Biosecurity and water, sanitation, and hygiene (WASH) interventions in animal agricultural settings for reducing infection burden, antibiotic use, and antibiotic resistance: a One Health systematic review

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Prevention and control of infections across the One Health spectrum is essential for improving antibiotic use and addressing the emergence and spread of antibiotic resistance. Evidence for how best to manage these risks in agricultural communities—45% of households globally—has not been systematically assembled. This systematic review identifies and summarises evidence from on-farm biosecurity and water, sanitation, and hygiene (WASH) interventions with the potential to directly or indirectly reduce infections and antibiotic resistance in animal agricultural settings. We searched 17 scientific databases (including Web of Science, PubMed, and regional databases) and grey literature from database inception to Dec 31, 2019 for articles that assessed biosecurity or WASH interventions measuring our outcomes of interest; namely, infection burden, microbial loads, antibiotic use, and antibiotic resistance in animals, humans, or the environment. Risk of bias was assessed with the Systematic Review Centre for Laboratory Animal Experimentation tool, Risk of Bias in Non-Randomized Studies of Interventions, and the Appraisal tool for Cross-Sectional Studies, although no studies were excluded as a result. Due to the heterogeneity of interventions found, we conducted a narrative synthesis. The protocol was pre-registered with PROSPERO (CRD42020162345). Of the 20 672 publications screened, 104 were included in this systematic review. 64 studies were conducted in high-income countries, 24 studies in upper-middle-income countries, 13 studies in lower-middle-income countries, two in low-income countries, and one included both upper-middle-income countries and lower-middle-income countries. 48 interventions focused on livestock (mainly pigs), 43 poultry (mainly chickens), one on livestock and poultry, and 12 on aquaculture farms. 68 of 104 interventions took place on intensive farms, 22 in experimental settings, and ten in smallholder or subsistence farms. Positive outcomes were reported for ten of 23 water studies, 17 of 35 hygiene studies, 15 of 24 sanitation studies, all three air-quality studies, and 11 of 17 other biosecurity-related interventions. In total, 18 of 26 studies reported reduced infection or diseases, 37 of 71 studies reported reduced microbial loads, four of five studies reported reduced antibiotic use, and seven of 20 studies reported reduced antibiotic resistance. Overall, risk of bias was high in 28 of 57 studies with positive interventions and 17 of 30 studies with negative or neutral interventions. Farm-management interventions successfully reduced antibiotic use by up to 57%. Manure-oriented interventions reduced antibiotic resistance genes or antibiotic-resistant bacteria in animal waste by up to 99%. This systematic review highlights the challenges of preventing and controlling infections and antimicrobial resistance, even in well resourced agricultural settings. Most of the evidence emerges from studies that focus on the farm itself, rather than targeting agricultural communities or the broader social, economic, and policy environment that could affect their outcomes. WASH and biosecurity interventions could complement each other when addressing antimicrobial resistance in the human, animal, and environmental interface.

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Introduction

Widespread antimicrobial use in human health care[1–3] and agriculture,[4] and subsequent environmental residues[5] are key drivers in the emergence and spread of antimicrobial resistance.[6] Antimicrobial use and antimicrobial resistance have increased in low-income countries and middle-income countries (LMICs) in which food production systems are intensifying.[7] Since 2000, meat production has grown by 68% in Africa, 64% in Asia, and 40% in South America,[7] and aquaculture is one of the fastest-growing sectors in Asia.[8,9] This agricultural intensification could increase microbial flows into wider food chains, especially enteric zoonotic pathogens,[10] such as pathogenic *Escherichia coli*, *Enterococcus* spp, *Salmonella* spp, *Klebsiella* spp, *Enterobacter* spp, and *Campylobacter* spp, which are known to harbour many antibiotic resistance genes that are easily disseminated through mobile genetic elements.[11,12]

In animal production, antimicrobial use contributes to the presence of antimicrobial residues in animal food products, antibiotic-resistant bacteria, and antibiotic resistance genes in animal waste. These residues, bacteria, and genes enter the environment through leachates from manure heaps, contaminating rivers, lakes, soil, and food crops.[12] Contaminated water sources can act as vehicles of resistant bacteria and antibiotic resistance genes, creating transmission cycles among humans, animals, and the environment. This is especially important for people living or working in close contact with animals. In domestic husbandry practices,[13] for example, free-ranging poultry increases human exposure to animal faeces[14] and zoonotic transmission. Globally, almost 45% of the population live in households

Panel: **Key messages**

- This systematic review comprehensively assessed the collective relevance of water, sanitation, and hygiene (WASH) and biosecurity interventions to the antimicrobial-resistance agenda in agricultural settings and appraised their reported effects on infection burden, antibiotic use, and antibiotic resistance in livestock production and aquaculture.
- By seeking to assess evidence not only in English but also in other languages, we were able to access an aggregate of literature and provide an overview of interventions that are reported to reduce antibiotic use, antibiotic resistance, infection burden, and microbial loads in animal agricultural settings that could be trialled at a broader scale and identify gaps and potential directions for future research.
- Successful interventions identified in this study commonly aimed to reduce antibiotic resistance genes in animal manure or applied farm-specific biosecurity protocols to reduce antibiotic use, suggesting these types of interventions could be explored further.
- Some interventions that could increase the risk of spreading antibiotic resistance and diarrhoeal disease in humans are not yet addressed (eg, sharing water resources between humans and animals).
- Some of the interventions included in this systematic review were antimicrobial resistance-sensitive, therefore their effect on antibiotic use and antimicrobial resistance was not directly measured. It would be necessary to test the magnitude of their effect on these outcomes across different settings to inform the assessment of their relevance to antimicrobial resistance.
- This systematic review curates the evidence for the impact of WASH and biosecurity interventions in animal agricultural settings and emphasises the relevance of accounting for not only animal faeces but also all animal fluids when aiming to reduce microbial loads and antimicrobial resistance.

dominated by agricultural activity.[15] Despite this increased exposure, there is no systematic assessment across the One Health spectrum of interventions to prevent infection and antimicrobial resistance in these populations.

Despite evidence of antibiotic-resistance gene transfer between bacteria affecting humans and animals,[16–19] the potential for water, sanitation, and hygiene (WASH), and on-farm biosecurity interventions as infection prevention and control measures to address antimicrobial resistance from a One Health perspective remains underexplored. Most WASH interventions focus on human populations, particularly reducing morbidity and mortality from diarrhoea in children in LMICs.[20,21] However, WASH interventions in animal production settings are also important to public health, as they can reduce the emergence and spread of resistant bacteria to consumers, farmworkers, and the surrounding farm environment. Likewise, biosecurity interventions[22] mainly focus on farmed animals, but their effect on protecting farmworkers from animal infections (other than the known zoonoses) is not always measured.

See **Online** for appendix

Recognising antimicrobial resistance as a development problem, the World Bank proposed the term antimicrobial resistance-sensitive to classify interventions that indirectly impact antimicrobial resistance by reducing multiple infections concurrently and the term antimicrobial resistance-specific for interventions aiming to curb antimicrobial resistance and antimicrobial use directly.[23] In this context, both WASH and biosecurity interventions can be antimicrobial resistance-sensitive—eg, improving access to clean water and sanitation facilities or supporting farmers to implement biosecurity measures. Both intervention types can be implemented at a system level, from which point they could influence risk factors embedded in social structures and address socioeconomic vulnerabilities. For example, structural interventions[24–26] promote health by aiming "to alter the structural context where health is produced or reproduced"[27] and can be highly effective in driving a positive effect on health. They have been proven successful in addressing other public health issues such as HIV, obesity, and chronic conditions.[25,26]

Despite the recognition of antimicrobial resistance as a global health priority, evidence of the effectiveness of interventions addressing antimicrobial resistance from a One Health perspective is scarce, making difficult the task of implementing effective policies. The overlapping aims and objectives between WASH and biosecurity concepts[28] warrant an investigation into their ability to prevent and control infections, reduce antimicrobial use, and reduce the emergence and spread of antimicrobial resistance in the One Health spectrum (people, animals, and the environment). In an overview of all systematic reviews related to antimicrobial resistance between database inception and Dec 31, 2019, we found that from 578 systematic reviews, 400 summarised knowledge on antimicrobial resistance, and 178 focused on interventions to prevent antimicrobial resistance. None of the systematic reviews covered WASH or biosecurity interventions' relevance to antimicrobial resistance (unpublished).

In this systematic review, we examined a range of WASH and biosecurity interventions that were implemented in animal production settings to reduce infection burden in animals or humans, microbial loads in the farm environment, antibiotic use, and antibiotic resistance. We aimed to identify what interventions were tested, any potential research gaps, and the enabling conditions or barriers for implementation across different settings. We also summarised the evidence of the interventions' effects and assessed their methodological quality.

Methods

Search strategy and selection criteria

We conducted a systematic review with a pre-published protocol[29] in accordance with PRISMA reporting standards,[30] registered with PROSPERO (CRD42020162345; appendix pp 3–5). We developed a search strategy in English covering five themes: populations (animal or humans), production systems (livestock, aquaculture, intensive farming, small-holders, subsistence, pastoralists), intervention types (WASH and biosecurity), study types (project, pilot, intervention, and policy), and countries (appendix pp 6–14) from database inception to Dec 31, 2019. A comprehensive literature search was conducted in Web of Science, PubMed, Ovid (CAB Abstracts, Global Health, Embase, MEDLINE, Veterinary Science, Social Work Abstracts, and PsycINFO), ProQuest, Epistemonikos, Trip, AgEcon, and Cochrane Library from May to August, 2020, with no language restrictions. We also performed searches in Spanish, Portuguese, and French in regional databases; namely, Scopus, Scielo, BIREME, E-Revistas, Redalyc, Lilacs, AfricaPortal, and Index Medicus for the South-East Asian and Western Pacific WHO regions. Manual searches were conducted in Access to Global Online Research in Agriculture (Food and Agriculture Organization of the UN), Agris, the Joint Programming Initiative on Antimicrobial Resistance, JSTOR, the Journal of Librarianship and Information Science, The World Bank database, the International Development and Research Centre Digital Library; and in Google Scholar and Open Grey for grey literature. Additional articles were identified through snowball searching references of relevant literature.

We included articles describing WASH and biosecurity interventions (table 1) measuring outcomes aiming to reduce infection or disease burden, microbial loads, antibiotic use, and antibiotic resistance in animal agricultural settings (including livestock, poultry, regional farm animals, and aquaculture). Included studies investigated bacterial and non-bacterial pathogens (eg, viruses or unicellular parasites) commonly treated with antibiotics, and that included an assessment of the intervention. No restrictions were applied to quantitative study designs. We excluded studies implementing interventions in human settings only with no connection to animals, applied outside farms (eg, disinfection of animal transport vehicles or carcasses), focusing on vaccinations or changes to animal nutrition, or other ways of improving animal husbandry not directly associated with WASH or biosecurity, or that tested disinfectants in vitro. Additionally, studies that were not in English, Spanish, Portuguese, French, German, Dutch, or unavailable in full text after contacting the authors were excluded.

Abstracts were downloaded and duplicates removed using EndNote X9. Searches were done by CEPJ. The selection process included independent screening of titles and abstracts of English articles, and full-text assessment by SK and PT. Any disagreements were resolved by CEPJ. The inter-rater agreement was moderate (κ 0·55). Articles not written in English were screened and assessed by authors with Spanish, German, or Dutch as their first-language or with fluency in the language (Portuguese and French); CEPJ for Spanish, Portuguese, and French, and SK for German and Dutch. CEPJ, SK, and PT checked and agreed on the articles included. All articles not meeting the eligibility criteria after full-text examination are listed in the appendix (pp 15–25), with the reasons for exclusion also stated. Data were extracted by SK and PT with a pre-designed form and 52 of 104 articles were randomly assigned for verification by CEPJ.

	Definition
WASH or biosecurity intervention typology[28]	
Water	
Water quantity	Provide infrastructure or improve water distribution systems, or implement policies to ensure access to water for drinking or cleaning, to safeguard human and animal health and welfare
Water quality	Remove or inactivate pathogens at source and at the point of use, or implement policies to ensure clean water for both humans and animals
Air	
Air quality	Prevent the dissemination of airborne pathogens among humans and animals
Sanitation	
Sanitation infrastructure	Provide or implement infrastructure for the safe disposal of human waste to reduce access by animals or vectors
Waste management	Establish strategies or policies to safely dispose of wastewater or fallen stock, or treat animal or human faeces to be used as fertilisers, to prevent the spread and dissemination of microbial threats to and from the environment
Hygiene	
Food or feed safety	Introduce hygiene strategies to safely manage and store food products including those of animal origin and animal feed, avoiding cross-contamination
Cleaning and disinfection	Promote hygienic practices, implement protocols, or enforce policies to facilitate good hygiene in the household, among individuals, and around animal dwellings, avoiding the introduction and spread of pathogens among humans, animals, and the environment
Other biosecurity measures complementing traditional WASH	
Barrier implementation	Preserve boundaries, implement barriers, or introduce policy strategies to limit exposure to microorganisms between animals and humans, and control potential vectors and fomites
Health protection	Implement strategies to boost immunity or manage infections in humans and animals, or improve access to health care, ensuring wellness, welfare, and productivity for humans and animals
Combined interventions	Interventions combining a set of strategies included in different categories of this type
Intervention level	
Interventions operating at a system level	
Structural	Operate at the social, political, and economic level and aim to change the structural context in which health is produced and reproduced (eg, policy on manure treatment, incentives for farmers to reduce antimicrobial use through biosecurity measures)
Interventions operating at an individual or community level	
Managerial	Focus on changing the management of the farm (eg, introducing new biosecurity protocols for visitors or implementing hygienic milking practices)
Educational or behavioural	Improve practices at an individual level through education or behaviour change strategies: people are not just applying the intervention but are the main focus for the change (eg, addressing handwashing or milking techniques)
Physical or infrastructural	Changing the physical environment to improve animal husbandry (eg, improved flooring and air filtration of animal facilities)
Biological or chemical	Focus on the microbial presence and burden (eg, implementing disinfection strategies, biological treatments, and cleaning products to eliminate pathogens)

(Table 1 continues next page)

Risk of bias

As we included different study designs, we selected the following tools: the Systematic Review Centre for Laboratory Animal Experimentation tool for randomised controlled trials (RCTs),[31] Risk of Bias in Non-Randomised Studies of Interventions for non-randomised trials,[32] and the Appraisal tool for Cross-Sectional Studies for cross-sectional and ecological studies.[33] Authors independently assessed the risk of bias for English (SK and PT), German (SK), Spanish (CEPJ), and Portuguese (CEPJ) studies.

	Definition
(Continued from previous page)	
Outcomes of interest	
Antimicrobial resistance-sensitive	
Relevant to microbial loads	Reduction of bacterial counts, positive microbiological culture, non-bacterial pathogens that may be treated with antibiotics, or reduction of bacterial counts isolated from animal facilities, or animal or human samples
Relevant to the burden of infections or diseases	Reduced incidence or prevalence of infections, disease, morbidity, or mortality rates
Antimicrobial resistance-specific	
Relevant to antibiotic use	Reduction in the number of veterinary visits or treatments, the quantity of antibiotics or medicated animal feed used, and antibiotic residue in animal products
Relevant to antibiotic resistance	Reduced presence of antibiotic resistant bacteria and antibiotic resistance genes

Adapted from Pinto Jimenez et al (2023).[28] WASH=water, sanitation, and hygiene.

***Table 1:* Typology of interventions used to classify studies**

Disagreements were resolved through discussion (SK, PT, and CEPJ). To calculate the overall risk of bias, we developed a criterion putting emphasis on bias due to confounding and randomisation, collection of results, and data reporting (appendix p 44). Additionally, as interventions in animal production settings tend to be complex, RCT methods are not always possible, and multiple pathways can generate the same outcome;[34] therefore, we did not exclude studies on the basis of the risk of bias assessment. Instead, we used this as a proxy to assess the strength of the evidence.

Data analysis

The heterogeneity of study designs used in the selected articles, including differences in strategies implemented, animal species, and outcomes of interest, precluded a meta-analysis. Thus, a narrative synthesis of interventions was done. Data extracted included type of publication, language, year, journal, country, region,[35] level of income (World Bank classification),[36] study design, intervention type, microorganism involved, animal species, production system, population type, livelihood system, outcomes, intervention results (reduction percentages, or measures of improvement), intervention effect (positive, negative, mixed, or neutral effect according to whether authors reported an improvement, a worsening of the situation, a combination, or no effect), statistical analysis, potential co-benefits, unintended consequences, and barriers.

Selected articles were classified depending on their relevance to WASH or biosecurity,[28] and the level of intervention at which the outcome was measured; namely, structural, educational–behavioural, managerial, physical–infrastructural, or biological–chemical. These categories were established by author consensus with input from existing intervention types and academic literature. Definitions of these types[28] and outcomes of interest were grouped by their impact on infection burden, microbial loads, antibiotic use, and antibiotic resistance (table 1). The narrative analysis involved cross-tabulations between the outcomes and intervention types and their reported effects.

Frequencies, percentages, p values, and CIs were extracted if available. We reported outcome effects in percentage reductions. Data from studies reporting logarithmic reductions in bacterial counts were transformed to percentage reductions using $P=(1–10^{-L})\times100$, where P is the percentage reduction, and L is the Log reduction.

Results

20 672 records were identified through primary searches and 102 articles through snowballing. After title and abstract screening, 320 full-text articles were assessed for eligibility. After 216 studies were excluded, 104 studies remained, representing 104 interventions (99 in English, three in Spanish, one in German, and one in Portuguese), published between database inception and Dec 31, 2019 (figure). A summary of selected studies with references is included in the appendix (pp 26–42).

Studies were from 39 countries in five geographical regions: 37 in Europe, 26 in Asia, 19 in North America, 11 in Africa, and 11 in Latin America (appendix p 43). 64 studies were done in high-income countries, 24 in upper-middle-income countries (UMICs), 13 in LMICs, two in low-income countries, and one study included both UMICs and LMICs. 48 studies focused on livestock (primarily pigs in 27 studies); 43 on poultry (primarily chickens in 36 studies), 12 on aquaculture, and one on livestock and poultry. 85 studies included interventions only focusing on animals, two focused only on humans (in animal production contexts), and 17 on both. In studies that focused on humans, 16 were done on farmworkers and three on household members. 19 studies also included the environment surrounding animal settings. 68 interventions took place in intensive farming environments and 22 in experimental settings. Only five studies were set in smallholder farms, five in subsistence farming, and four in mixed production systems (table 2). Overall, beyond information about production system type, little to no information about the physical, agroecological, socioeconomic, or cultural context in which the studies were conducted was provided.

Interventions targeted a range of pathogens that are commonly treated with antibiotics, but largely focused on bacteria. 44 studies investigated zoonotic pathogens, including 19 *Salmonella* spp, ten *Campylobacter* spp, five *Staphylococcus aureus* (including methicillin-resistant *S aureus* [MRSA]), seven *E coli* (including O157:H7), one *Leptospira*, one *Brucella*, and one highly pathogenic avian influenza. Eight studies targeted pathogens of animal health importance; three investigated mastitis-associated bacteria, two coliforms, two *Vibrio* spp, and one *Streptococcus agalactiae*. 30 studies analysed different bacterial species simultaneously (multibacteria), six focused on viruses, and two on unicellular parasites.

Six studies did not search for pathogens but investigated antibiotic resistance genes only, and eight studies assessed other indicators (table 2).

Studies were classified according to our WASH or biosecurity typology: two focused on water quantity, 21 water quality, three air quality, 24 waste management (sanitation), two food or feed safety (hygiene), 33 cleaning and disinfection (hygiene), 13 barrier implementation, four health protection, and two were combined interventions (table 3). When classified on the basis of the level at which the intervention took place, 51 studies were classified as biological–chemical, eight behavioural–educational, 26 physical–infrastructural, 18 managerial, and one was structural.

26 articles assessed outcomes related to infection or disease burden (reduced incidence or prevalence of infections, or reduced morbidity or mortality rates), 71 to microbial loads (reduced bacterial counts, non-bacterial pathogens, or positive microbiological culture), five to antibiotic use (reduced quantity of antibiotics used, number of treatments, veterinary visits, or antibiotic residues) and 20 to antibiotic resistance (reduced antibiotic-resistant bacteria or antibiotic resistance genes). As some studies assessed more than one relevant outcome of interest, they appear more than once in the subanalysis. Overall, 55 studies were assessed as high risk of bias, 28 were low, and 21 had a moderate risk (appendix pp 46–49). A summary table of study outcomes, their reported effect and the relevance to the One Health spectrum is provided in appendix (p 50). The interventions included, classified by type of animal farmed (poultry, livestock, or aquaculture), intervention typology, reported effect, and risk of bias is also reported (appendix p 51).

From 104 interventions, 57 reported positive effects on our outcomes of interest, 11 reported negative effects, 19 reported neutral effects, and 17 reported mixed effects. Differences in efficacy were seen based on the production setting: positive results were reported in 19 of 22 studies set in experimental settings, 32 of 68 interventions in intensive farming systems, and in only four of 11 interventions with smallholders and subsistence farmers. Among the WASH or biosecurity typology, positive results were reported for ten of 21 water quality interventions, all three air quality interventions, 15 of 24 waste management interventions, one of the two food or feed safety interventions, 16 of 33 cleaning and disinfection interventions, seven of 13 barrier implementation interventions, and all four health protection interventions (table 4). At the delivery level, positive effects were found for 28 of 51 biological–chemical interventions, four of eight behavioural–educational interventions, 13 of 26 physical interventions, 11 of 18 managerial interventions, and the one structural intervention (table 5).

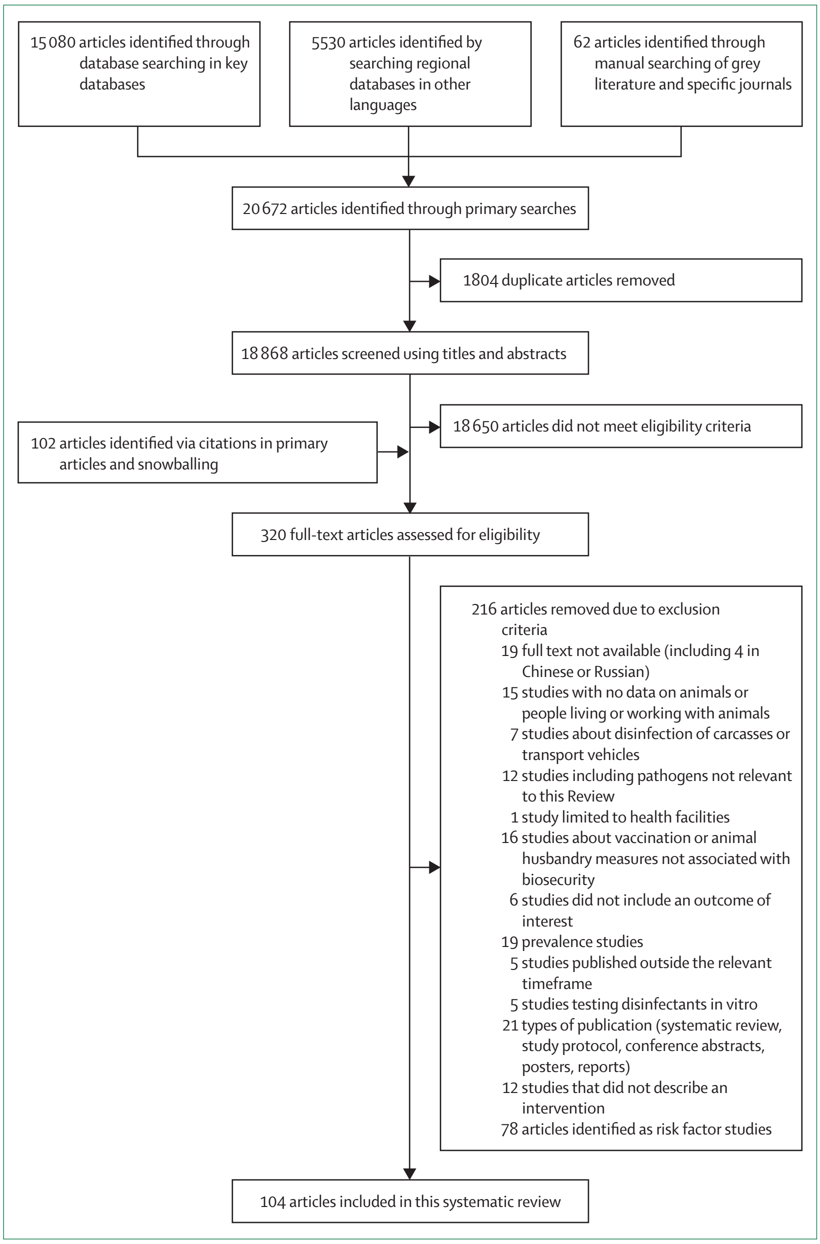

Figure: **Flow diagram of study selection**

From interventions aiming to reduce antibiotic use in animals, four targeted farmworkers in commercial farms in high-income countries.[37–40] Three were health protection interventions[37–39] focusing on the optimisation of health planning with a herd-specific approach in consultation with farmers, veterinarians, and other stakeholders. Although risk of bias was high[38,39] or moderate,[37] all three interventions had positive effects on reducing antibiotic use by 19%,[39] 47%,[37] and 52%.[38] Positive effects on costs of farm management[37,40] were reported throughout the studies, without negative effects on productivity,[38–40] health, or mortality.[37,38] A hygiene-oriented intervention[40] evaluating MRSA carriage by veal calves and farmworkers had mixed results as, unexpectedly, farms reducing antibiotic use in combination with

	Animal studies (n=102)*	Human studies (n=19)*
Study design		
Before and after study	15 (14·7%)	7 (36·8%)
Cross-sectional	5 (4·9%)	2 (10·6%)
Ecological	19 (18·6%)	0 (0·0%)
Interrupted time series analysis	23 (22·5%)	4 (21·1%)
Non-randomised trial	14 (13·7%)	1 (5·3%)
Randomised controlled trial	26 (25·5%)	5 (26·3%)
Country classification by income status		
High income	62 (60·7%)	10 (52·6%)
Upper-middle income	24 (23·5%)	2 (10·5%)
Lower-middle income	13 (12·7%)	4 (21·1%)
Low income	2 (1·9%)	2 (10·5%)
Including both upper-middle or lower-middle income	1 (1·0%)	1 (5·3%)
Region		
Europe	37 (36·3%)	4 (21·1%)
Asia	24 (23·5%)	6 (31·6%)
Northern America	19 (18·6%)	4 (21·1%)
Latin America and the Caribbean	11 (10·8%)	1 (5·3%)
Africa	11 (10·8)	4 (21·1%)
Population studied		
Cattle	18 (17·6%)	··
Sheep	1 (1·0%)	··
Pigs	26 (25·5%)	··
Horses	1 (1·0%)	··
Chickens	37 (36·3%)	··
Ducks	3 (2·9%)	··
Turkey	3 (2·9%)	··
Goose	1 (1·0%)	··
Fish or shellfish	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%)
Smallholders	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 or biosecurity typology)		
Water: water quantity	2 (1·9%)	··
Water: water quality	21 (20·6%)	··
Air: air quality	3 (2·9%)	··
Sanitation: waste management	23 (22·6%)	2 (10·6%)
Hygiene: food or feed safety	2 (1·9%)	··
Hygiene: cleaning and disinfection	33 (32·3%)	9 (47·3%)
Other biosecurity: barrier implementation	12 (11·8%)	6 (31·6%)
Other biosecurity: health protection	4 (3·9%)	2 (10·6%)
Combined interventions	2 (1·9%)	··
Type of intervention (classified by intervention target)		
Biological or chemical	50 (49·0%)	5 (26·3%)
Managerial	18 (17·6%)	4 (21·1%)
Educational or behavioural	15 (14·7%)	6 (31·6%)
Physical or infrastructural	26 (25·5%)	3 (15·8%)
Structural	··	1 (5·3%)
Sample studied		
Air	2 (2·0%)	··
Milk	8 (7·9%)	5 (26·3%)
Body or hand swabs	5 (4·9%)	1 (5·3%)
Compost samples	1 (1·0%)	··
Database on disease incidence	1 (1·0%)	··
Dermatitis scores	3 (2·9%)	··
Fallen stock	2 (2·0%)	··
Nasal swabs	2 (2·0%)	··
Litter	3 (2·9%)	··
Human faeces	··	1 (5·6%)
Animal faeces	20 (19·6%)	3 (15·8%)
Rectal or cloacal swabs	7 (6·9%)	1 (5·3%)
Tissues	3 (2·9%)	··
Blood	9 (8·8%)	2 (10·5%)
Personal protective equipment	3 (2·9%)	3 (15·8%)
Farm equipment and surfaces	16 (15·7%)	1 (5·3%)
Water	15 (14·7%)	··
Not relevant or not applicable	3 (2·9%)	3 (15·8%)
Environmental area		
Water	25 (24·5%)	··
Air	3 (2·9%)	··
Soil	7 (6·9%)	··
Farm environment	64 (62·7%)	16 (84·2%)
Household environment	3 (2·9%)	3 (15·8%)

(Table 2 continues in next column)

a specific cleaning and disinfection protocol had significantly higher loads of MRSA in the air than control farms (potentially due to MRSA aerosolisation during cleaning or co-selection because resistance to the biocide applied), and attained reductions in antibiotic use of 9–20% compared with control farms. This study reported that veterinary costs significantly increased with higher antibiotic use.[40]

From interventions aiming to reduce the dissemination of antimicrobial resistance (either by reducing antibiotic resistance genes or antimicrobial-resistant bacteria), eight had positive,[41–48] six mixed,[40,49–53] three negative,[54–56] and three neutral effects,[57–59] and 14 of 20 took place in intensive farming contexts. Ten studies aimed to reduce antibiotic-resistant bacteria in animals and four in the environment. A reduction of antibiotic resistance genes was measured in seven studies in animal waste and two in the environment. Six of the studies in waste management reduced antibiotic resistance genes in

	Animal studies (n=102)*	Human studies (n=19)*
(Continued from previous column)		
Microorganisms*		
Bacteria		
Enterobacteriaceae		
Escherichia coli‡	7 (6·9%)	1 (5·3%)
Salmonella spp	18 (17·6%)	1 (5·3%)
Coliforms	2 (2·0%)	··
Campylobacter spp	10 (9·8%)	3 (15·8%)
Vibrio spp	2 (2·0%)	··
Non-enteric bacteria		
Staphylococcus aureus	5 (4·9%)	1 (5·3%)
Streptococcus agalactiae	1 (1·0%)	··
Brucella spp	1 (1·0%)	1 (5·3%)
Leptospira spp	··	1 (5·3%)
Mastitis-associated bacteria	3 (2·9%)	3 (15·8%)
Multibacteria	30 (29·4%)	3 (15·8%)
Antibiotic resistance genes§	6 (5·9%)	··
Viruses		
Avian influenza virus	1 (1·0%)	1 (5·3%)
Porcine epidemic diarrhoea virus	2 (2·0%)	1 (5·3%)
Porcine reproductive and respiratory syndrome	2 (2·0%)	1 (5·3%)
Viral haemorrhagic septicaemia virus	1 (1·0%)	··
White spot virus	1 (1·0%)	··
Parasites		
Toxoplasma gondii	1 (1·0%)	··
Myxosoma cerebralis	1 (1·0%)	··
Indicators other than microorganisms (prevalence or incidence of infections or diseases; morbidity; or mortality)	8 (7·8%)	2 (10·5%)

WASH=water, sanitation, and hygiene. *Studies can be included in more than one category: from the 104 interventions included, 85 studies were only done in animals whereas two studies were only done in humans. The other 17 studies were done in both humans and animals. †Studies including more than one type of production system. ‡Includes three studies in *E coli* O157:H7. §Some studies only detected antibiotic resistance genes from microorganism DNA.

Table 2: **Summary of study characteristics**

wastewater through filtering[43,44] or by use of diverse experimental manure management, or composting methods,[41,42,45,46] with antibiotic-resistance gene removal ranging from 21–99%[41–44,46] (with various methods and targeted antibiotic resistance genes). Although these studies collectively suggest antibiotic resistance gene elimination is feasible with wastewater filtration or manure treatment, four took place in experimental settings[41–43,46] and only two took place on commercial farms.[44,45] The risk of bias in these studies was assessed to be low in three studies,[41,42,46] moderate in two,[43,44] and high in one.[45] Two additional studies,[51,52] only focusing on antibiotic resistance genes, had mixed results, but showed that it was possible to reduce the abundance of antibiotic resistance genes in manure by anaerobic digestion at different temperatures (25°C, 37°C, and 55°C,[52] albeit with an attenuated reduction at 55°C) and by farm treatment processes, reporting that microbial fermentation beds could reduce antibiotic resistance genes by 0–1·18 logs, whereas septic tanks, biogas digester, and natural drying increased some antibiotic resistance genes (ie, *tetC*, *tetG*, *sul1*, and *sul2*).[51] Both studies suggested that the existing bacterial communities could be essential in mitigating antibiotic resistance gene abundance and transfer. Further, six interventions[40,49–53] led to increased antibiotic resistance or increased abundance of specific antibiotic resistance genes, whereas other antibiotic resistance genes were significantly reduced, leading to mixed results. For example, a study evaluating the effects of different flooring designs on resistant *E coli* in turkeys treated with enrofloxacin paradoxically increased the abundance of ampicillin-resistant isolates despite no ampicillin being used in the trial.[53] The three interventions with negative effects were done in commercial intensive farming systems and aimed to reduce resistant bacteria by adjusting barn flooring,[54] implementing biosecurity measures,[56] and assessing the effect of integrated fish farming on antimicrobial resistance.[55] All found an increased prevalence of antibiotic-resistant isolates (including multidrug-resistant *E coli*, *Acinetobacter* spp, and *Enterococcus* spp), with resistance to clinically relevant antibiotics such as chloramphenicol, ciprofloxacin, erythromycin, oxytetracycline, streptomycin, sulfamethoxazole, tetracycline, and trimethoprim. In these studies, the authors hypothesised: that installing elevated slat platforms reduced birds' exposure to manure, but caused birds to prefer elevated areas, leading to high population density and fostering the transmission of antimicrobial resistance;[54] that seasonal variations could influence *Salmonella* prevalence and variations in the implementation of biosecurity practices due to paucity of reward for producers, could have influenced the results;[56] and that integrating fish farming with livestock manure could increase the selective pressure of antimicrobials in the pond environment, or introduce antimicrobial residues and antimicrobial resistance bacteria from animal manure.[55]

From the studies aiming to reduce microbial loads, 71 sought to reduce *Campylobacter*, *Salmonella*, or various other bacterial species in the farm environment or in animals or farmworker samples, of which 37 had positive,[47,60–95] ten mixed,[50,96–104] eight negative,[56,105–111] and 16 neutral[48,58,112–125] effects. Outcomes of interest were measured in farmworkers' hands or personal protective equipment (four studies), animal samples (34 studies), or animal facilities or the farm environment (58 studies). Of the 40 biological–chemical-based interventions, 21 were positive,[53,62,64,67,69–71,75,76,78–87,91,92] one was negative,[108] and 18 had either mixed or neutral effects.[48,96–103,112,114,116,120,122–125] The most frequently reported interventions applied chemical disinfection,[48,58,64,70,73,78,79,81–83,96,99–104,124] manure management or composting methods,[71,75,76,87,92,97,116] acidified drinking

	References
Water	
Water quantity (n=2)	
Interventions adjusting the quantity of water by lowering water levels to 50% (n=1) or by use of water troughs rather than pin-metered water lines (n=1)	106,107
Water quality (n=21)	
Interventions to improve the quality of drinking water provided to farm animals, including acidifying the water with the addition of products such as organic acids or vinegar for poultry: these products were added in water systems to lower the pH and improve water quality by preventing the growth of microbes (n=7)	80,84,91,108,114,122,123
Interventions that focus on providing clean drinking water systems through novel methods; applying low-frequency electromagnetic fields (n=1), filtration-treating wastewater (n=1), chlorination (n=1), or with nipple versus cup water troughs (n=1)	110,118,121,125
Interventions to improve the microbial water quality in aquaculture fishponds fed with animal manure or sewage (n=5) and interventions to provide clean water for aquaculture through changing the water temperature (n=1), or adding advanced oxidation processes (n=1), ultra-violet radiation (n=1), fish and shrimp polyculture (n=1), or Nile tilapia and filter-feeding bivalve mussles (n=1)	55,65,67,75,76,85,97,116,129,135
Air	
Air quality (n=3)	
Interventions to improve air quality on farms, including reducing airborne microorganisms with air filtration, super plasma ionising air purifiers, or with acidic electrolysed-water spray (n=3)	62,72,132
Sanitation	
Waste management* (n=24)	
A structural intervention implementing a livestock Manure Control Act that makes it compulsory for farms to be equipped with appropriate sludge process facilities (n=1)	140
Studies in manure composting methods: adding wet slurry to the cattle manure bedding (n=1), adding urea and ammonia treatments (n=1), or use of black soldier-fly larvae, bamboo charcoal, and high temperatures to prevent the persistence of antibiotic resistance genes in the manure (n=3); and interventions with microbial fermentation beds, septic tanks, biogas digesters, and natural drying methods to decrease antibiotic resistance genes in animal manure (n=3)	41-43,45,46,51,71,92
Interventions to reduce animal contact with their own excreta by adjusting farm flooring, heating of the barn floor, or the litter type or bedding used (n=7)	53,54,59,89,130,133,137
An intervention that enables biosecure disposal of infected pig carcasses to prevent pathogens escaping from the farm (n=1)	87
Studies implementing strategies to control pathogens in poultry litter (n=4), two studies looked at the safety in the repeated reuse of litter, and two studies used on-farm litter treatments with quicklime or litter tarping to reduce microbial counts	66,68,115,120
Intervention to control *Escherichia coli* O157:H7 faecal prevalence in feedlot cattle by adjusting the timeframe that artificial lighting is used in (n=1)	109
Integrated fish–farm investigations into the effect of filtering processes to removing antibiotic resistance genes from farm wastewater in flow-through aquaculture (n=1) and constructed wetlands (n=1)	44,52
Hygiene	
Food or feed safety (n=2)	
Introducing hygiene strategies to safely manage and store food products for humans and animals; one study investigated an intervention to address improved water and feed hygiene for cattle (n=1) and one study looked at the characteristics of bacteria in water in the troughs of litter-managed chicken systems (n=1)	61,112
Cleaning and disinfection (n=33)	
Interventions to test different disinfectant practices to eliminate or control infectious diseases in the farm environment, including the application and comparison of different detergents and commercially available disinfectants, the use of a high-pressure water rinse, and wet versus dry cleaning (n=17); one study compared cleaning and disinfection to competitive exclusion practices	40,48,57,64,70,73,78,81-83,96,99-103,124
Interventions that change farm hygiene practices, cleaning and disinfectant protocols, and cleaning products (n=2), including handwashing and the introduction of a hygienic barrier for footwear and overalls changing areas	86,98
Interventions that take measures to improve hygiene of the farm, ensuring a proper environment, including regular cleaning of animal facilities (n=2)	93,128
Interventions to improve staff hygiene practices on farms; testing protocols to improve hand hygiene in veterinary staff (n=1) or altering hygiene practices in animal production workers' shower facilities (n=1)	50,69
An intervention implementing a code of hygienic practices in poultry farms through a participatory staff training programme (n=1)	77
Interventions implementing a set of hygienic milking practices to prevent and control mastitis (n=5); changing milking order and technique, making use of disposable plastic gloves during milking, individual towels for wiping cow teats, and dipping cow teats in disinfectant post milking	49,63,95,113,138
Educational interventions to improve practices around mastitis (n=4), including the importance of hand washing before and after milking and hygienic farm management in mastitis prevention and control	60,117,131,136

(Table 3 continues on next page)

	References
(Continued from previous page)	
Other biosecurity measures complementing traditional WASH	
Barrier implementation (n=13)	
Interventions to preserve boundaries, implement barriers, or introduce policy strategies to limit exposure to microorganisms between animals and humans, and control potential vectors and fomites, including testing footbaths by looking at the bactericidal effects of commercial disinfectants to clean farm workers' boots (n=2)	[79,104]
Preventing pathogens from entering the farm and enhancing biosecurity compliance by improving existing practices, including changing clothes and showering before entering thefarm (n=1)	[56]
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 or household	[105,126]
Implementing a pond shutdown strategy in aquaculture (n=1) or a vacancy period in livestock (n=1) to manage disease outbreaks on commercial farms	[58,127]
Implementing animal movement strategies (n=3), including the strategic movement of animals after weaning to stop intergenerational pathogen transmission chains	[88,90,111]
Testing educational and behavioural change interventions to improve backyard poultry biosecurity, human protection, and disease management, yard, equipment, and poultry-pen cleaning, and the use of cages to protect chicks (n=1)	[139]
Interventions to prevent the transmission of pathogens through farm workers with management strategies that aim to disrupt the transmission cycle of existing pathogens on the farm (n=2), including changes to the movement patterns and biosecurity practices of farm staff regarding the use of personal protective equipment, clean footwear, and face shields	[74,94]
Health protection (n=4)	
Interventions to boost immunity, manage infections in humans and animals, or improve access to health care, ensuring wellness, welfare, and productivity for humans and animals, including herd-specific intervention strategies and health planning focusing on the optimisation of herd management to reduce antimicrobial use (n=3) and a study comparing organic antibiotic-free animal management practices to conventional farming methods (n=1)	[37,39,47]
Combined interventions (n=2)	
Interventions that make use of combined strategies related to hygiene or biosecurity to reduce the prevalence of bacterial disease in dairy cattle, including a questionnaire to evaluate a set of biosecurity practices to prevent digital dermatitis (n=1)	[119,134]

WASH=water, sanitation, and hygiene. *Interventions to establish strategies or policies to safely dispose of wastewater or fallen stock, or treat animal or human faeces, or mitigate the risk of antibiotic resistance genes in animal manure.

***Table 3*: Overview of WASH and biosecurity interventions reviewed**[28]

water sources for animals,[80,84,91,108,114,122,123] or improved air quality.[62] Mixed-effects interventions partly reduced the presence of bacteria, and inadvertently increased bacterial prevalence in other farm areas.[96,100,101,103] Barrier implementation strategies focusing on changing personal protective equipment or taking showers when entering farms,[94] use of disposable or bleach boot baths by farmworkers,[74] bag-in-a-box shipping methods, and strategic animal movement[88,90] were effective biosecurity strategies to lower[88,94] or eliminate[74,90] microbial transmission among animals because they reported positive effects. Three waste management studies[71,92] focusing on safely composting manure,[92] manure cultivation,[71] or fallen stock disposal[87] to reduce microbial loads in the environment, reported positive effects. One study showed a significant ($p<0\cdot05$) reduction of *E coli* via manure cultivation, whereas another ascertained the ability of urea and ammonia to remove *Salmonella* or *Yersinia enterocolitica*-contaminated pig slurry. The studies had a low[71] and moderate[87,92] risk of bias, respectively. Two studies applying fish polyculturing of Liza and Catfish,[65] or Nile tilapia[67] with shrimp or mussels successfully reduced *Vibrio* spp in a commercial farm in India and *Streptococcus agalactiae* in an experimental culture system in Malaysia by 80% and 87% (percentages converted from log reduction). Two studies, with commercial air filtration or purification products in commercial poultry in China, sought to improve air quality by spraying slightly acidic electrolysed water[62] or super plasma ionising air purifiers[72] to reduce microbial counts in air samples. Both interventions had positive results, reducing 25–50% (percentage converted from log reduction) of total indoor airborne bacterial counts. They also reported co-benefits, as the spray reduced airborne fungi by 35%, and the air purifiers reduced broiler mortality and significantly ($p=0\cdot003$) improved weight gain. Finally, two interventions addressing water quantity had negative outcomes. The use of water troughs to improve welfare and access to water for ducks,[107] or to reduce *E coli* O157:H7 faecal shedding in feedlot cattle by adjusting the water-to-cattle ratio in troughs,[106] caused a statistically significant increase of microbial loads of *E coli*, coliforms, and *Staphylococcus* ($p=0\cdot001$); greater duck mortality ($p=0\cdot008$)[107] and *E coli* O157:H7 shedding (odds ratio 1·6, 95% CI 1·2–2·0; $p=0\cdot02$);[106] and greater risk for farmworkers.

From interventions aiming to reduce infections or disease burden, 26 studies reduced the incidence or prevalence, or reduced morbidity or mortality rates in animals (24 studies)[37,49,63,64,67,72,77,86,107,113,126–139] or humans (two studies),[105,140] of which 18 had positive,[37,63,64,67,72,77,86,126–135,140] three mixed,[49,136,137] three negative,[105,107,139] and two neutral

	Positive 54·8% (n=57)*	Mixed 16·3% (n=17)*	Negative 10·6% (n=11)*	Neutral 18·3% (n=19)*
Water				
Water quantity (n=2, 1·9%)	..	..	2	..
Reduced microbial load	..	..	1	..
Reduced burden of infections or diseases	..	..	1†	..
Water quality (n=21, 20·2%)	10	1	3	7
Reduced microbial load	7	1	2	7
Reduced burden of infections or diseases	3†	..	..	..
Reduced antibiotic resistance	..	..	1	..
Air				
Air quality (n=3, 2·8%)	3	..	..	..
Reduced microbial load	1	..	..	..
Reduced burden of infections or diseases	2†	..	..	..
Sanitation				
Waste management (n=24, 23·1%)	15	4	2	3
Reduced microbial load	6	..	1	2
Reduced burden of infections or diseases	3	1	..	..
Reduced antibiotic use	1‡	..	..	..
Reduced antibiotic resistance	5	3	1	1
Hygiene				
Food or feed safety (n=2, 1·9%)	1	..	..	1
Reduction of microbial load	1	..	..	1
Cleaning and disinfection (n=33, 31·7%)	16	11	0	6
Reduced microbial load	10	7	..	2
Reduced burden of infections or diseases	6†	1	..	2†
Reduced antibiotic use	..	1‡	..	..
Reduced antibiotic resistance	..	2†	..	2
Other biosecurity measures complementing traditional WASH				
Barrier implementation (n=13, 12·5%)	7	1	4	1
Reduced microbial load	5	1	1	..
Reduced burden of infections or diseases	2	..	2†	..
Reduced antibiotic resistance	..	..	1†	1†
Health protection (n=4, 3·8%)	4	..	..	..
Reduced antibiotic use	3§	..	..	..
Reduced antibiotic use	1†	..	..	..
Combined interventions (n=2, 1·9%)				
Reduced microbial load	..	1	..	..
Reduced burden of infections or diseases	1	..	..	..

WASH=water, sanitation, and hygiene. *An intervention appears more than once if it addresses several relevant outcomes belonging to a different outcome grouping: 16 of 104 articles included outcomes relevant to different outcome groupings. †Includes one or more interventions that also measured microbial load reduction. ‡Includes one or more interventions that also measured reduction in antibiotic resistance. §Includes one or more interventions that also measured burden of infections or disease reduction.

Table 4: **Outcomes of WASH or biosecurity interventions with reported effect**

effects.[113,138] Seven interventions were classified as biological–chemical, eight physical–infrastructural, six managerial, four educational–behavioural, and one structural. Nearly all biological–chemical-based interventions (six of seven) had positive effects in reducing infectious diseases and mortality,[64,67,86,128,129,135] and one had no effect.[138] The successful interventions either applied chemical desinfectants and improved farm hygiene strategies in conventional agriculture, or improved water quality in aquaculture with ultraviolet irradiation, polyculture, or temperature control. The only study classified as structural[140] was a waste management intervention evaluating the effectiveness 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. This intervention attained a 33% (95% CI 13–53; $p<0·01$) decrease in human leptospirosis incidence during a 7-year post-implementation period. Three negative-effect interventions were classified as educational or behavioural (one study)[139] and physical or infrastructural (two studies),[105,107] with the intended outcome of reducing infections or diseases and mortality. Two of these were barrier implementation studies aiming to reduce backyard poultry biosecurity for subsistence farmers,[105,139] by corralling poultry and changing hygiene practices, which resulted in increased campylobacteriosis incidence in children,[105] and highly pathogenic avian influenza mortality rates.[139] The other study compared two water systems for ducks to reduce bacterial contamination and mortality.[107]

Discussion

This systematic review summarises the effect of WASH and biosecurity interventions on infection burden, microbial loads, antibiotic use, and antibiotic resistance in animal agricultural settings. From 104 selected studies, positive effects were reported for interventions implementing: strategies to improve water quality in aquaculture; waste management by preventing contamination of water bodies with antimicrobial resistance genes or enforcing policies to provide farmers with sludge processing facilities to reduce antibiotic resistance genes in manure; barriers to disrupt transmission cycles in farms by providing farmworkers with personal protective equipment; health protection measures involving farmers and veterinarians in discussions to reduce antibiotic use; or air-quality improvements in animal facilities.

Reduction of antibiotic use by 19–52%[37–39] was attained by interventions involving discussions among farmers, veterinarians, and facilitators with knowledge of antibiotics stewardship, although the impact of reducing antibiotic use and the abundance of antimicrobial resistant bacteria was not evaluated. A study[40] applying a pre-determined cleaning and disinfection protocol without previous consultation with relevant stakeholders attained a lower reduction in antibiotic use (9–20%). It is important to note that these studies were applied in different livestock production or intensification systems and countries, where the baseline situation could vary depending on the national policies related to antibiotic use already implemented.

The different metrics found across studies could have influenced the difference in reduction of antibiotic use. However, implementing problem-oriented approaches to reducing the use of antibiotics, in which stakeholders are consulted about their needs and goals, could improve alignment with interventions and contribute to the success of programmes to reduce antibiotic use. Similar reports from Denmark[141] and the Netherlands,[142] where bans on antibiotic use as growth promoters were introduced in the 2000s, indicate that the involvement of farmers and veterinarians in implementing health management plans within farms contributed to an effective reduction of antibiotic use.

Reductions in antibiotic resistance were mainly attained by studies focusing on antibiotic resistance gene mitigation in animal manure or farm wastewater. Collectively, these robustly designed studies showed that it is possible to reduce a broad range of clinically important antibiotic resistance genes by 21–99%, including tetracycline, sulfonamide, macrolide, vancomycin resistance genes, and mobile genetic elements. This result is particularly promising, as these are among the most frequently detected antimicrobial resistance genes in livestock waste.[143] Nonetheless, more research is needed to understand the factors influencing antibiotic resistance gene elimination and how best to manage manure outside commercial and experimental settings. Ultimately, approaches to reduce antibiotic gene resistance are crucial for reducing antibiotic resistance in other sectors, as the discharge of antibiotic resistance genes in animal waste represents a challenge of clinical importance not only for humans, but also for animals,[144] increasing the risk of hampering animal health and productivity.

Half of the interventions to reduce microbial loads in animals, humans, and the farm environment were successful. From these, interventions applied in aquaculture were especially relevant to antibiotic resistance, as prophylactic antibiotic use (particularly in LMICs) is considered a hotspot for the horizontal exchange of antibiotic resistance genes, which can easily contaminate nearby water resources.[145,146] These studies attempted to reduce initial microbial loads in the aquatic environment by deploying various interventions, including polyculture, feeding fish with fermented manure, shifting water temperatures, and installing ultra-violet light and oxidative processes. The efficacy of these interventions against a range of clinically relevant bacteria (eg, *E coli*, *Salmonella* spp, *Vibrio* spp, *Streptococcus* spp, *Staphylococcus* spp, and other coliforms) indicates that these strategies should be further explored to reduce excessive antibiotic use in aquaculture.

Interventions aiming to reduce infections in either animals or humans broadly took place in smallholder or subsistence farming settings, where curbing antibiotic use is particularly important as appropriate animal health-care services and diagnostic tools can be absent, inadequate, or inaccessible. These interventions often did not directly assess their effect on antibiotic use or antibiotic resistance. In these settings, an intervention including the free provision of cleaning tools to farming families[128] successfully reduced mortality rates in sheep. The evidence suggests that applying cleaning and disinfection regimens and products should supplement good hygienic practices rather than replace them outright,[83,101,124] as more than half of studies, including cleaning and disinfection protocols, reported mixed or neutral effects and changes in microbial loads that were transient.[50] Two-thirds of interventions that involved a combination of changing a farm personnel's hygiene practices with other measures reported positive results. However, these studies often did not measure adherence to introduced biosecurity practices and sustained changes in behaviour. When the studies did make these assessments, the simplicity and feasibility of biosecurity interventions influenced adherence by farmers,[38] especially for interventions that changed working habits and routines by improving hand and personal hygiene, changing needles, and implemented regular analysis of water quality.

	Positive 54·8% (n=57)*	Mixed 16·3% (n=17)*	Negative 10·6% (n=11)*	Neutral 18·3% (n=19)*
Structural (n=1, 0·9%)	1	..	..	..
Reduced burden of infections or diseases	1	..	..	..
Managerial (n=18, 17·3%)	11	2	2	3
Reduced microbial load	4	..	1	1
Reduced burden of infections or diseases	3†	..	..	1
Reduced antibiotic use	3	1‡	..	..
Reduced antibiotic resistance	1†	1§	1†	1†
Educational or behavioural (n=8, 7·7%)	4	2	1	1
Reduced microbial load	2	..	..	1
Reduced burden of infections or diseases	2†	1	1	..
Reduced antibiotic resistance	..	1†	..	..
Physical or infrastructural (n=26, 25%)	13	3	6	4
Reduced microbial load	6	..	3	3
Reduced burden of infections or diseases	5†	1	2†	..
Reduced antibiotic use	1‡	..	..	..
Reduced antibiotic resistance	1	2	1	1
Biological or chemical (n=51, 49·1%)	28	10	2	11
Reduced microbial load	18	9	1	8
Reduced burden of infections or diseases	6†	..	..	1
Reduced antibiotic resistance	4	1	1	2†

*An intervention appears more than once if it addresses multiple relevant outcome-types; 16 of 104 articles included outcomes relevant to different outcome groupings. †Includes one or more interventions that also measured microbial load reduction. ‡Includes one or more interventions that also measured reduction in antibiotics resistance. §Includes one or more interventions that also measured disease burden, infections, or disease reduction.

Table 5: **Reported effects organised by intervention level**

Interventions incurring high costs or introducing pronounced changes (such as implementing technical cleaning systems) were less common, and their effectiveness could be undermined by underapplication if the increased costs of implementing biosecurity co-occur with a (perceived) lack of reward.[38,56] Similarly, previous findings suggested that insufficient information on costs and revenues of implementing biosecurity practices can hinder the adoption of stringent preventive measures on farms.[147] Smallholders and subsistence farmers require evidence of the economic benefits to adopt biosecurity measures.[139] In this systematic review, most studies did not measure adoption, adherence, or cost-effectiveness. In addition to measuring adherence to changes by farmers, a cost-effectiveness analysis is needed to make the case for investing in an intervention.

The few studies reporting negative outcomes also offer important learning opportunities. Negative results often occurred when experimental designs failed to reproduce the same positive effect in a real-world setting. A previous modelling study suggested that reducing the volume of water in troughs by half would reduce *E coli* O157:H7 prevalence in feedlot cattle, yet the opposite effect occurred when tested on farms.[106] Similarly, a study based on previous epidemiological investigations hypothesised that corralling backyard free-ranging chickens in a peri-urban shantytown would reduce rates of *Campylobacter*-related diarrhoea in children exposed to those chickens;[105] however, children from corralling groups had twice the incidence of *Campylobacter*-related diarrhoea compared with control households, potentially due to less exposure to local *Campylobacter* strains, which affected children's immunity to external strains. Given the discrepancies between hypothesised effects and their imperfect translation into tangible improvements, especially in experimental studies (ie, more likely to report positive results), it is crucial to account for the settings and other relevant contextual factors when assessing interventions.

Several contextual factors that are also structural (such as the economic, social, and political contexts in which interventions are being implemented) can hinder or promote the adoption of measures and shape reliance on antibiotics. However, such factors are poorly documented in the studies included. It is commonly assumed that interventions are transferable beyond the research setting, but this is not always true. For WASH and biosecurity interventions the context is crucial, for example, the farm setting (ie, type of production system, location, population density, and infrastructure) strongly influences the risk of introducing pathogens and therefore the efficacy of any implemented measures. The lack of consideration of the influence of the context in the success or failure of these types of interventions may explain why fewer studies were performed in low-resource settings, where most farmers are smallholders or subsistence producers and do not engage in intensive farming practices, therefore the associated challenges of implementing interventions in these settings (ie, accessibility of the location, availability of funding, length of the study, ease of recruitment, and political and social barriers) make them less attractive for trialling interventions. In low-resource settings (smallholders and subsistence farmers), only three interventions addressed aspects such as the confinement of animals or educational interventions on biosecurity,[105,126,139] with mixed effects. To assess the enabling and limiting conditions for how interventions in these fields might, or might not, work in different settings, authors should document the context when reporting their results (eg, by applying a socioecological framework).[148] This documentation is especially important in animal production because the evidence suggests that the take-up of biosecurity measures by farmers and farmworkers (especially in LMICs) is shaped and influenced by context-related factors beyond psychosocial influences,[149] such as competing priorities, structural factors,[150] and perceived lack of support by governments.[151]

Only one intervention was classified as structural and successfully reduced the incidence of human leptospirosis by providing farmers with sludge processing facilities.[140] Although such measures could be essential in combating antimicrobial resistance, we found insufficient evidence to show this. In animal health, the introduction of regulations for the use of antimicrobials as growth promoters by some European countries,[152] together with a set of supportive measures (eg, the implementation of agriculture extension services, monitoring systems, farmer support programmes, farm treatment plans, farm health plans, and task forces with relevant stakeholders) successfully reduced antibiotic use. These are examples of how integrated structural changes promoted at a system level can positively reduce antibiotic use and affect antimicrobial resistance. In comparison, the introduction of regulations for antibiotic use in Mexico without additional supporting measures failed to produce positive results,[153] highlighting the importance of integrated measures addressing co-existent structural factors. Opportunities to pilot interventions in WASH and biosecurity in agricultural settings at a structural or system level might be most beneficial if focused on farm planning (eg, location, density, and size); the provision of incentives or safety nets for farmers to implement biosecurity measures focusing on disease prevention rather than control; and the implementation of WASH interventions that also address animal health problems and the safe disposal of animal waste.

Overall, the risk of bias was deemed high for 53% of selected studies. In our systematic review, most RCTs with high risk of bias contained few details about their randomisation methods and allocation concealment. Despite these flaws, almost all RCTs made conclusions with a low risk of reporting bias. Non-randomised trials with high risk of bias frequently showed issues with sample size, bias due to confounding, and selective reporting. Similarly, most ecological and cross-sectional studies with high risk of bias omitted sample size justification. We tried to minimise differences in bias assessment due to differences in study designs with various tools.

Important limitations of our systematic review include that (1) half of the selected studies reported positive effects—highlighting the need for assessment of publication bias; (2) the analysis of the context in which interventions were trialled was not possible as descriptions were often insufficient; (3) many authors did not provide sufficient information on their method and study designs for a confident assessment of the risk of bias and the interpretation of the results; (4) the heterogeneity and complexity of interventions included and the diversity of outcomes reported prevented us from conducting a meta-analysis; and (5) although we complemented our searches through snowballing, we did not include search terms for all countries beyond LMICs therefore some studies could have been missed.

The potential for WASH interventions, including an animal component to support antimicrobial resistance control strategies and the relevance of food-producing animals to WASH, has already been recognised,[28,144] particularly as water is a vehicle for spreading animal and human waste, and associated antimicrobial resistance genes. However, as WASH interventions often do not assess animals or the occupational risk of farmworkers or household members of farming or agricultural communities, many of these studies were excluded, creating a bias for studies focusing on animals only. Another important analysis would show whether reported positive effects were short term or sustained, which was often excluded by authors. Future studies in WASH and biosecurity should consider how the documentation and assessment of contextual factors influences the success or failure of interventions, and analyse the effects of combining interventions from these two fields (WASH and biosecurity), especially in agricultural communities.

Conclusion

Overall, this systematic review identifies several potentially effective interventions to reduce infection burden, microbial loads, antibiotic use, and antibiotic resistance in animal agricultural settings. Future studies in WASH and biosecurity could test some of these interventions or combinations of them, specifically in small-scale production systems and LMICs. Human health researchers must consider that most microorganisms important for animals are also important for human health as they can be a source of antibiotic resistance genes, especially in settings where humans and animals interact frequently. The provision of WASH is essential in creating the basic conditions for good health, but a One Health approach that recognises the closeness of people living or working with animals and the subsequent exchange of pathogens and contamination of the environment is often overlooked. The paucity of studies evaluating structural interventions in agricultural communities indicates an important gap to be filled by future research. Moreover, addressing antimicrobial resistance in populations engaging in animal agriculture (almost half of the world) requires drawing on and developing further evidence—such as in this systematic review—of findings from across the One Health spectrum.

Contributors

CIRC and CEPJ conceived the systematic review and wrote the protocol. OC provided feedback for the protocol. CEPJ developed the search strategies and executed the searches. SK and PT independently screened titles, abstracts, and full text of English articles. CEPJ conducted the screening of titles, abstracts and full text of articles written in Spanish, Portuguese, and French. SK and PT conducted the data extraction and bias assessment of English articles. SK extracted data and conducted the bias assessment from articles in German, and CEPJ in Spanish, Portuguese, and French. CIRC contributed to data interpretation and data analysis. SK, PT, and CEPJ synthesised the results and drafted the manuscript. CEPJ and CIRC reviewed and edited the manuscript. OC, AJP, and AM critically reviewed and edited the manuscript. All authors had full access to all data and can take responsibility for the integrity of the data and the accuracy of the data analysis. All authors have read and contributed to the latest version of this manuscript.

Declaration of interests

We declare no competing interests.

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References

1 Costelloe C, Metcalfe C, Lovering A, Mant D, Hay AD. Effect of antibiotic prescribing in primary care on antimicrobial resistance in individual patients: systematic review and meta-analysis. *BMJ* 2010; **340:** c2096.

2 Tangcharoensathien V, Chanvatik S, Sommanustweechai A. Complex determinants of inappropriate use of antibiotics. *Bull World Health Organ* 2018; **96:** 141–44.

3 Prendergast AJ, Gharpure R, Mor S, et al. Putting the "A" into WaSH: a call for integrated management of water, animals, sanitation, and hygiene. *Lancet Planet Health* 2019; **3:** e336–37.

4 Landers TF, Cohen B, Wittum TE, Larson EL. A review of antibiotic use in food animals: perspective, policy, and potential. *Public Health Rep* 2012; **127:** 4–22.

5 Gillings MR. Evolutionary consequences of antibiotic use for the resistome, mobilome and microbial pangenome. *Front Microbiol* 2013; **4:** 4.

6 Holmes AH, Moore LS, Sundsfjord A, et al. Understanding the mechanisms and drivers of antimicrobial resistance. *Lancet* 2016; **387:** 176–87.

7 Van Boeckel TP, Pires J, Silvester R, et al. Global trends in antimicrobial resistance in animals in low- and middle-income countries. *Science* 2019; **365:** eaaw1944.

8 Puspa Raj Khanal. Guido Santini, Merrey DJ. Water and the rural poor: interventions for improving livelihoods in Asia. Bangkok, Thailand: Food and Agriculture Organization of the United Nations—Regional Office for Asia and the Pacific, 2014.

9 Mustafa G, Hervas S, Khan G, Sharma BR, Rahman M. Assessing potential interventions to maximize fisheries-water productivity in the eastern Gangetic Basin (EGB) evaluation of constraints and opportunities for improvement: context Gorai-Madhumati (GM) sub-basin. Center for Natural Resources Studies, Bangladesh International Water Management Institute, World Fish Centre, International Water Management Institute Challenge Program on Water and Food, 2009.

10 Jones BA, Grace D, Kock R, et al. Zoonosis emergence linked to agricultural intensification and environmental change. *Proc Natl Acad Sci USA* 2013; **110:** 8399–404.

11 Aerts M, Battisti A, Hendriksen R, et al. Technical specifications on harmonised monitoring of antimicrobial resistance in zoonotic and indicator bacteria from food-producing animals and food. *EFSA J* 2019; **17:** e05709.

12 Cheng G, Ning J, Ahmed S, et al. Selection and dissemination of antimicrobial resistance in agri-food production. *Antimicrob Resist Infect Control* 2019; **8:** 158.

13 Zambrano LD, Levy K, Menezes NP, Freeman MC. Human diarrhea infections associated with domestic animal husbandry: a systematic review and meta-analysis. *Trans R Soc Trop Med Hyg* 2014; **108:** 313–25.

14 Marquis GS, Ventura G, Gilman RH, et al. Fecal contamination of shanty town toddlers in households with non-corralled poultry, Lima, Peru. *Am J Public Health* 1990; **80:** 146–49.

15 Alders RG, Campbell A, Costa R, et al. Livestock across the world: diverse animal species with complex roles in human societies and ecosystem services. *Anim Front* 2021; **11:** 20–29.

16 de Vries SPW, Vurayai M, Holmes M, et al. Phylogenetic analyses and antimicrobial resistance profiles of *Campylobacter* spp. from diarrhoeal patients and chickens in Botswana. *PLoS One* 2018; **13:** e0194481.

17 Richardson EJ, Bacigalupe R, Harrison EM, et al. Gene exchange drives the ecological success of a multi-host bacterial pathogen. *Nat Ecol Evol* 2018; **2:** 1468–78.

18 Richards VP, Velsko IM, Alam T, et al. Population gene introgression and high genome plasticity for the zoonotic pathogen *Streptococcus agalactiae*. *Mol Biol Evol* 2019; **36:** 2572–90.

19 Muloi D, Ward MJ, Pedersen AB, Fèvre EM, Woolhouse MEJ, van Bunnik BAD. Are food animals responsible for transfer of antimicrobial-resistant *Escherichia coli* or their resistance determinants to human populations? A systematic review. *Foodborne Pathog Dis* 2018; **15:** 467–74.

20 Piper JD, Chandna J, Allen E, et al. Water, sanitation and hygiene (WASH) interventions: effects on child development in low- and middle-income countries. *Cochrane Libr* 2017; **2017:** CD012613.

21 Fewtrell L, Kaufmann RB, Kay D, Enanoria W, Haller L, Colford JM Jr. Water, sanitation, and hygiene interventions to reduce diarrhoea in less developed countries: a systematic review and meta-analysis. *Lancet Infect Dis* 2005; **5:** 42–52.

22 Glossary OIE. OIE Terrestrial Animal Health Code. 2019. https://www.oie.int/en/what-we-do/standards/codes-and-manuals/terrestrial-code-online-access/?id=169&L=1&htmfile=glossaire.htm (accessed May 20, 2021).

23 World Bank. Pulling together to beat superbugs: knowledge and implementation gaps in addressing antimicrobial resistance. Washington DC: International Bank for Reconstruction and Development. The World Bank, 2019. http://documents.worldbank.org/curated/en/430051570735014540/pdf/Pulling-Together-to-Beat-Superbugs-Knowledge-and-Implementation-Gaps-in-Addressing-Antimicrobial-Resistance.pdf (accessed Oct 25, 2019).

24 Blankenship KM, Bray SJ, Merson MH. Structural interventions in public health. *AIDS* 2000; **14** (suppl 1): S11–21.

25 Sipe TA, Barham TL, Johnson WD, Joseph HA, Tungol-Ashmon ML, O'Leary A. Structural interventions in HIV prevention: a taxonomy and descriptive systematic review. *AIDS Behav* 2017; **21:** 3366–430.

26 Katz MH. Structural interventions for addressing chronic health problems. *JAMA* 2009; **302:** 683–85.

27 Blankenship KM, Friedman SR, Dworkin S, Mantell JE. Structural interventions: concepts, challenges and opportunities for research. *J Urban Health* 2006; **83:** 59–72.

28 Pinto Jimenez C, Keestra S, Tandon P, et al. One Health WASH: an AMR-smart integrative approach to preventing and controlling infection in farming communities. *BMJ Glob Health* 2023; **8:** e011263.

29 Pinto Jimenez C, Chandler IRC. Protocol: WASH and biosecurity interventions for reducing burdens of infection, antibiotic use and antimicrobial resistance: a One Health mixed methods systematic review. Project report. London School of Hygiene and Tropical Medicine, 2020. https://researchonline.lshtm.ac.uk/id/eprint/4657795/1/Pinto-Chandler-2020_WASH_and_biosecurity%20interventions_review.pdf (accessed Oct 10, 2022).

30 Page MJ, McKenzie JE, Bossuyt PM, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. *BMJ* 2021; **372:** n71.

31 Hooijmans CR, Rovers MM, de Vries RBM, Leenaars M, Ritskes-Hoitinga M, Langendam MW. SYRCLE's risk of bias tool for animal studies. *BMC Med Res Methodol* 2014; **14:** 43.

32 Sterne JA, Hernán MA, Reeves BC, et al. ROBINS-I: a tool for assessing risk of bias in non-randomised studies of interventions. *BMJ* 2016; **355:** i4919.

33 Downes MJ, Brennan ML, Williams HC, Dean RS. Development of a critical appraisal tool to assess the quality of cross-sectional studies (AXIS). *BMJ Open* 2016; **6:** e011458.

34 Byrne D. Evaluating complex social interventions in a complex world. *Evaluation* 2013; **19:** 217–28.

35 UN. World population prospects 2019: definition of regions. 2019. https://population.un.org/wpp/DefinitionOfRegions/ (accessed Dec 20, 2020).

36 World Bank. World Bank country and lending groups: country classification. 2020. https://datahelpdesk.worldbank.org/knowledgebase/articles/906519-world-bank-country-and-lending-groups (accessed Dec 20, 2020).

37 Collineau L, Rojo-Gimeno C, Léger A, et al. Herd-specific interventions to reduce antimicrobial usage in pig production without jeopardising technical and economic performance. *Prev Vet Med* 2017; **144:** 167–78.

38 Postma M, Vanderhaeghen W, Sarrazin S, Maes D, Dewulf J. Reducing antimicrobial usage in pig production without jeopardizing production parameters. *Zoonoses Public Health* 2017; **64:** 63–74.

39 Speksnijder DC, Graveland H, Eijck IAJM, et al. Effect of structural animal health planning on antimicrobial use and animal health variables in conventional dairy farming in the Netherlands. *J Dairy Sci* 2017; **100:** 4903–13.

40 Dorado-García A, Graveland H, Bos ME, et al. Effects of reducing antimicrobial use and applying a cleaning and disinfection program in veal calf farming: experiences from an intervention study to control livestock-associated *MRSA*. *PLoS One* 2015; **10:** e0135826

41 Cai M, Ma S, Hu R, et al. Rapidly mitigating antibiotic resistant risks in chicken manure by *Hermetia illucens* bioconversion with intestinal microflora. *Environ Microbiol* 2018; **20:** 4051–62.

42 Li H, Duan M, Gu J, et al. Effects of bamboo charcoal on antibiotic resistance genes during chicken manure composting. *Ecotoxicol Environ Saf* 2017; **140:** 1–6.

43 Huang X, Zheng J, Tian S, et al. Higher temperatures do not always achieve better antibiotic resistance gene removal in anaerobic digestion of swine manure. *Appl Environ Microbiol* 2019; **85:** e02878–18.

44 Kim YB, Jeon JH, Choi S, Shin J, Lee Y, Kim YM. Use of a filtering process to remove solid waste and antibiotic resistance genes from effluent of a flow-through fish farm. *Sci Total Environ* 2018; **615:** 289–96.

45 Zhou X, Qiao M, Su JQ, et al. Turning pig manure into biochar can effectively mitigate antibiotic resistance genes as organic fertilizer. *Sci Total Environ* 2019; **649:** 902–08.

46 Holman DB, Hao X, Topp E, Yang HE, Alexander TW. Effect of co-composting cattle manure with construction and demolition waste on the archaeal, bacterial, and fungal microbiota, and on antimicrobial resistance determinants. *PLoS One* 2016; **11:** e0157539.

47 Alali WQ, Thakur S, Berghaus RD, Martin MP, Gebreyes WA. Prevalence and distribution of *Salmonella* in organic and conventional broiler poultry farms. *Foodborne Pathog Dis* 2010; **7:** 1363–71.

48 Luyckx K, Millet S, Van Weyenberg S, et al. Comparison of competitive exclusion with classical cleaning and disinfection on bacterial load in pig nursery units. *BMC Vet Res* 2016; **12:** 189.

49 Karzis J, Petzer I-M, Donkin EF, Naidoo V. Proactive udder health management in South Africa and monitoring of antibiotic resistance of *Staphylococcus aureus*; in dairy herds from 2001 to 2010. *J S Afr Vet Assoc* 2018; **89:** e1–8.

50 Leedom Larson KR, Wagstrom EA, Donham KJ, et al. MRSA in pork production shower facilities: an intervention to reduce occupational exposure. *J Agric Saf Health* 2012; **18:** 5–9.

51 Ben W, Wang J, Pan X, Qiang Z. Dissemination of antibiotic resistance genes and their potential removal by on-farm treatment processes in nine swine feedlots in Shandong province, China. *Chemosphere* 2017; **167:** 262–68.

52 Huang X, Luo Y, Liu Z, et al. Influence of two-stage combinations of constructed wetlands on the removal of antibiotics, antibiotic resistance genes and nutrients from goose wastewater. *Int J Environ Res Public Health* 2019; **16:** 4030.

53 Chuppava B, Keller B, Meißner J, Kietzmann M, Visscher C. Effects of different types of flooring design on the development of antimicrobial resistance in commensal *Escherichia coli* in fattening turkeys. *Vet Microbiol* 2018; **217:** 18–24.

54 Chuppava B, Keller B, Abd El-Wahab A, Sürie C, Visscher C. Resistance reservoirs and multi-drug resistance of commensal *Escherichia coli* from excreta and manure isolated in broiler houses with different flooring designs. *Front Microbiol* 2019; **10:** 2633.

55 Petersen A, Andersen JS, Kaewmak T, Somsiri T, Dalsgaard A. Impact of integrated fish farming on antimicrobial resistance in a pond environment. *Appl Environ Microbiol* 2002; **68:** 6036–42.

56 Velasquez CG, Macklin KS, Kumar S, et al. Prevalence and antimicrobial resistance patterns of *Salmonella* isolated from poultry farms in southeastern United States. *Poult Sci* 2018; **97:** 2144–52.

57 Pletinckx LJ, Dewulf J, De Bleecker Y, Rasschaert G, Goddeeris BM, De Man I. Effect of a disinfection strategy on the methicillin-resistant *Staphylococcus aureus* CC398 prevalence of sows, their piglets and the barn environment. *J Appl Microbiol* 2013; **114:** 1634–41.

58 Luyckx K, Millet S, Van Weyenberg S, et al. A 10-day vacancy period after cleaning and disinfection has no effect on the bacterial load in pig nursery units. *BMC Vet Res* 2016; **12:** 236.

59 Chuppava B, Keller B, El-Wahab AA, Meißner J, Kietzmann M, Visscher C. Resistance of *Escherichia coli* in turkeys after therapeutic or environmental exposition with enrofloxacin depending on flooring. *Int J Environ Res Public Health* 2018; **15:** 1993.

60 Suranindyah Y, Wahyuni E, Bintara S, Purbaya G. The effect of improving sanitation prior to milking on milk quality of dairy cow in farmer group. *Procedia Food Sci* 2015; **3:** 150–55.

61 Ellis-Iversen J, Smith RP, Van Winden S, et al. Farm practices to control *E. coli* O157 in young cattle—a randomised controlled trial. *Vet Res* 2008; **39:** 3.

62 Hao X, Cao W, Li B, Zhang Q, Wang C, Ge L. Slightly acidic electrolyzed water for reducing airborne microorganisms in a layer breeding house. *J Air Waste Manag Assoc* 2014; **64:** 494–500.

63 Nagahata H, Ito H, Maruta H, et al. Controlling highly prevalent *Staphylococcus aureus* mastitis from the dairy farm. *J Vet Med Sci* 2007; **69:** 893–98.

64 Bragg RR, Plumstead P. Continuous disinfection as a means to control infectious diseases in poultry. Evaluation of a continuous disinfection programme for broilers. *Onderstepoort J Vet Res* 2003; **70:** 219–29.

65 Abraham TJ. Effect of polyculture of shrimp with fish on luminous bacterial growth in grow-out pond water and sediment. *J Coast Life Med* 2014; **2:** 438–41.

66 Lopes M, Leite FL, Valente BS, et al. An assessment of the effectiveness of four in-house treatments to reduce the bacterial levels in poultry litter. *Poult Sci* 2015; **94:** 2094–98.

67 Othman F, Islam MS, Sharifah EN, Shahrom-Harrison F, Hassan A. Biological control of streptococcal infection in Nile tilapia *Oreochromis niloticus* (Linnaeus, 1758) using filter-feeding bivalve mussel *Pilsbryoconcha exilis* (Lea, 1838). *J Appl Ichthyology* 2015; **31:** 724–28.

68 Roll VFB, Dai Prá MA, Roll AP. Research on *Salmonella* in broiler litter reused for up to 14 consecutive flocks. *Poult Sci* 2011; **90:** 2257–62.

69 Traub-Dargatz JL, Weese JS, Rousseau JD, Dunowska M, Morley PS, Dargatz DA. Pilot study to evaluate 3 hygiene protocols on the reduction of bacterial load on the hands of veterinary staff performing routine equine physical examinations. *Can Vet J* 2006; **47:** 671–76.

70 Kim JH, Kim KS. Hatchery hygiene evaluation by microbiological examination of hatchery samples. *Poult Sci* 2010; **89:** 1389–98.

71 Weinberg Z, Chen Y, Khanal P, Pinto R, Zakin V, Sela S. The effect of cattle manure cultivation on moisture content and survival of *Escherichia coli*. *J Anim Sci* 2011; **89:** 874–81.

72 Zhang G, Zhang Y, Kim Y, et al. Field study on the impact of indoor air quality on broiler production. *Indoor Built Environ* 2011; **20:** 449–55.

73 de Castro Burbarelli MF, do Valle Polycarpo G, Deliberali Lelis K, et al. Cleaning and disinfection programs against *Campylobacter jejuni* for broiler chickens: productive performance, microbiological assessment and characterization. *Poult Sci* 2017; **96:** 3188–98.

74 Dee S, Deen J, Pijoan C. Evaluation of 4 intervention strategies to prevent the mechanical transmission of porcine reproductive and respiratory syndrome virus. *Can J Vet Res* 2004; **68:** 19–26.

75 El-Shafai SA, Gijzen HJ, Nasr FA, El-Gohary FA. Microbial quality of tilapia reared in fecal-contaminated ponds. *Environ Res* 2004; **95:** 231–38.

76 Balasubramanian S, Rajan MR, Raj SP. Microbiology of fish grown in a sewage-fed pond. *Bioresour Technol* 1992; **40:** 63–66.

77 Taslima Akhter A, Islam SS, Sufian MA, et al. Implementation of code of practices (CoP) in selected poultry farms of Bangladesh. *Asian Australas J Food Saf Secur* 2018; **2:** 45–55.

78 Hancox LR, Le Bon M, Dodd CER, Mellits KH. Inclusion of detergent in a cleaning regime and effect on microbial load in livestock housing. *Vet Rec* 2013; **173:** 167.

79 Jang Y, Chang B, Myeong D, Chung H, Choe N. Evaluation of the efficacy of disinfectant footbaths against *Salmonella typhimurium*. *J Prev Vet Med* 2016; **40:** 144–47.

80 van Bunnik BAD, Katsma WEA, Wagenaar JA, Jacobs-Reitsma WF, de Jong MCM. Acidification of drinking water inhibits indirect transmission, but not direct transmission of *campylobacter* between broilers. *Prev Vet Med* 2012; **105:** 315–19.

81 Kloska F, Casteel M, Kump FW, Klein G. Implementation of a risk-orientated hygiene analysis for the control of *Salmonella JAVA* in the broiler production. *Curr Microbiol* 2017; **74:** 356–64.

82 Martelli F, Gosling RJ, Callaby R, Davies R. Observations on *Salmonella* contamination of commercial duck farms before and after cleaning and disinfection. *Avian Pathol* 2017; **46:** 131–37.

83 Martelli F, Lambert M, Butt P, et al. Evaluation of an enhanced cleaning and disinfection protocol in *Salmonella* contaminated pig holdings in the United Kingdom. *PLoS One* 2017; **12:** e0178897.

84 Chiriboga Chuchuca C, Sánchez Quinche ÁR, Vargas González ON, Hurtado Flores LS, Quevedo Guerrero JN. Uso de infusión de oreganón *Plectranthus amboinicus* (Lour.) spreng y del vinagre en la crianza de pollos “Acriollados” (*Gallus gallus domesticus*) mejorados. *Acta Agron* 2016; **65:** 298–303.

85 Poblete-Chavez R, Cortes-Pizarro E, Rojas R. Treatment of seawater for rotifer culture uses applying adsorption and advanced oxidation processes. *Lat Am J Aquat Res* 2016; **44:** 779–91.

86 Gibbens JC, Pascoe SJS, Evans SJ, Davies RH, Sayers AR. A trial of biosecurity as a means to control *Campylobacter* infection of broiler chickens. *Prev Vet Med* 2001; **48:** 85–99.

87 Vitosh-Sillman S, Loy JD, Brodersen B, Kelling C, Eskridge K, Millmier Schmidt A. Effectiveness of composting as a biosecure disposal method for porcine epidemic diarrhea virus (PEDV)-infected pig carcasses. *Porcine Health Manag* 2017; **3:** 22.

88 Nietfeld JC, Feder I, Kramer TT, Schoneweis D, Chengappa MM. Preventing *salmonella* infection in pigs with offsite weaning. *J Swine Health Prod* 1998; **6:** 27–32.

89 Skånseng B, Svihus B, Rudi K, Trosvik P, Moen B. Effect of different feed structures and bedding on the horizontal spread of *campylobacter jejuni* within broiler flocks. *Agriculture* 2013; **3:** 741–60.

90 Dahl J, Wingstrand A, Nielsen B, Baggesen DL. Elimination of *Salmonella typhimurium* infection by the strategic movement of pigs. *Vet Rec* 1997; **140:** 679–81.

91 Argüello H, Carvajal A, Costillas S, Rubio P. Effect of the addition of organic acids in drinking water or feed during part of the finishing period on the prevalence of *Salmonella* in finishing pigs. *Foodborne Pathog Dis* 2013; **10:** 842–49.

92 Bolton DJ, Ivory C, McDowell DA. The effect of urea and ammonia treatments on the survival of *Salmonella* spp. and *Yersinia enterocolitica* in pig slurry. *J Appl Microbiol* 2013; **114:** 134–40.

93 Fablet C, Fravalo P, Robinault C, Jolly JP, Eono F, Madec F. Reduction of *Salmonella* shedding of finishing pigs with the implementation of sanitary measures in a French farrow to finish farm. 12th International Congress on Animal Hygiene 2005; **1:** 351–55.

94 Kim Y, Yang M, Goyal SM, Cheeran MCJ, Torremorell M. Evaluation of biosecurity measures to prevent indirect transmission of porcine epidemic diarrhea virus. *BMC Vet Res* 2017; **13:** 89.

95 Abdalla MOM, Elhagaz FMM. The impact of applying some hygienic practices on raw milk quality in Khartoum state, Sudan. *Res J Agric Biol Sci* 2011; **7:** 169–73.

96 Mannion C, Leonard FC, Lynch PB, Egan J. Efficacy of cleaning and disinfection on pig farms in Ireland. *Vet Rec* 2007; **161:** 371–75.

97 Mlejnková H, Sovová K. Impact of fish pond manuring on microbial water quality. *Acta Univ Agric Silvic Mendel Brun* 2012; **60:** 117–24.

98 van de Giessen AW, Tilburg JJHC, Ritmeester WS, van der Plas J. Reduction of *Campylobacter* infections in broiler flocks by application of hygiene measures. *Epidemiol Infect* 1998; **121:** 57–66.

99 Kamal MA, Khalaf MA, Ahmed ZAM, Jakee JE. Evaluation of the efficacy of commonly used disinfectants against isolated chlorine-resistant strains from drinking water used in Egyptian cattle farms. *Vet World* 2019; **12:** 2025–35.

100 Carrique-Mas JJ, Marín C, Breslin M, McLaren I, Davies R. A comparison of the efficacy of cleaning and disinfection methods in eliminating *Salmonella* spp. from commercial egg laying houses. *Avian Pathol* 2009; **38:** 419–24.

101 Davies RH, Wray C. Observations on disinfection regimens used on *Salmonella enteritidis* infected poultry units. *Poult Sci* 1995; **74:** 638–47.

102 Battersby T, Walsh D, Whyte P, Bolton D. Evaluating and improving terminal hygiene practices on broiler farms to prevent *Campylobacter* cross-contamination between flocks. *Food Microbiol* 2017; **64:** 1–6.

103 White D, Gurung S, Zhao D, et al. Evaluation of layer cage cleaning and disinfection regimens. *J Appl Poult Res* 2018; **27:** 180–87.

104 Nasr SAE, Ismael E, Laban SE, et al. Effectiveness of some disinfectants commonly used in footbaths inside poultry farms multi-contaminant water treatment. *J Agric Vet Sci* 2018; **11:** 1–6.

105 Oberhelman RA, Gilman RH, Sheen P, et al. An intervention-control study of corralling of free-ranging chickens to control *Campylobacter* infections among children in a Peruvian periurban shantytown. *Am J Trop Med Hyg* 2006; **74:** 1054–59.

106 Beauvais W, Gart EV, Bean M, et al. The prevalence of *Escherichia coli* O157:H7 fecal shedding in feedlot pens is affected by the water-to-cattle ratio: a randomized controlled trial. *PLoS One* 2018; **13:** e0192149.

107 Schenk A, Porter AL, Alenciks E, et al. Increased water contamination and grow-out Pekin duck mortality when raised with water troughs compared to pin-metered water lines using a United States management system. *Poult Sci* 2016; **95:** 736–48.

108 De Ridder L, Maes D, Dewulf J, et al. Evaluation of three intervention strategies to reduce the transmission of *Salmonella typhimurium* in pigs. *Vet J* 2013; **197:** 613–18.

109 Edrington TS, Callaway TR, Ives SE, et al. Seasonal shedding of *Escherichia coli* O157:H7 in ruminants: a new hypothesis. *Foodborne Pathog Dis* 2006; **3:** 413–21.

110 Alcántara AB, Ramos A, Hernández GR, Herradora Lozano M, Pablos-Hach J, Gamba R. The effect of using separation/sedimentation/filtration-treated wastewater on the health of weaning pigs. *Tecnica Pecuaria en Mexico* 2008; **46:** 287–302.

111 Davies PR, Morrow WEM, Jones FT, Deen J, Fedorka-Cray PJ, Harris IT. Prevalence of *Salmonella* in finishing swine raised in different production systems in North Carolina, USA. *Epidemiol Infect* 1997; **119:** 237–44.

112 Folorunso OR, Kayode S, Onibon VO. Poultry farm hygiene: microbiological quality assessment of drinking water used in layer chickens managed under the battery cage and deep litter systems at three poultry farms in southwestern Nigeria. *Pak J Biol Sci* 2014; **17:** 74–79.

113 Omore AO, McDermott JJ, Arimi SM, Kyule MN. Impact of mastitis control measures on milk production and mastitis indicators in smallholder dairy farms in Kiambu district, Kenya. *Trop Anim Health Prod* 1999; **31:** 347–61.

114 Jansen W, Reich F, Klein G. Large-scale feasibility of organic acids as a permanent preharvest intervention in drinking water of broilers and their effect on foodborne *Campylobacter* spp. before processing. *J Appl Microbiol* 2014; **116:** 1676–87.

115 Sonoda LT, Moura DJ, Bueno LGF, Cordeiro DC, Mendes AS. Broiler litter reutilization applying different composting concepts. *Rev Bras Cienc Avic* 2012; **14:** 227–32.

116 Elsaidy N, Abouelenien F, Kirrella G. Impact of using raw or fermented manure as fish feed on microbial quality of water and fish. *Egypt J Aquat Res* 2015; **41:** 31.

117 Berg A. Does hygiene training among farmers in northeast India give healthier cows? With special focus on animal welfare, milk yield and *Brucellosis*. Swedish University of Agricultural Sciences Library. Swedish University of Agricultural Sciences, 2015.

118 Mateus-Vargas RH, Kemper N, Volkmann N, Kietzmann M, Meissner J, Schulz J. Low-frequency electromagnetic fields as an alternative to sanitize water of drinking systems in poultry production? *PLoS One* 2019; **14:** e0220302.

119 Dale EL, Nolan SP, Berghaus RD, Hofacre CL. On farm prevention of *Campylobacter* and *Salmonella*: lessons learned from basic biosecurity interventions. *J Appl Poult Res* 2015; **24:** 222–32.

120 Line JE, Bailey JS. Effect of on-farm litter acidification treatments on *Campylobacter* and *Salmonella* populations in commercial broiler houses in northeast Georgia. *Poult Sci* 2006; **85:** 1529–34.

121 Amaral L, Nader Filho A, Isa H, Barros L. Qualidade higiênico-sanitária e demanda de cloro da água de dessedentação de galinhas de postura coletadas em bebedouros tipo nipple e taça. *Rev Bras Cienc Avic* 2001; **3:** 249–55.

122 Haughton PN, Lyng J, Fanning S, Whyte P. Potential of a commercially available water acidification product for reducing *Campylobacter* in broilers prior to slaughter. *Br Poult Sci* 2013; **54:** 319–24.

123 De Busser EV, Dewulf J, Nollet N, et al. Effect of organic acids in drinking water during the last 2 weeks prior to slaughter on *Salmonella* shedding by slaughter pigs and contamination of carcasses. *Zoonoses Public Health* 2009; **56:** 129–36.

124 Argüello H, Rubio P, Jaramillo A, Barrios V, García M, Carvajal A. Evaluation of cleaning and disinfection procedures against *Samonella enterica* at swine farms, transport and lairage facilities. *SafePork* 2011; **2011:** 254–57.

125 Stern NJ, Robach MC, Cox NA, Musgrove MT. Effect of drinking water chlorination on *Campylobacter* spp. colonization of broilers. *Avian Dis* 2002; **46:** 401–04.

126 Agustina KK, Swacita IBN, Oka IBM, et al. Reducing zoonotic and internal parasite burdens in pigs using a pig confinement system. *Vet World* 2017; **10:** 1347–52.

127 Hernandez-Llamas A, Magallon-Barajas FJ, Perez-Enriquez R, Cabanillas-Ramos J, Esparza-Leal HM, Portillo-Clark G. Pond shutdown as a strategy for preventing outbreaks of white spot disease in shrimp farms in Mexico. *Rev Aquacult* 2014; **6:** 67–74.

128 Doko SY, Degla P, Edoun GO, Bosma RH. Effect of hygiene and medication on preweaning survival and growth of Djallonké sheep in Atacora, Benin. *Trop Anim Health Prod* 2013; **45:** 129–34.

129 Sano M, Ito T, Matsuyama T, Nakayasu C, Kurita J. Effect of water temperature shifting on mortality of Japanese flounder *Paralichthys olivaceus* experimentally infected with viral hemorrhagic septicemia virus. *Aquaculture* 2009; **286:** 254–58.

130 Zhao Y, Li X, Sun S, et al. Protective role of dryland rearing on netting floors against mortality through gut microbiota-associated immune performance in Shaoxing ducks. *Poult Sci* 2019; **98:** 4530–38.

131 Kaneene JB, Ssajjakambwe P, Kisaka S, Vudriko P, Miller R, Kabasa JD. Improving efficiency of the dairy value chain in Uganda; effect of action research-based interventions on milk quality and safety. *Livest Res Rural Dev* 2016; **28:** 9.

132 Dee S, Cano JP, Spronk G, et al. Evaluation of the long-term effect of air filtration on the occurrence of new PRRSV infections in large breeding herds in swine-dense regions. *Viruses* 2012; **4:** 654–62.

133 Abd El-Wahab A, Visscher CF, Beineke A, Beyerbach M, Kamphues J. Effects of high electrolyte contents in the diet and using floor heating on development and severity of foot pad dermatitis in young turkeys. *J Anim Physiol Anim Nutr* 2013; **97:** 39–47.

134 Oliveira VHS, Sørensen JT, Thomsen PT. Associations between biosecurity practices and bovine digital dermatitis in Danish dairy herds. *J Dairy Sci* 2017; **100:** 8398–408.

135 Hoffman GL. Disinfection of contaminated water by ultraviolet irradiation, with emphasis on whirling disease (*Myxosoma cerebralis*) and its effect on fish. *Trans Am Fish Soc* 1974; **103:** 541–50.

136 Ng L, Jost C, Robyn M, et al. Impact of livestock hygiene education programs on mastitis in smallholder water buffalo (*Bubalus bubalis*) in Chitwan, Nepal. *Prev Vet Med* 2010; **96:** 179–85.

137 Berk J. Foot pad dermatitis in male broilers depending on different kinds of litter. *Landbauforsch Völkenrode* 2007; **57:** 171–78.

138 Schiavon DBA, Schuch L, Oyarzabal MEB, Prestes LS, Zani JL, Hartwig C. Use of medicinal plants for antisepsis of cows' teat after milking. *Rev Cuba Plantas Med* 2011; **16:** 253–59.

139 Conan A, Goutard FL, Holl D, et al. Cluster randomised trial of the impact of biosecurity measures on poultry health in backyard flocks. *Vet J* 2013; **198:** 649–55.

140 Ryu S, Lau CL, Chun BC. The impact of Livestock Manure Control Policy on human leptospirosis in Republic of Korea using interrupted time series analysis. *Epidemiol Infect* 2017; **145:** 1320–25.

141 Wielinga PR, Jensen VF, Aarestrup FM, Schlundt J. Evidence-based policy for controlling antimicrobial resistance in the food chain in Denmark. *Food Control* 2014; **40:** 185–92.

142 Speksnijder DC, Mevius DJ, Bruschke CJ, Wagenaar JA. Reduction of veterinary antimicrobial use in the Netherlands. The Dutch success model. *Zoonoses Public Health* 2015; **62** (suppl 1)**:** 79–87.

143 He Y, Yuan Q, Mathieu J, et al. Antibiotic resistance genes from livestock waste: occurrence, dissemination, and treatment. *npj Clean Water* 2020; **3:** 4.

144 WHO. Technical brief on water, sanitation, hygiene (WASH) and wastewater management to prevent infections and reduce the spread of antimicrobial resistance (AMR). World Health Organization (WHO), Food and Agriculture Organization of the United Nations (FAO) and World Organisation for Animal Health. OIE, 2020. https://www.who.int/publications/i/item/9789240006416 (accessed Dec 18, 2020).

145 Cabello FC. Heavy use of prophylactic antibiotics in aquaculture: a growing problem for human and animal health and for the environment. *Environ Microbiol* 2006; **8:** 1137–44.

146 Watts JEM, Schreier HJ, Lanska L, Hale MS. The rising tide of antimicrobial resistance in aquaculture: sources, sinks and solutions. *Mar Drugs* 2017; **15:** 158.

147 Laanen M, Maes D, Hendriksen C, et al. Pig, cattle and poultry farmers with a known interest in research have comparable perspectives on disease prevention and on-farm biosecurity. *Prev Vet Med* 2014; **115:** 1–9.

148 Léger A, Lambraki I, Graells T, et al. Characterizing social-ecological context and success factors of antimicrobial resistance interventions across the One Health spectrum: analysis of 42 interventions targeting *E. coli*. *BMC Infect Dis* 2021; **21:** 873.

149 Mankad A. Psychological influences on biosecurity control and farmer decision-making. A review. *Agron Sustain Dev* 2016; **36:** 40.

150 Ebata A, MacGregor H, Loevinsohn M, Win KS. Why behaviours do not change: structural constraints that influence household decisions to control pig diseases in Myanmar. *Prev Vet Med* 2020; **183:** 105138.

151 Gunn GJ, Heffernan C, Hall M, McLeod A, Hovi M. Measuring and comparing constraints to improved biosecurity amongst GB farmers, veterinarians and the auxiliary industries. *Prev Vet Med* 2008; **84:** 310–23.

152 Cogliani C, Goossens H, Greko C. Restricting antimicrobial use in food animals: lessons from Europe: banning nonessential antibiotic uses in food animals is intended to reduce pools of resistance genes. *Microbe Wash DC* 2011; **6:** 274–79.

153 Zaidi MB, Dreser A, Figueroa IM. A collaborative initiative for the containment of antimicrobial resistance in Mexico. *Zoonoses Public Health* 2015; **62** (suppl 1)**:** 52–57.