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\section*{ABSTRACT}
Infectious diseases in farm animals triggered by pathogenic microorganisms affect the health and well-being of livestock and human populations. Pathogen detection is an important step for the successful diagnosis, treatment and control of infectious diseases in animals. Pathogens that persist in the poultry and livestock industries can be responsible for more than 70\% of emerging infections. Thus, rapid diagnostic tools are extremely important. In recent years, nanotechnology has emerged as a great opportunity to tackle this challenge and to develop fast, accurate and economical diagnostics for the detection of pathogens. Various nanostructures, due to the presence of unique characteristics shown in nanomaterials, have already been applied in biodiagnostics to detect specific molecular targets, including pathogen detection. In this context, this review focuses on the application, role and challenges of nanosensors in detecting disease-causing pathogens in agriculture. Several nanostructures are investigated for their utility in providing innovative solutions for pathogen detection in farm animals. This comprehensive examination seeks to unravel the intricate nanosensors landscape, shedding some light on their role in advancing diagnostic capabilities within the agricultural domain. By elucidating the challenges inherent in their application, the review contributes to the ongoing discourse on harnessing nanotechnology for the detection and management of infectious diseases in livestock, ultimately paving the way for developments in veterinary diagnostics.

\section*{1. Introduction}
Mirroring the immense increase in the world population, the demand for animal origin food products is increasing and projected to double by 2050 [1]. This increase in demand for food points toward an imbalance in relation to supply, which must be addressed if food supply and security is to be preserved [2]. Even temporary interruptions in animal production can have severe consequences, triggering a cascade of harmful events and impairing the supply balance of animal-based proteins for the human population [3,4]. In this scenario, infectious agents that can cause animal diseases are the ones which pose the largest threat to the stability of world production systems [5,6] and can impact animal welfare, reducing potential production and cost-effectiveness, in addition to having potential environmental and biodiversity consequences worldwide [7,8].

Pathogens can cause infectious diseases that display symptoms with onsets ranging from minutes to hours or even days after the original infection [9,10]. These infections can spread to healthy animals from an affected animal or vector [9]. Correct and efficient diagnosis of disorders is further precluded by the complexity and diversity of disease-causing microorganisms, as well as by the lengthy incubation periods of some of these agents prior to the onset of disease symptoms. Adding to this, a wide range of pathogens cause diseases, including bacteria, viruses, fungi, protozoa, parasitic worms, and prions, with bacteria having the greatest influence on the animal food supply chain [9]. Bacterial infections can present a threat to human and animal health and are one of the most common causes of death worldwide due to an increasing incidence and spread [11]. Bacterial infections were shown to be responsible for 7.7 million fatalities in 2019, which equates to one-eighth of all global deaths and elevates bacterial infections to the
world's second leading cause of death, trailing only ischemic heart disease [12]. Pathogenic animal infections, in addition to threatening animal production and food supply worldwide, are responsible for more than 70% of human emerging infections [13,14]. During the last three decades, emerging and re-emerging infectious diseases have been identified as one of the most important public health problems. Despite modern health care, infectious diseases remain one of the leading causes of global mortality, involved in 13-6% of global deaths [12], with resistant bacterial pathogens being the most common concern [15].

The epidemiology of antimicrobial-resistant microorganisms at the human-animal interface involves a multitude of transmission routes. These routes can include selective antimicrobial pressure, and other ecological factors, as well as the horizontal transfer of resistance genes between bacterial species and genera. Therefore, effective disease control is critical to reduce the socioeconomic impact of these microorganisms, in addition to conserving and maintaining the animal-origin food supply.

One of the ways to minimize the spread of animal diseases is early and accurate diagnosis, which is crucial for establishing the prevention and treatment of infections [7]. Animal disease diagnosis frequently necessitates laboratory confirmation, which is dependent on multiple polymerase chain reaction (PCR) techniques, immunoassays, and cell cultures. As such, diagnosis commonly demands long response times, a trained team, and well-equipped laboratories [8,16]. Therefore, it is also an expensive process, demands specific resources, and often does not correlate with disease progress due to the considerable turnaround periods from sampling process to results, which can range from days to weeks [8].

The major challenge for current research, in addition to controlling the spread of infectious diseases, is the search for rapid pathogen detection and identification technologies. Nanotechnology, due to the presence of distinct properties in nanoscale materials, is a great opportunity to solve this challenge and to develop fast, accurate and economical diagnostics for the detection of pathogens [17-19].

Different nanostructures have already been applied in biodiagnostics to detect specific molecular targets, including pathogen detection [18,20]. In this context, this review focuses on the application, role and challenges of nanosensors in detecting disease-causing pathogens in agriculture.

2. Nanosensors for detection and diagnosis of infectious diseases in livestock

Nanotechnology has already demonstrated its great potential and has an extensive assortment of applications in several areas, including agriculture and food industry [21]. In agriculture, problems such as low crop productivity, low agricultural input efficiency, fertile land loss, diseases and climate change susceptibilities have been detected and benefited by nanotechnology, mainly through the targeted delivery of nanocomponents such as additives, growth regulators, enzymes, vitamins, fertilizers and pesticides [22,23]. Another application of nanotechnology, which is gaining prominence in the agricultural sector, is the use of nanocapsules and nanodevices to detect and deal with diseases through in situ sensors for real-time monitoring of large areas in the field, enzymatic biosensors for specific sensing, luminescent nanocrystals, and quantum dots for fluorescent labeling in biological recognition of molecules and pathogens [24-26]. In addition, nanosensors developed to probe plant systems have the potential to increase our comprehension of plant biology. For example, intracellular nanosensors can detect metabolic precursors, signal ligands, and nutrients, allowing researchers to better understand the complicated roles of these chemicals in plant systems [27]. In food industry, in turn, nanosensors represent a promising technology for tackling challenges through novel solutions related to food safety, food processing, and food packaging [28]. Here, nanosensors represent a powerful tool to identify nutrient deficiencies, toxicity, plant and animal diseases in order to improve food quality and safety [29] (Tables 1 and 2). Biosensors have been developed to identify pathogen-related targets using highly sensitive and specific recognition features exhibited by antibodies, aptamers, glycans, and DNA probes [7]. These targeted entities, referred to as analytes and biomarkers, may include various types such as biomolecules, which can be enzymes, DNA/RNA, proteins, and organisms, such as bacteria, fungi and viruses [30]. The utilization of biomarkers is a growing trend in the animal health sector and has found application in assessing diverse health parameters. Specifically, biomarkers prove valuable in clinical contexts, playing a crucial role in diagnosing illnesses and predicting and/or monitoring responses to treatments [31].

Nanotechnology offers opportunities for the development of cheaper, faster and more accurate diagnostic tools, allowing better diagnosis with a positive effect on the cost of animal health care. Currently, nanomaterials are playing a key role in imaging and monitoring in early disease detection. Here, nanomaterials-based research has emerged as an exciting new field where different nanomaterials can be synthesized and functionalized to diagnose the target effectively [52]. The potential for agricultural applications can be substantially increased by the development of novel sensing methods for the local detection of metabolites in real time.

Nanomaterials used in nanosensor manufacturing include nanoparticles, carbon materials, metal & metal oxide nanoparticles, polymers, silica, and biomaterials [53]. Metallic nanoparticles have emerged as pivotal components in the development of nanosensors for veterinary diagnostics. Their unique optical properties, including surface plasmon resonance, enable the sensitive detection of specific biomolecules [54]. Carbon-based nanomaterials have garnered attention for their exceptional electrical conductivity and biocompatibility [55], making them valuable elements in veterinary nanosensors. Carbon nanotubes, with their high aspect ratio and large surface area, are employed for immobilizing biomolecules and facilitating electron transfer in electrochemical sensors, enabling rapid and sensitive detection with potential applications in point-of-care settings [56]. Polymeric nanomaterials play a pivotal role in nanosensor development tailored for targeted detection of specific animal diseases. Polymers properties, such as their surface charge, hydrophobicity, and biodegradability, make them versatile platforms for sensor construction [57]. These nanosensors offer enhanced sensitivity and specificity, crucial for distinguishing between different pathogens or disease states. Silica nanoparticles have found applications in veterinary nanosensors, particularly in encapsulating and protecting sensitive biomolecules, such as enzymes or DNA probes [58]. The porous nature of silica nanoparticles allows for the controlled encapsulated compounds release, contributing to the stability and longevity of nanosensors. Silica nanoparticles are also utilized as imaging agents for their compatibility with various imaging modalities [59].

Cutting-edge techniques, from top-down to bottom-up approaches, such as lithography, self-assembly and 3D printing, can be employed in the fabrication of nanosensors for accurate and early animal diseases detection [60]. Lithography-based techniques stand at the forefront of nanosensor fabrication, offering unprecedented control over nanoscale features [61,62]. Photolithography employs light to selectively modify a photosensitive material, enabling the creation of intricate patterns for sensor surfaces. Electron beam lithography, otherwise, utilizes a focused electron beam to directly write patterns at the nanoscale. These approaches provide a high degree of precision, ensuring enhanced sensitivity and specificity in disease detection [61,62]. One example consists in a sensor surface containing a hexagonal array of Au fabricated by nanosphere lithography for Pseudomonas aeruginosa detection [63]. Self-assembly strategies offer an elegant and efficient approach to nanosensor fabrication, capitalizing on the inherent properties of nanomaterials to spontaneously organize into functional structures [64], like self-assembled chiral gold nanostructures developed to detect avian influenza A (H5N1) [65]. Molecular self-assembly relies on the affinity of molecules to arrange themselves into ordered patterns [66], while
### Table 1
Nanosensors for animal infectious diseases detection.*

<table>
<thead>
<tr>
<th>Nanomaterial</th>
<th>Pathogen/Target</th>
<th>Recognition element</th>
<th>Detection method</th>
<th>Sensitivity</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Copper-doped nickel and zirconium oxide nanoparticle</td>
<td><em>Brucella abortus</em></td>
<td>Anti-lipopolysaccharide antibodies</td>
<td>Cyclic voltammetry, electrochemical impedance spectroscopy</td>
<td>$10^4$ CFU ml$^{-1}$</td>
<td>[32]</td>
</tr>
<tr>
<td>Gold nanoparticle complexes</td>
<td>Avian influenza A H5N1 virus</td>
<td>H5N1-specific aptamer</td>
<td>Electrochemical impedance spectroscopy</td>
<td>$10^4$ HAU</td>
<td>[33]</td>
</tr>
<tr>
<td>Gold nanoparticle</td>
<td><em>Salmonella</em> app.</td>
<td>Antibodies</td>
<td>Differential pulse voltammetry</td>
<td>$10^3$ CFU ml$^{-1}$</td>
<td>[34]</td>
</tr>
<tr>
<td>Gold nanoparticle</td>
<td>Foot and mouth disease (FMD) antibodies</td>
<td>FMD antigen</td>
<td>Dot-blot assay, visual observation</td>
<td>–</td>
<td>[35]</td>
</tr>
<tr>
<td>Gold nanoparticle</td>
<td><em>Brucella melitensis</em></td>
<td>Anti-brucella antibodies</td>
<td>Cyclic voltammetry, electrochemical impedance spectroscopy</td>
<td>$10^4$ CFU ml$^{-1}$</td>
<td>[36]</td>
</tr>
<tr>
<td>Gold nanoparticle and quantum dots</td>
<td>Muscovy duck parvovirus</td>
<td>ssDNA aptamer</td>
<td>Spectrophotometry or visual observation</td>
<td>3 EID$_{50}$</td>
<td>[37]</td>
</tr>
<tr>
<td>Gold nanoparticle and quantum dots</td>
<td>Porcine reproductive and respiratory syndrome virus (PRRSV)</td>
<td>Anti-PRRSV monoclonal antibody</td>
<td>Fluorescence resonance energy transfer</td>
<td>3 particles $\mu$L$^{-1}$</td>
<td>[38]</td>
</tr>
<tr>
<td>Gold nanoparticle and quantum dots</td>
<td>Avian influenza A H5N1 virus</td>
<td>Anti-H5N1 hemagglutinin antibody 2B7, anti-H5N1 neuraminidase polyclonal antibody</td>
<td>Circular dichroism spectra</td>
<td>1 pg.ml$^{-1}$</td>
<td>[39]</td>
</tr>
<tr>
<td>Gold and magnetic nanoparticle</td>
<td><em>Staphylococcus aureus</em></td>
<td>Anti-S. aureus antibodies</td>
<td>Colorimetry</td>
<td>$10^3$ CFU ml$^{-1}$</td>
<td>[40]</td>
</tr>
<tr>
<td>Gold nanowire</td>
<td>Bovine viral diarrhea (BVD) antibodies</td>
<td>BVD virus</td>
<td>Electrochemical impedance spectroscopy, cyclic voltammetry</td>
<td>–</td>
<td>[41]</td>
</tr>
<tr>
<td>Graphene oxide nanosheets</td>
<td>Negative energy balance (NEB)</td>
<td>Non-esterified fatty acids (NEFA) and beta hydroxy butyrate (βHBA) antibodies</td>
<td>Electrochemical analysis</td>
<td>0.111 mM</td>
<td>[42]</td>
</tr>
<tr>
<td>Ionic self-assembled multilayer</td>
<td><em>Brucella</em> DNA</td>
<td>Nucleotide probe</td>
<td>Optical spectrum analysis of the refractive index</td>
<td>$10^2$ CFU ml$^{-1}$</td>
<td>[43]</td>
</tr>
<tr>
<td>Magnetic nanoparticle</td>
<td>Gram-negative bacteria</td>
<td>Anti-LPS antibodies</td>
<td>Conductometry</td>
<td>$10^2$ CFU ml$^{-1}$</td>
<td>[44]</td>
</tr>
<tr>
<td>Magnetic nanoparticles</td>
<td><em>Listeria monocytogenes</em></td>
<td>Listeria protease</td>
<td>Colorimetry</td>
<td>$10^2$ CFU ml$^{-1}$</td>
<td>[45]</td>
</tr>
<tr>
<td>Phosphorous nanosheets</td>
<td>Haptoglobin</td>
<td>Anti-Hp antibody</td>
<td>Differential pulse voltammetry</td>
<td>0.01 mg.ml$^{-1}$</td>
<td>[46]</td>
</tr>
<tr>
<td>Platinum nanotubes</td>
<td>Porcine reproductive and respiratory syndrome virus (PRRSV)</td>
<td>Anti-PRRSV antibody</td>
<td>Fluorescence</td>
<td>2.4 ng.ml$^{-1}$</td>
<td>[47]</td>
</tr>
<tr>
<td>PtPd bimetal alloy nanoparticles</td>
<td><em>Streptococcus suis</em> serotype 2</td>
<td>Antibodies</td>
<td>Peroxydisulfate electrochemiluminescence</td>
<td>33 fg.ml$^{-1}$</td>
<td>[48]</td>
</tr>
</tbody>
</table>

* CFU: colony forming units; HAU: hemagglutination units; EID$_{50}$: 50% egg infection dose.

### Table 2
Commercially available biosensors and their characteristics [49-51].

<table>
<thead>
<tr>
<th>Company</th>
<th>Biosensor</th>
<th>Analyte</th>
<th>Sensitivity</th>
<th>Country</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meridian Bioscience</td>
<td>ImmunoCard STAT! E. coli O157 Plus</td>
<td><em>Escherichia coli</em></td>
<td>–</td>
<td>USA</td>
</tr>
<tr>
<td>Coris BioConcept</td>
<td>0157 Coli-Strip</td>
<td><em>Escherichia coli</em></td>
<td>–</td>
<td>Belgium</td>
</tr>
<tr>
<td>Coris BioConcept</td>
<td>0157 Coli Uni-Strip</td>
<td><em>Rotavirus</em></td>
<td>–</td>
<td>Belgium</td>
</tr>
<tr>
<td>Coris BioConcept</td>
<td>Quatro Vet Uni-Strip</td>
<td><em>Escherichia coli</em> k99</td>
<td>–</td>
<td>Belgium</td>
</tr>
<tr>
<td>Coris BioConcept</td>
<td></td>
<td><em>Coronavirus</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coris BioConcept</td>
<td></td>
<td><em>Vaccinia</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coris BioConcept</td>
<td></td>
<td><em>Franciscella tularensis</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coris BioConcept</td>
<td></td>
<td><em>Brucella burnetii</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ANP Tech</td>
<td>ANP NIDS (nano intelligent detection system) biothreat/biowarfare handheld assays</td>
<td>Botulinum toxin A</td>
<td>–</td>
<td>USA</td>
</tr>
<tr>
<td>ADVNT Biotechnologies</td>
<td>BADD – Biowarfare Agent Detection Devices</td>
<td><em>Yersinia pestis</em></td>
<td></td>
<td>USA</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Vibrio cholerae</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Salmonella</em></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td><em>Listeria</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Escherichia coli</em> O157</td>
<td>10 mg.ml$^{-1}$</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Bacillus anthracis</em></td>
<td>33 ng.ml$^{-1}$/500 ng.ml$^{-1}$</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Clostridium botulinum</em></td>
<td>105 CFU.ml$^{-1}$</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Yersinia pestis</em></td>
<td>10 ng.ml$^{-1}$</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Staphylococcus aureus</em></td>
<td>106 CFU.ml$^{-1}$</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Franciscella tularensis</em></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* CFU: colony forming units; HAU: hemagglutination units; EID$_{50}$: 50% egg infection dose.
colloidal self-assembly leverages the interactions between colloidal particles to form well-defined structures [67]. 3D printing technologies have revolutionized nanosensor fabrication by providing the capability to create intricate three-dimensional structures with precision and ease [68], such as the electrochemical sensor 3D printed for *Listeria monocytogenes* detection, based on loop mediated isothermal amplification, which shows a clear sensitivity with a limit of detection of 1.25 pg of DNA per reaction [69]. Chemical vapor deposition (CVD) and physical vapor deposition (PVD) techniques play a pivotal role in nanosensor synthesis by facilitating the controlled deposition of thin films and coatings [70]. CVD involves the chemical reaction of gaseous precursors to form a thin film on a substrate [71]. PVD, in contrast, relies on physical processes such as evaporation or sputtering to deposit materials onto a substrate [70]. Both methods contribute to the creation of functional sensor surfaces with enhanced sensitivity and stability [70]. Electrochemical fabrication approaches, including electrodeposition and electrospinning, harness electrochemical principles to deposit nanomaterials onto sensor surfaces.

Nanosensors are materials able to specifically detect analytes that are unique to a particular microorganism or condition. These sensors can be divided into immunosensors [72,73], genosensors [74,75], non-enzymatic receptor sensors [76], enzyme sensors [77,78] and whole cell sensors [79,80]. In terms of modes for signal transduction, nanosensors can be classified into electrochemical, optical, piezoelectric, magnetic, thermal, radioactive, and mechanical sensors [81]. Together, nanosensors and accentuators allow nanoparticle-based devices able to track and measure chemical, mechanical and physical changes related to a marker of interest. The biological specificity of nanosensors is generally conferred by means of targeting ligands, which attract a particular marker of interest, conjugated directly to nanoparticles that contribute to sensitivity and act as a detector for the generated signals [82].

Nanosensors, nanoprobes, as well as other nanoscale technologies, have increased the quality of chemical and biosensing structures significantly. These nanodevices have been engineered with rapid reaction times and low power consumption [83]. The bio-sensing of nanosensors involves surface functionalization of nanomaterials with specific recognition elements, such as antibodies or aptamers, enabling selective binding to target biomolecules associated with pathogens or interest molecules [60]. Upon encountering these biomolecules in a sample, binding events occur, inducing nanosensor’s properties modifications. Signal transduction mechanisms, including changes in electrical conductivity, optical properties, or mass, convert these binding events into measurable signals. Techniques such as electrochemical detection, quantum dots for fluorescence, and resonance-based methods are employed for signal readout [60]. Nanotechnology-based sensing systems hold significant importance across various fields due to their unique capabilities and potential impact, such as enhanced sensitivity, specificity and selectivity, miniaturization, multiplexing, real-time monitoring, improved biocompatibility, and reduced sample volume [83–85], which has allowed their use, e.g., in health monitoring [86,87], environmental monitoring [88], food safety [89] and quality assurance of food and water [90].

An efficient nanosensor should be able to identify the marker regardless of the origin or complexity of a biological sample [7,91]. The interactions between the marker and the bioreceptor are converted into a signal that is analyzed, allowing for both qualitative and quantitative measurement of the target of interest (Fig. 1). Immobilized sensing components, known as bioreceptors, assist in recognition. Monoclonal antibodies, RNA, DNA, glycans, lectins, enzymes, cofactors, tissues, and whole cells are all examples of bioreceptors [92]. There are several applications of nanosensors, including the detection of microorganisms and pathologies. In the following subsections, we will deal with nanosensors that are available for animal health.

### 2.1. Detection using antibody-antigen pairs

Immunology-based methods using nanoparticles are based on antibody-antigen relationships. These methods use antibodies or
antibody fragments as detection elements or as primary targets for diagnosis. Antibody and nanoparticle conjugation presents excellent nanosensors due to stability, biocompatibility, and their sensitivity to target antigens [93]. For the use of antibodies as targets and indicators of exposure and infection to pathogens, nanomaterials are functionalized with antigens in order to target these antibodies in several stages of infections [94,95] (Fig. 2B).

Nanosensors based on antibody-antigen recognition mechanisms have been described for efficient and specific detection of *Escherichia coli*. The development of immunosensors and immunoassays for rapid *E. coli* detection in infected samples is due to the availability of a wide variety of high-affinity antibodies against *E. coli* that bind to surface proteins, e.g., anti-*E. coli* O157:H7 [96], anti-LPS [97], and egg yolk antibody (IgY) [98]. Such nanosensors have furthermore been designed to allow amplification of measurable signals, including, e.g., magnetic functionalized nanoparticles [7].

For example, magnetic nanoparticles coupled to anti-LPS compose an immunonanosensor, with the purpose of capturing Gram-negative bacteria [99]. The use of immunomagnetic beads allows the detection of a biomarker in complex sample matrices, allowing the detection of *E. coli* and *Serratia marcescens* cultures, in addition to *Pseudomonas aeruginosa* and *Acinetobacter baumannii* isolates. In contrast, and confirming bacterial type specificity, Gram-positive bacteria were undetectable [44].

Additionally, an antifouling immunosensor based on Fe$_3$O/Au nanoparticles modified with hyaluronic acid and coated with polyethylene glycol was developed for immobilization of the membrane protein OMP31, a *Brucella* protein, and thus used for detection of anti-*Brucella* antibodies in bovine serum and milk samples [100].

Avian influenza viruses pose a worldwide threat to animal health and can be detected using nanosensors. A quartz crystal microbalance (QCM) immunosensor was recently functionalized with protein A and anti-influenza M1 antibodies conjugated to gold nanoparticles. The nanosensor demonstrated similar sensitivity to conventional molecular methods in detecting all influenza A virus serotypes [101]. In another study, using an electrochemical immunosensor, it was possible to detect and quantify influenza A virus PB1-F2 protein in alveolar epithelial infected cells [102,103].

Immunosensors can also be applied for toxin detection, where rapid identification is urgent, as these are potent biomolecules that can affect animal health, causing economic impacts including livestock production losses [104,105]. Nanotechnology provides a multitude of toxin detection technologies, that provide low detection and portability while remaining low-cost and easy-to-use setups [9]. For example, toxins produced by *Clostridium perfringens* were detected through the sensor produced on an epsilon toxin-specific monoclonal antibody immobilized on carbon nanotubes [106]. This immunosensor was adapted to detect relevant concentrations of the toxin, with sensitivities equivalent to those of ELISA, demonstrating sensitive detection of the *C. perfringens* epsilon toxin.

### 2.2. Detection using nucleic acids

Synthetic nucleic acid probes, such as aptamers and...
oligonucleotides, have been conjugated to, or otherwise incorporated into, nanomaterials in order to act as recognition components (Fig. 2D). Compared to antibodies, molecular-based recognition components have several advantages. Thus, antibodies suffer from low thermal stability, high production costs, and batch-to-batch volatility [107]. Aptamers, in contrast, are non-immunogenic and extremely stable under a variety of circumstances, maintaining their high affinity for the target analytes [108]. Thus, in contrast to antibody-based methods, aptamer-based nanosensors have the potential to reduce production costs by not requiring elaborate processes and infrastructure, such as animal care facilities [108].

Molecular-based nanosensors have been reported for detection of anthrax, a deadly contagious bacterial disease caused by *Bacillus anthracis* that is fatal to ruminants and humans and frequently spreads quickly through tainted food and water [109]. In a study focused on the prevalence of *B. anthracis* spores in small ruminants, these were found to be connected to the bodies of 30% of sheep and 27.5% of goats [110]. There are also other molecular-based diagnostic methods available for *B. anthracis* detection. One of these strategies employed a colorimetric assay with asymmetric PCR/AuNPs amplicon complexes to identify *B. anthracis* [111]. Another study employed a quartz crystal microbalance (QCM) biosensor that was 3.5 × 10^7 CFU.ml⁻¹ sensitive and had modified single-stranded AuNP probes that were specific for Ba813 and *B. anthracis* [112]. Similar to this, it has been reported that a fluorescent aptasensor based on multi-walled carbon nanotubes (MWCNTs) can identify domain 4 of recombinant *B. anthracis* protective antigen (rPAD4) in about 10 min [113].

The hybridization processes between nanomaterial-based probes and pathogens nucleic acids are frequently taken advantage of by molecular-based approaches. For example, the detection of *Brucella melitensis* in milk has been reported using aptamers in conjunction with QCM sensors [114]. Targeting the BCS3P1 outer membrane protein, an oligonucleotide-modified AuNP-based colorimetric test achieved a limit of detection of 10⁵ CFU.ml⁻¹ in bovine samples (urine and semen) and 10⁶ CFU.ml⁻¹ in milk with no cross-reactivity [115]. With unamplified *Brucella* genomic DNA and improved sensitivity of 1.09 pg.ml⁻¹, another target gene sequence, IS711, was found using the same method [116,117] without any cross-reactivity being noticed. The sensitivity was increased to 10 fg with a multiple cross displacement amplification and lateral flow test, using polymer nanoparticles modified with oligonucleotide probes that target the BSC3P1 gene [118].

Toxin detection can also be conducted through molecularly based nanosensors. Aflatoxin B1 (AFB1), a powerful carcinogen with hepatotoxic effects in poultry, cattle, and humans who consume infected animal products, is regarded as the most toxic metabolite [119]. It has already been detected based on variations in differential pulse voltammetry peak current, through an aptasensor comprising Au nanowires, graphene oxide, and an aptamer [119]. Another study created a molecular-based method, using surface-enhanced Raman scattering (SERS), which was more sensitive than other fluorescence techniques, with an increased limit of detection of 0.13 ng.ml⁻¹. This method was based on mesoporous silica nanoparticles modified with a combination of amino groups, aptamers, and Rh6G [120].

2.3. Detection using peptides

Antimicrobial peptides (AMPs) are used in a variety of bio-recognition techniques (Fig. 2C). For example, magnetic nanoparticles modified with the AMP bacitracin A are capable of capturing bacteria [121]. In doing so, it was demonstrated that pyrophosphate groups in the bacterium’s lipid targets, potentially strengthened by sodium and zinc ions, are responsible for the interactions between bacitracin A and bacteria. Surface-enhanced Raman spectroscopy (SERS) tags attached to the collected bacteria was employed for their detection after magnetic separation. It was possible to differentiate between *E. coli*, *P. aeruginosa*, and *Staphylococcus aureus* using SERS spectra.

Furthermore, fluorescence-labeled AMPs as a substitute for labeled antibodies in the detection of *E. coli* O157:H7 have been demonstrated to outperform antibodies [122]. Thus, the fluorescent dye cy5 was used to mark the AMPs cecropin P1, SMAP29, and PGQ before they were tested using a cell binding assay. It was discovered that cy5-cecropin P1 out-performed cy5 labeled with an anti-E. coli O157:H7 antibody in detecting target bacteria by a 10-fold factor [122].

Moreover, clavanin A is an AMP that has been investigated for its potential to recognize biological targets. Au nanoparticles that had been chemically modified and functionalized with clavanin A were used to build an electrochemical biosensor for detection of *Salmonella typhimurium* and *E. coli* [123]. Another study also developed an electrochemical biosensor based on Au nanoparticles functionalized with clavanin A for Gram-negative and -positive detection, achieving a limit of detection of 10⁵ CFU.ml⁻¹ for *E. coli*, Klebsiella pneumoniae and *S. typhimurium* [124]. As an impedimetric platform for bacterium detection, clavanin A was conjugated with carbon nanotubes as a label-free biosensor capable of detecting *K. pneumoniae*, Enterococcus faecalis, *E. coli* and Bacillus subtilis with a limit of detection lower than 10⁴ CFU.ml⁻¹ [125]. An electrochemical AMP-based biosensor was employed also to identify *Candida* strains quickly and accurately, 90. The sensor device, based on poly (3-thiophene acetic acid) nanofilms, titanium dioxide nanoparticles and clavanin A, was capable of identifying *Candida* species and whether these strains were in the haploid or diploid state. *Candida albicans* and *Candida tropicalis* presented the greatest response, with a limit of detection of 10⁶ CFU.ml⁻¹.

Mastoparans and ubiquicidin have also been investigated for their potential to detect pathogens. Synoeca-MP, an AMP from the mastoparan family, was evaluated as the recognition element in the detection of *E. faecalis*, *K. pneumoniae*, *P. aeruginosa*, and *C. tropicalis*. The electrochemical biosensor combined chitosan-coated iron oxide magnetic nanoparticles with the AMP and was able to detect bacteria and fungi with a limit of detection of 10⁴ CFU.ml⁻¹, presenting a promising tool for microbial detection in liquid samples [126]. A different study similarly found that a chemically altered form of ubiquicidin, in the form of a tri-branched dendrimeric scaffold, allowed fluorophore-enhanced detection using optical endomicroscopy, selectively killed pathogenic bacteria, such as *S. aureus*, *K. pneumoniae*, *E. coli*, *P. aeruginosa*, and the fungus *Aspergillus fumigatus*, with a limit of detection of 10⁵ CFU.ml⁻¹ [127].

2.4. Other recognition modes in nanosensors

As discussed above, antibodies, aptamers and peptides are frequently used as biorecognition components. Less frequent, but emerging as promising alternatives, are bacteriophages and enzymes, which have been proven to be efficient for biorecognition applications.

Bacteriophages have the potential to be used as diagnostic tools and treatments for bacterial infection (Fig. 2A) because of their great selectivity, which is conferred by receptor binding proteins (RBPs) present on the bacteriophage surfaces with which they target bacteria [126,129]. Bacteriophages have high selectivity due to an intrinsic targeting function that is comparable to that of antibodies. Bacteriophage-based sensing platforms are still in the early stages of development compared to other biorecognition elements, but their high target specificity makes them interesting candidates for biorecognition [130]. Exemplifying this, magnetic nanoparticles were functionalized with a high density of T4 bacteriophages [131] and then used to catch *E. coli*. T4 bacteriophages infect *E. coli* and, thanks to their tail fibers, have specificity to *E. coli* type B. The authors revealed the significant effects of tryptone in the medium and temperature of incubation used for growing *E. coli* on the capabilities of MNP capture probes. Enzymes are organic, naturally occurring molecules with an inbuilt capacity for achieving analyte specificity [132]. Enzymes use binding cavities in their structure to become specific to a bioanalyte. Red fluorescence gold nanoclusters (AuNCs) functionalized with lysozyme were created [133] via a microwave-assisted technique, and their potential
usage in an *E. coli* visual detection nanosensor was proven. Lysozymes are useful because it stabilizes the AuNCs by reducing Au ions. Furthermore, lysozymes are antibacterial enzymes that can recognize and eradicate a variety of bacteria [134]. The fluorescence increases in the lysozyme functionalized AuNCs, and the high *E. coli* lysozyme specificity allowed the nanosensor to detect *E. coli* quickly and accurately with reliable sensitivity. The benefits and drawbacks of utilizing enzymes as biorecognition components are comparable to those of using antibodies. They have superior affinity properties to targets due to their natural origin, but they are unstable and challenging to isolate and process in a laboratory setting. Due this difficulty, the use of enzymes as biorecognition elements in diagnostic applications remains limited and requires further research [130].

3. Translating nanosensors from proof-of-concept studies to agricultural industrial application

With all its recent developments, nanotechnology is emerging as one of the most important technologies in the recent agricultural business. This technology will serve as a driving economic force in changing current agricultural methods. Novel crop enhancement and productivity delivery methods have the potential to reduce the use of bulk agro-chemicals and hence provide a more cost-effective alternative, besides the rapid and selective plant pathogen and pesticide detection by nanosensors [135]. Nanosensors have the potential to provide precise measurements to optimize plant growth and productivity in agriculture, forestry, and research. Stakeholders, such as farmers and scientists, are eager to use these novel analytical tools to guide management decisions, but there are few examples of nano-based sensors on the market (Table 2). A network of nanosensors can measure agricultural productivity and identify numerous diseases in the field. Recently, there have been several ideas to link these nanosensors with information technology in order to communicate the results to far locations, hence benefiting farmers in rural places [136]. Otherwise, the use of nanosensors in the food business is rapidly expanding. Nanosensors, for example, have been utilized in the food industry to assure food safety throughout manufacturing and packaging [137] and to detect pathogens.

However, there are still barriers to extensive, real-world applications, especially related to the nano-sensing elements integration into analytical equipment and industrial-scale production. The lack of knowledge about the health consequences of nanomaterials, as well as the expensive costs of various raw materials, has harmed the commercial impact of nanosensors [27]. The accompanying expensive production and scale-up expenses of nanomaterials in a low-margin product industry have worsened this. Furthermore, the lack of defined markets required to make nanosensors appealing to investors and manufacturers may be contributing to the slow speed at which nanosensors progress from proof-of-concept to full-field deployment [27]. However, nanosensors continue to pique industry and public health authorities interest, and a number of projects are underway to generate nanomaterials utilizing more cost-effective methods and sources. Employing nanosensors in conjunction with information technology can aid farmers in rural places [138]. Connecting the nanosensor with the global positioning system can help farmers in remote areas manage the fertilizers, pesticides, insecticides, water levels, physical and chemical stresses, as well as pathogen detection to prevent the onset of many diseases in on-site crops in the fields [139].

4. Future perspectives

The future perspectives of using nanosensors in animal disease detection are promising, with anticipated advancements that may revolutionize veterinary diagnostics. Sensor-based technology have made significant contributions to reducing animal stress, enhancing animal well-being, and, as a result, eliminating economic losses [140]. By predicting future disease outbreaks, early identification of physiological reactions can assist farmers in taking targeted interventions to alleviate pressure on their animals. Several unique sensor-based approaches are still in the exploratory stage of development on the technological front, such as microRNA-based sensors for detecting bovine respiratory syncytial virus [141,142], sensors targeting salivary hormones such as Luteinizing Hormone for bovine estrus detection [143], and electrochemical sensors for detecting antibodies against influenza A and B [144]. For specific physiological situations, these rely on particular biomarkers. None of the commercially available commercial devices and sensor systems (Table 2) meet the requirements of the livestock sector in terms of size, functionality, and wearability, neither allow for animal movement while simultaneously measuring physiological parameters such as respiratory and heart rate. This disparity necessitates a study into the design and development of sensing systems for the next generation of precision livestock husbandry. Continued research is expected to yield nanosensors with enhanced sensitivity, enabling early disease detection and personalized diagnostics. Portable and user-friendly nanosensor devices could facilitate widespread adoption in point-of-care and in-field applications, transforming the landscape of animal health monitoring. Integration with artificial intelligence and targeted drug delivery systems may lead to real-time monitoring, predictive analytics, and precise therapeutic interventions.

5. Conclusions and outlook

The wellbeing of farm animals is crucial for human survival. Since most food products, such as milk, cheese, butter, meat, and eggs, come from animals, any infectious outbreaks among them have a direct and profound impact on human health. Designing inventive, distinctive, highly focused, and sensitive detection devices that can examine undesirable or disease-causing components, even at nanomolar quantities in complicated biological systems, is necessary. These technologies are accessible in clinics and on a laboratory scale. Researchers are currently working very hard to commercialize sophisticated nanosensors based on nanomaterials for humans and modifications for livestock, aiming to use them for the detection of infections, antibiotics, mycotoxins, body metabolites (glucose, uric acid, lactate, and others), bacteria, and other pathogens.

Nanosensors have demonstrated significant utility for various animal diseases diagnosis. These advanced sensing platforms have been successfully applied to detect pathogens and biomarkers associated with diseases such as avian influenza [33,144], bovine respiratory diseases [38,145], and bovine tuberculosis [56]. Nanosensors leverage specific recognition elements and transduction mechanisms to achieve highly sensitive and selective detection, offering promising solutions for precise diagnostics.

There is a considerable demand for development of portable, compact, multitargeting technologies that may be utilized directly in the field by veterinarians. To be employed outside of research laboratories, a biosensor must usually be adapted for usage in field conditions in order to limit the possibility of false positive or negative results. Among the detection technologies discussed, nucleic acid-based biosensors appear to be particularly well suited for rapid and sensitive testing. Their limitations are found in sample preparation stages such as nucleic acid extraction and amplification. The analytical properties of antibody- and aptamer-based biosensors are heavily dependent on the selectivity of these biomolecules as well as their stability following immobilization on the sensor surface. Moreover, biosensors technology incorporating nanotechnologies will eventually become a powerful systemic device for pathogen detection and monitoring system to manage and prevent animal disease outbreaks.

Nanosensors in animal disease detection offer notable benefits, including heightened sensitivity and rapid detection due to their nanoscale dimensions and tailored properties. Nanosensors, often functionalyzed with specific recognition elements, enhance selectivity by
precisely targeting biomolecules associated with diseases in animals. The nanoscale dimensions of these sensors contribute to heightened sensitivity, allowing for the detection of target analytes at low concentrations, which might go undetected by conventional methods. Nanosensors potency lies in their ability to provide rapid and real-time results, enabling timely intervention. Conventional methods, while well-established, may lack the precision and speed offered by nanosensors. The capacity for multiplexed detection and reduced sample volumes is advantageous, providing comprehensive analyses with minimal sample requirements. Furthermore, nanosensors may enable in vivo monitoring and integration with advanced technologies, enhancing diagnostic precision. However, challenges include potential high fabrication costs, complex functionalization processes, and concerns about biocompatibility and toxicity. Standardization issues and the need for ethical guidelines pose additional considerations. Balancing these benefits and limitations is critical for optimizing nanosensor applications in veterinary diagnostics, with ongoing advancements expected to address current challenges and unlock their full potential.

Nanotechnology will revolutionize veterinary science and technology in the future, increasing livestock output and filling knowledge shortages for animal welfare. Despite its numerous applications, nanotechnology has some limits in diagnostics, bioimaging, and food safety since it necessitates extremely sensitive, degradable, inexpensive, and cost-effective equipment, as well as less harmful nanoparticles. Overall, it is essential to continue studying different nanomaterials to develop promising new strategies for detection systems in order to achieve better results, as they can indirectly impact human health.

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The authors confirm their contribution to the paper as follows: T.R. wrote the main manuscript text and prepared figs. M.M. and G. F. prepared figs. O. F. was involved in planning and supervising the work. All authors provided critical feedback and helped shape the final version of the manuscript.

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Thuanny Borba Rios: Writing – original draft, Writing – review & editing. Mariana Rocha Maximiano: Writing – original draft, Writing – review & editing. Gabriel Cidade Feitosa: Writing – original draft. Martin Malmsten: Supervision. Octávio Luiz Franco: Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

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