



Assessment of the Effectiveness of Pre-harvest Meat Safety Interventions to Control Foodborne Pathogens in Broilers: a Systematic Review

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Abstract

Purpose of Review Ensuring broilers' meat safety is a priority to policy makers, producers, and consumers. This systematic review aims to update the recent knowledge on pre-harvest interventions to control main foodborne pathogens in broilers and to assess their effectiveness.

Recent Findings A total of 815 studies were retrieved from PubMed® and Web of Science for 13 pathogens. In total, 51 studies regarding *Campylobacter* spp., *Salmonella* spp., VTEC, ESBL-AmpC *Escherichia coli*, and *Clostridium perfringens* were included in this review.

Summary Research mostly focused on *Salmonella* spp. and *Campylobacter* spp. Biosecurity and management interventions had mixed outcomes, while the effectiveness of feed additives, though intensively researched, remains controversial. Research on other pathogens (i.e. ESBL-AmpC *E. coli*/*Salmonella*, and *Toxoplasma gondii*) was scarce, with publications focusing on epidemiology and/or on source-attribution studies. This is also true regarding research on *Listeria monocytogenes*, *Bacillus cereus*, *Clostridium botulinum*, *Clostridium perfringens*, and *Staphylococcus aureus* as these are frequently controlled by post-harvest interventions. Overall, studies on recent developments of novel pathogen-specific immunisation strategies are lacking.

Keywords *Campylobacter* · Farm practices · Poultry · *Salmonella* · Zoonoses

Introduction

Worldwide, poultry meat consumption is growing rapidly and has surpassed pork as the preferred animal protein source in

2016 [1]. Among poultry meat products, broiler meat is the most consumed. Therefore, ensuring the safety of broiler meat and broiler meat products is a priority to policy makers, producers, and consumers.

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In 2012, the European Food Safety Authority (EFSA) issued a scientific opinion on the public health hazards to be covered by inspection of poultry. This document contained a list of 13 biological hazards (Table 1) that can be transmitted to humans through the handling, preparation, and/or consumption of broiler meat and meat products [2]. Among these were *Campylobacter* spp. and *Salmonella* spp., which have been the two most frequently reported human gastrointestinal bacterial pathogens in Europe for the past decade [3]. Indeed, while several potential transmission routes for these two pathogens exist, broilers have been identified as the main reservoir for *Campylobacter* [4], and are also an important food vehicle for *Salmonella* [5].

Although less commonly reported as causes of human foodborne illnesses, *Bacillus cereus*, *Clostridium botulinum*, *Clostridium difficile*, *Clostridium perfringens*, verotoxin-producing *Escherichia coli* (VTEC), *Listeria monocytogenes*, *Staphylococcus aureus* enterotoxins, *Yersinia enterocolitica*, and *Toxoplasma gondii* are also liable to cause infections, intoxications, or toxicoinfections through the consumption of contaminated broiler meat [2]. Furthermore, extended spectrum β -lactamase (ESBL)/AmpC gene carrying *E. coli* were considered to be of medium to high relevance to public health, while ESBL/AmpC gene carrying *Salmonella* were considered to be of low to medium relevance [2].

As these 13 pathogens continue to pose a threat to public health, control options using a farm to fork approach are needed to minimise the risk and spread of foodborne diseases by consumption of broiler meat. Pre-harvest interventions take place on-farm and/or during transport of animals to slaughter

and aim to minimise the introduction, persistence, and transmission of foodborne pathogens into broiler flocks. Indeed, several studies have shown that reducing the prevalence and/or the concentration of pathogens in primary broiler production would result in greater public health benefits than interventions at later stages in the food chain [4–6].

Pre-harvest interventions can be divided into two main categories that aim to (1) reduce the prevalence of flocks contaminated with a specific pathogen, and (2) reduce the concentration of a pathogen in broilers belonging to contaminated flocks.

In the first group, the main intervention strategies are related to external biosecurity measures, which prevent the introduction of a pathogen into a farm. Key interventions focus on breeding animals free from selected pathogens at the top of the breeding pyramid, the control of feed and water supplies, and implementing physical barriers that restrict access to broiler houses and the external environment around farms [6, 7]. Internal biosecurity, where high hygiene is implemented through good cleaning and disinfection protocols, is also an important preventative measure [7].

In the second group, several interventions focus on increasing host resistance to reduce pathogen load in the caeca and on the use of antimicrobial alternatives to reduce or eliminate selected pathogens from colonised broilers. Research in this area has focused on studying and developing new control strategies such as the use of feed and water additives, the application of bacteriophages, vaccination, bacteriocins, and competitive exclusion [8–12].

Table 1 Keyword search and flow of information through the systematic review for 13 foodborne pathogens

Pathogen	Keyword searched	Records identified	Records after duplicate removal	Records retained after abstract screening	Records retained after full text screening
<i>B. cereus</i>	bacillus cereus	3	3	0	0
<i>Campylobacter</i>	campylobacter* OR “campylobacter jejuni” OR “campylobacter coli”	230	208	34	24
<i>C. botulinum</i>	clostridium botulinum OR botulism	3	2	0	0
<i>C. difficile</i>	clostridium difficile OR c. difficile OR clostridioides difficile	0	0	0	0
<i>C. perfringens</i>	clostridium perfringens OR c. perfringens OR clostridial diarrh*	23	22	5	3
ESBL-Amp C <i>E. coli</i>	((ESBL* OR AmpC) AND E. coli)	57	53	6	2
ESBL-Amp C <i>Salmonella</i>	((ESBL* OR AmpC) AND salmonella)	9	9	0	0
<i>Listeria monocytogenes</i>	listeria monocytogenes OR listeriosis	14	13	0	0
MRSA	methicillin resistant staphylococcus aureus OR MRSA OR resistant <i>s. aureus</i>	17	17	0	0
<i>Salmonella</i> spp.	salmon*	301	282	33	20
VTEC	Escherichia coli OR toxicoinfectious <i>E. coli</i> OR VTEC	201	191	8	1
<i>Y. enterocolitica</i>	Yersini*	6	6	0	0
<i>T. gondii</i>	toxoplasma gondii OR toxoplasmosis	10	9	0	0

This review of literature published between 2015 and 2020 aims to update the knowledge on pre-harvest interventions to control 13 public health hazards enlisted in EFSA's report, and to assess their effectiveness.

Materials and Methods

This systematic review was based on EFSA guidelines issued for "those carrying out systematic reviews" for food and feed safety assessments [13], and on the methodology proposed in the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) statement [14].

Literature searches were carried out on PubMed® and Web of Science on June 7th, 2020, including peer-reviewed studies written in English and published between 2015 and 2020 (until June 7th) on the effectiveness of pre-harvest meat safety interventions to control 13 foodborne pathogens. The 5-year timeframe was introduced as a Journal requirement and narrowed down this systematic review to focus on the most recent research tackling the control of foodborne pathogens on-farm or during transport. All searches were restricted to title and abstract. The structure of the search strings used in each database is shown in Fig. 1, and each search had pathogen-specific keywords, as shown in Table 1. The detailed search strings used for each database can be assessed in the supplementary material.

All records were imported into EndNote and duplicates were removed. One co-author screened abstracts using a defined set of inclusion and exclusion criteria (Table 2). In a second phase, full texts of all remaining references were retrieved and screened in parallel by two co-authors, using the same eligibility criteria (Table 2). For any record to be removed, both co-authors had to agree on its exclusion. If agreement was not attained, a third co-author reviewed the full text

and made the final decision. The flow of information through the systematic review process is shown in Table 1.

A Microsoft Office Excel spreadsheet documented the classification of studies based on the intervention described and other relevant information, such as follows: country and year where/when the study took place; type of experimental study (challenge or field studies); type (i.e. animal, flock, environmental) and number of experimental units; sample type; type of outcome measured; and estimate of the effectiveness of each intervention. For this systematic review, "study" was defined as any primary research peer-reviewed publication, in which the authors had collected, analysed, and reported their own data. On several occasions, we found that within the same study, authors evaluated the efficacy of different interventions. Therefore, within each study, several trials could be reported (defined as the unique treatment-to-control comparisons made), and whenever possible, trial-specific information was collected. Whenever the outcome of an intervention was measured through several time-points, data collected at the end of the study were preferred.

Results

A total of 815 unique studies were retrieved through the search strings run on PubMed® and Web of Science, for the 13 pathogens included in this study. *Salmonella* spp., *Campylobacter* spp., and VTEC were the pathogens for which the largest number of studies was found (Table 1), while *C. difficile*, *C. botulinum*, and *B. cereus* had the fewest, with zero, two, and three studies, respectively. After the abstract-based screening, only five pathogens remained. These were as follows: *Campylobacter* spp. ($n = 34$ studies), *Salmonella* spp. ($n = 33$ studies), VTEC ($n = 8$ studies), ESBL-AmpC *E. coli* ($n = 6$ studies), and *C. perfringens* ($n = 5$ studies). Full texts were then retrieved for further evaluation. Even though 86 studies passed

Fig. 1 Search string structure used for the searches conducted in PubMed® and the Web of Science databases on June 7th, 2020. The detailed search strings used are provided as supplementary material

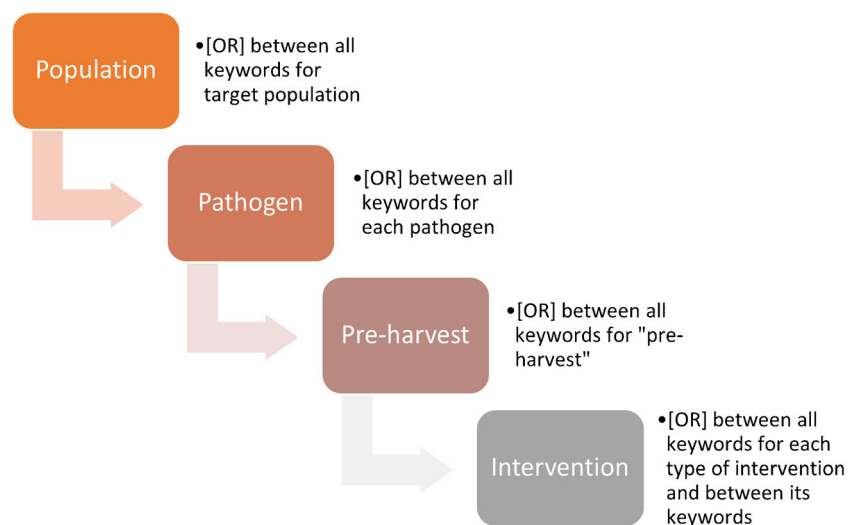


Table 2 Eligibility (inclusion and exclusion) criteria used for the screening of title/abstracts and full texts

PICO ^a	Inclusion	Exclusion
Population	Animal species being evaluated: must include (but not limited to) broilers Unit of study (animal, flock, house, farm and surfaces, food, water, environment, drinkers, feeder, other animals)	Does not include actual or theoretical <pathogen> infection/contamination in broilers Others
Intervention	Interventions to control/reduce/eradicate <pathogen> in broilers Interventions on-farm or during transport (pre-harvest) Field/experimental studies	Studies not mentioning control/reduce/eradicate interventions for <pathogen> in broilers Interventions on lairage, at slaughter and post-harvest Lab/bench studies
Comparison	Control group present (group subjected to no intervention)	Control group absent
Outcomes	Provides some measure of the efficacy of the intervention	Efficacy of the intervention not measured
Others	Language: English Peer reviews	Other languages Grey literature

^a PICO (participants, interventions, comparisons, and outcome(s)) constitutes the framework in which research questions are formulated, as proposed in the PRISMA statement [14]

the initial screening, one full text could not be retrieved, and 34 other studies were discarded during full text evaluation. Studies with the following characteristics were excluded ($n = 34$): studies not performed in the target population ($n = 14$, 41%), studies not mentioning a clear intervention ($n = 7$, 21%), studies with no control group ($n = 7$, 21%), studies which did not report a measurable outcome ($n = 3$, 9%), or in vitro studies ($n = 3$, 9%). Ultimately, a total of 51 studies (59% of the initial 86 studies) stratified by five different pathogens (Table 1) were included in this systematic review.

Within the reported studies (i.e. 24 studies regarding *Campylobacter* spp. and 20 studies regarding *Salmonella* spp.), a total of 71 and 62 trials on pre-harvest interventions to control *Campylobacter* spp. and *Salmonella* spp., respectively, were identified and included in this systematic review. Studies regarding *Campylobacter* spp. [15–34, 35, 36–38] and *Salmonella* spp. [15, 19, 39–56] were carried out in thirteen and eleven different countries, respectively. For both pathogens, most of the pre-harvest interventions were assessed in lab-based challenge trials (65% and 82%), while the remaining trials were field trials where no experimental infection was performed. Characteristics of these trials are summarised in Table 3.

Overall, the most common interventions to control *Campylobacter* were those evaluating the effect of feed additives (49%), followed by cleaning and disinfection programmes (23%). Regarding *Salmonella* spp., cleaning and disinfection programmes were the most frequent interventions applied (27%), followed closely by feed additives (21%).

All trials measured the effectiveness of each intervention by bacteriological isolation. For both pathogens, the most

common unit of measurement was log CFU/g, with 61% and 44% of trials reporting CFU/g for *Campylobacter* spp. and *Salmonella* spp., respectively. Still, many different units quantifying results were used. To overcome this, results were qualitatively characterised concerning the effectiveness of each intervention by considering whether a reduction in the outcome measured could be observed or not (Table 4).

Of the eight types of pre-harvest interventions to control *Campylobacter* spp. included in this review, only three had a positive effect. Biosecurity interventions proved to be most effective, with 71% of trials showing a reduction in *Campylobacter* spp. On the other hand, all biosecurity interventions were ineffective in reducing *Salmonella* spp., while all trials applying competitive exclusion and 80% of trials using bacteriophages proved to be effective in significantly reducing *Salmonella* spp.

Regarding *C. perfringens*, a total of 27 trials on pre-harvest interventions were identified in the three studies [16, 57, 58] included in this systematic review. Of those, 26 investigated the use of different feed additives to control this pathogen, while one trial used a drug-free programme with a combination of feed additives, improved water quality, and optimised brooding conditions. Of the 26 trials using feed additives, ten showed a significant reduction in bacterial counts (log CFU/g), while the drug-free programme proved ineffective.

One study, comprising two trials, investigated the use of feed additives and enrofloxacin to control ESBL-AmpC *E. coli*, showing that the first intervention was not effective in significantly reducing this pathogen, while enrofloxacin proved to be effective [59]. The second study investigated the effect of cessation of ceftiofur and its substitution with

Table 3 Descriptive characteristics of 72 trials from 24 studies and 62 trials from 20 studies investigating pre-harvest interventions to control *Campylobacter* spp. and *Salmonella* spp., respectively

Variable	Category	# trials, n (%)		
		<i>Campylobacter</i>	<i>Salmonella</i>	
On-farm vs. during transport intervention	On-farm	63 (89)	52 (84)	
	Transport coop	8 (11)	10 (16)	
Study setting	Commercial farm	20 (28)	11 (18)	
	Commercial plant	5 (7)		
	Research lab	46 (65)	51 (82)	
Type of intervention	Bacteriophages	1 (1)	5 (8)	
	Biosecurity	7 (10)	2 (3)	
	Cleaning & disinfection	16 (23)	17 (27)	
	Combination of measures	1 (1)	6 (10)	
	Competitive exclusion	-	3 (5)	
	Feed additives	35 (49)	13 (21)	
	Litter management	1 (1)	10 (16)	
	Oral immunisation	4 (6)	-	
	Vaccination	6 (9)	6 (10)	
	Sample type	Caecal	51 (72)	25 (40)
		Cloacal	1 (1)	6 (10)
Environmental		9 (13)	11 (18)	
Fresh faeces		1 (1)	-	
Litter		1 (1)	10 (16)	
Transport coop		8 (12)	10 (16)	

lincomycin-spectinomycin on the proportion of *E. coli* isolates harbouring ESBL-AmpC genes, which proved effective initially, but led to an increase in antimicrobial non- β -lactam resistance of ESBL-AmpC *E. coli* due to the use of this antibiotic [60].

Lastly, only one study regarding VTEC [61] was identified in this review. It investigated the effectiveness of feed supplementation with *Agaricus bisporus* in two different

concentrations (two trials) in the feed, but no statistical analysis was performed to assess if the reduction of VTEC counts reported in the trial using a higher concentration of the feed additive was significant.

Discussion

In the past 5 years, several studies have been published on pre-harvest interventions to control foodborne pathogens in broilers. These were mostly focused on two main hazards (*Salmonella* spp. and *Campylobacter* spp.), which reflects the high burden of disease associated with these pathogens.

Campylobacter and *Salmonella*

In this review, only two studies, in which biosecurity measures were applied to control *Campylobacter* spp. [15, 18] and *Salmonella* spp. [15], were identified, and both were carried out in a commercial setting. Dale et al. used a combination of pest control and hygienic actions for farm staff (e.g. hand disinfection, changing boots, house-specific working materials), which were insufficient measures to decrease the prevalence of either pathogen. The other study, focusing on *Campylobacter* control, encompassed five trials where a “biosecure cube” was installed and prevented contact between the farm-staff and the birds. All trials reported in this study

Table 4 Efficacy of pre-harvest interventions to control *Campylobacter* and *Salmonella* stratified by type. Effective interventions were those that significantly reduced a measurable outcome

Type of intervention	Efficacy of interventions %*	
	<i>Campylobacter</i> spp.	<i>Salmonella</i> spp.
Bacteriophages	0	80
Biosecurity	71	0
Cleaning & disinfection	50	77
Combination of measures	0	17
Competitive exclusion	-	100
Feed additives	31	31
Litter management	0	50
Oral immunisation	0	-
Vaccination	0	17

*Given as the number of trials with successful outcomes divided by the total number of trials for each intervention-pathogen

were successful in reducing *Campylobacter* loads. In another review focusing on pre- and post-harvest interventions to control *Campylobacter*, the implementation of biosecurity measures such as specific hygienic actions targeting farm-staff and drinking water and the restriction of other farm and wild animals were identified as successful interventions [62•]. A study also found the following variables to be independently associated with an increased risk of *Campylobacter* spp. colonisation using logistic regression analysis: (i) undisinfected water (having the greatest impact); (ii) tending to other poultry prior to entering the broiler house; (iii) tending to pigs before entering the house; (iv) geographic region; and (v) season (autumn vs. other seasons) [6].

On the other hand, Finland, Norway, and Sweden have documented, during more than 25 years, that stringent biosecurity measures targeting *Salmonella* spp. in poultry at herd level can be successful in controlling this pathogen [63, 64]. Furthermore, it has also been shown that epidemiological and biological differences between *Campylobacter* spp. and *Salmonella* spp. result in a greater likelihood of introduction of *Campylobacter* spp. into broiler flocks at any production stage if there are lapses in biosecurity standards, likely due to a lower number of organisms needed to infect broiler flocks than for *Salmonella* spp. and the lack of age-related reduced susceptibility [5].

Regarding cleaning and disinfection practices, most trials focused on transport coops, rather than on-farm interventions. For *Campylobacter* spp., the most successful intervention was implemented on a commercial plant and used a combination of disinfectant and water at 60°C, achieving reductions of up to 3.6 log₁₀ CFU per transport coop and a marked improvement in visual cleanliness [35•]. While for *Salmonella* spp., all trials were carried out in research institutions (controlled research setting), and the most effective intervention used a combination of slightly acidic electrolysed water and ultraviolet light, obtaining a complete 100% inactivation of *Salmonella* on plastic coop surfaces [53].

Overall, many studies tested the use of feed additives such as probiotics, prebiotics, and essential oils in controlling *Campylobacter* spp. [17, 20, 23, 25–30, 32–34, 65] and *Salmonella* spp. [42, 46, 51, 54]. Our results showed great variability in the effectiveness of this group of control measures (Table 4), which is in line with several reviews carried out in the past, which conclude that the exact impact of these feed additives is still unknown [8, 12, 62, 66].

Our results also show that, after excluding interventions to control pathogens through improved management, research is lacking on the development of targeted immunisation strategies for each pathogen. This type of intervention takes longer to develop and has the additional challenge of having to address the epidemiology and biological characteristics of each pathogen at study. However, this additional challenge is also what warrants a higher impact as a control intervention if successful.

Regarding vaccination strategies to control *Campylobacter* spp., we found only one study using an in ovo DNA vaccine, which presented ineffective results [31]. Furthermore, two studies reported different vaccination strategies for the control of *Salmonella enterica* subsp. *enterica* serovar Heidelberg [44, 49]. The first study tested the efficacy of a genetically modified live vaccine, which was able to partially decrease the bacterial load of *S. Heidelberg* in the caecum and its prevalence in the liver/spleen after oral challenge but had limited duration of immunity [44]. The second study investigated if a broiler breeders' vaccination protocol containing two live and two killed vaccines provided adequate protection in the broiler progeny, and if the level of maternal antibody would determine the protective status of broiler progeny. Results showed that broiler breeders' antibody titres wane over time, and therefore, broiler progeny is not protected from early *Salmonella* colonisation [49].

Given that most studies, including vaccination studies, were performed under controlled research settings, we highlight the need for more large-scale randomised, blinded trials conducted with different vaccination strategies on commercial farms. These would ascertain the efficacy of these interventions under field conditions.

As antimicrobial resistance raises concerns regarding the use of antimicrobials in livestock, interest in alternatives such as bacteriophage therapy has increased. Still, in our review, research on the use of bacteriophages to control *Campylobacter* and *Salmonella* was scarce. One study carried out on a commercial farm showed no significant impact of bacteriophage application on *Campylobacter* loads [37], while for *Salmonella*, studies carried out on research institutions (controlled research setting) reported that this type of intervention caused significant reductions of the pathogen loads [55, 56], which was not verified in a study held in a commercial setting [50]. While bacteriophages may be an alternative for the control of *Campylobacter* and *Salmonella*, one of the major disadvantages to widespread on farm bacteriophage application is due to the capacity of pathogens to become resistant to their bacteriophage, especially if the resistant bacteria can persist in the environment and replicate [11]. Indeed, replicable, and effective studies are lacking. Furthermore, the extrapolation of each trial's results to commercial farms may result in even less optimal outcomes due to the inherent difficulties in consistently implementing standard operating procedures (SOPs) in multiple farms/systems.

Other Pathogens

Interventions to control other less prominent hazards were much less frequent or non-existent, in spite of their relevance as stated in the 2012 EFSA report [2]. Several pathogen-specific factors should be taken into consideration.

Although broilers are considered a possible reservoir for ESBL-AmpC gene carrying *E. coli* [2], the public health burden associated with these animals as sources of human infection is still controversial [67]. Therefore, most studies published focus on the epidemiology of this pathogen and on source attribution, whereas interventions for its control are still not a common research topic. Regarding ESBL-AmpC gene carrying *Salmonella*, information is even more scarce.

Furthermore, human cases of VTEC infection have not been successfully attributed to broilers [2, 68, 69], and therefore, EFSA has considered that broiler meat constitutes a low risk for public health when considering this pathogen [2].

Although *Y. enterocolitica* has been the third most reported foodborne zoonoses in EU for several years [3], pigs are considered the main reservoir for this pathogen, and broilers have not been identified as a significant source of human infections [70].

In recent years, *T. gondii* has gained attention due to the severe repercussions it can cause in humans (e.g. neonatal or foetal losses and high disability in cases living permanently with the disease due to compromised vision and/or neurological disease; [71]). In a recent review, it was concluded that the risk of ingestion of *T. gondii* cysts in meat from commercially (indoor) reared broilers was low, but a high prevalence of this parasite was found in broilers from free-range farms [72]. In this study, we only considered studies that focused on indoor farms. Still, with the increase of consumers from high income countries who prefer meat from broilers in outdoor holdings as well as locally sourced products, there may be an increased risk to public health, and therefore need for implementation of preventive measures to control this parasite.

Finally, *L. monocytogenes*, *B. cereus*, *C. botulinum*, *C. perfringens*, and *S. aureus* are mainly controlled by post-harvest interventions [2], and therefore, it had to be expected that literature on pre-harvest interventions was not abundant.

Conclusion

The results of this systematic review reflect that the recent research on pre-harvest interventions to control foodborne zoonoses in broilers were mostly focused on *Salmonella* spp. and *Campylobacter* spp.

Biosecurity (i.e. pest control) and management (i.e. hygiene, cleaning, and disinfection) interventions had mixed outcomes in controlling these pathogens, and a strong emphasis on (1) adequately implementing the interventions and (2) combining multiple approaches is recommended across the literature for optimum results. In addition, the effectiveness of feed additives (probiotics, prebiotics, and essential oils) has been extensively researched but remains controversial with results showing great variability.

Recent research on other pathogens (i.e. ESBL-AmpC *E. coli*, ESBL-AmpC *Salmonella*, and *T. gondii*) was scarce, focusing mostly on the epidemiology of the disease and/or documenting source-attribution studies. This is also true regarding research on pre-harvest interventions for controlling *L. monocytogenes*, *B. cereus*, *C. botulinum*, *C. perfringens*, and *S. aureus* as these are mostly controlled by post-harvest interventions.

Overall, research is lacking on the development of targeted immunisation strategies for each pathogen. This is seen as an essential step to control some of the most prevalent pathogens. However, vaccination strategies should always be implemented in combination with other interventions, especially those which are related to best farming practices. Indeed, interventions such as good cleaning and disinfection and strict biosecurity may be enough to prevent the introduction and/or control less prevalent pathogens.

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Compliance with Ethical Standards

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