Targeted Drug Delivery for Veterinary Infections

1. Introduction
Domestic livestock are primary drivers for the livelihood of 600 million farmers in developing countries and contribute to about 30-35 per cent of agricultural gross domestic product. Devastating outbreaks of new diseases and re-emergence of old infections in animals with almost 61% of transmissible zoonotic infections are of paramount importance for livestock keepers. More than 200 zoonoses have been defined and attributed to ~2.2 million deaths/year and ~2.4 billion cases of human illness globally. Delayed detection is often associated with spread of the infection to entire herds and humans. Some zoonoses have spread beyond geographical boundaries and are globalised, e.g. H1N1, swine flu, Ebola, etc., and are threats for bio-security and bioterrorism. The World Organization for Animal Health adopts the policy of “Stamping out (Cull and Kill), vaccination, or a combination of the two”. In countries where such strategies are not legally permitted, livestock keepers are constrained to remain in close contact with the infectious animal, thereby endangering themselves. Drug resistance to existing therapies, insufficient drug potency, side-effects and drug residues in animal products compound the woes. The problems escalate further, when the infections are intracellular.

2. Intracellular Infectious Diseases
Intracellular infectious diseases are caused by pathogens that reside within the primary immune defence mechanism of the host, generally comprising macrophages from different reticuloendothelial organs (liver, spleen, lymphoid tissues, etc.). They are more difficult to eradicate. Extracellular pathogens in contrast survive on epithelial surfaces and extracellular spaces of the body that release specific proteins or toxins triggering body immune mechanism, and are generally more accessible to treatment. Interestingly, intracellular infection results when the organism outsmarts the body’s defence system. Phagocytosis of organisms following recognition is a natural defence strategy to kill the pathogen. Nevertheless, smart pathogens develop various adaptive mechanisms, survive destruction, and harbour safely within the reticuloendothelial system (RES). Common zoonotic infections and their major locations in the RES are listed in Table 1.

3. Adaptive Mechanism
A number of strategies are adopted by smart pathogens to evade death. Bypassing of normal phagocytosis and internalisation into macrophages by alternative pathways, parasitophorous vacuole or receptor-mediated pathways such as clathrin is an important approach. Secretion of endolysosomal lytic enzymes or endotoxins that break down endosome membrane favour direct entry into the nutrient-rich cytosol by passing phagolysosomal destruction. Interference with the phagolysosome formation by either preventing acidification of phagosome, delayed fusion with lysosome, enzymatic breakdown, reduced levels of proton ATPase, disturbances in forming lipid rafts or altering host signal is yet another possibility. Virulent pathogens also have general resistance that permits survival in low pH, lytic enzymes, oxidants and various other harsh conditions. These mechanisms are depicted in Figure 1.

4. Nanotechnology – A Solution
Conventional drug delivery strategies rely on diffusion of drug across cell membranes to build up intracellular concentrations. On the other hand, nanocarriers can be designed to follow the same pathway as the virulent organism to enable high drug payloads within the cell. More importantly, nanocarriers also overcome other confronting challenges, namely drug efflux through efflux pumps and Cyp-mediated metabolism. Targeted delivery of nanocarriers thus presents a promising therapeutic strategy in infectious diseases more specifically.

5. Targeted Drug Delivery
The challenges posed for the successful therapy of veterinary infections differ significantly from human infections, not so much in the eradication of the infections, but more so with drug concentration in non-target locations. Indeed, secretion of drug in milk of lactating animals and residual drug concentrations in meat pose significant hurdles in veterinary therapy. The concept of targeting goes back to the early twentieth century when the Nobel Laureate Paul Ehrlich proposed the magic bullet. The concept, however, has been exploited, although to a limited extent for human therapy, primarily for cancer treatment (e.g. Doxil®). AmBisome, a novel nanocarrier explored for leishmaniasis and fungal infections is the first success story, confirming the major role nanocarriers could play in the improved therapy of infections. Targeting to the RES can provide a key solution for most livestock intracellular infections.

Passive and active targeting strategies can be exploited to target macrophages. Passive targeting would rely on the body’s natural phagocytic ability to engulf the nanocarriers and would be significantly influenced by surface properties, mainly particle size, charge, shape and hydrophilicity. Active targeting using appropriate ligands to facilitate endocytic uptake can further enhance intracellular drug levels. Targeting...
## Diseases of bacterial origin

<table>
<thead>
<tr>
<th>Diseases</th>
<th>Causative organisms</th>
<th>Foci of infection</th>
<th>Adaptive mechanism</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Actinomycosis</td>
<td>Actinomyces bovis</td>
<td>Lung, bone and muscles, lymphatic systems</td>
<td>Block phagocytosis</td>
<td>10</td>
</tr>
<tr>
<td>Anthrax</td>
<td>Bacillus anthracis</td>
<td>Lymph node and spleen</td>
<td>Develops spores preventing breakdown</td>
<td>11, 12, 13</td>
</tr>
<tr>
<td>Brucellosis</td>
<td>Brucella spp.</td>
<td>Spleen, liver, lung, kidney, lymphatic system, bone marrow, intestine, CSF, seminal vesicles, testicles and epididymis</td>
<td>Forms beta-1, 2 glucans and disturbs normal formation of lipid rafts</td>
<td>14, 15</td>
</tr>
<tr>
<td>Ehrlichiosis</td>
<td>Ehrlichia spp.</td>
<td>Leukocytes and retain in systemic circulation</td>
<td>Inhibit phagolysosome fusion, and survive</td>
<td>16</td>
</tr>
<tr>
<td>Johne’s disease</td>
<td>Mycobacterium paratuberculosis</td>
<td>Lymph nodes, liver, GI tract</td>
<td>Replicates in macrophage phagosomes that fail to mature</td>
<td>17, 18</td>
</tr>
<tr>
<td>Mastitis</td>
<td>Streptococcus agalactiae</td>
<td>Alveolar cells and macrophages</td>
<td>Formation of capsule</td>
<td>19</td>
</tr>
<tr>
<td>Pseudotuberculosis</td>
<td>Yersinia pseudotuberculosis</td>
<td>Lung, liver and lymphatic system</td>
<td>Virulence inhibits phagocytosis</td>
<td>20, 21</td>
</tr>
<tr>
<td>Q fever</td>
<td>Coxiella burnetti, Clostridium spp.</td>
<td>Lung and liver</td>
<td>Survives in phagolysosomes, uptake by non-phagocytic pathway</td>
<td>4</td>
</tr>
<tr>
<td>Tuberculosis</td>
<td>Mycobacterium tuberculosis</td>
<td>Lung, lymph nodes</td>
<td>Disrupts phagolysosome and breakdown by enzymes</td>
<td>22, 23</td>
</tr>
<tr>
<td>Tularemia</td>
<td>Francisella tularensis</td>
<td>Bone, lung</td>
<td>Disrupts phagolysosome</td>
<td>24</td>
</tr>
</tbody>
</table>

## Diseases of viral origin

<table>
<thead>
<tr>
<th>Diseases</th>
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<th>Adaptive mechanism</th>
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</tr>
</thead>
<tbody>
<tr>
<td>Avian influenza (H5N1)</td>
<td>Influenza A, H5N1</td>
<td>Lungs</td>
<td>Compromised functional capacity</td>
<td>25</td>
</tr>
<tr>
<td>Ebola virus infection</td>
<td>Ebola virus</td>
<td>Kidneys, spleen, and liver</td>
<td>Utilises micropinocytosis through Rac-1/Pak-1–dependent membrane ruffling</td>
<td>5</td>
</tr>
<tr>
<td>Aujeszky’s disease</td>
<td>Pseudorabies virus</td>
<td>Liver, spleen, adrenal glands, lymph nodes, brain</td>
<td>Phagosome-lysosome fusion failure</td>
<td>26, 27</td>
</tr>
<tr>
<td>Malignant catarrhal fever</td>
<td>Herpes viridae</td>
<td>Peripheral blood leukocytes, liver, kidney, lymph node, liver and brain</td>
<td>Resistant to pH changes</td>
<td>28</td>
</tr>
<tr>
<td>Pseudorabies</td>
<td>Alpha herpes virus of swine</td>
<td>Lymphatic system, lungs, neurons</td>
<td>Phagosome-lysosome fusion failure</td>
<td>27</td>
</tr>
<tr>
<td>Rabies</td>
<td>Rabies virus</td>
<td>Spleen, lymphoid, brain</td>
<td>Virulence factors increases cytokine production</td>
<td>29</td>
</tr>
<tr>
<td>Severe Acute Respiratory Syndrome (SARS)</td>
<td>Severe acute respiratory syndrome (SARS) coronavirus</td>
<td>Liver, spleen, lymphatic</td>
<td>Enter a non-Interferon-α-production</td>
<td>30</td>
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## Diseases of parasitic origin

<table>
<thead>
<tr>
<th>Diseases</th>
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<th>Foci of infection</th>
<th>Adaptive mechanism</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Babesiosis</td>
<td>Babesia equi, Babesia microti</td>
<td>Blood and spleen</td>
<td>Activation of IL-12 and IFN-γ, decreasing macrophage population</td>
<td>31</td>
</tr>
<tr>
<td>Bovine besnoitiosis</td>
<td>Besnoitia besnoiti</td>
<td>Endothelial cells of blood vessels and lymphatic system</td>
<td>Development of cysts</td>
<td>32</td>
</tr>
<tr>
<td>Chagas disease</td>
<td>Trypanosoma cruzi</td>
<td>Liver, spleen and lymph node</td>
<td>Forms endocytic parasitophorous vacuole</td>
<td>33</td>
</tr>
<tr>
<td>Echinococcosis</td>
<td>Echinococcus granulosus</td>
<td>Liver, lung and CNS</td>
<td>Forms hydatid cyst</td>
<td>34</td>
</tr>
<tr>
<td>Leishmaniasis</td>
<td>Leishmania donovani</td>
<td>Spleen, liver, lymph node, bone marrow, liver</td>
<td>Prevents activation of macrophages, survives breakdown, alters signalling</td>
<td>35, 36</td>
</tr>
</tbody>
</table>

Table 1: Major zoonotic diseases and their major locations in the RES
strategy would therefore enable high drug concentrations at the site of infection and this can be achieved using a range of nanocarriers.

6. Nanocarriers for Targeted Drug Delivery

Attractive features of nanocarriers include enhanced bioavailability, desired tissue selectivity, and high drug payload at desired sites. More importantly, drug concentrations at other sites can be effectively decreased. This could provide a specific advantage in the therapy of veterinary infections, decreased secretion of drug in milk, and/or decreased accumulation in meat. A variety of nanocarriers may be resorted for targeted delivery. Some important carriers are summarised in Table 2.

7. Applications of Nanocarriers

a. Nanomedicine

Polymeric drug delivery systems and lipid drug delivery systems have been explored for infectious diseases. Depending upon the targeted site, properties of nanocarriers such as surface chemistry and size have been modulated. Modifying shape could direct nanocarriers to spleen, a major aspect of macropaghic infection. Various nanocarriers exploited for targeting livestock infections have been summarised in Table 3. Nano drug delivery systems have also been explored for delivery of minerals e.g. Selenium, for improving digestive process, animal production and improved immunity.

b. Nano-vaccines

Veterinary vaccines are different from human vaccines. Vaccination should increase the immunity in herd rather than the individual and control infections in animals. A less reactive and biodegradable vaccine is of high priority, especially if the animals are bred for human consumption. Besides, high genetic diversity with limited knowledge of targeting molecules (immune modulators and surface markers) in wildlife species is to be addressed. During epidemics, marker vaccines should also be able to differentiate vaccinated animals and infected ones. Subunit vaccines, DNA vaccines, vectored vaccines and recombinant vaccines have replaced the traditionally used killed or live modified vaccines. Earlier vaccines used adjuvants such as aluminum hydroxide to improve immune response, which has its own disadvantages of immunity, inflammation, etc. Nanoparticles for Trichinosis (caused by consumption of pork meat contaminated with Trichinella spiralis) revealed adequate induction against immune response in Trichinella-infected mice. Liposomes for oral and intranasal delivery of recombinant B subunit of cholera toxin or IgA have been studied in mice. Needle-free nanoeumulsion (droplet size ~ 40 nm) of hepatitis B antigen has been studied in mice, rats and guinea pigs. ISCOM (Immunostimululatory complexes) and ISCOMATRIXTM based adjuvants composed of antigen incorporated into lipid vesicles and admixtures with particulate adjuvant are widely researched for vaccines. Most of the research involved in study of vaccine delivery, target to the antigen presenting cells and Peyer’s patches. Incorporation of antigens within particulate delivery protect and provides enhanced immunogenicity, increased antigenic uptake, and control antigen release. It activates pattern recognition receptors (initiating innate immune response), up-regulates antigen-presenting cells (increasing T cells activation), control residence time, location and dose of antigen (maintaining immunity levels and translocation to lymph nodes) and maintains depot for prolonged release. It is reported that 40 animal diseases have nanoparticles developed or under development.

c. Nano-diagnostics

Early detection of pathogens with high accuracy is vital to prevent long-term complication and epidemic outbreaks, develop pandemicity, and preserve public health. Classical techniques of culturing and biochemical-based tests for pathogen detection, enzyme-linked immunosorbent assay and polymerase chain reaction are time-consuming with limited sensitivity. Nanomaterial-based devices have also been designed for detection of tuberculosis from sputum samples. Biosensors based on Au/Ag hetero-nanorod functionalised with anti-Salmonella antibodies have been used for detection of infectious diseases in food as well as in diagnosis. Change in estradiol blood level of animals can be determined by implanting carbon nanotube-based sensors. Carbon nanotubes bind to estradiol antibodies, track estrus

<table>
<thead>
<tr>
<th>Nanocarriers</th>
<th>Schematic representation</th>
<th>Features</th>
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</table>
| Liposome     | ![Schematic](Image)       | • Spherical phosphatidyl vesicles  
• Fluidity can be adjusted using combination of liposomes  
• Enclosing an internal aqueous volume with one or more concentric hydrophobic bilayers  
• Unilamellar (single bilayer) or multilamellar (many bilayers) or multivesicular  
• Encapsulates both hydrophilic and hydrophobic drug |
| Polymeric or lipid nanoparticles | ![Schematic](Image) | • Rigid nanoparticles made of natural / synthetic polymers or lipids  
• Drugs loaded within matrix are ‘nanocapsules’; Drugs loaded throughout matrix are ‘nanospheres’  
• Release of drug through matrix diffusion or degradation of matrix  
• Ease for surface modification |
| Lipid-polymer hybrid nanoparticles | ![Schematic](Image) | • Combination of lipid and polymer  
• Polymers enhance loading of hydrophilic drugs and lipids favour hydrophobic drug loading  
• Improved stability, ease to anchor ligands  
• Coating with lipsids mimic bio-interface |
| Dendrimer    | ![Schematic](Image)       | • Star polymers  
• Chemical polymers similar to bio-molecules  
• Surface functionalisation on surface to tailor drugs and ligand |
| Polymeric micelles | ![Schematic](Image) | • Amphiphilic block or graft copolymers  
• Self-assembly to form micelles, vesicles or gels  
• Enhances solubility of poorly soluble drugs |
| Carbon nanotubes | ![Schematic](Image) | • Single or multi-walled layer of rolled graphene/ graphite sheets capped by fullerene  
• Penetrate through barriers by ‘nano-needle’ mechanism |
| Layer double hydroxide (anionic clay) | ![Schematic](Image) | • Hydrotalcite [MII]nMIII(DH)2,6–n(2/3)OH·n(2/3)H2O(n=3–4)  
Where MII and MIII represents a divalent and trivalent metal cation respectively and M represents an anion  
• Cationic charge of LDH enhances loading of anionic actives |
| SPIONS       | ![Schematic](Image)       | • Super paramagnetic iron oxide nanoparticles (SPIONS) and ultra super paramagnetic iron oxide nanoparticles composed of maghemite, Fe3O4 or maghemite  
• Application directed by external magnetic field |
| Quantum dot  | ![Schematic](Image)       | • Semiconductors from II–VI or III–V of the periodic table  
• ZnS, ZnSe, ZnO, InAs, GaAs, CdTe, CdS  
• Broad absorption spectra, unique electronic luminescence and optical properties |
| Metallic nanoparticles | ![Schematic](Image) | • Gold, silver and platinum nanoparticles  
• Controlled optical properties tailored surface plasmon resonance  
• Anti-infective and other bioactivities of gold and silver itself |

Table 2: Different nanocarriers used in veterinary medicine

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in animals, and accordingly actuate breeding. Veterinary drug residues in milk, bovine urine such as sulfamethazine, progesterone, clenbuterol, etc., are being detected online using nano-based new optical biosensors working on the principle of surface plasmon resonance. Quantum dots, carbon nanotubes and nanoshells can be explored for imaging using fluorescence, X-rays, ultrasound or magnetic resonance. Nanosensors and cantilever array systems have also been used for diagnosis of a specific target sequence. Lab-on-chip recognises gene mutations, DNA mutation, pathogenic strains, and chemical pathogens with high sensitivity. Implanted sensors can also assist in gaining information about physiological parameters such as temperature, heart rates, blood pressure, etc.

8. Future Prospects

Targeted delivery of drugs for veterinary infections provides great promise for both short-term and long-term treatment strategy. The rate-limiting features concerned are the technology scale-up challenges and the possible new toxicities. Nevertheless, due recognition of the positive aspect of nanotechnology in targeted delivery could revolutionise the therapy of veterinary infections globally.

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