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Review

Farm Biosecurity Measures and Interventions with an Impact on Bacterial Biofilms

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Abstract: Farm biosecurity management includes a set of practical measures used to prevent and limit the spread of infections to humans and animals. Infections, predominantly caused by zoonotic agents, often occur due to a lack of safety standards monitoring on farms, but also because of the use of inappropriate antimicrobial products leading to bacterial resistance, tolerance to biocides and the emergence antimicrobial-resistant germs. To date, research was mainly focused on studying the antimicrobial resistance in bacterial biofilms and the mechanisms involved in their occurrence. At molecular level, the limited diffusion of biocides in the biofilm matrix, enzyme-mediated resistance, genetic adaptation, efflux pumps, and levels of metabolic activity inside the biofilm are some of the investigated biological mechanisms which can promote antimicrobial resistance in biofilms were also investigated. Interventions, based on the identification of novel antimicrobial compounds, that would exclude the occurrence of bacterial tolerance, including essential oils (oregano, cloves), organic acids (tannic & oleic acid) and natural plant compounds (e.g. alkaloids, flavonoids, tannins and coumarins) were also extensively studied and reviewed given their effectiveness against pathogen-produced biofilms. The aim of this review was emphasize the importance of biosecurity and farm management practices and to assess their impact on bacterial biofilm formation. Furthermore, we present the recent intervention strategies aimed at reducing and combating the formation of bacterial biofilms in livestock farms.

Keywords: farm biosecurity; disinfection; infection control and prevention; anti-biofilm strategies; sustainable livestock; biocides; pathogens

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1. Introduction

Reducing the spread of infectious diseases in livestock is an essential step in maintaining and improving livestock health status and standards. This goal is achieved through high biosecurity standards including a set of preventive measures aimed at reducing the presence of infectious agents and consequently the need of antibiotic over-prescription [1]. The reduction of antibiotic usage through high biosecurity standards will also prevent maintain the effectiveness of conventional antimicrobials designed to treat acute or chronic infections [2]. Likewise, the misuse of biocidal substances in animal production contributes to increasing antimicrobial resistance [3,4].

The set of measures designed to increase farm biosecurity are specifically aimed at preventing the introduction and spread of infectious diseases [5]. Furthermore, they are considered the "key factors" responsible for reducing disease incidence, increased farm productivity and reduced utilization of antimicrobial products, such as antibiotics [6]. Various other measures suggested by experts include constant animal testing for various diseases followed by isolation of these animals [7] or controlling 'visitors' access or requiring usage of personal protective equipment [8] however, the main preventive or treatment

option includes the use of antibiotics [9]. The presence of resistant bacteria in biofilms leads the development of bacterial communities with the strong resilience biocides and antibiotics [10,11]. The antibiotic resistance of the bacterial biofilms was also accentuated by the limited diffusion of antimicrobial agents through the biofilm matrix, enzyme-mediated antagonism to genetic adaptation, metabolic processes inside the biofilm, efflux pumps and outer membrane structures. Also, changes in bacterial phenotype and gene expression accompanied by known antibiotic resistance, metabolic activity and production of virulence-associated factors, are some of the features of biofilm-forming associated microorganisms [12]. Livestock and food sectors are severely affected by biofilm-producing bacteria, suffering severe economic losses, by causing food spoilage, disease outbreaks and even deaths [13]. The occurrence of antibiotic resistance in biofilm forming bacteria indicates the need to design and study effective, non-toxic anti-biofilm agents, and to further investigate the molecular targets related to biofilm generation, adhesion, chemotaxis and motility [14].

The implementation of farm biosecurity measures is highly dependent on farmers and there is a constant risk of improper implementation due to faulty management decisions [15]. For this reason, veterinarians should play a major role in farm management and become farmers' main source of information on animal wellbeing and animal health management [16] providing practical information to farmers on feasible biosecurity measures [17]. Therefore, the communication between veterinarians and farmers is of paramount importance to promote the preventive measures but also all stakeholders involved in the production chain should be engaged in implementing the biosecurity measures and more effective biosecurity practices [18]. Veterinarians and farm managers should first consider the farmers' requirements, priorities, motivations and objectives along with their perception of the effectiveness of the promoted measures [19]. They must also be proactive advisors and provide consistent and consensus messages related to ongoing monitoring and evaluation stages [15]. The solutions must be implemented in stages, and the first step in controlling the spread of disease and in the implementation of a biosecurity plan, should be the identification of risks specific to each farm. Such risks include the presence of zoonotic pathogens, wild animals, high-risk transmission agents and factors with a major impact on the welfare and management of farm animals. The second step in designing a biosecurity program is identifying critical control points or junctures where a type of hazard can be prevented or minimized by using a control measure [20].

Management practices and biosecurity were previously described as crucial for bacterial pathogen colonisation of poultry farms [21], however, in general it refers to the measures that can be implemented by farmers to manage the risks of infectious [22]. For this reason and for those mentioned above, we consider that it is necessary to review the farm management practices and interventions aimed at preventing biofilm formation and eradication in livestock farms. We describe some of the recent interventions designed and tested at farm level and the main bacterial pathogens targeted.

2. Mechanisms of Biofilm Formation and Intervention Strategies

Under certain environmental circumstances, bacteria can adhere to surfaces by creating three-dimensional multi-cellular fortifications, also known as "biofilms" [23,24]. The environmental conditions that affect biofilm development include temperature, pH, O_2 levels, hydrodynamics, osmolarity, specific ions, nutrients, and factors derived from the biotic environment [25]. Biofilms are usually incorporated into a self-produced extracellular polymeric substance (EPS), which can confer increased tolerance to environmental stress, and in the case of a high abundance of pathogenic bacteria, can provide resistance against antibiotics and the host immune system [26]. Biofilm is described as a complex comprising a highly hydrated three-dimensional structure in which the main component is represented by 97% water, along with polysaccharides, proteins, lipids, nucleic acids, amyloids, fimbriae, cells and flagella [27]. The development of a biofilm is a regulated process in several stages in which cell adhesion, EPS production and detachment of microorganisms from

the mature biofilm involve the expression of specific genes. Four general phases of biofilm formation are identified that are similar to most bacteria [23]. A reversible attachment of bacterial cells describes the first stage to a specific surface (e.g., glass, metal, plastic, wood, organic matter, etc.). Biofilms can also affect nutrient uptake, metabolite exchange, horizontal gene transfer, and cellular communication [28,29]. This can impact on the existing antibacterial therapies compared to planktonic bacteria as the capacity of forming biofilms on different surfaces and antibiotic resistance is among the major challenge in livestock farming, human and veterinary medicine [27].

2.1. Poultry

Salmonella, *Campylobacter*, *Staphylococcus*, *Pseudomonas*, *Clostridium*, *Bacillus*, *Listeria*, *Acinetobacter*, *Klebsiella*, *Enterococcus*, *E. coli* and *Aeromonas* spp. are the major biofilm-forming zoonotic pathogens in poultry farms posing a real threat to animal and human health [30–37]. The main risk factors, related to the contamination of poultry farming environment with biofilm-producing pathogenic bacteria, include contact with poultry feed, plants, pipes, dust, utensils, contact surfaces, faeces, and equipment [30,38]. In addition, contamination of poultry products, such as meat and eggs, can occur in multiple settings throughout the food chain, which includes production, processing, distribution, retail commerce, handling and preparation [31]. Specifically, in the water pipes biofilms are mainly formed by *Campylobacter jejuni*, *E. coli*, *Pseudomonas*, *Aeromonas* and *Salmonella* spp. [39] and the most common interventions to eradicate these biofilms are described in Figure 1.

As shown in Figure 1 most biosecurity measures and interventions are targeted the biofilms produce by on *Salmonella* and *Campylobacter*. The *Salmonella* genus has the ability to form strong and antimicrobial-resistant biofilms on floors, walls, pipes, and drain systems, including contact surfaces made of stainless steel, plastic, polystyrene, aluminium, rubber, and glass in poultry farms [31]. For example, *Salmonella* spp. recently isolated from poultry slaughterhouses, revealed a high antimicrobial resistance pattern and was classified as multidrug-resistant with an increased biofilm-producing capacity [37]. This increased capacity to form biofilm indicates that *Salmonella* is equipped with a set of specific genes such as *luxS*, *adrA* and *csgD*, which enables this pathogen to construct high-intensity structures associated with cellulose matrix and the fimbria adhesion factor, all elements which are participating in the stabilization and integrity of the sessile architecture [40]. Particularly, the *csgD* gene encrypts a protein that attributes improved rigidity of the biofilm structure under environmental stress conditions [40]. *Salmonella* is a genetically and phenotypically diverse genus, comprising more than 2500 serovars [41]. Isolates of the serovars *Enteritidis*, *Hadar*, *Heidelberg* and *Newport*, *Schwarzengrund*, *Braenderup*, *Hadar*, *Infantis* and *Typhimurium* were reported to produce strong biofilms on plastic surfaces at 25 °C and 15 °C and are mainly related to foodborne outbreaks linked to poultry products [41]. *Salmonella Typhimurium* is frequently associated with food outbreaks linked to consumption of contaminated eggs [42].

Other genes, including *adrA*, *csgD*, *csgB*, and *bapA* genes are commonly used as markers of biofilm detection in contaminated eggshells [49]. Recent research showed that cold nitrogen plasma, or plasma activation, could efficiently eliminate *S. Typhimurium* biofilm formed on the surfaces of hen eggs and duck eggshells without compromising the product's sensory quality [43]. The results showed that cold nitrogen plasma reacts with water molecules and induces hydroxyl radicals and the stimulation of bacterial cells to boost the levels of intracellular oxidative stress. Biofilm treatment by cold nitrogen plasma, at 600 W for 2 min, reduced the metabolic activity of *S. Typhimurium* by ≈ 82.2%, significantly declined the levels of extracellular proteins (from 68.5 to 12.3 μg/mL), extracellular polysaccharides (from 32.1 to 8.4) and extracellular DNA (from 26.5 to 8.6 μg/mL) and reduced the formation of bacterial with 3.13 logs CFU/cm^2 [43]. Moreover, such treatments were revealed as efficient against other pathogens including *Staphylococcus aureus*, *E. coli* and *Listeria monocytogenes* [30].

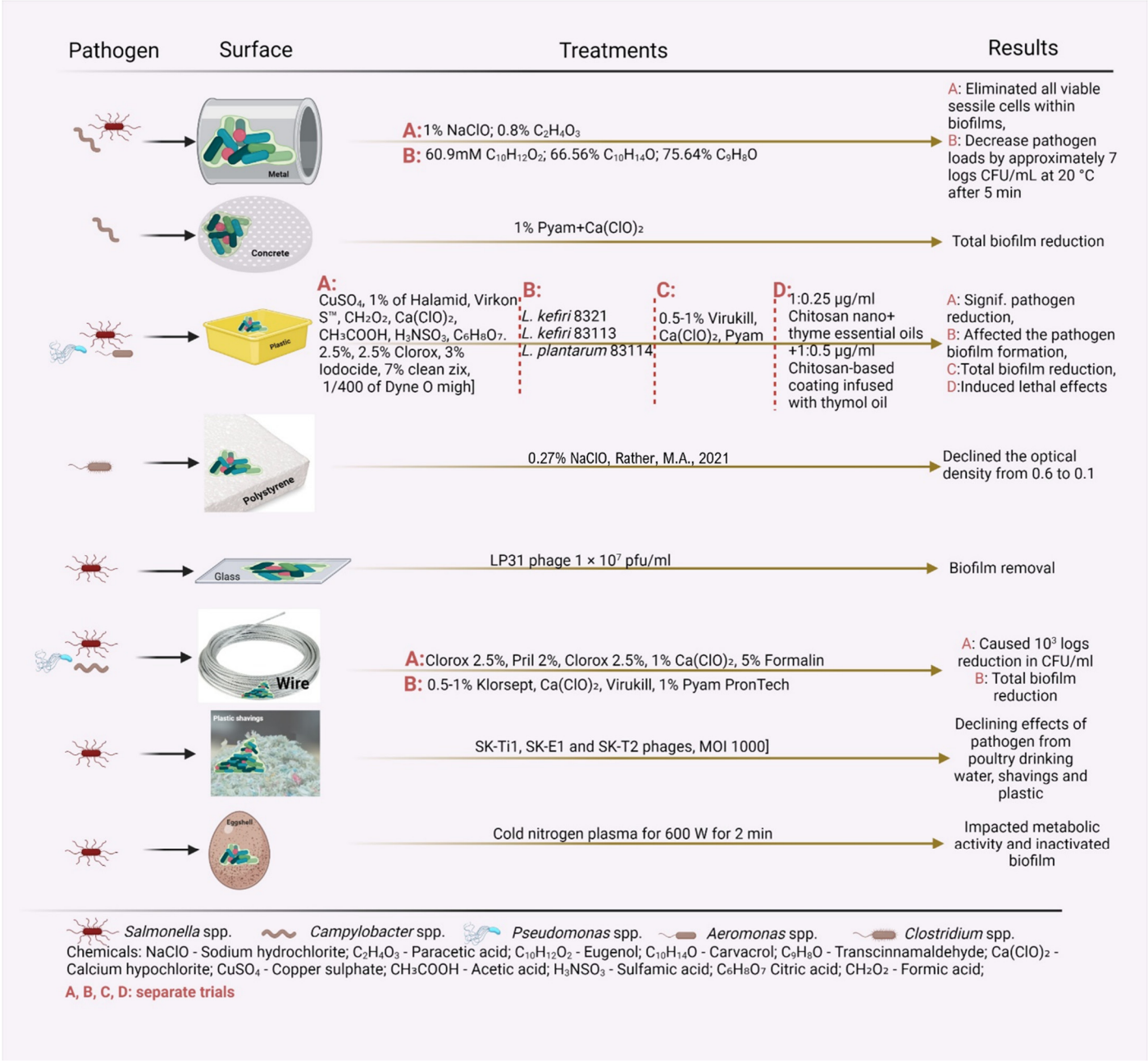

Figure 1. Poultry farm surfaces, biofilm forming pathogens and eradication solutions for *S. Minnsota* [40], *S. Typhimurium* [43], *P. aeruginosa* [44], *C. perfringens* [33], *S. Enteritidis* [45], *S. Pullorum* [34], *Salmonella* serovars [46], *C. jejuni* [47] and [48], *A. hydrophila* and *A. caviae* [36]. Created with Biorender.com.

As indicated in Figure 1, organic acids have also been tested for their capacity to reduce biofilm formed by *Salmonella enterica* serovars [42]. Results showed that acidity regulating salts (product A) applied at 0.4% concentration for 90 min, reduced the bacterial counts from a 3-day old biofilm by 4.90 logs CFU/peg and 5 days biofilm by 2.52 logs CFU/peg. At a similar concentration and in the same conditions, product C, described as a synergistic blend of free and buffered organic acids, induced a biofilm reduction between 2.98–3.76 logs CFU/peg. However, none of the products have completely eliminated 3 and 5-day-old *S. Typhimurium* biofilms and other strategies are considered [42]. Environmentally-friendly, bacteriophages and their derivatives are becoming popular strategies for reducing various bacterial pathogens in the food industries and other farm environments. Previous findings

showed that bacteriophages isolated from sewage environments successfully controlled biofilm-producing *Salmonella* spp. on glass, stainless steel and plastic surfaces [38]. Generally, antibacterial mechanisms of bacteriophages are ascribed due to their ability to adsorb and disperse in the biofilm matrix by generating specific degrading enzymes which facilitate biofilm destruction. An LP31 phage recently isolated from poultry faecal samples at a concentration of 1×10^7 pfu/mL showed a promising biofilm removal produced by *S. Enteritidis* and *S. Pullorum* from glass tube surfaces [34]. Furthermore, LP31 in drinking water application considerably reduced the bacterial counts of *S. Enteritidis* from chicken faeces and their environmental surroundings, suggesting its role as a successful candidate for *Salmonella* control in poultry farms.

Campylobacter jejuni and *Campylobacter coli*, are both species known to contaminate poultry equipment, scald tanks, plastic curtains, steel bolts, feather plucking machines, vent cutting, wastewater collection systems and evisceration equipment from slaughterhouses [50]. The environmental conditions at slaughterhouses can favour *Campylobacter* to persistence and adherence to different surfaces and form biofilms, especially at the bleeding and depluming section where temperatures can reach 30 °C [50]. Compared to other foodborne pathogens (e.g., *E. coli*, *S. aureus*, *S. enterica*), the genus *Campylobacter* is considered a more fastidious type of bacteria that requires more restricted growth conditions and lacks several stress-resilient genes, nevertheless, it is still a potent biofilm former [50]. The biofilm forming *C. jejuni* and *C. coli* strains, recently isolated from different poultry flocks, displayed an increased multidrug resistance profile to various antibiotics (ampicillin, nalidixic acid, ciprofloxacin, erythromycin, trimethoprim and sulfamethoxazole [50].

Currently available commercial biocides (Figure 1) are able to eliminate *C. jejuni* formed biofilm on various surfaces within poultry farms [47]. Environmentally friendly compounds, such as plant extracts could also have significant destructive effects in *C. jejuni*-formed biofilms from polystyrene and stainless steel surfaces from poultry farms at 20 °C and 37 °C, respectively [51]. The most efficient results were described for eugenol, carvacrol and trans-cinnamaldehyde, which when applied at 60.9, 66.56 and 75.64 mM concentrations, inactivated the biofilm by decreasing pathogen loads by approximately 7 logs CFU/mL at 20 °C after only 5 min of contact [48]. Eugenol, for example, also modulated the gene expression of *Campylobacter* oxidative stress regulators *ahpC* and *cosR*, including the cell-surface related gene *waaF*. Plant extracts, such as wheat extract, also inhibited *S. aureus* biofilm formation at concentrations of 0.29 mg/100 mL reducing 95.53% of *S. aureus* biofilms after 24 h of application inside the tubular structures used in the poultry farms [52]. These types of strategies could indeed be as efficient as the more industrial and non-environmentally friendly disinfectants given their above reflected efficiency and cost effectiveness in poultry farms.

2.2. Dairy

The processing equipment and devices in dairy farms are often considered surfaces to which organic (e.g., calcium phosphate and whey proteins) and biofouling substances facilitate the adhesion of microorganisms. Surfaces that come in contact with dairy products can adsorb organic and inorganic molecules from milk, forming a layer of organic matter that alters the physical properties of the food contact surface, leading to increased bacterial attachment [53]. Biofilm formation on the farm equipment could also lead to food spoilage due to the presence of spore-forming bacteria [54] and to an increased risk of foodborne disease transmission [55,56].

Listeria monocytogenes, resposible for causing listeriosis, is one of the most isolated pathogen in dairy industry because of its ability to form biofilm leading to increased persistance [57]. This ubiquitous pathogen survives, proliferates and produces biofilms in harsh conditions [58]. Some strains of *L. monocytogenes* may persist in food processing plants for long periods of time due to their biofilm formation capacity, resistance markers, disinfectant tolerance, and the presence of the stress survival islets (SSI) 1 and 2 [59]. The presence, absence and truncation of genetic markers (SSI-1, *inlA*, *inlL* and *actA*) and the biofilm phenotypes of different *L. monocytogenes* strains, indicate that SSI-1 is associated with biofilm formation in *L. monocytogenes*.

Staphylococcus aureus (MRSA) is known for causing intramammary infections in dairy cows, leading to significant milk losses, animal suffering and economic losses for the dairy industry [60]. Reducing its health impact is dependent on the capacity and the availability of solutions to impede biofilm formation and consequently reduce its capacity to fight antibiotics [61–63]. *S. aureus* has the ability to remain undetected in the udder, causing misdiagnosis of bovine intramammary infections (IMI) and becomes persistant in dairy herds [64]. The quest in identifying effcient interventions have startet long ago and an eraly study identified that hydrogenated hyperforin inhibited the growth and formation of biofilms in *S. aureus* Ig5, *S. aureus* ATCC 29213, MRSA, and *Enterococcus faecalis* ATCC 29212 [65] at a concentration of 37.5 μg/mL. Other anti-MRSA molecule tested was the synthesised 2-aminoimidazole triazole compound (2-AITs) with a 50% biofilm inhibition efficiency [66]. Tannic acid can also inhibit the development of *S. aureus* biofilm at a concentration of 20 μM through a molecular mechanism conditional upon the putative transglycosylase IsaA (immunodominant staphylococcal antigen A) involved in the cleavage of bacterial peptidoglycans [67]. Other extracts, including carvacrol and thymol essential oils, reduced *S. aureus* and *S. epidermidis* bacterial motility and virulence and inhibited biofilm formation. Carvacrol affected genes encoding quorum sensing (QS) and inhibited the production of acyl-homoserine lactones (AHLs) [68]. Oleic acid (primary unsaturated fatty acid) reduced the formation of *S. aureus* biofilm by blocking bacterial adhesion when added during the initial adhesion phase at concentrations of 2.5 nM [69].

As indicated in Figure 2, *Bacillus licheniformis* and *Pseudomonas aeruginosa* are two other pathogens considered extremely challenging for the dairy industry. Eradication or prevention of biofilm formation and maturation by these two pathogens has been achieved by modifying stainless steel surfaces with a peptide-based coating with no impact on dairy products [70]. The tripeptide-based coating containing elements that enables its assembly into coating, its absorption and antifouling activity were based on fluorinated phenylalanine residues [71] and remained intact after exposure to raw milk and retained its anti-biofilm activity [70]. Overall becomes clear that finding the correct interventions to reduce the presence of biofilms dairy farms or dairy processing as they can pose a threat the quality and safety of dairy products. Deeper understanding of the biofilm producing processes and the availability of resistant surfaces should become a priority for the industry to avoid the need for environmentally no-friendly products.

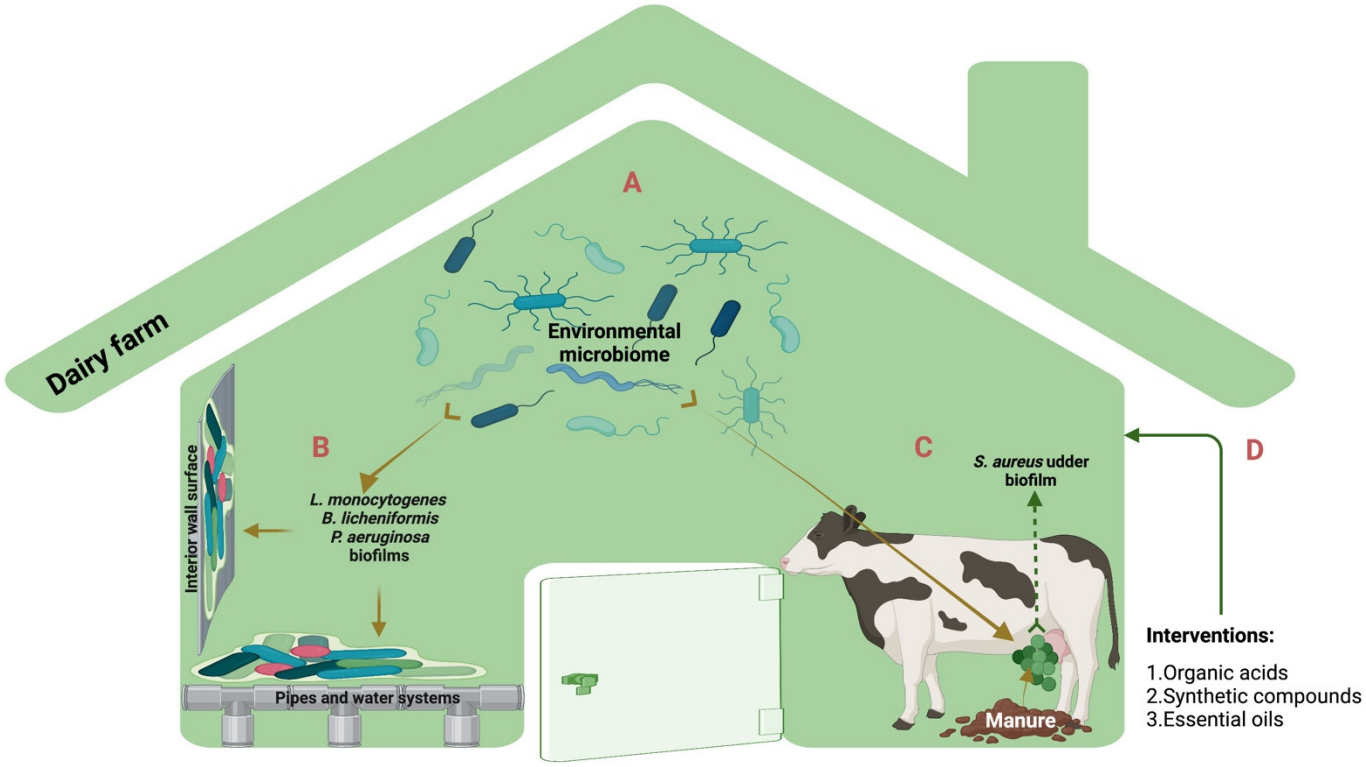

Figure 2. Examples of dairy farm biofilms and interventions. A. Inadequate conditions and poor disinfection in dairy farms leads to the growth and development of a pathogenic microbiome; B. *L. monocytogenes*, *B. licheniformis*, *P. aeruginosa* are the most widespread pathogens in dairy farms and identified in most biofilms; C. *S. aureus* due to its ability to "mask" and remain undetected causes significant economic damage due to its ability to form biofilms and cause mastitis and intramammary diseases in cows; D. Combating biofilm formation on the premises of dairy farms is an essential strategy, and currently tested compounds such as organic acids, synthetic compounds or essential oils gave promising results. Created with Biorender.com.

2.3. Pig Farms

Biological control and growth inhibition of bacterial biofilms in the pig rearing facilities is of major importance since they could ultimately impact on both pigs and human health (Figure 3). Water provided in pig farms can be a frequent route of pathogen transmission (D in Figure 3) as they can enter the water supply chain through external sources such as well waters or via routes inside the farm, including dispensers and nipple drinkers [72]. Such pathogens include *Salmonella*, *E. coli* [73], *Campylobacter* and *Yersinia enterocolitica* being responsible for severe food-borne diseases in developing countries with a temperate clime [74]. The disease caused by *Yersinia enterocolitica* was directly related to contaminated water supply or other food products [75].

These fecal bacteria have become and indicator group that should be avoided in the drinking water supplied to farm animals as they could lead to pig respiratory diseases, diarrhea, therefore impacting pig production and cause significant economic losses to farmers. Moreover, the listed pathogens were reported as high prevalent virulent strains and potent biofilm-formers in the farm water irrigation systems and environmental surfaces [76,77]. Unsanitary pig slaughtering environments and meat preparation surfaces with the equipment sheltering bacterial biofilm could also become as a source of pork meat contamination [78]. To avoid the uptake of undesired pathogens in pig farms and for improved biosafety status, various physical and chemical disinfection procedures should be applied.

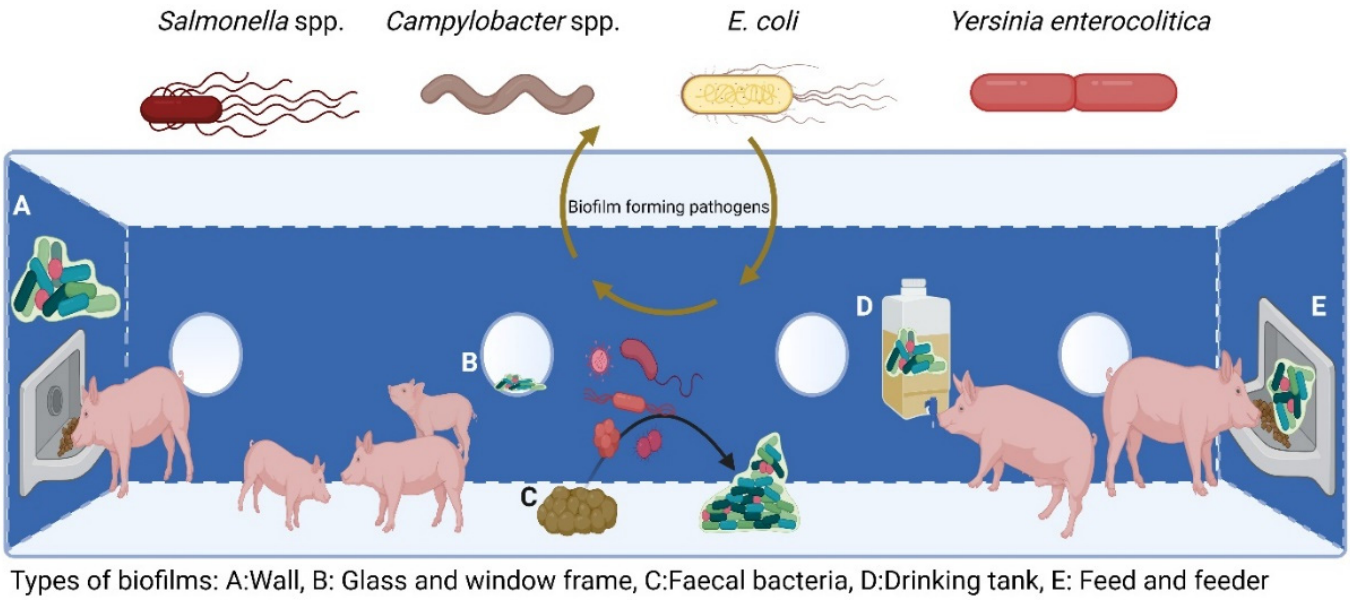

Figure 3. Examples of pig farm biofilms and interventions. The Pig farm environment is prone to form biofilms usually represented by *Salmonella* spp., *Campylobacter* spp., *E. coli* and *Y. enterocolitica*. A, B. The walls and glass surfaces should be subjected to frequent disinfection and cleaning procedures followed by the application of physical pressing wiping; C. Fecal bacteria have become indicator agents in the drinking water supplied to farm animals and they could lead to pig respiratory diseases and diarrhoea; D. The water supply in pig farms is a frequent route of pathogen transmission through external sources, dispensers or nipple drinkers; E. Feed or the feeders should be replaced as often as possible, and the feeders should be thoroughly wiped and cleaned with corresponding agents. Created with Biorender.com.

As described in Figure 3 pathogens can form biofilms on many areas of the pig farmhouse and efficient interventions (Figure 4) are designed by understanding the biological mechanisms involved in the observed anti-biofilm effects. One example is the constant struggle to control *Y. enterocolitica* biofilm formation in pig farms, known for causing yersiniosis in humans after consumption of contaminated products. A recent study has reported the effects of vanillic acid against biofilm-forming parameters of *Y. enterocolitica* on polystyrene (12-well plate) [74]. The in vitro and in silico results showed that different concentrations of vanillic acid had an anti-pathogenic and an anti-QS (quorum sensing) activity, inhibited 16% of cell-surface hydrophobicity, 52% of extra polymeric substances production and 60% of surfactant production [74]. More recently, it has been shown that 64 μg/mL of equol could inhibit biofilm formation and reduce *Y. enterocolitica* ATCC 9610 biofilm formation in polystyrene plates by 91.2% [75]. Mechanistically, through transcriptomic studies it has been shown that equol downregulated the expression of biofilm-associated *hms*T gene together with motility-related *flh*DC gene and attenuated metabolism-associated activities in *Y. enterocolitica* formed biofilms by approximately 86.6%.

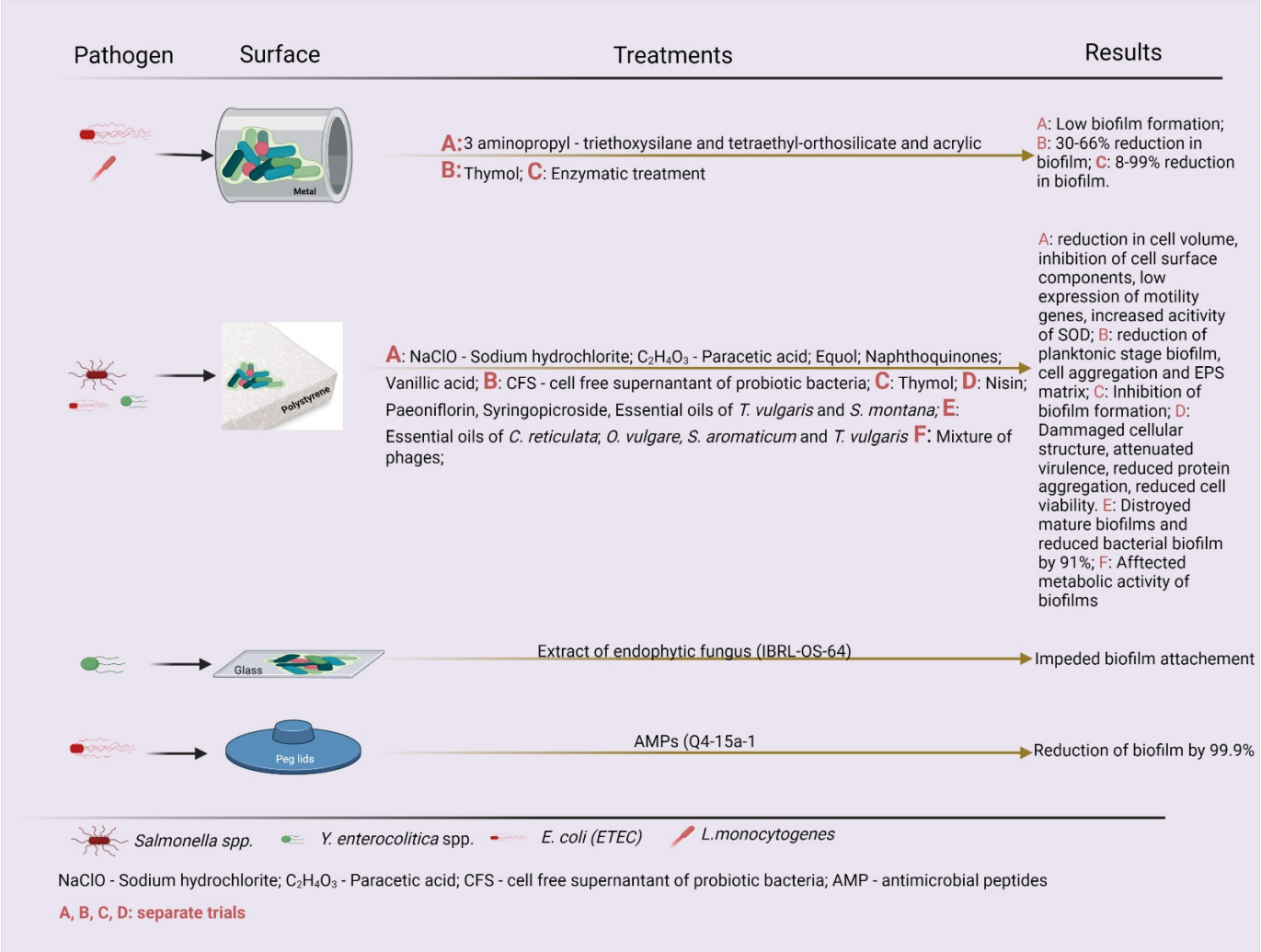

Figure 4. Poultry farm surfaces, biofilm forming pathogens and eradication solutions *Salmonella* app. [79–86]; *Y. enterocolitica* [74,75,77,87,88]; *E. coli* [89–92], and *L. monocytogenes* [93]. Created with Biorender.com.

3. Management and Prevention Strategies

Farm biosafety procedures include a set of measures and protocols that, once implemented, can reduce the risk of disease occurrence and transmission on farms [94,95]. The technologies and strategies (Figure 5), implemented by farmers, are required to achieve high biosecurity levels and control or prevent infections [96,97]. Pathogenic microorganisms are transmitted to farm animals through direct or indirect contact. Direct contact involves animal-to-animal or animal-to-farmworker contact. Indirect contact occurs when an infectious agent is transported by farm staff, or the contaminated objects from the infected animal are transmitted to an uninfected animal or person [98]. The preventive measures are intended to disrupt the possible pathogen transmission routes and are presented in Figure 4, each section being further discussed.

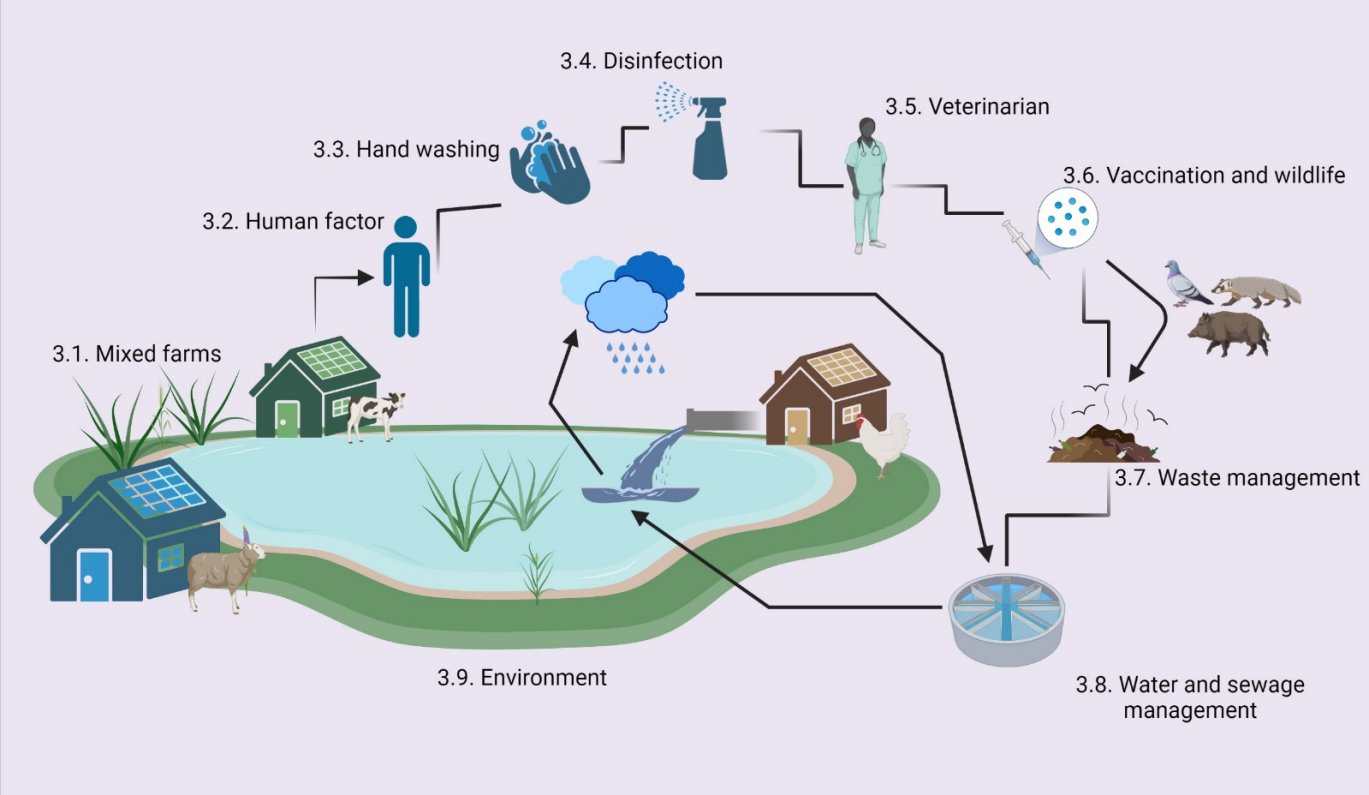

Figure 5. Farm management practice with an impact on bacterial biofilm formation and control. Important biosecurity steps essential for improved farm management: 3.1. Separation of domestic species, their removal and testing for the presence of possible diseases would be another important point of the biosecurity plan; 3.2. The human factor as a biosecurity measure aims to prevent indirect transmission by avoiding sharing equipment and promoting the effective disinfection of shared equipment, whilst the visitors should receive personal protective equipment for close contact with animals; 3.3 and 3.4. On-farm washing with detergents according will allow more effective removal of debris and biofilm; 3.5. The veterinarians should maintain a healthy environment; 3.6 Preventive environmental measures on farms should include the installation of physical barriers/fences, monitoring schemes, vaccination, including mandatory testing of wildlife fauna; 3.7. Wastes from sick animals, also including any biological wastes from wild animals should be subjected under appropriate disposal procedures; 3.8–3.9. Corresponding water management and environmental contamination prevents animals from accessing natural waters such as streams and creeks which could be a potential source of pathogens. Created with Biorender.com.

3.1. Mixed Farms

The presence of different species of animals on farms (e.g., cattle, goats, pigs or sheep) makes biosecurity management and the required strategies even more difficult to implement. Different livestock species can affect the control of other domestic species, causing damage of animal welfare standards and creates health problems and economic losses. Separation of domestic species, their removal and testing for the presence of possible diseases sharable with other species would be another suggestive point of the biosecurity plan. Managing livestock and biological waste is also necessary to reduce the risks of farm illness occurrence and circulation on farms [99].

3.2. The Potential of Human as a Vector for Disease Transmission

Transmission of biofilm forming pathogens and disease by visitors who come into close contact with animals (e.g., veterinarians, technicians, animal transporters) represents a major challenge in implementing efficient biosecurity measures [100]. Farm employees

who work on multiple farms and are in contact with many livestock species are at risk of spreading these pathogens in areas with animal circulation [101]. Exchanging or sharing equipment between farms (e.g., tractors) is another type of indirect contact [102] and biosecurity measures are required to prevent indirect transmission by avoiding sharing equipment and promoting the effective disinfection of shared equipment, whilst the visitors should receive personal protective equipment for close contact with animals [103]. Livestock transport vehicles should arrive cleared, clean and disinfected before entering another farm. Vehicles carrying animals from other farms are not advised to enter new farms without permission, and the drivers are not to be allowed to come into close contact with animals from the new farm [104].

3.3. Basic Hand Washing

Proper hand hygiene by soap and water washing or alcohol-based disinfectants is a proven solution in preventing the transmission of infectious agents and is widely accepted as one of the most important infection control measures [105]. The hand washing process mechanically reduces organic debris and transient/resident microorganisms from the skin, and by adding an antimicrobial compound except for antibiotics, a variety of microorganisms could be killed or inhibited [106]. It is also desirable that personnel who come into contact with animals keep their nails as shorter as possible to minimise the accumulation of contaminants under the nails to facilitate effective hand hygiene or alternatively simply utilise gloves. In addition, wearing jewellery should be avoided for safety and hygiene reasons. Hands should be sanitised before and after coming into contact with animals. Although hand washing effectively reduces the transmission of infectious agents, this can often compromise the integrity of the skin [107]. The utilisation of soap and water followed by the application of moisturising lotions are essential to promote healthy skin for the personal. Alcohol-based hand sanitisers can also provide a practical alternative. Studies recommend using hand sanitisers containing 60–95% ethyl or isopropyl alcohol [108]. Alternatively, ethyl and isopropyl alcohol are effective or even more useful than simple hand washing and can be utilised when soap and water are unavailable [109].

3.4. Cleaning and Disinfection

Cleaning and disinfection are multi-step processes that involve removing visible debris, washing the area with detergent, rinsing, and applying a suitable disinfectant at the correct dilution and for a recommended contact time. Although cleaning can reduce the bacterial load by approximatively 90% on a concrete surface, with the subsequent application of disinfection, the bacterial load will decline by up to 96% [110]. High concentrations of bacteria, persistent in an environmental niche, are very prone to form biofilms that adhere to various surfaces (e.g., glass, plastic, steel, wood). Biofilm formation significantly improves bacterial survival in different environmental niches, including unfavorable environments where disinfectants and antimicrobial treatments have been applied at inappropriate concentrations [24], hence, washing surfaces with detergents according to the manufacturer's instructions will allow a more effective removal of debris and biofilm [110]. Desinfection is also connected to quarantine as the latter is the most important measure in farms that aim to relocate animals and apply of quarantine rules appropiatelly [111–113]. In the case of a detected infection event, to avoid its spread, a biosecurity measure is recommended, consisting in the isolation of the sick animal and the elimination of the pathogen through the allocated disinfection of spaces [106].

3.5. Relevance of the Veterinarian

Veterinarians are prone to introduce various diseases and infections on farms including, for example, Bovine Viral Diarrhea (BVD) and Bovine Herpes Virus Type 1 (BHV). The veterinarian represents the highest risk in transmitting diseases between farms, as his main role includes visiting different farms on the same day and coming into direct contact with the animals [100]. Protective clothing and boots are the best-known measures of biosecurity

to minimise the likelihood of disease transmission [104]. The veterinarians should maintain a healthy environment and must be accountable for providing vital animal care. Their mission is also to ensure that their work is done in a clean environment and following well-installed standards. Previously, the equine influenza epidemic has emphasised the importance of biosecurity not only in protecting sick animals but also in protecting healthy animals on farms [114]. Veterinarians must remain vigilant to reduce the risk of introducing or spreading an infectious pathogen on farms.

3.6. Vaccination—A Sustainable Biosecurity Approach

In most farms, the strategic implementation of effective vaccination programmes (including sometimes of the neighbouring wildlife) is an attractive and efficient tool for preventing and controlling infectious diseases, improving animal health and welfare and reducing the transmission of zoonotic diseases [115]. For example, vaccination against *Brucella* spp. has effectively controlled brucellosis in the bovine population and reduced its impact on human health [116]. Disease control through vaccination campaigns can be problematic, as some diseases, such as *Mycoplasma bovis* pneumonia which cannot currently be controlled by vaccines, while others, such as pasteurellosis, require strain-specific vaccines [117]. Vaccination is only one aspect of the infectious disease control strategies and should be associated with strengthening on-farm biosecurity, expanding surveillance, and monitoring programs to raise disease awareness [118].

3.7. Waste Management

Waste management represents an important element of a comprehensive biosecurity program. Wastes from sick animals, also including any biological wastes from wild animals, may harbour pathogens that exhibit adverse effects to livestock animals and public health, and represent a specific concern for farms. Appropriate disposal procedures, such as autoclaving and incineration, are some of these ways depending on each infectious agent. Effective methods for disposing of the possible contagious agent may also include composting and the application of steam methods [99].

3.8. The Importance of the Water Management

Water poses a high risk for indirect transmission of several diseases, e.g., tuberculosis, due to its abundance and potential to retain mycobacteria. One of the biosecurity strategies includes preventing animals from accessing natural waters (streams and creeks), which are common on farms. They often dry out in summer and can retain water only in some earthy cavities. These water sources frequently flow through several locations on the farm, and preventing livestock access, by creating access to secure sources, could be one of the management tools to reduce the spread of infections and diseases in farms [119].

3.9. The Environment

Other on farm biosecurity management strategies include the reduction of interaction at wildlife–livestock interfaces. Recent studies have described the epidemiological relationships between wild and domestic species and concluded that wildlife presence within agricultural settings could promote the appearance of environmentally related diseases, such as the *Mycobacterium tuberculosis* complex and the persistence of *Staphylococcus aureus* [120–124]. Diseases and pathogens can also be transmitted through the various wild vectors including flies, ticks, rodents, mosquitoes [125]. Cats and dogs or other pets can also serve as biological or mechanical vectors for infectious agents and a source of antimicrobial-resistant bacteria [126]. Previous studies have identified a link between cats and outbreaks of *Salmonella enterica*, where the risk of *Salmonella* transmission has been highlighted based on the impact of cats on rodents and wild birds from farms [127]. As a specific example, the West Nile virus can be spread by mosquito bites, and flies emerge as carriers of Salmonella [128,129]. Therefore, implementing biosecurity strategies to reduce interactions between wild and farm animals could be efficient way to cut the transmission

of common pathogens. The set of strategies available could include actions to control the wild inhabitants (e.g. rodents, mites and insects) via preventive measures on farms (e.g. physical barriers-fences) or changes in farm management [120,130–133]. Other disease control options include vaccination or mandatory testing of wildlife fauna, but these measures are very expensive. One of the key requirement for wildlife disease control is to establish appropriate surveillance and a monitoring schemes to identify the epidemiological risks that lead to the transmission of pathogens at the interface [122]. Environmental monitoring may be more effective than observing an individual animal because it allows the possibility to identify reservoirs of critical pathogens [134]. Otherwise, regular monitoring is a less expensive alternative, but the attractive benefit would be assessing the biosecurity program's effectiveness.

4. Future Prospects

Biosecurity has become essential in animal husbandry as animal welfare largely depend on suitable management practices to ensure compliance. Compliance with control rules, limiting the spread of infectious agents and treating diseases on farms are some of the "pillars" that would contribute to better animal health and welfare prosperity. In-depth knowledge of the possible farm related diseases would help designing specific safety rules necessary for assessing critical control points and contribute to an informed selection of security measures. Failure to follow the recommendations can often lead to serious consequences, such as the persistence of diseases or even chronic diseases, which lead to animal deaths and significant economic losses for farmers. Usually, the source responsible for these damages are the bacteria resistant to antimicrobial products which can often be found in communities called biofilms. Knowledge of these fortified bacterial communities' structure, physiology and function would be essential to combat antimicrobial resistance. Therefore, there is a need for additional research on how farm management practices and tools can reduce bacterial biofilms and minimise the resistance and tolerance to different types of biocides and novel antimicrobials.

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