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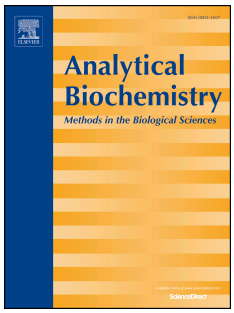

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RECENT PROGRESS IN ELECTROCHEMICAL BIOSENSORS AS POINT OF CARE DIAGNOSTICS IN LIVESTOCK HEALTH

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Abstract

Livestock are critical component for supporting the sustainable agriculture in the current global scenario. In the era of artificial intelligence and automation in field of livestock, sensors play an important role. Electrochemical sensor is the type of sensor which holds reliability and tremendous promise in raising the animal productivity in developing world.

An early and accurate diagnosis of the animal pathogen and metabolic status are the cornerstone for better animal productivity. The available diagnostic techniques require tedious sample preparation, sophisticated instrument, dedicated laboratory, trained personnel and it is time consuming also. The electrochemical biosensor technology might be a smart solution because of its sensitivity, simplicity, low cost, possible miniaturization and potential ability for real-time analysis. In the veterinary disease diagnostics, various biosensors including electrochemical biosensors have been developed recently, based on disease specific biomarkers. The main focus of article is on reviewing the research in detection of animal infectious and metabolic diseases, hormonal analysis and sweat analysis with electrochemical biosensor.

Keywords: Biosensors, Electrochemical, Livestock, Health, Bacteria, FMD Virus

1. INTRODUCTION

In the modern era, the emerging and re-emerging diseases threaten animals and public health

every year. Besides the infectious diseases, the metabolic and hormonal disturbances also lead to the economical impact on the farmer's profit and livelihood. Rapid detection of livestock diseases are non-existent in developing countries and also in few developed countries due to lack of veterinary infrastructure, expertise, diagnostic laboratories, availability of rapid, reliable and cost effective diagnostic tool especially for new diseases. The rapid detection of a new epidemiological event is therefore a key element for all policies to be developed. [1]. This challenge can be properly addressed with the biosensing technologies in an innovative way to provide a rapid and reliable detection of animal and public health threats within the agri-food livestock sector [2]. By 2050, food demand is expected to increase by 70%, and meat production will increase by 50%, making agri-food and livestock key industries for future growth [3]. The animal health threats might have an impact on animal product commerce by disrupting the food supply chain. Besides the animal diseases, the indiscriminate use of antibiotics and insecticides for treatment, and its residues in the animal products are also remain major concerns. Further, drug residues elicit the antimicrobial resistant microbes, which have become a serious international issue in recent years [4]. To ensure the supply of safe and quality animal food products, reliable screening methods for veterinary drug residues level is warranted. For fast, efficient and point of care analytical devices, biosensors are smart solution because of their simplicity, possible miniaturization and potential ability for real-time analysis [5]. By 2020 the value of biosensor market is expected to be of US$22.68 Billion. Furthermore, non-invasive health monitoring is also driving the growth and development of nanotechnology-based biosensors [6]. In veterinary diagnostics, the point-of-care diagnostics market is expected to increase at a compound annual growth rate (CAGR) of 18%, reaching US$6.71 Billion by 2021. In this context, electrochemical biosensors have proven to be useful tools to detect small sample volumes, low concentrations of biological components. Electrochemical biosensors, as a subclass of biological sensors, consist of analyte, receptors, electrochemical signal transducer, and data analysis system. When the analyte selectively reacts with the recognition element (enzymes, antibodies, aptamers, affimers, lectins or other biomolecules), an electrical signal is produced which is transduced via the transducer to the data analysis system.

Electrochemical biosensors are widely developed, and some of them have reached the commercial stage and are routinely used in environmental and agricultural applications, and especially, in clinical laboratory analysis [7] (Fig. 1). The electrochemical glucose biosensor has

wide commercial uses to monitor the blood glucose concentrations of diabetes mellitus patients. Further, these biosensors are being used to detect the celiac disease [7], breast cancer [8]; [9], prostate cancer [10], hepatitis B virus [11], etc. Electrochemical biosensor works on electrochemistry, a surface technique, and does not depend on the reaction volume. In the electrochemistry the reaction between the reactants produces the current (amperometry), a change in potential (potentiometry) or change in the charge transfer resistance (impedimetric) between working and counter electrodes, which is measured in the potentiostat. Furthermore, it offer advantages viz. high specificity, sensitivity and better signal to noise ratios.

This review focuses on the recent development of electrochemical biosensors for emerging and re-emerging diseases of veterinary public health importance along with the metabolic and reproductive disorder.

2. ELECTROCHEMICAL BIOSENSOR

Biosensors with electrochemical transducer are the common and versatile biosensor with point of care (POC) capability. The bio-interaction process over the transducer generates the electrochemical species and producing electrochemical signal, which is measured by the detector and analyzed with data analyzer (Fig. 2). Based on the electrochemical property involved, electrochemical sensors can be grouped into potentiometric, amperometric, voltammetric, impedimetric and conductometric sensors [12]. Potentiometric sensors measure the charge potential difference between working and reference electrode at equilibrium and monitor the ion activity in the reaction at electrode surface, which is described by Nernst equation[13]. The amperometric biosensors measures the generated current resulting from the electrochemical redox reaction of electroactive molecules at bio-receptor coated electrode at constant voltage. Amperometric changes are linearly proportionate on analyte concentration in the solution. The redox mediators have to be required during analysis, when the analytes are not redox partners[14]. They generally have higher sensitivities to the potentiometric biosensors. Impedimetric biosensors are sensitive for the analysis of the interfacial properties and measures impedance (both resistance and reactance). On the electrode surface, interaction of bio-receptor and analyte impedes the flow of electrons in an ac circuit resulting in ohmic resistance (real component) and capacitive resistance (imaginary component). The biorecognition can be carried out directly without incorporation of any label, makes impedimetric biosensors a suitable for

point of care diagnostic[15]. On the basis of the detection strategy the electrochemical biosensors may be of two type viz. label dependent and label free. Label dependent electrochemical biosensor require the biological molecule like enzyme, tagged antibodies or physiochemical molecule like metallic nanoparticles, conductive polymers etc to induce electrical impulse for measurement. Whereas the label free sensors are based on the immobilization of the target analyte over the bio-receptor electrode and measurement in the changes of capacitance and resistance over the electrode surface [16].

3. ELECTROCHEMICAL BIOSENSOR FOR ANIMAL DISEASES

3.1 Escherichia coli

E. coli is the normal inhabitant of the gastro intestinal tract of animals and the virulent strains causes disease range from gastroenteritis, septicemia, acute mastitis, encephalitis, peritonitis etc. This bacterium is also a concern for public health as the pathogenic form causes foodborne illness in humans. Furthermore it also has economic burden to the animal farmers. An outbreak in Europe in 2011, had $417 million loss due to food withdrawal and ban on exports [17]. The indiscriminate and improper uses of antibiotics to treat animal diseases spread the antibiotic resistance. E. coli is of zoonotic importance; hence the threat of antibiotic resistant infection to human is largely increased. The rapid and reliable diagnostic for virulent E. coli is vital, to treat the infection with suitable antibiotic and will also help to combat with drug resistance. The main issue in E. coli diagnostic is to separate the virulent strains from non-pathogenic strains. Bacterial culture, biochemical tests (catalase, oxidase, oxidative/fermentative, nitrate reduction etc), ELISA, PCR assay is widely used but they require longer detection time. Various biosensors, based on quartz crystal microbalance [18], surface plasmon resonance [19] and electrochemistry [20,21] has been developed across the globe. Most of the developed biosensors are designated as immuno sensor by functionality. A voltammetry based biosensor, using the enzyme-linked immunomagnetic electrochemistry (ELIME) for the rapid detection of E. coli had been developed [22]. They achieve a minimum detectable level of $5X10^3$ cells per ml in an assay time of 80 min. Lin et al fabricated a disposable amperometric biosensor using indirect ELISA (Enzyme linked immunesorbent assay) with double antibodies on AuNP (Gold nano particle)-modified screen printed carbon electrode (SPCE) and achieved the LOD (Limit of detection) for the detection of E. coli at 5×10^3 CFU (colony forming unit)/ml in milk [23].

Ferrocenedicarboxylic acid (FeDC) modified AuNP on SPCE and coupled HRPO (horse reddish peroxidase) activity greatly amplify the amperometric effect. Various researchers' uses the different label system over the same electrode surface for the development of integrated system for simultaneous rapid detection of multiple pathogens. Viswanathan and others reported multiplexed electrochemical immunosensor for detection of three important pathogens using nanocrystal bioconjugates and multiwall carbon nanotube (MWCNT) screen-printed electrode. The nano crystal bioconjugates has releasable metal ions for electrochemical measurement [24]. Maalouf et al develop a label-free electrochemical impedance spectroscopic detection method for E. Coli and achieved a LOD of 10 CFU/ml whole bacteria while 10^3 CFU/ml for lysed E. coli [25]. The biotinylated polyclonal anti-E. coli antibody was linked to a mixed self-assembled monolayer (SAM) on a gold electrode and analyze the polarization resistance (R_P). Conductometric immunosensor developed by El Ichi based on addressable magnetic nanoparticles coupled with anti-LPS (Lipo-polysaccharide) antibodies for detection of Gram negative bacteria including E.coli. [26]. These biosensor allowed real-time, sensitive detection of E coli cultures from 1 to 10^3 CFU/ml, further, Pseudomonas aeruginosa and Acinetobacter baumannii strains can be detected directly with 10-10^3 CFU/ml that were undetectable using standard immunoblot methods[26]. Aptamer based label free biosensor to detect E.coli was studied by Zelada-Guillen and workers. The aptamer was covalently immobilized on single walled carbon nano tube (SWCNT). The selective aptamer-target interaction significantly changes the electrical potential, thus allowing for both interspecies and inter-strain selectivity and enabling the immediate identification and detection of microorganisms in complex sample [27]. A novel electrochemical detection for E. coli was presented with the detection range of 10^1 to 10^5 CFUs by using L-Cysteine functionalized Iron (Fe_3O_4) Nanoparticles (L Cyst-Fe_3O_4 NPs). The Glassy carbon electrode (GCE) was modified with electrodeposition of L Cyst-Fe_3O_4 NPs. For the detection of E. coli cyclic voltammetry was performed. A linear response between signal current and E. coli concentration (r^2 = 0.879) was reported[28].

3.2. LISTERIA

Listeriosis is caused by Listeria monocytogenes, which is ubiquitous in nature. The microorganism mainly affect the ruminants, but it can infect non-ruminants including humans also. 100 colony forming units (CFU) in per gram of food is considered as a minimum infectious

dose for listeriosis [29]. However, most countries have a zero-tolerance policy towards the presence of L. monocytogenes in ready to eat foods. Therefore, a rapid, accurate and reliable technique is necessary for listeria detection. This will not only help to restrict the spread of listeriosis, provide the effective control measures, but also minimize the unnecessary recalls of food products [30].

Chai et al. investigated the impedimetric characteristic of an immunosensor on an aluminum surface insulated with an electrically resistive aluminum oxide layer. The anti-L. monocytogenes antibodies were immobilized on an aluminum surface which was insulated with aluminum oxide layer. The adsorption of L. monocytogenes produces physicochemical changes in the ionic layer which might help electrical current to flow and cause the resistance of impedance (R) value to decrease. The resistance of impedance (R) at 81 kHz decreased proportionally to the concentration of L. monocytogenes from 1.3 to 4.3 log CFU/ ml [31].

Cheng et al. described a direct sandwich type electrochemical immunosensor using self-assembled monolayer (SAM)-modified gold (Au) electrodes for the detection of Listeria. The polyclonal antibody for listeria conjugated to HRPO was used as enzyme label. In the presence of H_2O_2, HRP catalyzes the oxidation of thionine, whose back electrochemical reduction was detected on an Au electrode at −0.3 V. A linear relation between the response current and the amount of listeria (R^2= 0.9883) for 10^2 to 10^6 CFU/ml was reported, also there was no need of sample pretreatment when applied to detect Listeria in milk [32].

A microfluidic biosensor has been developed for fast detection of listeria integrating impedance analysis with urease catalysis. The MNP (magnetic nanoparticle) -Listeria-AuNP-urease sandwich complexes were captured in the separation chip by applying a high gradient magnetic field, then urea was injected to hydrolyze under the urease catalysis on the complexes into ammonium ions and carbonate ions, which were transported into a microfluidic detection chip with an interdigitated microelectrode for impedance measurement to determine the amount of the Listeria cells. A linear relationship between the impedance changes and the concentrations of Listeria from 1.9×10^3 to 1.9×10^6 CFU/ml was obtained. The limit of detection of this biosensor was 1.6×10^3 CFU/ml [33].

A label-free potentiometric aptasensor was reported for Listeria detection by Ding in 2014. They use the specific aptamer for internalin A of Listeria. The electrostatic interaction between protamin and aptamer was prevented by target-binding event, which is sensed on polycation-

sensitive membrane electrode. Using this method, L. Monocytogenes can be detected down to 10 CFU/ml [34].

A label-free impedimetric biosensor using DNA sensing probe on conducting polymer poly-5-carboxy indole (5C Pin) was developed for detection of Listeria hlyA gene. The probe were covalently immobilized on 5C Pin and further hybridized with the target DNA sequence. The charge transfer resistance and logarithmic concentrations of the target (genomic) DNA plot showed a linear range (1×10^{-4} to 1×10^{-12}M) [35].

3.3. BRUCELLA

Brucellosis is the most common bacterial zoonotic infection, primarily a reproductive disease of animals, affecting almost all animal species globally and transmitted through direct or indirect contact with infected tissues or body fluids. Proper diagnosis is one of the key obstacles for the complete eradication of brucellosis [36].

Wu et al had fabricated a label-free immunosensors by immobilizing Brucella melitensis antibody on gold nanoparticle-modified screen-printed carbon electrodes (GNP-SPCEs). A linear relationship between the change in electron-transfer resistance (Rct) and Brucella concentration from 4×10^4 to 4×10^6 CFU/ml in pure culture was demonstrated. The developed biosensor was able to detect as low as 1×10^4 and 4×10^5 CFU/ml of Brucella melitensis in pure culture and milk samples, respectively, in less than 1.5 h [37].

An amperometric immunosensors were made for detection of B. abortus cell envelop protein (CE-protein) antigen by Gupta et al. They have achieved a linear range of 1.56 µg/ml to100 µg/ml with detection limit of 0.1 µg/ml [38].

Rahi et al. develop a label free electrochemical genosensor using the electrodeposited palladium nanoparticles on a gold surface as a transducer to immobilize the Brucella-specific probe. The strategy used for detection of ssDNA has been depicted in Fig. 3. The genosensor could detect the complementary sequence with a sensitivity of 0.02 µA dm^3 mol^{-1}, a linear concentration range of 1.0×10^{-12} to 1.0×10^{-19} mol dm^{-3}, and a detection limit of 2.7×10^{-20} mol dm^{-3} [39].

Gupta et al. utilize the biocompatible CeO_2 nano-octahedra and chitosan for the Brucella immunosensors. They use the CV and EIS techniques and got a linear range of 6.25 µg/ml to 100 µg/ml antigen with detection limit of 0.1 ng/ml. [40].

Recently thick films of copper doped nickel and zirconium oxide nanoparticles was fabricate on gold-plated working electrodes for Brucella immunosensors by Khan et al. The fabricated electrochemical devices were tested with different Brucella abortus concentration in the range of 1×10^3 CFU/ml to 2×10^6 CFU/ml in phosphate buffer (pH = 7.2, 0.1 M) in presence and absence of antibody [41]. A novel Brucella immunosensors based on the low-fouling magnetic Fe_3O_4@Au@PEG@HA nanoparticles is fabricated with high selectivity, sensitivity and almost perfect protein-resistant properties by Lv et al. 2018. The Fig. 4 illustrates the strategy used for detection of antigen in electrochemical sensor. They shown a wide linear response range from 10^{-15} g/ml to 10^{-11} g/ml towards antibody and a low limit of detection (LOD) of 0.36 fg/ml [42].

3.4. CAMPYLOBACTER

Campylobacteriosis is an infectious disease typically affecting the digestive system of humans and animals including wild animals, pets, livestock species and birds. The WHO recommended, A control and surveillance of Campylobacter in poultry to reduce the risk for humans since this bacterium has a significant impact on public health [17]. The isolation of the organism from the clinical specimen is very difficult. Genus level assays including the 16S ribosomal RNA target amplification have begun more widely for campylobacter diagnosis [43].

For rapid detection of campylobacter, an electrochemical impedimetric immunosensors was developed by immobilizing the anti-FlaA monoclonal antibodies (2D12) on O-carboxymethylchitosan surface modified Fe_3O_4 nanoparticles (OCMCS-Fe_3O_4) by Huang et al. Under the optimized conditions, they achieve a linear impedimetric response in the range of $1.0x10^3$ to $1.0x10^7$ CFU/ml [44]. Ivnitski et al. suggested an experimental design of amperometric biosensor for campylobacter detection. The width of the current peak was correlated with the bacteria concentration, and suggested the LOD could be one cell per sample [45,46]. Viswanathan et al fabricate an immunosensors by immobilizing the campylobacter specific antibodies on multiwall carbon nanotube-polyallylamine modified screen printed electrode. The sandwich immunoassay was performed with campylobacter-PbS nanocrystal, which has releasable metal ions for electrochemical measurements. The square wave anodic stripping voltammetry (SWASV) was employed to measure released metal ions from bound antibody nanocrystal conjugates [24]. Morant-miñana and Elizalde (2015) fabricated an electrochemical genosensor based on thin-film gold electrodes deposited onto Cyclo Olefin

Polymer (COP) substrates. A good linear relationship was obtained for the concentrations of PCR amplicon of Campylobacter spp. between 1 and 25 nM with a limit of detection (LOD) of 90 pM. The developed hybrid polymer-metal device has a high potential to integrate electrochemistry and microfluidics for analysis of microorganism in food [47].

3.5. CLOSTRIDIUM PERFRINGENS

Clostridium perfringens is a common enteric pathogen of humans and animals. The organism produces several toxins, which causes animal disease. The pathogenesis of the disease commonly differs between host species. Thus specific research to develop pen side diagnostic for efficient control measures is required [48,49].

An electrochemical strategy based on molecular beacon (MB), streptavidin (SA), and hemin/G-quadruplex/Fe_3O_4 nanocomposites was demonstrated by Jiang et al (2014) as shown in Fig.5. Initially, the MB forms a stable hairpin, which blocks the binding capability of the Streptavidin aptamer. After incubating with target DNA, the hairpin opens and the SA aptamer is reactivated to capture the SA/alcohol dehydrogenase (ADH)/Fe_3O_4 nanocomposites. Through a "sandwich" reaction, the hemin/G-quadruplex is captured on the electrode surface, and the electrochemical signals as Differential Pulse Voltammetry were obtained. The results of differential pulse voltammetry showed a wide linear range from 10^{-12} to 10^{-6} M [50]. Quian et al utilizes the CeO_2 nanorod for label free genosensor as sensing material to achieve sensitive and selective detection of C. perfringens DNA sequence. The dynamic linear range of the proposed biosensor for detecting oligonucleotide sequence of Clostridium perfringens was from 1.0×10^{-14} to 1.0×10^{-7} mol/L. The detection limit was 7.06×10^{-15} mol/L. Moreover, the DNA biosensor could detect C. perfringens extracted DNA in dairy products and provided a potential application in food quality control [51].

3.6. FOOT AND MOUTH DISEASE

FMD is the highly contagious disease of cloven footed animals caused by FMD virus and considered as most economic devastating disease worldwide. Though the disease has low mortality but the morbidity is very high. There is great loss in the production efficiency of the cured animal. Clinically the disease could be diagnosed by presence of painful vesicles on dental pad, tongue, gums and drooling of saliva. The small ruminants are also affected by the virus but

the visible symptoms are very less. The animal is reluctant to walk, presence of vesicles on heel bulb, interdigital cleft etc are the major symptoms in small ruminants [52]. Various nations are declared FMD free by OIE (World Organisation for Animal Health) but sometimes the virus might re-incur and the outbreak causes huge economic loses. A suitable point of care device for FMD virus identification is required not only for the developed countries but also for the developing countries, as the countries that do not have the facility for the identification of virus, they have to send their samples to the international laboratories. Such transport of biological samples increases the time of analysis and also pose a serious biosecurity concern. Virus isolation, ELISA and RT-PCR test are the classical diagnostic test for FMD virus identification [53]. For rapid diagnosis in field condition Reid et al. developed a chromatographic strip [54]. These strips contain a specific monoclonal antibody against FMD virus. Waters in 2014 validate the lateral flow device coupled with the reverse transcription loop-mediated isothermal amplification [55]. ELISA based electrochemical detection of FMD virus has been developed by Longinotti and coworkers in 2010. A non structural protein 3ABC was used to selectively detect the antibodies. Further they use their biosensor to differentiate between the vaccinated and infected animals[56]. Cortina et al. (2016) presented an electrochemical magnetic microbeads-based biosensor (EMBIA) platform for PoC serodiagnosis of infectious animal disease. The developed biosensor can clearly differentiate the infected from non-infected animals. The antigen (3ABC) was cross linked on superparamagnetic COOH-modified microbeads. By sandwitch format the antibody bound microbeads were seperated, collected and placed on electrode surface. The peroxidase activity was amperometrically measured in substrate (H_2O_2) and hydroquinone. A difference in current values for negative and positive sera was presented during 20s time[57]. The research for the development of FMD biosensor has been going-on, still there are paucity in the field of electrochemical systems for FMD virus detection.

3.7. BOVINE VIRAL DIARRHOEA VIRUS (BVDV)

BVDV is OIE listed infectious disease of cattle, a common bovine respiratory disease. Within a few weeks, the full herd might get infected with the virus. BVDV infection leads to substantial economic impact due to direct and indirect losses. Delayed diagnosis may lead to animal death. In the US, BVD virus causes a total economic loss over US $2 billion annually to the cattle industry. For the effective control of the disease, an early diagnostic platform is essential.

Currently RT-PCR, ELISA, immunohistochemistry are used to identify the virus [58]. Sandwich-type SPR method using dual aptamer conjugated AuNP was developed for BVDV type 1detection with the limit of detection of 800 copies/ml [59].

A conductometric immunosensor has been developed by Luo et al. for detection of BVD antibodies. They fabricate the electrospun biosensor silver electrode using spray deposition for capillary separation and conductometric assay. The detection limit is 10^3 CCID (cell culture infectious dose)/ml for BVDV viral samples [60]. Montrose and coworkers developed an electrochemical immunosensor device for detection of BVD virus in serum [58]. They electrodeposited the BVD virus, as capture biomolecule, on single nanowire. The immunosensor is then applied for detection of bovine viral diarrhoea antibodies (10 μg/ml, 20 min) in both buffer and serum. The sensor clearly discriminates between positive and negative infected bovine sera. The nanowire based electrochemical sensors have the potential for on-farm diagnosis or therapeutic monitoring in animal health applications [2].

3.8. SUBCLINICAL MASTITIS

Mastitis is multifactorial disease of cattle and associated with the inflammation of the udder. The detection of subclinical mastitis is a major concern in dairy industry. Mastitis detection is primarily based on California mastitis test and the electrical conductivity test. The automated dairy farm operation requires a detection micro device that can allow the robotic milking operations. Various scientific groups are working on the development of biosensors for the mastitis detection, though there are very few electrochemical biosensor are available.

Mottram did some work in this field using N-acetyl glucosaminidase (NAGase), which is released in milk during intra-mammary infection. The paper proposed a novel method to detect elevated levels of NAGase using electrochemical biosensing platform. The NAGase convert 1-naphthyl N-acetyl-β-D glucosaminidine to 1-naphthol, which is detected by potentiometric analysis [61]. Okada et al. (2011) fabricate an electrochemical microdevice to identify mastitic cows based on the increased number of neutrophils in raw milk. As neutrophils produces superoxides (O_2^-) which was detected on a gold electrode using superoxide dismutase immobilized via a self-assembled monolayer of cysteine. Furthermore, a micropillar structure was fabricated on the gold electrode to trap and concentrate neutrophils around the electrode. The amperometric changes are dependent on the number of neutrophils. The micro device has an

applicability for rapid diagnosis of subclinical mastitis [62]. As mastitis is caused by various pathogenic organisms, so the biosensor for these organisms can be utilized for mastitis detection.

3.9. SUBCLINICAL KETOSIS

Bovine ketosis is an important metabolic disease during peri-parturient period. Subclinical ketosis can affect more than 40% of the cows in a herd though the incidence rate might go up to 80%. Each 0.1 mmol/L increase in β-hydroxy butyrate (BHB) at subclinical ketosis is associated with a decrease in milk production by 0.5 kg/day for the first 30 days in milk [63]. Further, the estimation of BHB remains a gold standard for subclinical ketosis diagnosis. Biosensors designed for detection of BHB provides precision, easiness and speedy diagnosis for subclinical ketosis. Khorsand et al. (2013) develop a BHB based electrochemical sensor. They covalently immobilize the NAD^+ on single-walled carbon nanotubes (SWCNT), on the surface of screen-printed electrode. LOD of 0.009 mM of BHB in sample was reported in the paper [64]. Weng and colleagues have developed an optical microfluidic biosensor using quantum dots (QDs) modified with cofactor nicotinamide adenine dinucleotide (NAD^+) with detection limit of 35µM [65]. Feng and liu developed a screen printed biosensor using ferrocenemethanol and thionine. The electrochemical oxidization behavior of BHB in the presence of β-hydroxybutyrate dehydrogenase and nicotinamide adenine dinucleotide (NAD) was investigated by researchers and proposes a chronoamperometric method to detect BHB, with a detection limit of 16µM [66].

3.10. SWEAT ANALYSIS

Sweat analysis could provide useful information about individual animal health, especially as a sign of physical stress in animals. These biosensors have ability to non-invasively analyze the metabolites, electrolytes and metals in sweat [67].

Kim et al described a stripping voltammetric biosensor using bismuth/Nafion film electrode for non-invasive monitoring of trace metals in sweat. Such detection method could readily be used to measure the other heavy metals in sweat [68]. Matzeu and colleagues developed a wearable potentiometric strips for real-time monitoring of sodium in sweat. The Solid-Contact Ion-Selective Electrode (SC-ISE) for Na+ detection and a liquid-junction-free Reference Electrode (RE), combined together on a single platform [69]. Multiplexed in situ sweat analysis is critical; the flexible and fully integrated system developed by Gao et al. conveys the levels of sodium,

potassium, lactate, glucose and skin temperature simultaneously [70]. Though the sweat analyzers are developed for human being, but with such technology animal health could be monitored in novel way.

CONCLUSION

In the past few years, the electrochemical sensor remains an active area of research for biomarker detection and disease diagnosis as evidenced by a large number of papers published. In this review, however, we emphasize the impact of electrochemical biosensors for animal pathogen, health and disease-related analysis (Table 1). The main focus was on the electrode fabrication, bioreceptor immobilization, measuring techniques and electrode surface modification that are used as analytical tools for biomarker detection. Aptamers, double-stranded DNA (dsDNA), single-stranded DNA (ssDNA), antibodies, enzymes and other biomolecules were used for animal disease diagnosis viz, E. coli, Brucellosis, Listeriosis, Ketosis, Mastitis. To immobilize the biomolecules on the modified electrode surfaces various immobilization methods like cross linking by EDC/NHS, conducting polymers being used. To observe the hybridization reactions over the electrode surfaces various indicators like methylene blue, ferrocene, daunomycin have been utilized. Different electrochemical techniques like DPV, CV, chronoamperometry, EIS, square wave anodic stripping voltammetry are being used to monitor the electrochemical responses for disease diagnosis and electrode surfaces as well. Electrode surface improvement, signal amplification and label free detection platforms significantly lower the detection limit along with the wide linear response range.

Separation and concentration of analyte of choice from the serum, milk, urine etc is a big problem. The use of Fe_3O_4 or superparamagnetic nanoparticles for Immunomagnetic separation and concentration over electrode surface could be a promising strategy for better electrochemical response. The conducting polymers like Cyclo olefins (COP), poly-5-carboxy indole (5C Pin) deposited to fabricate a metal-polymer hybrid, could enhance the electrochemical responses for detection of very low concentrations of biomolecules.

Electrochemical biosensors are emerging trend, though; there is still a long way to go to be accomplishing a place in clinical laboratory as a user friendly technique. Increase in the efficiency and reducing the time response is crucial for electrochemical biosensor optimization.

Further research is required for the electrode material, modification, stability, design to provide reproducible results with precision, to meet the requirement of point-of-care devices.

Table1: Electrochemical biosensors for livestock health management

Target analyte	Electrochemical Technique	Transducer type	Limit of detection	Analytical Range	Reference
E. coli	Square wave Voltammetric	Enzyme-linked immunomagnetic electrochemistry (ELIME)	5×10^3 cells/ ml	-	Gehring and Tu 2005
	Amperometric	AuNP - modified screen printed carbon electrode	50 CFU/ml	10^2 to 10^7 CFU/ml	Lin *et al.* 2008
	Impedimetric	Self-assembled monolayer (SAM) on gold electrode	10 CFU/ml	10 to 10^3 CFU/ml	Maalouf *et al.* 2007
	Conductometric	Gold interdigitated thin-film electrodes	1 CFU/ml	1 to 10^3 CFU/ml	El Ichi *et al.* 2014
	Cyclic Voltammetry	L-Cysteine-Fe_3O_4 NPs modified GCE	10^1 CFU/ml	10^1 to 10^5 CFU/ml	Panhwar *et al.* (2019)
Listeria	Impedimetric	Electrically resistive aluminum oxide layer	20 CFU/ ml	1.3 to 4.3 log CFU/ ml	Chai et al., 2014
	Chronoamperometric	Self- assembled monolayer-modified gold electrodes	10^2 CFU/ml	10^2 to 10^6 CFU/ml	Cheng *et al.* 2014
	Impedimetric	MNP-Listeria-GNP electrode	1.6×10^3 CFU/ml	1.9×10^3 to 1.9×10^6 CFU/ml	Wang et al. 2017
	Potentiometric	Polycation-sensitive electrode	10 CFU/ml	10-500 CFU/	Ding *et al.* 2014

				ml	
	Impedimetric	Screen printed gold electrode	1×10^{-4} M DNA	1×10^{-4} to 1×10^{-12} M DNA	Kashish *et al.* 2015
Brucella	Impedimetric	Gold nanoparticle-modified screen-printed carbon electrodes	1×10^{4} CFU/ml	4×10^{4} to 4×10^{6} CFU/ml	Wu *et al.* 2013
	Amperometric	Screen printed electrode	0.1 µg/ml Antigen	1.56–100 µg/ml Antigen	Gupta *et al.* 2014
	Impedimetric	Palladium nanoparticle modified gold disk electrode	2.7×10^{-20} mol dm^{-3}	1.0×10^{-12} to1.0×10^{-19} mol dm^{-3}	Rahi *et al.* 2016
	Cyclic voltammetry	Nano-octahedra CeO_2 modified GCE	0.1 ng/ml antigen	6.25 µg/ml–100 µg/ml antigen	Gupta *et al.* 2017
	Impedimetric	Copper doped zirconium oxide screen printed electrode	1×10^{3} CFU/ml	1×10^{3} to 2×10^{6} CFU/ml	Khan *et al.* 2018
	Differential pulse voltammetry	Fe_3O_4 @Au@PEG@HA NPs modified Glassy carbon electrode	0.36 fg/ ml antigen	10^{-15} g/ ml to 10^{-11} g/ ml antigen	Lv *et al.* 2018
Campylobacter	Impedimetric	O-carboxymethylchitosan-Fe_3O_4	$1.0x10^{3}$ CFU/ml	1.0×10^{3} to	Huang *et al.*

		Glassy carbon electrode		1.0×10^7 CFU/ml	2010
	Amperometric	Ion-channel steel electrode	one cell per sample	-	Ivnitski *et al.* 2000
	Square wave anodic stripping voltammetry	Multiwall carbon nanotube screen printed electrode	400 cells/ ml	1×10^3 -5×10^5 cells/ ml	Viswanathan *et al.* 2012
	Cyclic Voltammetry	Cyclo Olefin Polymer deposited thin film gold electrode	90 pM	1-25 nM	Morant-Miñana and Elizalde (2015)
Clostridium	Differential Pulse Voltammetry	Screen printed electrode	10^{-12} M DNA	10^{-12} to 10^{-6} M DNA	Jiang *et al.* 2014
	Differential pulse voltammetry	CeO_2/Chitosan modified Glassy carbon electrode	7.06×10^{-15} mol/L DNA	1.0×10^{-14} to 1.0×10^{-7} mol/L DNA	Qian *et al.* 2018
Bovine Viral diarrhoea	Conductometric	-	10^3 CCID/ml	-	Luo et al., 2010
	Cyclic voltammetry	Gold nanowires electrodes	10 µg/ml Antibody	-	Montrose *et al.* 2015
β-hydroxy butyrate (BHB)	Cyclic voltammetry	Single-walled carbon nanotubes screen printed electrode	0.009 mM	0.01-0.1 mM	Khorsand *et al.* 2013
	Chronoamperometric	Screen printed carbon electrode	16µM	50 µM to 5 mM	Feng and Liu 2015

MNP- Magnetic nano particle, CeO_2- Cerium oxide, GCE- Glassy carbon electrode, CFU- colony forming unit, PEG- poly (ethylene glycol), HA- hyaluronic acid, CCID- cell culture infectious dose, GNP- Gold nanoparticles

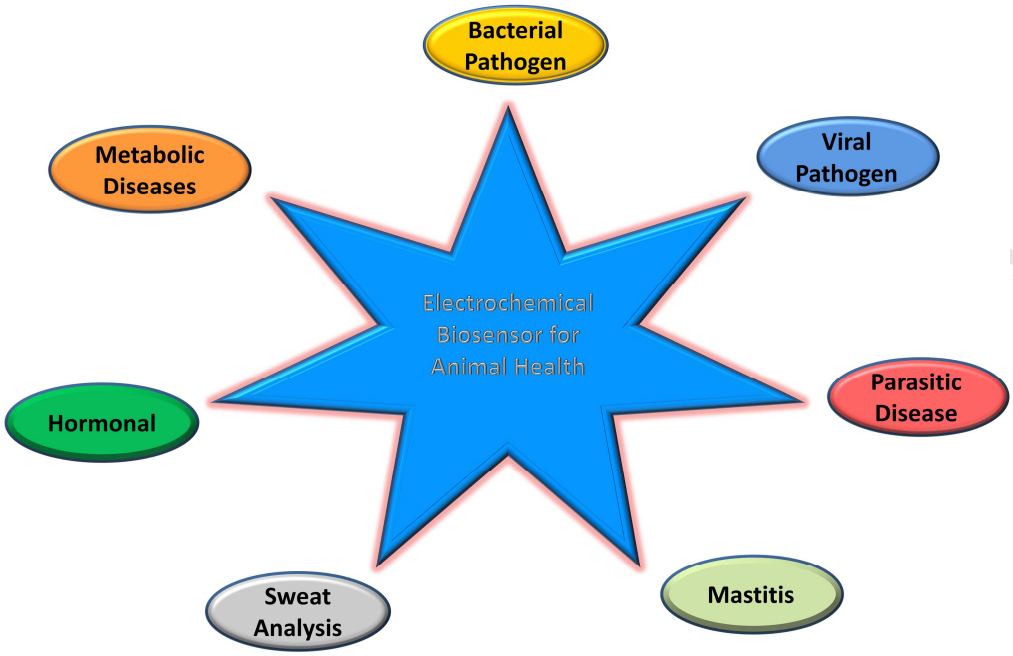

Fig 1. Applications of Electrochemical biosensors for animal health

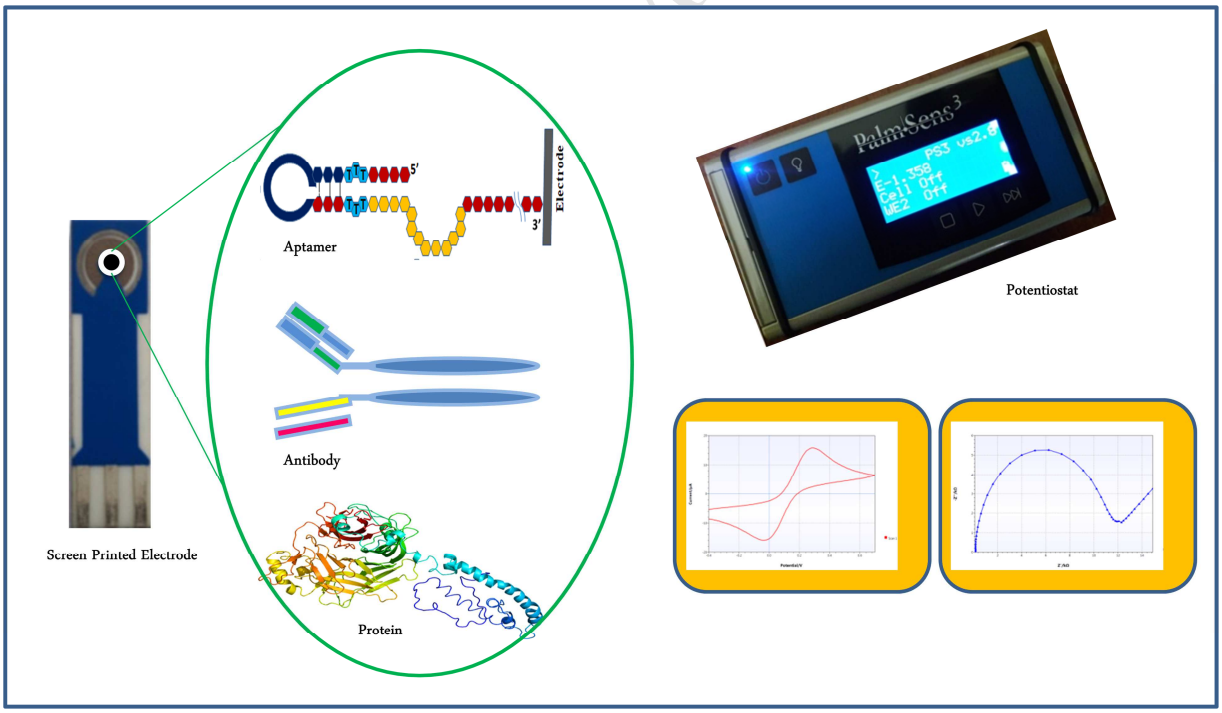

Fig.2. Portable electrochemical potentiostat with screen printed electrode showing the techniques and surface chemistry

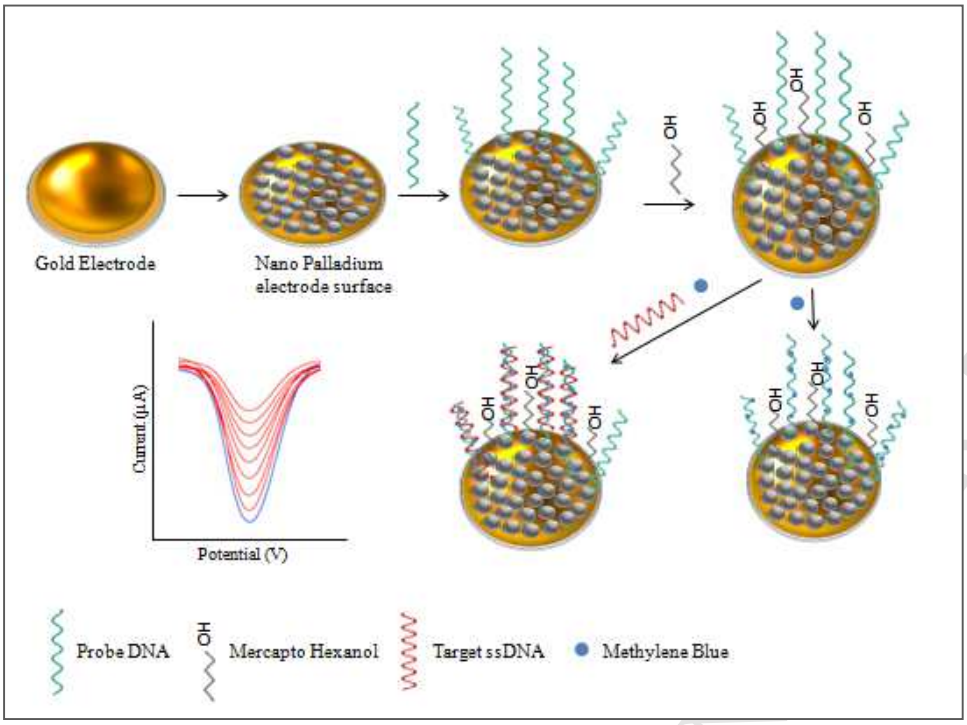

Fig. 3. Electrochemical Genosensor for detection of bacterial DNA. Adapted from Rahi *et al.*[39]

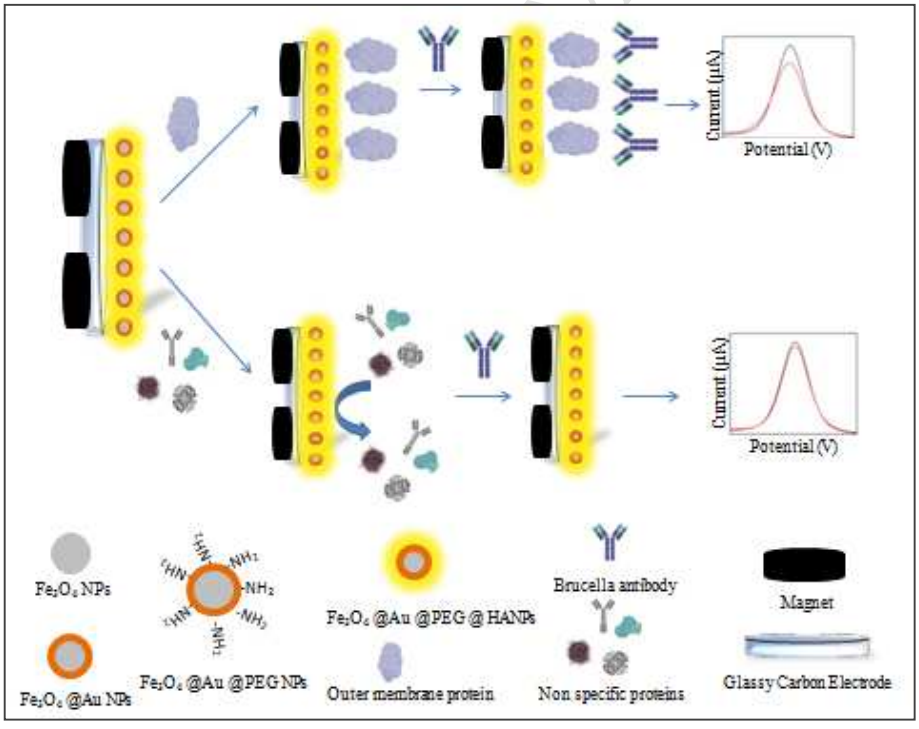

Fig. 4. Fabrication of electrochemical biosensor of brucellosis with Fe_3O_4@Au@PEG@HA Nano particles. Adapted from Lv *et al* [42].

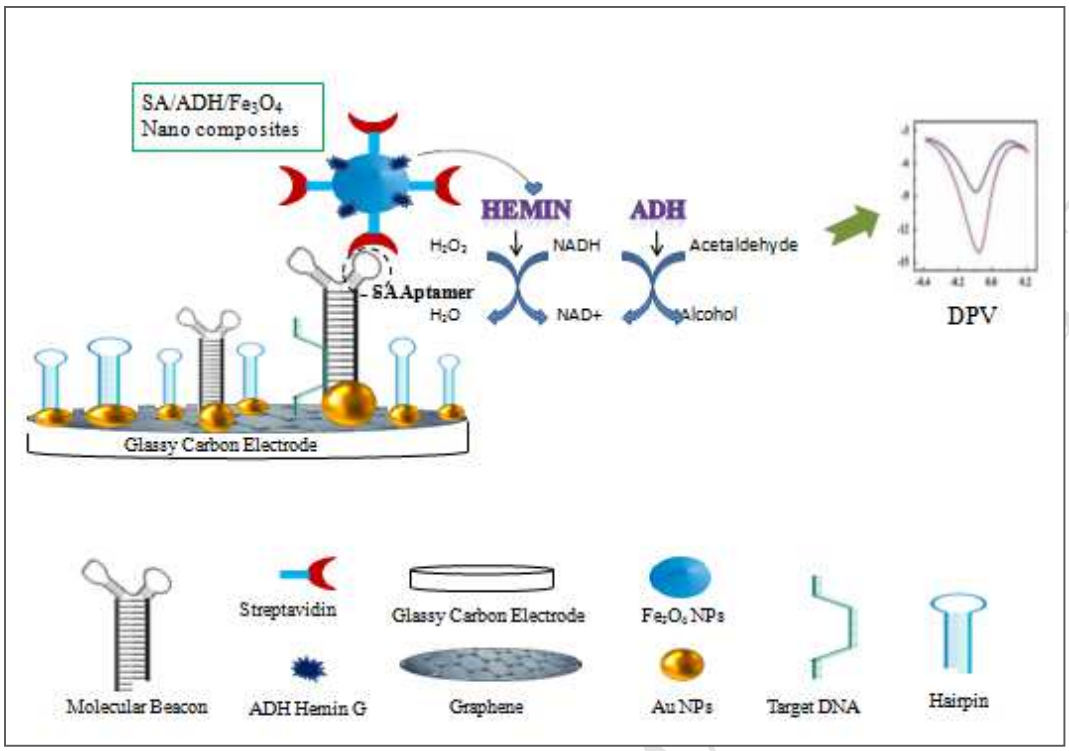

Fig. 5. An electrochemical aptasensor with molecular beacon and hemin/G-quadruplex for the pathogen detection. Adapted from Jiang *et al* [50].

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Livestock are critical component for supporting the sustainable agriculture in the current global scenario. In the era of artificial intelligence and automation in field of livestock, sensors play an important role. Electrochemical sensor is the type of sensor which holds reliability and tremendous promise in raising the animal productivity in developing world.

An early and accurate diagnosis of the animal pathogen and metabolic status are the cornerstone for better animal productivity. The available diagnostic techniques require tedious sample preparation, sophisticated instrument, dedicated laboratory, trained personnel and it is time consuming also. The electrochemical biosensor technology might be a smart solution because of its sensitivity, simplicity, low cost, possible miniaturization and potential ability for real-time analysis. In the veterinary disease diagnostics, various biosensors including electrochemical biosensors have been developed recently, based on disease specific biomarkers. The main focus of article is on reviewing the research in detection of animal infectious and metabolic diseases, hormonal analysis and sweat analysis with electrochemical biosensor.

The Review contains the very recent progress in use of electrochemical biosensors for Animal health and its management. It is unique and is done comprehensively. Corresponding author, Dr Praveen Singh is an expert in SPR biosensors field and also in development of point of care nano-diagnostics for animal diseases