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Review

Aptamer-based diagnostic and therapeutic approaches in animals: Current potential and challenges

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ABSTRACT

Fast and precise diagnosis of infectious and non-infectious animal diseases and their targeted treatments are of utmost importance for their clinical management. The existing biochemical, serological and molecular methods of disease diagnosis need improvement in their specificity, sensitivity and cost and, are generally not amenable for being used as points-of-care (POC) device. Further, with dramatic changes in environment and farm management practices, one should also arm ourselves and prepare for emerging and re-emerging animal diseases such as cancer, prion diseases, COVID-19, influenza etc. Aptamer – oligonucleotide or short peptides that can specifically bind to target molecules – have increasingly become popular in developing biosensors for sensitive detection of analytes, pathogens (bacteria, virus, fungus, prions), drug residues, toxins and, cancerous cells. They have also been proven successful in the cellular delivery of drugs and targeted therapy of infectious diseases and physiological disorders. However, the in vivo application of aptamer-mediated biosensing and therapy in animals has been limited. This paper reviews the existing reports on the application of aptamer-based biosensors and targeted therapy in animals. It also dissects the various modifications to aptamers that were found to be successful in *in vivo* application of the aptamers in diagnostics and therapeutics. Finally, it also highlights major challenges and future directions in the application of aptamers in the field of veterinary medicine.

Contents

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1. Introduction

Infectious diseases of livestock, particularly highly infectious diseases, almost always cause major economic losses to the livestock owners in particular and the country as a whole (Raboisson et al., 2020). Transmissible livestock diseases caused by pathogenic microorganisms such as viruses and bacteria have a negative impact on their health and well-being, limit their production, and often result in mortality, resulting in significant economic losses in the respective field.

Prompt diagnosis and proper treatment of livestock diseases is an integral aspect of disease management and prevention, and it decreases economic risk to dairy farmers. Precise diagnosis of animal diseases is an important step between the cause and cure of disease. The role of animal disease diagnostics is important not only for the welfare of animals and farmers but also for consumers' health. There are various animal diseases that can transmit to humans such as anthrax, rabies, tuberculosis, swine/bird flu, foot and mouth disease (FMD) and so on. The zoonotic diseases continue to be a concern today with recent outbreaks of severe acute respiratory syndrome corona virus (SARS-CoV) and influenza (H1N1). The SARS-CoV-2 of the current COVID-19 (Coronavirus Disease 2019) pandemic has also been detected in dogs, ferrets, cats (Shi et al., 2020)), and tigers, though infection from animal to human has not yet been verified. Viral and bacterial infections can cause serious diseases to livestock and humans. Precise and early detection of the pathogen is often crucial for clinical diagnosis and effective treatment.

An early diagnosis of the disease is a pre-requisite for effective treatment and management of animal diseases. In most cases the diagnosis and differential diagnosis of animal diseases are done based on symptoms and basic biochemical, microbial, parasitological and serological tests of biological samples. Additional confirmatory tests include ELISA, PCR, DNA chips and chromatographic techniques, which require sophisticated instruments, professional operators, and costly chemicals. More recently, newer POC biosensors have emerged as newer diagnostic tools but many of them suffer from low sensitivity, specificity and reliability (Sheikhzadeh et al., 2021; Wang et al., 2021). Biosensors are rapidly becoming a cutting-edge novel technology in disease diagnosis and treatment. Biosensors are analytical devices that use biological molecules to generate signals that can be used to detect a specific disease condition (Duarte et al., 2015). Biosensors have two important features such as selectivity and sensitivity (Seo and Gu, 2017). With the accumulating knowledge on the development of aptamers and aptasensors, they have been increasingly investigated and tested for the detection of various infectious agents, including bacteria and viruses, as well as their toxins.

Aptamers are single-stranded nucleic acids (DNA, RNA) having molecular recognition properties similar to antibodies that are usually 22–100 nucleobases long and consist of a variable region edged by constant regions, which allow for amplification and identification of sequences (Gu, 2016; Kanwar et al., 2011; Simmons et al., 2012). Aptamers have several applications like monoclonal antibodies such as biomarker identification, therapeutics, diagnostics, and high throughput screening (Green et al., 2001; Shigdar et al., 2011). Yet, by virtue of their biophysical properties, aptamers compete with antibodies in many aspects (Proske et al., 2005). Literature for the present review was collected through searched in PubMed, google using key words, aptamers, biosensors, animals disease diagnosis, drug residue, COVID19 etc. and manually from the institute library.

1.1. Aptamers synthesis technique

Aptamers can be chemically synthesized and have shown specificity comparable to antibodies for antigen capture and identification, making them excellent alternatives to antibodies (Thiviyanathan and Gorenstein, 2012). Thus, they are occasionally referred as "synthetic antibodies" and have been competing with antibodies in the research of disease diagnosis, prophylactics, and therapeutics. They offer advantages of reduced cost, smaller size, ease of production and modification, and improved stability over antibodies (Song et al., 2008). Consequently, a number of aptamer-based platforms have been developed in the form of biosensors (called aptasensors) for detection of pathogen and target analytes, immunophenotyping and management of animal health (Table 1).

The conventional SELEX (Systematic Evolution of Ligands by Exponential Enrichment) technology was described by Tuerk and Gold (1990). Formation of aptamers by this method lasts from a few weeks up to a month. A library of oligonucleotides containing unique but random sequences was designed by chemical method used to select aptamers and usually used to hybridize primers during polymerase chain reaction (PCR). Many steps of selection introduced during the conventional method of SELEX solely depend on the type of the desired aptamer (Fig. 1).

Nucleic-acid aptamers are paying attention towards intense interest and have found various applications in different areas. Extensive research is available on applying aptamers in the diagnosis of diseases in human beings, but very limited data is available in veterinary medicine. Therefore, this review is aimed to focus on the application of aptamers in the diagnosis of animal diseases, detection of drug residues and contaminants, and targeted drug delivery.

Table 1
Summary of aptamers/biosensors in veterinary medicine.

S. No.	Aptamer type/Biosensor type	Target/Disease condition	Reference
RNA aptamers			
1	RNA aptamer	N protein/sensitive detection of SARS coronavirus nucleocapsid protein	Ahn et al. (2009)
2	RNA aptamer	Fluorescence-based detection of the influenza A H1N1 virus using a sandwich-based aptamer assay	Tseng et al. (2016)
3	RNA aptamer	Diagnosis of FMD virus	Ellingham et al. (2006)
4	RNA aptamer	Detection of Shiga toxins of *E. coli.*	Challa et al. (2014)
5	RNA aptamer	Detection of capsid protein of the Porcine *circovirus type* 2.	Yoon et al. (2010)
6	RNA aptamer	Development of aptamers which specifically targets ATR/TEM8 Von Willebrand factor type A (VWA) domain.	Lee et al. (2015)
7	20′-F-RNA aptamer	Detection of *E. coli* strains	So et al. (2008)
8	20′-F-RNA aptamer	Detection of *E. coli* using dual strategy	Lee et al. (2009)
9	RNA Spiegelmer	NOX-B11-3, a RNA aptamer can suppress appetite by specifically binding to orexigenic hormone ghrelin and blocking its activity.	Becskei et al. (2008)
10	RNA Spiegelmer	Used to block the activity of anorectic and dipsogenic pancreatic hormone amylin.	Bilik et al. (2007)
DNA aptamers			
11	DNA aptamer	Detection of *avian influenza* viruses	Lee et al. (2020)
12	DNA aptamer	Detection of H5N1 *avian influenza* virus	Wu et al. (2008)
13	DNA aptamer	Detection of triple reassortant *influenza A* virus	Wongphatcharachai et al. (2013)
14	DNA aptamer	Diagnosis of *Bovine herpesvirus 1*	Xu et al. (2019)
15	DNA aptamer	Detection of FMD virus	Vidic et al. (2017)
16	DNA aptamer	Competitive fluorescence resonance energy transfer (FRET)-aptamer-based strategy for foot-and-mouth disease (FMD)	Bruno et al. (2008)
17	DNA aptamer	Detection of *E. coli* strains K88	Li et al. (2011)
18	DNA aptamer	Detection of *Salmonella enterica* serovar.	Joshi et al. (2009)
19	DNA aptamer	Nanoparticle-based two-stage aptasensing platform for *Campylobacter jejuni* and *Campylobacter coli.*	Kim et al. (2019)
20	DNA aptamer	Detection of a TB biomarker HspX in sputum.	Lavania et al. (2018)
21	DNA aptamer	Detection of prion diseases	Wang et al. (2011)
22	DNA aptamer	Detection of prion protein on a solid-phase membrane.	Hossain et al.(2010)
23	DNA aptamer	Detection of prion protein with very high sensitivity.	Wang et al. (2012)
24	DNA aptamer	Detected a prion protein of BSE in the CNS.	Antipin and Nadtochey (2019)
25	DNA aptamer	Prion proteins (PrP) detection through the formation of T-Hg(2+)-T configuration	Xiao et al. (2012)
26	DNA aptamer	Electrochemical aptasensor for the detection of sulfadimethoxine in veterinary medicine and milk.	Bai et al. (2019)
27	DNA aptamer	Competitive voltammetric aptasensor for azlocillin β-lactam antibiotic.	Chinnappan et al. (2020a)
28	DNA aptamer	Detection of *Bovine herpesvirus 1*	Xu et al. (2019; 2017)
ssDNA aptamers			
29	ssDNA aptamer	Detection of SARS coronavirus nucleocapsid protein	Cho et al. (2011)
30	ssDNA aptamer	HN protein of *Bovine Parainfluenza* Virus type 3	Cheng et al. (2018)
31	ssDNA aptamer	Detection of *avian influenza* H5Nx whole viruses	Nguyen et al. (2016)
32	ssDNA aptamer	Detection of Muscovy duck parvovirus	Lu et al. (2018)
33	ssDNA aptamer	Detection of *Staphylococcus aureus*	Baumstummler et al. (2014)
34	ssDNA aptamer	An enzyme linked aptamer for the detection of P48 protein of *M. bovis*	Fu et al. (2014)
35	ssDNA aptamer	Detection of TB from sputum samples	Rotherham et al. (2012)
36	ssDNA aptamer	Analytical assay for detection of brevetoxin.	Tian et al. (2016)
37	ssDNA aptamer	Combined the aptamer with electrochemical SPR to develop an aptasensing platform for detection of ampicillin in real time.	Blidar et al. (2019)
38	ssDNA aptamer	Aptamer based enzyme immunoassay was developed for the determination of enroflaxacin	Ni et al. (2014)
39	ssDNA aptamer	Developed an aptameric biosensor for the sensitive detection of tropomyosin allergen.	Chinnappan et al. (2020b)
Others			
40	Self-quenched probe based strategy	Sensitive detection of *Pseudorabies* virus	Shen et al. (2018)
41	Mannose-modified fluorescent carbon quantum dots (Man–CQDs)	Detection of bacteria using novel dual recognition strategy	Cheng et al. (2016)
42	NH_2 based aptamer	Impedimetric aptasensor for ultrasensitive detection of *Pseudomonas aeruginosa*	Roushani et al. (2019)
43			
44	Biotin tagged aptamer	Mastitis detection	Ashley and Li (2013)
45	Colorimetric biosensor	Detection of plasmin as biomarker in case of mastitis	Chinnappan et al. (2017)
46	Colorimetric aptasensor	Detection of *E. coli* strains	Wu et al. (2012)
47	Fluroscent probe system	Quantum dot-based aptamer beacon for tumor cells.	Hwang et al. (2016)
48	Electrochemical biosensor	Direct detection of *E. coli* O111.	Luo et al. (2012)
49	Photoelectrochemical (PEC) immunosensor	Nanoparticles based aptamer for detection of Ochratoxin A.	Chen et al. (2018)
50	SPR biosensor	Whole bacteria detection	Ahn et al. (2018)
51	Biotin labeled DNA	For detection of *Salmonella enterica* serovar *Typhimurium*	Dwivedi et al. (2013)
52	Prion protein aptamer	Determination of BoIFN-γ in TB	Crulhas et al. (2017)
53	Specific aptamers of antibiotics	Specific aptamers for detection of antibiotics such as Florfenicol, Cefquinome, Kenamycin, Oxytetracyclin, Tetracyclin and Chloramphenicol.	Sadeghi et al. (2018); Wang et al. (2018); Xu et al. (2015); Zhou et al. (2018); Yan et al. (2018)

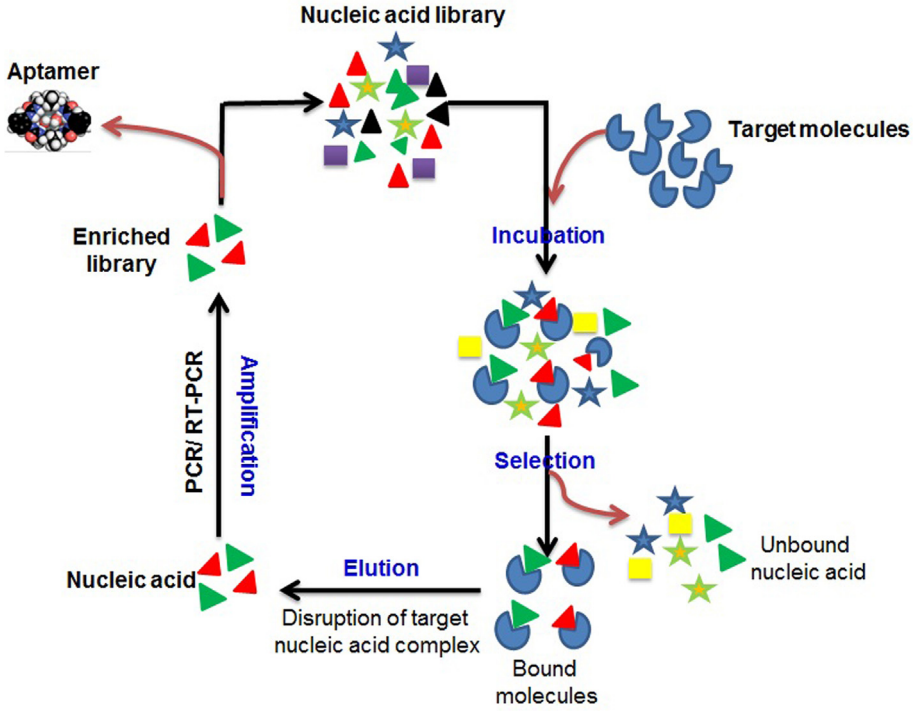

Fig. 1. Schematic representation is showing traditional SELEX procedure. A random nucleic acid (RNA/DNA) library is incubated with a target molecules and then unbound molecules are separated from the bound molecules by the process of selection. Eluted nucleic acid are amplified by PCR and serve as an enriched library for the next cycle.

2. Application of aptamers in diagnosis of animal diseases

There are various bacterial (e.g. mastitis, tuberculosis, enteritis or colibacillosis, tuberculosis etc.) and viral diseases (e.g. SARS CoV, influenza, FMD etc.) that have a high prevalence rate in animals and are affecting farmers' economies in many ways (Mendes et al., 2020; Puerto et al., 2021; Tsouloufi et al., 2020). Other conditions, such as bovine spongiform encephalopathy (BSE), cancer, and toxins, play important roles in veterinary medicine in addition to infectious diseases.

2.1. Diagnosis of viral diseases

2.1.1. Severe acute respiratory syndrome corona virus (SARS-CoV)

2.1.1.1. Influenza. Influenza viruses have various subtypes such as H1N1, H2N2, H5N1, H7N2, H9N2, which has global concern, and there is a need to develop methods for the early detection of these viruses to prevent loss in poultry and other agricultural sectors. The use of aptamers for the diagnosis of the virus has been extensively studied. Several aptamers have been reported to be effective in several viruses such as Muscovy duck parvovirus (Lu et al., 2018), H5Hx influenza viruses (Nguyen et al., 2016), H5N1 avian influenza virus (Wu et al., 2008), Triple reassortant influenza A virus (Wongphatcharachai et al., 2013), Bovine herpesvirus 1 (Xu et al., 2019), pseudorabies virus (Shen et al., 2018) etc.

In this connection, Cheng et al. (2018) proposed an indirect enzyme-linked aptamer assay (ELAA) for the detection of viruses in cattle. They developed ssDNA aptamers that can rapidly detect HN protein of Bovine Para-influenza Virus type 3 with high affinity and specificity. The positive detection rate of the ELAA was higher than that of the commercial ELISA kits, and the coincidence rate of ELAA and ELISA was 88%. A real time immuno-polymerase chain reaction (RT-I-PCR) method has also been developed for detecting viruses such as H9N2 influenza virus in poultry. In the assay, aptamers are applied as ligands to capture and detect the virus with higher sensitivity than standard ELISA (Hmila et al., 2017).

The particular diagnosis of avian influenza H5N2 whole viral particles has also been possible using a cognate pair of sandwich-type aptamers. In this strategy, a pair of aptamers were selected for whole avian influenza virus particles of H5N2 as the analyte of interest by using graphene-oxide-based SELEX (GO-SELEX) (Kim et al., 2019). The lateral flow strips could detect the particles as low as 6×10^5 in the buffer and 1.2×10^6 in the duck's feces by direct examination. Such aptamer-based test strips can thus, be useful for rapid screening and preventing pandemics. Sandwich-type SPR- based aptasensor has also been used to diagnose avian influenza H5Nx whole viruses (Nguyen et al., 2016). Seo and Gu (2017) have also critically reviewed the literature on aptamer-based sandwich-type biosensors.

Several studies have also used electrochemical aptasensors for the detection of avian influenza viruses. To avoid the electrochemical signal-based errors due to external interferences, Lee et al. (2020) utilized a self-calibrating dual-electrode system based electrochemical aptasensor to detect avian influenza viruses. They fabricated two electrodes using tungsten rods, and methylene blue was used as an electron mediator. One electrode was capped with the virus-specific aptamer, whereas the second electrode was capped with a control aptamer. This dual-electrode platform exhibited excellent output-signal stability compared to a conventional single-electrode platform and did not require purification and washing steps. Thus, using such dual electrodes for the detection of electrochemical signals from aptasensors offers a reliable and robust platform for detecting molecules.

A new approach was adopted for fluorescence-based detection of H1N1 virus by sandwich-based aptamer assay (Tseng et al., 2016), which is comparatively much faster than the conventional viral culture.

2.1.2. Foot and mouth disease (FMD)

FMD mainly affects the animals such as swine, cattle, goats, deer, and sheep. FMD affects extensive areas worldwide enlisted in the notifiable diseases to the World Organization for Animal Health (http://www.oie.int/eng/en index.htm). Since it was recognized as a significant epidemic disease in 16th century to yet it is a major global animal health problem (Longjam et al., 2011). FMD has caused numerous outbreaks in the US, UK and Asia including India. Owing to significant economic losses there is an urgent need to develop a fast and most unswerving test for the detection of FMD virus. In order to detect this disease, DNA aptamers were successfully screened (Vidic et al., 2017).

Aptamers have also been used for selective detection of pathogenic viruses. Bruno et al. (2008) developed a FRET based novel aptamer for FMD and labelled it with Black Hole Quencher-2 and Alexa Fluor 546-14-dUTP dye. The detection principle was based on competitive fluorescence energy transfer (FRET) and could detect FMD within 10 min with a sensitivity of 25–250 ng/mL of a FMD peptide. Ellingham et al. (2006)have been selected RNA-dependent RNA polymerase (3Dpol) enzyme for RNA aptamers specific to FMD.

2.1.3. Severe acute respiratory syndrome corona virus (SARS-CoV)

The recent pandemics and epizootics in various parts of the world have highlighted the public health importance of viruses such COVID-19 (SARS-CoV-2), influenza virus, herpes viruses etc. At the beginning of January 2020, a novel coronavirus (COVID-19) was identified in Wuhan, China as a causal agent of an outbreak of respiratory disease. SARS-CoV-2 (Severe Acute Respiratory Syndrome - Coronavirus-2) is a member of *Coronaviridae*, which is the responsible agent for COVID-19. SARS-CoV-2 is an enveloped virus measuring approximately 50–200 nm in diameter, having single strand RNA genome consists of 26–32 kb in length (Lu et al., 2020; Xu et al., 2020). WHO declared this disease a pandemic on 11 March 2020. COVID-19 has presently spread in the 222 countries/territories with the death of 3.27 millions out of total positive cases, which accounts for more than 156.75 millions upto 07 May 2021. While in India alone, 2.15 millions are infected, and 0.234 millions deaths have been reported. As per reports, strains of SARS-CoV-2 are showing mutations in different countries. A recent study at Peking University in Beijing reported two types of strains: "L-type" and "S-type". It has been suggested L-type strain is "more aggressive" and jumped from animals to humans.

Few studies have reported the possible connections with animals in the origin of SARS-CoV-2 and reverse zoonosis. However, a recent study in China investigated the possibilities of the vulnerability of ferrets and animals in close connection with humans to SARS-CoV-2(Shi et al., 2020). They found that replication of SARS-CoV-2 is very less in dogs, pigs, chickens, and ducks but efficiently in ferrets and cats. It has also been reported that the virus spreads in cats via respiratory droplets. Wildlife Conservation Society releases one report, the USA on 05 April 2020 that a tiger at the Zoo found positive for COVID-19.

Recently, WHO conducted a two-day meeting on COVID-19 and highlighted various issues related to the importance or possible linkage of COVID-19 with animals (WHO, 2020). Various researchers have also tried to develop aptamers for the diagnosis of SARS-CoV. Ahn et al. (2009) have been developed a RNA aptamer, is capable to bind with N protein with high affinity with a K(d) of 1.65 nM (nano Molar), using a SELEX and recombinant N protein. K(d) value denotes the equilibrium dissociation constant between the antibody and its antigen. The strength of a given interaction can be judged through the association constant K or the dissociation constant Kd. The nucleocapsid protein binding ssDNA aptamer was developed with Kd value of 4.93 ± 0.30 nM by SELEX procedure (Cho et al., 2011).

The aptamer can potentially be used in the diagnosis of COVID19 in humans as well as animals. A recent paper on POC diagnostic devices for rapid detection of novel coronavirus (SARS-nCoV19) has been reviewed all possible recent diagnostic tests including aptamer/biosensors based tests for COVID19 (Rao, 2021). About 900 SARS-nCoV-2 test kits are in pipeline, among 395 test kits are molecular bested test kits and only few test kits are developed using Aptamer technology https://www.finddx.org/covid-19/pipeline/. (Rao, 2021).

Rapid microbiology has developed "Pinpoint's Low-Cost Handheld COVID-19 Aptamer-based Diagnostic Device" which is an accurate COVID-19 diagnostic test that non-clinicians can execute in human being. Moreover, there is no widespread of COVID19 in the farm and pet animals except few studies of possible transmission from humans to animals; hence research in this direction is still awaited. Chen et al. (2018) have developed a novel method for the detection of SRAS-CoV-2N protein using DNA based aptamers. However, the diagnostic performance, such as sensitivity and specificity needs further investigation.

2.2. Diagnosis of bacterial diseases

2.2.1. Mastitis

Bovine mastitis can be well-defined as 'inflammation of mammary gland' and is one of the most common and dreadly diseases of dairy cows and poses substantial economic loss to dairy industry. The estimated total economic loss of US$ 2 billion per year in the United States of America Viguier et al. (2009) due to mastitis. The estimated annual economic loss due to both subclinical and clinical mastitis in India was US$ 98,228 million [7165.51 crore Indian Rupees] (Bansal and Gupta, 2009). It is among the most critical diseases of cattle and buffalo because it causes numerous complications to milk production, processing of milk and quality of milk and its products, resulting in severe economic losses to the dairy industry. Mastitis is caused by more than 200 infectious microorganisms and commonest pathogens are *Staphylococcus aureus*, *E. coli*, *Streptococcus* spp., *Pseudomonas* spp., *Mycoplasma* spp. etc. (Sharma and Maiti, 2010; Sharma et al., 2012; Zhang et al., 2009).

Mastitis poses major problem in dairy farms regardless of various prevention and control methods (Sharma et al., 2018). Delay in diagnosis and treatment of mastitis are the major factors in the failure of treatment protocols (Down et al., 2017; Vasquez et al., 2017). It has been shown that timely and accurate diagnosis of mastitis is a major key for prevention and control of mastitis and its effective treatment (Down et al., 2016). Various methods of diagnosis of mastitis have been developed (El-Sayed et al., 2017). Current methods for detecting clinical and particularly subclinical mastitis (e.g., California mastitis test, somatic cell count, and bacteriological culture) (Sharma et al., 2018; Sharma et al., 2011) are either insufficient, have low accuracy, or have low specificity (Robles et al., 2021; Windria et al., 2021; Zecconi et al., 2021). The various conformist methods for detection and identification of pathogens are generally either time-consuming or expensive for the dairy farmers at their farms in field conditions. Recent advances and developments in micro- and nanotechnologies have opened the window for the development of biosensors (Martins et al., 2019).

The sensor-based diagnostic systems help in detecting the intramammary infection with minimal stress on the animal (Martins et al., 2019). The various emerging technologies, including transcriptome and proteome analyses and nano- and microfabrication of handy portable devices, offer promising, effective methods for early diagnosis of mastitis (Duarte et al., 2015). Change et al. (2016) developed aptamer-coated magnetic beads and antibiotic-capped gold nanoclusters for the detection of bac-

teria. Baumstummler et al. (2014) identified SOMAmer (slow off-rate modified aptamer) to detect *S. aureus*. SOMAmers are made from ssDNA that contain pyrimidine residues and have quite long (>30 min) dissociation rates (Gold et al., 2010). They found that SpA and ClfASOMAmers are useful for the selective detection of *S. aureus*. Another study used ELONA (Enzyme-Linked OligoNucleotide Assay) to detect the Protein A-binding aptamer PA#2/8 in *S. aureus* (Stoltenburg et al., 2016). Roushani et al. (2019b) have also developed an impedimetric aptasensor for ultrasensitive detection of *Pseudomonas aeruginosa* using a glassy carbon electrode. The glassy carbon electrode was modified by electrodeposition of silver nanoparticles and NH_2aptamer was covalently attached to its surface. Using such an aptamer-coated electrochemical probe, *P. aeruginosa* could be detected in the range of 10^2–10^7 CFU/mL with a detection limit of 33 CFU/mL in serum samples.

Mycoplasma bovis (*M. bovis*) is a disease causing agent that causes mastitis, respiratory diseases and arthritis in cattle. Mycoplasmas do not have cell wall, which is the reason that many antibiotics are not effective against Mycoplasma. Various methods such as culture, FAT, and PCR are used for the diagnosis of Mycoplasmosis, but recently developed biosensors can detect Mycoplasma infection rapidly, easily and with a high specificity level. In a study, ssDNA aptamers were selected against P48 protein of *M. bovis* with high affinity and specificity (Fu et al., 2014).

During the inflammation of mammary gland various enzymes are released from the inflammatory cells, which may be used as mastitis markers. Guliye et al. (2002) emphasized that high levels of enzyme activity related to quality of milk are associated with damaged udder cells and have been used as an evidence of the degree of udder inflammation. Catalase acts as an indicator of increased milk somatic cell count (SCC) and mastitis, and also works as an antioxidant in the milk. It was demonstrated that catalase activity can be used as a marker of mastitis (Fox and Kelly, 2006).

An aptamer-based surface plasmon resonance (SPR) biosensor with a detection limit of 20.5 nM has been developed for detecting catalase in milk as a mastitis marker (Ashley and Li, 2013). Aptamer tagged with biotin was immobilized onto a gold platform by affinity capture. While Ashley et al. (2012) developed catalase aptamer by non-SELEX method for detecting catalase in bovine milk with a K_D value in the low micro molar range. A recent study of Chinnappan et al. (2017) developed magnetic nanoparticles-based colorimetric biosensor assay in which plasmin was used as biomarker with a high sensitivity (1 ng/mL).

2.2.2. Enteritis causing bacteria

Colibacillosisis considered as the most important problems faced in livestock production, causing significant losses related to economy in dairy farming, which is mainly caused by *Escherichia coli* (Bashahun and Amina, 2017). Some pathogenic strains of enteric bacteria cause various diseases in livestock and poultry such as gastrointestinal diseases, diarrhoea, mastitis, urinary tract infections, meningitis, septicaemia etc. In poultry, *E. coli* causes colibacillosis, which is categorized by the relocation of the virulent strains (e.g. O78:K80, O1:K1, O2:K1) from intestinal tract to other organ systems such as respiratory or urinary tracts. This infection in poultry has negative impact on egg production, chicken growth and increases mortality of birds and causes severe economic losses (Vidic et al., 2017). An important challenge in the diagnosis of *E. coli* is to differentiate between the closely related pathogenic and non-pathogenic species.

There are many conventional methods to detect the presence of *E. coli,* and various assays have been developed to detect these strains at the herald of the diseases. The conventional method to detect E. coli usually takes two to three days (Liu et al., 2020; Rani et al., 2021). This method is not enough to characterize the bacteria, and additionally requires bacterial identification either by PCR (Rajendhran and Gunasekaran, 2011), 16S rRNA sequencing, or by using biological probes, including antibodies and aptamers (Gopinath et al., 2014). These methods show drawbacks in terms of long identification time (typically few days), high labor and costly reagents (Fournier-Wirth et al., 2006).

Screening of *E. coli* strains using aptamer –functionalised Single-Walled carbon nano tube field effect transistors have been reported (So et al., 2008). Dual strategy combining immunomagnetic separation and real time PCR amplification of aptamers to detect *E. coli* was manufactured (Lee et al., 2009). New method for competitive FRET- aptamers applied to *E. coli* assay development (Bruno et al., 2010). Another group developed electrochemical based aptasensor for the detection of *E. coli* directly and calorimetrically (Wu et al., 2012). The DNA aptamers have also been developed for detection of *E. coli* strains K88 through binding with fimbriae protein of enteropathogenic *E. coli* (Li et al., 2011). Luo et al. (2012) have developed a sensitive and specific electrochemical biosensor for direct detection of *E. coli* O111, while showed less sensitivity to other bacteria such as *Salmonella typhimurium*, *S. aureus* and non-pathogenic *E. coli*.

Ahn et al. (2018) adopted whole-bacteria SELEX (WB-SELEX) strategy to isolate specific aptamer and develop surface plasmon resonance (SPR) biosensor. The SPR is highly sensitive to various molecular binding processes occurring onto planar metal surface or metal nanoparticles and is the basis of many label-free biosensors and lab-on-chip sensors. When combined with specific aptamers, the developed biosensor could specifically and selectively discriminate pathogenic *Vibrio parahaemolyticus*bacteria from other enteric species such as *Escherichia coli*, *Listeria monocytogenes*, *Sigellasonnei*, and *Vibrio fischeri*. Such SPR biosensors demonstrate the feasibility of using DNA aptamer platform for developing POC diagnostics. The WB-SELEX strategy has also been used for development of aptamers to capture and detect *Salmonella enterica* serovar *Typhimurium* (Dwivedi et al., 2013). The aptamer showed high binding affinity with Kd value of 1.73 ± 0.54 μM and low cross-reactivity with other food borne bacteria. In another study, Joshi et al. (2009) developed a DNA-based aptamer to detect *Salmonella enterica* serovar *Typhimurium* by selective enrichment against outer membrane proteins (OMPs).

A number of efforts have also been made to make the aptamer-based technologies label free and capable of being used at POC. Kim et al. (2019) developed a gold nanoparticle-based two-stage aptasensing platform for fast and on-site detection of *Campylobacter jejuni* and *Campylobacter coli* and detected by the change of colour from red to purple. The aptasensor were found to be highly specific when tested on chicken samples. Such calorimetric detection methods can provide an excellent and rapid screening tool for on-site detection of bacteria and may possibly be extended to other bacterial species. A novel aptamer-based strategy has recently been developed for label-free, in situ detection of bacteria by utilizing surface-enhanced Raman scattering (SERS). The SERS signals generated by bacteria-aptamer on silver nanoparticles should linear dependence on bacterial concentration and could be used to recognize bacteria quickly with a detection limit of 1.5 CFU/mL (Gao et al., 2017). Thus, this strategy offers an opportunity to create SERS-based biochips for quick in-situ detection of bacterial.

2.2.3. Tuberculosis

Bovine tuberculosis (BT) is a slow progressing disease and primarily caused by the *Mycobacterium bovis* (*M. bovis*), and to a lesser

extent by *M. caprae* and *M. tuberculosis*. Bovine tuberculosis is affecting the livelihoods and is hampering efforts to control and/or elimination by 2030 (WHO, 2018). The traditional diagnostic test i.e. tuberculin skin test, is a standard and the most popular test in the livestock. However, the bacterial culture of *M. bovis* is a time-consuming process and can take eight weeks or more. Aptamers are hopeful tools for developing POC diagnostic assays for TB. Mozioglu et al. (2016) developed ssDNA aptamers that recognize *M. tuberculosis* H37Ra using SELEX procedure.

Bovine tuberculosis caused by *M. tuberculosis* is difficult to be identified by the pathological symptoms. Release of bovine interferon gamma (BoIFN-γ) by T-cells can offers an diagnostic marker of *M. tuberculosis* (Crulhas et al., 2017). They developed an electrochemical aptasensor for sensitive and specific determination of BoIFN-γ. In this study, this biosensor can be used for on-site detection bovine TB (Crulhas et al., 2017). In another study, Rotherham et al. (2012) developed ssDNA aptamers to detect TB from sputum samples. It has reported that CSIR 2.11 aptamer have affinity to bind with CFP-10.ESAT-6 was developed to detect BT (Rotherham et al., 2012). Lavania et al. (2018) developed two DNA aptamer-based diagnostictests, namely aptamer linked immobilized sorbent assay (Aptamer ALISA) and electrochemical sensor (ECS), for the direct detection of a TB biomarker HspX in sputum.

2.3. Diagnosis prions diseases

Prion disease is a lethal and tragic neurodegenerative condition that affects both humans and animals. Transmissible spongiform encephalopathy is the common term assigned to all known prion diseases. Prions are infectious particles that lead Gerstmann-Straussler-Scheinker syndrome in humans, bovine spongiform encephalopathy (BSE) in cattle and there is a link between the transmission of prion diseases in humans and cattle (MacGregor, 2001). Prion diseases are neurodegenerative in nature and there is a great demand for sensitive and rapid diagnostic tools for these particles.

DNA aptamers have been used to detect transmissible spongiform encephalopathies in animals. The assay is based on their ability to detect conformationaly normal prion protein from abnormal isoforms in prion diseases. Using SELEX enrichment, Wang et al. (2011) identified DNA aptamers, which can distinguish normal and abnormal PrP isoforms. The aptamer can even distinguish different strains of prions in blood samples, which are usually not detectable by antibody-based detection procedure. Researchers have developed a high-throughput, inexpensive and convenient method for a specific and sensitive determination of prion protein on a solid-phase membrane (Hossain et al., 2010).

In another approach, a cellular prion protein (rhPrPC) based detection platform was developed using magnetic and gold-coated magnetic nanoparticles as support material (Kouassi et al., 2007) biotinylated aptamer. An effective aptamer-mediated chemiluminesence detection of prion protein has been developed (Zhang et al., 2014). More research led to the development of robust aptasensor for the detection of prion protein with very high sensitivity (Wang et al., 2012). Antipin and Nadtochey (2019) detected a prion protein of BSE in the CNS of BSE affected cow by using DNA aptamers specifically binded with amyloid epitops. A study established a sensitive method for the development of DNA aptamer for prion proteins (PrP) detection through the formation of T-Hg(2+)-T configuration (Xiao et al., 2012).

2.4. Diagnosis of cancer

In recent year, efforts have also been made to use aptsensors for diagnosis of cancer. Hwang et al. (2016) have applied quantum dot-based aptamer beacon for fluorescent imaging of circulating tumor cells. In this strategy, aptamers were designed for binding sequences of EpCAM/MUC1, which are known to be expressed in circulating metastasizing cancerous cells. The aptamers were labelled with Black Hole Quencher-2 and Cy5.5 fluorescent dye and, conjugated to quantum dots as aptamer linker beacon. The binding of aptamer to the EpCAM/MUC1 of circulating cancerous cells triggers the dissociation of quencher and generates fluorescence signal of Cy5.5 for its detection. The aptamer beacon was successfully tested in Panc02-implanted mouse model for the rapid monitoring of the circulating tumor cells.

Aptamers have also been explored for detection of blood analytes and replace biochemical analytical methods. One such example is the development of RNA aptasensor for detecting cyclic adenosine monophosphate (cAMP), which is related with the healthy state of the animal. Zhao et al. (2015) developed an RNA aptamer having principle of electrochemical impedance spectroscopy- enhanced by gold nanoparticles electrodeposited on the surface of gold electrode which can detect cAMP in a wide range with a detection limit of 50 nM in serum. Importantly, the aptasensor could be fabricated in cost effective way and could be used multiple times thereby demonstrating great potential as POC device for clinical test.

3. Sensing of toxins

Several approaches have also been made to find out selective aptamers for detecting bacterial, fungal and plant toxins. In a study, Challa et al. (2014) developed a RNA aptamer against Shiga toxins of by *E. coli*, which is known to cause life threatening hemolytic uremic syndrome. The RNA aptamers could block the binding of Shiga toxins to cells. Unfortunately, the developed aptamers could not neutralize the toxin-mediated cytotoxicity and death of the cells. An aptamer-based aptasensor for detecting Ochratoxin A has also been developed. Chen et al. (2018) hybridized Ochratoxin A aptamer onto dual gold nanoparticles and immobilized onto an electrode for electrochemical signal reporting. Due to high surface-to-volume ratio and excellent electro-conductivity of gold nanoparticles, the developed aptasensor was ultrasensitive to as low target concentration as 0.001–5000 ppb over six orders of magnitude. Tian et al. (2016) developed an aptamer-based analytical assay for detection of brevetoxin. The aptamers had a dissociation constant of 4.83 μM and IC50 of 73.81 ng/mL. The assay was as efficient as commercial ELISA.

Toxins present in feed and fodders are occasional cause of toxicity and death in animals. One such example is Ricin toxin resulting from the castor bean plant. The Ricin toxin is highly thermostable, active at acidic and alkaline pHs and is known to cause animal death due to toxicity. Aptamers against the B-chain of ricin was shown to detect ricin B in several liquid food and outcompeted ELISA kit with a detection limit of 25 ng/mL (Lamont et al., 2011). The aptamer could also be combined with surface enhanced Raman scattering technique for label free detection. Thus, such aptamers can serve as pre-analytical tool for screening of toxins in animal feed and fodder. Zhang et al. (2018) developed a fast and cost-effective ssDNA aptamer to detect zearalenone in animal feed with a detection limit of 12.5 nM.

4. Application of aptamers on detection of drug residues and contaminants

Aptamers have also been used for the purpose of detecting drug remains in milk, meat and farm waste. They have also been used successfully for detection of contaminants in drugs, allergens or environmental residues in various animal products. In particular, the rampant usage of antibiotics in animal feeds and veterinary

medicine had led to their widespread presence in various animal products and environment. Entry of these antibiotic residues into food chain has resulted in development of several new bacterial strains with multiple antimicrobial resistance and allergic reactions. Thus, identification of antibacterial residues is an important research area for animal industry to ensure consumer safety. A number of rapid screening tests have been developed for detection antibiotic residues in foods of animal origin and are based on immunological tests or physico-chemical methods. However, each of these tests suffers from disadvantages such as cost, specificity and reliability. Thus, several studies have investigated the applications of aptamers as possible tool for prompt and specific detection of antibiotic residues. Bai et al. (2019) have prepared a DNA aptamer-based electrochemical aptasensor to detect sulfadimethoxine in veterinary drugs and milk. In their unique approach, a sulfadimethoxine- specific DNA aptamer was developed, which upon binding with sulfadimethoxine, triggers the cleavage activity of nuclease PI to cause an electrochemical change that an electrode can detect. The developed aptasensor had a detection limit of 0.038 nmol/L and could specifically detect the sulfadimethoxine can with linear range of 0.1–500 nmol/L. A graphene-doped Bismuth sulphide nanorods has also been used as a visible-light photoelectrochemical aptasensing platform for detecting sulfadimethoxine in veterinary medicine and milk with a detection limit of 0.55 nM (Okoth et al., 2016). Blidar et al. (2019) combined the aptamer with electrochemical SPR to develop an aptasensing platform for detection of ampicillin in real time. The technique could detect ampicillin in the range of 2.5–1000 μmol/L with a limit of detection of 1 μmol/L. Such aptasensors could be useful for identifying the presence of ampicillin residues in the food and environment.

Specific aptamers have also been developed and used for detection of other antibiotics such as Florfenicol (Sadeghi et al., 2018), Cefquinome (Wang et al., 2018), Kenamycin (Xu et al., 2015), Oxytetracyclin (Xu et al., 2018), Tetracyclin (Zhou et al., 2018) and, Chloramphenicol (Yan et al., 2018), which allowed rapid and sensitive colorimetric detection by naked eye or using a colorimeter with detection limit of 0.0031 ng/mL. In order to detect chloramphenicol residues, Roushani et al. (2019a) developed an aptasensor by coating 3-aminomethyl pyridine functionalized GO on the surface of glassy carbon electrod, which was again coated with silver nanoparticle and chloramphenicol specific aptamer. The aptasensor allowed impedimetric ultrasensitive detection of chloramphenicol by glassy carbon electrode in the linear range of 1.0 pM to 1.0 nM with the detection limit of 0.3 pM in milk samples. DNA aptamer has also been used to construct a competitive voltammetric aptasensor for azlocillin β-lactam antibiotic (Chinnappan et al., 2020a). The developed aptasensor was highly selective, sensitive and cost-effective with a limit of detection of 1.2 pg/mL. Antibiotics such as enrofloxacin are widely used fluoroquinolone in poultry and are the main causative agent of bacterial drug resistance. A single stranded DNA aptamer based enzyme immunoassay was developed for the determination of enroflaxacin (Ni et al., 2014).

High doses of quinolones antibiotics are regularly used in livestock farming and they are one of the most commonly observed antibiotic residues in animal products and water bodies. Aptamer-based technologies have been shown to be a sensitive and effecting monitoring tool for quinolones. Reinemann et al. (2016) selected an aptamer pool, which could detect various quinolones such as ofloxacin with high specificity using the Capture-SELEX procedure. The aptamers were demonstrated to be effective in detecting ofloxacin in water and in effluents of sewage plants.

Components of animal products can cause allergic reaction to consumers and therefore, quick and sensitive methods for detecting various allergens in animal foods are essential to assist patients in managing their allergies. Currently, immunoassays, polymerase chain reaction and mass spectrometry are employed for analysing allergens. However, these methods are complex, costly and time consuming. Thus, aptamers are being investigated for rapid and specific detection of food allergen. Chinnappan et al. (2020b) developed anaptameric biosensor for the sensitive detection of tropomyosin allergen. The aptamer-based sensor detected the allergen within 30 min with a detection limit of 2 nM.

5. Application of aptamers in the targeted drug delivery and treatment of animal diseases

Current therapeutic strategies such as use of antibiotic or antiviral agents are ineffective in many cases and may even result in development of resistance upon their prolonged usage (Aminov, 2021; Caudell et al., 2020; Palma et al., 2020). Consequently, newer alternative therapeutic approaches are actively explored worldwide. In recent years, several oligonucleotide-based therapies such as short interfering RNAs (siRNA), microRNAs, aptamers, DNAzymes, and ribozymes have been used for diagnosis and/or treatment of viral diseases (Kim and Lee, 2021; Lundstrom, 2020). Among these nuclei-acid based approaches, DNA or RNA aptamers have been increasingly gaining wide attention due to their efficacy and specificity to pathogens and ease in their production by complete chemical synthesis methods. The first report on the use of aptamers for clinical application appeared in 1992 and since then it has been widely explored for human application using animal models (Fig. 2). Unfortunately, the development and application of aptamer-based diagnostics and therapeutics in veterinary field has been slow. Nevertheless, after successful demonstration on animal models, efforts are being made for application of aptamers on diagnosis and treatment of livestock and pet animals (Kim et al., 2020; Kim and Lee, 2021; Vidic et al., 2017). There are many studies of aptamers are going on in the different stages of testing and have provided researchers to address some of the disadvantages of antibodies (Table 2).

Various aptamers have been shown to successfully treat various infectious and non-infectious diseases by specifically targeting the pathogen, hormone or, enzymes or by allowing targeted delivery of another drug. In recent years, antimicrobial peptides such as beta-defensin have emerged as a promising new class of antibacterial and antiviral drugs. Unfortunately, most exogenously administered antimicrobial peptides are unstable in biological fluids and have low penetrating ability into mammalian cells. Several researchers have used DNA aptamers for effective and rapid delivery of antimicrobial peptides into the cells.

Aptamers such as FO24 and FO21 have been tested as preventive and/or therapeutic antiviral therapy against viruses. Studies have shown that injection of FO24 and FO21 ssDNA aptamers for 24 h prior to inoculation with street rabies virus FJ strain into mice resulted in 60–80% survival rate (Liang et al., 2014a; Liang et al., 2013). The aptamers could be used for both pre-exposure as well as post-exposure prophylaxis but the pre-exposure prophylaxis was superior to post-exposure prophylaxis. These aptamers were specific to street rabies virus and did not the replication of other viruses such as canine distemper virus and canine parvovirus (Liang et al., 2014b). However, clinical trials have not yet been done to use them as therapeutic molecules. Similarly, Xu et al. (2019; 2017) demonstrated that specific DNA aptamers can inhibit the production of Bovine herpesvirus 1 through blocking nucleocytoplasmic shuttling and inhibiting the infectivity of the virus by blocking viral entry. The developed aptamers exhibited high affinity and specificity for Bovine herpesvirus 1 with a Kd value of 3.519 nM and significantly reduced viral replication. Thus, DNA aptamers could be used as a novel tool for the treatment of Bovine

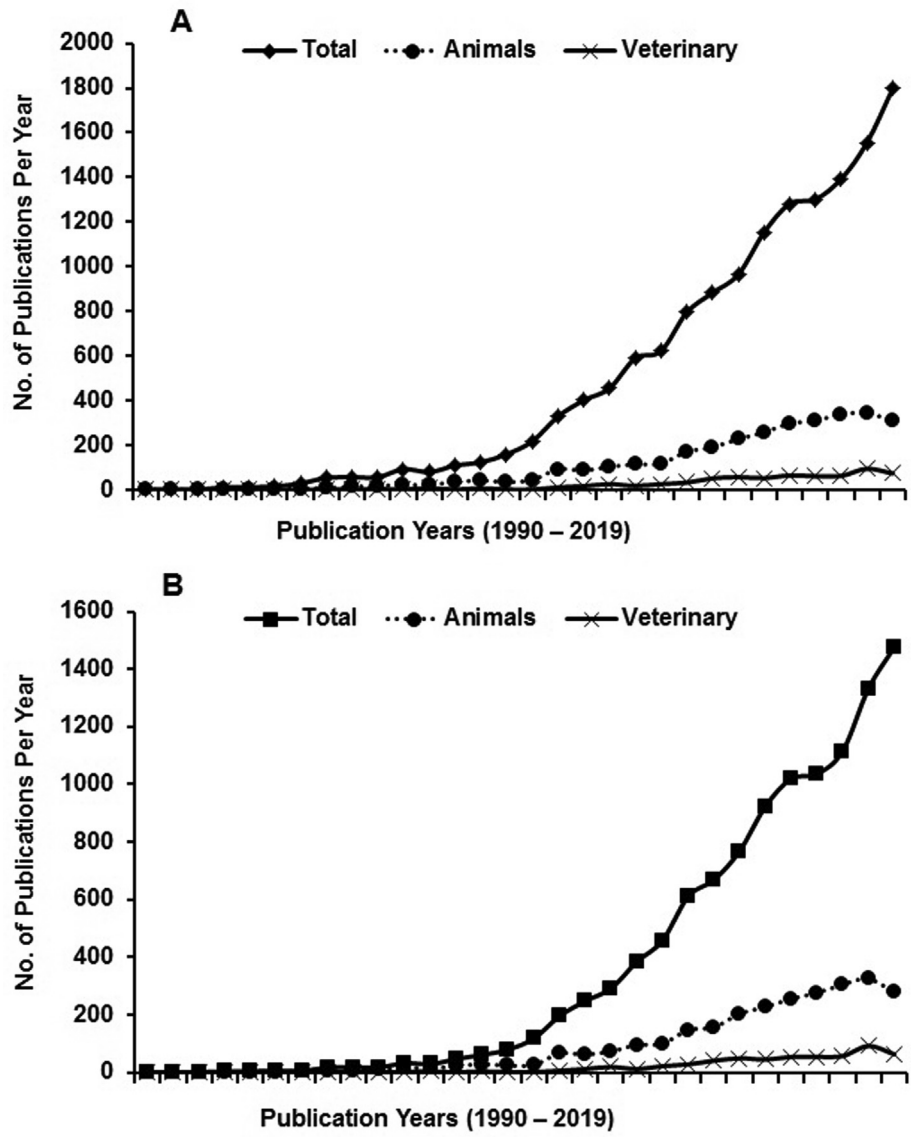

Fig. 2. Number of publications on aptamers published per year during 1990–2019. A: Overall publications on aptamers; B: Publications on the use of aptamers for diagnosis or therapy. Total number of publications (any species) and publications exclusively on animals or veterinary science are shown separately. First publication on aptamer appeared on 1992 whereas its application in animal or veterinary science appeared first on 1993.

herpesvirus 1 infection in cattle. In yet another study, Yoon et al. (2010) developed RNA aptamers that bound to the capsid protein of the Porcine circovirus type 2 with nanomole affinity and neutralized the viral infectivity in PK-15 cells in dose dependent manner.

Aptamers have also been investigated as novel anti-prion compounds. It was shown that peptide aptamers binding to PrP(C) prion protein can abrogate prion propagation (Corda et al., 2018). Binding of the designed anti-prion aptamer with PrP(C) prion protein increased α-cleavage and interfered with its internalization and inhibited prior replication. Aptamers can also used for neutralization of bacterial, viral, fungal and plant toxins. Lee et al. (2015) developed RNA aptamers which specifically targets ATR/TEM8 Von Willebrand factor type A (VWA) domain. Such RNA aptamers were shown to have an inhibitor efficiency (IC50) of 5 μM and could block the binding of Protective Antigen (PA) to its receptors in order to neutralize anthrax toxin.

Aptamers have also been used for non-infections conditions such as hormonal imbalance, intestinal motility, modifying apatite or thirst etc. Becskei et al. (2008) showed that NOX-B11-3, a RNA aptamer (called RNA Spiegelmers) can suppress appetite during food deprivation by specifically binding to orexigenic hormone ghrelin and blocking its activity. The NOX-B11-3 could effectively block the effect of endogenous ghrelin on neuronal activity in the hypothalamic arcuate nucleus. Interestingly, the NOX-B11-3 acted only on animal under fasting and not on normal-fed animals. Similarly, RNA Spiegelmer has been used to block the activity of anorectic and dipsogenic pancreatic hormone amylin (Bilik et al., 2007). Such RNA Spiegelmer may be beneficial for the treatment of complications arises due to high amount of plasma amylin, as in cancer anorexia.

6. Conclusion

This review paper has put forward an outline of novel progresses in developing aptamer-based sensors to diagnose and treat major animal diseases. In the light of current limitations in common detection techniques of early disease diagnosis, researchers

Table 2
Important patents on the development and application of aptamers in veterinary diagnostics and therapeutics.

No	Title	Inventors [Country]	Publication Number	Priority Date
1	Switch mode nucleic acid aptamer probe and uses thereof in detection of tumor living cell and living body	Guo Qiuping et al., [China]	WO2011134328A1	2010-04-26
2	Nucleic acid aptamer-based diagnostic methods with novel techniques for signal enhancement	Nussbaum Ofer [USA]	WO2012004790A2	2010-07-06
3	Carcinoembryonic antigen detection kit based on nucleic acid aptamer autocatalysis effect, and preparation method thereof	Zou Mingjing [China]	CN107064505A	2016-02-18
4	Aptamer-directed drug delivery	Bagalkot Vaishali et al., [USA]	WO2007137117A2	2006-05-17
5	Method for detecting N-hydroxyacetylneuraminic acid content based on aptamer	Gong Sheng et al., [China]	CN108709989A	2018-05-29
6	Nucleic acid aptamer specifically binding to enrofloxacin or ciprofloxacin	Jeong Sang Hee et al., [South Korea]	KR101526921B1	2013-06-28
7	Aptamer for binding bifidobacterium breve as well as screening method and application of aptamer	Chen Wei et al., [China]	CN105838719A	2016-04-13
8	Serum IgG antibody aptamer of tuberculosis patients and preparation method thereof	Cai Jiangli et al., [China]	CN103045600A	2011-10-11
9	Gene regulation with aptamer and modulator complexes for gene therapy	BUEHLER BERND et al., [UK]	US2005260164A1	2002-10-10
10	Aptamers for prion diagnostics and aptamer binding detection system	Mohanty Sarina [USA]	WO2014008440A2	2012-07-04
11	DNA aptamer binding to glyphosate with specificity	Gu Man Bock et al., [South Korea]	KR101338520B1	2011-10-25
12	Aptamer ELISA detection kit, preparation method, and application thereof	DONG ZAIZAI et al., [China]	CN108957001A	2018-06-27
13	Research and development of aptamer-based rapid detection technology for agricultural veterinary drug residues	Ling Qingyun	CN109596605A	2017-09-30
14	Aptamer specifically binding to nonylphenol and detecting method using thereof	Kim So Youn [South Korea] and Ren Shuo [China]	KR101822881B1	2014-03-31
15	Biosensing probe kit for detecting sulfadiazine on basis of nucleotide aptamer specificity, and application thereof	Sun Qi et al., [China]	CN107119054A	2017-05-04
16	Aptamer-based multiplexed assays	Katilius Evaldas et al, [USA]	SG10201701361WA	2012-06-07
17	DNA Insulin receptor-specific DNA aptamer and uses thereof	Ahn Ji Young et al., [South Korea]	KR101850793B1	2016-07-14
18	Target substance detection method using aptamer	Kim So Youn [South Korea]	WO2010093223A2	2009-02-16
19	Amine-terminated aptamer functionalized surface plasmon resonanace sensors, methods of making and methods of using same	Cameron Brent D et al., [USA]	US9562266B1	2012-01-14
20	Nucleic acids aptamer binding specifically to cucurbitacin	Choi Tae Young et al., [South Korea]	KR101953994B1	2017-09-26
21	Use of nucleic acid aptamer in alkaline phosphatase heterodimer recognition and binding or in tumor detection	Bing Tao et al., [China]	WO2019149115A1	2018-02-02
22	Application of nucleic acid aptamer in recognizing and binding alkaline phosphatase heterodimer	Bing Tao et al., [China]	CN109554369A	2018-02-02
23	Nucleic acid aptamer capable of specifically binding to tetracyclines compound and use thereof	Gu Man Bock et al., [South Korea]	KR101342710B1	2012-02-14
24	Aptamer nucleic acid probe kit for detecting doxycycline residue as well as preparation method and application thereof	He Hongqiu et al., [China]	CN104360077A	2014-11-25
25	Real-time and continuous measurement in vivo using aptamer-based biosensors	Arroyo Curras Netzahualcoyotl et al., [USA]	WO2017164982A1	2016-03-21
26	Nucleic acids aptamer binding specifically to Ingenol mebutate	Choi Tae Young et al., [South Korea]	KR101987591B1	2018-08-06
27	Antigen-binding fragment and/or aptamer for binding to an extracellular part of cd9 and therapeutic uses	Corbeil Denis et al., [USA]	WO2020044285A1	2018-08-29
28	Escherichia coli o157:h7 aptamer and applications thereof	Amraee Masoum et al.,[Iran]	WO2018189639A1	2017-04-15
29	Nucleic acid aptamer which specifically binds to bisphenol A	Kim So Youn [South Korea]	EP2397550A2	2009-02-16
30	Method for detecting mycoplasma gallisepticum antibody of enzyme-linked nucleic acid aptamer and kit special for mycoplasma gallisepticum antibody	Wang Feng and Wu Wenxue [China]	CN109762825A	2019-03-25
31	Target substance detection method using aptamer	Kim So Youn [South Korea]	US2015212038A1	2009-02-16
32	Nucleic acid aptamer capable of specifically binding to tebuconazole, mefenacet, and inabenfide, and use therof	Gu Man Bock et al., [South Korea]	WO2015056980A1	2013-10-16
33	Aptamer inhibition of thrombus formation	Li Minyong et al., [China]	AU2009281857A1	2008-08-15
34	Aptamer-based rapid kanamycin detection test paper as well as preparation method and application thereof	Liu Jing et al., [China]	CN106990237A	2017-05-10
35	Aptamer of enrofloxacin and preparation method and application thereof	Yu Xiaoyan et al., [China]	CN104450725A	2015-01-05
36	Aptamer-coated implant, process of production, and uses	Borck Alexander [Germany]	US2011150964A1	2009-12-21
37	DNA EGFR-specific DNA aptamer as incubation additives for promotion of mammalian cell growth and uses thereof	Ahn Ji Young et al., [South Korea]	KR101859615B1	2016-07-14
38	Aptamer-based device for detection of cancer markers and methods of use	Datta Bhaskar et al., [USA]	US2012088232A1	2010-09-30
39	DNA PDGFR-specific DNA aptamer as incubation additives for promotion of mammalian cell growth and uses thereof	Ahn Ji Young [South Korea]	KR101833231B1	2016-07-14

and scientists are turning their attention towards the development of aptamer based diagnosis for efficient rapid non-invasive detection of diseases in veterinary medicine. Aptamer based biosensors are attaining importance over other biosensors due to its several advantages. However, the development of biosensors for multiple disease detection is still a big challenge to the scientist and researchers. Integration of high multidisciplinary approaches and the use of nanomaterials in the development of aptamer biosensors will enhance the sensitivity of these devices and make it more efficient for early detection of disease, which is the need of the hour. In conclusion, while there are still some gaps in developing aptamers for clinical applications in veterinary medicine, aptamers will be widely used in early and most accurate disease diagnosis with the improvement of the relevant technologies.

7. Future perspectives and challenges in the application of aptamers

In veterinary medicine, there is still lot to be done for the developments in the diagnostic techniques to meet the demand of dairy farmers. We expect that this new field of aptamer-based biosensors in the diagnosis and treatment of animal diseases will eventually become a real-world tool. This would help in encountering challenges that could be difficult with currently available conventional technologies.

The success of aptamers in clinics depends on their stability in blood and other biological fluids upon injection into the body. The general use of aptamers is also hindered by their incapability of intracellular accumulation at the site of action. While several studies have shown success with the use of aptamers under in vitro conditions, their use in animal system requires special considerations. One such approach could be encapsulation of the aptamers for protecting them against nuclease activity of biological fluids. Bedi et al. (2013) established that fusion phage coat protein fpVIII, exhibiting cancer-targeting peptides, can commendably encapsulate siRNAs and could be used as nanophages to transport them into the cells leading to specific silencing of the model gene.

A yet another challenge in the application of aptamers is the complex optimization process of SELEX in screening and identifying the monoclonal aptamer candidate for target molecule of interest. Target molecules occasionally have isoforms or variants which may not be targeted by a single monoclonal aptamer, as is the case with FMD virus. The FMD virus has several strains and a monoclonal aptamer developed against conserved peptide region could selective identify few strains but were did not match the sensitivity of the polyclonal aptamer. Similarly, many toxins such as Shiga toxins have two antigenically distinct forms, which could not be identified by a single aptamer (Kaur et al., 2020).

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.

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