WASH and biosecurity interventions for reducing burden of infection, antibiotic use, and antimicrobial resistance in animal agricultural settings:

a One Health mixed methods systematic review

DESCRIPTIVE REPORT

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ACRONYMS

ABR	Antibiotic resistance
ABU	Antibiotic use
AMR	Antimicrobial resistance
AMU	Antimicrobial use
ARGs	Antimicrobial resistance genes
BRICS	Brazil, Russia, India, China and South Africa - emerging economies.
CIs	Complex interventions
C&D	Cleaning and disinfection
DNA	Deoxyribonucleic acid
EC	European countries
FAO	Food and Agriculture Organization of the United Nations
GAP	Global Action Plan
GBD	Global Burden of Disease
HACCP	Hazard Analysis and Critical Control Points
HCPs	Health care professionals
HICs	High-income countries
ICARS	International Centre for Antimicrobial Resistance Solutions
ILRI	International Livestock Research Institute
IPC	Infection prevention and control
JOLIS	World Bank and IMF library catalogue
LILACs	Latin American and Caribbean Literature on Health Sciences
LMICs	Low-and-middle income countries
LICs	Low-income countries
LSHTM	London School of Hygiene and Tropical Medicine
McREBEL	Management changes to Reduce Exposure to Bacteria to Eliminate Loses
MRSA	Methicillin-resistant *Staphylococcus aureus*
NAP	National Action Plan
NRTs	Non-randomised trials
OECD	Organisation for Economic Co-operation and Development
OIE	World Organization for Animal health
PICOS	Participants, Interventions, Comparators, Outcomes, Study designs
PRISMA-P	Preferred Reporting Items for Systematic review and Meta-analysis Protocols
PROSPERO	International Prospective Register of Ongoing Systematic Reviews
RCTs	Randomized Control Trials
RG	Resistance-genes
RNA	Ribonucleic acid
UMICs	Upper-middle income countries
SDGs	Sustainable Development Goals
SIs	Structural interventions
WASH	Water, sanitation and hygiene
WB	World Bank
WHO	World Health Organization

GLOSSARY

Animal facilities: a building or a separate zone inside a building consisting of rooms or installations for the housing and production of animals.

Animal dwelling: a shelter where an animal lives.

AMR-sensitive interventions[1]: interventions that indirectly address AMR, but which can contribute to reduce risk or create conducive conditions for AMR-specific interventions. They can be designed and delivered in such a way that they contribute indirectly to combating AMR. Their primary purpose is not AMR control, e.g., improving access to clean water and sanitation, thereby reducing the spread of infections.

AMR-specific interventions[1]: have as their main purpose to reduce AMR; for example, establishing and enforcing regulations to ensure people can only obtain antimicrobial medicines with a valid prescription.

Biosecurity[2]: combination of practices to reduce the risk of introduction and/or spread of diseases. It can be grouped in three categories:

1. Bio-exclusion: to prevent the introduction of a new pathogen into farms/animal facilities.
2. Bio-containment: to prevent escape of pathogens to neighbouring farms/animal facilities.
3. Bio-management: to control and manage pathogens already present in farms/animal facilities.

Complex interventions[3]: interventions with several interactive components. There are several dimensions of complexity: a range of possible outcomes, variability of target population, number or groups or organisational levels targeted by the intervention, degree of flexibility of the intervention.

Structural interventions[4]: interventions that seek to alter the context within which health and illness are produced and reproduced. They focus on structural factors – social, cultural, political, economic, and environmental – that shape individual, community and societal health outcomes.

Water, sanitation and hygiene (WASH)[5] interventions: interventions to improve access to these services. These can be grouped into four categories:

1. Water quantity or supply improvement: to provide or improve a water distribution system.
2. Water quality: to remove or inactivate pathogens “at source” and “at point of use”.
3. Sanitation: to provide or improve sanitation and waste disposal.
4. Hygiene: to promote or implement changes in hygiene conditions.

SUMMARY

Background Infection prevention and control (IPC) is recognised as essential to addressing the emergence and spread of antimicrobial resistance (AMR) in human health, food production and the environment. How best to address this through a One Health perspective remains a challenge. This systematic review addresses this gap by identifying and synthesising evidence from interventions designed to improve water, hygiene, sanitation (WASH), and biosecurity in animal agriculture and in people that live and/or work with animals. This review aimed to capture evidence of effects of all types of intervention and across different settings.

Methods We conducted a systematic search for studies that reported on WASH and biosecurity interventions with the potential to reduce the burden of infections and reliance on antibiotics in animal production for populations living with animals and/or involved in agriculture/aquaculture with a primary focus on LMICs. We searched the following databases: Web of Science, PubMed, OVID, ProQuest, Epistemonikos, Trip, AgEcon, and Cochrane Library. For articles in Spanish, we searched Scielo, BIREME, E-Revistas, Redalyc, Lilacs, AfricaPortal, IMSEAR and WPRIM. A hand search of literature was also conducted in relevant sources, and Google Scholar and Open Grey were used for grey literature. The main outcomes of interest were: (i) reduction of infections/cases, (ii) reduction of bacterial load, and (iii) reduction of antimicrobial use and AMR. We extracted data from selected studies, performed a narrative synthesis, and developed a framework.

Findings A total of 104 studies were included in this systematic review. The majority of studies (64/104) (61.5%) were conducted in HICs, especially in Europe and the USA. Only 13 (12.5%) studies took place in LMICs. The majority of studies (77) were animal based, followed by 12 targeting both animals and the environment, nine focused exclusively on the environment, and only one study was exclusively about humans. Most studies were conducted in poultry (36) and pigs (27), and assessed impacts on multiple types of bacteria (commonly *Salmonella spp.* and *Campylobacter spp.*). Eighty-seven (87) studies assessed impact on IPC, 3 on AMU, and 14 on AMR. The interventions were classified as follows: 57 applied biological or chemical products to eliminate pathogens; 26 modified infrastructure and apparatus; 15 were educational/behavioural and one was a structural intervention. Around 52.8% (55/104) studies included WASH interventions focused on water quality (20), water quantity (2), hygiene (30), and sanitation (3). Likewise, 47.1% (49/104) included biosecurity interventions focused on bio-management (34), bio-containment (10), and bio-exclusion (5). Positive impacts were reported for 64 (61.5%) interventions, on infection burden (54/87), antibiotic use (3/3) or AMR (7/14). The majority were non-randomised studies (55), although a quarter were randomised controlled trials (26). A total of 27 studies were classified as having low risk of bias, 21 moderate and 56 high risk of bias.

Interpretation This review identifies a number of effective interventions to reduce the burden of infections, antimicrobial resistance and antibiotic use in animal agricultural settings. Interventions which undertook bio-management and bio-containment measures appeared to have positive effects most often. These measures attempted to create and maintain a conducive environment for animal raising in terms of physical infrastructure and protocols. The few studies reporting sanitation measures - which were similar to bio-containment interventions - all reported positive effects. By contrast, efforts to impact water quantity, water quality, and hygiene had more mixed effects on the outcomes assessed. Bio-exclusion interventions had mostly negative effects. Risk of bias was high or moderate in many studies, however, and publication bias should also be considered. The paucity of studies evaluating structural interventions needs to be addressed. There are opportunities to learn from biosecurity Interventions for WASH and we propose the 'A' In WASH represents both 'Animals' and 'Air' in recognition of pathways of infection that can be addressed to also impact AMR.

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PROSPERO Registration: The protocol for the systematic review was registered at PROSPERO, registration number CRD42020162345.

INTRODUCTION

Antimicrobial Resistance (AMR) threatens a return to the pre-antibiotic era[6] when human and animal populations suffered a greater burden of infectious diseases. Although antibiotic resistance (ABR) is a natural phenomenon observed in bacteria, the widespread use of antibiotics in human medicine[7,8], agriculture (livestock, aquaculture, and crops)[9], and consequently, the contamination of the environment[10], is being signalled as a key driver of ABR[11]. Moreover, there is already evidence of the links between ABR in animals and humans, as several studies have found resistance-genes (RG) transfer between bacteria affecting humans and animals[12-16], though without certainty of the directionality of these interactions. Hence, efforts to reduce the emergence and spread of these genes in the human-animal-environmental interface are focused on minimizing the selective pressure put on bacteria, through strategies oriented to reduce the amount of antibiotics[17] that are used every day in health care and animal production[18].

AMR has been described as a **challenge with important material and social dimensions** from the micro to macro level. There are numerous routes through which animals and people living in agricultural communities may become affected by AMR. In domestic husbandry practices[19] free poultry scavenging increases the chances of children's and adult's exposure to animal faeces[20] which may serve as a source of zoonotic infections and contamination of water sources[21,22] and a source of potential antibiotic-resistant pathogens. On a larger scale, the intensification of agriculture can increase microbial flows into wider food chains. Enteric pathogens such as *E. coli*, *Enterococcus* sp., *Salmonella* spp., *Klebsiella* spp., *Enterobacter* spp., and *Campylobacter* spp., are amongst the most common bacteria present in human and animal faeces. Certain strains of these bacterial species are known to harbour many resistance genes that are easily disseminated through genetic interchange mechanisms. Moreover, these bacterial species are recognised as important pathogens affecting both human and animal health, and are amongst the main microorganisms monitored in food production[23]. Lack of access to clean water, and absence of hygiene and sanitation measures, are known as crucial contributors to the burden of infections and therefore to the dissemination of pathogenic microorganisms, including resistant bacteria, into the environment[24]. The widespread use of antimicrobials in animal agriculture and aquaculture contributes to the presence of antimicrobial residues, metabolites, presence of resistant bacteria, and resistance genes in animal waste. These then enter the environment through leachates from landfills, contaminating rivers, lakes, other water neighbouring sources, soil, and food crops. Likewise, the use of antibiotics in humans also leaves an environmental footprint as their residues and metabolites can also contaminate the environment through their presence in human excreta. Wastewater and contaminated water sources can act as vehicles of resistant bacteria and their functional resistant genes, creating a vicious cycle of transmission to humans, animals and back to the environment[24].

Antibiotics have been characterised as infrastructural - entangled with many areas of life, production, and economies[25]. Attention has been drawn to the way that they provide a quick fix[26] to compensate for deficiencies in health systems including care and hygiene, and in the animal health field to ensure productivity, standardisation, and to support intensified livestock farming. Antibiotic use and antibiotic resistance are rising in particular in low- and middle-Income countries (LMICs) in both humans, animals and in plant agriculture. Meat production trends indicate that this industry has grown by 68%, 64%, and 40% in Africa, Asia and South America, respectively[27]. Similarly, aquaculture is one of the fastest growing sectors in Asia[28,29]. Analyses suggest that in low resource settings, antibiotics are being used in place of good information, good husbandry practices, good access to veterinary services, good animal disease prevention programmes, and good biosecurity and management systems. Antibiotics to date, have been argued to have camouflaged deficiencies in socio-economic development plans, for both humans and animals[25]. To reduce antibiotic resistance, then, interventions may operate on a number of different domains that can impact infections and antibiotic use.

Recognising AMR as a development problem in many settings, the World Bank (2019) has described **'AMR-sensitive' interventions** as those that have an indirect but potentially important impact on AMR and with the capacity of addressing multiple diseases at the same time, and that can create favourable conditions for '**AMR-specific' interventions** (interventions with direct effect on AMR)[1]. Examples of interventions described as AMR-sensitive are those with the potential of reducing the spread of infections such as improving access to clean water and sanitation[1]. Interventions may also be characterised as structural, infrastructure/apparatus, educational/behavioural, or biological/chemical. **Structural interventions (SIs)** can be defined as interventions operating at the political and economic level that change the context where health is produced or reproduced. Blakenship et al., (2000) describe three types of contextual factors that determine health: availability, acceptability and accessibility[4], and three levels of action: society, social and physical environment, and population groups[30]. Several studies have highlighted the importance of SIs in addressing public health issues[4] such as: HIV/AIDS[31], chronic diseases[32], and health disparities[33]. **Infrastructure and apparatus interventions** focus on changing physical environment and protocols including for animal husbandry, such as flooring types and regimens. **Educational and behavioural interventions** focus on changing the practices of individuals such as farmers or residents through education or other behaviour change techniques, for example by changing husbandry practices, hand washing or food preparation. **Biological and chemical interventions** include technologies such as vaccines, strategies such as competitive exclusion, as well as applying products to eliminate pathogens.

How **WASH interventions** are conceptualised **for animal health and production is still an unexplored area**. Most research and development projects carried out focus on improving water, hygiene, and sanitation for humans and reduce diarrhoea morbidity mainly affecting children in LMICs[5,34]. Less is known about the potential of WASH interventions to reduce disease burden in animals and prevent the spread of zoonotic infections and the emergence or dissemination of antibiotic resistant bacteria. Many recommendations to reduce burden of infections in animals are based on creating or promoting clean environments within farms and animal facilities. Most recommendations related to WASH for animal agricultural settings are associated to the implementation of **biosecurity measures**. These measures are associated with water, by ensuring better water quality for animals, hygiene, when implementing hygiene protocols to handle animals, and sanitation, when safely disposing animal waste. Therefore, to acknowledge the overlaps existing between these two concepts is essential for a comprehensive approach to infection prevention and control, to reduce the amount of antibiotics used to treat infections, and decrease the likelihood of emergence of antibiotic resistance.

Currently biosecurity measures are oriented to ensure animal food product safety for human consumption, and to reduce the spread of animal diseases that are categorised as notifiable by the OIE[35] because of their influence in the international trade of goods. The underlying power of these types of interventions of promoting and ensuring animal welfare is commonly underestimated. In some animal production systems animals are just considered as *inputs* or *commodities* in the manufacturing system, neglecting their living nature. In contrast, improving animals' access to clean water is crucial to ensure their health and productivity, and is therefore critical for promoting global health and animal welfare. Furthermore, animal production systems can generate large amounts of waste, which when not treated appropriately can become a source of environmental contamination. Lack of access to water, hygiene, and sanitation measures in animal production systems is understood to create optimal conditions for the emergence and spread of dangerous pathogens (including antimicrobial resistant bacteria) to surrounding farms, facilitating the transmission of emergent zoonotic diseases and increasing the demand for antibiotic use.

In this systematic review, we assessed a range of WASH and biosecurity interventions implemented to address animal health or production issues for their impacts on infection burden, antibiotic use and antimicrobial resistance**.** We developed a framework to conceptualize how WASH interventions influence AMR and identified points of intervention at the structure and systems levels that can be addressed to reduce antibiotic use (ABU), assessing the potential impact of WASH and biosecurity interventions in animal production on reducing reliance on antibiotics and consequently ABR, exposing their potential co-benefits positively impacting other infectious diseases or public health issues.

CONCEPTUAL FRAMEWORK

We performed a quick search of relevant literature to create a framework to conceptualise WASH and biosecurity under the One Health umbrella (figure 1). We proposed several pathways to show how lack of WASH and biosecurity interventions could contribute to the increased interaction between humans, animals and the environment, and therefore to the emergence and dissemination of antimicrobial resistance genes (ARGs). We also assessed studies documenting risk factors associated to burden of infections to identify potential points for interventions.

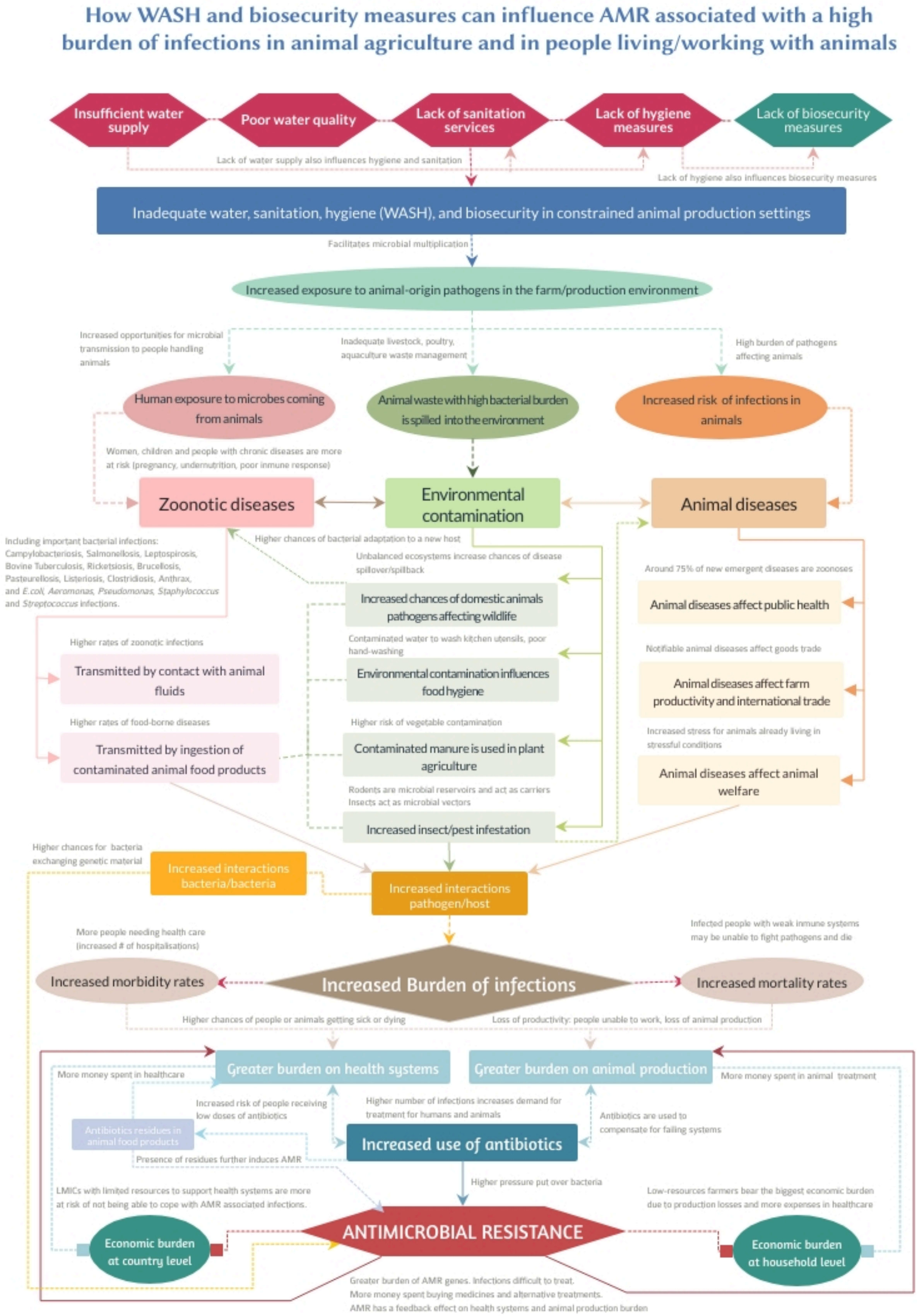

Figure 1: Framework to conceptualise the relevance of WASH and biosecurity interventions to AMR

Points for interventions identified in risk factors studies

To identify possible AMR points of intervention, we assessed 78 risk factors studies identified through our search strategy that were relevant to WASH and biosecurity. These studies, which represented research in thirty countries, reported risk factors relevant to human (n=18), animal (n=119) as well as environmental health (n=185). Of the studies which specified the target animal (n=71), 36 studies looked at livestock such as cows (n=10), pigs (n=19), as well as goats or sheep (n=7). A further 34 studies looked at chickens, ducks or turkeys. Only 1 study looked at risk factors in aquaculture. Of the 61 that looked at particular microorganisms, 21 were about *Salmonella*, 18 about *Campylobacter*, whereas other pathogens of interest included mastitis-causing bacteria (n=5), zoonoses such as *Coxiella burnetti* (n=5), and *Toxoplasma gondii* (n=5) amongst others. Despite estimations suggesting that two-thirds of the global faecal biomass originates at the level of households[154] only 2 of the relevant studies identified through our search investigated risk factors at the level of household production systems, with an additional 9 studies conducted amongst pastoralists and smallholders. All other studies were set in intensive or extensive commercial farming systems.

Risk factors that were significantly associated with increased infection included practices related to entering and exiting the farm by humans. For example, studies on Bangladeshi poultry farms identified a high number of staff (OR:5.1; CI95%: 2.2-12.2) and frequent weekly veterinary visits (OR: 3.0; CI95%:1.2-7.3) as key biosecurity related risk factors[155,156]. Having frequent contact with multiple farm workers was also found to be a risk factor in other contexts, such as in pig farms in Spain (OR:1.8; CI95%: 1.1-2.9)[157], whereas regular contact with animal health workers was a risk factor identified on Nigerian poultry farms as well (OR:1.06; CI95%:0.54-2.1)[158]. Hygienic practices of staff, for example whether they changed footwear when entering the farm[159], were important protective factors for *Campylobacter* in chicken (RR: 0.55; CI95%:0.35-0.85). Similarly, the disinfection status of farming equipment (OR:0.08; CI95%:0.028-0.203) and staff footwear (OR:0.26; CI95%: 0.068-0.952) were protective factors against Salmonella in pigs[160].

Animal health related risk factors included diverse points of intervention such as reducing the contact of poultry or livestock with wild animals (OR:6.573; CI95%: 2.148-20.115) or other herds (OR:1.95; CI95%:1.12-3.42) and limiting purchases of animals at live markets (OR:7.59; CI95%: 2.69-21.45)[156,161,162]. The presence of other animals such as cats, dogs, or rodents was also identified as a risk factor in contexts as diverse as goat farms in Thailand (OR:5.12; CI95%: 1.04-25.21)[163] and Danish mink farms (OR: 9.3)[164]. Significant environmental risk factors included the farm density in the region, for example having another farm less than 1 km away (OR: 1.97, CI95%: 1.15-3.37)[158] but also different components of farm infrastructure. In this context floor type (OR: 3.6; CI95%:1.93-6.8), lack of bedding (OR:1.9, CI95%: 1.05-3.58)[165], and adequate barn size (OR: 10.17)(e.g. Kivaria et al. 2005)[166], were all identified as potential AMR-sensitive points of intervention. To reduce subsequent disease in humans living in close proximity with animals, it was found in diverse context that households sharing a water source with animals were at higher risk of disease, both in Ethiopian pastoralists (OR: 4.403; CI95%: 2.42-8.00)[167] and in urban Arusha, Tanzania (OR:7.54; CI95%: 2.41-23.45)[168].

METHODS

A comprehensive electronic search of literature in relevant databases was performed from September 2019 to September 2020. We included all relevant articles published from inception to 2019. We developed this review following the PRISMA standards outlined in our pre-published protocol[36]. The scope of the literature was limited to WASH and biosecurity interventions oriented to reduce the burden of infections and reliance on antibiotics for animals and humans. We aimed to answer the following questions:

Q1) What type of WASH and biosecurity interventions play a role in reducing the burden of infections and therefore have the potential to reduce reliance on antibiotics and therefore ABR in animals and humans?

Q2) Under which enabling or limiting conditions are these interventions effective for reducing burden of infections and ABU in animals and humans?

The search was not restricted by language, and we also developed a search query in Spanish, French, and Portuguese to identify articles written in these languages in relevant regional databases.

Scope of the review

Our PICOS statement covered the following:

a. **Population:** We included studies that focused on people and animals that coexist together in animal production systems and aquaculture in LMICs and HICs. These included, for people: small-scale farmers, producers, fishermen, smallholders, community holders, pastoralists, and people/families raising animals at a domestic level; and for animals: livestock, poultry, aquaculture, pisciculture, and regional productive species (e.g. goats, camels, alpaca, llama, bison).

b. **Interventions:** We included WASH and biosecurity interventions to reduce burden of infections and therefore reduce antibiotic use in animal agriculture and in people living/working with animals. We classified interventions in structural or not structural according to their potential to work at a system level. Some of these types of interventions may also be classified by the World Bank as AMR-sensitive interventions[1], and include interventions such as providing/improving water and sanitation, wastewater management, and implementing biosecurity measures in animal facilities. Interventions such as vaccinations were excluded from the study as they have been extensively discussed in other reviews[37-39]. We used the following definitions to categorise the interventions:

- ***Water, sanitation and hygiene (WASH) interventions*** for animals[5], grouped into four categories:

  - *Water quantity or supply improvement*: to provide or improve water distribution systems. For this review, it included installation of water facilities (pumps, deposits, tanks) in farms, rainwater harvesting systems, access to water channels in production systems, or reduction of dry matter in animal production (increase use of silage – to reduce animals' water intake).
  - *Water quality*: to remove or inactivate pathogens "at source" and "at point of use". It included water treatments such as filtration, sedimentation, chemical treatment, chlorination, and UV treatment. Additionally, interventions that changed the composition of drinking water, for example through acidification, and those that changed the water quality in aquaculture were considered here.
  - *Sanitation*: to provide or improve sanitation and waste disposal. For this review, it included: installation of waste systems, composting methods, manure treatment, septic tanks, slurry treatment, rubbish management, or disposal of biological waste.
  - *Hygiene*: to promote or implement changes in hygiene conditions. For this review, it included: equipment to facilitate farmers/producers/animal owner's handwashing or showering, use of disinfectants, use of hot water, cleaning of animal facilities, use of chemical products, use of high-pressure cleaners, bedding, food storage conditions, footbath, housing, sanitizers, sterilisation, pasteurisation, pest control, maintenance of pets and birds outside animal production areas, or use of protective barriers by farm workers or animal owners.
- ***Biosecurity[2] interventions:*** combination of practices to reduce the risk of introduction and/or spread of diseases. They were grouped into three categories:
  - *Bio-exclusion*: to prevent the introduction of a new pathogen (e.g. shower in and out, monitor health status previous to introduction to the farm, control of vehicles, people movement, animal movement restrictions).
  - *Bio-containment*: to prevent escape of pathogens to neighbouring farms (e.g., corralling, cleaning, disinfection, manure removal).
  - *Bio-management*: to control and manage pathogens already present in the farm (e.g., McREBEL protocols, all-in-all out systems).
- ***Multiple interventions***: combination of strategies including WASH and biosecurity interventions to reduce the burden of infections in animal facilities or animal dwellings.

c. **Comparator:** We did not include a comparison group. Interventions performed in LMICs with (another intervention or no intervention) or without comparison groups were included, instead we also captured studies performed in HICs to be able to compare studies performed in different types of settings.

d. **Outcomes:** The primary outcomes of interest were (see figure 2):

(a) Reduction of number of infections
    i. Reduction of the incidence/prevalence of infections/diseases
    ii. Reduction of morbidity/mortality rates
(b) Reduction of bacterial loads
    i. Reduction of bacterial counts
    ii. Reduction on the number of bacteria isolated from animal facilities
    iii. Reduction of number of bacterial concentrations
    iv. Reduction of positive microbiological culture
(c) Reduction of AMU
    i. Reduction of the number of veterinary visits
    ii. Reduction of the quantity of antibiotics used
    iii. Reduction of antibiotic prescriptions
    iv. Reduction of medicated animal feed
    v. Reduction of antibiotic residues in animal products
(d) Reduction of AMR
    i. Reduction of AMR-bacteria isolates
    ii. Reduction of antibiotic-resistance genes
    iii. Increased sensitivity to the effects of antibiotics

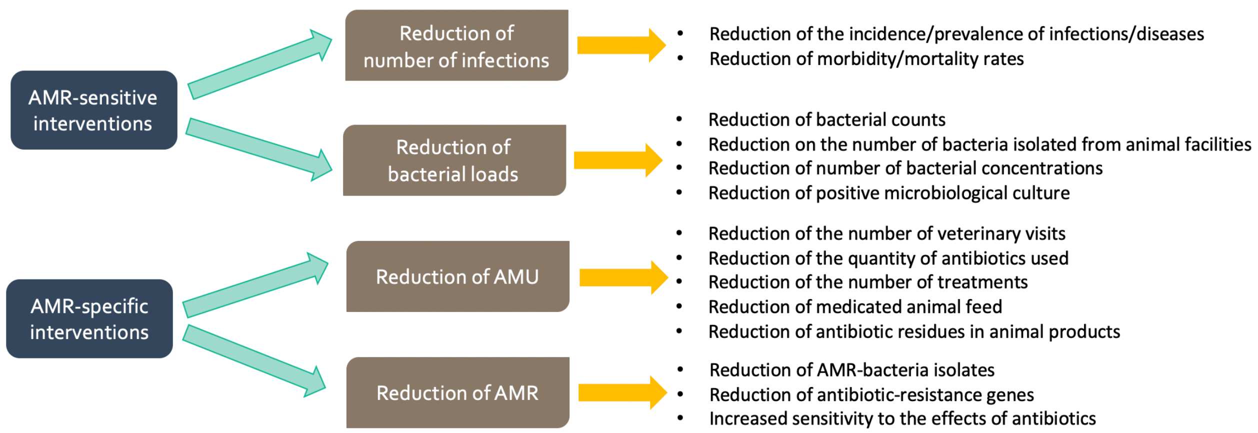

Figure 2: Algorithm to classify outcomes of interest

e. **Study designs:** to be as inclusive and comprehensive as possible we included the following study types: randomized control trials, non-randomized control trials, community-based studies, quasi experimental studies, before-and-after studies, uncontrolled studies, interrupted time series, matched control, regression-discontinuity, and epidemiological (case series, individual case reports, cross-sectional studies, ecological studies and longitudinal studies) study designs.

Search strategy and selection criteria

We performed searches in the following databases: Web of Science, PubMed, OVID (CAB Abstracts, Global Health, Embase, MEDLINE, Veterinary Science, Social Work Abstracts, PsycINFO®), ProQuest, Epistemonikos, Trip, AgEcon, and Cochrane Library. For other languages we performed the search in these relevant regional databases: Scopus, Scielo, BIREME, E-Revistas, Redalyc, Lilacs, AfricaPortal, IMSEAR (Index Medicus for the South-East Asian Region), and Western Pacific Region Index Medicus (WPRIM). A hand search of literature was also conducted in FAO Agora, Agris, JPIAMR platform, JSTOR, JOLIS, The World Bank database, International Development and Research Centre Digital Library (IDRC). Google Scholar and Open grey were used to search for grey literature. Additional articles were identified by performing a snowballing search in the references of relevant literature. Searches were run from May to August 2020.

Our search strategy was composed by keywords related to the topic and described five comprehensive search themes set: 1) animals and human population of interests, 2) WASH and biosecurity types of interventions, 3) the outcomes of interests - bacterial counts, reduction in AMU/AMR, reduction in infections/diseases/morbidity or mortality, 4) the types of studies, and 5) the countries of interests - LMICs, details of the search strategy can be seen in appendix I. The themes were combined using Boolean operator "OR" and "AND" and adapted to the requirements of each database. Database specific MESH terms and subject headings were adapted for some databases from the original strategy. The search terms and strategy were peer-reviewed by the librarian of the London School of Hygiene and Tropical Medicine.

Studies were eligible for inclusion if they met the following criteria:

- The study described a primary research study (original article).
- The study described a WASH or biosecurity interventions with the potential of reducing the burden of infections and therefore antimicrobial use.
- The study included an animal component in the intervention that analysed strategies to reduce burden of infections in livestock, poultry, aquaculture, and regional farm animals.
- The study covered WASH and biosecurity interventions implemented inside farms, or in animal production systems, or at community/household level aiming to reduce burden of infections.
- The study covered WASH and biosecurity interventions in animal production systems (including intensive farming, small-holders farming, subsistence farming, pastoralists, fishermen, pisciculture systems) and/or in people living/working in animals.
- The study covered any of the outcomes of interest mentioned above.

We excluded studies that:

- Included an intervention limited to health facilities.
- Tested disinfectants "in vitro" or at laboratories.

- Included interventions applied in human communities/settings with no attention paid to animal populations.
- Included interventions that were applied outside production systems (such as disinfection of animal transport vehicles or carcass disinfection).
- Relied on people changing their behaviour without including the assessment for an outcome of interest.
- Included interventions such as vaccinations, changes to nutrition, or other ways of improving animal husbandry measures not directly associated with WASH or biosecurity.
- Were unavailable in full-text, or unavailable in English/Spanish/Portuguese/French/German/Dutch and the author, after contact, did not provide the full-text.

We considered a broad range of infections to be of interest to this systematic review, including all studies that addressed bacterial or viral infections, or infections with the following unicellular parasites: Coccidias, Neospora, Cryptosporidum, and *Toxoplasma Gondii*. The reasoning behind this is three-fold. Firstly, interventions that reduce the transmission of pathogens, including viruses and some parasites, can be considered to be AMR-sensitive interventions according to the World Bank definition. Secondly, the diagnostic tools to differentiate between the pathogen causing disease in some veterinary settings may be absent, especially in LMIC contexts. As a result, infections with pathogens other than bacteria may erroneously be treated with antibiotics. Therefore, any interventions that reduce the spread or burden of pathogens that may be inadvertently treated with antibiotics are also of interest to this systematic review. Finally, infections with some unicellular parasites, such as the ones listed above, are treated using antibiotics that are also used to treat bacterial infections. Thus, any intervention that reduces infectious diseases burden also reduces antibiotic use, and therefore lessens the opportunity of resistant infections to emerge.

Study selection, risk of bias assessment and data extraction

The lists of articles retrieved from each database were downloaded into EndNote version X9 (Thomson Reuters, New York, USA) and duplicates were removed. The selection process was conducted in two stages. Firstly, two authors (SK and PT) screened titles and abstracts of all articles written in English independently, and decided if they fit the eligibility criteria. Any disagreements were resolved by a third author (CP). The interrater agreement between the two authors (SK and PT) stage was moderate (κ=0.55). Articles written in Spanish, Portuguese, and French were screened by one author (CP). Secondly, three authors (SK, PT, CP) reviewed the full-text. All articles that did not meet the eligibility criteria after full-text examination are listed in appendix II with the reasons for their exclusion.

Two review authors (SK, PT) independently assessed the risk of bias for the included English studies. One author assessed the risk of bias for the German study (SK) and one (CP) assessed the risk of bias for Spanish and Portuguese studies. We resolved disagreements through discussion between members of the research team until consensus was reached. Given the heterogenous nature of studies included within this systematic review, bias assessment tools were selected for use based on the specific methodology and experimental set up of the assessed interventions. The risk of bias was assessed by following (i) SYRCLE's rob tool[40], based on Cochrane Rob tool for articles with randomised-control trials (RCTs) designs, (ii) ROBINS-I[41] for non-randomised trials, (iii) AXIS[42] for cross-sectional and ecological studies, and (iv) MMAT[43] for articles using mixed methodologies. As this study aimed to evaluate interventions in multiple complex systems, no studies were excluded based on quality assessment. After bias examination studies were classified as having "low risk of bias", "moderate risk of bias" or "high risk of bias". Quality assessments commonly have RCTs as a 'gold standard' design for evaluating interventions, however RCTs normally assume a linear relationship between the intervention and the outcome, which in the case of complex interventions is not always relevant as multiple pathways can generate the same outcome[44]. Instead, quality issues were examined as a source of heterogeneity, accounting for differences in the study results.

Data were extracted using a predesigned form including information about the following: authors, aim, type of publication, language, year, source, journal, country, region (using the definition of geographic regions by United Nations[45]), type of collaborations (multidisciplinary, interdisciplinary, transdisciplinary, and no collaborations), type of study (quantitative or qualitative, observational, or experimental), study design, stakeholders involved, recruitment method, type of intervention, intervention aim, method of delivering (face-to-face, group-based, internet-based, community-based, print-based, or combined), methods employed, frequency, environmental element (soil, air, water, farm, or household environment), microorganism involved (if relevant), description of intervention, intervention groups, duration of intervention, animal species involved, type of production system (intensive farming, extensive farming, small-holders, community holders, pastoralism, or subsistence), type of feed utilised, agroecological situation, climate conditions, population type, livelihood system (farming-commercial, household farming-subsistence, mixed-farming + agriculture, labourer-farmworkers, fishing/hunting, or other occupations), type of setting (urban, peri-urban, rural, peri-rural, remote/isolate, or experimental), intervention outcomes, method of assessing outcomes, statistical analysis conducted, baseline results, intervention results, estimation of effects, beneficial outcomes, potential co-benefits, adverse events, unintended consequences, barriers identified, evaluation design, and research gaps identified.

We selected interventions working at different levels of the human-animal-environmental interface and that applied different strategies to mitigate the development of infections or the dissemination of ARGs. Therefore, we classified interventions into four categories: (i) structural interventions, which focus on changing the political-economic context where health is produced or reproduced; (ii) biological and chemical interventions which include strategies such as competitive exclusion, as well as applying products to eliminate pathogens; (iii) infrastructure and apparatus interventions, which focus on changing the physical environment and protocols included for animal husbandry, such as flooring types and regimens; and (iv) educational and behavioural interventions, which focus on changing the practices of individuals such as farmers or residents through education or other behaviour change techniques.

Data analysis

The heterogeneity of study designs included in the selected studies, the differences in physical and socio-cultural contexts, animal species, and outcomes of interest, prevented us from performing a meta-analysis, as grouping the quantitative results of the interventions was deemed not possible. Therefore, a narrative synthesis of interventions was performed and a descriptive report written up.

Synthesis methods

Information obtained from the articles was summarised in two steps:

1. An analysis of the risk factors that link different aspects of the (farm) environment to burden of infectious disease/antimicrobial use. This suggested points for interventions to reduce the emergence and spread of AMR in animals and informed the conceptual framework (included after the introduction)
2. A narrative synthesis of the evidence collected from interventional studies. We grouped interventions based on similarities, conceptualising WASH and biosecurity interventions and pathways to AMR by applying a One Health approach

Data extracted from the interventions were tabulated and summarized narratively, and summary statistics for analysed interventions were presented. Articles were categorized by intervention type, country of origin, region, and type of funding received, and were reported in tables according to their risk of bias assessment. We conceptualised WASH and biosecurity interventions for animal agriculture settings, considering that a one-policy-for-all approach might not be possible in different country settings. The results of the study were reported using the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) checklist[46] (appendix IX).

RESULTS

Search results

We identified a total of 20,672 studies for potential inclusion (20,610 through database searching, 62 through manual searches). Search strategies used in key databases are included in appendix I. A total of 1,804 duplicates were removed before abstract screening, and 18,868 articles were screened using titles and abstracts.

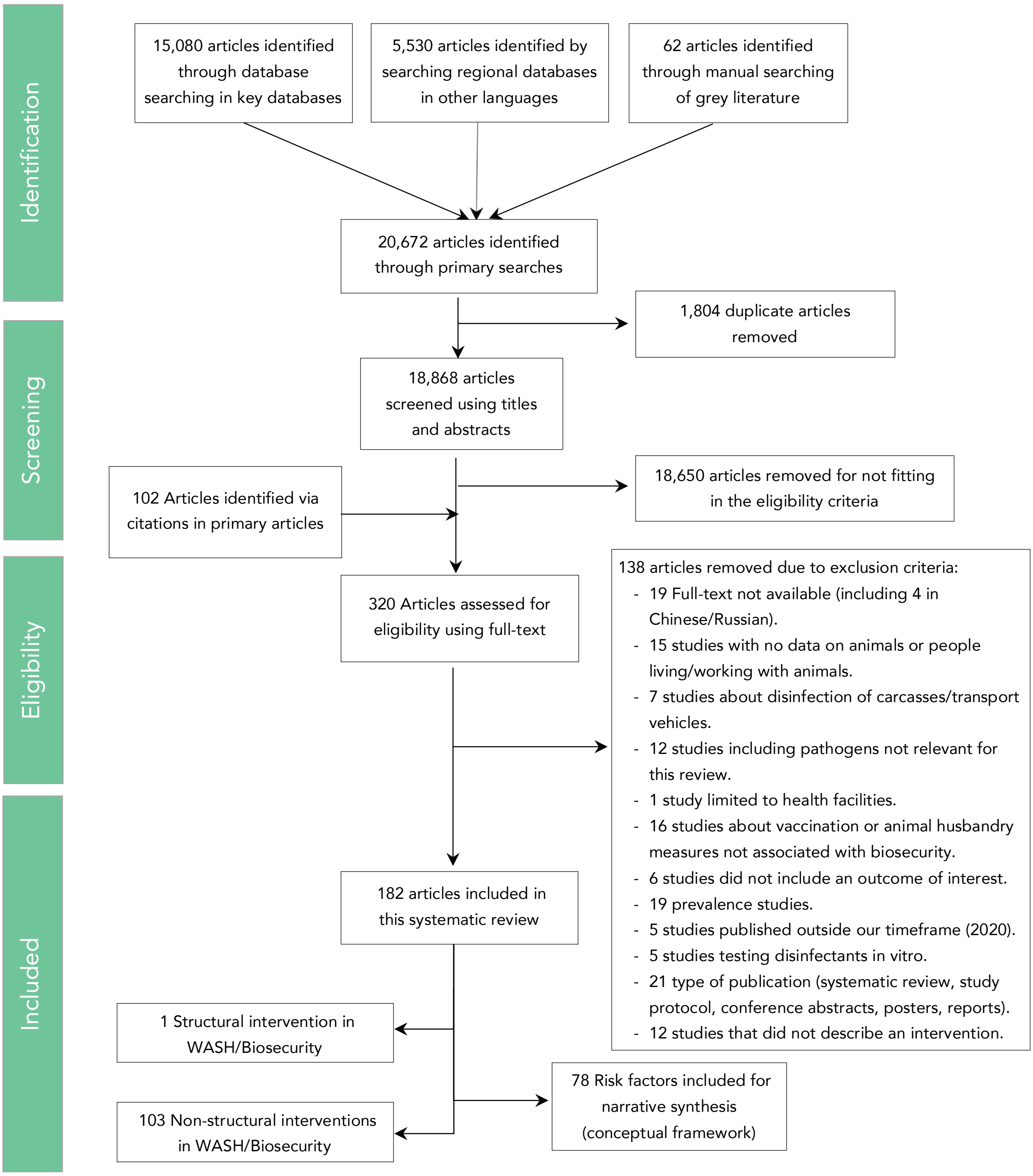

Figure 3: Literature review process and results

During abstract screening, 18,650 articles were removed for not fitting the inclusion criteria. A total of 102 further studies were identified by searching the references of relevant articles and added for full-text assessment. In total 320 studies were included in full-text assessment. After full-text examination 182 articles were eligible for inclusion in this review. Only 1 article was identified as a structural intervention and 103 articles were identified as non-structural interventions (biological/chemical, infrastructure and apparatus, and educational/behavioural). Seventy-nine studies collecting information about risk factors were included for narrative synthesis. A list of excluded studies and reason for exclusion can be found in appendix II. The study's selection process is outlined in figure 3.

Characteristics of included studies

Most studies 78.8% (82/104) had an experimental design, of which 56 were non-randomised trials (NRTs), including before and after studies and interrupted time series designs, and 26 were randomised-controlled trials (RCTs). There were 21.2% (22/104) studies with an observational design, including 5 cross-sectional, 15 ecological, and 2 case-control.

The majority of included studies 95% (99/104) were written in the English language, whereas 1% (1/104) of studies were in German, 1% (1/104) in Portuguese, and 3% (3/104) in Spanish. Of the 104 studies, 98% (102/104) were journal articles, 1 conference poster, and 1 thesis. We found relevant studies published from 1974 to 2019, 2017 being the year where most relevant studies 13.5% (14/104) were found (see figure 4). The duration of the studies ranged from 3 days[47,48] to 10 years[49]. Most studies had a duration of less than one year. Additional details about the characteristics of the included studies can be found in table 1.

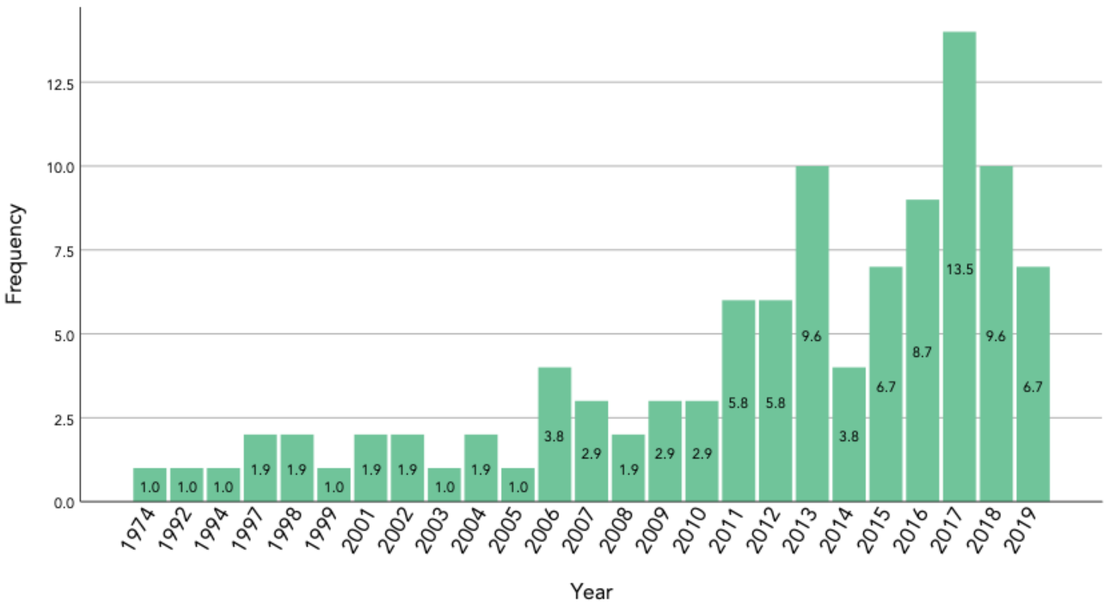

Figure 4: Number of studies published from inception to 2019

Type of populations

From the 104 studies included, 102 included animal populations and 19 included human populations. In total, 85 studies were exclusively performed in animals whereas 2 studies were exclusively performed in humans. The other 17 intervention studies reported outcomes for both animals and humans. Most intervention studies were performed in livestock 46.2% (48/104), and poultry 41.3% (43/104), only 11.5% (12/104) of studies focused on interventions relevant to aquaculture. The most common animal populations studied were chickens (especially broilers) 36, followed by 27 pigs, 18 cattle, and fish/crustaceans 12. Animal species less frequently studied were ducks, geese, turkeys, sheep, and horses. Amongst the human populations studied 16 were farm workers and 3 household members.

Table 1: Summary of study characteristics (n/%) by population type

Characteristic	Number of animal studies (n=102)*	Number of human studies (n=19)*
Study design		
Randomised control trial (RCT)	26 (25.5%)	5 (26.3%)
Non-randomised trial (NRT)	54 (52.9%)	12 (63.2%)
Cross sectional/Ecological	20 (19.6%)	1 (5.3%)
Case-control	2 (2%)	1 (5.3%)
Population studied		
Cattle	18 (17.6%)	..
Sheep	1 (1%)	..
Pigs	27 (26.4%)	..
Horses	1 (1%)	..
Poultry	36 (35.3%)	..
Ducks	3 (2.9%)	..
Turkey	3 (2.9%)	..
Goose	1 (1%)	..
Fish	12 (11.7%)	..
Farm workers	..	16 (84.2%)
Household members	..	3 (15.8%)
Type of production system		
Intensive farming	67 (65.7%)	7 (36.8%)
Small-holders	5 (4.9%)	2 (10.5%)
Subsistence	5 (4.9%)	3 (15.8%)
Mixed	4 (3.9%)	3 (15.8%)
Experimental set-up	21 (20.6%)	4 (21.1%)
Type of intervention (classification by WASH and biosecurity)		
Water quality	21 (20.6%)	..
Water quantity	2 (2%)	..
Hygiene	28 (27.4%)	5 (26.3%)
Sanitation	2 (2%)	1 (5.3%)
Bio-exclusion	5 (4.9%)	2 (10.5%)
Bio-management	34 (33.3%)	11 (57.9%)
Bio-containment	10 (9.8%)	..
Type of intervention (classified by intervention target)		
Biological/Chemical	56 (54.9%)	6 (31.6%)
Educational/behavioural	15 (14.7%)	10 (52.6%)
Infrastructure and apparatus	26 (25.5%)	1 (5.3%)
Structural	..	1 (5.3%)
Mixed	5 (4.9%)	1 (5.3%)
Sample studied		
Milk	9* (7.5%)	6 (33.3%)
Body swabs	6* (5%)	..
Human faeces	..	1 (5.6%)

Animal faeces	10* (8.3%)	1 (5.6%)
Nasal	1 (0.8%)	..
Rectal/cloacal swabs	22* (18.3%)	3 (16.7%)
Tissues	6* (5%)	..
Blood	10 (8.3%)	3 (16.7%)
Hands swabs	..	1 (5.6%)
PPE	2 (1.7%)	3 (16.7%)
Environmental element		
Farm equipment and surfaces	22* (18.3%)	..
Water	14* (11.7%)	..
Air	3* (2.5%)	..
Soil	1 (0.8%)	..
Plagues (including flies and rodents)	2 (1.7%)	..
Slurry	1 (0.8%)	..
Wastewater	1 (0.8%)	..
Compost	4 (3.3%)	..
Litter	6* (5%)	..
Microorganism studied*		
Bacteria		
Enterobacteriaceae		
Escherichia coli	7 (6.9%)	1 (5.3%)
Salmonella spp.	18 (17.6%)	1 (5.3%)
Coliforms	2 (2%)	..
Staphylococcus aureus	5 (4.9%)	1 (5.3%)
Streptococcus agalactiae	1 (1%)	..
Vibrio spp.	2 (2%)	..
Campylobacter spp.	10 (9.8%)	3 (15.8%)
Brucella spp.	1 (1%)	1 (5.3%)
Leptospira spp.	..	1 (5.3%)
Mastitis-associated bacteria	3 (2.9%)	3 (15.8%)
Multibacteria	30 (29.4%)	3 (15.8%)
Other unspecified	8 (7.8%)	2 (10.5%)
Viruses		
Avian influenza virus	1 (1%)	1 (5.3%)
Porcine Epidemic diarrhoea virus (PEDV)	2 (2%)	1 (5.3%)
Porcine reproductive and respiratory syndrome (PRRSV)	2 (2%)	1 (5.3%)
Viral haemorrhagic septicaemia virus	1 (1%)	..
White spot virus	1 (1%)	..
Parasites		
Toxoplasma gondii	1 (1%)	..
Myxosoma cerebralis	1 (1%)	..
Antibiotic Resistance genes (ARGs)[a]	6 (5.9%)	..

*Studies can be included in more than one category. From the 104 interventions' studies included, 85 studies were exclusively performed in animals whereas 2 studies were exclusively performed in humans. The other 17 studies were performed in both humans and animals so are counted for both humans and animals. [a] Some studies detected ARGs from microorganism's DNA.

Samples and types of microorganisms included

Studies measured the outcomes of interest by testing different types of samples taken either from the animal, from people, from surfaces in the farm environment, or from animal dwellings. Most studies searched for pathogens in rectal or cloacal swabs or faeces, blood, tissues, farm equipment, or water. Many studies examined more than one type of sample, including in their analysis samples taken from the animals, the farm environment, and from potential environmental contaminants such as compost, slurry, and wastewater (see table 1).

Selected interventions targeted a range of pathogens including mainly bacteria species, some viruses, and parasites. A significant number of interventions (30/104, 28.8%)

did not specifically target one type of microorganism, instead they looked at many bacterial species at the same time ('multibacteria'). Eight (7.7%) studies did not specify the type of microorganism studied. Pathogens with zoonotic potential such as *Salmonella 18.3%* (19/104), *Campylobacter 9.6%* (10/104), *Staphylococcus aureus* including MRSA 4.8% (5/104), *Escherichia coli* including O157:H7 6.8% (7/104), *Leptospira* (1/104), *Brucella* 1% (1/104), and Avian influenza 1% (1/104) were amongst the pathogens addressed with these interventions (Figure 5). Six (5.8%) studies did not search for pathogens but for their antimicrobial resistance genes (ARGs). Finally, 2.9% (3/104) studies also mentioned looking at other microorganisms like virus and parasites, whereas 6.7% (7/104) exclusively looked at viruses, and 1.95% (2/104) at parasites that were eligible for inclusion in this systematic review. Additional information regarding types of pathogens studied in animal and/or human population studies can be found in table 1.

Study settings

Types of production systems

Most studies were performed in intensive farming production systems (65.4%, 68/104), followed by 21.2% (22/104) in experimental set-ups, commonly implemented in university facilities. Only 4.8% (5/104) involved small-holders, and the same amount involved subsistence farmers, 3.9% (4/104) of studies included different types of productive systems, 1 was done in mixed systems (extensive and intensive farming), 2 in small holders and intensive farming, and 1 subsistence and small-holders.

Socioeconomic settings

Regarding the characteristics of the settings where studies took place, we found that half the studies (53/104) did not provide information about the type of settings where the study was performed, 21,2% (22/104) were done under experimental conditions in university facilities, 17.3% (18/104) were done in rural settings, 3.9% (4/104) in peri-urban settings, 2.9% (3/104) in peri-rural, two studies were performed across different types of settings, and just one study was done in urban, and remote/isolate settings each.

Livelihood systems

The livelihood system of the human population involved in the studies was mainly based on commercial farming (68 working in intensive production systems and 2 as small-holders, 5.8% (6/104) of studies were based on populations raising animals as their main source of income (4 subsistence farming and 2 small-holders), 4.8% (5/104) had mixed livelihood systems including commercial and subsistence or as labourers. For 21.2% (22/104) of studies the livelihood system was irrelevant as these were experimental set ups.

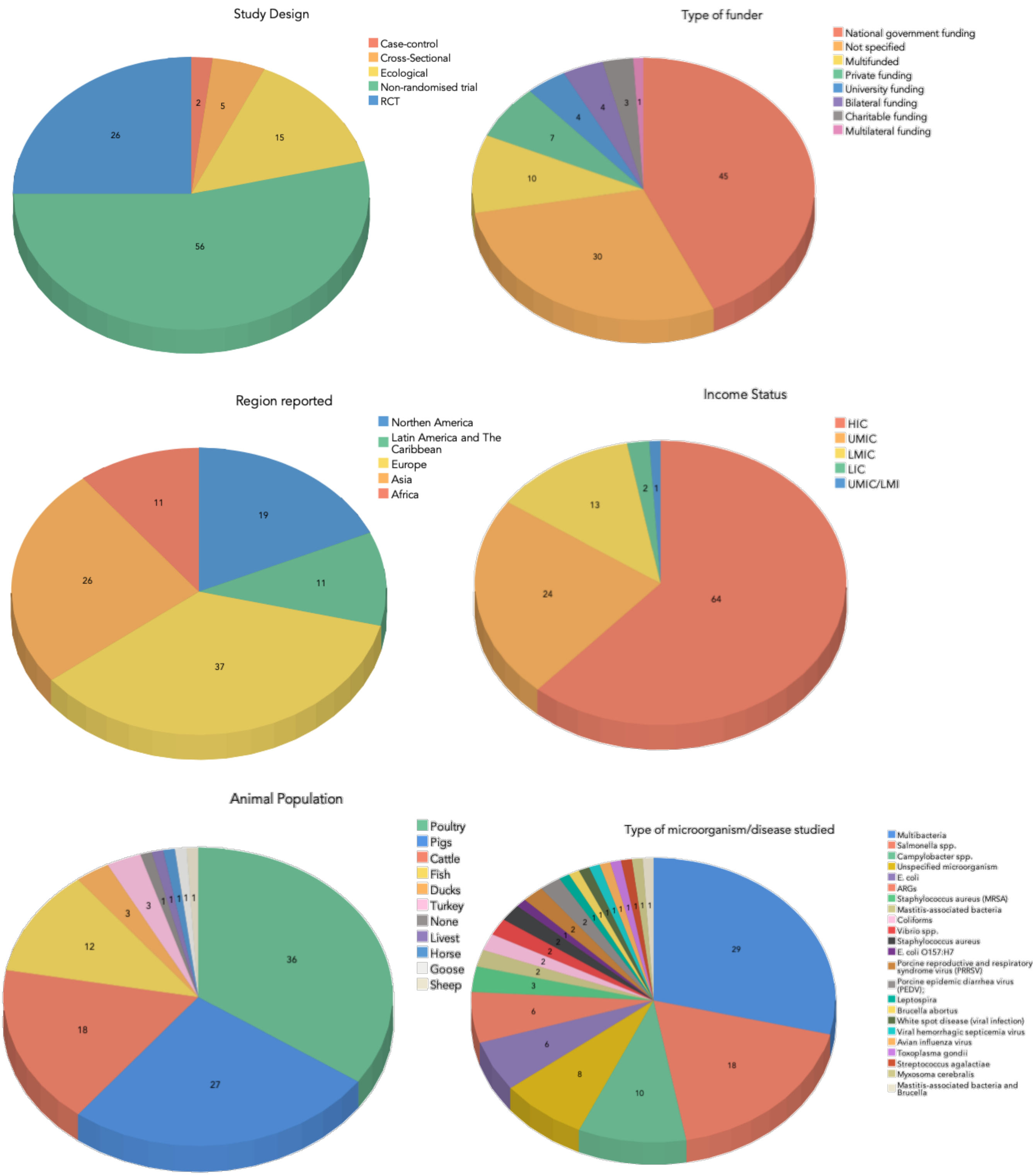

Figure 5: Pie charts showcasing some characteristics of included studies

Countries, regions, levels of income, and sources of funding

Included intervention studies came from a range of countries from five of the six geographical regions designated by the United Nations[45], no studies came from Oceania.

Most studies 36% (37/104) were performed in European countries, 25% (26/104) in Asia, 18% (19/104) in Northern America, 10.6% (11/104) in Africa, and 10.6% (11/104) in Latin America and The Caribbean. Most studies were performed in the United States 16.3% (17/104), China 8.7% (9/104), Germany 7.7% (8/104), and the United Kingdom 6.7% (7/104). The total number of studies per country can be seen in figure 6.

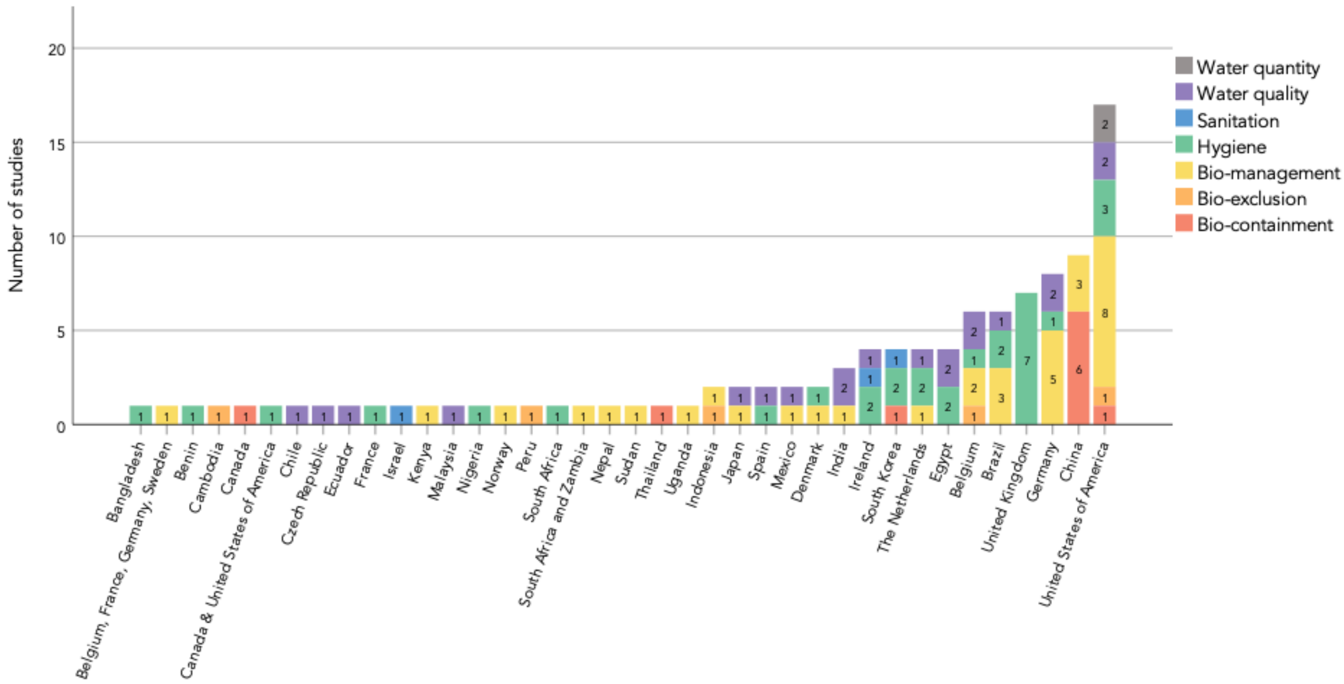

Figure 6: Total number of studies per country by type of intervention

Looking at the income status of the countries where the studies were conducted, one study was conducted in multiple countries (UMIC/LMIC). Despite using search terms specifically aiming to capture studies conducted in LMICs, 61.5% (64/104) of the studies were conducted in HICs, whereas 23.1% (24/104) were performed in UMICs, 12.5% (13/104) in LMICs, and only 1.9% (2/104) were conducted in contexts classified as LICs (see figure 5). Countries' levels of income were classified according to the World Bank Classification[50].

Of the 104 studies included, 28.8% (30/104) did not specify the funding source. A total of 128 funding sources were declared by the other 79 studies. Most studies 43.3% (45/104) received funding from national government bodies, whereas only 1% (1/104) was funded by multilateral funding bodies. Other sources of funding were bilateral bodies 3.8% (4/104), charitable sources 2.9% (3/104), private entities 6.7% (7/104), university funding 3.8% (4/104), and 9.6% (10/104) where funded by a combination of different funding bodies (see figure 5).

When analysing the types of collaborations established to apply the interventions, we found that most studies 33.7% (35/104) did not specify the type of collaboration or if there was any, and the majority of studies 53.8% (56/104) seem to not have collaborated with professionals from different disciplines or involved relevant stakeholders.

Types of interventions and outcomes

From the studies selected, one study[51] was classified as containing a structural intervention. This study included the assessment of the impact of a policy to control livestock manure for the purpose of reducing human Leptospirosis incidence in South Korea. The other 103 included studies were classified as non-structural interventions which were further classified into three categories: biological/chemical, educational/behavioural, and infrastructure and apparatus. From the non-structural studies, the majority 54.8% (57/104) were focused on the biological/chemical (such as applying products to eliminate pathogens) aspects of infection, followed by interventions focused on the modification of the infrastructure/apparatus of farms/animal dwellings in 25% (26/104), and the application of educational strategies focusing on the change of practices by farmworkers/household members in 14.4% (15/104) (figure 7). A descriptive summary of included interventions can be seen in appendix III.

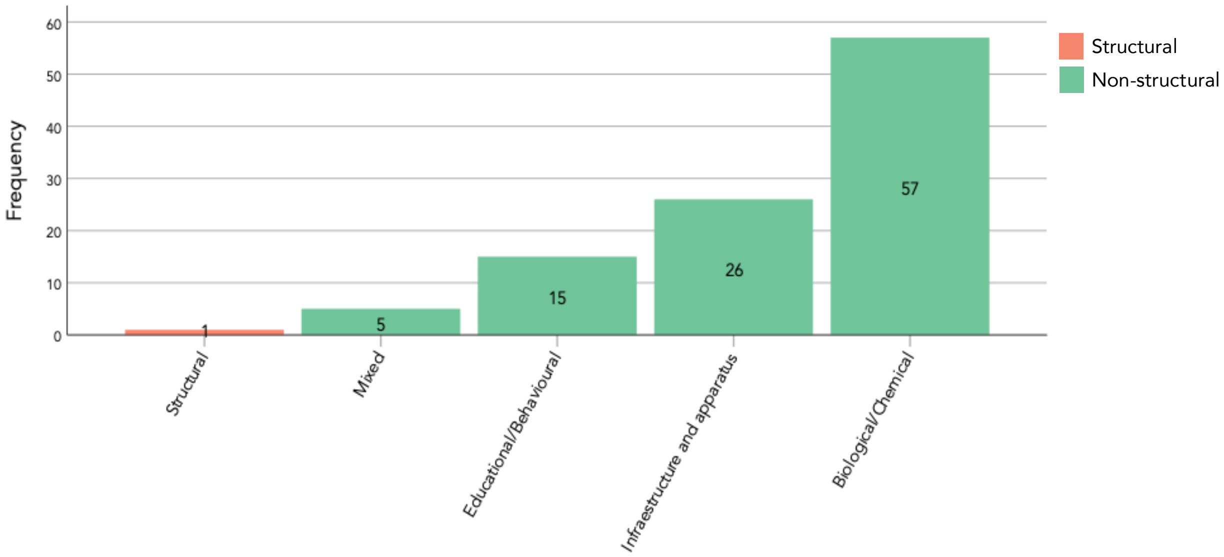

Figure 7: Interventions included classified according to the type of methodology used

WASH and Biosecurity interventions and their relevance to the AMR agenda

Interventions included in the selected studies were also classified into a WASH/biosecurity component (water, hygiene, sanitation, bio-exclusion, bio-management, and bio-containment) according to the typology described in the methods. Due to the overlaps between some of the WASH and biosecurity components some interventions could be classified into more than one component. For the purposes of our analyses, we classified interventions according to the most prominent WASH/Biosecurity component (table 2).

Around half 52.8% (55/104) of the studies included were relevant to WASH, with 19.2% (20/104) of studies focusing on water quality, 1.9% (2/104) on water quantity, 28.8% (30/104) on hygiene, and 2.9% (3/104) on sanitation. The other 47.1% (49/104) studies included interventions about biosecurity, where the majority of interventions 32.7% (34/104) focused on bio-management strategies, followed by 9.6% (10/104) on bio-containment, and 4.8% (5/104) on bio-exclusion. Within each component interventions were grouped and further categorised. A more detailed description of these classification and further subcategories can be seen in table 2. Additionally, a crosstabulation of both interventions' typologies can be seen in figure 8.

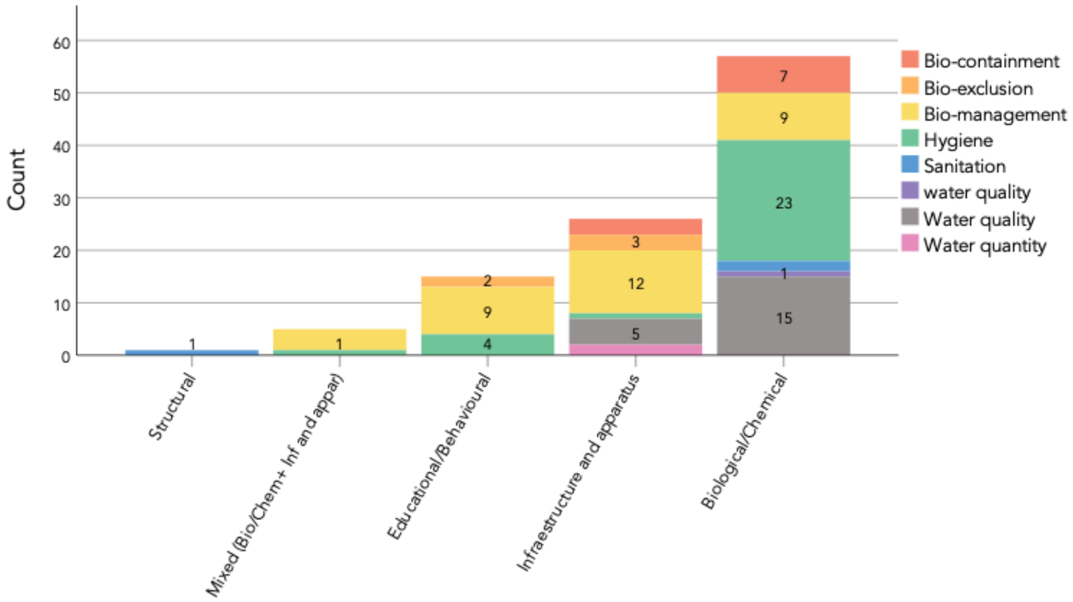

Figure 8: Crosstabulation of the interventions' typologies applied in this study

In figure 9 we represent the existing overlaps between biosecurity interventions and WASH components. For human health researchers and workers, measures to prevent and control infections at a population level are most often conceptualised as related to WASH, and for animal health researchers and workers some of the same type of measures are related to different components of biosecurity. We wanted to showcase how many of the interventions categorised in this review as belonging to one of the WASH or biosecurity components could also be considered an intervention related to the other group. Figure 9 shows that while water quantity, hygiene and sanitation have overlaps with biosecurity components, no interventions related to water quantity overlapped with biosecurity. In contrast, all the interventions categorised as sanitation overlap with biosecurity components, most prominently with bio-containment.

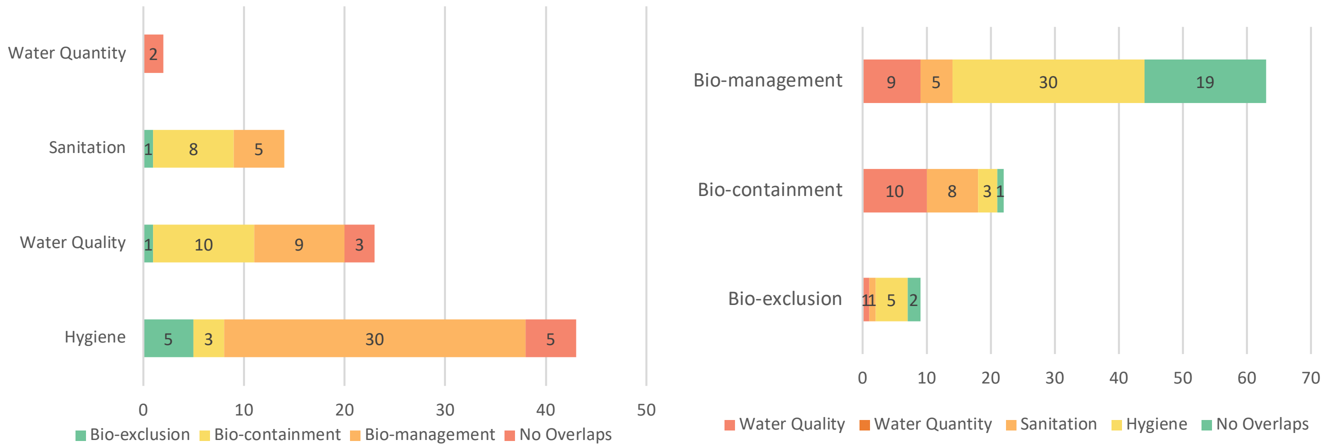

Figure 9: Graphic representation of overlaps between WASH (left) and biosecurity (right) interventions depending on the perspective from which it is seen.

Interventions were also classified according to the reported outcome of interest and their relevance to AMU, AMR, and the burden of infections. Some studies reported more than one outcome of interest, however for the typology we categorised the studies according to their primary outcome of interest. All the outcomes considered were those that had an impact on reducing the burden of infections/diseases that indirectly or directly could contribute to reduce AMU and AMR (figure 10).

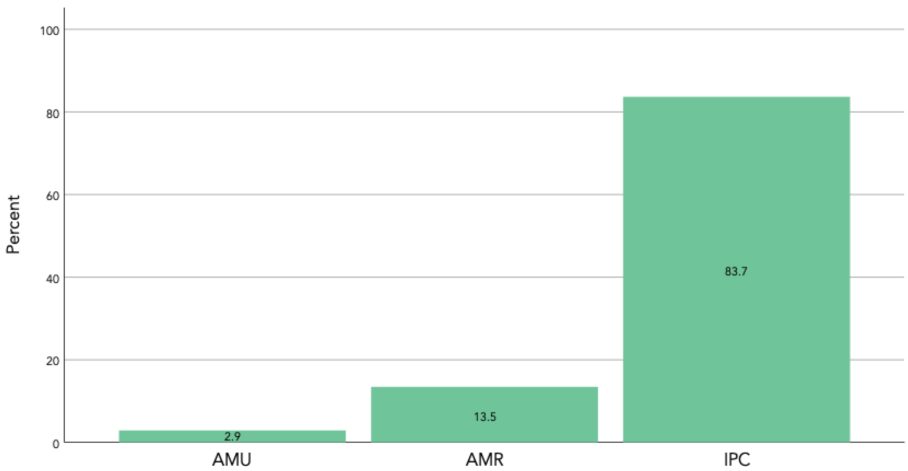

Figure 10: Relevance of selected interventions to burden of infections (infection prevention and control - IPC), AMU and AMR.

Table 2: Typology and description of interventions included in selected studies

Intervention type	Description	Study's references
Water quantity		
Adjusting the quantity of water	Interventions adjusting the quantity of water by lowering water levels to 50% (n=1) or by using water troughs rather than pin-metered water lines (n=1)	52,53
Water quality		
Acidification of drinking water	Interventions to improve the quality of drinking water provided to farm animals, particularly poultry, by acidifying it with the addition of products such as organic acids or vinegar. These products were added in water systems to lower the pH, thereby preventing the growth of microbes, improving water quality (n=7).	54-60
Cleaner drinking water systems on farms	Interventions that focused on providing clean drinking water systems through novel methods such as applying low-frequency electromagnetic fields (n=1) or separation/ sedimentation/ filtration-treated wastewater (n=1), or chlorination (n=1). One study looked at the progressive occurrence and characteristics of bacteria in water troughs for caged- and deep litter- managed layer chickens (n=1) and another at using nipple versus cup water troughs (n=1).	61-65
Improving water quality in aquaculture	Interventions aimed at improving the microbial water quality in aquaculture fishponds fed with animal manure or sewage (n=4), providing methods for providing clean water for aquaculture through changing water temperature (n=1), advanced oxidation processes (n=1), and UV-radiation (n=1), or by using polyculture with shrimp and fish (n=1) or Nile tilapia and filter-feeding bivalve muscle (n=1).	66-74
Hygiene		
Cleaning and disinfection (C&D) protocols and products	Studies that test different disinfectants and disinfection regimes and methods to eliminate and/or control infectious diseases in the farm environment (n = 17). These include the application and comparison of different detergents and commercially available disinfectants, but also the use of a high-pressure water rinse and wet versus dry cleaning.	75-91
Combined interventions in changing staff practices as well as cleaning and disinfection protocols.	Interventions that combine changing farm hygiene practices in with cleaning and disinfection protocols and products (n=4). These interventions included adjustments such as the introduction of a hygienic barrier where footwear and overalls must be changed, hand washing, cleaning and disinfection procedures of equipment and facilities.	92-95
Comprehensive measures to improve farm environment hygiene	Interventions that provide a comprehensive measures package to improve hygiene of the farm ensuring a proper environment, including addressing improved water and feed hygiene as well as regular cleaning of animal facilities (n=3).	96-98
Farmworkers changing personal hygiene practices	These interventions aimed to improve hygiene practices in the staff working on farms. This was done by testing protocols to improve hand hygiene in veterinary staff (n=1) or by altering hygiene practices in animal production workers' shower facilities (n=1).	99,100
Implementation of code of practices	Intervention through implementing the adoption of a code of hygienic practices through a participatory training program (n=1).	101
Use of footbaths	These studies evaluated the use of disinfectant footbaths, by looking at the bactericidal effects of commercial disinfectants to clean farmworkers' boots (n=2).	102,103

Table 2: Typology and description of interventions included in selected studies (continued)

Intervention type	**Description**	**Study's references**
Sanitation		
Policies to control livestock manure	Structural intervention at a policy level. This study evaluated the introduction of the Livestock Manure Control Act that makes it compulsory for farmers to be equipped with appropriate sludge process facilities on their farms (n=1).	51
Manure composting methods	Studies of manure composting methods such as manure cultivation whereby wet slurry is added daily to the cattle manure bedding (n=1) or the use of urea and ammonia treatments (n=1).	104,105
Bio-exclusion		
Preventing the introduction of pathogens at farms entrance	Interventions aimed at preventing pathogens from entering the farm and enhancing biosecurity compliance by improving existing practices including changing clothes and showering before entering the farm (n=1).	106
Competitive exclusion	Intervention that compares the efficacy of competitive exclusion with classical C&D to prevent the introduction of particular pathogens into farms (n=1).	107
Educational interventions for backyard poultry	Education and behavioural change interventions to improve backyard poultry biosecurity (n=1). This included distributing leaflets and holding sessions on raising awareness on the preventing pathogens from being introduced, human protection, disease management, importance of the cleaning of yards, equipment and poultry pens as well as using cages to protect chicks.	108
Confinement of free-ranging animals	Interventions promoting the use of pig confinement systems (n=1) or the corralling of free-range chicken (n=1) to replace the practice of animals roaming around freely in the community/household.	109,110
Bio-containment		
Controlling the contamination of water with ARGs	Studies on ARGs in different types of integrated fish-farms (n=1) and the effect of filtering process in flow-through aquaculture (n=1) and constructed wet-lands in removing ARGs from farm waste-water (n=1).	111-113
Mitigating the risk of ARGs from animal manure	Interventions aimed at mitigating the risk of ARGs from animal manure (n=6). Manure composting methods included using black soldier fly larvae, bamboo charcoal, and high temperatures to prevent the persistence of antibiotic resistant genes in the manure. Other interventions implemented the use of microbial fermentation bed, septic tank, biogas digester, natural drying methods to decrease ARG levels in animal manure.	114-119
Safe composting of fallen stock	Intervention to allow for the bio-secure disposal method of infected pig carcasses on the farm to prevent pathogens from escaping from the farm (n=1).	120
Bio-management		
Adjust flooring	Interventions that adjust farm flooring to reduce animal contact with their own excreta, the heating of the barn floor, the litter type or bedding used (n=7).	121-127
Air quality	Interventions aimed at improving air quality on farms. These included reducing airborne microorganisms for example through the use of air filtration, super plasma ionizing air purifiers, or the spraying of acidic electrolyzed water (n=3).	128-130

Table 2: Typology and description of interventions included in selected studies (continued)

Intervention type	**Description**	**Study's references**
Educational interventions on mastitis prevention and control	These educational interventions focused on delivering information, training and public health social mobilization training to improve practices around mastitis (n=4). Examples of these included: education on the importance of hand washing before and after milking, and hygienic farm management in mastitis prevention and control.	131-134
Herd-specific strategies	Reducing antimicrobial use through herd-specific intervention strategies and health planning focusing on the optimization of herd management (n=3).	135-137
Implementation of strategies to prevent and control mastitis in cows	Interventions that implemented a set of hygienic milking practices to prevent and control mastitis(n=4). Such adjustments of practices included changing milking order and technique, using disposable plastic gloves during milking and individual towels for wiping cow's teats, as well as post-milking teat dipping.	49,138-140
Lighting on the farm	Intervention adjusted length of artificial lighting in the farm environment to control O157:H7 prevalence (n=1).	141
Litter treatment methods	Application of on-farm treatments to safely reuse litter several times, but also using quicklime or litter tarping to reduce microbial counts in broiler litter (n=4).	142-145
Animal movement strategies	Interventions using strategic movements of animals as a bio-management strategy (n=3). These included an assessment of all-in-all-out management strategies as well as the strategic movement of animals after weaning to stop intergenerational pathogen transmission chains.	146-148
Organic farming practices	Study comparing organic antibiotic-free animal management practices versus conventional farming methods (n=1). Specifically, differences in raising animals with or without routine administration of antibiotics were noted.	149
Prevent transmission of pathogens through farm workers	Interventions that prevent the transmission of pathogens through farm workers by altered management and hygiene strategies that aim to disrupt the transmission cycle of existing pathogens on the farm (n=2). These may include changing the movement patterns and biosecurity practices of staff regarding the use of PPE, clean footwear, and face shields.	150,151
Vacancy period	Interventions using pond shutdown strategy in aquaculture (n=1) or a vacancy period in livestock (n=1) to manage disease outbreaks on commercial farms.	152,153

According to this classification, most interventions 83.7% (87/104) reported outcomes relevant to infection prevention and control such as reduction of bacterial counts (n=28), reduction of bacterial concentration (n=4), reduction of positive microbiological culture (n=14), reduction of bacteria isolated from animal facilities (n=18), reduction of morbidity/mortality rates (n=5), and reduction of incidence/prevalence of infections/diseases (n=18) either in animals or humans, whereas 13.5% (14/104) of studies reported outcomes relevant to the control of AMR-resistant bacteria or ARGs, reduction of AMR-bacteria isolates (n=6), reduction of antibiotic-resistance genes (n=8). Outcomes such as reduction of antibiotic residues in animal products (n=1), and reduction of quantity of antibiotics used (n=3) were reported as relevant to antimicrobial use (Figure 11). One study reported outcomes relevant for both antimicrobial use and antimicrobial resistance.

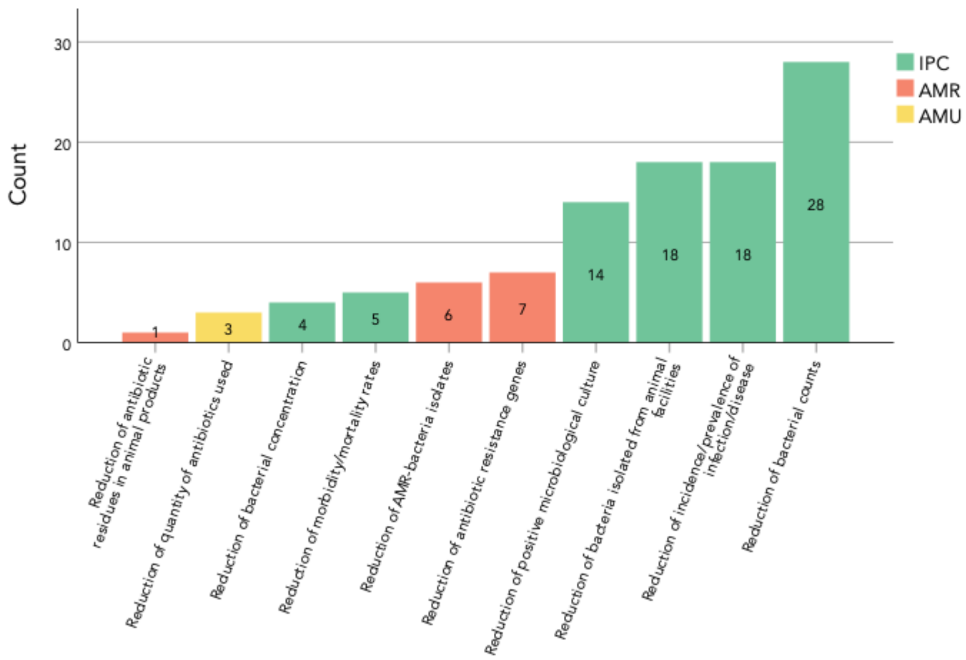

Figure 11: Number of studies that reported outcomes of interest categorised by their relevance to burden of infections, AMU and AMR.

The One Health triad

The relevance of the studies to the One Health triad was categorised upon explicit intention stated by authors (figure 12). The majority of studies 74% (77/104) included in this review were animal based, followed by studies relevant to animals and the environment 11.5% (12/104). Nine studies (8.7%) were exclusively relevant for the environment whereas only one study was exclusively relevant for humans. Unfortunately, we had to exclude a number of studies that included WASH interventions for agricultural communities and farmers because the authors did not include in their analysis the assessment of the relevance of the animal component in WASH interventions applied to farming communities.

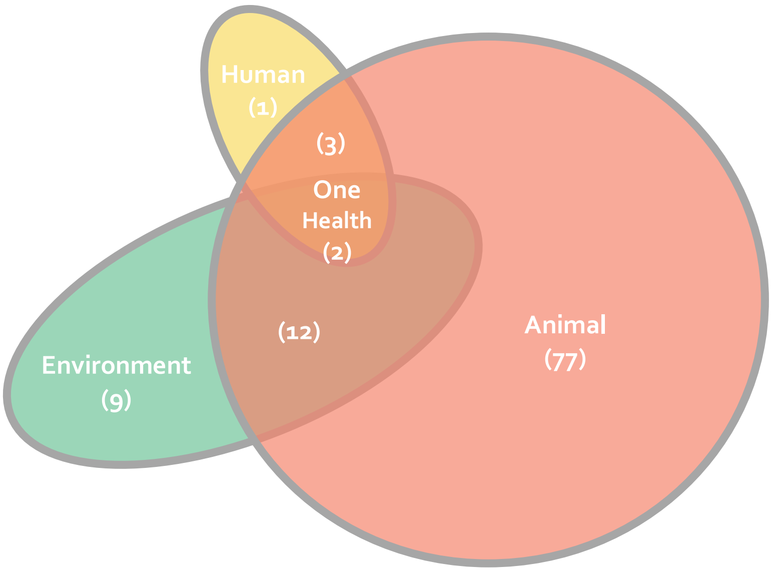

Figure 12: Article-reported sector relevance of interventions

Many of the studies identified as just relevant to animals by the authors could also have been classified as relevant to humans, because some of the microorganisms they focused on are considered zoonotic pathogens including *Campylobacter* spp.[81,94,95,125] and *Salmonella* spp.[55,84,86,103,145]. Likewise, all the studies identified as relevant for the environment were focusing on reducing the presence of ARGs in manure[114,117-119], animal slurry[105], or animal wastewater[113]. The two studies[92,103] identified as One Health[99,110] were studies that included the measurement of outcomes in every component of the triad.

Intervention impact

The impact of included studies was categorised as either positive, negative, no effect, or mixed on the basis of their reported results. Studies that reported both positive and negative impacts on different outcomes were categorised as mixed. The majority of studies 60.6% (63/104) reported a positive impact of the interventions on reducing the burden of infections, compared with only 9.6% (10/104) of the studies that reported a negative impact. A summary of the reported impacts per every WASH/biosecurity component can be seen in Table 3.

Overall, without considering the risk of biased results, the bio-management and bio-containment measures appeared to have positive effects most often. These measures attended to creating and maintaining a conducive environment for animal raising in terms of physical Infrastructure and protocols. The few studies reporting sanitation measures - which were similar in aims, scope, and outcomes to bio-containment Interventions - all reported positive effects. By contrast, efforts to impact water quantity, water quality and hygiene had more mixed effects. Bio-exclusion interventions included had mostly negative effects (figure 13). Interestingly, most bio-exclusion interventions relied on people "doing" or "changing" something in the animal's environment such as building confinement facilities for animals, or farmworkers and household members learning about how to better raise chickens in their backyards.

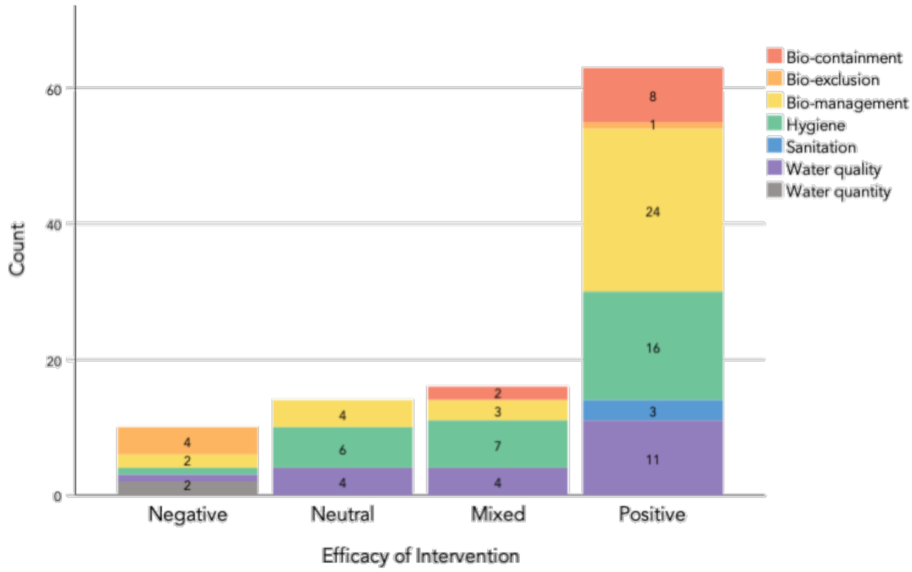

Figure 13: Efficacy of interventions by type of WASH/Biosecurity intervention.

When analysing interventions classified by their target and comparing those results with the reported effect we found that the distribution of effects was fairly even between the different types (figure 14). The only structural intervention found had a positive effect and multifaceted interventions (mixed) also had promising results. Just 2 biological/chemical interventions reported negative effects.

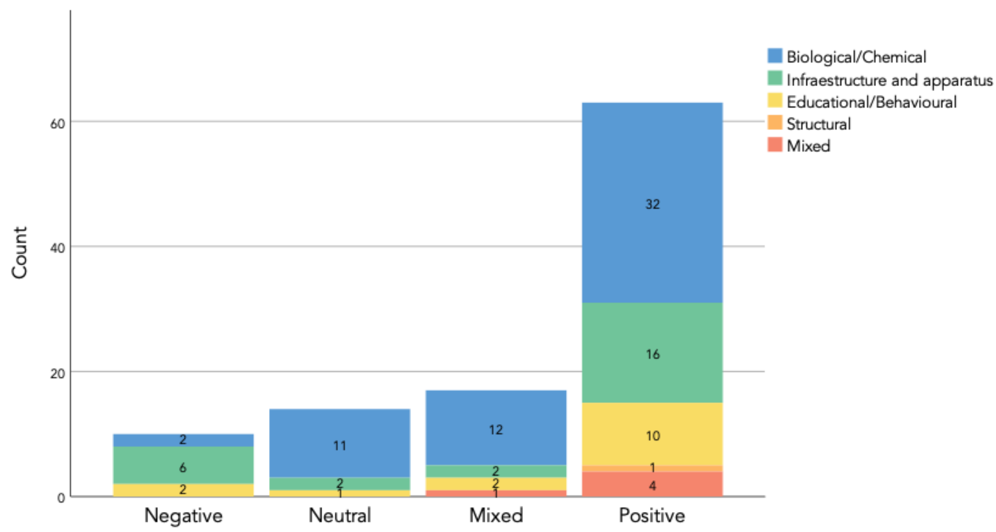

Figure 14: Reported efficacy of interventions by type of intervention target.

Table 3:: Summary of intervention impact as reported by study authors

Type of intervention	Reported impact				Total (n=104)
	Positive 60.6% (63/104)	Mixed 16.3% (17/104)	Negative 9.6% (10/104)	Neutral 13.5% (14/104)	
Water quantity (n=2)			**2**		
Adjusting the quantity of water			2		2
Water quality (n=21)	**12**	**4**	**1**	**4**	
Acidification of drinking water	3	2		2	7
Cleaner drinking water systems on farms	1	1	1	2	5
Improving microbial water quality in aquaculture	8	1			9
Hygiene (n=29)	**15**	**7**	**1**	**6**	
Cleaning and disinfection (C&D) protocols and products	8	5		4	17
Combined interventions in changing staff practices as well as cleaning and disinfection protocols.	2	1		1	4
Comprehensive measures to improve farm environment hygiene	2	1			3
Farmworkers changing hygiene practices	1			1	2
Implementation code of practices	1				1
Use of footbaths	1		1		2
Sanitation (n=3)	**3**				
Policies to control livestock manure	1				1
Manure composting methods	2				2
Bio-exclusion (n=5)	**1**		**4**		
Preventing the introduction of pathogens at farms entrance			1		1
Competitive exclusion			1		1
Educational interventions for backyard poultry			1		1
Confinement of free-ranging animals	1		1		2
Bio-containment (n=10)	**8**	**2**			
Controlling the contamination of water with ARGs	2	1			3
Mitigating the risk of ARGs from animal manure	5	1			6
Safe composting of fallen stock	1				1
Bio-management (n=34)	**24**	**4**	**2**	**4**	
Adjust flooring	4		1	2	7
Air quality	3				3
Educational interventions on mastitis prevention and control	3	1			4
Herd-specific intervention strategies	3				3
Implementation of strategies to prevent and control mastitis	3	1			4
Lighting on the farm		1			1
Litter treatment methods	2			2	4
Animal movement strategies	2		1		3
Organic farming practices	1				1
Prevent transmission through farm workers	1	1			2
Vacancy period	2				2

When comparing the reported effects of the intervention, the overall risk of bias and the interventions classified by what they were impacting (figure 15), we found that the interventions oriented to reduce antimicrobial use that reported a positive effect (n=3) had either moderate or high risk of bias. Conversely, there were various infection prevention and control interventions aiming to reduce burden of infections that reported a positive effect and were assessed as low risk of bias (n=18). Interventions implemented to decrease and control the dissemination of ARGs directly impacting AMR had mostly positive results.

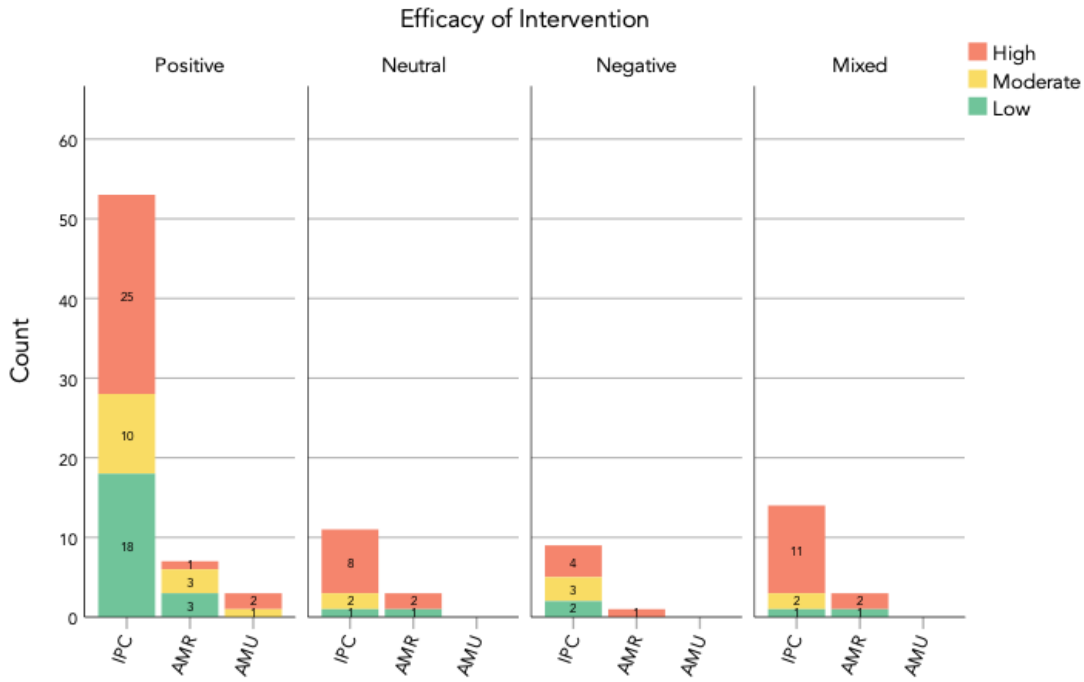

Figure 15: Number of interventions classified by what they are impacting (infection prevention and control, antimicrobial use or antimicrobial resistance) vs. the reported efficacy and the overall risk of bias

Risk of bias assessment

The Risk Of Bias In Non-randomised Studies of Interventions (ROBINS-I) tool was used to assess non-randomized trials (NRTs, including interrupted time series analyses and before and after studies)[41]. The Systematic Review Centre for Laboratory Animal Experimentation (SYRCLE) Risk of Bias tool was used to assess randomized control trials (RCTs)[40]. The Appraisal tool for Cross-Sectional Studies (AXIS) was used to assess cross-sectional and ecological studies[42]. Lastly, the Mixed Methods Appraisal Tool was used to assess case-control and cohort studies[43]. A summary of the tools used can be seen in appendix VIII.

Domains within each bias tool were judged as high, low, or unclear risk of bias. We presented an overall risk of bias score for each study. While bias tools did not explicitly state to indicate overall risk of bias, our aim was to broadly gauge the strength of the evidence base, and therefore developed criteria to guide overall judgement and decision-making. The criteria we used to assess bias for the 104 interventions included in this review places special emphasis on bias due to experimental design (including confounding and randomization), bias due to collection of results, and bias due to reporting of data. Refer to appendix VIII for more details on how domains were scored.

Just over half of the studies were assessed to be at a "high" risk of bias 53.8% (56/104) while fewer were assessed to be at low 25.9% (27/104) and moderate 20.1% (21/104) risk of bias (Figure 14). Surprisingly, 22/26 (85%) of included RCTs presented a high risk of bias. The likely cause for this was that although study authors reported a randomized component within their study design, most authors did not include details about methods of randomization (e.g. random sequence generation). Therefore, the selection bias domain was often judged to be at high or unclear risk of bias for RCTs. Concealment of allocation of study subjects to treatment arms was rarely discussed in the context of these studies either, raising more questions about the quality of the selection process and randomness of allocation to treatment arms in studies. Notably, 25/26 (96%) of included RCTs made conclusions justified by the presented data and were therefore assessed to be at low risk for reporting bias (figure 16).

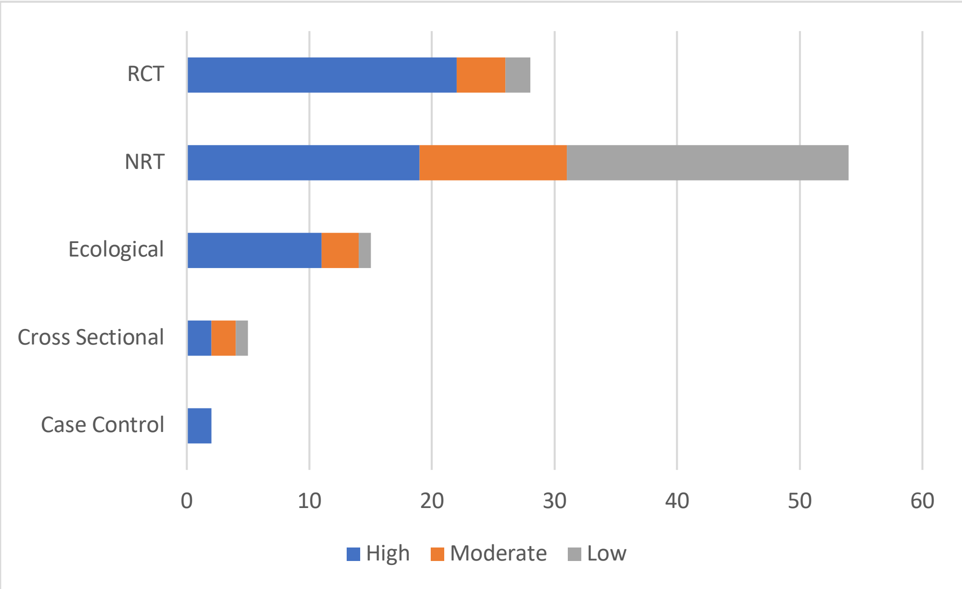

Figure 16: Risk of bias overall scores of selected interventions

NRTs were most commonly found as a study design used within the included studies (54/104). Of these NRTs, 23/54 (43%) were evaluated to be at low risk of bias. Of those assessed to be at moderate or high risk of bias, frequently seen issues were related to bias due to confounding, for example in place and time, and bias due to selective reporting of study results. As these studies by their nature were non-randomized, often assessed without control groups or only compared to baseline results, there was sometimes a high risk for confounding of variables without sufficient adjustment for such biases by study authors. Additionally, in some studies, we noticed that authors sometimes drew conclusions selectively on the basis of (sometimes insignificant) results from multiple outcome measurements within the same outcome domain, multiple analyses of the intervention-outcome relationship, or from different subgroups. For instance, in an intervention aimed to evaluate the occurrence of *Campylobacter* spp. on chicken farms after implementation of hygiene practices, van de Giessen et al. (1998)[92] conclude that "the application of hygiene measures significantly reduced [*Campylobacter*] infections of broiler flocks on both farms”. However, the sample size of data used to justify this claim is limited, the intervention is implemented on the two farms included in the study at different times within the broiler production cycle, and data are missing for one production cycle from one of the two farms. Studies that we similarly assessed to be reporting unjustified conclusions were judged being at high risk for reporting bias.

Most ecological, cross sectional, and case control studies were assessed to have a high risk of bias, as was expected for observational studies. Ecological and cross-sectional studies most often failed to justify the sample sizes included within studies, resulting in the study conceptualization domain judged to be at a high risk of bias. With observational studies, it was often unclear whether the chosen sample would be representative of the target population (due to experimental set-up, convenience sampling, etc.).

We recognize that the risk of bias reported for each study included within the review is based upon the authors’ judgements and independent review. We do not aim to make conclusions about the merit of studies, but rather to understand the possible strength of evidence they suggest to complement our analysis of where gaps in evidence remain. The risk of bias assessment of all included interventions can be seen in appendixes IV- VII.

DISCUSSION

Our systematic review assessed WASH and biosecurity interventions with the potential to reduce burden of infections, AMU and AMR in animal agricultural settings.

Summary of findings

This review found 104 intervention studies from around the world that intended to impact infections, antibiotic use or antibiotic resistance through implementing WASH or biosecurity measures at farms or in agricultural communities. Of these, only one was considered a structural intervention; 15 were educational or behavioural; 26 Involved modification of infrastructure or apparatus, and 57 focused on the application of biological or chemical products to eliminate pathogens. The majority of studies were from high income country settings in Europe and Asia, most were conducted in poultry and pigs, and assessed impacts on multiple bacteria. Eighty-seven (87) studies assessed impact on infection levels, 3 on antibiotic use, and 14 on antibiotic resistance. The majority were non-randomised studies, although a quarter were randomised controlled trials. A total of 27 studies were classified as having a low, 21 moderate and 56 high risk of bias.

Of the 104 studies included, 64 showed positive impacts - on infection burden (n=54), antibiotic use (n=3) or AMR (n=7). Without considering the risk of bias results, the bio-management and bio-containment measures appeared to have positive effects most often. These measures attempted to create and maintain a conducive environment for animal raising in terms of physical infrastructure and protocols. The few studies reporting sanitation measures - which were similar to bio-containment interventions - all reported positive effects. By contrast, efforts to impact water quantity, water quality, and hygiene had more mixed effects on the outcomes assessed. When considered in terms of intervention target, 33 interventions classified as biological/chemical showed positive impacts. Likewise, 10 educational/behavioural interventions, 16 infrastructure and apparatus, 1 structural and 4 using mixed interventions (biological chemical and infrastructure and apparatus) also had positive impacts.

It is important to consider though that the fact that many interventions reported a positive effect may be an indication of publication bias[173]. This is a type of bias that is common in academia where authors have historically had greater incentives to publish research that has produced positive effects, including that journals may be more likely to publish these types of studies.

Which Interventions work?

Although the risk of bias was high/moderate, all three studies that evaluated interventions to address AMU showed positive effects. Most directly linked with AMR, the primary outcome of interest for the interventions evaluated by Collineau et al. (2017)[135], Postma et al. (2017)[136], and Speksnidjer et al. (2017)[137] was reduction of antibiotic usage on farms. In all three studies, a herd-specific approach was used, and an organized planning-based approach was taken in consultation with farmers. In all three studies, antibiotic use was reduced and animal production parameters were improved, suggesting a possible advantage in using context-specific and collaborative approaches which include input from farmers and veterinarians, as well as facilitators with knowledge of promoting prudent usage of antibiotics. In contrast, Dorado Garcia et al. (2015)[82] reported an intervention wherein farms were assigned to pre-determined protocols for reducing ABU with or without concurrent changes in cleaning and disinfection. While the experimental arm that reduced ABU was successful in reducing prevalence of methicillin-resistant Staphylococcus aureus (MRSA) at the completion of the intervention compared to control farms, farms included in the experimental arm that also altered cleaning and disinfection practices had similar MRSA levels as control, resulting in an overall mixed efficacy for the study. Of note, however, was the finding that ABU was positively associated with MRSA across study arms for both humans and animals, suggesting that reducing ABU may be a promising strategy in curbing spread of resistant bacteria, but may only be fully effective in a context-specific tailored approach. It should be noted that this intervention, unlike those reported by Collineau et al. (2017), Postma et al. (2017), and Speksnidjer et al. (2017), did not include a facilitated approach to reducing antibiotic usage on farms, but rather assigned farms to a pre-determined protocol. This lends further support to the idea that a facilitated approach to reducing antibiotic use on farms - one that includes sufficient consultation with farmers (including discussion of their goals) and with animal health experts - may be critical in ensuring success of AMU reduction programs.

Studies reporting interventions that sought to reduce the presence of antibiotic resistance genes in the environment were often successful in achieving their intended aims. Most studies mitigated the presence of ARGs by using animal manure composting protocols at different temperatures. Of the 8 studies that included reduction of antibiotic resistance genes, 7 reported positive results, while 1 reported mixed results. Collectively, these studies investigated a broad range of common antibiotic resistance genes, including tetracycline (*tet*), sulfonamide (*sul*), macrolide (*erm*), vancomycin (*van*) integrase (*int*) genes as well as mobile genetic elements (MGEs). This is a particularly promising finding as these genes are the most frequently detected ARGs in livestock waste[169]. Discharge of ARGs in livestock waste represents a significant challenge of clinical importance not only for humans, but also for animals increasing the risk of hampering animal health and productivity[170,171]. Of these studies, 4 were assessed to be at a low risk of bias, while 3 were at a moderate risk of bias, and 1 was at high risk of bias. Collectively taken, these findings suggest that these interventions are effective, trialled within relatively robustly-designed studies, and target clinically relevant ARGs for humans and livestock alike.

Another subset of interventions that were generally met with success included studies that sought to improve water quality in aquaculture. These studies sought to reduce bacterial contamination of water using biological/chemical/physical interventions (including polyculture with other species, feeding fish ponds with raw or fermented manure, shifting water temperatures, and use of UV light and oxidative processes). Most interventions reported a reduction in the number of bacteria isolated from water samples and/or in mortality of fish, with the majority (8/9) reporting a positive effect and one reporting a mixed effect. Within these studies, there was a considerable degree of variability in risk of bias as 4/9 studies were at low risk of bias, 1 at moderate risk, and 4 at high risk. Nonetheless, the efficacy of these interventions against a broad range of clinically relevant bacteria (*E. coli*, *Salmonella spp.*, *Vibrio spp.*, *Streptococcus spp.*, *Staphylococcus spp.*, and other coliforms) and viral haemorrhagic septicaemia virus indicates that these approaches may be broadly effective and should be further explored.

When evaluated with the definition posited by Blankenship et al. (2000)[172] (i.e. interventions directed "to change the social context where health is produced or reproduced."), only one of 104 studies included within this systematic review was considered to be "structural" in nature[51]. In their study, Ryu et al. (2017) analysed the effect of the 2007 Korean Livestock Manure Control Act, which made it compulsory for livestock farmers to be equipped with appropriate sludge process facilities on their farms. Although the study had its limitations (i.e. retrospective interrupted time series analysis based on data from national disease databases, and potential confounding due to altered exposure to rodents), the authors appropriately reported such barriers and accounted for them in their analyses and interpretation of data. Ultimately, there was "a significant association between the enforcement of the Livestock Manure Control Policy with a 33% (95% CI: 13–53, p<0.01) decrease in leptospirosis incidence [in humans] during the post-enforcement period"[51]. This study was assessed to be at a low risk of bias. While this was the only intervention implemented at a "structural" level, and assessed outcomes only in humans who work with livestock without assessment of livestock health, it offers encouraging results that are supported with evidence. We recommend that additional structural interventions are piloted in the future with assessment of both human and animal health outcomes.

While here we have outlined patterns, further analyses of the included studies should be conducted to assess the size of impacts on different AMR-specific and AMR-sensitive outcomes. Another critical level of analysis would seek to determine whether effects reported within these studies reporting positive effects were short term or sustained.

Which Interventions do not work?

The heterogeneity of the interventions reviewed and potential publication bias indicated that most categories and subsets showed positive or mixed effects. Only bio-exclusion interventions showed to be generally ineffective (4/5). Careful review of results and evidence presented in the included studies indicated that several interventions (9/17) led to mixed (i.e. a positive change in one outcome with a negative change in another) or neutral (no net change) outcomes. For instance, many interventions that applied cleaning and disinfection (C&D) regimes reported mixed or neutral (no effect) results. A very wide range of cleaning and disinfection products were used across the included studies, directed towards the disinfection of an array of pathogens (including both bacterial and viral). While some C&D interventions significantly reduced bacterial contamination of environmental surfaces, many of these studies reported these changes to be transient. Overall, a recurring conclusion presented by authors was that success of C&D regimens was highly variable and dependent on a broad range of factors, including: biochemical profiles of disinfectants, specific characteristics of the pathogen(s), frequency of disinfection regimen, method of disinfectant administration, surfaces present in the environment, and adherence to the C&D protocols[83,85,87,90].

Given the variability in the efficacy of C&D measures, authors recommend using C&D protocols to supplement good hygienic biosecurity practices, rather than to replace them[78,87,90]. Some examples of promising biosecurity practices that authors suggest are strategic movement of animals and rodent control, as well as hygienic measures by farm personnel such as the use of personal protective equipment (PPE) or the changing of boots. Of the 104 included studies, 4 interventions use this combined approach to reducing prevalence of pathogens/infection[92-95]. Of these, two studies report a positive outcome, 1 reports a neutral outcome, and the last reports a mixed outcome. Given the numerous recommendations by authors to attempt similar integrated approaches and the limited evidence base on interventions that supplement C&D regimens with hygiene protocols, we recommend that further research be done in this area.

There is also a concern that many studies did not report a method with which to measure adherence to introduced biosecurity practices and changes in behaviours. Indeed, Postma et al. (2016) noted that simplicity and feasibility of management and biosecurity interventions determined adherence by farmers[136]. Specifically, more easily adopted measures were those that changed the working habits and routines of the farmer, for instance by improving hand and personal hygiene, changing needles, and regular analysis of water quality. However, the authors noted that interventions incurring high costs and/or more pronounced changes, such as introducing a new hygiene lock where clothes and boot need to be changed were implemented less frequently. Similarly, Velasquez and colleagues (2018) did not find that the introduced biosecurity measures recommended in the intervention were effective, but suggested that increased cost for implementing biosecurity and a (perceived) lack of reward for doing so might underlie lack of implementation[106]. This echoes previous findings by Laanen et al. (2014) who suggested that insufficient information on the cost and revenues gained by implementing biosecurity practices may hinder the adoption of more stringent preventive measures on farms[174]. The need for evidence on economic benefit may also limit the adoption of biosecurity measures in other production systems, such as smallholders and backyard poultry holders[108].

Which Interventions need still to be piloted?

When the range of interventions included in this evidence base is compared with the risk factors identified by our search strategy, there is overlap in some areas, in particular, regarding interventions focussing on hygiene and bio-management. Interventions in these areas included improving dairy farmers' knowledge and practices regarding hygienic milking and udder health, such as not using the same towel for all animals or complying with a strict milking order that minimizes infection of healthy cows. Such interventions had the potential to significantly reduce incidence of mastitis and were found to be effective[139,175,176]. Additionally, addressing the importance of bedding and floor type[165] as a risk factor for cattle health and keeping bedding dry was found to be a determining factor as part of a comprehensive biosecurity package on cattle farms in an intervention study[96]. Cleaning and the use of disinfectants had previously been identified as reducing risk of infection with particular zoonoses such as *C. burnetii*[162] or *Salmonella spp.*[177], and accordingly several included studies looked at optimising cleaning and disinfection protocols for animal facilities[75]. Other interventions addressing previously identified risk factors were often in the area of bio-management, for example by introducing a longer vacancy or clean out period before restocking of the animal facilities[178], as well as introducing the strategic movement of animals[148,179] during the production cycle. However, in others, there was limited evidence that interventions addressed known risk factors. For example, risk factor data suggest that the number of staff on the farm and frequency of visits by technical personnel or animal health workers may increase risk of infection[179,180]. Despite this, we found a dearth of interventions minimising the number of part-time staff on the farm and number of times personnel entered animal facilities. Other risk factors that remained unaddressed were the density of animals in the animal facilities and number of houses on the farm[181,182].

There was a dearth of interventions being trialled in the context of subsistence farming and household animal ownership, as well as pastoralism or mixed forms of production systems at a small-scale level. Particularly within LMIC contexts, only a limited set of interventions focussing on aspects such as the confinement of animals or educational interventions on biosecurity have been trialled[108-110]. These have been met with mixed effects. Examining risk factor studies from these contexts suggests that certain points of interventions, such as the sharing of water resources between humans and animals - which may increase risk of AMR-carriage and diarrhoeal disease in humans - have yet to be addressed[168,183]. Furthermore, only one study by Oberhelman et al (REF) attempted to intervene at the household level to reduce diarrhoea incidence in children, although several studies show that exposure to larger numbers of poultry in the household may carry significant implications for disease incidence in children[184]. One study by Bikes Destaw et al (2017)[183] set in a nomadic community in Ethiopia found that drinking from water sources not protected from animal contact significantly increased the risk of childhood diarrhoeal disease. Interventions in the area of WASH in human health, especially aimed at decreasing incidence of diarrhoea in children, could therefore benefit from taking a One Health approach in their design, acknowledging the animal component when designing WASH interventions in farming, agricultural or pastoral communities in LMIC contexts in particular.

Finally, social research has drawn attention to the structural factors that may reduce infection incidence and shape reliance on antibiotics. However, as mentioned previously, we found only one study that assessed a structural intervention attempting to change practices at a systems level by introducing a law that makes changing practices and farm infrastructure mandatory at a nation-wide level[51]. One opportunity to pilot intervention at a structural level may be the planning of farm location, density, and size. For livestock[185], poultry farms[158,186], and aquaculture[187], farm density in the region, location of the farm, and proximity to nearby farms may affect risk of infection and/or antimicrobial use. Furthermore, size of the farm is another important risk factor where no interventions have been piloted[163,188]. As no interventions in the evidence-based planning of farms at a policy level has been trialled before, this is a particular area where further empirical research is needed.

What needs to be considered for future evaluation studies?

The evidence base for which interventions are successful or not was weak in places with poor quality of study designs, and lack of contextual information that could have enabled the assessment of the intervention in a wider setting. The lacking description of the settings, interdisciplinary collaborations, living conditions of human and animal communities, specific agroecological situation, and the type of climates where the studies were implemented make it difficult to build an evidence-base on how and why certain interventions work. Additionally, this hinders the creation of an understanding of which enabling or limiting conditions allow interventions to make a positive impact on the burden of infections, AMU and AMR.

The majority of the included studies were performed in intensive farming settings which is in line with the fact that most studies were done in HIC contexts. Limited information about small-holders and subsistence farmers explain why just a few studies were performed in LMICs settings, as in these settings, the majority of farmers in rural areas are either small-holders or subsistence producers and do not engage in large scale intensive farming practices. Therefore, most interventions may be of limited relevance to these particular localities. However, in some LMICs, there are currently substantive efforts to encourage people to shift their small production systems towards more industrialised animal production systems. Moreover, just one study[135] included an economic analysis of the intervention they were proposing, limiting the possibilities of widely recommending the intervention, as money is frequently scarce and the main constraint in LMICs settings. This is particularly relevant, given the possibility that lack of improvements of biosecurity practices may partially be economically motivated[108,136].

Overall, future studies should consider the documentation and assessment of how contextual factors influence the success or failure of the intervention. As well as specific data about the economic conditions of the population involved, the social context where study participants live, the political environment where the intervention is being implemented, and cultural practices and beliefs that may hinder or promote the adoption of intervention measures should be considered. This would enable the assessment of how and why interventions work. Additionally, more research in LMICs -specifically in small-holders and subsistence farmers -is needed to complement the gaps in information in this area, and to reduce the enormous effect that AMR has on small-producers and their family members.

Human health researchers must consider that most microorganisms important for animals are also important for human health as they can be a source of ARGs for human pathogens when humans and animals live in close proximity or interact frequently. Likewise, the assessment of the impact of IPC interventions into AMR is critical to find alternatives that could help to reduce the AMR problem.

The future of WASH and biosecurity interventions in the AMR agenda

The provision of adequate water, sanitation, and hygiene (WASH) services is essential to providing the basic conditions that can enable people to live their lives in good health. WASH interventions reduce risk of water-borne infections occurring and may stem their spread in face of an infectious disease outbreak[189]. Starting with the famous intervention by John Snow, who removed the Broad Street pump handle In 1854 to confirm his suspicions of the water-borne nature of an ongoing Cholera outbreak[190], water, sanitation and hygiene interventions have been vital for enhancing human health and wellbeing.

To date, WASH interventions have been circumscribed to the provision of facilities and services to human populations commonly living in remote or rural areas, working in agriculture and in closely contact with animals. However, most WASH interventions have failed to address the risk imposed by the closeness of people living/working with animals. Some authors have already highlighted the importance of putting the 'A' (from animals) into WASH[191] (Water, Animals, Sanitation and Hygiene), calling for the reduction of the exposure to animals and their faeces. This is especially important for those whose main source of livelihood is working with livestock, poultry or fish. In challenging settings, the lack of access to services and medicines leave people vulnerable to infections, not just physically but economically, as they are frequently unable to afford medical treatment. As such, farmers whose livelihoods depend on their animals are placed in precarious situations when they bear the economic losses associated with infected animals (i.e. cost of treatment, loss of productivity, etc.) Moreover, some authors have also recognised the potential of WASH to contribute to the AMR agenda[192,193]. This is especially due to the importance of water sources as a vehicle of animal and human waste, providing the necessary opportunities for the dissemination and emergence of AMR-bacteria to the environment and back to people and animals.

The concept of biosecurity is well disseminated amongst veterinarians and animal production systems. Both biosecurity as well as WASH itself aim to prevent and control infections. However, unlike WASH, the management of the air quality as a biosecurity measure is crucial to reduce the exposure of animals to pathogens. Many biosecurity interventions focus on improving the quality of the air in farms and animal facilities by applying various technologies that purify the air, thereby reducing the likelihood of the transmission of pathogens through aerosols. In this sense, the One Health aspects of this review prompt us to call for the integration of the "air component" into WASH. We propose that the 'A' in WASH stands for both 'Animals' and 'Air'.

This is especially important in the current context where the emergence of SARS-Cov-2 (potentially from contact animals) has also exposed the human vulnerability to airborne pathogens and the importance for ensuring air quality for human health as well.

Limitations

Interventions of potential importance had to be excluded because they did not report one of the outcomes of interest, or omitted the animal component in WASH interventions aimed at farming or agricultural communities. Therefore, an understanding of how those interventions could have been applied to the topic of our research was not possible. WASH interventions performed in agricultural communities did not evaluate any outcome related to the presence of animals in the communities or the occupational risk that household members or farmworkers are exposed when working in close contact with animals. This created a bias of the types of studies selected towards studies focusing more on animals without any outcome of interest being measured in humans.

We did not perform a meta-analysis due to the breadth of the research topic, the heterogeneity of the elements involved, and the diversity of the outcomes analysed. Moreover, due to the complexity of the interventions analysed, different combinations of causal conditions could have produced the same outcome, making data drawn from these studies unsuitable for meta-regression analysis.

Although we covered a wide range of study types and designs, we recognised that it was challenging to identify the study design as many authors did not state the design they were using, nor did they provide sufficient information about the methodology applied. This could have affected the conclusions drawn based on the tools that were used to assess the risk of bias and, consequently, the interpretation of the value of the intervention to reduce burden of infections.

We acknowledge that analysing these types of interventions can be challenging as in some cases descriptions of all the actors involved are insufficient, contextual factors are not always documented, and authors fail to provide useful information when writing their manuscripts impacting the results of our review. All these factors could have hindered a deeper understanding of the interventions analysed.

We also recognise that measuring the benefits and unintended consequences of these interventions and their relevance to the AMR agenda might be limited as even when it is logical to suggest that reducing burden of infections might reduce the need for antibiotic treatment, measuring the direct effect of these types of interventions in empirically reducing ABU is challenging in different animal production contexts

CONCLUSIONS

This review identifies a number of effective interventions to reduce the burden of infections, antimicrobial resistance and antibiotic use in animal agricultural settings. However, the number of studies in each subcategory of WASH and biosecurity components was small and given the heterogeneous nature of studies included, the reported effects were dispersed. This makes it challenging to draw conclusions from the evidence found, yet we were able to identify several areas in which further interventions in WASH and biosecurity could be trialled. WASH and biosecurity strategies are essential to the prevention and control of infections and therefore, to reducing AMU and incidence, spread, and burden of AMR. Overall, there was a paucity of studies promoting structural changes (SI) to the way that agricultural communities and farmworkers interact with animals, and the AMR-related risks to which they are exposed, which indicates an important gap to be filled by future research. There remains potential for further learning and integration between the WASH field and biosecurity approaches in agriculture. This review represents a starting point for such integration and experimentation.

CONTRIBUTORS

CC and CP conceived the review and wrote the protocol. CP developed the search strategies and executed the searches. SK and PT conducted the screening of titles, abstracts, and full-text of English articles. CP conducted the screening of titles, abstracts and full-text of articles written in Spanish, Portuguese, and French. SK, PT, and MPJ conducted the data extraction and bias assessment of English articles. SK extracted data and conducted the bias assessment from articles in German, and CP in Spanish, Portuguese, and French. CP, SK, PT synthetized the results. CP prepared the original draft of the manuscript. CC reviewed and edited the manuscript. All authors contributed to the latest version of this report.

DECLARATION OF INTERESTS

The authors declared no conflicts of interest.

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APPENDIXES

Appendix I: Search terms and results of database search in Web of Science

Web of Science - Search configuration

Date: 05/05/2020 – Web of Science/All databases 1900-2019: Web of Science Core Collection, BIOSIS Citation Index, Data Citation Index, Derwent Innovations Index, KCI-Korean Journal Database, MEDLINE®, Russian Science Citation Index, SciELO Citation Index, Zoological Recor. Auto-suggest publication names: OFF. No language restriction

Set	Results	Save History / Create Alert Open Saved History
# 8	8,035	#7 AND #6 AND #5 AND #4 AND #3 *Databases= WOS, BCI, DRCI, DIIDW, KJD, MEDLINE, RSCI, SCIELO, ZOOREC Timespan=1900-2019* *Search language=Auto*
# 7	8,963,921	#2 OR #1 *Databases= WOS, BCI, DRCI, DIIDW, KJD, MEDLINE, RSCI, SCIELO, ZOOREC Timespan=1900-2019* *Search language=Auto*
# 6	6,115,540	TS=(LMICs OR LMIC OR "low-and-middle income countries" OR "developing countries" OR "Latin America" OR Africa OR Asia OR "South America" OR "Central America" OR Afghanistan OR Albania OR Algeria OR Angola OR "Antigua and Barbuda" OR Argentina OR Armenia OR Azerbaijan OR Bangladesh OR Belarus OR Belize OR Benin OR Bhutan OR Bolivia OR "Bosnia and Herzegovina" OR Botswana OR Brazil OR "Burkina Faso" OR Burundi OR "Cabo Verde" OR Cambodia OR Cameroon OR "Central African Republic" OR Chad OR China OR Colombia OR Comoros OR "Democratic Republic of Congo" OR Congo OR "Cook Islands" OR "Costa Rica" OR "Côte d'Ivoire" OR Cuba OR Djibouti OR Dominica OR "Dominican Republic" OR Ecuador OR Egypt OR "El Salvador" OR "Equatorial Guinea" OR Eritrea OR Ethiopia OR Fiji OR Gabon OR Gambia OR Georgia OR Ghana OR Gre nada OR Guatemala OR Guinea OR "Guinea-Bissau" OR Guyana OR Haiti OR Honduras OR India OR Indonesia OR Iran OR Iraq OR Jamaica OR Jordan OR Kazakhstan OR Kenya OR Kiribati OR "Democratic People's Republic of Korea" OR Kosovo OR Kyrgyzstan OR Lao People's Democratic Republic OR Lebanon OR Lesotho OR Liberia OR Libya OR "Former Yugoslav Republic of Macedonia" OR Madagascar OR Malawi OR Malaysia OR Maldives OR Mali OR "Marshall Islands" OR Mauritania OR Mauritius OR Mexico OR Micronesia OR Moldova OR Mongolia OR Montenegro OR Montserrat OR Morocco OR Mozambique OR Myanmar OR Namibia OR Nauru OR Nepal OR Nicaragua OR Niger OR Nigeria OR Niue OR Pakistan OR Palau OR Panama O R "Papua New Guinea" OR Paraguay OR Peru OR Philippines OR Rwanda OR "Saint Helena" OR Samoa OR "São Tomé and Príncipe" OR Senegal OR Serbia OR Sierra Leone OR "Solomon Islands" OR Somalia OR "South Africa" OR "South Sudan" OR "Sri Lanka" OR "Saint Lucia" OR "Saint Vincent and the Grenadines" OR Sudan OR Suriname OR Swaziland OR "Syrian Arab Republic" OR Tajikistan OR Tanzania OR Thailand OR "Timor-Leste" OR Togo OR Tokelau OR Tonga OR Tunisia OR Turkey OR Turkmenistan OR Tuvalu OR Uganda OR Ukraine OR Uzbekistan OR Vanuatu OR Venezuela OR Vietnam OR "Wallis and Futuna" OR "West Bank and Gaza Strip" OR Yemen OR Zambia OR Zimbabwe) *Databases= WOS, BCI, DRCI, DIIDW, KJD, MEDLINE, RSCI, SCIELO, ZOOREC Timespan=1900-2019* *Search language=Auto*

# 5	61,032,731	TS=(intervention$ OR implementation OR experiment OR monitor* OR program* OR pilot$ OR initiative$ OR strateg* OR polic* OR method$ OR measures OR technique OR legislation$ OR r egulation$ OR effectiveness OR "cost-effectiveness" OR "cost-benefit" OR "cost-analysis" OR "cost-utility" OR "cost effectiveness" OR "cost benefit" OR "cost analysis" OR "cost utility" OR "economic evaluation" OR impact) *Databases= WOS, BCI, DRCI, DIIDW, KJD, MEDLINE, RSCI, SCIELO, ZOOREC Timespan=1900-2019* *Search language=Auto*
# 4	507,922	TS=((reduc* NEAR/5 "burden of infections") OR (decrease* NEAR/5 "burden of infections") OR "low* burden of infections" OR (reduc* NEAR/5 "burden of disease$") OR (decrease* NEAR/5 "burden of disease$") OR "low* burden of disease$" OR (reduc* NEAR/5 "disease$ burden") OR (decrease* NEAR/5 "disease$ burden") OR "low* disease$ burden" OR (reduc* NEAR/5 "infection$ burden") OR (decrease* NEAR/5 "infection$ burden") OR "low* infection$ burden" OR (reduc* NEAR/5 "resistant bacteri*") OR (decrease* NEAR/5 "resistant bacteri*") OR "low* resistant bacteri*" OR (reduc* NEAR/5 "microb* infection") OR (decrease* NEAR/5 "microb* infection") OR "low* microb* infection$" OR (reduc* NEAR/5 "microb* coloni?ation") OR (decrease* NEAR/5 "microb* coloni?ation") OR "low* microb* coloni?ation" OR (reduc* NEAR/5 "bacteri* coloni?ation") OR (decrease* NEAR/5 "bacteri* coloni?ation") OR "low* bacteri* coloni?ation" OR (reduc* NEAR/5 "bacteri* contamination") OR (decrease* NEAR/5 "bacteri* contamination") OR "low* bacteri* contamination" OR (reduc* NEAR/5 "bacteri* concentration") OR (decrease* NEAR/5 "bacteri* concentration") OR "low* bacteri* concentration" OR (reduc* NEAR/5 "bacteri* count$") OR (decrease* NEAR/5 "bacteri* count$") OR "low* bacteri* count$" OR (reduc* NEAR/5 "bacteri* load$") OR (decrease* NEAR/5 "bacteri* load$") OR "low* bacteri* load$" OR (reduc* NEAR/5 infection$) OR (decrease* NEAR/5 infection$) OR "low* NEAR/5 infection$" OR (reduc* NEAR/5 incidence) OR (decrease* NEAR/5 incidence) OR "incidence reduction" OR "low* incidence" OR (reduc* NEAR/5 prevalence) OR (decrease* NEAR/5 prevalence) OR "low* prevalence" OR "prevalence reduction" OR (reduc* NEAR/5 seroprevalence) OR (decrease* NEAR/5 seroprevalence) OR "low* seroprevalence" OR "seroprevalence reduction" OR (decrease* NEAR/5 mortality) OR (reduc* NEAR/5 mortality) OR "low* mortality" OR "mortality reduction" OR (reduc* NEAR/5 morbidity) OR (decrease* NEAR/5 morbidity) OR "morbidity reduction" OR "low* morbidity" OR (reduc* NEAR/3 "antibiotic prescription$") OR (decrease* NEAR/3 "antibiotic prescription$") OR (low* NEAR/3 "antibiotic prescription$") OR (reduc* NEAR/3 "antimicrobial prescription$") OR (decrease* NEAR/3 "antimicrobial prescription$") OR (low* NEAR/3 "antimicrobial prescription$") OR (reduc* NEAR/3 "antimicrobial prescribing") OR (decrease* NEAR/3 "antimicrobial prescribing") OR (low* NEAR/3 "antimicrobial prescribing") OR (reduc* NEAR/3 "antibiotic prescribing") OR (decrease* NEAR/3 "antibiotic prescribing") OR (low* NEAR/3 "antibiotic prescribing") OR (reduc* NEAR/5 "veterinary visit$") OR (decrease* NEAR/5 "veterinary visit$") OR (low* NEAR/3 "veterinary visit$") OR (reduc* NEAR/5 "veterinary service$") OR (decrease* NEAR/5 "veterinary service$") OR (low* NEAR/3 "veterinary service$") OR (reduc* NEAR/5 "antibiotic residues") OR (decrease* NEAR/5 "antibiotic residues") OR (low* NEAR/3 "antibiotic residues") OR (reduc* NEAR/5 "antimicrobial residues") OR (decrease* NEAR/5 "antimicrobial residues") OR (low* NEAR/3 "antimicrobial residues") OR (reduc* NEAR/5 "antimicrobial resistan*") OR (decrease* NEAR/5 "antimicrobial resistan*") OR (low* NEAR/3 "antimicrobial resistan*") OR (reduc* NEAR/5 "antibiotic resistan*") OR (decrease* NEAR/5 "antibiotic resistan*") OR (low* NEAR/3 "antibiotic resistan*") OR (reduc* NEAR/5 AMR) OR (decrease* NEAR/5 AMR) OR (low* NEAR/3 AMR) OR (reduc* NEAR/5 ABR) OR (decrease* NEAR/5 ABR) OR (low* NEAR/3 ABR) OR (reduc* NEAR/5 "drug-resistan*") OR (decrease* NEAR/5 "drug-resistan*") OR (low* NEAR/3 "drug-resistan*") OR (reduc* NEAR/5 "drug$ resistan*") OR (decrease* NEAR/5 "drug$ resistan*") OR (low* NEAR/3 "drug$ resistan*") OR (reduc* NEAR/5 "multidrug-resistan*") OR (decrease* NEAR/5 "multidrug-resistan*") OR (low* NEAR/3 "multidrug-resistan*") OR (reduc* NEAR/5 "multiple-drug resistan*") OR (decrease* NEAR/5 "multiple-drug-

		resistan*") OR (low* NEAR/3 "multiple-drug-resistan*") OR (reduc* NEAR/5 "multiple drug resistan*") OR (decrease* NEAR/5 "multiple drug resistan*") OR (low* NEAR/3 "multiple drug resistan*") OR (reduc* NEAR/5 "antimicrobial use") OR (decrease* NEAR/5 "antimicrobial use") OR (low* NEAR/3 "antimicrobial use") OR (reduc* NEAR/5 AMU) OR (decrease* NEAR/5 AMU) OR (low* NEAR/3 AMU) OR (reduc* NEAR/5 "antibiotic use") OR (decrease* NEAR/5 "antibiotic use") OR (low* NEAR/3 "antibiotic use") OR (reduc* NEAR/5 "antibiotic usage") OR (decrease* NEAR/5 "antibiotic usage") OR (low* NEAR/3 "antibiotic usage") OR (reduc* NEAR/5 "antimicrobial usage") OR (decrease* NEAR/5 "antimicrobial usage") OR (low* NEAR/3 "antimicrobial usage") OR (reduc* NEAR/5 ABU) OR (decrease* NEAR/5 ABU) OR (low* NEAR/3 ABU) OR (reduc* NEAR/3 "use of antimicrobials") OR (decrease* NEAR/3 "use of antimicrobials") OR (low* NEAR/3 "use of antimicrobials") OR (reduc* NEAR/3 "use of antibiotics") OR (decrease* NEAR/3 "use of antibiotics") OR (low* NEAR/3 "use of antibiotics") OR (reduc* NEAR/3 "antimicrobial drug$") OR (decrease* NEAR/3 "antimicrobial drug$") OR (low* NEAR/3 "antimicrobial drug$") OR (reduc* NEAR/3 "veterinary drug$") OR (decrease* NEAR/3 "veterinary drug$") OR (low* NEAR/3 "veterinary drug$")) *Databases= WOS, BCI, DRCI, DIIDW, KJD, MEDLINE, RSCI, SCIELO, ZOOREC Timespan=1900-2019* *Search language=Auto*
# 3	27,098,735	TS=(WASH OR WATSAN OR "water access" OR "water quality" OR "clean water" OR "water-sharing" OR watering OR freshwater OR groundwater OR "ground water" OR borehole OR "water conditioner" OR rainwater OR "pipe water" OR pipewater OR "water source" OR "household well$" OR "water treatment" OR "water bodies" OR "waterways" OR "tubewell$" OR "water supply" OR "water safety" OR filtration OR "safe water" OR pond$ OR "river$ diversion" OR "hydraulic structure$" OR chlorination OR "irrigation channels" OR "irrigation canals" OR "canal water" OR "water mills" OR pump OR (water NEAR/5 dam$) OR (water NEAR/3 pit) OR "irrigation system$" OR swamp$ OR "flood recession" OR "water harvest*" OR "water system$" OR "water storage" OR "potable water" OR "electroly?ed water" OR "catch* rainwater" OR "harvest* rainwater" OR "water salinity" OR "drinking water" OR "wastewater management" OR sanitation OR slurry OR "dirty water" OR manure OR "waste management" OR "waste disposal" OR compost* OR "septic tank" OR excre* OR faeces OR feces OR fecal OR def$ecation OR sewage OR sewerage OR "litter treatment$" OR (litter NEAR/2 treatment) OR sanitizer$ OR hygiene OR cleaning OR washing OR disinfect* OR "hygienic measures" OR antibiocides OR chemicals OR "water rinse" OR autoclaving OR sterilisation OR decontaminat* OR "boot? scrubbing" OR biosecurity OR biosafety OR "bio-exclusion" OR "bio-containment" OR "bio-management" OR bioexclusion OR biocontainment OR biomanagement OR "protective barriers" OR "protective equipment" OR "protective clothing" OR "McREBEL protocol$" OR crossfostering OR "safe handling" OR "ASEAN GAHP" OR "good animal husbandry practices" OR isolation OR fencing OR corralling OR "building fence$" OR thinning OR bedding OR depopulation OR "empty days" OR "all-in-all-out production" OR "stocking density" OR ventilation OR "dry bedding" OR "feed storage" OR "tank cleaning" OR fumigation OR "pest control" OR "fly screen$" OR cull* OR "litter* system" OR (housing NEAR/5 animal$) OR corral* OR cage$ OR "on-farm carnivorous pets" OR "second-hand equipment" OR foothbath OR footwear OR quarantine OR "animal movement" OR "pasteuri?ation") *Databases= WOS, BCI, DRCI, DIIDW, KJD, MEDLINE, RSCI, SCIELO, ZOOREC Timespan=1900-2019* *Search language=Auto*
# 2	6,741,829	TS=(farm$ OR farming OR livestock OR "animal agriculture" OR "food animal$" OR "farm animal$" OR "animal production" OR "food production" OR "food producing animal$" OR "food-producing animal$" OR "farm-level" OR "backyard

		poultry" OR flock$ OR herd OR "market animal$" OR "dairy farming" OR ruminant$ OR bovine OR cow$ OR cattle OR calf OR calves OR heifer OR "beef animal$" OR beef OR "dairy animal$" OR goat$ OR caprine OR porcine OR pig$ OR swine OR pork OR sow$ OR piglet$ OR ovine OR sheep OR ewe OR mutton OR lamb$ OR camelids OR alpaca OR cria OR llama OR tui OR rabbit OR aquaculture OR aquafarming OR pisciculture OR fish OR seafood OR crustacean$ OR mollusc$ OR shellfish OR "fish farm*" OR "fish hatcher*" OR fisheries OR polyculture OR "marine aquaculture" OR "freshwater aquaculture" OR mariculture OR finfish OR "aquatic organism$" OR "aquatic animal$" OR pond$ OR chicken$ OR chick$ OR poultry OR aviculture OR broiler$ OR chook OR hen$ OR "laying hen$" OR "egg laying" OR cock OR pullet$ OR rooster OR roaster$ OR duck$ OR duckling$ OR turkey OR geese OR equine OR horse$) *Databases= WOS, BCI, DRCI, DIIDW, KJD, MEDLINE, RSCI, SCIELO, ZOOREC Timespan=1900-2019* *Search language=Auto*
# 1	3,837,388	TS=(farmer$ OR pastoralist$ OR "small-hold*" OR smallhold* OR "small hold*" OR "small-scale" OR "small scale" OR "small farmer" OR "small grower" OR "rural farm$" OR "indigenous farmer$" OR agribusiness OR agricultur* OR aquacultur* OR "agricultural worker$" OR "animal breeder$" OR producer OR villager$ OR "farm worker$" OR farmworker$ OR farmhand OR "household farm*" OR "farming household$" OR "household farm$" OR (animal$ NEAR/3 household?) OR "family farming" OR "family-based farm?" OR farming OR shepherd$ OR farmland OR cooperative OR (animal$ NEAR/3 backyard) OR "free-range" OR "organic farming" OR "sub-sistence farm*" OR "subsistence farm*" OR "family farm*" OR "artisanal farm*" OR (artisanal NEAR/5 farm*) OR fisherman$ OR fisher$ OR "fishing communit*" OR "artisanal fisherman" OR "artisanal fish*" OR "artisanal aquaculture" OR (nomad$ NEAR/3 production system) OR "semicommercial farm$" OR "semi-commercial farm$" OR "noncommercial farm$" OR "non-commercial farm$" OR "animal owner" OR "animal health worker$" OR "commercial farm$" OR "intensive farming" OR "intensive system" OR "commercial-scale production") *Databases= WOS, BCI, DRCI, DIIDW, KJD, MEDLINE, RSCI, SCIELO, ZOOREC Timespan=1900-2019* *Search language=Auto*

Appendix II: List of excluded studies and reasons for exclusion

Article name	Authors	Type of publication	Language	Country	Region	Reason exclusion
Hygienic evaluation of working conditions and indicators of health status of workers in poultry farms	Ladnova, 1994	Journal article	Russian	Russia	Asia	Full text not available in English/Spanish/Portuguese/German/French/Dutch
Evaluation of effectiveness of comprehensive control for diarrhoea diseases in rural areas of east Fujian and analysis of its cost-benefit	Xiao, 1997	Journal article	Chinese	China	Asia	Full text not available in English/Spanish/Portuguese/German/French/Dutch
Studies on prevention measure of white spot disease of kuruma shrimp Marsupenaeus japonicus	Satoh, 2012	Journal article	Japanese	Japan	Asia	Full text not available in English/Spanish/Portuguese/German/French/Dutch
The partial replacement of antibiotics with biologically active substances at treatment of cows' mastitis	Aziamov, 2018	Journal article	Russian	Russia	Asia	Full text not available in English/Spanish/Portuguese/German/French/Dutch
The efficacy of cleaning and disinfection on pig farms	Mannion, 2005	Conference abstract	English	Ireland	Europe	Full-text not available
Participation of feed industry personnel and pig farms to reduce risk of disease spread between farms	Bottoms, 2014	Conference abstract	English	NA	NA	Full-text not available
Interventions for prevention and control of anthrax according to the one health approach in South Omo zone, Ethiopia	Braus, 2019	Conference abstract	English	Ethiopia	East Africa	Full-text not available
A Randomised Controlled Trial to Reduce Salmonella Infection in Finisher Pigs	Cook, 2003	Conference abstract	English	NA	NA	Full-text not available
Antimicrobial resistance against critical antibiotics in the environment of intensive and organic Turkey farms	Di Martino, 2019	Conference abstract	English	NA	NA	Full-text not available
Effects of broiler stocking density and poultry litter reuse in broiler performance and poultry litter production	dos Santos, 2005	Journal article	Portuguese	NA	NA	Full-text not available
Effect of chlorination, antibiotics and UV radiation on Vibrio population in the hatchery system of Macrobrachium rosenbergii (DEMAN)	Krishnika, 2013	Journal article	English	NA	NA	Full-text not available
Causes of mortality in calves and methods of making the calves more resistant	Naziha, 1987	Report	English	NA	NA	Full-text not available

Appendix II: List of excluded studies and reasons for exclusion (continued)

Article name	Authors	Type of publication	Language	Country	Region	Reason exclusion
An observation on the influence of management environment on the infection rates of bovine cutaneous *Streptothricosis kirchi*	Ologun, 1987	Journal article	English	NA	NA	Full-text not available
Application of bacterial product for zero-liquid-discharge pig waste management under tropical condition	Ong, 1993	Journal article	English	NA	NA	Full-text not available
Seroprevalence of toxoplasmosis in pastoral goat herds and attendants in a neglected tropical region of Pakistan	Ahmad, 2014	Conference abstract	English	Pakistan	Asia	Full-text not available
Experiment on bactericidal efficacy in drinking system using slightly acidic electrolyzed water in large-scale poultry houses	Wang, 2017	Journal article	English	NA	NA	Full-text not available
Shrimp (Pacific White Shrimp) Farm Biosecurity: Practical Methods To Prevent Virus Entering Farm And Quarantine If Infected To Prevent From Spreading	Taw, 2010	PowerPoint presentation	English	NA	NA	Full-text not available
Effect of chlorination of drinking-water on water quality and childhood diarrhoea in a village in Pakistan	Jensen, 2003	Journal article	English	Pakistan	Asia	Intervention applied in human communities/settings with no attention to animal populations.
Learning from the Nation-Wide Rural Sanitation Capacity Building Initiatives in Indonesia for a Robust ASEAN Community	Kasri, 2015	Conference paper	English	Indonesia	Asia	Intervention applied in human communities/settings with no attention to animal populations.
Interim evaluation of a large-scale sanitation, hygiene and water improvement programme on childhood diarrhoea and respiratory disease in rural Bangladesh	Huda, 2012	Journal article	English	Bangladesh	Asia	Intervention applied in human communities/settings with no attention to animal populations.
Effectiveness of Large-Scale Water and Sanitation Interventions: The One Million Initiative in Mozambique	Elbers, 2012	Journal article	English	Mozambique	East Africa	Intervention applied in human communities/settings with no attention to animal populations.
Impact Evaluation of a Large-Scale Rural Sanitation Project in Indonesia The World Bank Sustainable Development Network Water and Sanitation Program Impact Evaluation Series No. 83	Cameron, 2013	Policy paper	English	Indonesia	Asia	Intervention applied in human communities/settings with no attention to animal populations.
Small-Scale Sustainable Water Project Decreases Infections, Complements Short-Term Medical Missions	Johanson, 2018	Journal article	English	Guatemala	Central America	Intervention applied in human communities/settings with no attention to animal populations.

Appendix II: List of excluded studies and reasons for exclusion (continued)

Article name	Authors	Type of publication	Language	Country	Region	Reason exclusion
Water Access, Sanitation, and Hygiene Conditions and Health Outcomes among Two Settlement Types in Rural Far North Cameroon	Gorham, 2017	Journal article	English	Cameroon	West Africa	Intervention applied in human communities/settings with no attention to animal populations.
Hand washing with soap and WASH educational intervention reduces under-five childhood diarrhoea incidence in Jigjiga District, Eastern Ethiopia: A community-based cluster randomized controlled trial	Hashi, 2017	Journal article	English	Ethiopia	East Africa	Intervention applied in human communities/settings with no attention to animal populations.
Acceptance and Impact of Point-of-Use Water Filtration Systems in Rural Guatemala	Larson, 2017	Journal article	English	Guatemala	South America	Intervention applied in human communities/settings with no attention to animal populations.
Reduction of enteric infectious disease in rural China by providing deep-well tap water	Wang, 1989	Journal article	English	China	Asia	Intervention applied in human communities/settings with no attention to animal populations.
Probabilistic quantitative microbial risk assessment model of farmer exposure to Cryptosporidium spp. in irrigation water within Kumasi Metropolis-Ghana	Sampson, 2017	Journal article	English	Ghana	West Africa	Intervention applied in human communities/settings with no attention to animal populations.
Diarrhoeal diseases among adult population in an agricultural community Hanam province, Vietnam, with high wastewater and excreta re-use	Phuc, 2014	Journal article	English	Vietnam	Asia	Intervention applied in human communities/settings with no attention to animal populations.
Faecal contamination of water and fingertip-rinses as a method for evaluating the effect of low-cost water supply and sanitation activities on faeco-oral disease transmission. II. A hygiene intervention study in rural north-east Thailand	Pinfold, 1990	Journal article	English	Thailand	Asia	Intervention applied in human communities/settings with no attention to animal populations.
The Relationship Between Acute Malnutrition, Hygiene Practices, Water and Livestock, and Their Program Implications in Eastern Chad	Marshak, 2017	Journal article	English	Chad	Central Africa	Intervention applied in human communities/settings with no attention to animal populations.
The epidemiology of hepatitis E virus and the relationship between infection in pigs and humans in a community of agricultural-food system in Nan Province, Thailand	Hinjoy 2012	Journal article	English	Thailand	Asia	Intervention applied in human communities/settings with no attention to animal populations.
Using health education intervention to improve knowledge and practice of prevention of avian influenza among bird handlers in Sokoto, Nigeria	Oche, 2013	Journal article	English	Nigeria	West Africa	Intervention applied outside production systems (such as disinfection of animal transport vehicles or carcass disinfection).

Appendix II: List of excluded studies and reasons for exclusion (continued)

Article name	Authors	Type of publication	Language	Country	Region	Reason exclusion
The synergistic effects of slightly acidic electrolyzed water and UV-C light on the inactivation of Salmonella enteritidis on contaminated eggshells	Bing, 2019	Journal article	English	China	Asia	Intervention applied outside production systems (such as disinfection of animal transport vehicles or carcass disinfection).
Efficacy of electrolyzed oxidizing water and lactic acid on the reduction of Campylobacter on naturally contaminated broiler carcasses during processing	Rasschaert, 2013	Journal article	English	Belgium	Europe	Intervention applied outside production systems (such as disinfection of animal transport vehicles or carcass disinfection).
Effect of lactic acid administration in the drinking water during preslaughter feed withdrawal on Salmonella and Campylobacter contamination of broilers	Byrd, 2001	Journal Article	English	United States	North America	Intervention applied outside production systems (such as disinfection of animal transport vehicles or carcass disinfection).
Biocide and antibiotic susceptibility of Salmonella isolates obtained before and after cleaning at six Danish pig slaughterhouses	Gantzhorn, 2014	Journal article	English	Denmark	Europe	Intervention applied outside production systems (such as disinfection of animal transport vehicles or carcass disinfection).
Impact of cleaning and disinfection procedures on microbial ecology and Salmonella antimicrobial resistance in a pig slaughterhouse	Bridier, 2019	Journal article	English	France	Europe	Intervention applied outside production systems (such as disinfection of animal transport vehicles or carcass disinfection).
Intervention strategies to improve the safety of pork	McMullen, 2000	Journal article	English	NA	NA	Intervention for pathogens not included in this systematic review
Evaluation of comprehensive measures of schistosomiasis control in Caidian District, Wuhan City	Xu, 2014	Journal article	Chinese	China	Asia	Intervention for pathogens not included in this systematic review
Research and demonstration of comprehensive measures of schistosomiasis prevention and control technology. II. Longitudinal evaluation on control effect	Wu, 2011	Journal article	Chinese	China	Asia	Intervention for pathogens not included in this systematic review
Integrated control trial of schistosomiasis at Nakiwogo fishing village near Entebbe, Uganda	Odongo-Aginya, 1996	Journal article	English	Uganda	East Africa	Intervention for pathogens not included in this systematic review
Sustainable floor type for managing turkey production in a hot climate	Farghly, 2018	Journal article	English	Egypt	North Africa	Intervention for pathogens not included in this systematic review

Appendix II: List of excluded studies and reasons for exclusion (continued)

Article name	Authors	Type of publication	Language	Country	Region	Reason exclusion
The effect of cellulose added to straw bedding on turkeys' welfare and production results	Slobodzian-Ksenicz, 2010	Journal article	English	Poland	Europe	Intervention for pathogens not included in this systematic review
Biological aerobic treatment of pig slurry in France: Nutrients removal efficiency and separation performances	Béline, 2004	Journal article	English	France	Europe	Intervention for pathogens not included in this systematic review
Preliminary evaluation of Community-Led Total Sanitation for the control of Taenia solium cysticercosis in Katete District of Zambia	Bulaya, 2015	Journal article	English	Zambia	South Africa	Intervention for pathogens not included in this systematic review
Assessing the Effect of an Integrated Control Strategy for Schistosomiasis Japonica Emphasizing Bovines in a Marshland Area of Hubei Province, China: A Cluster Randomized Trial	Hong, 2013	Journal article	English	China	Asia	Intervention for pathogens not included in this systematic review
Effects of Environmental Factors on Death Rate of Pigs in South Korea	Lee, 2012	Journal article	English	South Korea	Asia	Intervention for pathogens not included in this systematic review
A health-education intervention trial to reduce porcine cysticercosis in Mbulu District, Tanzania	Ngowi, 2008	Journal article	English	Tanzania	East Africa	Intervention for pathogens not included in this systematic review
Evaluation of the effects of footwear hygiene protocols on nonspecific bacterial contamination of floor surfaces in an equine hospital	Stockton, 2006	Journal article	English	United States	North America	Intervention limited to health facilities.
Poultry rearing on perforated plastic floors and the effect on air quality, growth performance, and carcass injuries—Experiment 1: Thermal Comfort	Almeida; 2017	Journal article	English	Brazil	South America	Intervention such as vaccination and improving animal husbandry measures not associated with biosecurity.
Influence of a turkey stable with a veranda on performance, behaviour and health of tom turkeys	Berk, 2006	Journal article	German	Germany	Europe	Intervention such as vaccination and improving animal husbandry measures not associated with biosecurity.
A study of the effects of the use of a veranda for the housing of turkeys on animal health, performance and carcass quality	Berk, 2007	Journal article	German	Germany	Europe	Intervention such as vaccination and improving animal husbandry measures not associated with biosecurity.
The impact of best practice health and husbandry interventions on smallholder cattle productivity in southern Cambodia	Young, 2014	Journal article	English	Cambodia	Asia	Intervention such as vaccination and improving animal husbandry measures not associated with biosecurity.
A cluster-randomised controlled trial to compare the effectiveness of different knowledge-transfer interventions for rural working equid users in Ethiopia	Stringer, 2011	Journal article	English	Ethiopia	East Africa	Intervention such as vaccination and improving animal husbandry measures not associated with biosecurity.

Appendix II: List of excluded studies and reasons for exclusion (continued)

Article name	Authors	Type of publication	Language	Country	Region	Reason exclusion
Evaluation of strategies to improve village chicken production-controlled field trials to assess effects of Newcastle disease vaccination and altered chick rearing in Myanmar	Henning, 2009	Journal article	English	Myandmar	Asia	Intervention such as vaccination and improving animal husbandry measures not associated with biosecurity.
Validation of a traditional preparation against multi-drug resistant Salmonella Typhi and its protective efficacy in S. Typhimurium infected mice	Chattopadhyay, 2018	Journal article	English	India	Asia	Intervention such as vaccination and improving animal husbandry measures not associated with biosecurity.
Commercial field trial evaluation of mucosal starter culture to reduce Salmonella incidence in processed broiler carcasses	Bailey, 2000	Journal article	English	United States	North America	Intervention such as vaccination and improving animal husbandry measures not associated with biosecurity.
Anticoccidial effects of Morinda lucida acetone extracts on broiler chickens naturally infected with Eimeria species	Ola-Fadunsin, 2014	Journal article	English	Nigeria	West Africa	Intervention such as vaccination and improving animal husbandry measures not associated with biosecurity.
Have You Disinfected Your Boots? A Case Study of Food Safety and Biosecurity Practices of a Salmon Farm in Chile	Soon, 2015	Journal article	English	Chile	South America	Intervention such as vaccination and improving animal husbandry measures not associated with biosecurity.
A Biosecurity measures application with proper treatment to overcome the risk factors that limit effective control of subclinical mastitis in dairy buffalo farms-A field study	Shawky, 2013	Journal article	English	Egypt	North Africa	Intervention such as vaccination and improving animal husbandry measures not associated with biosecurity.
On-farm biosecurity as perceived by professionals visiting Swedish farms	Nöremark, 2014	Journal article	English	Sweden	Europe	Intervention such as vaccination and improving animal husbandry measures not associated with biosecurity.
Effect of wheat bran and wheat: barley ratio in pelleted feed on Salmonella prevalence and productivity of finishers	Jorgsen, 2001	Journal article	English	Denmark	Europe	Intervention such as vaccination and improving animal husbandry measures not associated with biosecurity.
Heat Destruction of Salmonella in Poultry Feed: Effect of Time , Temperature , and Moisture	Himathongkham, 2018	Journal article	English	United States of America	North America	Intervention such as vaccination and improving animal husbandry measures not associated with biosecurity.
Serological, clinical, and risk factors of the Newcastle disease on broilers flocks in Algeria	Messai, 2019	Journal article	English	Algeria	North Africa	Intervention such as vaccination and improving animal husbandry measures not associated with biosecurity.

Appendix II: List of excluded studies and reasons for exclusion (continued)

Article name	Authors	Type of publication	Language	Country	Region	Reason exclusion
Assessment of biosecurity and control measures to prevent incursion and to limit spread of emerging transboundary animal diseases in Europe: An expert survey	Léger, 2017	Journal article	English	Italy, UK, Switzerland	Europe	Observational/prevalence study only
Disease management and biosecurity measures of small-scale commercial poultry farms in and around Debre Markos, Amhara Region, Ethiopia	Melkamu, 2016	Journal article	English	Ethiopia	East Africa	Observational/prevalence study only
Investigation of the interaction between the fate of antibiotics in aquafarms and their level in the environment	Zhong, 2018	Journal article	English	China	Asia	Observational/prevalence study only
Salmonella Weltevreden in integrated and non-integrated tilapia aquaculture systems in Guangdong, China	Li, 2017	Journal article	English	China	Asia	Observational/prevalence study only
A longitudinal observational study of Salmonella shedding patterns by commercial turkeys during rearing and fattening, showing limitations of some control measures	Morris, 2015	Journal article	English	United Kingdom	Europe	Observational/prevalence study only
Persistence of Mycobacterium avium subsp. paratuberculosis in soil, crops, and ensiled feed following manure spreading on infected dairy farms	Fecteau, 2013	Journal article	English	United States	North America	Observational/prevalence study only
Non-typhoidal Salmonella serovars in poultry farms in central Ethiopia: prevalence and antimicrobial resistance	Tadesse, 2018	Journal article	English	Ethiopia	East Africa	Observational/prevalence study only
Determination of the sources and antimicrobial resistance patterns of Salmonella isolated from the poultry industry in Southern Ethiopia	Abdi, 2017	Journal article	English	Ethiopia	East Africa	Observational/prevalence study only
Does physical state of farm housing and milking practices affect total bacteria and somatic cell count of cow milk?	Paraffin, 2018	Journal article	English	Zimbabwe	South Africa	Observational/prevalence study only
Trends and correlates of antimicrobial use in broiler and turkey farms: a poultry company registry-based study in Italy	Caucci, 2019	Journal article	English	Italy	Europe	Observational/prevalence study only
Association of smallholder dairy farmers management and milking practices with bacterial quality of milk in Mbeya, Tanzania	Massawe, 2018	Journal article	English	Tanzania	East Africa	Observational/prevalence study only
Prevalence of subclinical mastitis and associated risk factors in dairy farms in urban and peri-urban areas of Thika Sub County, Kenya	Mureithi, 2016	Journal article	English	Kenya	East Africa	Observational/prevalence study only

Appendix II: List of excluded studies and reasons for exclusion (continued)

Article name	Authors	Type of publication	Language	Country	Region	Reason exclusion
Ecology of Staphylococcus aureus and its antibiotic resistance genes in dairy farms: Contributing factors and public health implications	Elmonir, 2019	Journal article	English	Egypt	North Africa	Observational/prevalence study only
Poultry husbandry, water, sanitation, and hygiene practices, and child anthropometry in rural Burkina Faso	Gelli, 2019	Journal article	English	Burkina Faso	West Africa	Observational/prevalence study only
Prevalence and risk factors for multi-drug resistant Escherichia coli among poultry workers in the Federal Capital Territory, Abuja, Nigeria	Aworh 2019	Journal article	English	Nigeria	West Africa	Observational/prevalence study only
Effects of carbon source addition on microbial community and water quality in recirculating aquaculture systems for Litopenaeus vannamei	Chen, 2020	Journal article	English	China	Asia	Published outside of the timeframe of this systematic review
Effect of Sanitation Improvements on Pathogens and Microbial Source Tracking Markers in the Rural Bangladeshi Household Environment	Fuhrmeister, 2020	Journal article	English	Bangladesh	Asia	Published outside of the timeframe of this systematic review
Pilot study assessing the possible benefits of a higher level of implementation of biosecurity measures on farm productivity and health status in Belgian cattle farms	Renault, 2020	Journal article	English	Belgium	Europe	Published outside of the timeframe of this systematic review
The fate of antibiotic resistance genes during co-composting of swine manure with cauliflower and corn straw	Li, 2020	Journal article	English	China	Asia	Published outside of the timeframe of this systematic review
Impact of participatory training of smallholder pig farmers on knowledge, attitudes and practices regarding biosecurity for the control of African swine fever in Uganda	Dione, 2020	Journal article	English	Uganda	East Africa	Published outside of the timeframe of this systematic review
Evaluation of commonly-used farm disinfectants in wet and dry models of Salmonella farm contamination	McLaren, 2011	Journal article	English	United Kingdom	Europe	Tested disinfectants “in vitro” or at laboratories.
Efficacy of a novel foot pan in biosecurity protocols for control of salmonellae in poultry farms	Bashandy, 2017	Journal article	English	Egypt	North Africa	Tested disinfectants “in vitro” or at laboratories.
Efficacy of some Disinfectant compounds against porcine bacterial pathogens.	Thomson, 2007	Journal Article	English	United Kingdom	Europe	Tested disinfectants “in vitro” or at laboratories.
Inhibition of Escherichia coli in cultivated cattle manure	Weinberg, 2014	Journal article	English	Israel	Asia	Tested disinfectants “in vitro” or at laboratories.
Efficacy of disinfectants and detergents intended for a pig farm environment where Salmonella is present	Gosling, 2017	Journal article	English	United Kingdom	Europe	Tested disinfectants “in vitro” or at laboratories.

Appendix II: List of excluded studies and reasons for exclusion (continued)

Article name	Authors	Type of publication	Language	Country	Region	Reason exclusion
Risk factors associated with Salmonella prevalence on swine farms	Funk, 2004	Journal article	English	United States of America	North America	Type of publication, literature review
Economic Analysis of Johne's Disease Control Strategies in Dairy Herds	Cho, 2010	Poster	English	NA	NA	Type of publication, modelling study
The effect of alternative testing strategies and bio-exclusion practices on Johne's disease risk in test-negative herds	More, 2013	Journal article	English	NA	NA	Type of publication, modelling study
The Transmissibility of Highly Pathogenic Avian Influenza in Commercial Poultry in Industrialised Countries	Garske, 2007	Journal article	English	NA	NA	Type of publication, modelling study
Method for raising domestic fowls such as hen, turkey, duck, quail, pheasant, ostrich, goose and silky fowl and improving mortality rate and meat quality of domestic fowls, involves providing hot spring water	Kiyomoto, NA	Patent application	English	NA	NA	Type of publication, patent application
Livestock Waste Management in East Asia Project	Independent Evaluation Group, 2013	Report	English	East Asia (Thailand, China, Vietnam)	Asia	Type of publication, report without any primary data on an intervention
Training Communities in Livestock-Derived Food Safety and Hygiene in Chitwan District, Nepal	Jost, 2005	Final report	English	Nepal	Asia	Type of publication, report without any primary data on an intervention
Report of the water and sanitation intervention	International Centre for Diarrhoea Disease, Bangladesh, 1985	Report	English	Bangladesh	Asia	Type of publication, report without any primary data on an intervention
A final report on environmental management seminar	Campos, 2017	Report	English	NA	NA	Type of publication, report without any primary data on an intervention
Interventions for improving bio-security of small-scale poultry producers in Egypt	ECTAD/AGAP FAO, 2007	Report	English	Egypt	North Africa	Type of publication, report without any primary data on an intervention
Impact of participatory training on biosecurity protocols on the knowledge, attitudes and practices of smallholder pig farmers in Uganda	Dione; 2017	Research Brief	English	Uganda	East Africa	Type of publication, study protocol only

Appendix II: List of excluded studies and reasons for exclusion (continued)

Article name	Authors	Type of publication	Language	Country	Region	Reason exclusion
Integrated Interventions to Tackle Antimicrobial Usage in Animal Production Systems: The ViParc Project in Vietnam	Carrique-Mas, 2017	Journal article	English	Vietnam	Asia	Type of publication, study protocol only
Impact of improved duck rearing on sale and consumption of ducks in rural households of flood-prone areas of Bangladesh	NA, 2018	Trial registration	English	Bangladesh	Asia	Type of publication, study protocol only
One Health disease surveillance and community engagement in Sierra Leone	Maarten 2017	Trial registration	English	Sierra Leone	East Africa	Type of publication, study protocol only
Cluster-randomised controlled trials of individual and combined water, sanitation, hygiene and nutritional interventions in rural bangladesh and Kenya: The WASH benefits study design and rationale	Arnold, 2013	Journal article	English	Kenya	East Africa	Type of publication, study protocol only
Biosecurity-based interventions and strategies to reduce Campylobacter spp. on poultry farms	Newell, 2011	Journal article	English	USA	North America	Type of publication, systematic review
Evaluation of the relationship between the biosecurity status, production parameters, herd characteristics and antimicrobial usage in farrow-to-finish pig production in four EU countries	Postma, 2016	Journal article	English	4 countries in Europe	Europe	Observational/prevalence study only
A framework for targeting water, sanitation and hygiene interventions in pastoralist populations in the Afar region of Ethiopia	Whitley, 2019	Journal article	English	Ethiopia	East Africa	Observational/prevalence study only
Seroprevalence of leptospirosis in human groups at risk due to environmental, labor or social conditions.	Meny, 2019	Journal article	Spanish	Uruguay	South America	Observational/prevalence study only
Aprovechamiento de los ambientes reducidos para la producción de organismos acuáticos susceptibles a cultivo, para el consumo humano.	Sánchez 2009	Journal article	Spanish	Mexico	South America	Type of publication, report without any primary data on an intervention
Efecto de la densidad poblacional y temperatura en truchas arco iris (Oncorhynchus mykiss) inoculadas con Piscirickettsia salmonis	Larenas, 1997	Journal article	Spanish	Chile	South America	Type of publication, report without any primary data on an intervention
Estudio y caracterización de las prácticas de manejo sanitario y bioseguridad en granjas avícolas de pequeños y medianos productores de cuatro zonas de alta producción en el Ecuador.	Cevallos 2010	D.V.M Thesis	Spanish	Ecuador	South America	Type of publication, report without any primary data on an intervention
Mejora en la eficiencia del flujo en piscinas destinadas a la cría de peces mediante simulación numérica bidimensional.	Fraga, 2017	Abstract on Conference	Spanish	Spain	Europe	Full-text not available
Evaluación de la efectividad de la desinfección con formaldehido mediante tres métodos de control bacteriologicos.	Cepero 2007	Journal article	Spanish	Cuba	Central America	Type of publication, report without any primary data on an intervention

Appendix II: List of excluded studies and reasons for exclusion (continued)

Article name	Authors	Type of publication	Language	Country	Region	Reason exclusion
Efecto de dos Tipos de Material de Cama sobre la Carga Parasitaria de Cerdos en Crecimiento y Engorde Alojados en Cama Profunda.	Rondón 2014	Journal article	Spanish	Venezuela	South America	Intervention for pathogens not included in this systematic review
Caracterización de las medidas de bioseguridad de las granjas avícolas en la provincia de Coronel Portillo, Ucayali - Perú.	Germany 2019	Journal article	Spanish	Peru	South America	Type of publication, report without any primary data on an intervention
Gestion sanitaire des villages cambodgiens comme moyen de réduction de transmission de maladies infectueuses entre volailles et de la volaille à l'homme	Conan, 2013	PhD thesis	French	France	Europe	Full-text not available
A community-based education trial to improve backyard poultry biosecurity in rural Cambodia	Conan, 2013	Journal article	English	Cambodia	Asia	No outcome of interest included
Evaluation of strategies to enhance biosecurity compliance on poultry farms in Québec: Effect of audits and cameras	Racicot, 2012	Journal article	English	Canada	North America	No outcome of interest included
Understanding the failure of a behavior change intervention to reduce risk behaviors for avian influenza transmission among backyard poultry raisers in rural Bangladesh: a focused ethnography	Rimi, 2016	Journal article	English	Bangladesh	Asia	No outcome of interest included
The effects of different knowledge-dissemination interventions on the mastitis knowledge of Tanzanian smallholder dairy farmers	Bell, 2005	Journal article	English	Tanzania	Eastern Africa	No outcome of interest included
Uncovering outcomes and challenges of using an Ecohealth approach for better human and animal waste management in Hanam Province, Vietnam	Pham; 2016	Brief	English	Vietnam	Asia	No outcome of interest included
Evaluation of the effectiveness of formaldehyde disinfection by means of three methods of bacteriological control	Cepero 2007	Journal article	Spanish	Cuba	Centre America	No outcome of interest included
Prevalence and antimicrobial resistance of Campylobacter from antibiotic-free broilers during organic and conventional processing	Bailey, 2018	Journal article	English	USA	North America	Intervention applied outside production systems (such as disinfection of animal transport vehicles or carcass disinfection).
Antimicrobial-Resistant Campylobacter in Organically and Conventionally Raised Layer Chickens	Kassem, 2017	Journal article	English	USA	North America	observational/prevalence study only
A comparative study of production performance and animal health practices in organic and conventional dairy systems	Silva, 2014	Journal article	English	Brazil	South America	Intervention such as vaccination and improving animal husbandry measures not associated with biosecurity.

Appendix III: Summary of studies including WASH and biosecurity interventions

Study	Country	Study design	Type of production system	Setting	Animal of interest	Main objective	Outcome of interest	Intervention Content	Results	Overall Risk of Bias
Water quantity										
Beauvais *et al.* 2018[52]	United States of America	RCT	Intensive farming	Not specified	Cattle: Beef	To assess the effect of reducing the level of water in automatically refilling water troughs on faecal shedding of E. coli O157:H7 in feedlot cattle.	Faecal samples were collected both at baseline and three weeks after the intervention, and tested for the presence of E. coli O157:H7 using immunomagnetic bead separation and selective culture.	Intervention in which the level of water in the trough of feedlot cattle was adjusted to 50% of the baseline level (i.e. the level that was maintained by the feedlot before the trial began) for the duration of the trial. All troughs in the trial had automated filling, which ensured that the trough water level remained consistent throughout the trial. In control pens, no adjustments were made to the level of water in the trough at baseline.	A statistically significant association between reduced water levels in the trough and increased prevalence of E. coli O157:H7 in the faeces was observed (OR = 1.6; p = 0.02).	High
Schenk *et al.*, 2016[53]	United States of America	RCT	Intensive farming	Rural	Pekin Duck	To determine whether water troughs would show improved duck body conditions and environmental quality compared to pin-metered water lines.	Barn environment measures (temperature, humidity, ammonia, water use), duck biological measures (body weight, quality, plasma hormone, microbiome), and duck production (food intake, feed conversion ratio, mortality) were all assessed.	Intervention in which ducks were randomly housed in 2 barns: one using water lines and another using water troughs. The intervention was replicated using a crossover design and watering systems were switched into the opposite barns.	Water troughs showed higher bacterial growth ($p < 0.001$), including higher ($p < 0.001$) E. coli, coliforms, and Staphylococcus in the water troughs. Water lines typically showed no bacterial growth in culture-based assays. Ducks housed with water troughs used greater ($p = 0.001$) volumes of water compared to ducks housed with water lines. Ducks with water troughs also showed a greater percent ($p = 0.008$) mortality at all ages compared to ducks with water lines.	High

Appendix III: Summary of studies including WASH and biosecurity interventions (continued)

Study	Country	Study design	Type of production system	Setting	Animal of interest	Main objective	Outcome of interest	Intervention Content	Results	Overall Risk of Bias
Water quality										
Miejnková and Sovová, 2012[66]	Czech Republic	Ecological	Intensive farming	Peri-Rural	Fish	To evaluate the effect of applying semi-liquid pig manure to a fish pond on faecal pollution and related potential health risks.	Microbial water quality was determined based on assessment of indicator microorganisms, including faecal coliform bacteria, Escherichia coli, enterococci, and heterotrophic plate counts at 22°C.	Intervention looking at the effects annual manuring of ponds. For fertilizing, semiliquid pig manure was used. Manuring of the ponds was carried out once a year in spring with manure dose from 50 to 200 t dependent on the cubic content of pond and presence of other nutrient sources.	Microbial analyses of fish pond sediments revealed the presence of neither faecal bacteria nor pathogens (Salmonella, E. coli O157) in fish pond sediments before and after manuring. No statistics presented.	High
Jansen et al., 2014[54]	Germany	Non-randomised trial	Intensive farming	Rural	Poultry: Broilers	To evaluate the effect of a commercially available organic acid water additive in conventional broiler production on Campylobacter species.	Samples were taken from drinking water, feed, boot swabs, cloacal samples, and carcases. Campylobacter spp. were then enumerated.	Intervention in which treatment group flocks received acidified drinking water during the whole rearing cycle via a commercially available water additive based on short-chain organic acids and medium-chain fatty acids which is supposed to reduce the pH in the drinking water synergistically and consecutively in the stomach and gut of the animals. The control group flocks were reared without acidifying the drinking water.	A significant (p<0.05) difference was observed in only one of six independent analyses. The preventive application of organic acids under commercial field conditions did not have a significant effect on the qualitative colonization profile of a flock.	Moderate
Abraham, 2014[73]	India	Ecological	Intensive farming	Not specified	Fish, Shrimp	To study the distribution of marine luminous bacteria in shrimp culture systems of West Bengal and the potential of shrimp/fish polyculture to reduce luminous bacteria.	Luminous bacterial counts were enumerated by spread plating on seawater complex agar from shrimp grow-out pond water and pond sediment samples.	Intervention in which shrimp (P. monodon) were grown in ponds supplemented with chemical and biological products both with and without fish. After polyculture, pond water and sediment were sampled to determine luminous bacterial count.	Log luminous bacterial count in shrimp ponds with fish (pond water = 2.33/mL; pond sediment = 2.96/mL) and without fish (pond water = 3.01/mL; pond sediment = 3.45/mL)	High
Othman et al., 2015[74]	Malaysia	Non-randomised trial	Experimental set-up	Not specified	Fish: Nile tilapia	To assess the effect of filter-feeding bivalve mussel Pilsbryoconcha exilis in controlling streptococcal infection in Nile tilapia Oreochromis niloticus.	Nile tilapa were assessed for bacterial counts of S. agalactiae, mortality, and specific growth rate across the four treatment groups.	Intervention in which Nile tilapia, bivalve mussels, and bacteria were co-cultures in different combinations: treatment-1: mussel and bacteria but no fish, treatment-2: tilapia and mussel but no bacteria, treatment-3: tilapia and bacteria but no mussel, and treatment-4: tilapia, mussels, and bacteria.	Addition of mussel with tilapia and bacteria (T4) resulted in significantly ($p < 0.001$) lower mortality (43.3%) and higher specific growth (0.83) of tilapia compared to tilapia and bacteria without addition of mussel (T3).	Low

Appendix III: Summary of studies including WASH and biosecurity interventions (continued)

Study	Country	Study design	Type of production system	Setting	Animal of interest	Main objective	Outcome of interest	Intervention Content	Results	Overall Risk of Bias
Sano et al., 2009[70]	Japan	RCT	Experimental set-up	Not specified	Fish: Japanese flounder	To assess the effect of increasing the water-rearing temperature on the mortality of Japanese flounder *Paralichthys olivaceus* experimentally infected with viral hemorrhagic septicemia virus.	Viral titer of Japanese flounder viral hemorrhagic septicemia virus (VHSV) along with histopathological change and mortality rate of Japanese flounder infected with a VHSV isolate was measured.	Intervention in an experimental setup, in which Japanese flounder reared in aquariums were infected with viral hemorrhagic septicemia virus isolate JF00Ehi1 and the water temperature was subsequently shifted from 14 °C to 20 °C (Experiment I) and from 20 °C to 15 °C (Experiment II). In experiment III, fish inoculated with the virus were reared at 20 °C or at 25 °C (a viral non-permissive temperature).	In Experiment I, earlier shifting resulted in lower cumulative mortality rates (p <0.05). In Experiment II, the group of fish reared for a longer period at 20 °C showed lower cumulative mortalities (p<0.05). In experiment III, no mortality was observed in either group.	High
De Ridder et al., 2013[55]	Belgium	RCT	Intensive farming	Not specified	Pigs	To evaluate the effect of three intervention strategies on S. typhimurium transmission in pigs.	Post-mortem ileum, caecum, ileocaecal lymph nodes, and tonsils were sampled, along with ileal, caecal and rectal contents, and tested for the presence of Salmonella spp.	Intervention compared the effect of using 3 strategies (feed supplemented with coated calcium-butyrate (group A), oral vaccination with a double attenuated Salmonella typhymurium strain (group B), and water acidified with a mixture of organic acids (group C)) on the transmission of S. typhimurium among pigs.	The proportion of pigs that excreted fecal Salmonella was significantly higher in group C (58%, P < 0.0001) and the positive control group (41%, P = 0.03), compared to group B (15%), and the proportion in group C was also significantly higher than in group A (23%, P = 0.01). Group A had the lowest proportion of positive post-mortem samples (18%), followed by group B (31%), the positive control group (41%) and group C (64%) (P < 0.03). The highest transmission was seen in the positive control group and group C (R = +Inf with 95% CI [1.88; +Inf]), followed by group B (R = 2.61 [1.21; 9.45]) and A (R = 1.76 [1.02; 9.01]).	High
Elsaidy et al., 2015[67]	Egypt	Non-randomised trial	Experimental set-up	Not specified	Fish: Nile tilapia	To evaluate the impact of using raw or fermented manure as fish feed on microbial quality of water and fish.	Water quality and microbiological quality of fish samples assessed by measuring total bacterial counts, total coliform counts, and specific prevalence of E. coli O157:H7 and Salmonella.	Intervention in which Nile tilapia were fed different mixtures of chicken manure or fermented chicken manure with fish ration (FR) in the following ratios: 0:100, 25:75, 50:50 and 100:0 (%CM or FCM:% FR).	Total bacterial count (TBC) and total coliform count (TCC) were significantly higher (p < 0.05) in CM compared to both FCM and FR. E. coli and Salmonella were isolated from CM but not from FCM or FR. Additionally, TBC and TCC were significantly higher (p < 0.05) in water and fish samples from CM ponds, followed by FCM ponds, and FR-treated ponds.	Low

Appendix III: Summary of studies including WASH and biosecurity interventions (continued)

Study	Country	Study design	Type of production system	Setting	Animal of interest	Main objective	Outcome of interest	Intervention Content	Results	Overall Risk of Bias
El-Shafai et al., 2004[68]	Egypt	Non-randomised trial	Experimental set-up	Not specified	Fish: Nile tilapia	To evaluate and compare the microbial quality of tilapia stocked in different sewage contaminated ponds.	The fecal coliform count in fish organs was evaluated across treatment ponds in addition to water quality parameters in fish ponds.	Intervention investigating the microbial quality of tilapia reared in four different fecal-contaminated ponds. One of the fishponds received treated sewage and was fed fresh duckweed grown on treated sewage. The second fishpond received treated sewage and was fed wheat bran. The third fishpond received freshwater and was fed duckweed. The fourth pond received only settled sewage.	The average counts in the fishponds were 2.2 x 10e3, 1.7 x 10e3, 1.7 x 10e2, and 9.4 x 10e3 cfu/100 mL in TDP, TWP, FDP, and SSP, respectively. FDP had a significantly ($p < 0.05$) lower fecal coliform count than the treated sewage-fed ponds and SSP.	Low
Folorunso et al., 2013[63]	Nigeria	Ecological	Intensive farming	Rural	Poultry	To investigate the progressive occurrence and characteristics of bacteria in water troughs for caged- and deep litter- managed layer chickens.	Samples were taken from all water troughs using sterile swabs and bacteria were isolated. Bacteria were identified based on morphology, stain, and biochemical characteristics.	Intervention monitored the effect of not cleaning water troughs on 3-tier cages system or in deep litter design layer chicken farms on the microbial load and occurrence of different microbial species.	No differences between farms on day 1 and 3 but a significant difference on day 5 ($p < 0.01$) and on day 7 ($p < 0.05$), indicating poor hygienic status of Farm B and C.	High
Balasubramanian et al., 1992[69]	India	Cross-Sectional	Experimental set-up	Not specified	Fish	To find out the bacterial load in different fish species cultured in a sewage-fed pond.	Samples of skin, muscles, gills, and gut content were screened for the presence of total bacteria, coliform, salmonella and faecal streptococci. The microbiological status of the sewage from the culture pond was also studied. Fish were also cooked for 20minutes and samples were screened for pathogens.	Intervention looked at reducting microbial load in six different fish species raised in a sewage-fed ponds after a depuration period of 20 days in fresh water.	The bacterial load was higher in the gut contents than in skin, gills and muscle. Detritivorous fish species had a higher bacterial count than the filter feeders. The bacterial load was reduced during the depuration period (20 days in fresh water) of the fishes. The fish-sauce preparation examined revealed the complete elimination of microbes.	High
van Bunnik et al., 2012[56]	The Netherlands	Non-randomised trial	Intensive farming	Not specified	Poultry: Broilers	To investigate the effect of acidification of the drinking water on both the direct and indirect transmission of Campylobacter between broilers.	Cloacal swabs were taken on a daily basis, incubated, and examined for presence of Campylobacter.	Intervention consisted of a direct (4 groups of n=23 animals) and an indirect transmission experiment (4 groups of n=9 animals). Each trial was conducted in duplicate and consisted of one group receiving acidified drinking water (A commercially available acid (Forticoat®, Selko BV) was diluted until a final pH of 4), whereas control groups received tap water.	For the direct transmission group, no significant differences in the transmission parameter were found between control and treatment groups ($p = 0.9$). However, for the indirect transmission group, the difference between the control and treatment groups was significant ($p < 0.05$), indicating that acidification of the drinking water reduced the transmission parameter.	Moderate

Appendix III: Summary of studies including WASH and biosecurity interventions (continued)

Study	Country	Study design	Type of production system	Setting	Animal of interest	Main objective	Outcome of interest	Intervention Content	Results	Overall Risk of Bias
Chiriboga et al., 2016[57]	Ecuador	Non-randomised trial	Intensive farming	Not specified	Poultry: Broilers	To determine the efficacy of vinegar and infusion of oregánon Plectranthus amboinicus in acriollados bred broilers.	The outcomes of interest studied in this trial were bacterial count, bird weight, feed intake and feed conversion of the broilers.	Intervention in which 170 Broiler chickens were randomized to one of four water treatments (i.) vinegar, (ii.) vinegar + oregano infusion, (iii.) oregano infusion, (iv.) control.	The bacteriostatic efficiency of oreganon was higher than that of vinegar or a mixture of both (vinegar and oreganon). Oregano and vinegar infusion had a significant effect (p<0.05) associated with the reduction of bacterial count in chicken feces. Oregano has also previously been reported to control Eimerias (coccidia) infestation in birds.	High
Poblete-Chávez et al., 2016[71]	Chile	Non-randomised trial	Experimental set-up	Not specified	Fish: Rotifers	To evaluate the prospect of using different advanced oxidation processes to inactivate Vibrio species and total bacteria cultures present in seawater.	Samples were withdrawn each 20 min to measure bacterial concentrations, pH, ozone, bromine, H2O2, dissolved oxygen percentage saturation (DO%), oxide reduction potential (ORP), temperature and conductivity.	Intervention looking at the application of different advanced oxidation processes (UVc/O3/H2O2, UVc/H2O2 and O3/H2O2) were applied to inactivate Vibrio spp. and total bacteria present in seawater sourced from a fish farming tank.	It was possible to inactivate 100% of bacteria present in the seawater when UVc/ozone/hydrogen peroxide and UVc/hydrogen peroxide were applied, needing around 20 and 80 min for Vibrio spp. and total culturable bacteria inactivation, respectively.	Low
Amaral et al, 2001[64]	Brazil	Ecological	Small-holders	Not specified	Poultry: Laying hens	To evaluate the influence of two kinds of water troughs nipple and cup, used for laying hen water intake and the increasing demand of chlorine from the water offered by both kinds of water troughs.	The outcome of interest was a reduction of bacterial counts in the drinking fountains.	Intervention on one farm two compare water troughs nipple and cups in layer hens. Sampling of 20 samples from each reservoir in periods of rain and drought and, fortnightly during the same periods, samples were taken from three drinking fountains of each type, at different points of the line (beginning, middle and end), making a total of 30 samples for each type of drinking fountain studied during the rainy season, and 30 samples in the dry season.	Results showed that both, water trough and tank water, were microbiologically contaminated before passing through the water trough. The contamination of faecal bacteria was greater in the cup water trough samples and they were the responsible for a greater organic material accumulation. This caused an elevated chlorine demand and greater depreciation in the water quality offered to the laying hens. From both water trough studied, nipple showed to be less harmful concerning water quality	High
Alcántara et al., 2008[62]	Mexico	Non-randomised trial	Small-holders	Rural	Pigs	To determine the effect of separation/ sedimentation/filtration, on the prevalence of Enterobacteraeceae and Salmonella spp and the health of nursery pigs.	Outcomes of interest were daily feed intake, total feed intake, daily water intake, total water intake, feed conversion rate, body temperature, body weight gain, and clinical conditions of the pig. Additionally, swabs were taken during necropsies.	Intervention in which chlorinated and or filtered water was compared to normal potable water. In Stage 1, a total of 40 samples were collected (i.e.: 2 weekly samples at each of four points [sedimentation pit and three filters] for 5 wk). In Stage 2, 24 pigs were assigned to three treatment groups (T1= filtered/chlorinated water; T2=filtered water; T3=potable water)	In Stage 1 no differences (P>0.05) were found in enterobacteria among water treatment stages, but *S. enterica* was isolated from 15 % of the samples. During Stage 2, daily feed intake differences (P<0.05) were found from day 4 to day 9 in T2 as compared to T1 and T3. Total feed intake was higher (P<0.05) in T1. Body temperature was higher (P<0.05) in T2 on d 3 and 4. Clinical condition differences (P<0.05) were found among treatments on d 4, 5, 8 and 11. *S. enterica* was re-isolated from the ileocecal valve and the liver of two T1 pigs, and from the ileocecal valve of one T2 pig. This treatment type does not warrant the use of this water as drinking water. Furthermore, increased amount of chlorine in water reduced water intake in pigs.	High

Appendix III: Summary of studies including WASH and biosecurity interventions (continued)

Study	Country	Study design	Type of production system	Setting	Animal of interest	Main objective	Outcome of interest	Intervention Content	Results	Overall Risk of Bias
Hoffman, 1974[72]	United States of America	Non-randomised trial	Experimental set-up	Not specified	Fish: Rainbow Trout Fry	To determine whether UV irradiation would prevent infection of rainbow trout exposed to water containing *M. cerebralis.*	Mortality, average weight, and number of Rainbow Trout Fry containing *M. cerebralis* spores were measured.	Intervention in which water contaminated with *Myxosoma cerebralis* (causative agent of Whirling disease) was irradiated with 2537 Angstrom units of ultraviolet light.	Ultraviolet irradiation at a wavelength of 2537 A, for 35,000, 43,000 and 112,000 microwatt sec per squared cm following filtration through 25-gm-pore cartridge-type filters killed or removed *Myxosoma cerebralis* such that there was no incidence of *M. cerebralis* spores or Whirling Disease in rainbow trout.	Moderate
Haughton et al., 2013[58]	Ireland	Non-randomised trial	Intensive farming	Not specified	Poultry: Broilers	To investigate the potential of a commercially available acidified water treatment for reducing Campylobacter in vitro and other bacteria in the gut of live broilers.	Total microorganisms in water and Campylobacter in caecal contents of broilers was enumerated.	Intervention took place on 4 broiler farms, where PWT (a commercially available acidified water treatment) was added to broiler drinking water. One of the treatment groups received PWT in accordance with the manufacturers' instructions, which involved administering PWT in their drinking water for the first 7 d, 2 d before and 2 d after each feed change and at feed withdrawal prior to slaughter. The second treatment group received PWT in their drinking water at feed withdrawal for 24 h prior to slaughter. The remainder of the birds in the broiler house served as the control group and received non-acidified water.	Addition of PWT to the broiler drinking water for the first 7 d, 2 d before and 2 d after each feed change and at feed withdrawal prior to slaughter or only after feed withdrawal had no effect on the number of Campylobacter in caecal samples on farm before thinning and depopulation compared to untreated controls.	Moderate
De Busser et al., 2009[59]	Belgium	RCT	Intensive farming	Not specified	Pigs	To assess the effect of adding organic acids to drinking water on Salmonella shedding by pigs during the last two weeks prior to slaughter	Pigs were randomly selected and sampled for blood, contents of ileum and rectum, mesenteric lymph nodes and carcass swabs. Samples were assessed for Salmonella positivity.	Intervention in which the treatment group received acidified drinking water (a mixture of different organic acids (INVE Nutri-Ad) was added using a dose-measuring pump until a pH of maximum 4 was achieved. This mixture of organic acids contained formic acid, propionic acid, acetic acid, sorbic acid and a liquid carrier. All other factors including housing and feeding were identical in both groups.	The results did not reveal a significant difference between the treatment and control groups for the different slaughterhouse samples. The strategic application of organic acids during the last 2 weeks prior to slaughter was insufficient to decrease Salmonella shedding and contamination shortly before and during slaughter.	High

Appendix III: Summary of studies including WASH and biosecurity interventions (continued)

Study	Country	Study design	Type of production system	Setting	Animal of interest	Main objective	Outcome of interest	Intervention Content	Results	Overall Risk of Bias
Arguello et al., 2013[60]	Spain	Non-randomised trial	Intensive farming	Not specified	Pigs	To assess the effect of adding organic acids in drinking water or feed during part of the finishing period on the prevalence of Salmonella in finishing pigs.	Samples consisted of blood and rectal feces from pigs, feces from pens, and cecal contents from the gastrointestinal tracts of slaughtered pigs. Samples were subject to processing and bacteriological analysis to establish Salmonella seroprevalence.	Intervention in which organic acids were added to water or feed of finishing pigs: Pigs received either a mixture of acids (lactic, formic, propionic, and acetic) added to their drinking water at a concentration of 0.035% (trial A), or a basal diet containing 0.5% potassium-diformate, KH(COOH)2 (trials B and C), during the last 6–7 weeks of the finishing period.	In Trial A, a lower prevalence of Salmonella shedders was detected in the experimental group pigs (7/40) compared to the control group pigs (20/39) ($p<0.01$; RR=2.34, 95% CI (1.41–6.25)) at the end of the finishing period. Salmonella isolated from ceca and mesenteric lymph nodes was also lower among experimental group pigs compared to control group pigs, though these differences did were not statistically significant ($p = 0.18$). In trial B, the prevalence of Salmonella shedders was significantly higher in the control group (9 of 40) compared to the experimental group (1/40) ($p = 0.017$). No significant differences were detected in the frequency of shedding between the experimental and control group in any of the samplings performed during trial C.	Moderate
Mateus-Vargas et al., 2019[61]	Germany	Non-randomised trial	Intensive farming	Not specified	Poultry	To evaluate the effect of the application of LF-EMF on bacterial concentrations and biofilms at scale-models of different drinking systems conventionally used in poultry holdings.	Water and biofilm samples were subject to microbiological analysis, as well as pH and temperature measurements.	Intervention utilised of low-frequency electromagnetic fields (LF-EMF) on bacterial concentrations and biofilms at scale-models of different drinking systems (circulating and non-circulating) conventionally used in poultry holdings. Treated systems were equipped with commercial devices producing pulsed electromagnetic signals of low frequency up to 10,000 Hz; max. 21 mT.	Colony counts of water differed significantly neither between trials nor between the drinking systems studied.	Low
Stern et al., 2002[65]	United States of America	Non-randomised trial	Experimental set-up	Not specified	Poultry: Broilers	To compare the rate of intestinal colonization with Campylobacter in chickens provided chlorinated drinking water in relation to the frequency of colonization in chickens given non-supplemented drinking water.	Cecal and faecal samples were taken from broilers and assessed for Campylobacter spp. prevalence.	Intervention looking at the effect of drinking water chlorination on Campylobacter spp. colonization of broilers was assessed in both experimental and field settings. Within the experimental unit, water was provided to the chicks by nipple waterers holding either untreated water or water supplemented with 2 parts per million (ppm) chlorine. In the field trial, broiler drinking water was provided by drilled wells. Broilers in the treatment group received a constant 2-5 ppm chlorine in drinking water using the in-line medicator of the drinking water system.	Under both experimental and field conditions, no significant difference ($p > 0.05$) was detected in prevalence of Campylobacter spp. in birds provided chlorinated drinking water and control birds provided water without supplemental chlorine.	Moderate

Appendix III: Summary of studies including WASH and biosecurity interventions (continued)

Study	Country	Study design	Type of production system	Setting	Animal of interest	Main objective	Outcome of interest	Intervention Content	Results	Overall Risk of Bias
Sanitation										
Ryu et al., 2017[51]	South Korea	Non-randomised trial	Intensive farming	Urban, Rural	Humans	To evaluate the impact of Livestock Manure Control Policy on human leptospirosis in Republic of Korea using interrupted time series analysis	Monthly data of leptospirosis and HFRS incidence were collected from the Database of National Notifiable Infectious Diseases of the Korea Centers for Disease Control and Prevention.	Intervention consisted of the 2007 Korean Livestock Manure Control Act, which makes it compulsory for livestock farmers to be equipped with appropriate sludge process facilities on their farms.	The annual incidence of leptospirosis in South Korea decreased by 33% after policy enforcement of the policy. A significant change in the slope of human leptospirosis cases was observed after the policy enforcement (**β** = −0·09, p < 0.001). Moreover, there was an association between the size of the rice paddy fields and the decrease in leptospirosis incidence in provinces (r = 0·817, p = 0·01).	Low
Weinberg et al., 2011[104]	Israel	Non-randomised trial	Experimental set-up	Not specified	Cattle: Dairy	To examine the effect of manure cultivation on the persistence of E. coli in a model system.	Manure was analysed for moisture content, and total endogenous E. coli was enumerated.	Intervention in a model system, wherein a cow manure-derived E. coli strain was tagged with green fluorescence protein and antibiotic resistance markers and was used to inoculate cow manure in 10-L buckets. After 3 successive cycles of inoculation and cultivation, wet slurry was added during an additional 2 cycles.	Throughout the experiment, the counts of the tagged E. coli were less (p < 0.05) and disappeared faster in the cultivated than in the no cultivated manure.	Low
Bolton et al., 2013[105]	Ireland	Non-randomised trial	Experimental set-up	Peri-Urban	Pigs	To investigate the survival of Salmonella and Yersinia enterocolitica strains in pig slurry and evaluate urea and ammonia as disinfection strategies.	Samples from each slurry cocktail were tested regularly for bacterial counts (specifically Salmonella spp. and Yersinia enterocolitica).	Intervention in which strain cocktails were inoculated into fresh pig slurry and then treated with urea or ammonia. These cocktails consisted of Salmonella Anatum, Salmonella Derby, Salmonella Typhimurium DT19 and Y. enterocolitica bio-serotypes 4, O:3, 2, O:5,27 and 1A, O:6,30, and divided into 4 groups where each group is a different bacterial 'cocktail'.	There was no significant difference (p > 0 01) between the different serotypes within a given treatment. However, for each serotype, the control decimal reduction dose values were significantly higher (p < 0 01) than the ammonia-treated samples, which were in turn significantly greater (p < 0 01) than those obtained with urea-treated slurry.	Moderate

Appendix III: Summary of studies including WASH and biosecurity interventions (continued)

Study	Country	Study design	Type of production system	Setting	Animal of interest	Main objective	Outcome of interest	Intervention Content	Results	Overall Risk of Bias
Hygiene										
Nasr et al., 2018[102]	Egypt	Non-randomised trial	Intensive farming	Not specified	Poultry	To determine bactericidal efficacy of disinfectants commonly used in foot bath inside poultry farms.	Shoes were swabbed and samples were plated on agar and bacteria were CFU enumerated.	Intervention that compared the bactericidal effectiveness of foot baths vessels kept at room temperature for three days of experimentation, calculating the bacterial log reduction, a daily sampling before and after immersion of contaminated rubber shoes for 1 minute.	After one-minute contact time, none of the disinfectants were effective in reducing the bacterial load of the contaminated shoes. Although going through the motions of stepping in a boot bath can help increase employee awareness of biosecurity and maintain a clean workplace, this study indicates that this is an insufficient biosecurity measure that potentially increase the risk for infection spread through contaminated boots by farm personnel. This is because organic matter on shoes' soles acted as a physical barrier that protected bacteria from contact with the disinfectants. Recommendations were previously set to scrub and rinse foot wears with a detergent, before contact with a proper disinfectant, provided that contact time should not be less than 15 to 30 minutes. But expectations to follow these recommendations in commercial poultry husbandry environments were practically hard. These results emphasize the need to use freshly prepared disinfectant with regular cleaning of the foot baths. Furthermore, the shoes should be strongly scrapped before foot bath immersion to reduce the organic load; otherwise, prolonged foot bath contact time should be applied.	Low
Mannion et al., 2007[75]	Ireland	Ecological	Intensive farming	Rural	Pigs	To assess the efficacy of washing and disinfecting (pig) finisher units on category 1 and category 3 farms in reducing or eliminating the levels of contamination.	Samples were taken from the pen floors, feeders and drinkers of seven category 1 and seven category 3 farms, and Enterobacteriaceae and Salmonellae were enumerated after plating samples.	Intervention assessed the efficacy of washing and disinfecting finisher units on Salmonella category 1 and category 3 farms in reducing or eliminating the levels of contamination. Cleaning procedures included high- versus low pressure wash, the use of disinfectant, and how long the pens were rested for.	Category 1 Farms: In most cases, there was a moderate reduction in the counts of Enterobacteriaceae following cleaning, and this reduction was significant ($p = 0.01$). In cleaned feeders, there was little or no reduction on many of the farms, and on three of them there was an increase in the counts of Enterobacteriaceae, although the increase was not significant. Category 3 Farms: There was a significant reduction in the levels of Enterobacteriaceae after cleaning ($p = 0.01$). There was a large increase in the numbers of Enterobacteriaceae detected in the feeders on the category 3 farms after cleaning. These cleaned feeders were significantly more contaminated than dirty feeders on the same farms.	High

Appendix III: Summary of studies including WASH and biosecurity interventions (continued)

Study	Country	Study design	Type of production system	Setting	Animal of interest	Main objective	Outcome of interest	Intervention Content	Results	Overall Risk of Bias
White et al., 2018[76]	United States of America	Non-randomised trial	Intensive farming	Not specified	Poultry: Laying hens	To determine the efficacy of commonly used disinfection products on soiled layer cages.	Swabs of cage floors were collected and assessed for the presence of total aerobes, coliforms, *Staphylococcus spp*, and *Pseudomonas spp.*	Intervention assessed the efficacy of high- and low- pressure water rinse (power washer versus garden hose), as well as the use of different disinfectants to clean the cages of layer hens. Disinfectants included were a soap, a chlorinated cleaner, a quaternary ammonium, a glutaraldehyde, a peroxyacetic acid, a phenolic, a potassium peroxymonosulfate, a hydrogen peroxide, and a quaternary/glutaraldehyde blend product.	No treatment in the first trial decreased ($p < 0.05$) coliforms or Staphylococcus spp. when compared to untreated control cages and the high-pressure water rinse. However, reduction ($p < 0.05$) of coliforms and Staphylococcus spp. were observed with all disinfectants in trial two. Two disinfectant products reduced ($p < 0.05$) Pseudomonas spp. in trial one, and 5 disinfectant products reduced *Pseudomona spp.* in trial two.	Low
Ellis-Iversen et al., 2008[96]	United Kingdom	RCT	Intensive farming	Rural	Cattle: Dairy, Beef	To investigate the effect of three complex management intervention packages to reduce the burden of E. coli O157 in groups of young-stock on cattle farms in England and Wales.	Faecal samples were collected and analysed for the presence of E. coli O157 by immuno-magnetic separation (IMS).	Intervention included comprehensive hygiene and biosecurity measures and consisted of four groups. Package A included no new animals brought in or contact with other cattle; keeping bedding dry and animals clean; use boot-dip and overcoat. Package B included the same herd management measures but additionally focused on the weekly cleaning and emptying of water troughs and no sharing of water resources. Package C combined all measures in package A and B, whereas in the control package hygiene practices were not altered.	The significant reduction in E. coli O157 observed in intervention group A (RR 0.14; $p = 0.032$) was a result of applying several control measures. The most important intervention variables were "dry bedding" and "keep animals in same groups" to apply to ensure effectiveness of the intervention package. Other measures of slightly less importance were whether new animals were bought into the herd during the study period or whether the cattle had direct contact with animals from other farms (both $p < 0.1$). The change in proportion of E. coli O157 positive samples was an overall decline in all four groups over the four and a half trial months. Intervention package A reduced the number E. coli O157 positive samples within a group of young-stock over a four and a half months period more effectively than the control group albeit this reduction was not statistically significant (RR = 0.26; CI95:0.05- 1.43, $p = 0.122$). The RCT provided no evidence of an effect of intervention packages B (RR = 1.37, $p = 0.631$) or intervention package C (RR = 1.27, $p = 0.671$) on E. coli O157, when compared to the control group.	Moderate
Martelli et al., 2017a[77]	United Kingdom	Non-randomised trial	Intensive farming	Rural	Ducks	To assess the effectiveness of cleaning and disinfection programmes used on duck farms in the UK, and to compare farms where terminal formaldehyde disinfection was in use with those where it was not.	Samples were collected from the anteroom, drinkers, dust, faeces, feeders, fixtures, floor (including floor cracks), and walls and subsequently assessed for the proportion of Salmonella-positive samples.	. All farms started the cleaning and disinfection process with a mucking out and washing stage followed by the application of various disinfectant products. The disinfection programmes were subdivided into two main categories: (1) programmes that included a final formaldehyde disinfection step; and (2) programmes that did not include a final formaldehyde disinfection step.	There was a statistically significant ($p < 0.001$) reduction in the percentage of Salmonella-positive samples between before cleaning and disinfection (41.1%) and after cleaning and disinfection (3.1%). Farms in which disinfection programme 1 was used were 5.34 times less likely to have samples positive for Salmonella after cleaning and disinfection than farms which implemented programme 2. After restocking, the number of Salmonella-positive samples increased significantly ($p < 0.001$), with 65.3% of the samples tested being positive for Salmonella.	High

Appendix III: Summary of studies including WASH and biosecurity interventions (continued)

Study	Country	Study design	Type of production system	Setting	Animal of interest	Main objective	Outcome of interest	Intervention Content	Results	Overall Risk of Bias
Bragg and Plumstead, 2003[79]	South Africa	RCT	Intensive farming	Rural	Poultry: Broilers	To evaluate a continuous disinfection programme for broilers under experimental and field conditions.	Production parameters, such as growth rate, feed conversion ratio and feed consumption, of the birds In the three groups were monitored. In addition, all mortalities in the different groups were recorded and classified into diseases of an infectious nature, non-infectious nature and unknown category. Bacterial counts were also done on a weekly basis from the different pens.	Intervention assessing disinfection treatments. Treatment 1 consisted of a full pre-disinfection of the pens with Virukill and pre-fogging with a 1 % solution of Virukill prior to placement of birds. The pens also received a full continuous disinfection programme, which consisted of continuous application to the drinking water of the chickens with a 100 ppm dilution of Virukill, as well as daily spraying of the chickens with a knapsack sprayer at a 1 % dilution of Virukill. The second set of treatments consisted of a full pre-disinfection process which consisted of a washing stage with a soap solution followed by disinfection with a 1 % dilution of commercially available glutaraldehyde-based disinfection. The control group consisted of no pre-disinfection and no continuous disinfection.	The lowest mortalities were recorded in the pens receiving the full pre-disinfection with Virukill as well as the full continuous disinfection programme. Significantly ($p < 0.05$) lower bacterial counts were obtained in the pens on the full continuous disinfection programme when compared to those of the other two treatments.	High
Doko et al., 2012[97]	Benin	Non-randomised trial	Subsistence farming	Not specified	Sheep	To assess the effect of hygiene and medication on preweaning survival and growth of Djallonké sheep in Atacora, Benin	Survival and growth were observed in lambs. Additionally, weights, disease prevalence and type, treatments and, mortality, and information about owner and the herd (composition and management) were recorded. The value of the production was estimated by multiplying the weight gain with the average market price of a 3-month-old sheep.	Intervention to compare hygiene versus medication measures to improve preweaning survival of animals. The first treatment focused on hygiene, the second, on medicinal prevention, and the third combined both. Hygienic measures consisted of daily cleaning of stable, and water and feed troughs. The animals in the control group did not receive any preventive or curative treatment. In case of diarrhoea, the animals of the three intervention groups were treated with drugs. Chemicals, medicines, a bucket, and a broom were provided for free.	Good housing and hygiene reduced mortality and increased growth of suckling lambs ($p < 0.05$). Good housing plus medication reduced mortality ($p < 0.05$), but growth was not higher than the control. The combination of both treatments increased growth and benefits compared with the hygiene treatment, but decreased the internal rate of return.	High
Leedom Larson et al., 2012[99]	United States of America	Non-randomised trial	Intensive farming	Urban	Pigs	To summarize, implement and evaluate common MRSA prevention guidelines to determine their effectiveness in pork production operations.	Biochemical tests were performed to confirm the presence of *S. aureus* and MRSA, multi-locus sequence typing was used to determine the presence of pvl & spa genes, and antimicrobial susceptibility testing was performed to determine antibiotic resistance.	Intervention to prevent MRSA infection in pork producers. These included educating employees about good hand hygiene, general infection control, and MRSA transmission. Encourage use of alcohol-based hand sanitizers when hands not visibly dirty, liquid soap dispensers and the use of warm water and soap and a clean towel. Discourage sharing of personal items including soap and razors. Proper cuts or wound care and reporting as well as washing and cleaning of clothing and equipment.	Of the shower room samples, 7/50 were positive for MRSA pre-intervention but became negative post-intervention. 4/50 of samples that were negative for MRSA pre-intervention became positive post-intervention. For the remaining samples, no change in status was observed post-intervention; 14 samples (28%) remained negative and 2 (4%) samples remained positive.	High

Appendix III: Summary of studies including WASH and biosecurity interventions (continued)

Study	Country	Study design	Type of production system	Setting	Animal of interest	Main objective	Outcome of interest	Intervention Content	Results	Overall Risk of Bias
Traub-Dargatz et al., 2006[100]	Canada & United States of America	Non-randomised trial	Experimental set-up	Not specified	Horses	To determine the reduction of bacteria on the hands of students performing a routine equine physical examination associated with 3 hand hygiene protocols.	Bacterial colony count per hand at the prephysical, postphysical, and postprotocol stages was enumerated and reduction factors were calculated for the three hand hygiene protocols.	Intervention comparing three hygiene protocols were performed on the hands of veterinary staff performing routine equine physical examinations: washing with soap, ethanol gel application, and chlorohexidine-ethanol application.	The reduction factors were significantly different ($p < 0.0001$) between the hand-washing group and the other 2 treatment groups (the alcohol-gel and the chlorhexidine-alcohol lotion).	Low
Kim and Kim, 2010[80]	South Korea	Non-randomised trial	Intensive farming	Not specified	Poultry	To investigate the inhibitory effects of formaldehyde application methods on aerosol bacterial counts in a commercial hatchery.	Bacterial contamination (aerobic bacteria and coliforms) of equipment, facilities, and air samples in the hatchery environment was enumerated. Designated sites in the hatchery were serologically tested for Salmonella.	Intervention in which formaldehyde was administrated in two ways in a hatchery to investigate inhibitory effects on aerosol bacterial counts. In the conventional method, 37% formaldehyde was administrated only once into a basin at the time of transfer, whereas in the CRI method, the 37% formaldehyde was administrated at the same rate during hatching.	The experimental group receiving formaldehyde by constant rate infusion during hatching had a significantly superior inhibitory effect on aerosol bacterial count 4 h before hatching as compared with the group receiving formaldehyde into a basin and the negative control group ($P < 0.05$).	Low
De Castro Burbarelli et al., 2017[81]	Brazil	Ecological	Intensive farming	Peri-Urban	Poultry: Broilers	To evaluate 2 cleaning and disinfecting programs regarding their influence on productive performance, elimination of Campylobacter, and characterization of *C. jejuni* strains when applied to broiler chickens' facilities.	The effectiveness of the treatments was evaluated by microbiological analysis of the environment and equipment (total bacterial counts) performed before and after treatment applications, as well as by broiler performance results (live weight, feed intake, feed conversion ratio, livability).	Intervention applied two cleaning and disinfection programs on two groups of 960 broiler chickens each. The regular program consisted of sweeping facilities, washing equipment and environment with water and natural detergent whereas the proposed cleaning program consisted of dry and wet cleaning, application of 2 detergents (one acid and one base), and 2 disinfectants.	After cleaning and disinfection, there was a smaller occurrence of Campylobacter spp. in drinkers and floors with the proposed program ($p <0.05$). There were no other significant decreases in Campylobacter prevalence.	High
Dorado-García et al., 2015[82]	The Netherlands	RCT	Intensive farming	Urban, Peri-Urban	Cattle: Beef	To evaluate two cleaning and disinfection strategies to curb livestock-associated methicillin resistant S. aureus.	Nasal swabs from calves were tested for MRSA and confirmed by PCR. Antimicrobial use was determined by calculating daily doses per animal per cycle.	Intervention in which fifty-one veal calf farms were assigned to one of 3 study arms: RAB farms reducing antimicrobials by protocol; RAB-CD farms reducing antimicrobials by protocol and applying a cleaning and disinfection program; and Control farms without interventions. MRSA carriage was tested in week 0 and week 12 of 2 consecutive production cycles in farmers, family members and veal calves.	The rise in MRSA prevalence over time was significantly flattened in RAB farms as compared to Control farms while RAB-CD farms showed an intermediate trend. The differences between study arms were statistically significant for the comparison between RAB and Control farms in week 12. RAB-CD farms did not significantly differ from Control farms.	High

Appendix III: Summary of studies including WASH and biosecurity interventions (continued)

Study	Country	Study design	Type of production system	Setting	Animal of interest	Main objective	Outcome of interest	Intervention Content	Results	Overall Risk of Bias
Taslima Akhter et al., 2018[101]	Bangladesh	Cross-Sectional	Intensive farming	Not specified	Poultry	To understand the impact of food safety control measures towards developing a food control guideline for the poultry value chain.	Farm data (i.e. farm practices in light of biosecurity training) were recorded and scored by trained veterinarians. A convenience sampling technique was applied to collect samples from live broiler, broiler meat, LBM environmental sample, broiler carrying vehicles to test for microbial presence and antimicrobial residues.	Intervention consisted of the delivery of an intensive participatory training program focussing on Good Agriculture Practices (GAP) and Good Hygiene Practices (GHP) related five (microbial) plus five (chemical) Code of Practices (CoP) were adopted at the farm level at 500 poultry farms in Bangladesh.	CoP adapted farms had significantly lower likelihood for contamination by Salmonella spp. (in feed samples) and Campylobacter spp. (in cloacal swab, feed, water, and whole carcass samples) than non-CoP farms.	High
Van de Giessen et al., 1998[92]	The Netherlands	Non-randomised trial	Intensive farming	Not specified	Poultry: Broilers	To evaluate the occurrence of Campylobacter species on chicken farms and assess the potential of hygiene practices in reducing its prevalence.	Samples of faeces were collected at random, cultivated and then tested using PCR and RAPD analysis for Campylobacter spp.	Intervention focusing on hygiene measures including cleaning and disinfection of broiler houses, construction of a hygiene barrier where footwear and overalls must be changed, washing of hands, banning of children and pets into broiler houses, separate tools to be used, control of rodents.	After introduction of the control measures, the percentage of Campylobacter positive flocks decreased from 66 % (12/18) to 22 % (2/9) at farm C and from 100% (4/4) to 42% (5/12) at farm D.	High
Pletinckx et al., 2013[89]	Belgium	Non-randomised trial	Intensive farming	Not specified	Pigs	To assess the efficacy of an animal disinfection strategy in reducing the livestock-associated methicillin resistant Staphylococcus aureus prevalence in sows, their offspring and the barn environment.	Swabs were taken at various points during the sow round of both the farm and pigs and subsequently analysed by DNA extraction and multiplex PCR for S. aureus.	Intervention consisted of washing the sows with a shampoo followed by disinfection of the skin with a solution containing chlorhexidine digluconate and isopropanol.	On the first day of disinfection and 6 days after stopping the disinfection, a significant decrease ($p < 0.01$) in sow MRSA prevalence was observed on both farms, whereas no decrease was seen in the control groups. However, this did not remain significant ($p = 0.20$) 21-28 days after disinfection. The MRSA prevalence of the piglets in the test groups was significantly lower ($p < 0.01$) 6 days after the end of disinfection. In the swine nursery unit, no significant difference (P = 0 99) was seen between both groups.	Low
Hancox et al., 2013[83]	United Kingdom	RCT	Intensive farming	Not specified	Pigs	To determine the effect of a detergent soaking period in a cleaning regime by monitoring total aerobic and Enterobacteriaceae counts on different materials in livestock housing.	Swab samples were taken from concrete, metal (galvanised steel slats) and stock board surfaces at several stages throughout the cleaning and enumerated for total aerobic count and Enterobacteriaceae.	Intervention in pig pen cleaning regime compared to regular protocol. This included scraping, soaking with or without detergent (treatment and control), pressure washing, disinfection and natural drying. There were two groups, control (n=4) and the proposed cleaning regimen (n=4).	There were significant reductions in both TAC and ENT after disinfection of concrete (1.6 log cfu per cm squared, $p < 0.005$ and 0.7 log cfu per cm squared, $p < 0.05$, respectively) and stock board (1.1 and 0.6 log cfu per cm squared respectively, $p < 0.05$), but no significant change in TAC or ENT on metal.	Low

Appendix III: Summary of studies including WASH and biosecurity interventions (continued)

Study	Country	Study design	Type of production system	Setting	Animal of interest	Main objective	Outcome of interest	Intervention Content	Results	Overall Risk of Bias
Oliviera et al., 2017[93]	Denmark	Cross-Sectional	Intensive farming	Not specified	Cattle: Dairy	To evaluate the relationship between biosecurity measures and within-herd digital dermatitis prevalence in dairy cattle herds.	All lactating cows were scored as negative or positive for DD at the hind legs during milking in the milking parlor. Information about biosecurity was obtained through questionnaires addressed to farmers, on-farm observations, and information from the Danish Cattle Database.	Intervention consisted of biosecurity measures i.e. cleaning and disinfection procedures of equipment and facilities, personal hygiene of farm staff, and strategies for improved hoof health of cows.	Poor external biosecurity measures associated with higher prevalence of DD were recent ani-mal purchase, access to pasture, lack of boots available for visitors, farm staff working at other dairy farms as well, hoof trimming without a professional attending, and animal transporters having access to cattle area. For internal biosecurity, higher DD prevalence were associated with infrequent hoof bathing, manure scraping less than 8 times a day, manure removal direction from cows to heifers, animal pens' exit without water hoses, manure-handling vehicle used in other activities, and water troughs contaminated with manure.	Low
Dale et al., 2015[84]	United States of America	Non-randomised trial	Intensive farming	Not specified	Poultry: Broilers	To identify what realistic, cost-effective biosecurity and pest management interventions could be performed at the farm level to mitigate infection of commercial poultry flocks with Campylobacter and Salmonella.	Campylobacter and Salmonella prevalence were measured across 3 flocks via weekly environmental sampling inside and outside of the 2 houses. In addition to environmental monitoring, the shavings preplacement, chick hatch debris, fly and beetle traps, waterlines, and preprocessing cecal samples were cultured during Flock 2 and Flock 3.	Intervention in the cleaning and disinfection of poultry houses, which entailed full removal of all litter and debris down to the dirt pad, followed by a hot water wash of the house, subsequently followed by disinfection with formaldehyde. All water lines were cleaned and disinfected with a chlorine bleach product. Farmers were required to change footwear before entering bird spaces, use hand-gel before and after entering, and use separate equipment for the bird spaces. There were three Groups (n=18,900 per group): 1 - control, 2 and 3 - treatment.	Prevalence of Campylobacter did not vary greatly between all 3 flocks. Only 2 statistically significant differences were found. The inside boot sock samples of Flock 3 were significantly higher ($p < 0.001$) than Flocks 1 and 2, which did not differ significantly from each other. The outside boot socks of Flock 2 had significantly higher levels than Flock 1 ($p < 0.001$), but did not differ significantly from Flock 3.	High
Jang et al., 2016[103]	South Korea	Non-randomised trial	Experimental set-up	Not specified	Cows, pigs, poultry	To evaluate the efficacy of six commercial disinfectants, as used in footbaths on farms, for reducing S. typhimurium contamination of footwear.	Rubber boots were sampled for aerobic bacterial counts using selective media.	Intervention assessed the efficacy of six commercial disinfectants used for foothbaths for cleaning boots after contamination with animal faeces on Salmonella Typhimurium prevalence.	When used at the recommended concentrations, most disinfectants showed over 91% reduction efficacy in absence of organic matter. The greatest reductions were seen with oxidizing agents including potassium peroxymonosulfate-based products and sodium dichloroisocyanurate.	High

Appendix III: Summary of studies including WASH and biosecurity interventions (continued)

Study	Country	Study design	Type of production system	Setting	Animal of interest	Main objective	Outcome of interest	Intervention Content	Results	Overall Risk of Bias
Kamal et al., 2019[85]	Egypt	Ecological	Intensive farming	Not specified	Cattle: Dairy, Beef	To investigate drinking water bacterial profile, determine chlorine-resistant strains, and draw statistical correlations with the used disinfectants and disinfection processes.	A structured questionnaire was used to identify commonly used disinfection process, disinfectant types, disinfectants frequency, and rate of use. Water samples were collected for microbiological analysis to obtain water bacterial profile and testing resistance to chlorine.	Intervention identified the effects of commonly used disinfection process, disinfectant types, disinfectants frequency, and rate of use on water samples' bacterial profile. Resistance to chlorine was also tested. The study took place in three groups: 1 beef cattle farms (n=60), 2 dairy cattle farms (n=60), 3 dairy beef mixed farms (n=12)	Experimental evaluation of the bactericidal effect of the eight selected disinfectants on the chlorine-resistant isolated strains revealed that peroxymonosulfate killed 19/19 isolated strains per 15 minutes of contact time, and quaternary ammonium compounds killed only 3/19 strains/15min of contact time. The qacE resistance gene was detected in 3/4 isolated chlorine-resistant E. coli strains.	Moderate
Carrique-Mas et al., 2009[86]	United Kingdom	Ecological	Intensive farming	Not specified	Poultry: Laying hens	To determine the comparative effectiveness of disinfection programmes in Salmonella-positive cage and non-cage (i.e. free-range, barn) houses in the field.	Hand held gauze swabs were used to sample a range of surfaces (cage interior, drinker cups, feed troughs, dropping boards, house floor, and egg belts) in the house. Soil from paddocks was additionally sampled from free-range houses. Salmonella was isolated using selective media and serotyped using the Kauffmann-White typing scheme.	Intervention in the cleaning and disinfection routine. Regimes consisted either of (1) a compound disinfectant consisting of a mixture of formaldehyde, glutaraldehyde and quarternary ammonium applied at the recommended concentration; (2) a 10% (vol/vol) dilution of the standard 37% commercial formalin, applied by a contractor; and (3) other disinfection procedures selected and applied by the farmer.	The proportion of positive samples from floor and dropping board were significantly greater than that obtained from any other type of sample ($p < 0.05$). Moreover, the proportion of positive samples from scratching areas was greater than any other type of sample ($p < 0.01$). The differences in prevalence of Salmonella between cage houses and non-cage houses were not statistically significant ($p = 0.454$)	High
Davies and Wray, 1994[87]	United Kingdom	Ecological	Intensive farming	Not specified	Poultry	To describe observations made during studies of survival of *S.enteritidis* following cleansing and disinfection of naturally contaminated poultry houses.	Samples collected and tested for *S. enteritidis*, including from litter, faeces, feed, dust and surfaces such as cleaned floors, walls, and mice droppings.	Intervention using various disinfection regimes in three groups Groups: 1 - Trial breeder house study (n=350 broiler breeders), 2 -broiler house study (n= unknown), 3 - broiler breeder and layer breeder houses (n=20 houses). For the trial breeder house Pen A was disinfected with a commercial product blend of peroxygen compounds and organic acids (diluted 1: = 280), Pen B with a commercial product blend of naturally occurring tar oils (diluted 1:200), and Pen C with a peroxygen compound (1:100); for the broiler house study a commercial product blend of peroxygen compounds and organic acids, applied at a concentration of 1:90, was used for disinfection; for broiler breeder and layer breeder houses a variety of approaches using formaldehyde or peroxygen were used.	Trial Breeder House Study: Overall disinfection produced a significant reduction in the prevalence of Salmonella ($p < 0.00001$). In Pen A, there was a reduction in the prevalence of Salmonella contamination but this was not statistically significant. No Salmonella was isolated from Pen B ($p < 0.05$) or Pen C ($p < 0.005$) after disinfection, indicating a significant reduction in contamination in Pens B and C. Broiler House Study: the prevalence of Salmonella contamination was significantly increased ($P < .05$) by the ineffective cleansing and disinfection.	High

Appendix III: Summary of studies including WASH and biosecurity interventions (continued)

Study	Country	Study design	Type of production system	Setting	Animal of interest	Main objective	Outcome of interest	Intervention Content	Results	Overall Risk of Bias
Kioska et al., 2017[88]	Germany	Ecological	Intensive farming	Not specified	Poultry: Broilers	To implement a risk-orientated hygiene analysis for the control of S. Java in broiler houses.	Samples were taken from numerous places (including water lines, feed lines, feed suppliers, walls, air supply system, ceilings, etc.) As well, neck skin and cecal content samples were taken from each batch of animals at slaughter. Samples were tested using qPCR to determine S. Java presence.	Intervention in cleaning and disinfection procedures. This new regime consisted of: barns dry-cleaned, washed with hot water and commercial foam-producing soaking liquid, feeding troughs and drinking cups disinfected, barn re-rinsed and left to dry. First disinfection of farm A was carried out with a product containing peracetic acid. Farm B was fogged with a formalin-based product. On both farms, final disinfection was done with a sodium hypochlorite solution. This study was carried out in three farms, Farm 1A (n=40,000 broilers), Farm 1B (n=40,000 broilers, Farm 2 (n=30,000).	Before cleaning, all checkpoints were tested positive for Salmonella DNA. Salmonella reduction of 66% of the sampled points could be achieved by intensive cleaning. A first disinfection on farm A and B failed to completely eradicate S. Java. A second disinfection followed and finally achieved a Salmonella-free status of the barns.	High
Martelli et al., 2017b[78]	United Kingdom	RCT	Intensive farming	Not specified	Pigs	To evaluate the effectiveness of cleaning and disinfection between batches of pigs as a measure to reduce the transmission of Salmonella.	Both pooled and individual faecal samples were collected from pigs and Salmonella isolation was carried out according to an ISO6579:2002 Annex D-based method.	Intervention in which ten buildings were cleaned and disinfected by contractors according to a standardised protocol comprising a series of steps. This included removal of faeces, foaming, washing, disinfecting and cleaning portable equipment. Ten other buildings featured as a control.	The intervention buildings were significantly less likely ($p = 0.004$) to be positive for Salmonella after cleaning and disinfection. The pre-restocking pigs had the highest likelihood ($p < 0.001$) of being Salmonella positive (often with multiple serovars) and there was no significant difference between intervention and control buildings in Salmonella prevalence at the post-restocking visit ($p = 0.199$). However, the pigs housed in the intervention buildings were significantly less likely ($p = 0.004$) to be positive for Salmonella at slaughter age.	High
Schiavon et al., 2011[91]	Brazil	Non-randomised trial	Intensive farming	Not specified	Cattle: Dairy	To evaluate the use of antiseptics in post-milking teat disinfection.	Outcomes of interest were number of new infections and bacterial isolation, which were assessed with California Mastitis Test results and Microbiological culture	Intervention to place on a dairy farm, using two treatment groups: one using a commercially available iodine solution (Iodo Mastin), and the second one an extract of T. Minuta plus linseed extract. These treatments were applied to cows' teats as antiseptics.	There were four cases of clinical mastitis during the experiment, two cases in each treatment. The weekly prevalence of California Mastitis Test ranged from 29.5 to 17.1 % in group 1 and from 29.7 to 19.6 % in group 2 but no significant difference was found in any week. The incidence of positive culture for Staphylococcus/Streptococcus was 3.93 and 6.96/1 000 quarters/day for groups 1 and 2 respectively, being $p= 0.057$. The results of this intervention were neutral.	High

Appendix III: Summary of studies including WASH and biosecurity interventions (continued)

Study	Country	Study design	Type of production system	Setting	Animal of interest	Main objective	Outcome of interest	Intervention Content	Results	Overall Risk of Bias
Battersby et al., 2017[95]	Ireland	Non-randomised trial	Intensive farming	Not specified	Poultry: Broilers	To evaluate current cleaning practices in broiler houses by testing a range of broiler house sites after cleaning and disinfection and to evaluate the most commonly used methods in a commercial broiler house after flock harvesting	Samples were collected from a range of points (including feeders, drinkers, walls, aprons) and tested for total viable count total, Enterobacteriaceae count, and Campylobacter spp. after cleaning and disinfection by using selective media.	Intervention assessing the most common methods of cleaning and disinfection methods for the poultry farm environment, including application of a detergent followed by rinsing with a power hose as well as disinfectant treatment using thermal fogging. The intervention was conducted in ten farms, each consisting of twenty broiler houses.	The results of the first study demonstrated that critical areas in 12 of the 20 broiler houses were not effectively cleaned and disinfected between flocks as the tarmac apron, ante-room, house door, feeders, drinkers, walls, columns, barriers and/or bird weighs were Campylobacter positive. Thermal fogging with the combination of potassium peroxymonosulfate, sulfamic acid and sodium chloride (5%, v/v) or the glutaraldehyde and quaternary ammonium complex (0.3%, v/v) were the most effective treatments while other disinfectant treatments were considerably less effective.	Low
Arguello et al., 2011[90]	Spain	Ecological	Intensive farming	Not specified	Pigs	To assert the efficacy of routinely cleaning and disinfection procedures performed at three points of the pork production chain: finishing farms, transport and lairage.	Environmental samples were collected from pens (floors, walls, corridors, and dust), from trucks, and from abattoir holding pens. Samples were confirmed for Salmonella using bacteriological methods and serotyped using the White-Kauffmann scheme.	Intervention assessing the efficacy of routine cleaning and disinfection protocols at various stages of pig production. Three Groups were sampled: Finishing farms (n=36), Transport (n=8 trucks), Holding Pens (n=66).	22.2% of the farms, 62.5% of the slaughter trucks and 63.6% of the holding pens tested were positive for Salmonella after cleaning and disinfection procedures. Routine cleaning and disinfection procedures performed at the farm, slaughter truck, and slaughterhouse level are not able to eliminate Salmonella properly	High
Gibbens et al., 2001[94]	United Kingdom	RCT	Intensive farming	Not specified	Poultry: Broilers	To assess whether the risk of a broiler flock becoming infected with Campylobacter could be reduced by biosecurity measures.	Cloacal swabs were collected weekly from chickens and microbiologically cultured to identify *Campylobacter*.	Intervention investigating the effect of a biosecurity protocol (pre-defined standard method of cleansing and disinfecting poultry houses prior to stocking) compared to control on Campylobacter infection of broiler chickens.	Intervention flocks survived longer without Campylobacter infection than control flocks (Wilcoxon test, p = 0.1) Campylobacter infection was lower in intervention flocks compared to control flocks (Fisher's exact test, p = 0.08). The adjusted estimate of the intervention on the risk of Campylobacter infection at 42 days was 0.16 (p = 0.2) when Company C flocks were excluded.	Moderate
Fablet et al., 2005[98]	France	Non-randomised trial	Intensive farming	Urban, Rural	Pigs	To evaluate the effect of the implementation of a preventive programme based on the results of a previous survey on Salmonella shedding of fattening pigs.	Environmental samples were collected from pens and were tested for *Salmonella* presence. Serum samples were taken from pigs and submitted to antibody detection, with the following infections being investigated: *Lawsonia intracellularis*, Swine influenza, PRRS and PRCV. All Salmonella isolates were serotyped using the Kauffman-White scheme.	Intervention evaluated a Salmonella control programme was developed based on risk factors for Salmonella shedding by fattening pigs and implemented on a farm. The control program implemented modifications related to hygiene procedures: washing, cleaning and disinfection strategies in post-weaning and fattening phases. Measurements were taken before and after implementation of the program.	On the 5 followed batches of pigs, no one was found to be Salmonella contaminated at the end of the finishing phase. Serologically, pigs tested negative for swine influenza and PRRS. 2/5 Batches tested positive for *L. intracellularis* and 3/5 tested positive for PRCV.	High

Appendix III: Summary of studies including WASH and biosecurity interventions (continued)

Study	Country	Study design	Type of production system	Setting	Animal of interest	Main objective	Outcome of interest	Intervention Content	Results	Overall Risk of Bias
Bio-containment										
Cai et al, 2018[114]	China	Non-randomised trial	Experimental set-up	Not relevant	Poultry	To investigate the impact of black soldier fly larval bioconversion of chicken manure on the persistence of associated antimicrobial resistance genes.	Composted chicken manure was sampled and nine tetracycline resistance genes were measured (tet A, tet C, tet G, tet M, tet O, tet Q, tet T, tet W, tet X).	Intervention looked at ARG dynamics during composting of fresh chicken manure to reduce ARGs using black soldier fly larvae. Both normal and sterile black soldier fly larvae were compared with traditional composting methods.	Compared with traditional composting or sterile larval treatments (by 48.4% or 88.7%), non-sterile BSF larval treatments effectively reduced ARGs and integrin genes by 95.0% after 12 days. After larval treatments, bacterial community composition differed significantly ($p < 0.05$), with the percentage of Firmicutes possibly carrying ARGs reduced by approximately 65.5%. On average, human pathogenic bacteria populations declined by 70.7%–92.9%.	Low
Li et al., 2017[115]	China	Non-randomised trial	Experimental set-up	Not relevant	Poultry	To investigate the effects of adding different proportions of bamboo charcoal on antimicrobial resistance genes during chicken manure composting.	Four tetracycline resistance genes (tet: tetC, tetG, tetW, and tetX), four sulfonamide resistance genes (sul:sul1, sul2, dfrA1, and dfrA7), four macrolide resistance genes (erm:ermB, ermF, ermQ, and ermX), and an integron gene (intI1) were analysed by PCR and agarose electrophoresis. Bio-available heavy metals (bio-Cu and bio-Zn) were extracted with diethylenetriamine-pentaacetic acid (DPTA) at a solid: liquid ratio of 1:5 (w/v) and analysed using aflame atomic absorption spectrometer.	Intervention aimed to assess the ARG dynamics of composting chicken manure using different proportions of bamboo charcoal (BC) in experimental conditions for 26 days. The four composting experiments were designated as: CK (chicken manure+wheat stalk), BC5 (chicken manure+wheat stalk+5% BC), BC10 (chicken manure+wheat stalk+10% BC), and BC20 (chicken manure +wheat stalk+20% BC). The pile of compost was turned over on days 0, 2, 4, 7, 14, and 26, and water was added as necessary to maintain 60% humidity.	The average relative abundance reductions with 0%, 5%, 10%, and 20% BC were 0.85, 1.05, 1.08, and 1.15 logs, respectively. Temperature was the most important environmental factor for ARG profiles, according to redundancy analysis. BC significantly decreased the bio-Cu and bio-Zn levels, thereby reducing the co-selection pressure from heavy metals. Different proportions of BC had no significant effects on the removal of tetG, tetW, tetX, sul2, drfA1, and ermB.	Low
Petersen et al., 2002[111]	Thailand	Case-control	Intensive farming	Peri-Rural	Fish	To determine whether integrated fish farming affects the levels of antimicrobial-resistant bacteria in the aquatic environments of fish ponds.	Water-sediment samples were collected from the bottom of the fish pond. Acinetobacter spp. and Enterococcus spp. were isolated using selective media and antimicrobial resistance was determined using the disk diffusion method.	Intervention looking at the microbial quality of the fish on integrated fish farms, where the chickens, pigs, and ducks were fed animal feed containing growth promoters. Additionally, the animals received antimicrobials in the drinking water prophylactically and for treatment of diseases. None of the fish in the ponds were given any antimicrobial treatment or given any traditional fish feed. The fish on the control farms were fed rice bran and other agricultural waste products.	Although no significant temporal variations in resistance were found, the levels of resistance to most antimicrobials were higher among *Enterococcus spp.* isolated from the water-sediment samples from the integrated farms than among Enterococcus spp. isolated from the control fish farms. The differences were significant for resistance to erythromycin, oxytetracycline, and streptomycin among isolates from integrated broiler-fish farms and for ciprofloxacin resistance among isolates from integrated layer-fish farms.	High

Appendix III: Summary of studies including WASH and biosecurity interventions (continued)

Study	Country	Study design	Type of production system	Setting	Animal of interest	Main objective	Outcome of interest	Intervention Content	Results	Overall Risk of Bias
Ben et al., 2017[116]	China	Ecological	Intensive farming	Urban	Pigs	To determine the dissemination of antimicrobial resistance genes from swine feedlots to adjacent environments at a regional scale and examine the performance of waste treatment processes currently in use for eliminating ARGs.	Samples were taken from piglet manure, sow manure, fattening pig manure, fertilized soil, unfertilized soil, swine house wastewater, discharged wastewater, river sediment, and treated wastes. DNA extractions were performed, microbial communities were assessed via PCR, and ARGs were quantified using qPCR.	Intervention investigated the use of four on-farm treatments (Microbial fermentation bed, septic tank, biogas digester, and natural drying) in nine swine feedlots on the dissemination of antibiotic resistance genes encoding resistance to sulphonamide and tetracycline.	Microbial fermentation bed (MFB) could reduce the relative ARG abundances by 0-1.18 logs. However, septic tank, biogas digester and natural drying methods were relatively ineffective for ARG removal, and the relative abundances of some ARGs (i.e., tetC, tetG, sul1, and sul2) even increased by 0.74-3.90 logs in treated wastes. Bacterial diversity analysis indicates that the evolution of bacterial communities in the MFB played a crucial role in eliminating the ARGs.	Moderate
Huang, 2019b[117]	China	Non-randomised trial	Experimental set-up	Not relevant	None	To assess the effect of temperature and residual antibiotics on the dynamics of antibiotic resistance genes (ARGs) and microbial communities during anaerobic digestion of swine manure.	High-throughput quantitative PCR and 16S rRNA sequencing were used to evaluate the presence of antibiotic resistance genes (ARGs) and microbial communities during anaerobic digestion of swine manure.	Intervention using swine manure sourced from a farm, which was subsequently anaerobically digested under four treatment conditions (25°C, 37°C, and 37°C with 50 mg of wet weight antibiotics of body weight, and 55°C).	Anaerobic digestion significantly decreased 16S rRNA gene abundance for all the treatments ($p < 0.05$). Antibiotic spiking did not significantly affect the abundance of 16S rRNA genes. The abundances of most ARG types were significantly correlated with those of 16S rRNA genes and transposase genes, except that the abundance of vancomycin-resistant genes was significantly correlated with that of integrase gene ($p < 0.01$)	Low
Huang, 2019a[113]	China	Non-randomised trial	Experimental set-up	Not relevant	Geese	To evaluate the ability of three configurations of two-stage hybrid constructed wetlands to remove high-concentration antibiotics (tilmicosin and doxycycline), antimicrobial resistance genes, and nutrients from goose wastewater.	Wastewater was sampled in each experimental setup and tested for the presence of tilmicosin (TMS) and doxycycline (DOC), antibiotic resistance genes (ARGs)(seven tet genes and three erm genes), intI1, 16S rRNA, and nutrients.	Intervention to reduce ARGs contamination in goose wastewater using a model system. Three configurations of two-stage hybrid constructed wetlands were operated (horizontal subsurface flow-down-flow vertical subsurface flow CWs (HF-DVF); horizontal subsurface flow-up-flow vertical subsurface flow CWs (HF-UVF); down-flow vertical subsurface flow-up-flow vertical subsurface flow CWs (DVF-UVF)).	All three hybrid CWs could remove more than 98% of TMS and DOC from wastewater, without significant difference among treatments ($p > 0.05$). For ARGs, DVF-UVF showed significantly higher removal efficiencies of intI1, ermB, ermC, ermF, tetW, and tetG compared to HF-UVF ($p < 0.05$).	Moderate
Kim et al., 2018[112]	South Korea	Ecological	Intensive farming	Peri-Urban	Fish	To investigate reduction in antibiotic resistance genes by targeting solid waste in water effluent from a flow-through aquaculture in South Korea.	Effluents were sampled and microbial profiles were determined using 16s rRNA analysis and antibiotic resistance genes determined using PCR.	Intervention in which filtering tests were conducted using polyethylene, non-woven fabric filters (having four different pore sizes of 100, 50, 25 and 5 μm) on the effluent of a flow-through fish farm.	Levels of ARGs in the filtrates were reduced to approximately 60.5% of those of the ARGs in the effluents. With a filter pore size of 25 μm, a maximum removal efficiency of 66.0% was achieved. In particular, the relative abundance of detected tetracycline resistance genes decreased only after passing through the filters.	Moderate

Appendix III: Summary of studies including WASH and biosecurity interventions (continued)

Study	Country	Study design	Type of production system	Setting	Animal of interest	Main objective	Outcome of interest	Intervention Content	Results	Overall Risk of Bias
Zhou et al., 2019[118]	China	RCT	Intensive farming	Not specified	Pigs	To determine whether converting composted pig manure into biochar could reduce the dissemination of antimicrobial resistance genes into the soil in comparison with a compost amendment.	The distributions of the antibiotic resistome, mobile genetic elements (MGEs) and bacterial community composition in soils during cultivation were evaluated by high-throughput qPCR and Illumina sequencing.	Composted pig manure was converted into biochar using an industrial scale production oven and an experiment was performed using two pig manure-based composts and the biochar (derived from composted pig manure) as organic fertilizer. The dissemination of antimicrobial resistance genes and mobile genetic elements in soil was subsequently determines using qPCR and Illumina sequencing.	The total ARGs and MGEs abundance in the biochar-treated soils were significantly ($p < 0.05$) lower than those in the compost-amended soils during cultivation. The total ARGs abundance in the biochar-amended soils was similar to that in the control soils during cultivation.	High
Holman et al., 2016[119]	Canada	Non-randomised trial	Experimental set-up	Not relevant	Cattle: Beef	To determine whether the addition of construction and demolition waste would affect the persistence of antimicrobial resistance genes in the compost microbiota.	Compost samples were taken representing different manure types, times, and depths. DNA was extracted from compost samples and processed using the QIIME software package and archaeal, bacterial, and fungal microbiota were determined. AMR genes were determined using qPCR.	Intervention in the composting of cattle manure. This study assessed the dynamics of the archaeal, bacterial, and fungal microbiota of two different types of composted cattle manure and one co-composted with construction and demolition waste over a 99-day composting period.	Neither the source of the manure nor the addition of construction and demolition waste was a significant factor in determining the structure of the compost microbiota. The concentrations of all but two resistance genes were lowered by day 99.	Low
Vitosh-Sillman et al., 2017[120]	United States of America	Non-randomised trial	Experimental set-up	Not relevant	Pigs	To evaluate the persistence of porcine epidemic diarrhoea virus RNA in matrices and temperature conditions representative of composting in order to determine the potential effectiveness of this method for PEDV mortality disposal.	Samples were collected from the windrow composting bin and assessed for presence of PEDV RNA via RT-qPCR.	Intervention on the safe decomposting of fallen stock. Porcine epidemic diarrhoea virus-infected piglet carcasses were incorporated into compost windrow sections and monitored for temperature and virus degradation over two composting cycles using qRT-PCR.	At all temperatures, viral RNA copies declined over time, with the decline most marked and rapid at 65 and 70°C. Detectable RNA did persist throughout the trial in all but the most extreme condition, where two of three samples incubated at 70°C yielded undetectable viral RNA after 14 days.	Moderate
Bio-exclusion										
Agustina et al., 2017[109]	Indonesia	Non-randomised trial	Subsistence farming	Remote/Isolate	Pigs	To validate the effectiveness of a pig confinement system (PCS) in reducing the prevalence of zoonotic and internal parasite burdens in pigs.	Five pigs were monitored per household every 3 months for 15 months and blood and faeces collected. Pigs received a single dose of oxfendazole at 30 mg/kg at baseline. Qualitative faecal examinations for intestinal parasite stages were performed, and serum was tested for antibodies to cysticercus of *Taenia solium*, *Trichinella spp.*, and *Toxoplasma gondii*.	Intervention compared a traditional pig production system based on free-range scavenging to the confinement of pigs. Farmers in the intervention group were given assistance in constructing a confinement system and received advise on feed. Non-PCS farmers were encouraged to continue to produce pigs in the traditional non-confined way.	The seroprevalence of T. gondii antibodies increased from 6%±0.240 to 10%±0.303 in PCS pigs and from 7%±0.248 to 24%±0.505 in non-PCS pigs. These results demonstrate the potential of a PCS to reduce the prevalence of pigs infected with zoonotic and internal parasites and thus reduce the risk to human and pig health.	High

Appendix III: Summary of studies including WASH and biosecurity interventions (continued)

Study	Country	Study design	Type of production system	Setting	Animal of interest	Main objective	Outcome of interest	Intervention Content	Results	Overall Risk of Bias
Oberhelman et al., 2006[110]	Peru	RCT	Subsistence farming	Peri-Urban	Poultry: Broilers	To describe the epidemiology of endemic Campylobacter infections in a Peruvian peri-urban shantytown with large numbers of free-ranging chickens and to analyze chicken-to-child transmission of *C. jejuni* strains.	The entire study group included 137 participants (71 in the corral group [45 less than three years of age and 26 3–5 years of age] and 66 in the no corral group (40 less than three years of age and 26 3–5 years of age]). These participants corresponded to a total of 55 families (27 with corrals and 28 without corrals). Outcomes assessed were rates of all diarrhoea episodes and Campylobacter-associated diarrhoea.	Intervention testing the efficacy of chicken coops or corrals (donated and installed by the study team). Control group families could continue to use homemade corrals for the duration of the study in homes where they were already in place or continue to have free-ranging chicken. Major damage was repaired by someone from the study team.	Corralling of chickens in the household was not associated with reduced risk of Campylobacter-related diarrhoea in this study. Rates of all diarrhoea episodes and Campylobacter-associated diarrhoea were significantly higher among children living in homes with corrals versus children living in homes without corrals. Rates of Campylobacter- associated diarrhoea among children in households with corrals were approximately twice the rates found among children in houses without corrals, in both the entire study group (0.57 epy with corral versus 0.27 epy without corral; P=0.006) and in children less than 36 months of age (0.77 epy with corral versus 0.46 epy without corral; P = 0.08). In children living in homes with more than 21 chickens, differences were even more striking (approximately seven-fold differences) in both the overall study group (0.77 epy with corral versus 0.077 without corral; P=0.002) and in children less than 36 months of age (1.15 epy with corral versus 0.15 epy without corral; P=0.021). Family contacts of culture-positive cases also tended to be culture positive more frequently in the corral group (12 [43%)] of 28 specimens) than in the non-corral group (2 [22%] of 9 specimens). Consequently, the children in households with corrals did not experience fewer overall Campylobacter infections (and their rate of Campylobacter-related disease was significantly higher).	Moderate
Velasquez et al., 2018[106]	United States of America	Non-randomised trial	Intensive farming	Not specified	Poultry	To determine changes in Salmonella prevalence and antimicrobial resistance patterns on poultry farms following implementation of recommended changes in biosecurity practices.	Four poultry farms were sampled by collecting cloacal swabs, drag swabs, and litter samples. Salmonella was identified via PCR, serotyped, and antimicrobial resistance profiles were analysed.	Intervention aimed at changing biosecurity practices in four poultry farms. Recommendations consisted of visitors changing clothing before entering the farm, showering before and after entering the farm, maintaining records of entry and exit to the farm, recording house entry, implementing a rodent and insect control program, and the use of coveralls and shoe covers. All farms were sampled for Salmonella before and after implementation of recommendations.	Prevalence of Salmonella was 3 to 4% during pre-recommendations, while the prevalence was higher (P > 0.05), ranging from 5 to 14% during post recommendations. Higher Salmonella prevalence was observed for pre- and post-recommendation phases by sample type in cloacal and drag samples −5% for farm 1, drag swab −6% on farm 2, cloacal swab −6% for farm 3, and drag swab −17% on farm 4. A total of 7% isolates exhibited resistance to at least one of 8 antimicrobials. Based on these prevalence results, it can be inferred that, irrespective of implementation of improved biosecurity practices, seasonal variation can cause changes in the prevalence of Salmonella on the farms.	Low

Appendix III: Summary of studies including WASH and biosecurity interventions (continued)

Study	Country	Study design	Type of production system	Setting	Animal of interest	Main objective	Outcome of interest	Intervention Content	Results	Overall Risk of Bias
Luyckx et al., 2016b[107]	Belgium	Non-randomised trial	Intensive farming	Not specified	Pigs	To compare the effectiveness of a commercial competitive exclusion protocol with a classical cleaning and disinfection protocol in decreasing Salmonella, E. coli, faecal coliforms, Enterococcus species, and MRSA contamination of nursery units.	Samples were collected from the synthetic grid floor, concrete wall, synthetic wall, drinking nipples and feeding trough and subjected to multiplex PCR to test for microbiota composition. Piglet weight, feed intake, and faecal consistency was also evaluated.	Intervention to reduce prevalence of Salmonella, E.coli, , Enterococcus spp. and MRSA in pig nursery units comparing the effectiveness of competitive exclusion or cleaning and disinfection of pig units. Pigs were divided into two groups: one group was control, receiving C&D protocol (n=3 units), and the treatment group received competitive exclusion (n=3 units). The intervention lasted a total of 18 weeks (6 weeks per production cycle).	No significant differences were found between feed intake of piglets raised in competitive exclusion (CE) and control pens and there was no significant differences in scores of faecal consistency between protocols. More countable E. coli samples were found for CE units after cleaning compared to control units after disinfection ($p < 0.01$). Detection results showed that the number of MRSA positive samples was the highest (90 %) for CE units compared to the control units (81 %) ($p < 0.01$)	Moderate
Conan et al., 2013[108]	Cambodia	RCT	Subsistence farming	Rural	Ducks, Chickens	To evaluate the effectiveness of low-cost biosecurity interventions in backyard poultry flocks in Cambodia.	The main end-point of the trial was household poultry mortality rates considering one duck flock and one chicken flock per household. In the same 39 households, blood samples were collected from ducks on three occasions: November 2009 (M0, before the training), August 2010 (M9) and February 2011 (M15). Serological testing was performed on pooled samples from each household using hemagglutination inhibition tests for ND and H5 avian influenza viruses.	Intervention consisting of an educational package focussing on backyard poultry health including cleaning yards and equipment and quarantine of newly introduced and sick animals and burning dead birds. This included holding sessions for raising awareness, posters, leaflets and emphasis focusing on the following messages: (i.) cleaning yards, equipment and poultry pens daily; (ii.) use of cages to protect chicks; (iii.) prevention of pathogen introduction (limitation of yard entries from middlemen and use of cages to quarantine new birds); (iv.) diseases management (daily observation of animals, quarantine of sick animals, burying/burning of carcasses) and (v.) human protection (i.e. personal basic hygiene).	Mortality rates in intervention villages were higher both for chicken and duck flocks ($p>0.001$).In multivariate analyses, intervention was not correlated with mortality trends in ducks or chickens as a protective factor, whereas intervention was correlated with an increase in mortality rates in several individual study months for both ducks and chickens Mortality rates in chicken flocks in intervention villages (mean 6.3%, range 3.5–13.8%, per month) were significantly higher. Mortality rates in duck flocks in intervention villages (mean 4.1%, range 1.9–7.9%, per month).	High

Appendix III: Summary of studies including WASH and biosecurity interventions (continued)

Study	Country	Study design	Type of production system	Setting	Animal of interest	Main objective	Outcome of interest	Intervention Content	Results	Overall Risk of Bias
Bio-management										
Suranindyah et al., 2015[131]	Indonesia	Non-randomised trial	Small-holders	Rural	Cattle: Dairy	To investigate the effect of environmental and pre-milking sanitation on milk quality of dairy cow in the farmers.	Quality of milk was assessed by measuring alcohol content, pH, density, and fat percentage. Microbial content was evaluated via methylene blue reduction test.	Intervention consisted of (i.) delivering information about hygienic practices to increase quality of milk, (ii.) demonstrating by example a clean and hygienic barn, milking process, and manure handling, (iii.) ensuring the implementation of good bio-management by cleaning floor of stable, water and feed trough, washing the cow and proper pre-milking and post-milking practices.	Improving sanitation significantly ($p < 0.05$) decreased milk acidity from 0.19% to 0.14% and number of bacteria in milk.	High
Hernandez-Llamas et al., 2014[152]	Mexico	Ecological	Intensive farming	Not specified	Shrimp	To analyse the relationship between the length of pond shutdown and the prevalence of the white spot disease in shrimp.	Prevalence of white spot disease as the percentage of the Local Aquaculture Health Boards (LAHBs) affected by the disease in relation to the total (exposed) number of LAHBs, both for different Length of Pond Shutdown (LPS) or different years.	Intervention assessed the efficacy of pond shutdown as an on-farm biosecurity measure in aquaculture. By drying and exposure this to sunlight the goal was to eliminate residual pathogens, particularly white spot disease.	The Length of Pond shutdown (LPS) increased significantly from 2.1 months in 2005 to 3.1 months in 2011. The prevalence of White Spot Disease (WSD) diminished significantly from as LPS increased in 2005 through to 2009 and 2005 through to 2011 ($P < 0.05$). When analysed on an annual basis, the prevalence decreased significantly in relation with LPS in 2006 ($P = 0.048$), 2007 ($P = 0.002$), 2008 ($P = 0.0001$) and 2009 ($P = 0.001$); no evidence for decreasing the prevalence occurred in 2005 ($P = 0.1$), 2010 ($P = 0.17$) or 2011 ($P = 0.52$).	Low
Kim et al., 2017[150]	United States of America	RCT	Experimental set-up	Urban	Pigs	To evaluate the effectiveness of biosecurity procedures directed at minimizing transmission of Porcine Epidemic Diarrhoea Virus via personnel following different biosecurity protocols using a controlled experimental setting.	Disrupting the Porcine Epidemic Diarrhoea Virus (PEDV) transmission cycle through graded biosecurity measures, and as a result cause a reduction of incidence/prevalence of infection/disease.	Intervention looked at biosecurity measures to prevent personnel spreading PEDV across animal facilities. PEDV negative pigs were inoculated with PEDV and housed with uninfected pigs to assess transmission by contact. To prevent subsequent spread of PEDV, four biosecurity protocols were applied: (i.)low biosecurity protocol [movement through soiled corridor; no changes of clothes or footwear; no washing of hands or face]; (ii.) medium biosecurity protocol [movement through clean corridor only after procedures were followed; wash hands and face; change clothes and footwear;]; (iii.) high biosecurity protocol [movement through clean corridor only after procedures were followed; shower; change clothes and footwear;]; (iv.) negative control protocol [no movement of people or fomites between the different rooms of the animal facilities; dedicated study personnel different from personnel attending the other groups; shower, clean clothes and footwear each time entering the room].	Low biosecurity: PEVD spread to other rooms in the first 24 hours. Medium and high biosecurity no such spread occurred for the duration of the trial. These results indicate the indirect transmission of PEDV through contaminated personnel PPEs occurs rapidly under modelled conditions and to prevent transmission between groups of pigs, changing PPE and/ or taking a shower is recommended as an effective option to lower the risk of virus spread.	High

Appendix III: Summary of studies including WASH and biosecurity interventions (continued)

Study	Country	Study design	Type of production system	Setting	Animal of interest	Main objective	Outcome of interest	Intervention Content	Results	Overall Risk of Bias
Karzis et al., 2018[49]	South Africa and Zambia	Non-randomised trial	Mixed	Not specified	Cattle: Dairy	To investigate trends of resistance of S. aureus to antibiotics administered to dairy cows and to identify possible contributing factors.	The resistance of *S. aureus* strains to eight commonly used antibiotics in South Africa from 2001 to 2010 was evaluated via antimicrobial susceptibility testing. S. aureus isolates were selected from cows with subclinical mastitis in 20 herds routinely sampled as part of the proactive udder health management programme.	Intervention implemented a proactive udder health management programme, using the Milk Sample Diagnostic (MSD) computer programme (Abaci Systems, Aretsi SA, Pretoria), was developed to sample all lactating cows in a herd for both microbiological and cytological evaluations in order to identify S. aureus-positive cows. This information assisted managers and advisors in making management decisions. cows with udder infections caused by *S.aureus* could be separated from those not infected and milked last or if necessary be removed from the herd separate camp and milked last. Selection of the antibiotic for treatment was based on antibiotic susceptibility testing results, microbiological cure was monitored and chronic cases were culled as soon as possible. Milking hygiene practices were introduced including disinfection of milker's hands or gloves, pre- and post-teat dipping, application of disinfectants, and testing of milking systems. A veterinarian visited the herd if there was no improvement and the protocol was immediately adjusted according to findings and observations made during the visit.	There was a significant ($p < 0.01$) increase in percentage of susceptible S. aureus isolates over time for all antibiotics tested (except ampicillin). The overall prevalence of mastitis did not change during the study period.	High
Chuppava et al., 2018b[123]	Germany	RCT	Intensive farming	Rural	Turkey	To evaluate the effects of different types of flooring designs on antimicrobial resistance in commensal Escherichia coli from turkeys treated with enrofloxacin.	*E. coli* in cloacal swabs and the turkey manure and resistance of isolates to enrofloxacin and ampicillin.	Intervention limited contact intensity with excreta through adjusting flooring design and a changed environment after using antimicrobials (enrofloxacin). The flooring designs of the pens were assigned to four groups; G1 – entire floor pen covered with litter, G2 – floor pen with heating, G3 – partially slatted flooring including an area that was littered, G4 – fully slatted flooring with a sand bath.	In trial 1 the group with floor heating (G2; average floor temperature in all trials: G1 = 27.0/G2 = 30.5/G3 = 26.5/G4 = 26.0C) showed a significantly higher number of resistant E. coli isolates than the other groups. Enrofloxacin resistance in E. coli isolates from all samples did not show any differences between the groups during the BT stage. Overall, frequencies of enrofloxacin resistance were significantly different between the unchanged and changed environment. At the same time, the proportion of ampicillin-resistant isolates increased in the first trial, although no ampicillin was applied in this study. During the after-trial stage, cloacal swab isolates from G4 showed significantly lower resistance than the other groups. E. coli isolates from samples of G1 demonstrated lower resistance means than in the other groups.	High

Appendix III: Summary of studies including WASH and biosecurity interventions (continued)

Study	Country	Study design	Type of production system	Setting	Animal of interest	Main objective	Outcome of interest	Intervention Content	Results	Overall Risk of Bias
Chuppava et al., 2018a[122]	Germany	RCT	Intensive farming	Rural	Turkey	To evaluate the effect of double antibiotic treatment with enrofloxacin or solely environmental exposition on resistance against antibacterial agents in commensal E. coli, depending on different flooring.	A total of 864 Escherichia coli isolates were obtained from cloacal swabs and poultry manure samples at days 2, 9, 15, 21, and 35. The broth microdilution method (MIC) was used to determine the resistance of isolates to enrofloxacin and ampicillin.	Intervention evaluated the effect of double antibiotic treatment with enrofloxacin or solely environmental exposition on resistance against antibacterial agents in commensal E. coli, depending on different flooring. Animals were kept on dry wood shavings or floor pen covered with litter combined with an electrical floor heating system. Animals in these two groups continuously had full contact with manure. Additionally, in the third group pens were divided into two equal parts consisting of 50% solid flooring with wood shavings and 50% plastic slatted flooring. In the last group (G4), plastic slatted flooring with a sand bath was used, the bath being disinfected and the sand replaced on a daily basis. Animals in the fourth group had no contact with litter except in the sand bath.	A double antibiotic treatment with enrofloxacin reduced the proportion of susceptible Escherichia coli isolates significantly in all flooring designs. Simulation of water losses had no significant effect, nor did the flooring design. Ampicillin-resistant isolates were observed, despite not using ampicillin.	High
Ng et al., 2010[132]	Nepal	Case-control	Subsistence farming	Rural	Water buffalo	To measure the impact of a livestock hygiene education program on mastitis in smallholder water buffalo.	Indicators of knowledge and practice for the prevention and control of mastitis in buffalo and reduction of incidence/prevalence of mastitis. The California Mastitis Test was used to diagnose sub-clinical mastitis from milk samples, and the IDEXX SNAP test to identify the presence of tetracycline residues.	Intervention based on informational handouts and public health social mobilization training to make changes in women's food production and preparation, including hand washing before and after milking, safe food storage, proper boiling of milk, and changes in water supply to improve livestock and human hygiene for mastitis prevention and management in smallholders of water buffalo	The prevalence of mastitis in trained households (39.4%) was 43.78% of that in untrained households (60.4%), lower but not significantly so (p = 0.08, 95% CI 0.17–1.12). Thirteen (52.0%) indicators were positively influenced by training, four (16.0%) significantly so (p < 0.05). Trained households were 3.41 times (p = 0.001, 95% CI 1.72–6.73) more likely to remove milk from sick buffalos from household consumption. Trained households were 2.35 times (p = 0.01, 95% CI 1.21–4.48) more likely to remove the milk of antibiotic treated buffalos from household consumption. Trained households were 3.50 times (p = 0.001, 95% CI 1.73–7.07) more likely to wash their hands with soap before milking. Finally, trained households were 4.19 times (p = 0.03, 95% CI 1.15–15.24) more likely to have brick, wood or concrete flooring in their barn. Two (8.0%) indicators were not positively influenced by training, and counter-intuitively one of these was significantly associated with untrained households. Trained households were significantly less likely (OR 0.28) to know to collect stripped milk in a container rather than to strip onto the ground (p = 0.001, 95% CI 0.13–0.59)."	High

Appendix III: Summary of studies including WASH and biosecurity interventions (continued)

Study	Country	Study design	Type of production system	Setting	Animal of interest	Main objective	Outcome of interest	Intervention Content	Results	Overall Risk of Bias
Hao et al., 2014[128]	China	Non-randomised trial	Intensive farming	Peri-Urban	Poultry: Laying hens	To determine whether slightly acidic electrolyzed water spray is a potential method for reducing microbial presence in layer houses.	The air temperature, relative humidity, dust concentration, and microbial population were measured at the sampling points in the five zones during the study period.	Intervention used a tunnel-ventilation system, with an evaporative cooling. Slightly acidic electrolyzed water spray (SAEW) was sprayed by workers in the whole house. The BC (1:2000) and PVP-I (1:1000) disinfectant solutions were used as controls. The experimental area was divided into five zones along the length of the house, with zone 1 nearest to an evaporative cooling pad and zone 5 nearest to the fans. A six-stage air microbial sampler was used to measure airborne microbial population.	The population of airborne bacteria and fungi were sharply reduced by 0.71x10e5 and 2.82x10e3 colony-forming units (CFU) per cubic metre after 30 minutes of exposure to SAEW, respectively. Compared with the benzalkonium chloride (BC) solution and povidone-iodine (PVP-I) solution treatments, the population reductions of airborne fungi treated by SAEW were significantly ($p < 0.05$) more, even though the three disinfectants can decrease both the airborne bacteria and fungi significantly ($p < 0.05$) 30 minutes after spraying.	High
Nagahata et al., 2007[138]	Japan	Non-randomised trial	Intensive farming	Rural	Cattle: Dairy	To assess the impact of a rigorous mastitis control program on the prevalence of S. aureus, mastitis, and milk quality on a dairy farm.	S. aureus was isolated from milk samples, plated on blood agar, and biochemically identified. Additionally, the somatic cell count in the bulk milk of the herd was determined.	Intervention changing milking practices to prevent mastitis: (a) maintenance of the correct milking order, i.e., normal cows first and infected cows or cows showing elevated SCC last; (b) use of disposable plastic gloves for the workers, and individual towels for wiping the teats of each cow; (c) post-milking teat dipping with approved disinfectants; (d) treatment of infected quarters by antibiotic infusion at drying-off; and (e) culling of cows infected with S. aureus in two or more quarters or cows having chronic S. aureus infection.	Eighteen months after the control program was started, the rate of S. aureus infection in the quarter milk decreased dramatically ($p < 0.05$), and no S. aureus isolates were found in the milk of the remaining cows. The SCC in the bulk milk of the herd dropped to a monthly mean of <20 × 10e4 cells/ml.	Moderate
Omore et al., 1999[139]	Kenya	RCT	Small-holders	Rural	Cattle: Dairy	To assess the technical and financial impact of mastitis and mastitis control in smallholder dairy farms.	There were five objectives that relate to outcome of interest: 1. Improve mean milk yield per farm 2. Reduce SCC 3. Reduce prevalence of mastitis causing pathogens 4. Reduce mastitis incidence 5. Minimise financial losses	Intervention compared three types of mastitis control programmes: (i.) Group (A)improved management practices (a) hand and udder washing with disinfectant before milking; (b) post-milking teat dipping; and (c) proper milking technique (d) Iodophor udder wash and teat dip; (ii.) Group B: Therapy of subclinical cases, (iii.) Group C: Improved management practices and therapy of subclinical cases combining i. and ii.; Group D: Non-intervention group.	The overall incidence of clinical mastitis was 10.8 per 100 cow-years at risk and was not significantly different between the groups ($p=0.05$). Contagious and environmental pathogens were negatively associated with the combined interventions of milking hygiene and therapy of subclinical cases ($p<0.05$). The biggest impact was the reduction in the prevalence of contagious pathogens by 18% (1.60/9.06) under the combined intervention of milking hygiene and therapy of subclinical cases. There were no significant increases in milk yield brought about by the interventions. The average total cost of drugs, fees and discarded milk incurred for each case of clinical mastitis was estimated to be Ksh 1010, which at the annual incidence of clinical mastitis of 10.8%, this translates into Ksh 109 per cow per year.	High

Appendix III: Summary of studies including WASH and biosecurity interventions (continued)

Study	Country	Study design	Type of production system	Setting	Animal of interest	Main objective	Outcome of interest	Intervention Content	Results	Overall Risk of Bias
Luyckx et al., 2016a[153]	Belgium	Non-randomised trial	Intensive farming	Peri-Rural	Pigs	To evaluate the effect of a 10-day vacancy period in pig nursery units on bacterial load in pig nursery units.	The microbiological load was measured for total aerobic flora, Enterococcus spp., E. coli, faecal coliforms and methicillin resistant S. aureus (MRSA) using selective media.	Intervention evaluated the effect of a 10-day vacancy period in pig nursery units on total aerobic flora, Enterococcus spp., Escherichia coli, faecal coliforms and methicillin resistant Staphylococcus aureus (MRSA). Three vacancy periods of 10 days were monitored, each time applied in 3 units. The microbiological load was measured before disinfection and at 1, 4, 7 and 10 days after disinfection.	No significant decrease or increase in *E. coli*, faecal coliforms, MRSA and *Enterococcus spp.* was noticed.	Low
Lopes et al., 2015[142]	Brazil	RCT	Experimental set-up	Urban, Peri-Urban	Poultry	To evaluate the application of quicklime or tarping, alone versus together, on the prevalence of bacteria in poultry litter.	Poultry litter was periodically assessed for internal temperature, as well as microbiological analysis (total bacteria, Staphylococcus, and Enterobacteriaceae).	Intervention using a randomized design, in which four in-house litter treatments were implemented in poultry farms for 12 days: no treatment (control), quicklime (300 g per metre squared), tarping, or tarping + quicklime (300 g per metre squared).	The use of quicklime alone or quicklime + tarping was more effective ($p < 0.05$) in reducing bacteria when compared to litter tarping. Except for the control group, all treatments resulted in a more than 84% reduction in the count of colony-forming units (CFUs) at the end of fallowing.	High
Roll et al., 2011[143]	Brazil	Non-randomised trial	Intensive farming	Varied	Poultry	To verify the occurrence of *Salmonella* in broiler litters reused up to 14 times in Brazilian poultry farms.	*Salmonella* presence was identified in broiler litter samples using antiserum containing specific Salmonella antibodies.	Intervention on the safety of reusing poultry litter. Litter samples (reused up to 14 times) from Brazilian broiler farms were analysed to verify the occurrence of Salmonella through serological testing.	There is a significant decrease ($p < 0.05$) in the count of samples positive for Salmonella with the reuse of litter. After the sixth reuse of the litter, values of samples positive for *Salmonella* are significantly ($p < 0.0001$) lower than expected.	Low
Sonoda et al., 2012[144]	Brazil	RCT	Experimental set-up	Not relevant	Poultry	To evaluate two methods of broiler litter fermentation based on composting concepts and their effect on litter and the air quality during fermentation in small-scale broiler houses.	Before beginning the treatment, six litter samples were collected from each house and analysed for total nitrogen content, humidity, pH and microbial counts. Litter humidity, gas emission (NH_3 and CO_2), environmental temperature, air relative humidity, and air velocity were determined during and after composting.	Intervention wherein litter from the same grow-out (one, two or three) was distributed in two experimental houses, where it was either piled or spread.	Bacterial population, especially of Salmonella spp., was higher when the litter was piled compared with spread litter. Fungi population decreased after composting. Neither piling, not spreading were able to significantly reduce bacterial counts, specifically Salmonella spp., when the population before and after fermentation were compared.	High

Appendix III: Summary of studies including WASH and biosecurity interventions (continued)

Study	Country	Study design	Type of production system	Setting	Animal of interest	Main objective	Outcome of interest	Intervention Content	Results	Overall Risk of Bias
Zhang, 2011[129]	China	Non-randomised trial	Intensive farming	Not specified	Poultry: Broilers	To investigate the impact of indoor air quality on the production of broilers.	The weight gain, mortality rate and serum cholesterol level of broilers were monitored as the benefit indicators of air quality and antibiotics.	Intervention in the air quality of broiler houses. Three broiler houses of different air quality environments with different controlled ventilation rates and uses of super plasma ionizing (SPI) air purifiers were investigated. The broilers were fed with antibiotic or non-antibiotic feeds to determine if the air quality control could be sufficient to keep broilers healthy without using antibiotics. The weight gain, mortality rate and serum cholesterol level of broilers were monitored as the benefit indicators of air quality and antibiotics.	Higher weight gain of broilers was observed in the broiler house with a higher ventilation rate. SPI air purifiers improved weight gain of broilers, however, the significance of this change varied. The uses of higher ventilation rate, SPI air purifier and antibiotics significantly reduced the mortality rate of broilers.	Low
Zhao et al., 2019[124]	China	RCT	Intensive farming	Not specified	Ducks	To investigate the protective role of dryland rearing on netting floors on Shaoxing duck mortality.	Laying rates, feed-egg ratios, and mortality rates were assessed. Serum immune parameters, including thymus index, spleen index, levels of immunoglobulin G (IgG), interleukin 1**β** (IL-1**β**), and tumoral necrosis factor **α** (TNF-**α**), were determined. Sequencing of the 16S rRNA gene was used to analyse the variability of gut microbiota in the duck ileum and cecum.	Intervention in which Chaoxing ducks were divided into two groups; the control group was raised in a dry-haulm bedding duck house accompanied by an open-air swimming pool, while in another duck house, the treatment group was kept on a 45 cm-high netting floor, without any swimming pool.	Dryland rearing on netting floors significantly reduced the mortality rate of the ducks and increased the thymus index ($p < 0.05$), compared to the control. No other significant differences were detected in productional and immune indices ($p > 0.05$).	High
Dee et al., 2004[151]	United States of America	Non-randomised trial	Experimental set-up	Not specified	Pigs	To test four intervention strategies for their ability to prevent the mechanical transmission of porcine reproductive and respiratory syndrome virus.	Swabs were collected from selected sites and tested by TaqMan polymerase chain reaction for PRRSV RNA and by swine bioassay to confirm the presence of infectious PRRSV.	Intervention in which four strategies were tested for their ability to prevent the mechanical transmission of porcine reproductive and respiratory syndrome virus (PRRSV): the use of disposable plastic boots to prevent contamination of personal footwear, the use of boot baths to disinfect PRRSV-contaminated plastic boots, the use of plastic slatted (Polygrate) flooring in the anteroom to prevent PRRSV contamination of incoming personal footwear, and the use of bag-in-a-box shipping methods to prevent PRRSV contamination of the contents of a container destined for a swine farm.	The use of disposable boots, bleach boot baths or bag-in-a-box shipping methods was highly efficacious in preventing mechanical transmission of PRRSV. The use of Polygrate flooring in the anteroom did not prevent contamination of personal footwear (no significant difference, $p = 0.24$).	High

Appendix III: Summary of studies including WASH and biosecurity interventions (continued)

Study	Country	Study design	Type of production system	Setting	Animal of interest	Main objective	Outcome of interest	Intervention Content	Results	Overall Risk of Bias
Kaneene et al., 2016[133]	Uganda	Non-randomised trial	Small-holders	Not specified	Cattle: Dairy	To measure the effectiveness of AgShare (biosecurity program) in improving milk quality and safety.	Farm hygiene scores, milk, and sera were collected to measure milk quality and safety at the beginning of AgShare and 11 months after participation. Milk quality was determined by somatic cell count scores, and milk safety was measured by the prevalence of mastitis and brucellosis. Qualitative assessments ofAgShare impact were conducted by an external reviewer at the end of the program, and through interviews with study participants.	Intervention focused on AgShare university–led action research–based approach to educating farmers in the dairy value chain. Based on findings from the needs assessments and baseline data describing dairy farms in PhaseI, three issues were identified for interventions: diseases affecting milk production; milk quality and safety and mastitis and brucellosis prevention and treatment. Educational materials were developed for student training to aid farmers in improving dairy production, quality, and safety, by emphasizing dairy production hygiene and the importance of subclinical mastitis.	Prevalence of mastitis decreased after participation in AgShare ($p < 0.05$). Improved milk quality and safety were significantly ($p < 0.05$) associated with increasing hygiene scores. Qualitative assessments indicated that over 90% of participants indicated that AgShare was beneficial, and farmers' communities benefitted by employing similar interventions and methods in their own dairy production systems.	High
Edrington et al., 2006[141]	United States of America	Non-randomised trial	Intensive farming	Not specified	Cattle: Beef	To determine whether seasonal shedding patterns observed for *E.coli* O157:H7 are a result of changing day length and subsequent physiologic changes, such as hormone profiles, within the host.	To understand the impact of day length and artificial lighting times on *E.coli* O157 prevalence in faecal matter of intervention and control pens	Intervention in the lighting times in a commercial feedlot. Four pens of cattle received approximately 5 hours of artificial lighting daily (in addition to the natural light) for 60 days and four pens served as controls.	*E.coli* prevalence in the control pens was lower after 25 and 53 days of artificial lighting. There was a higher correlation between day length and E. coli O157:H7 prevalence ($r = 0.67$) than temperature and prevalence ($r = 0.43$). After 53 days the study found three-fold higher faecal prevalence of E. coli O157 when compared to the unlighted control pens ($P= 0.008$). Early results indicate that the hormone melatonin, which is secreted seasonally, similar to patterns of E. coli O157:H7 shedding, may play a role in this pathogen's populations and/or shedding in beef cattle (unpublished results).	High
Dee et al., 2012[130]	United States of America	Non-randomised trial	Intensive farming	Not specified	Pigs	To evaluate the correlation between air filtration and incidence of PRRSV infection among pigs.	To reduce rate of PRRSV infection incidence by filtering air in pig farms.	Intervention in the air filtration in pig herd housing. Participating herds ($n = 38$) were organized into 4 independent cohorts and the effect of air filtration on the occurrence of new PRRSV infections was analysed at 3 different levels from September 2008 to January 2012 including the likelihood of infection in contemporary filtered and non-filtered herds, the likelihood of infection before and after implementation of filtration and the time to failure in filtered and non-filtered herds.	New infections, and time until new infections, were lower in filtered breeding herds compared to those without filtration; 8 infections in the filtered herds versus 89 in non-filtered herds. Therefore, new infections in filtered breeding herds were significantly lower ($P<0.01$), odds of new infection before filtration were 7.97 times more likely than after ($P<0.01$) and median time until new infection was significantly longer in filtered herds ($P<0.01$).	High

Appendix III: Summary of studies including WASH and biosecurity interventions (continued)

Study	Country	Study design	Type of production system	Setting	Animal of interest	Main objective	Outcome of interest	Intervention Content	Results	Overall Risk of Bias
Alali et al., 2010[149]	United States of America	Ecological	Intensive farming	Not specified	Poultry	To compare the prevalence and resistance profiles of Salmonella among organic and conventional broiler poultry farms.	To understand the effects of chickens raised without antibiotics (RWA) on the prevalence of antimicrobial resistance (AMR) of Salmonella. Samples of faeces, food, and water were collected and analysed for salmonella presence using enrichment techniques. Phenotypic (antimicrobial susceptibility) and genotypic testing was done using pulsed-field gel electrophoresis (PFGE).	Intervention comparing prevalence of salmonella among organic or conventionally raised chicken populations to assess the impact of administering antibiotics on AMR. In this study there were two groups - 1 Chickens raised with antibiotics (n=400) 2 Chickens raised without antibiotics (n=300).	Prevalence of faecal Salmonella was lower in certified-organic birds than in conventionally raised birds. In addition, the prevalence of antimicrobial-resistant Salmonella was higher in conventionally raised birds than in certified-organic birds. The prevalence of Salmonella in faecal and feed samples adjusted for the dependence of isolates within farms was significantly ($p < 0.05$) higher (OR = 11.9 and 7.2, respectively) in conventional farms than in organic farms. The study indicates that organic rearing techniques (such as feeding) are linked to a lower prevalence of salmonella, and AMR salmonella.	High
Berg, 2015[134]	India	Cross-Sectional	Mixed	Rural	Cattle: Dairy	To investigate whether the International Livestock Research Institute hygiene training project affected the health status of the animals and the economy of the farmers.	To improve overall health, quality, and hygiene of cattle in rural dairy farms and reduce positive microbiological culture. Methods of assessment included clinical examinations to check body condition, hygiene status, and hoof status of cows. Milk sampling (using the Brucella milk ring test) was used to test milk seroprevalence of brucellosis. A questionnaire was used to interview farmers about hygiene routines, health status, milk yield, etc.	Intervention consisted of a training programme by the International Livestock Institute on hygiene and quality aimed at informal dairy farmers in North India. The farmers took part in a 5-day training course with daily lectures and practical exercises concerning topics such as good husbandry, hygienic milking routines and milk handling.	The results showed that IRLI hygiene training had positive effects in some areas, including a mean increase of 0.67 litres of milk per cow per day in trained farms. While no difference was seen between trained and non-trained farms regarding body condition, hygiene, and hoof status, a majority of the trained farmers experienced improvement in the overall health status of animals (87.5%). At least half of the tested cows in Assam were positive for brucellosis and there was no difference between trained and non-trained farmers, unsurprising as hygiene training did not focus on disease control. The results showed that the ILRI hygiene training was associated with a significant increase in the milk yield ($p=0.003$). There was also statistical significance in the general knowlede of diseases ($p=0.008$) and in milk hygiene ($p=0.001$).	Moderate
Chuppava et al., 2019[121]	Germany	RCT	Intensive farming	Not specified	Poultry: Broilers	To examine the occurrence and development of antimicrobial resistance and prevalence of multi-drug resistant *E. coli* isolated from excreta and manure samples from large-scale broiler housings with special consideration of the effects of different flooring designs.	To reduce the prevalence of AMR *E. coli* among chicken populations. Collection of excreta and manure samples took place at different points of time during the experiment. Swabs were processed on Gassner agar plates and incubated, then tested for AM presence. Antibiotics were also used to test samples for antibiotic susceptibility.	Intervention compared the effects of chicken coop flooring and the prevalence of AMR *E. coli* in faeces. The study consisted of two groups: 1 - elevated flooring (n=15,000), 2 - control group (n=15,000).	Despite less contact with manure, the experimental group (on elevated platforms) had a significantly higher prevalence of resistant *E. coli* due to the birds preferring elevated areas and clustering here with high population density, leading to greater animal-to-animal contact. The study concluded that animal-to-animal contact was more important than excreta-contact for spreading AMR. The study indicated that reduction of AMR *E. coli* is not likely to be achieved by changing coop flooring but instead by preventing animal-to-animal contact and ensuring animals not carrying resistance at the start of life are obtained.	High

Appendix III: Summary of studies including WASH and biosecurity interventions (continued)

Study	Country	Study design	Type of production system	Setting	Animal of interest	Main objective	Outcome of interest	Intervention Content	Results	Overall Risk of Bias
Abdalla and Elhagaz., 2011[140]	Sudan	Non-randomised trial	Mixed	Not specified	Cattle: Dairy	To evaluate the efficiency of hygienic practices prior to milking in improving raw milk quality.	To minimize microbiological, chemical and physical contamination in milking practices to improve health of cattle and quality of their milk. Samples taken before and after each treatment, and tested for total bacterial count, coliform count, *Staphylococcus aureus* count and titratable acidity.	Intervention consisted of implementing different hygiene practices: 1=no hygienic practices, 2= milker rubs udders and hands with clean wet towels before milking, 3= milker washed hands with soap and water, put on a clean coat, covered his hair, rubbed udders, teats, and hands with clean wet towel. The intervention consisted of one group (n=60) exposed to all three treatments.	Total bacterial, *S. aureus* and coliform counts were lower after hygienic practices were applied; total bacterial count reduced to about 9% when treatment No.2 was applied and to about 20% when treatment No. 3 was applied. There was a significant effect of application of hygienic practices prior to milking on total bacterial count, *S. aureus* count and coliform count (P=0.001). However, the titratable acidity did not show any significant variation concerning the application of hygienic practices (P>0.05). The application of hygienic practices associated with milking routine, cleanliness of cow's udders. teats and cleanliness of milker's hands, wearing clean clothes and hair covering, were very efficient and effective in improving raw milk quality and reduced bacterial contamination	Low
Abd El-Wahab et al., 2013[127]	Germany	RCT	Intensive farming	Not specified	Turkey	To test the effects of poultry diets with normal levels of electrolytes versus a surplus level of electrolytes, both with and without using floor heating.	To reduce the development and severity of foot-pad dermatitis in turkeys. External assessment of footpads took place at weekly intervals, killing of all birds at the end of experiment for histopathological scoring, sampling of excreta, measurement of body weight and calculation of feed conversion ratio.	Intervention testing different feed-types and flooring for their impact on prevalence of food-pad dermatitis in turkeys. The study consisted of four groups: 1 - normal electrolye feed and floor heating (n=20), 2-normal electrolyte feed and wet litter (n=20), 3-surplus electrolyte feed and floor heating (n-20), 4- surplus electrolyte feed and wet litter (n=20).	Combining low Na and K levels with a floor heating system reduced the scores of Foot pad dermatitis (FPD) by approximately 60%, compared with high electrolyte levels without floor heating. Feeding the high electrolyte diet resulted in significantly higher external and histopathological FPD scores in comparison with those for birds being fed normal electrolyte levels ($p < 0.05$). Furthermore, using floor heating resulted in significantly lower external and histopathological FPD scores compared with groups without floor heating ($p < 0.05$). The study indicated that both dietary electrolyte levels and floor heating markedly affected FPD via litter moisture, suggesting that moisture is a key factor in the development and severity of FPD.	Moderate
Collineau et al., 2017[135]	Belgium, France, Germany, Sweden	Non-randomised trial	Intensive farming	Not specified	Pigs	To assess, across four countries, the technical and economic impact of herd-specific interventions aiming at reducing antimicrobial usage in pig production.	Outcomes of interest were analysis of annual farm costs, calculation of antimicrobial treatment incidence in herds, and analysis of farm technical performance including both reproductive parameters (i.e. litter size and farrowing index) and growth parameters (i.e. feed conversion ratio and daily weight gain during the fattening period and final weight of the finisher pigs), as well as mortality rates in suckling pigs, weaners and fatteners.	Intervention included herd-specific adjustments that were different per farm but related to six measures: (i) improvement of external biosecurity status; (ii) improvement of internal biosecurity status; (iii) modifications of the herd vaccination scheme; (iv) changes in feed or drinking water composition, safety or quality; (v) better pig health care or welfare; and (vi) pig stable climate and other zootechnical measures. This study involved four groups of farms in different countries: 1 - Belgian (n=16), 2 - French (n=20), 3 - German (n=25), 4 - Swedish (n=9).	A median reduction of 47.0% of antimicrobial usage was achieved across four countries when expressed in terms of treatment incidence from birth to slaughter, corresponding to a 30.5% median reduction of antimicrobial expenditures. Farms with higher level of compliance with the predefined plan tended to achieve bigger reduction, but the association was not statistically significant (Spearman's rank correlation =-0.18, $p = 0.162$). Reduction of TI200d and reduction of antimicrobial expenditures were highly correlated (Spearman's rank correlation =0.63, $p < 0.001$). No significant change was observed in the mortality rates of suckling piglets, weaners and fatteners before and after intervention.	Moderate

Appendix III: Summary of studies including WASH and biosecurity interventions (continued)

Study	Country	Study design	Type of production system	Setting	Animal of interest	Main objective	Outcome of interest	Intervention Content	Results	Overall Risk of Bias
Postma et al., 2017[136]	Belgium	Non-randomised trial	Intensive farming	Not specified	Pigs	To evaluate the potential reduction in antimicrobial use by promoting prudent usage combined with farm management optimization through the guidance of farmers.	Data were collected by means of a visual herd inspection during the visit of the herd and a paper and pencil questionnaire about the production results and herd management characteristics. Technical parameters were recorded (including growth performance and mortality). The level of biosecurity was scored for each herd using the Biocheck.UGent risk-based scoring system.	Intervention in which herd-specific intervention strategies were produced, focussing on the optimization of herd management, biosecurity status, vaccination strategy, anthelmintic therapy and advice on prudent AMU (n=61 herds).	A significant (p < 0.05) improvement of 2.4 points external, and 7 points internal biosecurity on the herds was obtained, combined with additional vaccination, anthelmintic therapy and prudent AMU. As well, there was significant reduction in the AMU with a decrease of 52% for the pigs from birth till slaughter and 32% for breeding animals. Production parameters significantly improved, with the number of weaned piglets per sow per year (+1.1), daily weight gain (+5.9 g/day) and mortality in the finisher period (-0.6%).	High
Line and Bailey, 2006[145]	United States of America	RCT	Intensive farming	Not specified	Poultry: Broilers	To determine the effect of two acidifying litter treatment chemicals on *Salmonella* and *Campylobacter* prevalence in commercial broiler houses.	Samples were collected from each broiler house and assessed for Salmonella and Campylobacter. Samples included faecal, litter, environmental drag swabs, chick transport pads, and on-farm broiler carcass rinses.	Intervention using commercially available litter treatments - aluminum sulfate and sodium bisulfate. The study consisted of three groups: 1 (n=5) aluminium sulfate-treated houses, 2 (n=5) sodium bisulfate-treated houses, 3 (n=10) untreated control houses.	No significant difference (P > 0.05) in mean Campylobacter populations was observed between treated and control houses for either treatment; likewise, no significant effect on Salmonella populations was indicated (P > 0.05). There was no significant effect (P > 0.05) of sodium bisulfate treatment on Salmonella prevalence in the broiler houses, nor was there a significant effect (P > 0.05) of aluminum sulfate treatment on Salmonella prevalence in the faecal samples.	High
Speksnijder et al., 2017[137]	The Netherlands	RCT	Intensive farming	Not specified	Cattle: Dairy	To evaluate the effects of a facilitated animal health planning program (developed by an advisory team consisting of a dairy farmer, his veterinarian, and his feed adviser) on animal health, production parameters, and antimicrobial use.	Various antimicrobial use parameters and animal health parameters were recorded pre- and post-intervention. Additionally, data were collected and scored on the following parameters; udder health, metabolic health and transition management, fertility, young stock rearing, housing and animal nursing, nutrition and production, collaboration within the advisory team, antimicrobial reduction, and miscellaneous.	Intervention in which the advisory team developed a farm-specific animal health planning program with support from the facilitator. They compared 20 intervention farms with 19 control farms.	In the intervention group, AMU was significantly lower at the end of the study period compared with the start of the study (−19%; p = 0.026), whereas in the control group the AMU was not significantly different from the initial situation (−14%; p = 0.091). Animal health parameters were not significantly different between the intervention and control groups at the start of the study.	High

Appendix III: Summary of studies including WASH and biosecurity interventions (continued)

Study	Country	Study design	Type of production system	Setting	Animal of interest	Main objective	Outcome of interest	Intervention Content	Results	Overall Risk of Bias
Nietfeld et al., 1998[146]	United States of America	RCT	Intensive farming	Not specified	Pigs	To evaluate a strategy to prevent Salmonella infection in pigs from a herd with an ongoing clinical problem with *S. cholerasuis* by weaning pigs offsite.	On the day the pigs were purchased, milk and rectal swab samples were collected from sows in the herd of origin. On days 1, 43, 83, and 109 of the study, rectal and oropharyngeal swabs were collected for Salmonella spp. culture and sera were collected for serologic testing for Salmonella antibodies from all offsite pigs.	Intervention assessing the strategic movement of piglets in combination with different antibiotic treatments. In this study piglets were randomly allocated into one of two experimental groups: piglets that received daily intramuscular injections of 0.3 mL (15 mg) ceftiofur on days 1–5, and piglets that received no parenteral antibacterial agents throughout the trial. On day 43, both groups of pigs were moved to an indoor finisher facility with a partially slatted concrete floor. Pigs were housed according to treatment, seven pigs to a pen. On day 140, pigs were sold to slaughter. Meanwhile, on day 61 of the study, 15 pigs from the same farrowing cohort as the offsite pigs were moved from an inside nursery, ear tagged for identification, and moved to an outdoor grow-finish pen. These 15 pigs served as onsite controls.	In the offsite pigs, no Salmonella spp. were isolated and there was no serological evidence of Salmonella exposure. On the farm, Salmonella spp. were isolated from rectal swabs from 7 of the 15 onsite, age-matched pigs. Between days 61 and 99 of the study, mean anti-Salmonella titers for onsite pigs increased, indicating ongoing exposure to Salmonella spp.	High
Skanseng et al., 2013[125]	Norway	Non-randomised trial	Experimental set-up	Not specified	Poultry: Broilers	To investigate the effects of different feed structures and beddings (with or without litter) on the spread of *C. jejuni* in broiler flocks and the effect on the cecal microbiota.	Samples from the cloacal mucosa of all birds were taken daily for *C. jejuni* quantification and cecum samples were collected at the end of the experiment for *C. jejuni* quantification and microbiota analyses.	Intervention in which broiler chickens were raised in 24 eight-bird group cages on either rubber mat or wood shavings and were fed either a wheat-based control diet (Control), a diet where 50% of the ground wheat was replaced by whole wheat prior to pelleting (Wheat), or a wheat-based diet, such as the control diet diluted with 12% oat hulls (Oat).	Increased feed structure (inclusion of oat hulls and whole wheat) delayed the spread of *C. jejuni* in broiler flocks. This effect was significantly stronger in birds kept on rubber mats. At Day 3–5 pi, there was a statistically significant difference in the spread of *C. jejuni* between chickens given different feeds, with the highest spread in flocks given control diet. The effect of bedding was only statistically significant at Day 5 pi, where the spread was lower in birds on rubber mats. Only at Day 4 pi, with rubber mat as bedding, there was a statistically significant difference ($p = 0.004$) between diets oat hulls and whole wheat.	Low
Davies et al., 1997[147]	United States of America	Cross-Sectional	Intensive farming	Not specified	Pigs	To assess the effects of raising pigs in different production systems on Salmonella prevalence in finishing swine.	Faecal samples were assessed for prevalence of Salmonella spp.	Intervention compared farms that were either finishing sites using all-in-all-out-management of buildings in multiple-site systems (14 farms) or farrow-to-finish systems using continuous flow management of finishing barns (15 farms) and looked at differences in Salmonella prevalence as a result of difference in bio-management practices.	The proportion of herds which were positive for Salmonella differed ($p < 0.04$) between groups A and B, with all five negative herds in the farrow-to-finish, continuous sow group (group B). Moreover, mean prevalence was lower ($p < 0.06$) for herds where pigs were housed on slotted floors (16.5%) compared with herds raising pigs on other surfaces (36.7%).	Moderate

Appendix III: Summary of studies including WASH and biosecurity interventions (continued)

Study	Country	Study design	Type of production system	Setting	Animal of interest	Main objective	Outcome of interest	Intervention Content	Results	Overall Risk of Bias
Dahl et al., 1997[148]	Denmark	Non-randomised trial	Intensive farming	Not specified	Pigs	To assess the effects of strategically moving pigs at different ages on the presence of S. typhimurium in three pig herds.	*S. typhimurium* was detected either serologically (using ELISA) or in faeces (bacteriologically) in three pig herds.	Intervention focusing on the strategic movement of pigs. In the experimental group, pigs were moved either at weaning, from the nursery, or from the grower unit to newly built or rigorously cleaned and disinfected finishing units with no known history of Salmonella infection an Salmonella-controlled feed. Control groups were pigs living on normal finisher farms. Additional measures aimed at avoiding the transfer of infectious material from the old finishing unit, including the use of separate boots and tools for each unit. This included cleaning and disinfection before first arrival pigs, avoid faecal contact between pens.	No detectable infection was observed at slaughter either serologically or bacteriologically by random testing of the pigs which had been moved, whereas a proportion of the pigs raised at the same time in the continuous systems on the farms were found to be infected ($p < 0.0044$).	High
Berk, 2007[126]	Germany	Non-randomised trial	Intensive farming	Not specified	Poultry: Broilers	To assess the effects of 5 different kinds of litter on the development of foot pad dermatitis and performance of male broilers.	Randomly selected broilers were weighed and assessed for foot pad dermatitis on a weekly basis.	Intervention comparing five types of flooring in relation to foot pad dermatitis. The different types of flooring used were Dinkelspelzen, Häckselstroh, Hobelspäne, Hygiene Holz-Späne, and Pelletinos	The results showed that Pelletinos and Hygiene Wood- Shavings as alternative litter could reduce the prevalence and severity of foot pad dermatitis. Chopped straw as used in our investigation led to the highest percentage of foot pad dermatitis. The best results were found for Pelletinos (trial 1: 16.3 %; trial 2: 57.5 % without lesions) and Hygiene Wood-Shavings (17.5 and 18.7 %, respectively). The percentages of animals without foot pad dermatitis were for wood shavings 2.5 and 6.2 % and for dinkel glumes 1.3 % in both trials, respectively.	Low

Appendix IV: Risk of bias assessment of Randomised Control Trials (RCTs) with the SYRCLE tool

Study (author/year)	Selection Bias (Sequence Generation)	Selection Bias (Baseline)	Selection Bias (Allocation Concealment)	Performance Bias (Random Housing)	Performance Bias (Blinding)	Detection Bias (Random Outcome Assessment)	Detection Bias (Blinding)	Attrition Bias (Incomplete Data)	Reporting Bias (Selective Outcome Reporting)	Other	Overall
Beauvais et al., 2018[52]	High	High	High	Low	Unclear	High	Low	Unclear	Low	High	**High**
Bragg and Plumstead, 2003[79]	High	High	High	Low	High	High	High	High	Unclear	High	**High**
Chuppava et al., 2018a[123]	High	Low	High	Low	Unclear	Low	Low	Low	Low	Low	**High**
Chuppava et al., 2018b[122]	High	Low	High	Low	Unclear	Not Relevant	Unclear	Low	Low	Low	**High**
Chuppava et al., 2019[121]	Low	Low	High	Low	High	Unclear	Low	High	Low	Low	**High**
Conan et al., 2013[108]	Unclear	High	High	High	High	High	High	Unclear	Low	High	**High**
De Busser et al., 2009[59]	High	Low	High	Unclear	High	Low	Unclear	High	Low	High	**High**
De Ridder et al., 2013[55]	High	Low	High	Low	High	Not Relevant	Unclear	Low	Low	High	**High**
Dorado-Garcia et al., 2015[82]	High	Low	High	Not Relevant	High	Not Relevant	Low	Unclear	Low	Low	**High**
El-Wahab et al., 2011[127]	Low	Low	Low	Low	High	Low	Unclear	Low	Low	Low	**Moderate**
Ellis-Iversen et al., 2008[96]	Low	Low	Low	Not Relevant	Unclear	Unclear	Low	Low	Low	Low	**Moderate**
Gibbens et al., 2001[94]	Low	High	Low	Low	Low	Not Relevant	Low	Low	Low	High	**Moderate**
Hancox et al., 2013[83]	Low	Low	Low	Not Relevant	Low	Not Relevant	Unclear	Not Relevant	Low	Low	**Low**
Kim et al., 2017[150]	High	Low	High	Not Relevant	High	Not Relevant	Unclear	Low	Low	Low	**High**
Line and Bailey, 2006[145]	High	Low	High	High	High	High	Low	Low	Low	Low	**High**
Lopes et al., 2015[142]	High	Low	High	Low	High	Not Relevant	Low	Low	Low	Low	**High**
Martelli et al., 2017[78]	High	Low	Unclear	Low	Unclear	Low	Low	High	Low	Low	**High**
Nietfeld et al., 1998[146]	High	Low	High	Low	High	Not Relevant	Low	High	Low	High	**High**
Oberhelman et al., 2006[110]	Low	Low	Low	Not Relevant	High	Unclear	Unclear	High	Low	Low	**Moderate**
Omore et al., 1999[139]	High	High	High	High	High	Not Relevant	Unclear	Low	Low	High	**High**
Sano et al., 2009[70]	High	Low	High	Low	High	Low	Low	Low	Low	Low	**High**
Schenk et al., 2016[53]	High	High	High	Low	Low	Low	Low	Low	Low	High	**High**
Sonoda et al., 2012[144]	High	Low	Not Relevant	Not Relevant	High	Not Relevant	Low	Not Relevant	Low	Low	**High**
Speksnijder et al., 2017[137]	High	High	High	Not Relevant	High	Unclear	Unclear	Unclear	Low	High	**High**
Zhao et al., 2019[124]	High	Unclear	High	Not Relevant	High	Low	Low	Low	Low	High	**High**
Zhou et al., 2019[118]	High	Low	High	Low	High	Not Relevant	Low	Not Relevant	Low	Low	**High**

Appendix V: Risk of bias assessment of Non-randomised Trials (NRTs) assessed with the ROBINS tool

Study (author/year)	Bias Due to Confounding	Bias in Selection of Participants	Bias in Classification of Interventions	Bias Due to Departure from Intended Interventions	Bias Due to Missing Data	Bias in Measurement of Outcomes	Bias in Selection of Reported Results	Overall
Abdalla et al., 2011[140]	Low	Unclear	Low	Low	Low	Low	Low	**Low**
Agustina et al., 2017[109]	High	Low	Low	High	Low	High	Low	**High**
Alcantara et al., 2008[62]	High	Low	High	Low	High	Unclear	Unclear	**High**
Arguello et al., 2013[60]	High	Low	Low	Low	Low	Low	Low	**Moderate**
Battersby et al., 2017[95]	Low	Low	Low	Low	Low	Low	Low	**Low**
Berk, 2007[126]	Low	Low	Low	Low	Low	Low	Low	**Low**
Bolton et al., 2013[105]	High	Low	Low	Low	Low	Low	Low	**Moderate**
Cai et al., 2018[114]	Low	Low	Low	Low	Low	Low	Low	**Low**
Chiriboga et al., 2016[57]	High	Low	Unclear	Low	Unclear	High	High	**High**
Collineaeu et al., 2017[135]	High	Low	High	Unclear	Low	Low	Low	**Moderate**
Dahl et al., 1997[148]	High	Low	Low	Low	Low	Low	High	**High**
Dale et al., 2015[84]	High	Low	Low	Low	Low	Low	Low	**Moderate**
Dee et al., 2004[151]	High	Unclear	Low	Low	Low	High	High	**High**
Dee et al., 2012[130]	High	Low	Low	Unclear	Low	Unclear	Low	**High**
Doko et al., 2012[97]	High	Low	Low	Low	Low	Low	High	**High**
Edrington et al., 2006[141]	High	Low	Low	Unclear	Unclear	Low	High	**High**
El-Shafai et al., 2004[68]	Low	Low	Low	Low	Low	Low	Low	**Low**
Elsaidy et al., 2015[67]	Low	Low	Low	Low	Low	Low	Low	**Low**
Fablet et al., 2005[98]	High	Low	High	Low	High	Low	High	**High**
Hao et al., 2014[128]	High	Low	Unclear	Unclear	Low	High	Low	**High**
Haughton, 2013[58]	Low	Low	Low	Low	Low	Low	High	**Moderate**
Hoffman, 1974[72]	Low	Low	Low	Low	Low	Low	High	**Moderate**
Holman et al., 2016[119]	Low	Low	Low	Low	Low	Low	Low	**Low**
Huang et al., 2019a[117]	Low	Low	Low	Low	Low	Low	Low	**Low**
Huang et al., 2019b[113]	High	Low	Low	Low	Low	Low	Low	**Moderate**
Jang et al., 2016[103]	High	Low	Low	Low	Low	High	High	**High**

Appendix V: Risk of bias assessment of Non-randomised Trials (NRTs) assessed with the ROBINS tool (continued)

Study (author/year)	Bias Due to Confounding	Bias in Selection of Participants	Bias in Classification of Interventions	Bias Due to Departure from Intended Interventions	Bias Due to Missing Data	Bias in Measurement of Outcomes	Bias in Selection of Reported Results	Overall
Jansen et al., 2014[54]	High	Low	Low	Low	Unclear	Low	Low	**Moderate**
Kaneene et al., 2016[133]	High	Unclear	High	High	High	High	Low	**High**
Karzis et al., 2018[49]	High	High	Low	High	Low	High	Low	**High**
Kim and Kim, 2010[80]	Low	Low	Low	Low	Low	Low	Low	**Low**
Leedom et al, 2012[99]	High	Low	High	Unclear	Low	Low	High	**High**
Li et al., 2017[115]	Low	Low	Low	Low	Low	Low	Low	**Low**
Luyckx et al., 2016[107]	High	Low	Low	Low	High	Low	Low	**Moderate**
Luyckx et al., 2016b[153]	Low	High	Low	Low	Low	Low	Low	**Low**
Martelli et al., 2017b[77]	High	Unclear	High	Low	High	Low	Low	**High**
Mateus-Vargas et al., 2019[61]	Low	Low	Low	Low	Low	Low	Low	**Low**
Nagahata et al., 2007[138]	High	Unclear	Low	Low	Low	Low	Low	**Moderate**
Nasr et al., 2018[102]	Low	Low	Low	Low	Low	Low	Low	**Low**
Othman et al., 2015[74]	Low	Low	Low	Low	Low	Low	Low	**Low**
Pletinckx et al., 2013[89]	Low	Low	Low	Low	High	Low	Low	**Low**
Poblete-Chavez et al., 2016[71]	Low	Low	Low	Low	Low	Low	Low	**Low**
Postma et al., 2017[136]	Low	Low	High	Unclear	High	High	Low	**High**
Roll et al., 2011[143]	Low	Low	Low	Low	Low	Low	Low	**Low**
Ryu et al., 2017[51]	Low	Low	Low	Low	Unclear	Low	Low	**Low**
Schiavon et al., 2011[91]	High	Unclear	High	High	High	High	Unclear	**High**
Skanseng et al., 2013[125]	Low	Low	Low	Low	Low	Low	Low	**Low**
Stern et al., 2002[65]	High	High	Low	Low	Low	Low	Low	**Moderate**
Suranindyah et al., 2015[131]	High	Unclear	High	Unclear	unclear	Low	High	**High**
Traub-Dargatz et al., 2006[100]	Low	Low	Low	Low	Low	Low	Low	**Low**
van Bunnik et al., 2012[56]	High	Low	Low	Low	Unclear	Low	Low	**Moderate**
Van De Giessen et al., 1998[92]	High	High	Low	Low	High	Low	High	**High**
Velasquez et al., 2018[106]	Low	Low	Unclear	Low	Low	Low	Low	**Low**

Appendix V: Risk of bias assessment of Non-randomised Trials (NRTs) assessed with the ROBINS tool (continued)

Study (author/year)	Bias Due to Confounding	Bias in Selection of Participants	Bias in Classification of Interventions	Bias Due to Departure from Intended Interventions	Bias Due to Missing Data	Bias in Measurement of Outcomes	Bias in Selection of Reported Results	Overall
Vitosh-Sillman et al., 2017[120]	Low	Low	Low	Low	Low	High	Low	**Moderate**
Weinberg et al., 2011[104]	Low	Not Relevant	Low	Low	Low	Low	Low	**Low**
White et al., 2018[76]	Low	Low	Low	Low	Low	Low	Low	**Low**
Zhang et al., 2011[129]	Low	Low	Low	Low	Low	Low	Low	**Low**

Appendix VI: Risk of bias assessment of case-control studies assessed with the MMAT tool for descriptive quantitative

Study (author/year)	Adequacy of Sample Strategy	Representativeness of Sample	Appropriateness of Measurements	Non-Response Bias	Appropriateness of Statistical Test	OVERALL
Ng et al., 2010[132]	Low	Unclear	Low	High	Low	**High**
Petersen et al., 2002[111]	Low	Unclear	Low	Unclear	Low	**High**

Appendix VII: Risk of bias assessment of cross-sectional/ecological studies assessed with the AXIS tool

Study (author/year)	Study Conceptualization	Target Population	Outcome Measurement	Adequacy of Data Analysis	Response Bias	Internal Consistency	Reporting Bias	Other	Overall
Abraham et al., 2014[73]	High	Low	Low	High	Not Relevant	Low	Low	Low	**High**
Akhter et al., 2018[101]	High	Low	Low	High	Unclear	Low	Low	High	**High**
Alali et al., 2010[149]	High	Unclear	Low	Low	Low	Low	Low	Low	**High**
Amaral et al., 2001[64]	High	Unclear	High	High	Not Relevant	Unclear	High	High	**High**
Arguello et al., 2011[90]	High	High	Low	High	Unclear	Unclear	Low	Unclear	**High**
Balasubramanian et al., 1992[69]	High	High	Low	High	Not Relevant	Low	High	High	**High**
Ben, 2017[116]	High	Low	Low	Low	Not Relevant	Low	Low	Low	**Moderate**
Berg, 2015[134]	High	Low	Low	Low	Unclear	Low	Low	High	**Moderate**
Carrique-Mas et al., 2009[86]	High	High	Low	Low	Unclear	Unclear	Low	Low	**High**
Davies and Wray, 1994[87]	High	Unclear	Low	Low	Not Relevant	Low	Low	Unclear	**High**
Davies et al., 1997[147]	Low	High	Low	Low	Low	High	Low	Low	**Moderate**
De Castro Burbarelli et al., 2017[81]	High	Low	Low	High	Unclear	High	High	Low	**High**
Folorunso et al., 2013[63]	High	Unclear	Low	Low	Not Relevant	Low	Low	Unclear	**High**
Hernandez-Llamas et al., 2015[152]	Low	Low	Low	Low	Low	Low	Low	Low	**Low**
Kamal et al., 2019[85]	High	Low	Low	Low	High	Low	Low	Low	**Moderate**
Kim et al., 2018[112]	High	Low	Low	High	Not Relevant	Low	Low	Low	**High**
Kloska et al., 2017[88]	High	Low	Low	High	Low	Low	Low	Low	**High**
Mannion et al., 2007[75]	High	High	Low	Low	High	Low	Low	Low	**High**
Mlejnkova and Sovova, 2012[66]	High	High	Low	High	Not Relevant	High	High	Low	**High**
Oliveira et al., 2017[93]	Low	Low	Low	Low	Low	Low	Low	High	**Low**

Appendix VIII: Summary of tools used to assess the risk of bias

(A) ROBINS-I

Question Series	Bias Type
1*	Bias Due to Confounding
2	Bias in Selection of Participants
3	Bias in Classification of Interventions
4	Bias due to departure from intended interventions
5	Bias due to Missing Data
6*	Bias in Measurement of Outcomes
7*	Bias in Selection of the Reported Results

* Denotes a "critical" domain. High or unclear risk in 2 critical domains was assigned an overall score of high risk, 1 was assigned moderate risk, and 0 was assigned low risk. High risk in 3 or more overall domains was assigned an overall score of high risk, up to 2 was assigned moderate risk, and up to 1 was assigned low risk. The "worse" of the two scores was taken to be the overall risk of bias score for the study.

(B) SYRCLE

Bias Domain	Bias Type
1*	Selection Bias (Sequence Generation Q1)
2	Selection Bias (Baseline Q2-4)
3*	Selection Bias (Allocation concealment Q5)
4	Performance Bias (Random Housing Q6-7)
5*	Performance Bias (Blinding Q8)
6	Detection Bias (Random Outcome Assessment Q9)
7	Detection Bias (Blinding 10-11)
8	Attrition Bias (Incomplete Data Q12-15)
9	Reporting Bias (Selective Outcome Reports Q16-17)
10	Other (Q18-22)

* Denotes a "critical" domain. High or unclear risk in 2 critical domains was assigned an overall score of high risk, 1 was assigned moderate risk, and 0 was assigned low risk. High risk in 3 or more overall domains was assigned an overall score of high risk, up to 2 was assigned moderate risk, and up to 1 was assigned low risk. The "worse" of the two scores was taken to be the overall risk of bias score for the study.

(C) AXIS

Bias Domain†	Bias Type
1	Study Conceptualization (Q1-3)
2	Target Population (Q4-7)
3	Outcome Measurement (Q8-9)
4	Adequacy of Data Analysis (Q10-12)

5	Response Bias (Q13-14)
6	Internal Consistency (Q15)
7	Reporting Bias (Q16-18)
8	Other (Q19-20)

† Denotes that questions were sorted into bias domains by study authors. * Denotes a "critical" domain. High or unclear risk in 2 critical domains was assigned an overall score of high risk, 1 was assigned moderate risk, and 0 was assigned low risk. High risk in 5 or more overall domains was assigned an overall score of high risk, up to 3 was assigned moderate risk, and up to 2 was assigned low risk. The "worse" of the two scores was taken to be the overall risk of bias score for the study.

(D) MMAT

Question	Bias Type
Screen 1*	Clarity of Research Question
Screen 2*	Ability of Collected Data to Address Research Question
1	Adequacy of Sample Strategy
2	Representativeness of Samples
3	Appropriateness of Measurements
4	Non-Response Bias
5	Appropriateness of Statistical Methods

*All included studies passed the initial screening questions and were analysed with the five questions listed above. High or unclear risk in 2 or more domains was assigned an overall score of high risk, 1 was assigned moderate risk, and 0 was assigned low risk.

Appendix IX: PRISMA (Preferred Reporting Items for Systematic review and Meta-Analysis) 2009 checklist[46]

Section/Topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	Cover
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	8
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	10,11
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	10
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	15
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	16,18
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	18
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	18
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	19
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	20
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	20
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	19
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	21

Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	22
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	23
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	39
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	29
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	Not done
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	Not done
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	42
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	49
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	50
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	3