

Microorganisms Control in Animal Effluents- A One Health Approach

Ana Sofia Pereira Carvalho Soares

Researcher at CITAB - Centre for the Research and Technology of Agro-Environmental and Biological Sciences, University of Trás-os-Montes e Alto Douro, 5001-801 Vila Real, Portugal

Carla Isabel da Silva Miranda

Researcher at CITAB - Centre for the Research and Technology of Agro-Environmental and Biological Sciences, University of Trás-os-Montes e Alto Douro, 5001-801 Vila Real, Portugal

Researcher at University of Trás-os-Montes e Alto Douro, 5001-801 Vila Real, Portugal

Henrique Manuel da Fonseca Trindade

Researcher at CITAB - Centre for the Research and Technology of Agro-Environmental and Biological Sciences, University of Trás-os-Montes e Alto Douro, 5001-801 Vila Real, Portugal

Professor at University of Trás-os-Montes e Alto Douro, 5001-801 Vila Real, Portugal

Ana Cláudia Correia Coelho

Researcher at CECAV - Animal and Veterinary Research Center. University of Trás-os-Montes e Alto Douro, 5001-801 Vila Real, Portugal

Professor at University of Trás-os-Montes e Alto Douro, 5001-801 Vila Real, Portugal

Publication Month and Year: June 2021

Pages: 26

E-BOOK ISBN: 978-81-952994-2-3

Academic Publications

C-11, 169, Sector-3, Rohini, Delhi, India

Website: www.publishbookonline.com

Email: publishbookonline@gmail.com

Phone: +91-9999744933

Index

S. No.	Section	Page No
	Abstract	01
1.	Introduction	02-03
2.	Microorganisms in manure, slurry and effluents	04-08
2.1	Escherichia coli	06
2.2	Salmonella sp.	07
2.3	<i>Enterococcus spp.</i>	08
3.	Manure treatments	09-12
4.	Cattle manure and slurry as an emergent concern in the One Health approach	13-14
5.	Conclusions	15
	Acknowledgements	
	References	

List of Figures

Figure No.	Title	Page No.
1.	Diagram summarizing animal effluents disposal and consequences without treatment application	09
2.	Diagram summarizing animal effluents disposal and consequences when there is a previous treatment application	10
3.	Diagram of the One Health concept	13

Abstract

Utilization of organic manures such as cow slurry is the most economic, practical, environmentally beneficial and useful option for improving soil quality and fertility. In the specific case of land-spreading of bovine manure, that is a common fertilization practice, this effluent is recognised to contain human pathogenic bacteria, which may persist for several weeks in soil, so this practise needs to be done correctly. Spreading fresh manures directly to land presents complex risks in many levels, including a higher risk of pathogen transfer to the food chain for example the liquid fraction of bovine slurry contains an important part of the total bacteria of slurry (>80%). This information was being used during the years to improve guidelines on the management of manures to minimise the risks of pathogen transfer from animal effluents to the human food chain or water systems. The effectiveness of manure management systems needs to concern environmental transmission pathways by which zoonotic pathogens presents in animal manures may be transported to the environment. So, these management systems are planned to reduce the concentrations of microbes found in manure (by 90 to 99% or more). Antibiotics used in farms are excreted by the animals and end up in their effluents, being animal effluents (manure or slurry) used as fertilizers, the antibiotics residues will be transferred to the soil and in some cases can contaminate ground water. With the amount of slurry applied on the soils as fertiliser every year, there is a need of studies to measure the leaching of pathogenic agents, antibiotics residues normally present in slurry, and their fate in the environment using for that a One Health approach. This manuscript is a review of the scientific literature on the viability of some microorganisms in animal effluents and the effects of different manure treatments on the microbial concentrations.

Keywords: Microorganisms; Animal effluents; Manure; One Health

1. Introduction

One of the main problems of intensive animal production is the quantity of animal effluents created in small areas. Every year, one billion tons of animal manure and slurry are produced in the Europe, therefore, the management of slurry was been converted in one of the central dairy activities in farms ^[1]. Manure represents one of the most valuable organic fertilizers, but it could contain a large number of microorganisms that can have pathogenic potential as *Escherichia coli*, *Enterococcus* spp. or *Salmonella* sp. ^[2, 3]. Livestock effluents have long been used as organic fertilizers. However, in the last years, the growth of application of animal production in certain areas has been responsible for the production of large volumes of livestock effluents that if where not managed properly can have significant risks that can have consequences to humans, animals and the environment ^[4, 5]. Introduction of new intensive animal housing practices results in the increased amount of manure produced with a higher share of slurry ^[6]. Utilization of organic manures such as cow slurry is the most economic, practical, environmentally beneficial and useful option for improving soil quality and fertility, and at the same time providing an extra source of nutrients for growing plants ^[7, 8]. Effluents from farming processes include raw manure, untreated slurry (a mixture of manure, urine, split feed, and water that is held without aeration), and treated slurry (that is filtered to separate the solid fraction from the liquid fraction). Livestock manure is constituted by animal excreta (faeces and urine), water, bedding, secretions, blood and skin, nutrients and organic matter used to improve soil properties, but may also contain a variety of pathogens with potential negative impact on human health including zoonosis. Animal effluents can be a reservoir of a huge number of microorganisms as faecal streptococci, *E. coli*, particularly *E. coli* O157:H7 and *Salmonella* sp. but it is also possible the isolation or detection of other pathogenic microorganisms such as multiple virus like rotavirus group A (RV-A), hepatitis E virus (HEV), *Brucella*, *Mycobacterium*, *Leptospira*, *Chlamydia*, *Campylobacter* as well parasites ^[3, 9-13]. The concerns about its management are in the most of the cases only fixated in the environmental effects of its nutrients (Nitrogen, Phosphate, Carbon) and the presence of microorganisms is the most of the cases not must studied ^[14, 15]. Areas with a high density of animal production are

documented as having a higher risk for contaminating fresh produce, however, the specific pathway of contamination remains unproven. Suspected sources include inappropriately composted manure/wastewater and wild animals ^[16]. It is possible that surface application of manure may decline the risk of contamination of groundwater and plant roots compared to injection of slurry ^[8]. Furthermore, it has been shown that *E. coli* O157:H7 and *Salmonella* serovar Typhimurium may be detected in the surface of plants growing in soil corrected with contaminated manure ^[17, 18] and could even be internalized by the plants ^[8,19]. Animal wastes like manure are largely recycled to agricultural land as the most economical and environmentally sustainable means of treatment and reuse. These materials have a beneficial fertilizer value (nitrogen-phosphate-potassium) and can help maintain soil quality and fertility. However, animal manures frequently contain enteric pathogenic microorganisms and land spreading can lead to pathogen entry into the food chain ^[18]. In consequence of this, with the amount of slurry applied on the soils as fertiliser every year, there is a need of studies to measure the leaching of microbial agents normally presents in slurry ^[13, 20].

2. Microorganisms in manure, slurry and effluents

The danger of pathogen transmission by land application of untreated animal manures is documented for more than 100 years. Around 150 pathogens are identified with capacity to be transmitted from animals to humans by different ways. Once manure is correctly treated it is a safe and effective fertilizer, but when it is untreated or incorrectly treated, it may contain pathogens like *Bacillus anthracis*, *Brucella* spp., *Campylobacter* spp., *Chlamydia* spp., *Escherichia coli*, *Leptospira* spp., *Listeria monocytogenes*, *Mycobacterium* spp., *Salmonella* sp., or *Yersinia* spp. that are capable of contaminate the field or even water supplies ^[15]. In the specific case of land-spreading of bovine manure, that is a common fertilization practice. This effluent is recognised to occasionally contain human pathogenic bacteria, such as *Escherichia coli* O157:H7 and *Salmonella* sp. that may persist for several weeks in soil, so the practise of spread manure in the land needs to be done correctly ^[21]. In the past, *E. coli* O157:H7 and *Campylobacter jejuni* liberated to the environment, originating from contaminated manure, resulted in a large waterborne disease outbreak in Walkerton, Canada, related to the consume of contaminated drinking water ^[13, 22]. Determining the environmental fate of pathogens from manure is very difficult. Biological variables include pathogen liberation by individual animals; microbial interactions with the stored manure; inoculation of stored manure each time an animal liberates pathogens; interactions with water, organic matter, exchanges with plants, nematodes, and soil microorganisms after land application. Physical variables that can influence include type of manure storage, temperature and humidity during storage, soil type, temperature, moisture, water, pH, salinity, and rainfall events. There is a need for research to determine the factors that affect the environmental survival and persistence of microbial pathogens in manures ^[11, 23]. Other studies demonstrate that pathogens prevalence and their levels in manures are affected by animals age (with higher levels in the faeces of young animals), dietary variations (for example when cattle are moved between housing and grazing), and with fasting or other forms of stress ^[7]. Other studies have revealed that inoculum cell conditions, exposure of inoculated soil to sunlight, protozoan predation, frost and temperature may also be important to pathogen survival ^[21]. Studies show that *E. coli* O157:H7, *Salmonella*, *Campylobacter* and *Listeria* are capable of survive in

stored slurries and dirty water for up to three months. However, all these pathogens survived for less than one month in solid manure heaps where temperatures are superior to 55 °C [7, 16]. Survival of pathogens may range from several days (turned composted manure) to more than a year (nonaerated manure) [8]. Food-borne illness affects lots of people around the world including in developed countries, between the main causative agents are bacteria, mainly Verocytotoxic *Escherichia coli* (VTECs), *Salmonella*, *Campylobacter* and *Listeria*. One-way explanation for this is the application of contaminated animal effluents to agricultural soil [7]. The anaerobic conditions in the slurry storage have revealed ability to increase survival for *E. coli* O157:H7. Though, for *S. Typhimurium* identical survival in aerobic and anaerobic slurry was similarly appreciated [24]. Also, *S. Typhimurium* has been shown to be more resistant to environmental stresses and survive for longer periods of time in natural substrates compared to *E. coli* O157:H7 [16,25,26]. Survival and transport of pathogens from manure to the environment depends on a number of multifaceted phenomena, like seasonal effects. After infiltrating the soil, the retaining of bacteria depends on the physical structure of soil, the soil chemistry, and the properties of the bacterial cells [17,21,23]. Between soil types, the sandy soil showed to be the best for *E. coli* survival and the nastiest for *Enterococcus* spp. survival. However, *Enterococcus* spp. survived best in the loamy soil. For both bacterial species, an inferior incubation temperature (5 °C) and a higher soil moisture content (100% Field Capacity) improved survival [27]. While the threat of contamination by nutrients leaching from manure-treated fields is well recognized, the threat by leaching of zoonotic pathogens from the manure has received much less attention [13]. This information was being used during the years to improve guidelines on the management of manures to minimise the risks of pathogen transfer from animal effluents to the human food chain or water systems, to reduce the potential costs and environmental concerns (e.g. on ammonia and nitrous oxide emissions to air, nitrate leaching losses to water) or to implement practical measures to control the spread of manure-borne pathogens [7]. Studies refer also that to lower the risk of manure-borne pathogens, such as *Salmonella* serovar Typhimurium and *E. coli* O157:H7 contaminate vegetables grown in manure-fertilized soil, it is necessary to establish appropriate time limits between the application of the manure (if non treated) and the vegetable harvest [17]. To avoid all of these problems, farm effluents should be stored in holding tanks with proper aeration for suitable lengths of time (1 to 3 months or as required) before being used as fertilizers. Improperly incubation and/or storage of slurry can serve as a way for environmental spread and dissemination of pathogens [28].

2.1 *Escherichia coli*

The liquid fraction of slurry contains an important part of the total bacteria of slurry (>80%), including the pathogen indicator organisms such as *Escherichia coli* (*E. coli*). This bacteria is predominant in animal wastes and consequently serves as an indicator of faecal contamination [28], with epidemiological data indicating that the serotype *E. coli* O157:H7 may be existing in up to 8.3% of dairy and beef cattle and that being asymptotically in the faeces [19, 29]. Since it was first recognised as a human pathogen in 1982, Vero cytotoxin-producing *Escherichia coli* (VTEC) serogroup O157 has been gradually recognized as a cause of haemorrhagic colitis, including severe abdominal pain, diarrhoea and blood in the stools. Transmission of VTEC O157 is often foodborne mainly from undercooked contaminated beef or raw milk, and contact with farmed or companion animals it has also caused human infections. The source of VTEC O157 is the gastrointestinal tract of animals, including humans, cattle have been recognised as a main reservoir [30]. Bovine faeces are a potential way for transmitting *E. coli* O157:H7 to cattle, food and the environment, with the pathogen holding its ability to produce verotoxins. *E. coli* is capable to survive much longer in soil than in the manure representing a major risk to contaminate water or vegetables [18, 31]. Studies refereed also that *E. coli* survives longer than other slurry pathogens on slurry storage processes [32]. A significant increase of *E. coli* was observed when slurry was applied to grassland via shallow injection compared to surface application and the decay rates were significantly higher for slurry applied to grassland in spring relative to summer and autumn [20]. Some experiments showed, that the survival rate of *E. coli* in the soil was related to the local pH. Moreover, *E. coli* O157:H7 owns systems for survival at low pH [33]. The animal manures including poultry, cattle, and swine are the primary source of water resources contamination by water-borne pathogens, as well as *E. coli* or *Salmonella* sp. [4, 5]. Research has established the long-term survival of *E. coli* O157:H7 in manure. It may survive at least 19 weeks at 9±21 °C in bovine manure/soil. This survival held under a variety of conditions and may result from the application of manure containing viable *E. coli* O157:H7 to production fields [21, 34]. Another way by which *E. coli* O157:H7 may be introduced in the environment is flood irrigation with water contaminated with cattle faeces or contact with contaminated surface runoff, studies have also demonstrated the capacity for this pathogen to survive for extended periods in water [30, 35]. Some *E. coli* O157:H7 outbreaks have been linked to contaminated water by the application of contaminated manure to the production field or irrigation with contaminated water, because these bacteria can enter the lettuce plant

over the root system and migrate to the edible part of the plant ^[19]. An outbreak of *E. coli* O157:H7 with 23 confirmed cases was related with the drinking of freshly pressed apple cider that was made from fallen apples at a farm. It was suspected that the apples had fallen in to soil that had been contaminated by cow manure ^[36].

2.2 *Salmonella* sp.

Salmonellosis, caused by *Salmonella enterica* with several serovars is considered one of the most widespread zoonosis in the world. Such as the transmission cycle of *Salmonella* involves practically all vertebrates, their control poses a test to public health in both developed and developing countries. Most *Salmonella* serovars associated with pathologies in animals are the strains: *S. Dublin* (bovines), *S. Choleraesuis* and *S. Typhisuis* (swine), *S. Abortusequi* (equines), *S. Pullorum* and *S. Gallinarum* (birds). However, since *Salmonella* is a zoonotic agent, some of its strains cause undifferentiated infection between humans and other animal species ^[37]. Being *Salmonella* sp. the agent of an infection associated with production animals, it have a negative economic impact on the commercialization of these animals and their products, because when an animal present in a herd is considered positive, normally, the entire herd is treated as positive ^[38, 39]. As this infection have long periods of latency, it is possible that release of bacterial cells from faeces of the infected animals can occurs, contaminating environment, food and water ^[40, 41]. Cattle is considered one of the main disseminator of this pathogen, because of that, among the years some studies have been done to better understand the survival of this pathogen on cattle manures ^[42]. In a previous study, *S. Typhimurium* was survived in dairy effluents with various particle sizes after a 30 days of storage under greenhouse condition ^[43]. Dairy compost can provide long-term survival conditions for *Salmonella enterica*, including survival for more than 168 days, at 5 °C with high inoculum levels ^[44]. In other study was showed a clear relation among the temperatures of storage and the *Salmonella* survival ^[32]. In last decades, numerous studies have been done and showed that *Salmonella* sp. survival is influenced by storage temperature, suspended solids content and pH value of the slurry ^[9, 12, 32, 45, 46]. Being, high temperature of storage the factor that presents better results in the elimination of *Salmonella* sp. ^[46, 47, 48, 49]. Outbreaks of this pathogen are progressively related to consumption of fresh produce. An understanding of soil survival of *Salmonella* and transfer to water and to fresh produce is needed to control salmonellosis ^[16]. Numerous studies have isolated

Salmonella sp. together in farm animals and environmental samples [33, 50, 51, 52]. What proves that this pathogen can persist in the farm environment for prolonged periods of time, circulating inside the farm between different reservoirs such as animals, stools, soil and plants [15, 53]. The content of this pathogen in farm environment is dependent of several factors including: herd health, herd size, age and housing environment [54-56]. *Salmonella* sp. may contaminate the soil from various ways, however application of contaminated manure remains the most common source [16]. After being introduced to the soil, the pathogen survival has been revealed to be mainly influenced by method of introduction, temperature, predation by soil protozoa, temperature, moisture, soil type, presence of plants, exposure to sun (UV) light, and the initial number of microorganisms present. *Salmonella* sp. has been described to survive from a few days up to 332 days in manure-amended soils [18, 57]. Surface spreading of manure is leading to air pollution problems and therefore injection of manure into the soil has been in the last years studied. This application method might increase the survival of *Salmonella* sp. [16].

2.3 *Enterococcus* spp.

Enterococcus spp. have long been known as commensals of the gastrointestinal tract and as one of the present bacteria in the environment. Between the multiple set of bacteria that constitutes the intestinal microbiota of animals and humans the most of these microorganisms are beneficial to intestinal motility [58-60]. *Enterococcus* spp. are facultative anaerobic organisms that can survive in high salt concentrations and survive in pH extremes (as low as 4.8 and as high as 9.6) [59, 58, 61]. Although these bacteria do not produce toxins, have virulence factors in the form of aggregation substances, so they can cause disease based in the way they adhere to the host tissues. Based on this virulence factors production and their high antibiotic resistance *Enterococcus* are now considered emerging pathogens [62-65]. Based on their abundance in faeces of mammals and their long survival in the environment *Enterococci* are commonly used as faecal indicators in water management studies and in studies focused in the source of faecal pollution. The presence and abundance of *Enterococcus* in slurry is a known fact [66], because of that, when slurry, resultant from animal production is used to fertilize fields, this bacteria and its antibiotic resistance genes presents in the slurry can be transmitted to the environment [67, 68].

3. Manure treatments

Soil adjustment with animal slurry (a mixture of urine, feces and water), is a cost-effective and viable option for reloading nutrients and organic matter in cropped soils, constituting a sustaining option for recycling nutrients at the farm level ^[69]. With the crescent demands from de worldwide population, the selection of proper treatment and manure disposal methods constitutes a challenge. In order to properly utilise cattle manure, this waste needs to be characterised to evaluate its value as feedstock, elemental composition, heavy metal content, and GHG emissions ^[70].

Spreading fresh manures directly to land presents complex risks in many levels, including a higher risk of pathogen transfer to the food chain ^[7]. The contaminants of slurry may take different pathways to groundwater and/or surface water bodies via direct runoff, subsurface flow, leaching and percolation depending on the local hydrology ^[71, 72]. The effectiveness of manure management systems need to concern not only GHG emissions but also environmental transmission pathways by which zoonotic pathogens presents in animal manures may be transported in environment including to water resources (**Figure 1**).

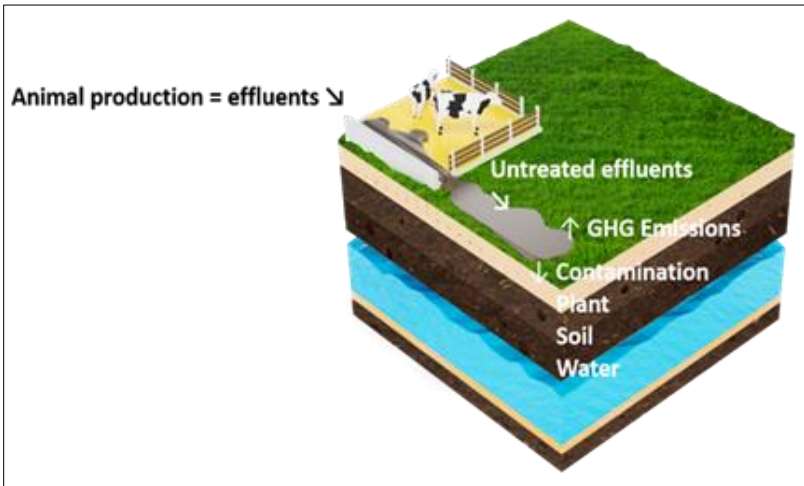


Fig 1: Diagram summarizing animal effluents disposal and consequences without treatment application (Soares JA®). Legend: GHG - greenhouse gas

This management systems are planned to reduce the concentrations of microbes found in manure (by 90 to 99% or more) and to prevent off-farm transport of manure materials (e.g., no discharge systems) [11]. In the past, animal wastes and residues from bedding were composted for several days, with the compost reaching temperatures of 70 °C or more before being used as fertilizer.

Composting and drying of manure is recognised to be effective in reducing the number of viable pathogens that could be present in the effluent, there continued a small risk of pathogen survival in the cooler exterior or drier parts of manure heaps [7]. However, composting is not an effective approach for processing cattle manure. Progressions in mechanized farming have led to large numbers of animals per farm and most of those large farms wash animal faeces, urine, and spilt feed into a slurry mixture. The most common practise for storing slurry is storage in tanks, away from the animal housing and under anaerobic degradation processes (untreated slurry) for more than one month before disposal (**Figure 2**).

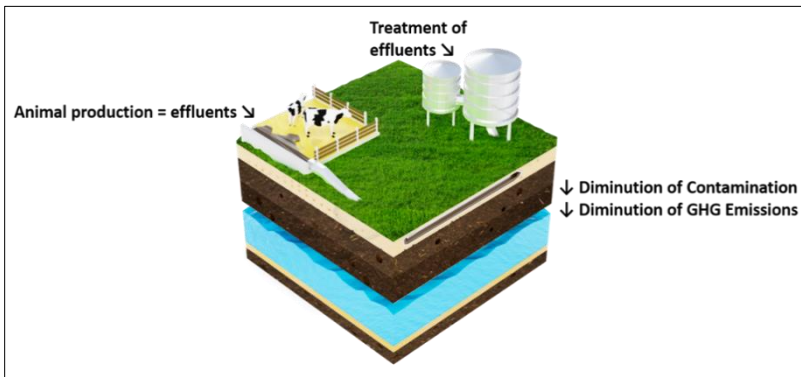


Fig 2: Diagram summarizing animal effluents disposal and consequences when there is a previous treatment application (Soares JA®). Legend: GHG - greenhouse gas

In addition, some farmers reduce the amount of untreated slurry by using a mechanical/aeration technique that separates the solid and liquid portions of the slurry. With the main propose of recycling some of the solids to made beds and to composting [70]. Slurry differs from manure by having less dry matter, lower pH, less nitrogen, more dissolved organic matter and less diversity in microbial community. Previous studies showed that pathogens have been shown to survive longer in the slurry than in farm yard manure [7, 8, 16]. The pathogen declined for example of *Salmonella*, *Mycobacterium paratuberculosis*, *E. coli* O157:H7 and some viruses (rotavirus, herpesvirus) in stored effluents depends on management and

storage conditions, temperature, aeration, pH, dry matter content and the length of time that manure or slurry is stored before it is applied to soil [7, 73, 74]. Studies show that even at low to moderate temperatures pathogen numbers can decline over time if there are presence of dry conditions and UV light radiation exposure. The bacteria can survive up to three months in slurry stored in laboratory conditions that have been reported [7, 49]. Pathogen survival in slurry stored outdoors is longer during winter than in summer, because of inferior ambient temperature [47]. In recent years, slurry has achieved a greatest importance between the natural fertilizers. Yet, incorrect practices of management makes it a potential microbiological risk for environmental and public health [3, 75]. The long-term capacity of survival of microorganisms in animal effluents highlights the need for appropriate farm waste management to limit environmental spread of this microorganism. Because of that, since a few years manure-handling guidelines stated that a composting/storage period before application of the effluent to a field as fertilizer needs to be done [19]. Slurry treatment has been used as a solution to minimize its environmental impact, greater convenience because of farms storage capacity and to increase its agronomic value for crop utilization [75]. Environmental problems may rise on livestock farms, which produce an excess of slurry in relation to field requirements. Separation of this slurry into a liquid fraction and a solid fraction, followed by transferring the one of the fractions to farms with less animals, may lighten the problem [76]. To guarantee that the slurry applied to fields matches the nutrient necessities of the crops, some treatment techniques have been established. Slurry separation is now used in some dairy farms to improve slurry management, specifically the recycling of slurry nutrients on farms. After the use of slurry separation systems, the high dry-matter solid fraction (SF) obtained may be transferred out of the farm and may be composted while the low dry-matter liquid fraction (LF) may be applied on farm for example may be used for fertigation instead of the raw slurry [69, 75]. Mechanical slurry separation has been extensively used in Asia [69]. On the other hand, such practise was less established in Europe and North America where slurry treatment has been considered as cumulative in production costs [77]. Studies refer that treatment costs can be reduced if the amount of manure that treated is augmented, both by having a larger livestock production on farms or by processing manure from several farms at a centralized separation system [76]. The particle size of the slurry fractions obtained depends on the type of the mechanical separation technique (centrifugation, screening, or screw-press), the filter size and the slurry type [78].

Biochar, is a carbonaceous solid resultant from pyrogenic material that have a substantial scientific attention in line for to its potential in climate change mitigation. But also for waste management, soil fertility development and crop growth promotion [79-81]. When biochar is applied to soil its major benefits are associated with increased carbon (C) sequestration and suppression of greenhouse gas (GHG) emissions [82, 83]. Studies show that 90% of the biochar used in Europe goes into animal farming. Wherever less studied, when it is used in feeding, litter or for slurry treatment, the farmers rapidly notice less smell [84] making it a new technique to be applicate directly in to slurry. Application of concentrated acid to slurry has been a common practice in some countries since 2010 based on its efficiency to the minimization of NH_3 emissions [1]. This minimization is based on the decrease of pH of the effluent, and has the propose to reduce the greenhouse gases emissions, and improve the nutrient value of the slurry [1, 85]. Lowering pH may affect chemical and biological routes in slurry, as well as slurry composition. In result, the fertilizer value of slurry as well as the N dynamics from acidified slurry may differ for non-acidified slurry after soil application [69,7 5, 86,]. A study reported also a postponement in nitrification of ammonium (NH_4^+) and subsequent reduction in nitrous oxide (N_2O) emissions from soils amended with acidified slurries relative to non-acidified ones [78]. A study described that slurry acidification is capable to reduce the microbial activity [86], however, other study was mentioned that in some conditions, acidification can have a weaker reducing effect on pathogenic bacteria [87].

4. Cattle manure and slurry as an emergent concern in the One Health approach

The “One Health” approach is based on the premise that man shares habitat with animals resulting in a measure between human medicine and veterinary medicine to promote interdisciplinary collaboration to achieve knowledge about common diseases. But also promoting interventions about health as something more than the absence of disease. The American Veterinary Medical Association ^[88] has defined “One Health” as an effort to collaborate across disciplines - working locally, nationally and globally - to achieve health for humans, animals and the environment (**Figure 3**).

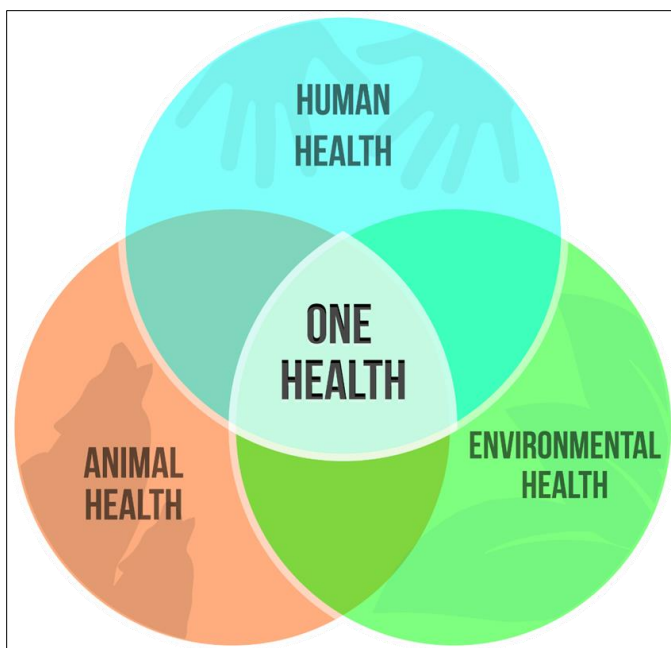


Fig 3: Diagram of the One Health concept (Soares JA®)

Understanding and addressing the health problems that rise in the middle of these links is the basis of this concept. The extensive use of antibiotics in animal production has started the concern of antibiotic resistance spread from animals to humans, and studies estimate that the most

common route of bacterial transmission is through the food chain, characteristically due to an absence of hygiene while handling animals or animal products. The use of high doses of antibiotics in animal feed increases the number of antibiotic-resistant bacteria that may spread their genes into the environment after slurry disposal on fields ^[89]. Because of that, animal manure is considered a major source of antimicrobial resistant bacteria entering the environment ^[90-92]. Related do that, studying resistance to different groups of antibiotics in a sentinel species like *E. coli* can give an indication of the reservoir of resistances that is present in the farm bacterial population ^[93]. Also, Enterobacteriaceae owning genes for producing extended-spectrum β -lactamases (ESBL) are an emerging problem, and with great importance for public health. Because these bacteria show resistance to β -lactam antimicrobials, including third- and fourth-generation cephalosporins, which its categorized by the World Health Organization ^[94] as critically significant for human medicine. Antibiotics used in farms for veterinary purposes or as growth promoters are excreted by the animals and end up in their effluents. Being animal effluents manure or slurry used as fertilizers, the antibiotics residues that can be presents will be transferred to the soil and in some cases can contaminate ground water ^[95]. Soil antibiotic resistances could be related with clinical incidences of resistant infections ^[96]. It's not clear what is the fate of antimicrobials released in the environment and the mechanisms to contain or detoxify the environment from antimicrobials. However, it's clear that farmers have an important paper, being their education on the subject essential. The One Health approach may play a catalytic role for new research discoveries, the achievement of effective prevention responses, the development of health care education and better preparedness for zoonotic disease surveillance ^[97, 98].

5. Conclusions

Adult milking cows can produce 7-8% of their body weight as manure per day ^[70]. Cattle manure ends mostly in the form of slurry. The best way to recycle this effluent is its application into the soil. This process has many advantages, but also can be a source of contaminations, the effectiveness of management systems needs to concern not only the reduction of GHG emissions but also environmental transmission of pathogens that can be present in animal effluents and transmitted to the environment. Because of that, proper manure management systems must be used to minimize environmental impacts. The best way to fix and to do research on this field is to consider the interdisciplinary collaboration to achieve health for humans, animals and the environment.

Acknowledgements

This research was supported by Project I&D INTERACT - Integrative Research in Environment, Agro-Chain and Technology, NORTE-01-0145-FEDER-000017, included at the research line entitled Innovation for Sustainable Agro-food Chains – ISAC, co-financed by the European Regional Development Fund (ERDF) through NORTE 2020 (Regional Operational Programme North 2014–2020). This work is supported by National Funds by FCT - Portuguese Foundation for Science and Technology, under the project UID/AGR/04033/2019.

References

1. Fangueiro, D., Surgy, S., Napier, V., Menaia, J., Vasconcelos, E., and Coutinho, J. (2014). Impact of slurry management strategies on potential leaching of nutrients and pathogens in a sandy soil amended with cattle slurry. *Journal of Environmental Management* 146, 198–205.
2. Ferens, W.A., and Hovde, C.J. (2011). *Escherichia coli* O157:H7: animal reservoir and sources of human infection. *Foodborne Pathogens and Disease* 8, 465–487.
3. Nolan, S., Waters, N.R., Brennan, F., Auer, A., Fenton, O., Richards, K., Bolton, D.J., Pritchard, L., O’Flaherty, V., and Abram, F. (2018). Toward assessing farm-based anaerobic digestate public health risks: Comparative investigation with slurry, effect of pasteurization treatments, and use of miniature bioreactors as proxies for pathogen spiking trials. *Frontiers in Sustainable Food Systems* 2, 41.
4. Collins, R., Elliott, S., and Adams, R. (2005). Overland flow delivery of faecal bacteria to a headwater pastoral stream. *Journal of Applied Microbiology* 99, 126–132.
5. Cardoso, F., Shelton, D., Sadeghi, A., Shirmohammadi, A., Pachepsky, Y., and Dulaney, W. (2012). Effectiveness of vegetated filter strips in retention of *Escherichia coli* and *Salmonella* from swine manure slurry. *Journal of Environmental Management* 110, 1–7.
6. Uvarov, R., Oblomkova, N., and Freidkin, I. (2019). Slurry acidification techniques: first steps towards comprehensive study in Russian conditions. *Engineering for Rural Development* 538-542.
7. Nicholson, F.A., Groves, S.J., and Chambers, B.J. (2005). Pathogen survival during livestock manure storage and following land application. *Bioresource Technology* 96, 135–143.
8. Semenov, A. V., van Overbeek, L., and van Bruggen, A.H.C. (2009). Percolation and survival of *Escherichia coli* O157:H7 and *Salmonella enterica* Serovar Typhimurium in soil amended with contaminated dairy manure or slurry. *Applied and Environmental Microbiology* 75, 3206–3215.
9. Guan, T.Y., and Holley, R.A. (2003). Pathogen survival in swine manure environments and transmission of human enteric illness - A review. *Journal of Environment Quality* 32, 383.
10. Bornay-Llinares, F.J., Navarro-i-Martínez, L., García-Orenes, F., Araez,

- H., Pérez-Murcia, M.D., and Moral, R. (2006). Detection of intestinal parasites in pig slurry: A preliminary study from five farms in Spain. *Livestock Science* 102, 237–242.
11. Ziemer, C.J., Bonner, J.M., Cole, D., Vinjé, J., Constantini, V., Goyal, S., Gramer, M., Mackie, R., Meng, X.J., Myers, G., *et al.* (2010). Fate and transport of zoonotic, bacterial, viral, and parasitic pathogens during swine manure treatment, storage, and land application. *Journal of Animal Science* 88, 84–94.
 12. Olszewska, H., and Skowron, K. (2013a). Effect of storage temperature and type of slurry on survivability of *Salmonella*. *Journal of Central European Agriculture* 14, 369–375.
 13. Krog, J.S., Forslund, A., Larsen, L.E., Dalsgaard, A., Kjaer, J., Olsen, P., and Schultz, A.C. (2017). Leaching of viruses and other microorganisms naturally occurring in pig slurry to tile drains on a well-structured loamy field in Denmark. *Hydrogeology Journal* 25, 1045–1062.
 14. Pell, A.N. (1997). Manure and microbes: Public and animal health problem? *Journal of Dairy Science* 80, 2673–2681.
 15. Gerba, C.P., and Smith, J.E. (2005). Sources of pathogenic microorganisms and their fate during land application of wastes the opinions expressed in this article are those of the authors and do not necessarily reflect those of the USEPA. *Journal of Environmental Quality* 34, 42–48.
 16. Jacobsen, C.S., and Bech, T.B. (2012). Soil survival of *Salmonella* and transfer to freshwater and fresh produce. *Food Research International* 45, 557–566.
 17. Natvig, E.E., Ingham, S.C., Ingham, B.H., Cooperband, L.R., and Roper, T.R. (2002). *Salmonella enterica* serovar Typhimurium and *Escherichia coli* contamination of root and leaf vegetables grown in soils with incorporated bovine manure. *Applied and Environmental Microbiology* 68, 2737–2744.
 18. Islam, M., Morgan, J., Doyle, M.P., Phatak, S.C., Millner, P., and Jiang, X. (2004). Persistence of *Salmonella enterica* serovar Typhimurium on lettuce and parsley and in soils on which they were grown in fields treated with contaminated manure composts or irrigation water. *Foodborne Pathogens and Disease* 1, 27–35.
 19. Solomon, E., Matthews, K.R., Solomon, E.B., Yaron, S., and Matthews, K.R. (2002). Transmission of *Escherichia coli* O157: H7 from contaminated manure and irrigation water to lettuce plant tissue and its subsequent internalization. *Applied and Environmental Microbiology* 68, 397–400.

20. Hodgson, C.J., Oliver, D.M., Fish, R.D., Bulmer, N.M., Heathwaite, A.L., Winter, M., and Chadwick, D.R. (2016). Seasonal persistence of faecal indicator organisms in soil following dairy slurry application to land by surface broadcasting and shallow injection. *Journal of Environmental Management* 183, 325–332.
21. Lau, M.M., and Ingham, S.C. (2001). Survival of faecal indicator bacteria in bovine manure incorporated into soil. *Letters in Applied Microbiology* 33, 131–136.
22. Hrudey, S.E., Huck, P.M., Payment, P., Gillham, R.W., and Hrudey, E.J. (2003). Walkerton: Lessons learned in comparison with waterborne outbreaks in the developed world. *Journal of Environmental Engineering and Science* 1, 397–407.
23. Unc, A., and Goss, M.J. (2004). Transport of bacteria from manure and protection of water resources. *Applied Soil Ecology* 25, 1–18.
24. Semenov, A. V., Van Overbeek, L., Termorshuizen, A.J., and Van Bruggen, A.H.C. (2011). Influence of aerobic and anaerobic conditions on survival of *Escherichia coli* O157:H7 and *Salmonella enterica* serovar Typhimurium in Luria-Bertani broth, farm-yard manure and slurry. *Journal of Environmental Management* 92, 780–787.
25. Franz, E., Diepeningen, A.D. van., Vos, O.J. de., and Ariena HC, van B. (2005). effects of cattle feeding regimen and soil management type on the fate of *Escherichia coli* O157:H7 and *Salmonella enterica* Serovar Typhimurium in manure, *Manure-Amended Soil, and Lettuce*. 71, 6165–6174.
26. Sinton, L.W., Braithwaite, R.R., Hall, C.H., and Mackenzie, M.L. (2007). Survival of indicator and pathogenic bacteria in bovine feces on pasture. *Applied and Environmental Microbiology* 73, 7917–7925.
27. Cools, D., Merckx, R., Vlassak, K., and Verhaegen, J. (2001). Survival of *E.coli* and *Enterococcus* spp. derived from pig slurry in soils of different texture. *Applied Soil Ecology* 17, 53–62.
28. Stocker, M.D., Pachepsky, Y.A., Hill, R.L., and Martinez, G. (2018). Export from manured fields depends on the time between the start of rainfall and runoff initiation. *Journal of Environment Quality* 47, 1293.
29. Faith, N.G., Shere, J.A., Brosch, R., Arnold, K.W., Ansary, S.E., Lee, M.S., Luchansky, J.B., and Kaspar, C.W. (1996). Prevalence and clonal nature of *Escherichia coli* O157:H7 on dairy farms in Wisconsin. *Applied and Environmental Microbiology* 62, 1519–1525.
30. Chalmers, R.M., Aird, H., and Bolton, F.J. (2000). Waterborne *Escherichia coli* O157. *Symposium Series (Society for Applied Microbiology)* 124S-132S.
31. Hruby, C.E., Soupir, M.L., Moorman, T.B., Pederson, C., and Kanwar,

- R. (2018). *Salmonella* and fecal indicator bacteria survival in soils amended with poultry manure. *Water, Air, and Soil Pollution* 229.
32. Biswas, S., Pandey, P.K., and Farver, T.B. (2016). Assessing the impacts of temperature and storage on *Escherichia coli*, *Salmonella*, and *L. monocytogenes* decay in dairy manure. *Bioprocess and Biosystems Engineering* 39, 901–913.
 33. Sandvang, D., Jensen, L.B., Baggesen, D.L., and Baloda, S.B. (2000). Erratum: Persistence of a *Salmonella enterica* serotype Typhimurium clone in Danish pig production units and farmhouse environment studied by pulsed field gel electrophoresis (PFGE). *FEMS Microbiology Letters* 189, 321.
 34. Wang, G., Zhao, T., and Doyle, M.P. (1996). Fate of Enterohemorrhagic *Escherichia coli* O157:H7 in Bovine Feces. *Applied and Environmental Microbiology* 62, 2567–2570.
 35. Wang, G., and Doyle, M.P. (1998). Survival of enterohemorrhagic *Escherichia coli* O157:H7 in water. *Journal of Food Protection* 61, 662–667.
 36. Besser, R.E., Weber, J.T., Barrett, T.J., Wells, J.G., Griffin, P.M., Lett, S.M., and Doyle, M.P. (1993). An outbreak of diarrhea and hemolytic uremic syndrome from *Escherichia coli* O157:H7 in fresh-pressed apple cider. *JAMA: The Journal of the American Medical Association* 269, 2217–2220.
 37. Maurer, J. (2007). *Salmonella* species. In *Food Microbiology: Fundamentals and Frontiers, Third Edition* (pp. 187-236). American Society of Microbiology.
 38. Warnick, L.D., Kaneene, J.B., Ruegg, P.L., Wells, S.J., Fossler, C., Halbert, L., and Campbell, A. (2003). Evaluation of herd sampling for *Salmonella* isolation on midwest and northeast US dairy farms. *Preventive Veterinary Medicine* 60, 195–206.
 39. Magwedere, K., Rauff, D., De Klerk, G., Keddy, K.H., and Dziva, F. (2015). Incidence of nontyphoidal *Salmonella* in food-producing animals, animal feed, and the associated environment in South Africa, 2012-2014. *Clinical Infectious Diseases* 61, S283–S289.
 40. Wakchaure, R., and Ganguly, S. (2015). Marker Assisted Selection (MAS) in Animal Breeding: A Review. *Journal of Drug Metabolism & Toxicology* 6.e127
 41. Tohidi, R., Javanmard, A., and Idris, I. (2017). Immunogenetics applied to control salmonellosis in chicken: a review. *Journal of Applied Animal Research* 2119, 1–9.
 42. Shinohara, N. K. S., Barros, V. B. D., Jimenez, S. M. C., Machado, E. D. C. L., Dutra, R. A. F., & Lima Filho, J. L. D. (2008). *Salmonella*

- spp., important pathogenic agent transmitted through foodstuffs. *Ciencia & Saude Coletiva* 13(5), 1675-1683.
43. Diao, J., Chen, Z., Gong, C., and Jiang, X. (2015). Factors affecting pathogen survival in finished dairy compost with different particle sizes under greenhouse conditions. *Foodborne Pathogens and Disease* 12, 749–758.
 44. Chen, Z., Kim, J., and Jiang, X. (2018). Survival of *Escherichia coli* O157:H7 and *Salmonella enterica* in animal waste-based composts as influenced by compost type, storage condition and inoculum level. *Journal of Applied Microbiology* 124, 1311–1323.
 45. Arrus, K.M., Holley, R.A., Ominski, K.H., Tenuta, M., and Blank, G. (2006). Influence of temperature on *Salmonella* survival in hog manure slurry and seasonal temperature profiles in farm manure storage reservoirs. *Livestock Science* 102, 226–236.
 46. Côté, C., Massé, D.I., and Quessy, S. (2006). Reduction of indicator and pathogenic microorganisms by psychrophilic anaerobic digestion in swine slurries. *Bioresource Technology* 97, 686–691.
 47. Plachá, I., Venglovský, J., Sasáková, N., and Svoboda, I.F. (2001). The effect of summer and winter seasons on the survival of *Salmonella typhimurium* and indicator micro-organisms during the storage of solid fraction of pig slurry. *Journal of Applied Microbiology* 91, 1036–1043.
 48. Olszewska, H., and Skowron, K. (2013b). Wpływ temperatury składowania oraz typu gnojowicy na przeżywalność pałeczek z rodzaju *salmonella*. *Journal of Central European Agriculture* 14, 369–375.
 49. Soares, A.S., Miranda, C., Teixeira, C.A., Coutinho, J., Trindade, H., and Coelho, A.C. (2019). Impact of different treatments on *Escherichia coli* during storage of cattle slurry. *Journal of Environmental Management* 236, 323–327.
 50. Baloda, S. B., Christensen, L., and Trajcevska, S. (2001). Persistence of a *Salmonella enterica* serovar typhimurium DT12 clone in a piggery and in agricultural soil amended with *Salmonella*-contaminated slurry. *Applied and Environmental Microbiology* 67(6), 2859–2862.
 51. Dorr, P.M., Tadesse, D.A., Zewde, B.M., Fry, P., Thakur, S., and Gebreyes, W.A. (2009). Longitudinal study of *Salmonella* dispersion and the role of environmental contamination in commercial swine production systems. *Applied and Environmental Microbiology* 75, 1478–1486.
 52. Weigel, R.M., Nucera, D., Qiao, B., Teferedegne, B., Suh, D.K., Barber, D.A., Bahnson, P.B., Isaacson, R.E., and White, B.A. (2007). Testing an ecological model for transmission of *Salmonella enterica* in swine production ecosystems using genotyping data. *Preventive Veterinary*

53. Kupriianov, A.A., Semenov, A.M., and Van Bruggen, A.H. (2010). Transition of enteropathogenic and saprotrophic bacteria in the eco-niche cycle: animals-excrement-soil-plants-animals. *Izvestiia Akademii Nauk. Serii Biologicheskaja/Rossiiskaia Akademiia Nauk* 37, 318–323.
54. Ojha, S., and Kostrzynska, M. (2007). Approaches for reducing *Salmonella* in pork production. *Journal of Food Protection* 70, 2676–2694.
55. Payne, J.B., Osborne, J.A., Jenkins, P.K., and Sheldon, B.W. (2007). Modeling the growth and death kinetics of *Salmonella* in poultry litter as a function of pH and water activity. *Poultry Science* 86, 191–201.
56. Rajić, A., Waddell, L.A., Sargeant, J.M., Read, S., Farber, J., Firth, M.J., and Chambers, A. (2007). An overview of microbial food safety programs in beef, pork, and poultry from farm to processing in Canada. *Journal of Food Protection* 70, 1286–1294.
57. You, Y., Rankin, S.C., Aceto, H.W., Benson, C.E., Toth, J.D., and Dou, Z. (2006). Survival of *Salmonella* enterica serovar Newport in manure and manure-amended soils. *Applied and Environmental Microbiology* 72, 5777–5783.
58. Fisher, K., and Phillips, C. (2009). The ecology, epidemiology and virulence of *Enterococcus*. *Microbiology* 155, 1749–1757.
59. van Schaik, W., and Willems, R.J.L. (2010). Genome-based insights into the evolution of enterococci. *Clinical Microbiology and Infection* 16, 527–532.
60. John, U. V., and Carvalho, J. (2011). *Enterococcus*: Review of its physiology, pathogenesis, diseases and the challenges it poses for clinical microbiology. *Frontiers in Biology* 6, 357–366.
61. Huycke, M.M., Sahm, D.F., and Gilmore, M.S. (1998). Multiple-drug resistant *enterococci*: The nature of the problem and an agenda for the future. *Emerging Infectious Diseases* 4, 239–249.
62. Vu, J., and Carvalho, J. (2011). *Enterococcus* : review of its physiology, pathogenesis, diseases and the challenges it poses for clinical microbiology. *Frontiers in Biology* 6, 357–366.
63. Aslam, M., Diarra, M.S., Checkley, S., Bohaychuk, V., and Masson, L. (2012). Characterization of antimicrobial resistance and virulence genes in *Enterococcus* spp. isolated from retail meats in Alberta, Canada. *International Journal of Food Microbiology* 156, 222–230.
64. Guerrero-Ramos, E., Molina-gonzales, D., Blanco-Moran, S., Igrejas, G., Poeta, P., Alonso- Calleja, C., and Capita, R. (2016). Prevalence, antimicrobial resistance, and genotypic characterization of Vancomycin-Resistant *Enterococci* in meat preparations. *Journal of Food Protection*

65. Ahmed, M.O., and Baptiste, K.E. (2017). Vancomycin-Resistant Enterococci: A review of antimicrobial resistance mechanisms and perspectives of human and animal health. *Microbial Drug Resistance*, 24(5), 590-606.
66. Manero, A., Vilanova, X., Cerdà-Cuéllar, M., and Blanch, A.R. (2002). Characterization of sewage waters by biochemical fingerprinting of *Enterococci*. *Water Research* 36, 2831–2835.
67. Sengeløv, G., Agersø, Y., Baloda, S.B., and Jensen, L.B. (2001). Bacterial antibiotic resistance levels in agricultural soil as a result of treatment with pig slurry. *ISME-9 - Interactions in the Microbial World* 28, 587–595.
68. Wallace, J.S., Garner, E., Pruden, A., and Aga, D.S. (2018). Occurrence and transformation of veterinary antibiotics and antibiotic resistance genes in dairy manure treated by advanced anaerobic digestion and conventional treatment methods. *Environmental Pollution* 236, 764–772.
69. Hjorth, M., Christensen, K. V., Christensen, M.L., and Sommer, S.G. (2010). Soli-liquid separation of animal slurry in theory and practice. A review. *Agronomy for Sustainable Development* 30, 153–180.
70. Font-Palma, C. (2019). Methods for the treatment of cattle manure - A review. *Journal of Carbon Research*, 5(2), 27.
71. Kjær, J., Olsen, P., Bach, K., Barlebo, H. C., Ingerslev, F., Hansen, M., and Sørensen, B. H. (2007). Leaching of estrogenic hormones from manure-treated structured soils. *Environmental Science & Technology* 41(11), 3911-3917.
72. Bech, T.B., Rosenbom, A.E., Kjaer, J., Amin, M.G.M., Olsen, P., and Jacobsen, C.S. (2014). Factors influencing the survival and leaching of tetracycline-resistant bacteria and *Escherichia coli* through structured agricultural fields. *Agriculture, Ecosystems & Environment* 195, 10-17.
73. Kudva, I.T., Hunt, C.W., Williams, C.J., Nance, U.M., and Hovde, C.J. (1997). Evaluation of dietary influences on *Escherichia coli* O157:H7 shedding by sheep. *Applied Environmental Microbiology*. 63, 3878–3886.
74. Himathongkham, S., and Riemann, H. (1999). Destruction of *Salmonella typhimurium*, *Escherichia coli* O157:H7 and *Listeria monocytogenes* in chicken manure by drying and/or gassing with ammonia. *FEMS Microbiology Letters* 171, 179–182.
75. Owusu-Twum, M.Y., Loick, N., Cardenas, L.M., Coutinho, J., Trindade, H., and Fangeiro, D. (2017a). Nitrogen dynamics in soils amended with slurry treated by acid or DMPP addition. *Biology and Fertility of*

76. Møller, H.B., Lund, I., and Sommer, S.G. (2000). Solid-liquid separation of livestock slurry: *Efficiency and cost*. *Bioresource Technology* 74, 223–229.
77. Petersen, S.O., Sommer, S.G., Béline, F., Burton, C., Dach, J., Dourmad, J.Y., Leip, A., Misselbrook, T., Nicholson, F., Poulsen, H.D., et al. (2007). Recycling of livestock manure in a whole-farm perspective. *Livestock Science* 112, 180–191.
78. Fangueiro, D., Gusmão, M., Grilo, J., Porfírio, G., Vasconcelos, E., and Cabral, F. (2010). Proportion, composition and potential N mineralisation of particle size fractions obtained by mechanical separation of animal slurry. *Biosystems Engineering* 106, 333–337.
79. Chan, K. Y., Van Zwieten, L., Meszaros, I., Downie, A., and Joseph, S. (2008). Agronomic values of greenwaste biochar as a soil amendment. *Soil Research*, 45(8), 629-634.
80. Hossain, M. K., Strezov, V., Chan, K. Y., Ziolkowski, A., and Nelson, P. F. (2011). Influence of pyrolysis temperature on production and nutrient properties of wastewater sludge biochar. *Journal of environmental management*, 92(1), 223-228.
81. Jeffery, S., Bezemer, T. M., Cornelissen, G., Kuypers, T. W., Lehmann, J., Mommer, L., Sohi, S., Voorde, T., Wardle, D., and van Groenigen, J. W. (2015). The way forward in biochar research: targeting trade-offs between the potential wins. *Global Change Biology Bioenergy*, 7(1), 1-13.
82. Lin, X. W., Xie, Z. B., Zheng, J. Y., Liu, Q., Bei, Q. C., and Zhu, J. G. (2015). Effects of biochar application on greenhouse gas emissions, carbon sequestration and crop growth in coastal saline soil. *European Journal of Soil Science*, 66(2), 329-338.
83. Subedi, R., Taupe, N., Ikoyi, I., Bertora, C., Zavattaro, L., Schmalenberger, A., Leahy J.J., and Grignani, C. (2016). Chemically and biologically-mediated fertilizing value of manure-derived biochar. *Science of the Total Environment*, 550, 924-933.
84. Schmidt, H. P., and Wilson, K. (2014). 55 uses of biochar. *The Biochar Journal* 286-288.
85. Owusu-Twum, M.Y., Polastre, A., Subedi, R., Santos, A.S., Mendes Ferreira, L.M., Coutinho, J., and Trindade, H. (2017b). Gaseous emissions and modification of slurry composition during storage and after field application: Effect of slurry additives and mechanical separation. *Journal of Environmental Management* 200, 416–422.
86. Ottosen, L.D.M., Poulsen, H. V., Nielsen, D.A., Finster, K., Nielsen, L.P., and Revsbech, N.P. (2009). Observations on microbial activity in

- acidified pig slurry. *Biosystems Engineering* 102, 291–297.
87. Zhang, D., Yuan, X., Guo, P., Suo, Y., Wang, X., Wang, W., and Cui, Z. (2011). Microbial population dynamics and changes in main nutrients during the acidification process of pig manures. *Journal of Environmental Sciences* 23, 497–505.
 88. King, L.J., Anderson, L.R., Blackmore, C.G., Blackwell, M.J., Lautner, E.A., Marcus, L.C., Meyer, T.E., Monath, T.P., Nave, J.E., Ohle, J., Pappaioanou, M., Sobota, J., Stokes, W.S., Davis, R.M., Glasser, J.H., Mahr, R.K. (2008). Executive summary of the AVMA One Health Initiative Task Force report. *Journal of the American Veterinary Medical Association* 233(2), 259-261.
 89. Manyi-Loh, C., Mamphweli, S., Meyer, E., and Okoh, A. (2018). Antibiotic use in agriculture and its consequential resistance in environmental sources: potential public health implications. *Molecules (Basel, Switzerland)*, 23(4), 795.
 90. Aarestrup, F. M., Bager, F., Jensen, N. E., Madsen, M., Meyling, A., and Wegener, H. C. (1998). Resistance to antimicrobial agents used for animal therapy in pathogenic, zoonotic-and indicator bacteria isolated from different food animals in Denmark: a baseline study for the Danish Integrated Antimicrobial Resistance Monitoring Programme (DANMAP). *Journal of Pathology Microbiology and Immunology* 106(7-12), 745-770.
 91. Binh, C. T. T., Heuer, H., Gomes, N. C. M., Kotzerke, A., Fulle, M., Wilke, B. M., Schlöter, M. and Smalla, K. (2007). Short-term effects of amoxicillin on bacterial communities in manured soil. *FEMS Microbiology Ecology*, 62(3), 290-302.
 92. Ghosh, S., and LaPara, T. M. (2007). The effects of subtherapeutic antibiotic use in farm animals on the proliferation and persistence of antibiotic resistance among soil bacteria. *The ISME Journal-Multidisciplinary Journal of Microbial Ecology* 1(3), 191.
 93. Ibrahim, D. R., Dodd, C. E., Stekel, D. J., Ramsden, S. J., & Hobman, J. L. (2016). Multidrug resistant, extended spectrum β -lactamase (ESBL)-producing *Escherichia coli* isolated from a dairy farm. *FEMS Microbiology Ecology*, 92(4).
 94. WHO, 2012. Critically important antimicrobials for human medicine, third revision. Accessed november 4, 2020. <http://www.who.int/foodsafety/publications/antimicrobials-third/en/>
 95. Xie, W.Y., Shen, Q., and Zhao, F.J. (2018). Antibiotics and antibiotic resistance from animal manures to soil: a review. *European Journal of Soil Science* 69, 181–195.
 96. Graham, D.W., Knapp, C.W., Christensen, B.T., McCluskey, S., and

- Dolfing, J. (2016). Appearance of β -lactam Resistance genes in agricultural soils and clinical isolates over the 20th Century. *Scientific Reports* 6, 1–8.
97. Osburn, B., Scott, C., and Gibbs, P. (2009). One world - One medicine - One health: Emerging veterinary challenges and opportunities. *OIE Revue Scientifique et Technique* 28, 481–486.
98. Rabozzi, G., Bonizzi, L., Crespi, E., Somaruga, C., Sokooti, M., Tabibi, R., Vellere, F., Brambilla, G., and Colosio, C. (2012). Emerging zoonoses: The “One health approach.” *Safety and Health at Work* 3, 77–83.