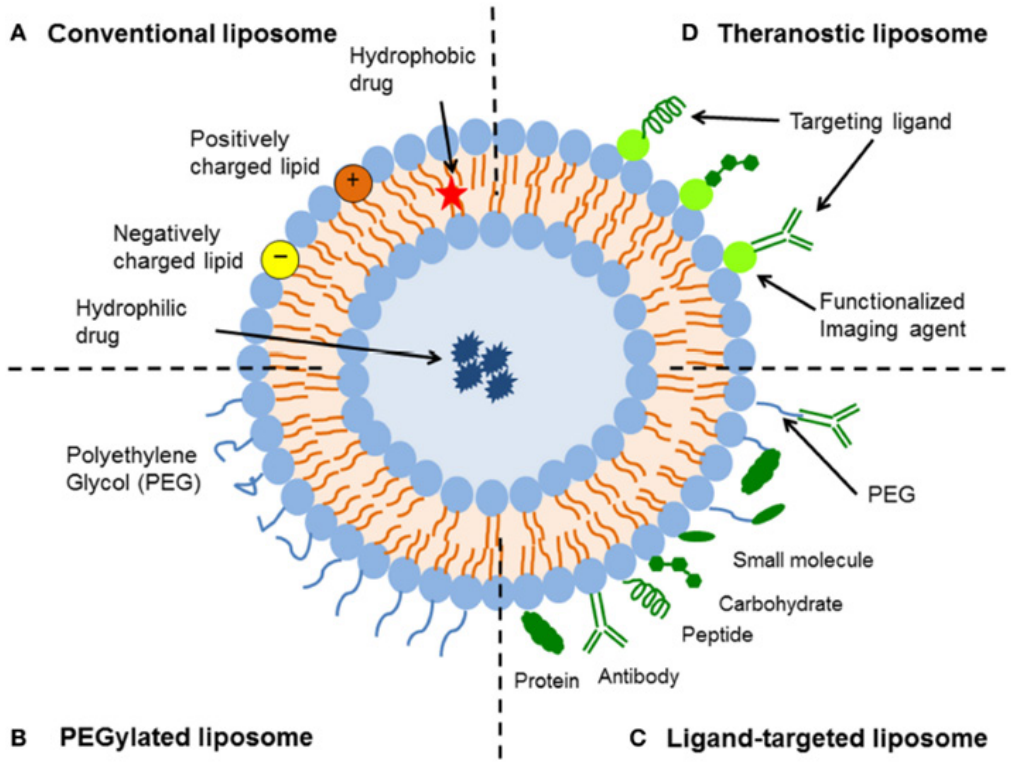


Figure 1. Schematic representation of the different types of liposomal drug delivery systems (Sercombe et al., 2015)

Table 1. Characteristics, advantages, disadvantages, and specific veterinary applications for conventional, stimulus-responsive, stealth, ligand-targeted, and multifunctional liposomal delivery systems

Type of liposome	Description	Advantages	Disadvantages
Conventional liposome	First-generation liposomes for drug delivery, composed of neutral, cationic, or anionic phospholipids without surface modification, often supplemented with cholesterol for enhanced bilayer stability (Bulbake et al., 2017)	Enhance drug delivery and decrease toxicity (Sercombe et al., 2015)	Rapid clearance from the bloodstream, instability in the plasma leading to a brief blood circulation half-life (Guimarães et al., 2021), degradation, and coalescence (Olusanya et al., 2018). Often identified as alien particles by the opsonins, leading to their subsequent destruction by the mononuclear phagocyte system's (MPS) phagocytes (Riaz et al., 2018)
Stimulus-responsive liposome	Liposomes are designed to release their contents in response to specific stimuli such as pH, temperature, or enzymes (Pham et al., 2020; Castelli et al., 2014)	Targeted release contents in response to specific triggers, hence can reduce the side effects and enhance efficacy (Paul et al., 2023)	Complex formulation which can elevate the manufacturing cost. Non-specific release of drug risk if the stimuli are not precisely controlled leads to a reduction in therapeutic efficacy and causes unwanted side effects (Saravanakumar et al., 2019)
Stealth liposome	Second generation of liposomes incorporates a surface modification strategy by applying a coating of hydrophobic polymer conjugates like polyethylene glycol (PEG) on the surface (Kang et al., 2017)	Enhances blood circulation which leads to reduced immunogenicity, decreased macrophage uptake, improved stability in circulation, extended blood half-life, and ultimately, reduced systemic toxicity (Hatakeyama et al., 2013; Madni et al., 2014; Sogut et al., 2021)	Extensive body biodistribution, which hampers selective delivery to specific target cells (Saraswat and Maher, 2020)
Ligand-targeted liposome	Third-generation of liposomes conjugated with specific ligands (antibodies, peptides, small molecules) that target specific cells or tissues (Le et al., 2019; Fathi and Oyelere, 2016)	Enhances targeted delivery to various cell types and tissues, enhances uptake by target cells (Islam Shishir et al., 2019)	High complexity and production cost. The attachment of ligands has the potential to provoke an immune response that can reduce the effectiveness of liposomes (Robson et al., 2018).
Multifunctional liposome	Fourth-generation liposomes are engineered to have multiple functionalities (e.g., targeting ligands, imaging agents, and stimuli-responsive elements) through surface modification techniques (Riaz et al., 2018)	Versatile, can combine targeting, controlled release, and imaging capabilities, hence improving the systematic therapeutic effects (Cheng et al., 2022)	High cost due to complex formulation and also production (van der Meel et al., 2013). May have a higher risk of toxicity due to the combination of different functional components (Sercombe et al., 2015)

Application in veterinary medicine

Recent advancements have seen liposomes utilized in various veterinary therapeutic areas. Liposome-based delivery systems have emerged as a promising approach in veterinary medicine, demonstrating improved therapeutic efficacy and safety for antibiotics, anti-inflammatory drugs, and antiparasitic agents. Furthermore, their ability to encapsulate vaccines enhances immunogenicity and facilitates targeted delivery to immune cells. Among various existing nanocarriers, liposomes stand out as well-studied and widely used for targeted drug delivery (Liu et al., 2021). Research and development of liposome-based delivery systems for veterinary applications have witnessed a significant surge over the past decade since liposomes offer significant advantages as discussed before (Liu et al., 2022).

Liposomes in drug delivery system

Liposome-based cancer therapeutics

Cancer remains the primary cause of mortality among domestic animals, despite the availability of several approved veterinary anti-cancer medications (Biller et al., 2016). Modern veterinary oncology offers a spectrum of treatment options including surgical procedures (Boston and Henderson, 2014), radiation therapy (Meier et al., 2019), conventional and targeted chemotherapy (Kent, 2013; Turner et al., 2017), and electrochemotherapy (Dos Anjos et al., 2019), or in combination with the treatment to achieve optimal outcomes. Despite the wide use of anti-tumor drugs in modern cancer therapy (Yao et al., 2020), the *in vivo* effectiveness remains significantly hampered by several limitations. These limitations include the drugs' inability to specifically target cancer cells, insufficient drug availability at the tumor site, extensive drug distribution throughout the body, and harmful effects on healthy, non-dividing tissues (Gyanani et al., 2021; Sapino et al., 2022). For example, chemotherapy should always be suggested before surgery to reduce the tumor's size, after surgery to prevent the disease relapse, or as palliative care. While chemotherapy is a common treatment for various cancers, it is often plagued by side effects and rendered ineffective due to multidrug resistance (MDR) (Zabielska-Koczywąs and Lechowski, 2017) and low specificity for neoplastic cells (de Faria Lainetti et al., 2020). In addition, the management of highly recurrent cancers like feline injection-site sarcomas (FISS) remains a critical hurdle for veterinary oncologists, with recurrent rates ranging from 14% to 69% (Zabielska-Koczywąs et al., 2017).

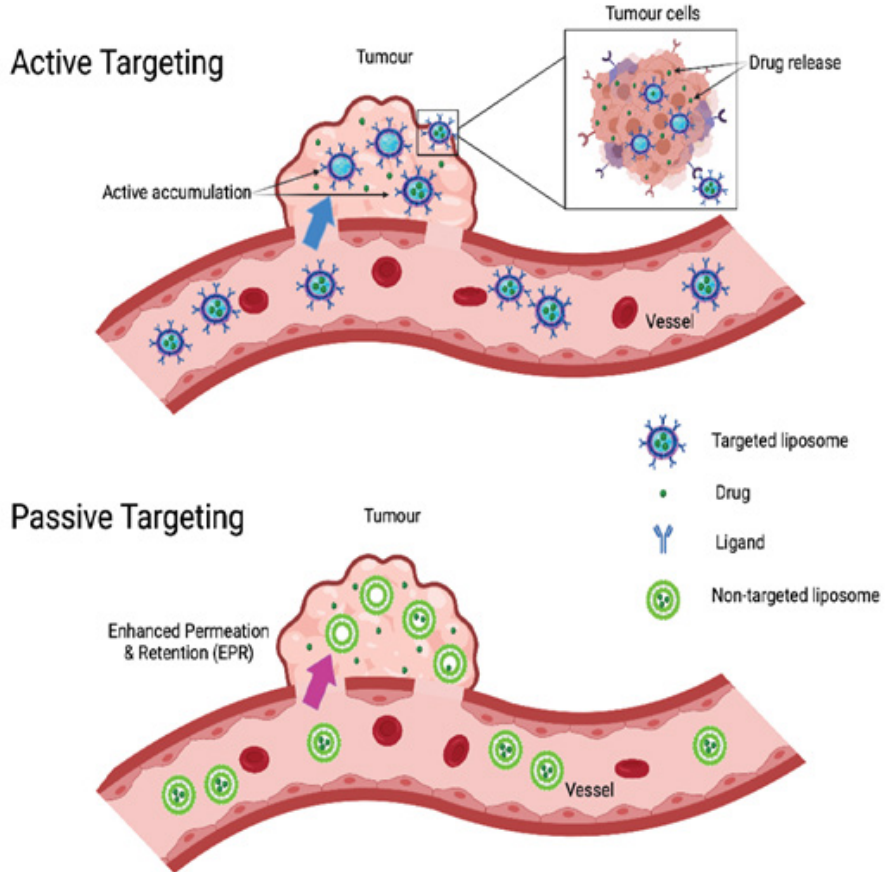


Figure 2. Schematic representation of the mechanism of active targeting and passive targeting (Agarwal, 2022)

Table 2. Liposome use in the delivery of anti-tumour agents in the veterinary field

Anti-tumor agent	Type of cancer	Target species	Mechanism	Outcome	Reference
Doxorubicin	Brain tumor	Canine	Temperature-sensitive liposome-encapsulated doxorubicin with localized hyperthermia triggers the release of the drug specifically at the brain tumor site	Temperature-sensitive liposome-encapsulated doxorubicin with localized hyperthermia has the potential to be a safe and effective method for delivering therapeutic doxorubicin doses to brain tumors in dogs, which reduces systemic toxicity and enhances tumor response	(Bredlau et al., 2018)
Curcumin	Canine cancer (primary and metastatic neoplasia within the lung)	Canine	Liposome encapsulation increases the bioavailability and stability of curcumin	*In vitro*: Liposome-encapsulated curcumin inhibited the viability of canine osteosarcoma, melanoma, and mammary carcinoma cell lines more effectively than free curcumin. *In vivo*: The pilot trial showed that liposome-encapsulated curcumin was well-tolerated by the dogs and led to a reduction in tumor size in dogs, with minimal side effects.	(Withers et al., 2018)
Panobinostat-loaded folate	Canine B-cell lymphoma	Canine	Both targeted and non-targeted liposomes induced cancer cell death through apoptosis and histone acetylation, a mechanism linked to tumor suppression	Panobinostat-loaded folate-targeted liposomes offer an effective and safer treatment option for canine B-cell lymphoma. Further preclinical investigations using canine B-cell lymphoma xenograft murine models are necessary to assess its efficacy and safety *in vivo* before advancing to clinical trials in canine patients. In addition, since this study was conducted in cell lines and mice, more research in dogs is required to confirm the effectiveness in the real world	(André et al., 2023)

A comprehensive understanding of the fundamental aspects of nanoparticle-based cancer therapy is well-documented in the literature. Liposomes as a drug delivery system offer a promising solution to address the limitations of current cancer treatments in animals. Their unique properties, including efficient cellular uptake, high drug encapsulation capacity, and small size for targeted tumor delivery, enable them to overcome various challenges associated with conventional therapies (Dessale et al., 2022; Verma et al., 2023). Furthermore, liposomes can take advantage by accumulating within the tumor due to the unique properties of the tumor tissues such as leaky microvessels and severely compromised lymphatic drainage (Subhan et al., 2021; Chehelgerdi et al., 2023). Upon being administered, liposomal drugs travel via the bloodstream to reach and accumulate at the site of pathological lesions. The mechanism of action in these drugs affects tumors, begins with their accumulation at the specific location, their absorption by the tumor cells, and the subsequent release of the free drugs. Liposomes essentially have two methods of accessing the tumor which are passive and active targeting. Passive targeting utilizes the unique features of tumor tissues, such as leaky blood vessels and the enhanced permeability and retention (EPR) effect, to accumulate liposomes within the tumor without requiring specific targeting ligands (Deshpande et al., 2013; Kumari et al., 2016). In contrast, active targeting involves attaching ligands to the liposomal surface, allowing them to interact with specific receptors overexpressed on tumor cells (Petros and DeSimone, 2010). It is generally intended to minimize the negative impacts of liposomal nanoparticles on healthy cells and also non-target tissues (Deshpande et al., 2013). The mechanism of action for active and passive targeting is presented in Figure 2.

Veterinary oncology has witnessed the use of liposomes as a delivery system for the administration of chemotherapeutic drugs such as doxorubicin (DOX), cytokines like interleukin 2 (IL-2), small molecule inhibitors, and immune active reagents like muramyl tripeptide phosphatidyl ethanolamine (MTP) (Lin et al., 2015). Doxil® is the first PEGylated liposomal encapsulated with DOX, for pharmaceutical application (Barenholz, 2012), which is currently used to treat various human cancers (Kumari et al., 2016) and has also been studied in veterinary oncology (Barenholz, 2012). Although the utilization of liposomes in animals has received less attention compared to humans, there is ample evidence suggesting that they can enhance the delivery of veterinary anti-tumor agents (Table 2).

Liposome-based drug delivery holds immense promise for revolutionizing veterinary oncology. By overcoming the limitations of conventional cancer therapies through enhanced drug delivery, targeted tumor cell action, and potential reductions in side effects, liposomes pave the way for improved treatment options for our beloved animals. However, continued research and development efforts, specifically addressing existing species-

specific challenges and conducting larger-scale clinical trials, are crucial to unlocking the full potential of liposomal therapies and optimizing their application for effective veterinary cancer management.

Liposome-based analgesic therapeutics

Treatment for acute and chronic pain is an important part of veterinary medicine for laboratory animals, pets, and livestock. Veterinarians prioritize pain management, making it an essential part of care, not just an added service. This aligns with their ethical duty to alleviate animal suffering and contributes significantly to successful treatment and stronger relationships between veterinarians, pet owners, and their animals (Epstein et al., 2015). Chronic pain is a significant cause of reduced well-being in animals and often stems from prevalent conditions like osteoarthritis, intervertebral disc disease, and cancer (Lopez and Schachner, 2015; Riley et al., 2021). While non-steroidal anti-inflammatory drugs (NSAIDs) are frequently used for managing chronic pain in animals (Muller et al., 2018), their efficacy can be limited, and they may pose potential side effects (Belshaw et al., 2016). For managing acute pain in small animals, opioids are highly used and effective (Epstein et al., 2015), but they may cause negative side effects like dysphoria (Becker et al., 2013), nausea, regurgitation, and also vomiting (Torrente et al., 2017).

Recent advances in veterinary medicine introduce liposome-based analgesics, offering a novel and promising approach to pain management. These microscopic carriers deliver medication directly to the source of pain in animals, potentially leading to stronger pain relief and fewer side effects compared to traditional methods. As research progresses, liposomal analgesics hold the potential to revolutionize veterinary pain management, significantly improving animal comfort and well-being. As early as 1997, a rat study on the wound infiltration potential of liposomal bupivacaine showed liposomes could function as depot formulations for analgesics. Since then, the pharmacokinetics and pharmacodynamics of liposomes as delivery systems for analgesic drugs in numerous veterinary species have been studied. Modern technology in recent years has allowed liposomes to incorporate a wide variety of analgesic drugs (Sadozai and Saeidi, 2013), which give advantages including low toxicity to the central nervous systems and cardiovascular systems, lower plasma concentrations, sustained release, and longer duration of action (Hua and Wu, 2013).

In 2016, Nicota® (liposomal formulation encapsulated with bupivacaine) was approved for use in dogs (FDA, 2016), and in 2018, it was approved for use in cats (FDA, 2018), which provides 72 hours of pain relief by slowing the release of local anesthetic prior to surgery (Martin et al., 2022). The use of dogs was initially labeled for cranial cruciate ligament surgery, while cats were labeled for forelimb onychectomy, both as part of a four-point forelimb block for declawing. For tissue closure in dogs, the 13.3 mg/mL veterinary formulation is injected into the tissue layers at a dose of 5.3 mg/kg (0.4 mL/kg) per injection site. Cats receive the same dose as dogs per forelimb, for a total dose of 10.6 mg/kg (0.8 mL/kg). For tissue closure in dogs, the 13.3 mg/mL veterinary formulation is injected into the tissue layers at a dose of 5.3 mg/kg (0.4 mL/kg) per injection site. Cats receive the same dose as dogs per forelimb, for a total dose of 10.6 mg/kg (0.8 mL/kg) (Bailey, 2022). Beyond its approved purpose, bupivacaine liposome injectable suspension (BLIS) finds widespread off-label use in surgeries like amputations and tumor removals for both dogs and cats. Notably, its analgesic effect necessitates post-incision tissue infiltration, differing from standard bupivacaine which can be administered pre-operatively due to its conventional formulation, as its liposome structure is susceptible to disruption earlier in the process. BLIS also is frequently employed off-label for a multitude of such blocks (dental/oral, auriculopalpebral, etc.) in both cats and dogs. Pre-emptive injection of BLIS for these blocks is feasible due to the remote administration of its liposomes, thereby avoiding disruption by the surgical incision or tissue debridement. However, the large size of these liposomes limits the release of the pain medication locally, rendering BLIS less suited for blocks requiring the local anesthetic to migrate to distant tissues or where the drug is dispersed throughout a tissue rather than delivered to a specific site for localized liposomal bupivacaine release (Grubb and Lobprise, 2020).

Martin et al. (2022) found that a single injection of bupivacaine liposome as a cornual nerve block matched the pain-relieving efficacy of a combined lidocaine and meloxicam regimen during and after dehorning of the calves. This simpler approach offers an attractive alternative for minimizing pain in livestock, addressing both perioperative and postoperative discomfort (Martin et al., 2022). A pilot study with 46 dogs compared BLIS tissue infiltration to saline in dogs undergoing CCL surgery. Results indicated that BLIS provided significantly superior pain relief over 72 hours (Lascelles et al., 2016). A more recent study showed BLIS efficacy against other medications in dogs undergoing tibial plateau leveling osteotomy (another type of leg surgery). Utilizing subjective pain scales, pressure sensitivity test (measuring pain sensitivity), and opioids used after surgery (giving additional pain medication if needed), demonstrated that BLIS significantly reduced the requirement for additional pain medication compared to other drugs, highlighting its superior efficacy in managing postoperative pain (Reader et al., 2020). However, research on BLIS in cats is limited. A single noninferiority study evaluated BLIS compared to 0.5% bupivacaine hydrochloric acid (HCl) administered as incisional blocks followed by daily nonsteroidal anti-inflammatory drug (NSAID) injections in cats undergoing ovariohysterectomy. The level of postoperative pain was recorded repeatedly for up to 42 hours and the results were compared across the two groups. The findings suggested that BLIS performed on par with bupivacaine. This was indicated by

the observation that most pain assessments resulted in a median score of 0, indicating minimal discomfort. Moreover, average pain scores were considerably low (less than 0.5 out of a possible score of 20) with negligible differences between the groups, slightly in favor of BLIS. These findings raise questions regarding the validity of the pain assessment protocol employed and whether the level of postoperative pain experienced by the participants was sufficient to adequately test the noninferiority hypothesis. Further research with larger sample sizes and refined pain assessment methodologies is warranted to definitively establish the efficacy of BLIS in feline postoperative analgesia (Gordon-Evans et al., 2019).

Subcutaneous administration of liposomal cannabidiol provided sustained pain relief in a dog with a chronic pain case report. This promising therapy demonstrates high drug exposure while maintaining lower plasma concentrations over an extended period (28 days), where this formulation presents a potential alternative to oral cannabidiol administration. However, limitations should be acknowledged, including the fact that this report involved only one subject, and further investigation is necessary to corroborate its efficacy and safety profile in broader groups. While cannabis products show potential for pain management in animals, their legal restrictions in many countries hinder their widespread use in veterinary medicine (Shilo-Benjamini et al., 2022).

On the other hand, liposomal encapsulation with buprenorphine has been studied and it could make the drug's effect last longer and potentially improve pain management in cats. Buprenorphine is a medication often used for pain relief, and in this case, it is being studied for potential use in perioperative and chronic pain management in cats. This research presents a stepping stone for further studies to refine the drug formulation and eventually conduct a multicenter efficacy study (Johnson et al., 2017). While research has largely focused on trapping opioids within liposomes for pain relief, studies have also explored the potential of encapsulating nonsteroidal anti-inflammatory drugs (NSAIDs) within these delivery systems. A cream containing 1% diclofenac sodium encapsulated in liposomes has been developed for skin application in horses. This formulation was designed to be easy to apply and to limit systemic side effects, which are typically seen with injectable forms of diclofenac sodium. The liposomal design allows the drug to quickly penetrate the skin and underlying tissues, with the highest concentration of the drug being reached approximately 18 hours after application. In a research experiment involving inflammation in the horses' necks induced by carrageenan, the topical use of this 1% diclofenac sodium liposomal cream reduced a key inflammatory marker (prostaglandin E2) in the fluid that had leaked from capillaries, compared to the horses that were not treated. In addition to reducing lameness, this cream, when applied topically, reportedly caused less hardening of the radial carpal bone and fewer visible erosions of cartilage in horses, compared to the effects seen with phenylbutazone given orally (Levine et al., 2009).

The performance of liposomes in both systemic and topical applications justifies further investigation, underscoring their ongoing clinical relevance in managing pain in the field of veterinary medicine. Biomedical research greatly benefits from the dual utility of controlled-release formulations for pain-relieving drugs, as they facilitate effective pain control in a range of domestic and exotic animal species. This, in turn, provides an opportunity to use these species as models for human nanomedicine studies.

Liposome-based antimicrobial therapeutics

Veterinary antibiotics are the most widely used drugs in animal medicine, which are crucial for treating diseases, preventing infections, and promoting animal growth. While these drugs benefit animal farming and food production, their overuse has led to severe consequences. This includes increased treatment failures, the rise of antibiotic-resistant bacteria, as well as adverse effects on livestock health (Luo et al., 2019). The global threat of rising antibiotic resistance, acknowledged by organizations like the UN and WHO, demands innovative solutions. As developing new antibiotics takes time, research is crucial to improve existing medications (Hassan et al., 2020).

This highlights the growing significance of knowledge-based nanoparticles in veterinary medicine for treating infectious diseases, potentially enhancing effectiveness and reducing side effects (Hassan et al., 2020). Liposomes offer several advantages for antibiotic delivery. They protect the medication from premature breakdown by enzymes and the immune system, while precisely delivering it to the site of infection in a controlled and sustained manner. This minimizes distribution to healthy tissues, potentially reducing side effects (Bai et al., 2018). Additionally, liposomes may directly interact or merge with bacterial cell walls, further enhancing the effectiveness of the encapsulated antibiotic (Gonzalez Gomez and Hosseinidoust, 2020).

Liposome-encapsulated antibiotics hold promise in overcoming some bacterial resistance mechanisms such as impermeable outer membranes, efflux processes, and enzymatic degradation, thus improving overall antibiotic effectiveness (Ferreira et al., 2021), as shown in Figure 3. Studies show that liposomes enhance antibiotic stability and safety, allowing for longer circulation in the bloodstream and targeted delivery to infection sites (Eleraky et al., 2020). The interaction between liposomes and bacterial cells involves several fundamental mechanisms, namely adsorption, fusion, lipid exchange, and endocytosis as shown in Figure 4. This selective delivery increases the speed of antibiotic uptake by target cells, leading to improved therapeutic efficacy and reduced side effects. Consequently, fewer antibiotic doses may be

needed, further minimizing potential toxicity (Sercombe et al., 2015; Nsairat et al., 2022). Liposomes also offer a promising strategy to combat biofilms. These microscopic structures can deliver high doses of antibiotics directly to the site of infection. Biofilms are notoriously difficult to treat due to their complex structure and ability to withstand much higher antibiotic concentrations (approximately 1000-fold) compared to planktonic bacteria (Rukavina and Vanić, 2016; Algburi et al., 2017). Table 3 provides a summary of experimental studies exploring liposomal use in delivering antimicrobial agents for veterinary applications.

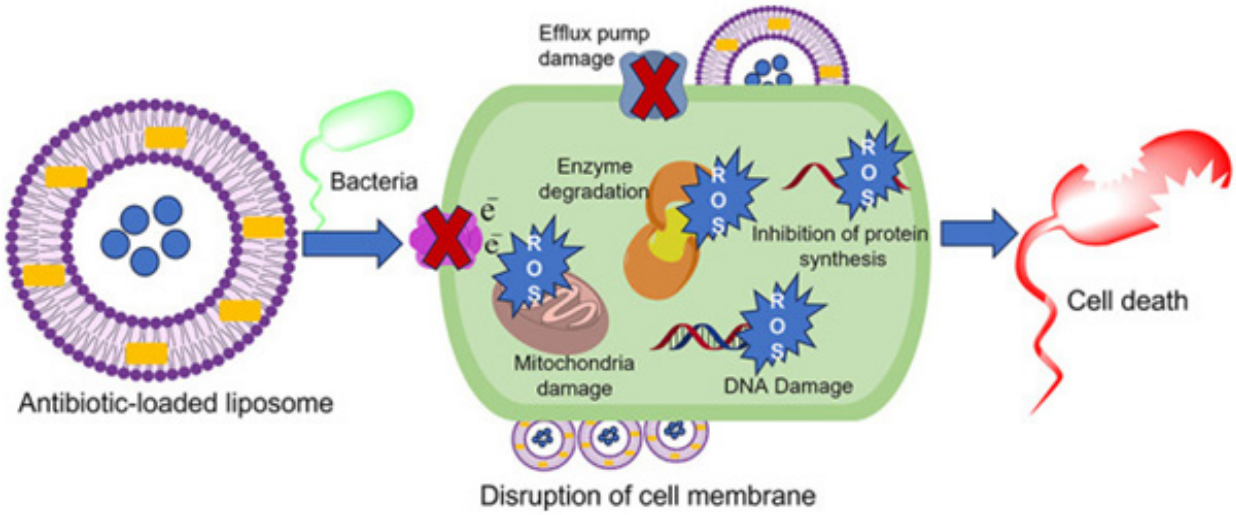


Figure 3. Mechanism action of liposome-encapsulated antibiotics. They work by disrupting the bacterial cell wall and membrane, leading to the overproduction of reactive oxygen species (ROS) that induce oxidative stress and damage crucial intracellular macromolecules. They also alter membrane permeability, damage mitochondria, and DNA, inhibit protein synthesis, and degradation of enzymes that lead to cell death (Ghosh and De, 2023)

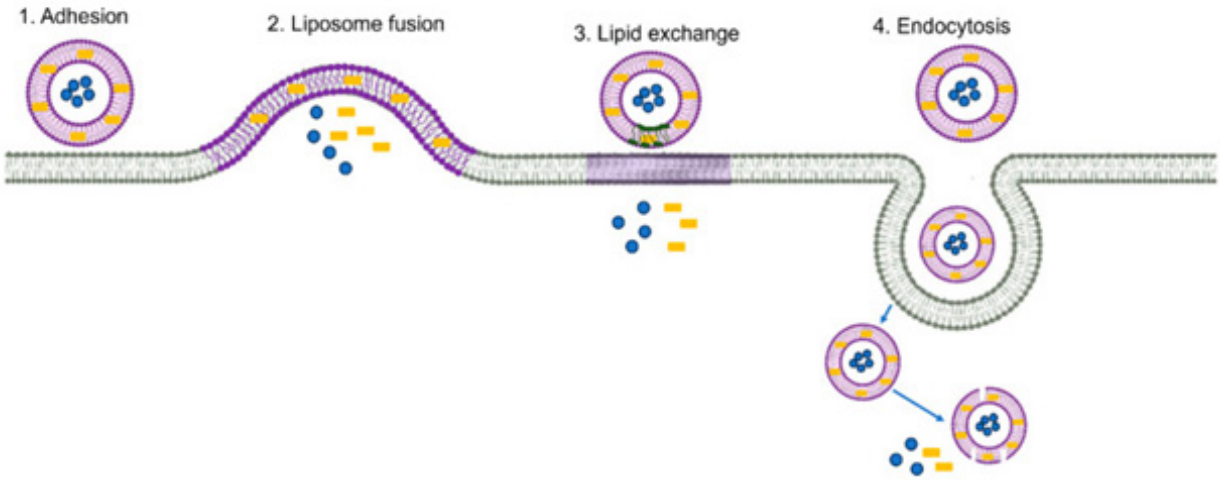


Figure 4. The mechanisms of antibacterial resistance involving antibiotic-loaded liposomes are visually represented schematically. The interaction between liposomes and the lipid bilayer of eukaryotic cells can occur through adhesion, where electrostatic forces attract the liposomes toward the cell membrane, leading to the eventual release of the encapsulated cargo. Additionally, fusion can occur, resulting in the fusion of lipids present in the liposome with those in the cell membrane. Furthermore, lipid exchange occurs as the lipids from both the liposome and the cell membrane undergo a transfer exchange between them (Ghosh and De, 2023)

Table 3. Liposome use in the delivery of antimicrobial agents in the veterinary field

Liposomal drug/agent	Target species	Targeted infection	Mechanism	Outcome	Reference
Liposomal gentamicin	Quarter Horse foals	Pneumonia caused by *Rhodococcus equi*		Intravenous liposomal gentamicin showed an effective treatment for *Rhodococcus equi* pneumonia. However, the nephrotoxicity result indicates that an alternative dosing or route is needed before the liposomal gentamicin is considered safe for clinical use	(Cohen et al., 2016)
PEGylated liposome encapsulated meglumine antimoniate	Canine	Canine visceral leishmaniasis (CVL)		Mixed liposomal formulation (conventional+PEGylated liposome+allopurinol containing meglumine antimoniate) showed a significantly greater parasite load reduction in the liver, spleen, and bone marrow compared to other groups, especially after four months of treatment	(dos Santos et al., 2020)
Vancomycin	Beagle dog	Septicemia	Targeted delivery to the bloodstream	Cell-penetrating peptides-liposome encapsulated vancomycin is more effective in delivering the drugs to the bloodstream and helps to overcome the intestinal barrier and deliver the drug more efficiently.	(Uhl et al., 2023)

Antimicrobial therapeutics based on liposomes are emerging as a significant strategy to combat the ever-increasing issue of drug-resistant infections in the field of veterinary science. The evolution of liposome technologies, along with deeper insights into their mechanisms of action against pathogens, provides an opportunity to transform the approach to treating animal diseases caused by infectious agents. Continued research and the execution of clinical trials stand as critical steps for optimizing the use of liposomal delivery systems in veterinary medicine and confirming their safety and effectiveness as innovative therapeutic tools.

Liposome-based vaccine delivery system

Veterinary vaccines offer a powerful tool to prevent a staggering number of diseases in animals (Knight-Jones et al., 2014), which significantly reduces the disease burden in these populations (Hussain, 2019; DeFrancesco, 2020). They can even eliminate or control major animal diseases when combined with other strategies like quarantine, improved hygiene, and better animal care. The focus of vaccination varies depending on the animal group. Livestock vaccinations primarily aim to boost overall production for farmers, with cost-effectiveness being a key factor. In wildlife, vaccinations target diseases transmissible to humans (zoonoses), with a growing emphasis on animal welfare. For zoonotic and foodborne infections, vaccinations aim to minimize or eradicate risks to consumers, while sometimes also improving individual animal productivity (Meeusen et al., 2007).

Vaccines come in two main types: prophylactic and therapeutic. Prophylactic vaccines, administered before disease onset, prepare the immune system to recognize and combat potential pathogens. Therapeutic vaccines, on the other hand, aim to bolster the immune response after an infection has already occurred, with a particular focus on tumors. Prophylactic vaccines work by delivering antigens to antigen-presenting cells (APCs), such as dendritic cells (DCs). These APCs then process the antigens and migrate to lymph nodes, where they present them to B cells, $CD4^+$ T cells, and $CD8^+$ T cells, priming the immune system to recognize and combat potential invaders. Therapeutic vaccines, particularly those targeting tumors, focus on enhancing the delivery of tumor antigens and stimulating cellular immunity. Adjuvants can be strategically injected at the tumor site to attract DCs. As DCs mature, they process and present tumor antigens to $CD4^+$ and $CD8^+$ T cells. Subsequently, these activated T cells and natural killer (NK) cells proliferate to identify and eliminate cancer cells at the primary tumor, metastatic sites, and potentially even distant locations within the body (Zhu et al., 2014).

Recent advancements highlight liposomal derivatives, including liposomal-polymer derivatives, positively charged (cationic) liposomes, liposome-protein complexes, and primary lipoplexes as promising tools for vaccine delivery and adjuvant development. These carriers offer several advantages over traditional methods. Unlike non-functional nanocarriers and conventional protein carriers, liposomes can transform weakly immunogenic substances into potent immunogens as they demonstrate a superior ability to stim-

ulate the immune systems (Gao et al., 2017). Additionally, their surface charge, size, and composition can be readily tailored to suit specific antigens. They also promote efficient antigen presentation by dendritic cells, crucial immune system players. These versatile carriers leverage their chemical properties to encapsulate various water-soluble antigens like proteins, peptides, nucleic acids, and sugars within their core. Additionally, lipophilic components such as antigens, adjuvants, and linker molecules can be integrated into the liposomal membrane itself (Schwendener, 2014). Alternatively, antigens can be attached to the liposome surface through electrostatic interactions or by incorporating acyl chains (Giddam et al., 2012) as in Figure 5.

The potential of liposome-based vaccines to prevent various diseases in animals has driven significant research efforts in recent decades. This surge in interest is fueled by the unique properties of liposomes, particularly their ability to deliver antigens precisely and enhance immune response (Sadozai and Saeidi, 2013). Table 4 provides a summary of experimental studies exploring liposomal vaccines for veterinary applications.

Challenges for the industrial market

Nowadays, the world's growing number of pets and farm animals creates a demand for new veterinary drugs that not only enhance the health of animals but also simplify the administration process, attenuating discomfort and potential side effects for the animals (Carvalho et al., 2020). Concurrently, there's also a focus on keeping drug residues out of meat and other products in the food industry to safeguard consumer health and align with the goals of veterinary drug companies (Hill and Li, 2017). In the realm of veterinary medicine, the majority of therapeutics in use are originally formulated for humans, a practice that often overlooks the distinct anatomical, physiological, and metabolic variations across animal species. Thus, the synthesis of novel veterinary-specific pharmaceuticals is paramount to ensure the well-being of these animals, presenting a potentially profitable venture for research laboratories. Hence, nanotechnology and nanomedicines are promising tools for this, allowing for the development of safe and powerful medications (El-Sayed and Kamel, 2020).

The development pathway of nanomedicines such as liposome, however, is accompanied by intricate cost-management considerations, as nanocarrier-based therapeutics are associated with a higher intrinsic value when compared with traditional medications.

A challenge arises as the functionality of the liposomal delivery system becomes more complex, especially when incorporating surface modification through coating and/or ligands. The integration of multiple components into a single nanosized carrier necessitates multiple chemical synthesis steps and formulation processes (Tinkle et al., 2014). The liposome manufacturing process involves complex techniques such as high-pressure homogenization, extrusion, and lyophilization, which are cost-intensive and require specialized equipment and also expertise (Karunakaran et al., 2023). Consequently, these processes pose problems for large-scale good manufacturing (cGMP) production, leading to increased production costs and making the evaluation of such products more challenging (Tinkle et al., 2014). The transition from small-scale production to mass manufacturing of nanomedicines entails substantial financial investment, with the scaling and manufacturing processes being particularly complex, time-intensive, and financially demanding (Ventola et al., 2017; Farjadian et al., 2019).

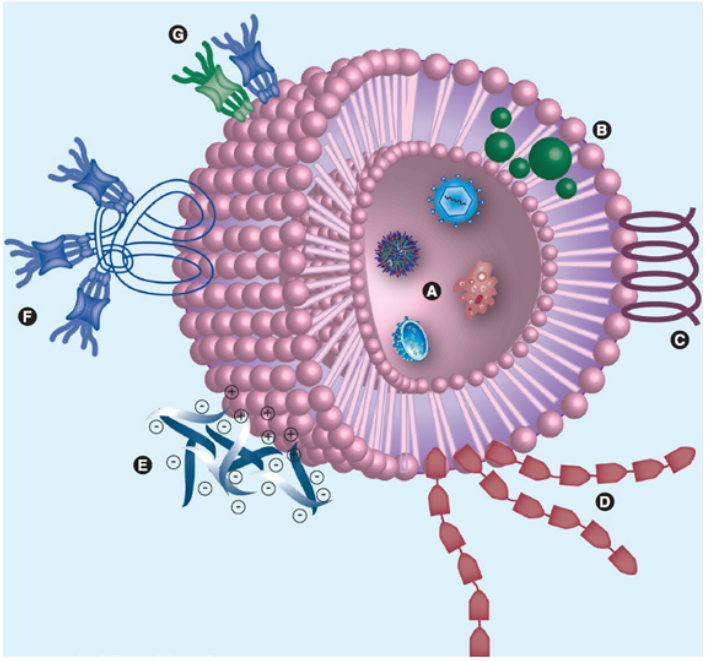


Figure 5. Different ways of connecting liposomes with antigens include: (A) Encasing live-attenuated/inactivated parasites, microorganisms, or soluble antigens within the hydrophilic center. (B) Trapping them inside the liposomal bilayer. (C) Adsorbing them onto the membrane surface. (D) Attaching antigenic peptides to the liposomal membrane through acyl chain modifications. (E) Forming electrostatic complexes between negatively charged antigens and positively charged liposomal membranes. (F) Linking immunostimulatory ligands using a polymer. (G) Directly coupling immunostimulatory ligands to the surface (Giddam et al., 2012)

Table 4. Liposomal-based vaccines in the veterinary application

Type of liposome	Antigen vaccine	Vaccine target	Animal species application	Outcome	Reference
Liposomal Foot-and-Mouth Disease vaccine (lecithin/cholesterol mixture containing poly I: C)	Inactivated FMD virus (FMD vaccine strains, type O (IND/R2/1975), A (IND/40/2000) and Asia-1 (IND/63/1972))	Foot-and-mouth disease	Cattle, intramuscular	Intramuscular route injection of liposome adjuvants in the FMD vaccine induces a strong immune response compared to the subcutaneous route, provides sustained immunity, and potentially reduces the booster frequency needed to maintain herd immunity.	(Saravanan et al., 2015)
Cationic liposome DPPC/DOOPAC/ Cholesterol	IBD vaccine	Infectious bursal disease	Chicken, subcutaneous	Enhance the delivery of the IBD vaccine to targeted organs, resulting in high and protective levels of IBD antibody titer with mild bursal lesions.	(Wahab et al., 2017)
Soy lecithin/Cholesterol/Alpha-tocopherol	Conserved peptides influenza vaccine (M2e, HA87-120, HA101-134, HA115-149, HA59-92, NP44-51, PB1347-355, PB1591-599, NA449-457, PA455-464)	Swine influenza A virus (H1N1)	Piglet, intranasal	Elicit robust mucosal IgA and cell-mediated immune responses, leading to superior protection compared to soluble peptides alone.	(Dhakal et al., 2018)
TOCOP/DPPC/SA/cholesterol	Inactivated FMD virus (A/ Argentina/2001 serotype)	Foot-and-mouth disease	Cattle, subcutaneous	Improved both humoral and cellular immune responses, which elicit a higher specific T-cell response against the virus characterized by an increased production of interferon-gamma (IFNγ)+/$CD8^+$ lymphocytes.	(Bidart et al., 2020)
Cationic liposome Cholesterol/PC/SA	H9N2, *Mycoplasma gallisepticum* (MG), *Echinacea* extract (EE)	Low pathogenic avian influenza virus (LPAIV). Infection by the H9N2 and MG	Chicken, intranasal	A positive correlation was observed between the highest mean levels of H9N2 and MG-specific IgA and IgG antibodies and several favorable outcomes.	(Kumosani et al., 2020)
Unilamellar liposome (PC/cholesterol)	Newcastle disease vaccine	Newcastle disease	Chicken, oral	Liposomal entrapped Newcastle vaccine provided faster onset protection and long-lasting immunity compared to conventional vaccine.	(Jaafer et al., 2021)
Cationic liposome (DOTAP/PC/cholesterol)	Newcastle disease vaccine	Newcastle disease	Chicken, oral	Enhanced antibody titers and strengthened cellular immune response, notably enhancing the activation and proliferation of T cells specific to the antigen.	(Okechukwu et al., 2023)

FMD: foot-and-mouth disease; TOCOP: alpha-tocopherol; DPPC: 1,2-dipalmitoyl-*sn*-glycero-3-phosphatidyl choline; PC: phosphatidylcholine; SA: sterylamine;, DOTAP: 1,2-dioleoyl-3-trimethyl ammonium propane; IBD: infectious bursal disease; IFN: interferon; Ig: immunoglobulin.

Unlike the human drug market, affordability is a major concern in veterinary medicine. This makes justifying the high development costs of liposome-based medications particularly challenging, since the majority of pet owners and farmers are price-sensitive, potentially limiting the use of these more expensive treatments (Carrique-Mas et al., 2019).

In addition, liposome-based veterinary drugs are difficult to get approval from regulatory bodies due to strict regulations if compared to conventional small molecule medications. Since there was rapid liposomal drug development, the FDA and European Medicine Agency (EMA), both offer a regulatory guideline for the evaluation of novel liposomal drug applications (Wang and Grainger, 2022). These regulations require long and expensive testing to prove the safety and effectiveness of the drug. The process involves a lot of paperwork and needs to follow the high standards. Additionally, the regulations can differ between countries, thus making global marketing a challenge.

Another problem is to optimize the liposomal compositions and formulations for various animal species. According to a review published in ISRN Veterinary Science in 2013, different species of animal require customized liposome designs for the best possible drug administration and efficacy due to the species-specific physiological difference (Sadozai and Saeidi, 2013). In addition, the challenge in liposome-based medication is their long-term stability. Maintaining optimal stability of liposomes in terms of chemical (oxidation reactions that may occur during the obtaining process) and physical factors (keeping ideal manufacturing and storage conditions) presents a significant challenge for liposome production at the industrial level. It is undeniable that liposomes have a moderate degree of instability as a colloidal system (Sainaga Jyothi et al., 2022). Liposome stability can be maintained by carefully taking into consideration the storage conditions, lipid selection, preparation methods, and additional pertinent investigations (Sainaga Jyothi et al., 2022). It is vital to consider that the technological procedure for generating liposomes must be cost-effective, utilizing a minimal quantity of non-toxic chemical reagents (Pasarin et al., 2023).

To overcome this hurdle, innovations in cost-effective manufacturing and strategies to highlight the clear benefits of liposomal drugs for animals are crucial. Collaboration between researchers, industry players, and regulators is vital to address these issues and unlock the full potential of liposome technology in veterinary medicine.

Risks and considerations of liposome-based applications in veterinary medicine

These days, safety concerns are becoming increasingly important in the various uses of nanomaterials. Despite the promise of liposomes for delivering drugs precisely in animals, there are potential safety concerns and practical limitations that require close evaluation. The primary focus for the safety is the potential immunogenicity and biocompatibility. Although liposomes are generally considered safe, certain liposome components may elicit mild immune reactions or inflammation at the site of injection. Some liposome ingredients, like phospholipids and PEG, might trigger immune responses called accelerated blood clearance (ABC) phenomenon and allergies in animals. This phenomenon can reduce the safety and effectiveness of the encapsulated therapeutic agents (Mohamed et al., 2019). One recent study published in Frontiers in Veterinary Science (2023) emphasizes the need for further research and investigation into the long-term safety profiles for particular liposomal formulations, specifically in relation to the possible chronic inflammatory reactions (André et al., 2023). Careful studies before and during development are needed to minimize these risks and ensure animal safety.

Another concern about liposome-based medication is its stability. This is because their large surface area compared to their volume makes liposomes have a lot of energy on their surface, which can lead to physical problems like clumping (aggregation) or merging (fusion) of the particles (Degobert et al., 2021). This instability problem will increase the size of liposomes and allow the leakage of the carried drug (De Sousa Lobato et al., 2013). Additionally, chemical changes like the breakdown of molecules attached to the liposome surface can harm its ability to target specific locations (Gatto et al., 2024). Several approaches can be utilized to enhance the stability of liposomes as a delivery system. These include the implementation of a protective coating, alteration of the liposomal membrane structure, addition of cryoprotectants prior to liposome lyophilization, and utilization of surfactants or specialized polymer gels (Sepúlveda et al., 2021; Subramanian et al., 2021). The importance of the stability of liposomes is to ensure that the delivery of therapeutic agents effectively reaches the intended targeted site. Low stability can result in degradation of the therapeutic agents along the way, while high stability may prevent the release of the therapeutic agents. Thus, the stability of the liposomes plays a critical role in the successful delivery of encapsulated therapeutic agents to the targeted site (Pasarin et al., 2023).

While liposome-based applications hold immense promise for veterinary medicine, a thorough comprehension and understanding of the potential risks and considerations associated with this technology is of utmost importance. By addressing concerns related to immunogenicity, off-target effects, and the stability of the formulation, researchers across the world can ensure the safety and effectiveness of translation of liposomal therapies in veterinary practice, thus ultimately resulting in enhanced animal health and welfare.

The future trend of liposomes in veterinary medicine

In the future, liposome-based drug and vaccine delivery in veterinary medicine is set to experience substantial growth, driven by innovations in nanotechnol-

ogy and personalized medicine. One area of research with great potential is the creation of theranostic liposomes, merging both therapeutic and diagnostic functions into a single system. These systems can transport drugs to the target location while concurrently assessing the therapeutic response, which enables individualized treatment plans. Moreover, the investigation of stimuli-responsive liposomes presents an exciting prospect. These liposomes release their encapsulated cargo upon encountering specific triggers within the body, such as changes in pH, temperature, or exposure to particular enzymes. This inventive method could greatly enhance drug delivery effectiveness while mitigating side effects. Furthermore, incorporating nanotechnology in liposome production presents enormous possibilities. Nanoliposomes exhibit increased stability, better drug encapsulation, and more effective delivery, making them an appealing instrument for forthcoming applications in veterinary medicine. With these new developments and ongoing research to make producing liposomes more reliable and efficient, veterinary medicine is poised for a major transformation. More effective treatment with fewer side effects can be customized to each animal's unique needs.

Conclusion

Liposomal drug and vaccine delivery systems possess enormous potential to transform the field of veterinary medicine by providing efficient, safe, and targeted treatment therapies for a wide variety of animals. The compatibility with biological systems, adaptability, and potential to increase treatment effectiveness give these systems considerable benefits when compared to traditional delivery methods. Recent advancements in liposome technology have further broadened their capabilities, and upcoming innovations suggest even greater developments in this area. While there are hurdles to overcome related to cost, scalability, and species specificity, the benefits of liposome technology in veterinary applications are vast. By enhancing the drug-delivery, the possibility for next-generation vaccines, and also for targeted disease control are immense. With the continuity of research and development of liposomes, it will hold the potential to revolutionize veterinary medicine in the industrial sector, which can lead to improved animal health, reducing the economic burden on the industry and promoting a more sustainable food system. As technology advances and regulatory support strengthens, these inventive delivery systems present tremendous opportunities to enhance animal health and welfare in the future.

Authors' contributions

All authors made a significant contribution to the work; gave final approval of the version to be published; have agreed on the journal to which the articles have been submitted; and agreed to be accountable for all aspects of the work.

Disclosure statement

The authors declare that there is no conflict of interest.

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