Biosensors for animal health and meat safety monitoring: farm-to-slaughterhouse continuum

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Abstract. The meat supply chain needs to be managed for sufficient levels of consumer protection. Healthy animals are an essential precondition for a safe food supply, since zoonotic diseases, including meatborne pathogens, are a major threat to public health. Information about the livestock’s general health, animal welfare and prevalence of major meatborne hazards such as Salmonella, Campylobacter, STEC and Listeria monocytogenes is of utmost importance for effective biosecurity control on farm. Early detection of these hazards in faecal samples, monitoring blood levels of metabolites relevant for animal welfare (hormones) and animal health (acute phase proteins) can provide high-level control in the animal farming industry. Multiplex biosensors for pathogens and metabolites in the farm-to-slaughterhouse continuum constitute a practical and cost-efficient tool for early detection of signs related to meat safety. Point-of-care multiplex biosensors are an advantage versus commonly used methods ELISA and RT-PCR, since they provide possibilities for early detection and do not require expensive equipment, trained personnel or significant time for sample transfer and analyses. Biosensors can improve meat inspection and meat safety controls, and can serve as a primary tool for monitoring food safety parameters and contribute to the modernization of veterinary inspection and risk-based meat safety assurance system.

1. Introduction
Meat safety is always at the forefront of public health and social-economic concerns [1]. Major meat safety challenges are associated with hazards that can be considered as traditional, new or emerging. This involves increased virulence and/or low infectious dose with antimicrobial resistance or resistance to other food-related stresses [1]. These hazards enter the meat chain in multiple points along the farm-slaughterhouse continuum. On the other hand, current, traditional meat inspection protocols (ante-mortem and post-mortem), based on visual inspection, palpation and incision, were not changed since the end of the nineteenth century, and were not fully efficient in terms of the current needs for consumer protection [2,3,4,5], since these protocols are intended for detection of traditional hazards (e.g. Trichinella spp., Brucella spp., Mycobacterium bovis, Bacillus anthracis and Taenia solium/bovis - cysticercosis) and can even increase cross-contamination due to palpation and/or incision procedures. The emerging hazards affecting safety of raw meat and poultry are bacterial pathogens such as Shiga toxin-producing Escherichia coli (STEC) O157:H7 and non-O157 [6], Salmonella, e.g. the big five: S. Typhimurium, S. Enteritidis, S. Infantis, S. Virchow, S. Hadar [7], Campylobacter jejuni, Yersinia enterocolitica and Toxoplasma gondii, while Listeria monocytogenes remains a concern in ready-to-eat (RTE) processed meat products [1,8]. These hazards cannot be detected by traditional meat inspection methods, and therefore, there is a growing need for development of on-site, user-friendly and rapid
testing and pathogen detection methodologies in the farm-to-slaughterhouse continuum and with sufficient sensitivity and specificity, as are biosensors as point-of-care (PoC) devices. These biosensors are devices that have the potential to detect and quantify physiological, immunological and behavioural responses of livestock and multiple animal species [9]. PoC solutions are an advantage in comparison to the commonly used methods ELISA and RT-PCR, as well as other sensors available on the market that do not provide possibilities of early detection and require expensive equipment, trained personnel and significant time for sample transfer and analyses.

Novel biosensing methodologies offer highly specialised monitoring devices for the specific measurement of individual and/or multiple parameters covering an animal's physiology as well as monitoring an animal's environment. In addition to that, information on animal welfare and general animal health status are valuable to supplement the implementation of harmonized epidemiological indicators (HEI) and food chain information (FCI) flow, from farm to slaughterhouse (bottom-top) and backwards, from slaughterhouse to farm (top-down), setting up the foundation for effective implementation of risk-based meat safety assurance system (RB-MSAS).

2. Biosensor application for animal health and meat safety control: current status

Biosensors in livestock farm management provide significant benefits and applications in animal health and welfare monitoring, including detection of reproductive cycles [9, 10]. With the development of integrated systems and the Internet of Things (IoT), continuously monitoring sensing devices are expected to become affordable. The data generated from integrated livestock monitoring should assist farmers to improve animal productivity. A biosensor is a device that recognizes a target biomarker characteristic for a particular pathogen and/or animal welfare or animal health molecules (indicators), via an immobilized sensing element called a bioreceptor (monoclonal antibody, RNA, DNA, aptamer, glycan, lectin, enzyme, tissue, whole cell) (Figure 1). The bioreceptor is an essential component as its biochemical characteristics assure high sensitivity and specificity of the biomarker detection and allow avoidance of interferences with other microorganisms or molecules present in the tested sample [11, 12]. It is challenging to provide high levels of sensitivity and specificity of biosensors for quantitative detection of biomarkers (pathogen, animal welfare & animal health indicator) in complex media such as the matrices collected from the production environment on farm and at slaughterhouse (e.g. faeces, saliva, blood, serum). Therefore, there is a need for PoC and/or automatic reliable detection and quantification tools that can foresee when disease is likely to occur before that any clinical sign appears in animals [11].

![Figure 1. Biosensor mode of action][12]

2.1. Biosensors on farm
Biosensors can provide accurate and real-time detection for a wide range of conditions related to animal health and welfare on farm [9], such as: lameness in solipeds from acceleration data provided by ear tags, automatic 3D vision locomotion monitoring for cows, monitoring of physiological and behavioural stress in animals, monitoring of vocalisation sounds to assess response of broilers to environmental variables, cattle and pig cough monitoring as indicator of respiratory disease and environmental conditions, drinking behaviour of animals, automatic detection of health (body temperature) with a video-based infrared thermography camera, evaluating hormone profiles to improve automated oestrus detection [9] and faecal shedding of food borne pathogens [10].

2.1.1. Sensor for detection of metabolites

**Mechanical sensors (pressure sensors).** Designed to be used specifically in pastures and stables. The noseband and an electronic interface are connected to record, analyse and store data at 20 Hz at computer [13]. For example, the jaw movement is identified as a pressure peak through the transmission of the movement to the halter and the change in the tube pressure. The software can identify bites and/or chews [13].

**Acoustic sensors.** Used for the analysis of the jaw movement and grazing behaviour, precisely identifying chewing and biting to enable the estimate of the food intake in cattle [14].

**Acceleration sensors.** Used for monitoring of jaw movement and feeding behaviour. The static acceleration due to gravity and dynamic acceleration due to animal movement are measured [15].

**Breath analyses biosensors.** Enable non-invasive method used for disease diagnostics by detection and characterization of volatile organic compounds (VOCs) [9]. VOCs can be found in breath, blood, faeces, skin, urine and vaginal fluids of animals and humans [9]. Breath metabolites encompass gasses, (e.g. hydrogen and methane) and fatty acids, all of which can be used as specific biomarkers for detection of metabolic and pathologic processes. For example, a high level of glucose in blood is detected by presence of specific VOCs, e.g. ketones, ethanol and methanol [16]. In livestock, these biosensors can accurately identify bovine respiratory diseases (BRD) [17], brucellosis [18], bovine tuberculosis [19], Johne’s diseases [20], ketoacidosis [21] and even foot and mouth (FMD) disease [22].

**Perspiration metabolite biosensors.** They have been developed mostly for human health monitoring, such as analysis of sweat for sodium concentration and lactate levels [23]. Such sensors can be also adapted to be used for animal welfare control, e.g. physical stress.

**Tear fluid biosensors.** The level of certain metabolites in tears can provide information about the concentration of these metabolites in blood. For example, a glucose sensor has been developed [24].

**Progesterone analyses biosensor.** This sensor was developed by integrating a selected aptamer specific for its binding properties with progesterone [9].

**Salivary detection of metabolites.** The metabolites detected in saliva can provide valuable information on animal welfare and disease. This is a non-invasive method where biomarkers in saliva are used instead of taking the blood samples. For example, a high level of uric acid in saliva could be connected with a metabolic syndrome, renal syndrome or physical stress, or salivary cortisol, which reflects the level of animal stress, can be monitored [25].

2.1.2. Sensors for detection of animal diseases

**Bovine Respiratory Disease (BRD).** This PoC biosensor is made to be sensitive and specific to anti-IgE present in commercial anti-BHV_1 (bovine Herpes Virus-1, the cause of BRD) and in real serum samples from cattle [26].

**Bovine Viral Diarrhoea (BVD).** The sensor can detect BVD antibodies in serum of cattle [27]. The detection time is 8 min, with detection limit of 10^3 CCID/ml in BVD samples.

**Avian Influenza virus (AIV).** The sensor is based on detection of immobilised H7N1 antibodies, providing low level of detection [28].

**Foot and Mouth Diseases (FMD).** The developed sensor includes a lateral flow immunochromatographic platform for the detection of antibodies against FMD proteins [29].
Mastitis. An indirect on-line sensor system based on the automated California mastitis test (CMT) in milk has been developed [30], or the recently developed sensor for detection of mastitis based on haptoglobin (Hp) [31].

Other. There are also other developed biosensors enabling PoC detection of ketosis and porcine reproductive and respiratory syndrome (PRRS) virus,

2.2. Biosensors in slaughterhouses
There is no wide commercial and routine use of biosensors in slaughterhouse for the purposes of meat safety monitoring, so far. On the other hand, several biosensors for detection of food(meat)bore pathogens are available. For example, lateral flow aptamer-based biosensors for PoC detection of Salmonella Enteritidis and Escherichia coli O157:H7 were developed with sensitivity level of $10^1$ CFU/ml and 10 CFU/ml, respectively [32, 33]; DNA-based sensor for detection of Campylobacter in meat (poultry) samples with detection level of $1.5 \times 10^1$ CFU/g [34]; Cell-based sensors which have mammalian cells as sensing elements to detect the pathogens or toxins of Clostridium perfringens [35]; Antibody-based biosensors for detection of Escherichia coli [36], or; conductometric-based biosensors for E. coli at detection level from 1 to $10^3$ CFU/mL [37]. However, the performance and detection limit of above mentioned biosensors were mainly tested with enriched bacterial suspension (in vitro) with scarcity of data when using a matrix from the production environment (e.g. straw, faeces, blood).

2.3. Biosensors in environmental control (slaughterhouse wastewater)
Slaughterhouses generate a substantial amount of wastewater, and the treatment protocols and post-treatment purity level of waste waters are regulated. Biochemical oxygen demand (BOD) is a widely used parameter to describe the level of organic pollution in water and wastewaters. Just recently, BOD biosensors were developed, based on detection of refractory compounds [38]. Namely, natural waters and slaughterhouse wastewaters have several specific refractory compounds that microorganisms are not able to use and degrade within the biosensor’s short measuring time. A semi-specific biosensor is manufactured that uses Aeromonas hydrophila P69.1 to estimate BOD in high fat and grease content wastewaters, while a universal biosensor is manufactured that uses non-specific Pseudomonas Fluorescens P75. Service life of A. hydrophila and P. Fluorescens biosensors are 110 and 115 days, respectively. The measuring time is 20 minutes, and the biosensor based on A. hydrophila proved to be more accurate in measuring the fat content of the meat industry wastewaters [39].

3. Need for development of biosensors for use in the farm-slaughterhouse continuum (F2SC)
For the purposes of meat safety monitoring and control in the meat supply chain continuum, there is a need for development and optimization of multiplex biosensors which will be effectively used as PoC devices in F2SC [10]. Namely, these biosensors should preferably detect and quantify several key target molecules relevant for farm biosecurity (e.g. selected pathogens), animal welfare (e.g. selected hormones) and general animal health (e.g. acute phase proteins) and, thus, serve as excellent food safety management tools to improve the consumer protection (Figure 2). The major challenges are related to the functionalization and increase of biosensors’ sensitivity, together with optimization of sampling protocols to enable accurate detection of key molecules in matrixes available in production environments (farm, slaughterhouse).

Healthy animals are an essential precondition for a safe food supply, since zoonotic diseases are a major threat to public health. Concerning livestock, early information about the prevalence of major health hazards of bacterial origin is of utmost importance for effective control on the farm. Early detection of Salmonella, Campylobacter, STEC O157, Listeria monocytogenes etc. in faecal samples, as well as monitoring blood levels of metabolites relevant for animal welfare and animal health, can provide high-level control in the animal farming and meat industry. Integration of such multiplex biosensors for pathogens and metabolites in the F2SC is a practical and cost-efficient tool for early detection of signs that meat safety is jeopardized.
The availability of relevant information, together with HEI for cattle, pigs, poultry (as well as other meat producing animals) will contribute to the FCI flow from farm-to-slaughterhouse (bottom-up) and vice versa, from slaughterhouse-to-farm (top-down) and enable implementation of a RB-MSAS [4].

**Figure 2.** A model for practical implementation of multiplex, point-of-care biosensor in the farm-to-slaughterhouse continuum for animal health, animal welfare and meat safety monitoring

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