

Evaluation of cleaning and disinfection strategies to control *Escherichia coli* aggregates and
biofilms in swine transportation

by

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Abstract

Livestock transport trailers can act as important vehicle of microbial persistence. After transport, trailer surfaces may retain fecal material and moisture, which can reduce the effectiveness of cleaning and disinfection. *Escherichia coli* can survive under these conditions and persist as aggregates or biofilm microbial communities, making disinfection more difficult to achieve. Innovative and effective cleaning and disinfection strategies are therefore needed to reduce microbial persistence and limit the risk of cross-contamination in live-haul environments.

The objective of this research was to evaluate cleaning and disinfection strategies to control *E. coli* aggregates and biofilms associated with swine transport environments and to evaluate a selected treatment under field conditions. Two complementary studies were conducted.

In the first study, *E. coli* TVS 353 was used to form aggregated cells and biofilms on aluminum coupons. Coupons were subjected to one of three mechanical cleaning procedures, scraping, vacuuming, or air, followed by disinfection treatments: 3% hydrogen peroxide (30 s), 400 ppm quaternary ammonium compounds (1 min), 400 ppm sodium hypochlorite (1 min), steam (100 °C, 30 s), carbon dioxide (30 s), and chlorine dioxide gas (500 ppm, 10 min). Treatments were evaluated at 30, 18, and 10 °C to represent seasonal transport conditions. Treatment efficacy was influenced by cleaning methods, disinfection treatment, temperature, and bacterial cell state ($P < 0.05$). Aggregated cells were more susceptible than biofilm cells ($P < 0.001$). The greatest reductions for aggregated cells ranged from 4.50 to 5.06 log CFU/cm², while the greatest reductions for biofilm cells ranged from 3.78 to 3.87 log CFU/cm².

In the second study, 3% hydrogen peroxide applied for 30 s after pressure washing was evaluated in a naturally contaminated swine trailer. Aerobic counts were measured before cleaning, after

pressure washing, and after hydrogen peroxide application. Aerobic counts were affected by intervention step ($P < 0.001$), surface type ($P < 0.001$), and their interaction ($P = 0.004$).

Hydrogen peroxide produced additional significant reductions of 1.36 to 1.80 log CFU/cm² across trailer sections and 1.13 to 1.95 log CFU/cm² across surfaces after pressure washing. Total reductions from initial counts to after hydrogen peroxide application ranged from 1.67 to 2.52 log CFU/cm² across trailer sections and were 1.76 and 2.27 log CFU/cm² on the floor and wall. However, counts were higher on the floor than on the wall initially and after hydrogen peroxide application.

Overall, the laboratory study identified treatment combinations that reduced *E. coli* aggregates and biofilms, while the field study showed that hydrogen peroxide further reduced aerobic counts after pressure washing, although its effect varied by surface type. These findings emphasize that C&D performance depends on microbial cell state, cleaning method, disinfectant, temperature, and surface characteristics.

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Dedication

I dedicate this thesis to my family in Honduras, especially my parents, siblings, and grandmother, who have always believed in me. I also dedicated it to my friends at Kansas State University, who became like a second family and supported me throughout this journey.

Chapter 1- Literature review

Cleaning and disinfection strategies to control aggregates and biofilm *Escherichia coli* in trailer models for the swine transportation industry

Search strategy

The literature included in this review was identified through searches conducted in Google Scholar, PubMed, and ResearchGate, as well as through direct access to peer-reviewed journals. Relevant articles were sourced from journals in microbiology, food safety, and veterinary science (e.g., *Applied and Environmental Microbiology*, *Journal of Food Protection*, *Frontiers in Microbiology*, *Porcine Health Management*, *Preventive Veterinary Medicine*, *Transboundary and central Emerging Diseases*). The search combined keywords such as cleaning, disinfection, livestock transportation, biofilms, aggregates, *Escherichia coli*, organic matter and surface contamination. Artificial intelligence-assisted tools helped refine search terms and identify relevant documents. Articles were screened for peer-review status, direct relevance to cleaning and disinfection, biofilm or aggregate formation, microbial persistence in livestock or food systems, publication between 2012 and 2026. Approximately 1,200 articles were initially identified across databases through searches using different combinations of keywords. In total, 93 references were included, incorporating additional governmental and international guidelines (e.g. USDA, CDC, WOA, Codex Alimentarius) and institutional sources to provide regulatory and practical context. When necessary, older foundational studies were considered.

1.1 Introduction to pathogen transmission in the animal transportation industry

Pathogen transmission in animal production systems is a key research area within the One Health framework, which emphasizes how animal, human, and environmental health are connected (CDC, 2025). Modern pork production operates through phases: breeding, farrowing, nursery, and grow–finish, designed to optimize efficiency and animal performance (Ciuoderis-Aponte et al., 2022; Nakrachata-Amon et al., 2024). However, the movement of pigs among these stages requires frequent transport between facilities, generating repeated contact points among farms, vehicles, and processing plants. These routine movements open pathways for pathogens to disseminate across the supply chain and create repeated connections that would otherwise remain independent (Dee et al., 2020; Lowe et al., 2014).

The importance of biosecurity systems has grown as high-consequence diseases, including those caused by bacterial pathogens like *Escherichia coli* (*E.coli*). In response, international organizations such as the World Organization of Animal Health (WOAH) and the Food and Agriculture Organization of the United Nations (FAO), along with industry groups, have developed standardized and practical guidelines to help producers assess risk, train employees, and prepare for emergencies. Resources like the *National Farm Biosecurity Manual for Pork Production*, outline expectations for managing people, animals, and equipment on farms. Likewise, programs such as the U.S. *Secure Pork Supply Plan* offer producers tools to build site-specific biosecurity plans, improve traceability, and support business continuity during disease outbreaks (Animal Health Australia & Australian Pork Limited, 2021; FAO, 2023; National Pork Board, 2025; WOAH, 2024).

Biosecurity practices refer to the set of protocols and management strategies applied on a farm to minimize the risk of diseases being introduced or spread, both within the farm and

between different farms. These practices include measures aimed at preventing pathogenic *E.coli* from entering the herd, known as external biosecurity, as well as actions designed to limit the spread of pathogenic *E.coli* within the herd, referred to as internal biosecurity (Ciuoderis-Aponte et al., 2022). External biosecurity focuses on practices such as controlling animal purchases, enforcing visitor restrictions, quarantining incoming animals, and regulating vehicle entry (Animal Health Australia & Australian Pork Limited, 2021). Internal biosecurity incorporates measures like age-group separation, all-in/all-out management, structured cleaning and disinfection, and consistent protocols for handling sick animals (Alarcón et al., 2021). These measures fall into four categories: herd management, facility hygiene, cleaning and disinfection, and personnel practices (Alarcón et al., 2021; Bernaerdt et al., 2023). The most widely used approach to evaluating biosecurity has been the development of scoring systems. In most cases, these scores rely on values assigned to different biosecurity practices based on the judgment of expert panels (Alarcón et al., 2021). Nowadays, tools like Biocheck.UGent™ and ATP bioluminescence help quantify different aspects of farm biosecurity status. Biocheck.UGent™ evaluates biosecurity management practices through a structured questionnaire. In contrast, ATP bioluminescence provides a rapid surface-level assessment of hygiene. It measures adenosine triphosphate, an indicator of organic contamination after cleaning and disinfection procedures (Bernaerdt et al., 2023; Letsch et al., 2024). However, the best way to maintain biocontainment integrity is to avoid direct and indirect contact between groups of animals, especially high-risk groups (Divya et al., 2025).

Biosecurity must be viewed across the entire supply chain, not just at the farm level. To evaluate cleaning and disinfection (C&D) practices, it is important to understand how animals, people, and equipment move through the pork supply chain. Transport trailers, loading docks,

and lairage areas are key points of frequent movement where contamination can build up and spread quickly between farms (Alarcón et al., 2021; Letsch et al., 2024; Lowe et al., 2014). Transportation plays a particularly influential role, as trucks and trailers routinely move between production sites, buying stations, and slaughterhouses. Without effective C&D, these vehicles can become major sources of pathogen dissemination (Alarcón et al., 2021; Animal Health Australia & Australian Pork Limited, 2021; National Pork Board, 2025). Even well-designed on-farm programs can be compromised by a single contaminated trailer.

1.2 Role of transportation in disease dissemination

In this section, we focus on the role of transport trailers in the dissemination of disease, an aspect of biosecurity that is often overlooked. According to the National Pork Producers Council, most of the approximately 66,000 pork farms in the United States are family owned. Many of these farms rely on shared or externally managed transportation services. (Cook & Lee, 2023; National Pork Producers Council, 2023). Recent studies by Galvis et al. (2022) using GPS-based tracking of livestock transport vehicles have improved our understanding of farm connectivity and disease spread. Analyses of vehicle movement networks have shown that trucks visiting multiple farms create connected structures. These networks facilitate the indirect transmission of pathogens (Galvis et al., 2022). Increased frequency of visits by vehicles previously exposed to infected farms has been associated with higher odds of infection. Moreover, epidemiological models that incorporate transportation networks have improved the ability to explain and predict outbreak patterns. They help identify highly connected farms that may act as superspreaders, using network analysis of vehicle movement data collected through GPS- tracking systems to quantify connectivity between farms (Yang et al., 2025).

During transport, pigs shed feces, urine, saliva, and other residues that settle on trailer floors, walls, gates, and corners (HIPRA, 2025). Handling, feed withdrawal, mixing with unfamiliar pigs, and environmental changes can disrupt intestinal homeostasis. This could increase fecal shedding of enteric bacteria (Lee et al., 2024). Under these conditions, the probability of pathogen exposure increases. This applies to pen mates and subsequent loads (Barros et al., 2023). Furthermore, debris provides protection for microorganisms, allowing them to remain viable (Galvis et al., 2022). If trailers are not thoroughly cleaned and disinfected between loads, residual contamination can be carried from one farm to the next. Evidence from epidemiological studies shows that fomites (objects able to convey infectious agents) play a significant role in dissemination. Routine veterinary visits may also contribute to the spread of infectious agents within and between livestock operations (Rossi et al., 2017).

Industry guidelines identify transportation as a critical point for disease prevention. Some systems promote the use of heat or drying steps to improve pathogen inactivation inside trailers (Animal Health Australia & Australian Pork Limited, 2021). However, compliance varies. This is often due to short turnaround times, weather limitations, or limited access to proper washing facilities (Galvis et al., 2022; Lowe et al., 2014). High-pressure washing (power washing) remains one of the most commonly used methods for cleaning livestock transport trailers due to its ability to remove visible organic debris and manure (USDA, 2026). Even so, studies in agricultural environments have shown that high-pressure cleaning can generate bioaerosols (Madsen & Matthiesen, 2013). The mechanical force of pressurized water may contribute to the dispersion of microorganisms within the surrounding environment. This risk increases when contamination is not adequately contained (USDA, 2026).

1.3 Importance of *Escherichia coli* in swine health and implications for food safety

E. coli is a normal inhabitant of the intestinal tract of pigs. Some *E. coli* strains are broadly classified into intestinal (diarrheagenic) pathotypes and extraintestinal pathotypes (ExPEC). Collectively, these conditions are referred to as colibacillosis (Fratamico et al., 2016). In swine production, colibacillosis is associated with increased mortality, growth retardation, treatment costs, and animal welfare concerns, leading to important economic losses at the farm level (Barros et al., 2023). Some strains may also exhibit antimicrobial resistance, increasing their impact on swine herds (Kim et al., 2022).

The most relevant *E. coli* strains for the swine industry include enterotoxigenic *E. coli* (ETEC), Shiga toxin–producing *E. coli* (STEC), and enteropathogenic *E. coli* (EPEC). Among these, ETEC is considered the most significant in swine production (Barros et al., 2023). ETEC strains use fimbria adhesins such as F4 and F18 to attach receptors on the small intestinal epithelium. They also secrete enterotoxins that disrupt fluid and electrolyte balance (Fairbrother et al., 2005; Kim et al., 2022). Post-weaning diarrhea often leads to increased use of antimicrobials because preventive and treatment-based medications are still widely used in many production systems (Barros et al., 2023; Kim et al., 2022).

Outbreaks of ETEC-associated colibacillosis can generate considerable economic impact. In severe outbreaks, the estimated cost per sow has been reported to range from approximately \$45 to \$360 in Europe (Luppi, 2017). STEC strains producing Shiga toxin type 2e are associated with edema disease in grower pigs, a condition characterized by neurological signs and sudden death (Van Hoorde et al., 2025). Some diarrheagenic and extraintestinal *E. coli* strains also have zoonotic potential. They can carry virulence gene combinations similar to those found in human cases and, in some situations, can colonize both pigs and people (Barros et al., 2023; Colello et

al., 2016). These pathogenic strains coexist with commensal *E. coli*, forming a reservoir that enables the exchange and maintenance of virulence and antimicrobial-resistance genes (Tawfick et al., 2022).

E. coli has also been detected in the feed manufacturing environment and in finished swine feed, highlighting feed as a potential vehicle for introducing enteric bacteria within production systems (Magossi et al., 2020). In the U.S., a study of Midwestern swine feed mills collected a large set of environmental and product-associated samples. They reported that *E. coli* was detected across facilities, emphasizing that contamination can occur at multiple points in feed-mill operations. Additional evidence from U.S. commercial feed-manufacturing sampling shows that *E. coli* contamination levels may be higher before thermal processing, with reductions observed after pelleting and possible recontamination (Munoz et al., 2021).

At the level of the finished product, generic *E. coli* is commonly used as an indicator organism and remains one of the most detected bacterial groups in pork meat. A systematic review and meta-analysis of studies along the pork chain estimated an average prevalence of generic *E. coli* in around 35.6% samples, and pathogenic *E. coli* in around 4.7 %, confirming that contamination can occur at several points from farm to retail (Blanco-Lizarazo & Sierra-Cadavid, 2023). Other investigations that followed STEC through the pork production chain recovered these strains from farm, slaughter, and boning room samples. This illustrates how pigs can act as reservoirs and how failures in the process may allow zoonotic *E. coli* to reach consumers (Colello et al., 2016).

Detection of pathogenic *E. coli* in swine farms typically involves both traditional culture methods and molecular techniques targeting virulence factors. On-farm detection operations typically start with targeted sampling during clinical events. Common on-farm sample types

include individual rectal/fecal samples from clinically affected piglets, as well as pen-level composites (pooled feces) to capture within-pen variability and reduce costs (Paiva et al., 2025). Samples such as feces or intestinal contents are first cultured on selective media, like MacConkey or blood agar, to isolate *E. coli*, and colonies can be differentiated based on their growth and biochemical characteristics (Hariri, 2022). Once isolates are obtained, pathotypes are identified by detecting specific virulent genes such as enterotoxins, fimbrial adhesins, or Shiga toxins using polymerase chain reaction (PCR). This combined approach enables differentiation of ETEC, STEC, and other disease-associated strains (Son & Kim, 2025).

1.3.1 Survival and persistence of *E. coli* on surfaces

The ability of *E. coli* to persist outside the host is a key factor of concern within animal production and transport environments. Once shed in feces, *E. coli* can remain viable on a variety of environmental surfaces, particularly when moisture and organic material are present (Remfry et al., 2021). Experimental studies have shown that *E. coli* survives for extended periods of time on nonporous materials, including metal surfaces, and that environmental conditions such as humidity play an important role in pathogen persistence (Lim et al., 2020).

Surfaces commonly used in livestock transportation, including aluminum and stainless steel, are considered easier to clean than porous materials. However, surface irregularities, joints, and areas shielded from mechanical action can retain organic residues and moisture. Figure 1 illustrates protected microenvironments that may allow *E. coli* cells to survive after routine washing procedures, particularly when fecal material is not completely removed (Dhiman et al., 2024). As a result, surfaces that appear visually clean may still harbor viable bacteria capable of contaminating subsequent animal loads (Parker et al., 2025).

In addition, *E. coli* can form biofilms, which significantly enhance its persistence in transport and processing environments. Biofilms are structured microbial communities embedded within a self-produced extracellular matrix that adheres strongly to surfaces (Beloïn et al., 2008; Flemming et al., 2016). This matrix protects bacterial cells from environmental stresses such as desiccation, temperature fluctuations, and exposure to disinfectants, making biofilm cells considerably more difficult to eliminate than aggregate bacteria (Flemming et al., 2016; Liu et al., 2024). Biofilm development is a dynamic and highly regulated process that typically occurs in sequential stages (Flemming et al., 2016; Kostakioti et al., 2013). It begins with the reversible attachment of planktonic cells to a surface, mediated by physicochemical interactions such as van der Waals forces, hydrophobic interactions, and electrostatic forces (Berne et al., 2018). This is followed by irreversible attachment, during which cells produce extracellular polymeric substances (EPS) that anchor them to the substrate and to neighboring cells (Flemming et al., 2016). The EPS matrix, composed primarily of polysaccharides, proteins, lipids, and extracellular DNA, provides structural stability and facilitates nutrient retention and intercellular communication (Seviour et al., 2019). As the biofilm matures, cells proliferate and form complex three-dimensional architectures characterized by microcolonies and water channels that enable nutrient and waste transport (Flemming et al., 2016). In the final stage, dispersion occurs, allowing cells to detach and return to a planktonic state, contributing to colonization of new surfaces (Kostakioti et al., 2013).

More recently, the concept of dry surface biofilms (DSB) has gained attention in scientific literature. These biofilms tend to be thinner and can survive under conditions of low moisture and limited nutrient availability (Ledwoch et al., 2022). Despite their potential relevance, dry surface biofilms remain poorly understood, and further research is needed to

clarify their role in pathogen persistence and their potential impact on the swine industry (Amaeze et al., 2024).

1.4 Cleaning and disinfection strategies in the animal transport industry

While various methods exist for assessing C&D procedures in human healthcare facilities and the food industry, their evaluation in farm environments remains challenging (Makovska et al., 2025). This difficulty is driven by the wide variability among farm settings, the high presence of organic material, and the difficulties of implementing C&D procedures. These conditions are especially challenging in winter, when low temperatures can affect cleaning procedures and limit disinfectants performance (Juszkiewicz et al., 2025).

Effective cleaning is a prerequisite for successful disinfection. Cleaning refers to the physical removal of organic matter such as feces, bedding material, feed residues, and dirt from the surface (Codex Alimentarius, 2023). The United States Department of Agriculture (USDA) Food Safety and Inspection Service (FSIS) defines disinfectant as a “substance used on inanimate surfaces that destroys or eliminates a specific species of infectious or other public health microorganism, but not necessarily bacterial spores”. Without proper cleaning, disinfectants work poorly because organic material can inactivate them or shield bacteria from exposure (Rutala & Weber, 2014; USDA FSIS, 2016).

An effective C&D program involves a series of sequential steps designed to progressively reduce microbial contamination on surfaces. The process begins with the removal of visible organic matter through cleaning, followed by the application of detergents to loosen adhered residues during a defined contact period. Surfaces must be adequately dried before disinfectants are applied to prevent dilution and ensure optimal antimicrobial activity. Finally, the effectiveness of the entire procedure should be verified through appropriate surface sampling.

Verification methods include microbiological swabs or ATP bioluminescence testing, both of which determine whether contamination has been successfully reduced (APHIS, 2023; Makovska et al., 2025; USDA, 2018).

1.4.1 Cleaning strategies in animal transportation

As illustrated in Figure 2, mechanical scraping is one of the most applied physical cleaning methods in livestock trailers. This technique involves the manual removal of dried or compacted residues from floors, ramps, and corners using scrapers or shovels. Scraping is particularly effective for removing bulk material that adheres strongly to surfaces and would otherwise reduce the efficiency of subsequent cleaning steps (USDA, 2018; WOA, 2021).

Compressed air may be used to loosen organic material from surfaces, especially in areas that are difficult to reach with manual tools. However, guidance documents caution that the use of compressed air can redistribute organic materials and microorganisms rather than remove them. This redistribution may deposit contaminants onto previously clean surfaces or equipment, increasing the risk of cross-contamination if containment measures are not in place (OSHA, 2025).

Vacuuming represents an alternative physical cleaning approach that removes loose debris while minimizing particle dispersion (Figure 3). This method is particularly useful in enclosed spaces or during cold conditions when wet cleaning is impractical (Fonstad, 2015; USDA, 2018; WOA, 2021).

1.4.2 Disinfection methods in the animal transportation industry

Selecting the appropriate disinfectant is one of the most important steps in the animal transport industry (USDA APHIS, 2025). The goal is to choose a product capable of effectively inactivating microorganisms to prevent contamination of the subsequent load. Several factors

must be evaluated when making this decision. Key questions include: What is the required contact time? Does the product have an acceptable toxicity rating? Does it remain effective in the presence of organic matter? Is it water-soluble? And is the overall cost reasonable? These considerations align with the five components proposed by Rutala & Weber (2014).

Disinfectants are classified into two major groups: oxidizing and non-oxidizing agents. Oxidizing disinfectants include compounds that release halogens. In contrast, non-oxidizing disinfectants are those that act by binding to cellular structures, such as quaternary ammonium compounds (Jefri et al., 2022). The effectiveness of disinfectants depends not only on the intrinsic susceptibility of the target microorganism but also on environmental factors such as temperature, surface type, moisture, and the presence of organic material (Cheng et al., 2025; Chowdhury et al., 2019). Microorganisms embedded within biofilms often exhibit reduced susceptibility to disinfectants due to limited penetration and physiological adaptations (Weber et al., 2007). Among the different disinfectant classes, oxidizing agents are commonly used in livestock production and transport systems because of their broad antimicrobial activity and practical applicability in these environments (Kamal et al., 2019).

1.4.2.1 Hydrogen peroxide

Hydrogen peroxide is an oxidizing disinfectant that causes damage to essential cellular components such as membranes, proteins, and nucleic acids (McLaren et al., 2011). It is effective against a wide range of bacteria, viruses, and fungi and breaks down into water and oxygen, leaving no chemical residues (CDC, 2023; McDonnell, 2014).

1.4.2.2 Sodium hypochlorite

Sodium hypochlorite is one of the most widely used disinfectants in animal production systems due to its low cost and strong antimicrobial activity (Fukuzaki, 2006). Its main limitation is its sensitivity to organic matter, which can quickly reduce the availability of free chlorine. In

addition, its effectiveness depends on pH, temperature, and proper pre-cleaning of surfaces (Chen & Hung, 2017; Fukuzaki, 2006).

1.4.2.3 Chlorine dioxide

Chlorine dioxide is used as an oxidizing disinfectant. Its antimicrobial action against bacteria, fungi, and viruses is well established; however, researchers still have not fully determined the exact cellular components it targets during the inactivation process (Jiang et al., 2024). Its main advantage is that it reduces the formation of harmful organic chlorinated compounds and minimizes the production of by-products (Jefri et al., 2022; Sharma & Sohn, 2012). Chlorine dioxide remains effective over a wide pH range and shows better tolerance to organic matter than other disinfectants (Obinata, 2022; Rougé et al., 2018). Both liquid and gaseous forms of chlorine dioxide have been studied for surface and environmental disinfection. Gaseous chlorine dioxide, in particular, has been shown to reach difficult-to-clean areas, making it relevant for enclosed spaces (Jefri et al., 2022; Meireles et al., 2016).

1.4.2.4 Quaternary ammonium compounds

Quaternary ammonium compounds (QACs) are widely used disinfectants in livestock production due to their ease of application, relatively low cost, and broad activity against Gram-negative and Gram-positive bacteria (Wesgate & Maillard, 2026). Their antimicrobial effect results from the disruption of the bacterial cell membrane, which compromises cell integrity and leads to cell death (Buffet-Bataillon et al., 2016; Hora et al., 2020). QACs are biodegradable under aerobic conditions, which enhances their environmental appeal compared to other chemical disinfectants (Tezel & Pavlostathis, 2015).

1.4.2.5 Dry carbon dioxide

Dry carbon dioxide (dry CO₂) has been studied as a non-chemical physical approach for microbial reduction (Witte et al., 2017). Its antimicrobial effect is mainly attributed to oxygen

displacement, CO₂ -induced stress, and temperature changes, rather than direct chemical damage to microbial cells (Vo et al., 2013). The decontamination with supercritical CO₂ is considered attractive because it leaves no chemical residues, is non-flammable, non-toxic, physiologically safe, and readily available (Tarafa et al., 2010).

Experimental studies evaluating pressurized CO₂ treatments have shown that microbial inactivation is dependent on process parameters such as pressure, temperature, and exposure time (Vo et al., 2013). These findings indicate that CO₂-based treatments alone may result in variable levels of microbial reduction, highlighting limitations when considered as a sole disinfection approach (Koutsoumanis et al., 2021; Maillard, 2018). Therefore, dry CO₂ has been used as a physical carrier facilitating the delivery and dispersion of alcohol-based disinfectant. Pressurized CO₂ has been described as an effective medium for transporting active compounds, enhancing surface contact and penetration without leaving chemical residues (Tarafa et al., 2010).

1.4.2.6 Steam

Thermal treatments based on steam applications have been evaluated as an effective non-chemical approach for microbial inactivation (Rana et al., 2022). Steam acts primarily through rapid heat transfer, leading to protein denaturation, membrane damage, and loss of cellular functionality in vegetative microorganisms (Bhana et al., 2013). Dry steam is effective against biofilms (Song et al., 2012). In food processing environments, steam has been used for the disinfection of equipment, floors, and hard surfaces, demonstrating > 6 log CFU/coupon reduction of bacterial pathogens, including *Escherichia coli* O157:H7, *Salmonella* Typhimurium, and *Listeria monocytogenes* (Park & Kang, 2014). The efficacy of steam depends on factors such as the surface temperature achieved, exposure time, and how easily the area can be reached (Park & Balasubramaniam, 2025). In addition, steam application alone does not remove organic debris,

highlighting the importance of prior mechanical cleaning to ensure effective heat transfer to contaminated surfaces (Yang et al., 2023).

1.5 Recent studies on disinfection in livestock transportation

In recent years, several studies summarized in Table 1 have evaluated C&D practices in livestock transport systems under commercial conditions. Rather than focusing only on laboratory efficacy, these studies examined how C&D performed in real-world settings. Such evaluations are important because routine transport operations involve practical challenges. Taken together, studies report incomplete microbial elimination after C&D. Bacteria such as *Campylobacter*, *Salmonella*, and generic *E. coli* have been detected. Studies also highlight variability in cleaning practices and compliance. This variability may contribute to inconsistent biosecurity outcomes in livestock transport systems.

1.6 Conclusion

Based on the literature reviewed, transportation represents a critical point for pathogen dissemination within swine production systems. Transport trailers frequently accumulate fecal material and microorganisms during animal movement. Inadequate C&D allows contaminated vehicles to facilitate indirect transmission between farms. *E. coli*, commonly present in swine environments, can persist on metal surfaces once shed in feces. It may also form aggregates or biofilms that increase resistance to disinfectants. Multiple chemical and physical disinfection strategies are available; however, their performance can be influenced by factors such as surface characteristics, environmental temperature, and the consistency of cleaning practices. These considerations highlight the importance of optimizing and standardizing C&D protocols in livestock transportation to maximize their effectiveness and reduce the risk of pathogen spread throughout the production chain.

Figures and tables

Figure 1. Interior of a livestock transport trailer highlights typical structural features relevant to cleaning and disinfection.



Figure 2. Manual removal of bedding and organic material from a livestock transport trailer (Karcher, 2026)



Figure 3. Vacuum cleaning in a livestock transport trailer (Hein, 2015).



Table 1. Recent experimental studies focused on evaluating cleaning and disinfection in live-haul transport trailers.

Article	Main Findings	Reference
Cleaning and disinfection of transport crates for poultry	Integration of chemical disinfectants with drying achieved greater microbial reductions of <i>Campylobacter</i> .	(Dzieciolowski et al., 2022)
Comparison of transport crates contamination with <i>Campylobacter</i> spp. before and after the cleaning and disinfection procedure in broiler slaughterhouses	<i>Campylobacter</i> was detected in 46% of crates with no significant reduction after C&D. The study suggests that ineffective cleaning and disinfection facilitate the spread of bacteria in poultry establishments.	(Morgan et al., 2022)
Disinfection reduces but does not eliminate drug resistant <i>Escherichia coli</i> from livestock trailers following transport of calves	The study found frequent contamination of livestock trailers with antibiotic-resistant <i>E. coli</i> .	(Urbaniec et al., 2025)
The role of short journey transportation in the spreading of swine pathogens and antimicrobial-resistant bacteria	Antibiotic-resistant <i>E. coli</i> was detected after C&D of transport trailers. It highlights that ineffective C&D procedures may represent a breach in the biosecurity of the farms.	(Masserdotti et al., 2024)
Prevalence and characterization of <i>Salmonella</i> species in entire steps of a single integrated broiler supply chain	The study identified that broiler transporting trucks may play a critical role in disseminating <i>Salmonella</i> between broiler farms and slaughterhouses.	(Choi et al., 2014)

Isolation of <i>Salmonella</i> spp. in pigs during transport, lairage, slaughter line and quartering	<i>Salmonella</i> was detected during transport and lairage, supporting the role of these stages in pathogen persistence and potential cross-contamination.	(Hernández et al., 2011)
Cleaning practices of transport vehicles by commercial and non-commercial livestock markets users in Scotland	Several respondents declared hiring a private haulage vehicle to transport their animals and leave the responsibility of C&D the vehicle up to the driver. The study reported that 65% of respondents were not using disinfectants.	(Allen et al., 2024)

References

- Alarcón, L. V., Allepuz, A., & Mateu, E. (2021). Biosecurity in pig farms: A review. *Porcine Health Management*, 7(1), 5. <https://doi.org/10.1186/s40813-020-00181-z>
- Allen, R., Boden, L. A., Hutchinson, I., Stirling, J., & Porphyre, T. (2024). Cleaning practices of transport vehicles by commercial and non-commercial livestock markets users in Scotland. *Research in Veterinary Science*, 180, 105413. <https://doi.org/10.1016/j.rvsc.2024.105413>
- Amaeze, N. J., Akinbobola, A. B., Kean, R., Ramage, G., Williams, C., & Mackay, W. (2024). Transfer of micro-organisms from dry surface biofilms and the influence of long survival under conditions of poor nutrition and moisture on the virulence of *Staphylococcus aureus*. *Journal of Hospital Infection*, 150, 34–39. <https://doi.org/10.1016/j.jhin.2024.03.023>
- Animal Health Australia & Australian Pork Limited. (2021). *National Farm Biosecurity Manual for Pork Production*. Animal Health Australia. https://www.farmbiosecurity.com.au/wp-content/uploads/2021/11/Pork-Biosecurity-Manual_update_2021.pdf
- APHIS. (2023). *Cleaning and Disinfection Procedures*. <https://www.aphis.usda.gov/sites/default/files/ac-aid-cleaning-and-disinfection-procedures.pdf>
- Barros, M. M., Castro, J., Araújo, D., Campos, A. M., Oliveira, R., Silva, S., Outor-Monteiro, D., & Almeida, C. (2023). Swine Colibacillosis: Global Epidemiologic and Antimicrobial Scenario. *Antibiotics*, 12(4), 682. <https://doi.org/10.3390/antibiotics12040682>

Beloin, C., Roux, A., & Ghigo, J.-M. (2008). *Escherichia coli* Biofilms. In T. Romeo (Ed.), *Bacterial Biofilms* (Vol. 322, pp. 249–289). Springer Berlin Heidelberg.

https://doi.org/10.1007/978-3-540-75418-3_12

Bernaerdt, E., Díaz, I., Piñeiro, C., Collell, M., Dewulf, J., & Maes, D. (2023). Optimizing internal biosecurity on pig farms by assessing movements of farm staff. *Porcine Health Management*, 9(1), 11. <https://doi.org/10.1186/s40813-023-00310-4>

Berne, C., Ellison, C. K., Ducret, A., & Brun, Y. V. (2018). Bacterial adhesion at the single-cell level. *Nature Reviews Microbiology*, 16(10), 616–627.

<https://doi.org/10.1038/s41579-018-0057-5>

Bhana, N., Zanzwar, A. S., Trivedi, V., & Jain, D. (2013). A Review: Steam Sterilization a Method of Sterilization. *Journal of Biological & Scientific Opinion*, 1(2), 138–141.

<https://doi.org/10.7897/2321-6328.01222>

Blanco-Lizarazo, C. M., & Sierra-Cadavid, A. (2023). Prevalence of *Escherichia coli* generic and pathogenic in pork meat: Systematic review and meta-analysis. *World Journal of Microbiology and Biotechnology*, 39(9), 247. <https://doi.org/10.1007/s11274-023-03687-0>

CDC. (2023). *Chemical Disinfectants*. https://www.cdc.gov/infection-control/hcp/disinfection-sterilization/chemical-disinfectants.html?CDC_AAref_Val=https://www.cdc.gov/infectioncontrol/guidelines/disinfection/disinfection-methods/chemical.html#cdc_generic_section_5-hydrogen-peroxide

CDC. (2025, July). *About One Health*. About One Health. <https://www.cdc.gov/one-health/about/index.html>

Chen, X., & Hung, Y.-C. (2017). Effects of organic load, sanitizer pH and initial chlorine concentration of chlorine-based sanitizers on chlorine demand of fresh produce wash waters.

Food Control, 77, 96–101. <https://doi.org/10.1016/j.foodcont.2017.01.026>

Cheng, H., Chen, J., Yu, H., Sun, B., Zhou, J., & Wu, G. (2025). Environmental factors and antimicrobial efficacy: The impact of temperature and humidity on material surfaces.

Microbiology Spectrum, 13(11), e00960-25. <https://doi.org/10.1128/spectrum.00960-25>

Choi, S.-W., Ha, J.-S., Kim, B.-Y., Lee, D.-H., Park, J.-K., Youn, H.-N., Hong, Y.-H., Lee, S.-B., Lee, J.-B., Park, S.-Y., Choi, I.-S., & Song, C.-S. (2014). Prevalence and characterization of *Salmonella* species in entire steps of a single integrated broiler supply chain in Korea. *Poultry Science*, 93(5), 1251–1257. <https://doi.org/10.3382/ps.2013-03558>

Chowdhury, D., Rahman, A., Hu, H., Jensen, S. O., Deva, A. K., & Vickery, K. (2019). Effect of disinfectant formulation and organic soil on the efficacy of oxidizing disinfectants against biofilms. *Journal of Hospital Infection*, 103(1), e33–e41.

<https://doi.org/10.1016/j.jhin.2018.10.019>

Ciuderis-Aponte, K., Diaz, A., Muskus, C., Peña, M., Hernández-Ortiz, J., & Osorio, J. (2022). Farm management practices, biosecurity and influenza a virus detection in swine farms: A comprehensive study in Colombia. *Porcine Health Management*, 8(1), 42.

<https://doi.org/10.1186/s40813-022-00287-6>

Codex Alimentarius. (2023). *General principles of food hygiene* (pp. 6–20). FAO; WHO; <https://doi.org/10.4060/cc6125en>

Colello, R., Cáceres, M. E., Ruiz, M. J., Sanz, M., Etcheverría, A. I., & Padola, N. L. (2016). From Farm to Table: Follow-Up of Shiga Toxin-Producing *Escherichia coli* Throughout

the Pork Production Chain in Argentina. *Frontiers in Microbiology*, 7.

<https://doi.org/10.3389/fmicb.2016.00093>

Cook, H., & Lee, S. (2023). *The United States Pork Industry 2023*. <https://nppc.org/wp-content/uploads/2024/06/U.S.-Economic-Contribution-Report-6.28.24.pdf>

Dee, S. A., Niederwerder, M. C., Patterson, G., Cochrane, R., Jones, C., Diel, D., Brockhoff, E., Nelson, E., Spronk, G., & Sundberg, P. (2020). The risk of viral transmission in feed: What do we know, what do we do? *Transboundary and Emerging Diseases*, 67(6), 2365–2371. <https://doi.org/10.1111/tbed.13606>

Dhiman, S., Kumar, A., Kaur, G., Mukherjee, G., Rustagi, S., Shreaz, S., Negi, R., & Yadav, A. N. (2024). Bacterial biofilms: Pathogenesis, monitoring, treatment approaches and associated challenges. *Biologia*, 79(10), 3161–3181. <https://doi.org/10.1007/s11756-024-01767-6>

Divya, K., Ponnuvel, P., Rajaganapathy, V., & Venugopal, S. (2025). Modern biosecurity measures in pig farming. *International Journal of Veterinary Sciences and Animal Husbandry*, 10(1), 165–172. <https://doi.org/10.22271/veterinary.2025.v10.i1c.2017>

Dzieciolowski, T., Boqvist, S., Rydén, J., & Hansson, I. (2022). Cleaning and disinfection of transport crates for poultry – comparison of four treatments at slaughter plant. *Poultry Science*, 101(1), 101521. <https://doi.org/10.1016/j.psj.2021.101521>

Fairbrother, J. M., Nadeau, É., & Gyles, C. L. (2005). *Escherichia coli* in postweaning diarrhea in pigs: An update on bacterial types, pathogenesis, and prevention strategies. *Animal Health Research Reviews*, 6(1), 17–39. <https://doi.org/10.1079/AHR2005105>

- FAO. (2023). *Progressive Management Pathway for Terrestrial Animal Biosecurity*.
<https://openknowledge.fao.org/items/flb248b5-0c60-400d-824c-e068b5cf1987>
- Flemming, H.-C., Wingender, J., Szewzyk, U., Steinberg, P., Rice, S. A., & Kjelleberg, S. (2016). Biofilms: An emergent form of bacterial life. *Nature Reviews Microbiology*, 14(9), 563–575. <https://doi.org/10.1038/nrmicro.2016.94>
- Fonstad, H. . L. (2015). Vacuum System for Cleaning Livestock Trailers. *Applied Engineering in Agriculture*, 31(6), 857–861. <https://doi.org/10.13031/aea.31.11481>
- Fratamico, P. M., DebRoy, C., Liu, Y., Needleman, D. S., Baranzoni, G. M., & Feng, P. (2016). Advances in Molecular Serotyping and Subtyping of *Escherichia coli*†. *Frontiers in Microbiology*, 7. <https://doi.org/10.3389/fmicb.2016.00644>
- Fukuzaki, S. (2006). Mechanisms of Actions of Sodium Hypochlorite in Cleaning and Disinfection Processes. *Biocontrol Science*, 11(4), 147–157. <https://doi.org/10.4265/bio.11.147>
- Galvis, J. A., Corzo, C. A., & Machado, G. (2022). Modelling and assessing additional transmission routes for porcine reproductive and respiratory syndrome virus: Vehicle movements and feed ingredients. *Transboundary and Emerging Diseases*, 69(5).
<https://doi.org/10.1111/tbed.14488>
- Hariri, S. (2022). Detection of *Escherichia coli* in Food Samples Using Culture and Polymerase Chain Reaction Methods. *Cureus*. <https://doi.org/10.7759/cureus.32808>
- Hein, T. (2015). *Manure in Trailers*. <https://www.manuremanager.com/manure-in-trailers-17939/>

Hernández, M., Gómez-Laguna, J., Luque, I., Herrera, S., Amaro, M., Reguillo, L., & Astorga, R. J. (2011). Isolation of *Salmonella* spp. In pigs during transport, lairage, slaughterline and quartering. *International Conference on the Epidemiology and Control of Biological, Chemical and Physical Hazards in Pigs and Pork*, 225–227. <https://doi.org/10.31274/safepork-180809-628>

HIPRA. (2025). *Transmission within herds*. <https://www.hipra.com/en/animal-health/knowledge/1-transmission-within-herds>

Jefri, H., Rashidah, S., Khan, A., Chee Lim, Y., Seng Lee, K., Bin Liew, K., Walid Kassab, Y., Choo, C.-Y., Mohammed Al-Worafi, Y., & Chiau, L. (2022). A systematic review on chlorine dioxide as a disinfectant. *Journal of Medicine and Life*, 15(3), 313–318. <https://doi.org/10.25122/jml-2021-0180>

Jiang, Y., Qiao, Y., Jin, R., Jia, M., Liu, J., He, Z., & Liu, Z. (2024). Application of chlorine dioxide and its disinfection mechanism. *Archives of Microbiology*, 206(10), 400. <https://doi.org/10.1007/s00203-024-04137-7>

Juszkiewicz, M., Walczak, M., Woźniakowski, G., Pejsak, Z., & Podgórska, K. (2025). The Influence of the Temperature on Effectiveness of Selected Disinfectants Against African Swine Fever Virus (ASFV). *Viruses*, 17(2), 156. <https://doi.org/10.3390/v17020156>

Kamal, M. A., Khalaf, M. A., Ahmed, Z. A. M., & El Jakee, J. (2019). Evaluation of the efficacy of commonly used disinfectants against isolated chlorine-resistant strains from drinking water used in Egyptian cattle farms. *Veterinary World*, 12(12), 2025–2035. <https://doi.org/10.14202/vetworld.2019.2025-2035>

Karcher. (2026). *Cleaning a cattle trailer*.

<https://www.kaercher.com/int/professional/know-how/cleaning-a-cattle-trailer.html>

Kim, K., Song, M., Liu, Y., & Ji, P. (2022). Enterotoxigenic *Escherichia coli* infection of weaned pigs: Intestinal challenges and nutritional intervention to enhance disease resistance.

Frontiers in Immunology, 13, 885253. <https://doi.org/10.3389/fimmu.2022.885253>

Kostakioti, M., Hadjifrangiskou, M., & Hultgren, S. J. (2013). Bacterial Biofilms: Development, Dispersal, and Therapeutic Strategies in the Dawn of the Postantibiotic Era. *Cold Spring Harbor Perspectives in Medicine*, 3(4), a010306–a010306.

<https://doi.org/10.1101/cshperspect.a010306>

Koutsoumanis, K., Allende, A., Álvarez-Ordóñez, A., Bolton, D., Bover-Cid, S., Chemaly, M., Davies, R., De Cesare, A., Herman, L., Hilbert, F., Lindqvist, R., Nauta, M., Ru, G., Simmons, M., Skandamis, P., Suffredini, E., Argüello, H., Berendonk, T., Cavaco, L. M., ... Peixe, L. (2021). Role played by the environment in the emergence and spread of antimicrobial resistance (AMR) through the food chain. *EFSA Journal*, 19(6).

<https://doi.org/10.2903/j.efsa.2021.6651>

Lee, B., Luttman, A. M., Ernst, C. W., Raney, N. E., Oh, S., & Siegford, J. M. (2024). Assessing the relationship between pigs' stress resilience and their behavior in response to weaning. *Frontiers in Animal Science*, 5, 1461526. <https://doi.org/10.3389/fanim.2024.1461526>

Letsch, F. G., Welch, M. W., Meyer, M., Hedblom, G. A., Parr, E., Classen, D. M., Dillard, M., & Boler, D. D. (2024). Evaluation of ATP bioluminescence for rapid determination of cleanliness of livestock trailers after a commercial wash. *Translational Animal Science*, 8, txae052. <https://doi.org/10.1093/tas/txae052>

Lim, S.-M., Lim, E. S., Kim, J.-S., Paik, H.-D., & Koo, O. K. (2020). Survival of foodborne pathogens on stainless steel soiled with different food residues. *Food Science and Biotechnology*, 29(5), 729–737. <https://doi.org/10.1007/s10068-019-00705-6>

Liu, H. Y., Prentice, E. L., & Webber, M. A. (2024). Mechanisms of antimicrobial resistance in biofilms. *Npj Antimicrobials and Resistance*, 2(1), 27. <https://doi.org/10.1038/s44259-024-00046-3>

Lowe, J., Gauger, P., Harmon, K., Zhang, J., Connor, J., Yeske, P., Loula, T., Levis, I., Dufresne, L., & Main, R. (2014). *Role of Transportation in Spread of Porcine Epidemic Diarrhea Virus Infection, United States—Volume 20, Number 5—May 2014—Emerging Infectious Diseases journal—CDC*. <https://doi.org/10.3201/eid2005.131628>

Luppi, A. (2017). Swine enteric colibacillosis: Diagnosis, therapy and antimicrobial resistance. *Porcine Health Management*, 3(1), 16. <https://doi.org/10.1186/s40813-017-0063-4>

Madsen, A., & Matthiesen, C. (2013). *Exposure to aerosols during high-pressure cleaning and relationship with health effects*. *Ann Agric Environ Med.*, 20(3), 420–425. 420–425.

Magossi, G., Lambertini, E., Noll, L., Bai, J., Jones, C., Nagaraja, T. G., Phebus, R., Woodworth, J., & Trinetta, V. (2020). Potential risk-factors affecting *Salmonella* sp. And *Escherichia coli* occurrence and distribution in Midwestern United States swine feed mills. *Journal of Applied Microbiology*, 129(6), 1744–1750. <https://doi.org/10.1111/jam.14758>

Maillard, J.-Y. (2018). Resistance of Bacteria to Biocides. *Microbiology Spectrum*, 6(2), 6.2.19. <https://doi.org/10.1128/microbiolspec.ARBA-0006-2017>

Makovska, I., Biebaut, E., Dhaka, P., Korniienko, L., Jerab, J. G., Courtens, L., Chantziaras, I., & Dewulf, J. (2025). Methods for assessing efficacy of cleaning and disinfection in livestock farms: A narrative review. *Frontiers in Veterinary Science*, *12*, 1581217. <https://doi.org/10.3389/fvets.2025.1581217>

Masserdotti, M., Formenti, N., Donneschi, A., Guarneri, F., Scali, F., Romeo, C., Giacomini, E., Bertasio, C., Boniotti, M. B., Alborali, G. L., & Luzzago, C. (2024). *The role of short journey transportation in the spreading of swine pathogens and antimicrobial-resistant bacteria*. In Review. <https://doi.org/10.21203/rs.3.rs-4251132/v1>

McDonnell, G. (2014). The Use of Hydrogen Peroxide for Disinfection and Sterilization Applications. In Z. Rappoport, *Patai's Chemistry of Functional Groups* (1st ed., pp. 1–34). Wiley. <https://doi.org/10.1002/9780470682531.pat0885>

McLaren, I., Wales, A., Breslin, M., & Davies, R. (2011). Evaluation of commonly-used farm disinfectants in wet and dry models of *Salmonella* farm contamination. *Avian Pathology*, *40*(1), 33–42. <https://doi.org/10.1080/03079457.2010.537303>

Meireles, A., Giaouris, E., & Simões, M. (2016). Alternative disinfection methods to chlorine for use in the fresh-cut industry. *Food Research International*, *82*, 71–85. <https://doi.org/10.1016/j.foodres.2016.01.021>

Morgan, R. B., Sierra–Arguello, Y. M., Perdoncini, G., Borges, K. A., Furian, T. Q., Gomes, M. J. P., Lima, D., Salle, C. T. P., Moraes, H. L. S., & Nascimento, V. P. (2022). Comparison of transport crates contamination with *Campylobacter* spp. Before and after the cleaning and disinfection procedure in broiler slaughterhouses. *Poultry Science*, *101*(7), 101909. <https://doi.org/10.1016/j.psj.2022.101909>

Munoz, L. R., Pacheco, W. J., Hauck, R., & Macklin, K. S. (2021). Evaluation of commercially manufactured animal feeds to determine presence of *Salmonella*, *Escherichia coli*, and *Clostridium perfringens*. *Journal of Applied Poultry Research*, 30(2), 100142.

<https://doi.org/10.1016/j.japr.2021.100142>

Nakrachata-Amon, T., Vorasayan, J., Pitiruek, K., Arunyanart, S., Niyamosoth, T., & Pathumnakul, S. (2024). Optimizing vertically integrated pork production supply chain: A Lagrangian heuristic approach. *Heliyon*, 10(6), e26407.

<https://doi.org/10.1016/j.heliyon.2024.e26407>

National Pork Board. (2025). *Strategic Tools for Pork Production*.

<https://porkcheckoff.org/pork-production-management/strategic-tools-for-sustainable-pork-production>

National Pork Producers Council. (2023). *NPPC Economic Update Q2 2023*.

<https://nppc.org/wp-content/uploads/2023/04/2023-Q2-Econ-Update-Report.pdf>

Obinata, K. (2022). New Countermeasures Against Infections with/after COVID-19: Is Chlorine Dioxide a Useful and Safe Disinfectant? *Juntendo Medical Journal*, 68(5), 465–472.

<https://doi.org/10.14789/jmj.JMJ22-0030-R>

OSHA. (2025). *Hand and portable powered tools and equipment, general*. | *Occupational Safety and Health Administration*. <https://www.osha.gov/laws-regs/regulations/standardnumber/1910/1910.242>

Paiva, R. C., Burrough, E. R., Macedo, N., Silva, A. P. S. P., De Lagarde, M., Fairbrother, J. M., Piñeiro, P. E., & Almeida, M. N. (2025). Description of a contemporary pathogenic

Escherichia coli isolated from pigs with post-weaning diarrhea in the United States from 2010 to 2023. *Veterinary Research*, 56(1), 130. <https://doi.org/10.1186/s13567-025-01568-y>

Park, H. W., & Balasubramaniam, V. M. (2025). Infrared Thermal Image Processing Technique for Evaluating Superheated Steam as a Dry Sanitation Method. *Food and Bioprocess Technology*, 18(2), 1417–1430. <https://doi.org/10.1007/s11947-024-03529-3>

Park, S.-H., & Kang, D.-H. (2014). Inactivation of biofilm cells of foodborne pathogens by steam pasteurization. *European Food Research and Technology*, 238(3), 471–476. <https://doi.org/10.1007/s00217-013-2121-8>

Parker, T. B., Meiklejohn, K. A., Machado, G., Rahe, M., Darrow, B. S., & Ferreira, J. B. (2025). Evaluation of porcine epidemic diarrhea virus RNA contamination on swine industry transportation vehicles. *Preventive Veterinary Medicine*, 237, 106447. <https://doi.org/10.1016/j.prevetmed.2025.106447>

Rana, Y. S., Chen, L., Balasubramaniam, V. M., & Snyder, A. B. (2022). Superheated steam effectively inactivates diverse microbial targets despite mediating effects from food matrices in bench-scale assessments. *International Journal of Food Microbiology*, 378, 109838. <https://doi.org/10.1016/j.ijfoodmicro.2022.109838>

Remfry, S. E., Amachawadi, R. G., Shi, X., Bai, J., Tokach, M. D., Dritz, S. S., Goodband, R. D., Derouchey, J. M., Woodworth, J. C., & Nagaraja, T. G. (2021). Shiga Toxin–Producing *Escherichia coli* in Feces of Finisher Pigs: Isolation, Identification, and Public Health Implications of Major and Minor Serogroups. *Journal of Food Protection*, 84(1), 169–180. <https://doi.org/10.4315/JFP-20-329>

Rossi, G., Smith, R. L., Pongolini, S., & Bolzoni, L. (2017). Modelling farm-to-farm disease transmission through personnel movements: From visits to contacts, and back. *Scientific Reports*, 7(1), 2375. <https://doi.org/10.1038/s41598-017-02567-6>

Rougé, V., Allard, S., Croué, J.-P., & Von Gunten, U. (2018). In Situ Formation of Free Chlorine During ClO₂ Treatment: Implications on the Formation of Disinfection Byproducts. *Environmental Science & Technology*, 52(22), 13421–13429. <https://doi.org/10.1021/acs.est.8b04415>

Rutala, W. A., & Weber, D. J. (2014). Selection of the Ideal Disinfectant. *Infection Control & Hospital Epidemiology*, 35(7), 855–865. <https://doi.org/10.1086/676877>

Seviour, T., Derlon, N., Dueholm, M. S., Flemming, H.-C., Girbal-Neuhauser, E., Horn, H., Kjelleberg, S., Van Loosdrecht, M. C. M., Lotti, T., Malpei, M. F., Nerenberg, R., Neu, T. R., Paul, E., Yu, H., & Lin, Y. (2019). Extracellular polymeric substances of biofilms: Suffering from an identity crisis. *Water Research*, 151, 1–7. <https://doi.org/10.1016/j.watres.2018.11.020>

Sharma, V. K., & Sohn, M. (2012). Oxidation of Amino Acids, Peptides, and Proteins by Chlorine Dioxide. Implications for Water Treatment. In E. Lichtfouse, J. Schwarzbauer, & D. Robert (Eds.), *Environmental Chemistry for a Sustainable World* (pp. 237–254). Springer Netherlands. https://doi.org/10.1007/978-94-007-2439-6_5

Son, J., & Kim, S. W. (2025). Post-weaning diarrhea caused by F18⁺ *Escherichia coli* and its impact on mucosa-associated microbiota and immune responses in the jejunum of nursery pigs. *Microbiome Research Reports*, 4(3). <https://doi.org/10.20517/mrr.2025.14>

Song, L., Wu, J., & Xi, C. (2012). Biofilms on environmental surfaces: Evaluation of the disinfection efficacy of a novel steam vapor system. *American Journal of Infection Control*, 40(10), 926–930. <https://doi.org/10.1016/j.ajic.2011.11.013>

Tarafa, P. J., Jiménez, A., Zhang, J., & Matthews, M. A. (2010). Compressed carbon dioxide (CO₂) for decontamination of biomaterials and tissue scaffolds. *The Journal of Supercritical Fluids*, 53(1–3), 192–199. <https://doi.org/10.1016/j.supflu.2010.02.006>

Tawfick, M. M., Elshamy, A. A., Mohamed, K. T., & El Menofy, N. G. (2022). Gut Commensal *Escherichia coli* , a High-Risk Reservoir of Transferable Plasmid-Mediated Antimicrobial Resistance Traits. *Infection and Drug Resistance*, Volume 15, 1077–1091. <https://doi.org/10.2147/IDR.S354884>

Urbaniec, J. W., Davies, P. L., & Neary, J. M. (2025). Disinfection reduces but does not eliminate drug resistant *Escherichia coli* from livestock trailers following transport of calves. *Journal of Dairy Research*, 1–6. <https://doi.org/10.1017/S0022029925101581>

USDA. (2018). *Standard operating procedures: cleaning and disinfection*. https://www.aphis.usda.gov/sites/default/files/sop_cd.pdf

USDA. (2026). *NVAP Reference Guide: Cleaning and Disinfection*. Importance of Cleaning and Disinfection. <https://www.aphis.usda.gov/nvap/reference-guide/cleaning-disinfection>

USDA FSIS. (2016). *Sanitation Performance Standards Compliance Guide | Food Safety and Inspection Service*. <http://www.fsis.usda.gov/guidelines/2016-0003>

Van Hoorde, S., Vereecke, N., Sperling, D., He, X., Vanbeylen, E., Van Denberghe, E., Cox, E., & Devriendt, B. (2025). External factors influence intrinsic differences in Stx2e production by Porcine Shiga Toxin-producing *Escherichia coli* strains. *PLOS Pathogens*, 21(10), e1013616. <https://doi.org/10.1371/journal.ppat.1013616>

Vo, H., Imai, T., Teeka, J., Sekine, M., Kanno, A., Van Le, T., Higuchi, T., Phummala, K., & Yamamoto, K. (2013). Comparison of disinfection effect of pressurized gases of CO₂, N₂O, and N₂ on *Escherichia coli*. *Water Research*, 47(13), 4286–4293. <https://doi.org/10.1016/j.watres.2013.04.053>

Weber, D. J., Rutala, W. A., & Sickbert-Bennett, E. E. (2007). Outbreaks Associated with Contaminated Antiseptics and Disinfectants. *Antimicrobial Agents and Chemotherapy*, 51(12), 4217–4224. <https://doi.org/10.1128/AAC.00138-07>

Wesgate, R., & Maillard, J.-Y. (2026). Impact of Wiping Materials on the Elimination from Surfaces of Dry Surface Biofilm of Bacteria of Food Safety Concern. *Journal of Food Protection*, 89(3), 100700. <https://doi.org/10.1016/j.jfp.2026.100700>

Witte, A. K., Bobal, M., David, R., Blättler, B., Schoder, D., & Rossmanith, P. (2017). Investigation of the potential of dry ice blasting for cleaning and disinfection in the food production environment. *LWT*, 75, 735–741. <https://doi.org/10.1016/j.lwt.2016.10.024>

WOAH. (2021). *Terrestrial animal health code* (Twenty-ninth edition). World Organization for Animal Health. <https://www.woah.org/en/what-we-do/standards/codes-and-manuals/>

WOAH. (2024). *Addressing African Swine Fever: Protocols and Guidelines for Laboratory Diagnosis*. WOA (World Organisation for Animal Health).

<https://doi.org/10.20506/asf.3475>

Yang, Q., Martínez-López, B., Moon, S. A., Gomez-Vazquez, J. P., & Scoglio, C. (2025). Animal movement estimation and network-based epidemic modeling: Illustration for the swine industry in Iowa (US). *PLOS One*, 20(6), e0326234.

<https://doi.org/10.1371/journal.pone.0326234>

Yang, Y., Wang, L., Zeng, C., Li, C., Hu, J., & Zheng, J. (2023). Evaluation of the decontamination effectiveness of a steam mop for hospital floors. *Environmental Pollutants and Bioavailability*, 35(1), 2212128. <https://doi.org/10.1080/26395940.2023.2212128>

Chapter 2. Research gap and thesis objective

Most available studies addressing disinfection efficacy focus on food processing facilities or on-farm environments, leaving a clear knowledge gap regarding transport systems. Livestock transport trailers are recognized as critical points for the dissemination of bacterial pathogens between farms and production stages (Lowe et al., 2014). Much of the existing research evaluating cleaning and disinfection (C&D) efficacy has been conducted using aggregate bacterial cells grown under simplified laboratory conditions, which do not adequately represent the complexity of real-world sanitation environments (Bridier et al., 2011; Penesyan et al., 2021). These laboratory-based approaches often fail to account for critical factors such as bacterial attachment to surfaces, biofilm formation, and the presence of organic matter, which substantially influence microbial survival and resistance to disinfection (Maillard, 2018). Consequently, results derived from aggregate-based assays may overestimate the true effectiveness of C&D strategies when applied to complex settings like livestock transport trailers (Rutala & Weber, 2014; Simões et al., 2010). Environmental variables, such as temperature, are often overlooked despite their impact on disinfection outcomes. Even when C&D are implemented, evidence shows that cross-farm contamination risk can remain high due to bacterial persistence (Galvis & Machado, 2024; Täubel et al., 2024). Considering these limitations, this thesis aims to evaluate and compare the efficacy of selected C&D strategies against *E. coli* aggregates and biofilm experimentally inoculated on a laboratory-scale trailer model while also assessing the influence of disinfection methods, bacterial physiological state, and temperature on microbial reduction. By addressing these factors under conditions that better reflect real-world scenarios, this research seeks to generate experimentally grounded evidence to

support the development of more effective and practical disinfection solutions for livestock transport trailers.

References

- Bridier, A., Briandet, R., Thomas, V., & Dubois-Brissonnet, F. (2011). Resistance of bacterial biofilms to disinfectants: A review. *Biofouling*, 27(9), 1017–1032.
<https://doi.org/10.1080/08927014.2011.626899>
- Lowe, J., Gauger, P., Harmon, K., Zhang, J., Connor, J., Yeske, P., Loula, T., Levis, I., Dufresne, L., & Main, R. (2014). *Role of Transportation in Spread of Porcine Epidemic Diarrhea Virus Infection, United States—Volume 20, Number 5—May 2014—Emerging Infectious Diseases journal—CDC*. <https://doi.org/10.3201/eid2005.131628>
- Maillard, J.-Y. (2018). Resistance of Bacteria to Biocides. *Microbiology Spectrum*, 6(2), 6.2.19. <https://doi.org/10.1128/microbiolspec.ARBA-0006-2017>
- Rutala, W. A., & Weber, D. J. (2014). Selection of the Ideal Disinfectant. *Infection Control & Hospital Epidemiology*, 35(7), 855–865. <https://doi.org/10.1086/676877>
- Simões, M., Simões, L. C., & Vieira, M. J. (2010). A review of current and emergent biofilm control strategies. *LWT - Food Science and Technology*, 43(4), 573–583.
<https://doi.org/10.1016/j.lwt.2009.12.008>
- Täubel, M., Castagnoli, E., Salthammer, T., Morawska, L., & Salonen, H. (2024). The impact of cleaning on the microbiomes of indoor surfaces. *Indoor Environments*, 1(3), 100021.
<https://doi.org/10.1016/j.indenv.2024.100021>

Chapter 3 - Evaluation of cleaning and disinfection strategies to control *Escherichia coli* aggregates and biofilms using a lab-scale trailer model

Abstract

Livestock transport trailers represent critical points where microorganisms can persist and transfer between animals. *Escherichia coli* can survive for extended periods and form aggregates and biofilms, which increase their resistance to cleaning and disinfection. There is a need to develop and evaluate effective cleaning and disinfection strategies to reduce microbial persistence and mitigate the risk of pathogen transmission in live-haul environments. *E. coli* TVS 353 was incubated at 37 °C for 24 h to form aggregated cells and 48 h for biofilms on aluminum coupons. Coupons underwent one of three mechanical cleaning procedures (scraping, vacuuming, or air) followed by disinfection treatment: 3% hydrogen peroxide (30 s), 400 ppm quaternary ammonium compounds (1 min), 400 ppm sodium hypochlorite (1 min), steam (100 °C, 30 s), carbon dioxide (30 s), and chlorine dioxide gas (500 ppm, 10 min). Treatments were conducted at 30, 18, and 10 °C to simulate seasonal transport conditions. Surfaces were sampled using swab sponges, neutralized, diluted, and plated for enumeration. Treatments were performed in triplicate with appropriate controls. The efficacy of cleaning and disinfection interventions was dependent on cleaning, disinfection, temperature, and bacterial physiological state ($P < 0.05$). For aggregated cells, the greatest reductions (4.50 - 5.06 log CFU/cm²) were observed with vacuum cleaning combined with chlorine dioxide gas at 10 °C, vacuum cleaning with carbon dioxide at 18 °C, and air with sodium hypochlorite at 30 °C. Biofilm-associated cells were more resistant. The highest biofilm reductions (3.76 – 3.87 log CFU/cm²) occurred with

vacuum combined with chlorine dioxide gas at 10 °C, vacuum cleaning with dry carbon dioxide at 18 °C, and vacuum with hydrogen peroxide at 30 °C. These findings highlight the importance of evaluating cleaning and disinfection to control microbial contamination and reduce cross-contamination risks in livestock transport environments.

3.1 Introduction

Transportation plays a critical role in swine production systems because pigs are frequently moved between breeding, nursery, grow-finish, and processing facilities (Ciuoderis-Aponte et al., 2022). However, the continuous movement of animals, personnel, and equipment also creates multiple opportunities for pathogen dissemination throughout the production chain (Dee et al., 2020; Lowe et al., 2014). Among the microorganisms associated with these transmission routes, *Escherichia coli* (*E. coli*) is of particular concern due to its importance in swine health, food safety, and environmental persistence (Barros et al., 2023). Within the One Health framework, transportation has become an important area of concern for controlling the spread of pathogens throughout animal production systems (CDC, 2025).

Although many *E. coli* strains are commensal inhabitants of the intestinal tract of pigs, pathogenic strains are responsible for enteric and systemic diseases collectively referred to as colibacillosis (Fratamico et al., 2016). These infections are associated with diarrhea, edema disease, reduced growth performance, increased antimicrobial use, and economic losses in swine herds (Barros et al., 2023; Luppi, 2017). In addition, generic *E. coli* is commonly used as an indicator organism for fecal contamination and hygienic status in livestock and food production environments (Blanco-Lizarazo & Sierra-Cadavid, 2023). During transport, pigs shed feces, urine, saliva, and organic debris onto trailer floors, ramps, gates, and walls, allowing microorganisms to persist on contaminated surfaces (HIPRA, 2025). If C&D procedures are

incomplete or inconsistently applied, contaminated trailers may facilitate indirect pathogen transmission between farms (Alarcón et al., 2021; Lowe et al., 2014). Previous epidemiological investigations demonstrated that vehicles visiting multiple production sites contribute to disease dissemination and increase connectivity among farms (Galvis et al., 2022; Yang et al., 2025). Furthermore, practical limitations such as short turnaround times, low environmental temperatures, and restricted access to washing facilities may compromise disinfection effectiveness and biosecurity compliance (Galvis et al., 2022; Lowe et al., 2014).

The persistence of *E. coli* in livestock environments is strongly influenced by its ability to survive on surfaces and form biofilms (Beloin et al., 2008). Biofilms are structured microbial communities attached to surfaces and embedded within a self-produced extracellular polymeric substances (EPS) matrix that promotes adhesion and protection against environmental stressors (Flemming et al., 2016). Biofilm cells are generally more tolerant to disinfectants, desiccation, and temperature fluctuations than aggregate cells, making them more difficult to eliminate during routine disinfection procedures (Flemming et al., 2016; Liu et al., 2024). Environmental factors such as moisture, organic material, temperature, and surface characteristics may further influence bacterial attachment and persistence (Lim et al., 2020). In livestock trailers, structural irregularities, joints, and protected corners may retain moisture and organic residues even after washing procedures, allowing microorganisms to survive despite routine disinfection (Dhiman et al., 2024; Parker et al., 2025). Recent attention has also focused on dry surface biofilms, which may persist under low-moisture conditions commonly encountered in transportation environments (Amaeze et al., 2024).

Effective C&D procedures are essential for reducing microbial contamination in livestock transportation systems. Cleaning focuses on the physical removal of feces, feed residues, and

organic material, whereas disinfection aims to inactivate remaining microorganisms on cleaned surfaces (Codex Alimentarius, 2023; USDA, 2018). However, disinfectant efficacy may be reduced in the presence of organic matter (Chowdhury et al., 2019). Studies conducted under commercial conditions demonstrated that C&D procedures often reduce, but do not completely eliminate, microorganisms from livestock trailers (Masserdotti et al., 2024; Urbaniec et al., 2025).

Although transportation is recognized as an important component of farm biosafety, limited information is available on the effectiveness of different C&D strategies against aggregate and biofilm *E. coli* under conditions representative of those in livestock trailers. One of the few studies evaluating trailer disinfection was conducted by Rajkowski et al., (1998), who assessed the reduction of *E. coli* in swine transport trailers disinfected during different seasons of the year. Their results showed that all trailers remained positive for *E. coli* after C&D, with some surfaces exceeding 5 log CFU/cm². Later, Böhm (1998) proposed a target of <3 log CFU/cm² for total viable counts (TVC) following C&D in animal housing facilities; however, no universally accepted standard currently exists for live haul trailer disinfection. More recent studies evaluating C&D practices in live haul trailers have also reported that current protocols are often insufficient to achieve adequate microbial reductions or meet the targets proposed by Böhm (Perrucci et al., 2026). In addition, most available studies have focused primarily on viral pathogens, while information regarding bacterial biofilms in environments relevant to live haul trailers remains scarce (Martinez et al., 2025; Parker et al., 2025). Given these gaps in the literature, this study evaluated *E. coli* in different physiological states, including aggregates and biofilms, to compare the efficacy of selected C&D strategies under different temperature

conditions and generate information that may support the development of more effective disinfection practices for swine transportation systems.

3.2 Materials and methods

3.2.1 Bacteria strain

A Biosafety Level 1 (BSL-1) *Escherichia coli* TVS 353 strain was used in this study. The strain was originally isolated by Dr. Trevor Suslow at the University of California, Davis (McKenzie-Reynolds et al., 2025). Two days before the experiment, the strain was recovered from frozen stock stored at -80°C , streaked onto Tryptic Soy Agar (TSA; BD Difco, Sparks, MD), and incubated at 37°C for 24 h. One day prior to the experiment, a single colony of *E. coli* was inoculated into Tryptic Soy Broth (TSB; BD Difco, Sparks, MD) and incubated at 37°C for 24 h to obtain approximately 9 log CFU/mL.

3.2.2 Aggregate and biofilm formation

To form aggregated cells and biofilms, 10×10 cm aluminum textured and smooth coupons (Figure 4) were obtained from Technology and Development Institute (TDI) at Kansas State University. Coupons were previously autoclaved. Each coupon was placed into a sterile stomacher bag (15.24×22.86 cm; Whirl-Pak, Pleasant Prairie, WI), followed by the addition of 200 mL of Tryptic Soy Broth (TSB), 2 mL of an overnight culture of *E. coli* TVS 353, and 2 g of autoclaved (121°C , 60 min) swine feces. Coupons were completely submerged in the inoculated media. The bags were sealed to create a closed system and incubated under static conditions at 37°C for 24 h to promote aggregate formation and an additional 48 h to allow biofilm development. Swine feces were obtained from the Swine Research Center at Kansas State University. Fresh feces were collected directly from the floor where finished pigs were housed (4–6 months old). After autoclaving, the swine feces were stored at -20°C .

3.2.3 Lab scale trailer model

A lab-scale trailer model illustrated in Figure 5 was used to perform experiments. The trailer dimensions were 0.50 m wide $\times$ 0.70 m tall $\times$ 0.60 m long. The trailer walls were constructed of smooth aluminum panels with multiple circular perforations along the sides. The floor consisted of diamond-plate aluminum, providing a textured surface. The unit was constructed in collaboration with TDI. For each experiment, inoculated aluminum coupons were clipped onto the interior surfaces of the trailer model (Figure 5). Previously sprayed with 70% ethanol binder clips (32 mm) were used to attach the smooth coupons to the left wall (L1) and right wall (L2). Textured coupon was placed on the trailer floor (L3).

3.2.4 Cleaning

Inoculated coupons were retrieved from the stomacher bags using sterile tweezers and placed in sterile glass trays inside a biosafety cabinet (Labculture Reliant, ESCO, Horsham, PA) for 15 min to allow surface drying. After the 15-min drying period, coupons were clipped into the trailer model. The trailer was then placed inside an incubator and allowed to reach the target temperatures of 30 °C, 18 °C, or 10 °C. Surface temperature was monitored using a thermocouple (Traceable, Weber, TX) in direct contact with the aluminum surface. The trailer took 30 min to decrease to 10 °C and 10 minutes to reach 30 °C, while 18 °C corresponded to room temperature. Once the desired temperature was reached, cleaning treatments were applied. For scraping, a stainless steel 10 cm wide scraper (Greenbrier International, Canada) was used. One pass was performed covering the entire surface area of the coupon. For vacuuming, a portable 12-volt vacuum (Meloh, China) with a 5 cm wide attachment was used. One vertical pass was applied, covering the full surface area of the coupon. For air cleaning, an electric air

blower of 40,000 rpm was used (Afmatic, China). Air was applied for 10 s at 20 cm from each coupon.

3.2.5 Disinfection

Following cleaning procedures, several disinfectants were evaluated. These disinfectants were selected based on their common use in farm biosecurity programs, differences in mode of action, compatibility with a range of environmental temperatures, broad microbial spectrum and practical applicability under field conditions. The liquid disinfectants were applied using a handheld sprayer (Chapin 2000, China). The evaluated treatments included: 3% hydrogen peroxide (HP; Aqua Solutions, Deer Park, TX; 30 s contact time); 400 ppm sodium hypochlorite (SH; Dibac, Charlotte, NC; 1 min contact time), with concentration confirmed using a chlorine test kit (ChemWorld, Kennesaw, GA); 400 ppm quaternary ammonium compounds (Quat; Diversey, Charlotte, NC; 1 min contact time), verified using test strips (LaMotte, Chestertown, MD); chlorine dioxide gas (CDG; Environmental, Allentown, PA) at 500 ppm for 10 min, monitored using a portable gas detector (Analytical Technology Inc., Collegeville, PA); steam (100 °C) applied for 30 s, monitored with thermocouples (Traceable, Weber, TX); and CO₂, a commercial formulation containing 58% isopropyl alcohol and 0.015% quaternary ammonium compounds and applied with 800 psi CO₂ (IQC; Biospray D2, Stamford, CT) for 30 s. To ensure reproducibility across runs, all C&D procedures were performed by the same operator using a consistent application technique. This included maintaining a fixed distance of 30 cm between the nozzle and the laboratory trailer surface during disinfectant application and following a triangular spray pattern. The spray sequence began at L1, moved to L3, and then to L2, with spray location changing every second. This sequence was repeated throughout the disinfectant application period.

3.2.6 Recovery and enumeration of *E. coli* cells.

Immediately after disinfection treatments, coupons were swabbed for bacterial recovery. Neutralizing sponges (Neogen, Lansing, MI) containing 10 mL of a neutralizing solution in a 15.24×27.94 cm bag were used for swabbing. An additional 10 mL of the corresponding neutralizing solution was aseptically added to each sponge prior to sampling. For SH, Dey-Engley neutralizing broth (Difco, Sparks, MD) was used. For Quat, HP, CDG and CO₂, Neutralizing Buffer (Difco, Sparks, MD) was used. After sample collection, sponges were individually processed in a stomacher (400 Lab Blender, West Sussex, United Kingdom) for 30 s. Following homogenization, serial dilutions were prepared in 0.1% peptone water (Bacto, Sparks, MD) and spread plated onto MacConkey Agar (BD BBL, Sparks, MD). Plates were incubated at 37 °C for 24 h. Colonies were enumerated using an automated colony counter (Interscience, Woburn, MA), and results were reported as log CFU/cm². The limit of detection (LOD) was 0.2 CFU/cm², based on the detection of 1 colony from 1 mL of undiluted sample, a recovery volume of 20 mL, and a sampling area of 100 cm². All observed values were above this threshold. The limit of quantification (LOQ) was 5 CFU/cm², based on the quantification of 25 colonies from an undiluted sample. All samples collected were above this limit.

3.2.7 Experimental design and statistical analysis

The experiment consisted of a $3 \times 2 \times 3 \times 3 \times 6 \times 3$ factorial treatment structure. The factors included three temperatures (10, 18, and 30 °C), two cell states (aggregates and biofilms), three sampling locations (L1, L2, and L3), three cleaning methods, six disinfection treatments and three repetitions. The design structure was a split-plot in an incomplete block design, with the combination of temperature-cleaning-disinfectant factors as the whole-plot factor and the

location as the split-plot factor. Days were considered as blocks to account for variability among experimental runs.

A run consisted of one cleaning and disinfection application performed under a specific combination of temperature and cell state (Figure 6). Aggregate and biofilm experiments were conducted in separate runs, and treatment repetitions were performed across different experimental days. Each run included its own positive and negative controls, and positive controls were used to calculate log reductions. Sampling locations L1, L2, and L3 were placed within the same laboratory trailer and received the same treatment simultaneously. This led to 972 observations between aggregate cells and biofilm.

A linear mixed-effects model was fitted to the data. Log reduction (RED; log CFU/cm²) was the response variable. All factors and their interactions were included as fixed effects: temperature, cell state, location, cleaning, and disinfection, including their interactions, were treated as fixed effects, and day and temperature:cleaning:disinfectant as random effects. Analysis of variance (ANOVA) was performed to evaluate the significance of fixed effects. When significant interactions were detected, estimated marginal means (emmeans) were calculated. Statistical significance was defined at $P < 0.05$. All analyses were conducted in R version 4.5.0 (R Core Team, 2026, Vienna, Austria). The following packages were used: lme4, lmerTest, emmeans, multcomp, dplyr, tidyr, ggplot2, ggpubr, ggtext, scales, stringr, readxl, writexl, multcompView, and MuMIn.

3.3 Results

The ANOVA indicated that all evaluated factors, except for location, significantly influenced the log reduction of *E. coli* (Appendix A Table 4). Significant interactions are

presented in Table 2. Double interactions were significant, as well as three-way interactions. Biofilms and aggregate cells were analyzed separately.

In commercial settings, organic matter, limited disinfectant contact times, and difficult-to-clean surfaces can reduce disinfection efficacy, making consistent 5-log reductions difficult to achieve (Martelli et al., 2017). Although 5-log reductions are commonly used as a benchmark under controlled laboratory conditions, trailer C&D in commercial production systems is performed under more variable environments where lower, yet meaningful, reductions may still contribute to reducing microbial loads and limiting cross-contamination risks. Therefore, based on previous studies and the practical limitations associated with trailer C&D, reductions greater than 3 log CFU/cm² for biofilm cells and greater than 4 log CFU/cm² for aggregate cells were considered indicative of adequate treatment performance under field-relevant conditions (Clayton et al., 2021; Martelli et al., 2017).

3.3.1 Aggregate cells

3.3.1.1 Influence of cleaning and disinfection

C&D influenced aggregate cell reductions, with responses depending on the treatment combination evaluated (Table 2; Figure 7). The positive control for aggregate cells was 7.31 ± 0.37 log CFU/cm², while treated samples showed reductions from 1.93 to 5.06 log CFU/cm². The lowest estimated reduction was observed with steam combined with vacuuming at 18 °C, whereas the highest reduction was obtained with CO₂ combined with vacuuming at 18 °C. This broad range shows that aggregate cell removal was strongly influenced by how the cleaning method and disinfectant were paired.

Differences among cleaning methods were observed for several disinfectants ($P < 0.001$). At 10 °C, differences were observed for SH and HP; at 18 °C, for steam, CO₂, and CDG; and at

30 °C, for steam, SH, Quat, CO₂ and CDG. CO₂ was less affected by cleaning methods at 10 °C ($P > 0.05$), but differences were observed at 18 and 30 °C. Scraping and vacuuming increased reductions compared with air cleaning, particularly for Quat, HP, and CDG. However, this improvement was not the same for every disinfectant. Air cleaning supported higher reductions with SH at 30 °C, and with CO₂ at 18 °C.

The emmeans showed different reduction patterns across disinfectants, temperatures, and cleaning methods. CO₂ was associated with high estimated reductions across several conditions. For CDG, estimated reductions exceeded 4 log CFU/cm² for all cleaning methods at 10 °C. High values were also observed for HP and SH under some cleaning conditions. Quat showed intermediate estimated reductions, with the highest values observed with scraping. Steam showed lower estimated reductions at 10 and 18 °C. Statistical differences among treatment combinations are indicated by the letter groupings in Figure 7.

3.3.1.2 Influence of temperature

Temperature affected aggregate cell reduction through its interaction with cleaning and disinfection, as indicated by the significant interaction observed in the model ($P < 0.05$). Temperature did not simply increase or decrease reductions across all treatments; instead, it changed the response of each disinfectant differently (Figure 7). CDG showed its highest estimated reductions at 10 °C; however, at 30 °C, reductions decreased. Steam showed the opposite tendency. Reductions were lower at 10 and 18 °C but increased at 30 °C, particularly when steam was combined with scraping. At 30 °C, steam did not differ from the chemical disinfectants when combined with scraping ($P > 0.05$).

HP reductions changed less across temperatures than most other disinfectants. CO₂ maintained high reductions at 10 and 18 °C, including the highest reduction observed in the study with vacuuming at 18 °C. At 30 °C, CO₂ estimates with air cleaning and scraping were

lower than at 10 °C, while the reductions with vacuum remained above 4 log CFU/cm². The patterns for SH and Quat across temperatures were influenced more by the cleaning method used. Together, these patterns show that the cleaning-disinfectant combinations with the highest estimated reductions varied across temperatures, rather than following one common response.

3.3.2 Biofilms

3.3.2.1 Influence of cleaning and disinfection

Compared with aggregate cells, biofilm cells showed a lower and narrower reduction range (Table 2; Figure 8). The positive control for biofilm cells was 7.35 ± 0.41 log CFU/cm², while treated samples showed reductions from 1.87 to 3.87 log CFU/cm². The lowest reduction was observed with steam combined with vacuuming at 18 °C, as also seen for aggregate cells. The highest biofilm reduction was observed with HP combined with vacuuming at 30 °C, with CO₂ under the same cleaning condition also among the highest reductions. All biofilm reductions remained below 4 log CFU/cm². Averaged across temperature, cleaning, disinfection, and location, estimated reductions were 0.648 log CFU/cm² higher for aggregates than for biofilms ($P < 0.001$), indicating that biofilms were more difficult to reduce.

The role of cleaning was also more selective for biofilms. Differences among cleaning methods were observed for SH at 10 °C; for CDG, CO₂, and HP at 18 °C; and for SH, Quat, HP, and CO₂ at 30 °C ($P < 0.05$). In contrast to aggregate cells, steam did not show differences among cleaning methods at any temperature ($P > 0.05$).

Enhanced cleaning supported several chemical treatments, but the benefit depended on the disinfectant. HP and CO₂ showed some of the strongest biofilm reductions when paired with scraping or vacuuming. SH also achieved high reductions with scraping at 10 and 30 °C. As observed for aggregate cells, the reductions for CDG were among the highest at 10 °C across all

cleaning methods. Quat showed intermediate estimated reductions, with higher values in specific scraping or vacuuming combinations. Steam was among the treatments with the lowest estimated biofilm reductions, with all values below 3 log CFU/cm². Thus, the biofilm results did not simply mirror the aggregate cell pattern at a lower scale. The pattern of reductions differed between cell states: CO₂ and CDG achieved higher reductions for aggregate cells, whereas HP and CO₂ reductions became more prominent for biofilms when paired with enhanced cleaning. This contrast highlights the greater resistance of biofilm cells and the need for cleaning-disinfection combinations that can act beyond surface-level removal (Galié et al., 2018).

3.3.2.2 Influence of temperature

Temperature influenced biofilm reductions through its interaction with C&D, as indicated by the significant interaction observed in the model ($P < 0.05$). Across temperatures, the emmeans showed treatment-specific patterns (Figure 8). These patterns partly overlapped with those observed for aggregate cells, but some important differences were evident. CDG showed its highest reductions at 10 °C in both aggregate and biofilm cells and decreased at warmer temperatures. Steam, compared to the other disinfectants, showed the lowest reductions for biofilms at 10 °C and 18 °C, although it did not significantly differ from every chemical disinfectant within each cleaning method. Unlike aggregate cells, where steam improved log reductions at 30 °C with scraping, this increase was not enough for steam to approach the reductions observed with SH or CO₂.

HP was one of the more stable treatments across temperatures and reached the highest biofilm reduction with vacuuming at 30 °C. CO₂ also maintained high reductions in several combinations, particularly at 10 °C and when paired with vacuuming or scraping at warmer temperatures. However, CO₂ log reductions decreased at 30 °C when only air cleaning was used, indicating that its biofilm performance at warmer temperatures relied more strongly on the

cleaning step. SH and Quat showed temperature responses that were closely tied to cleaning methods. SH improved log reductions with air cleaning or scraping at 30 °C, while Quat showed its strongest log reduction with vacuum at 10 °C. These results show that the descriptive temperature trends varied with the specific cleaning-disinfectant pairing rather than following one common response. Compared with aggregate cells, effective biofilm reduction required both an effective disinfectant and a cleaning method that improved access to attached cells.

3.4 Discussion

3.4.1 Factors influencing *E.coli* reduction

As shown in Table 2, multiple factors influenced the reduction of *E. coli*. These findings are consistent with what would be expected under real-world scenarios, where temperature, surface characteristics, presence of organic matter, disinfectant type, and prior cleaning all play a role in microbial reduction (Arthur et al., 2025). One of the clearest findings was the difference between aggregate and biofilm cells. Several aggregate cell treatments exceeded 4 log CFU/cm² and reached reductions above 5 log CFU/cm², whereas biofilm reductions remained below 4 log CFU/cm², with the highest response close to 3.9 log CFU/cm². This difference is consistent with the protective role of the EPS, which can limit disinfectant diffusion and reduce chemical access to embedded cells (Flemming et al., 2016; Galié et al., 2018; Liu et al., 2024). In addition, biofilm attachment to surfaces makes physical removal more difficult than removal of loosely associated aggregate cells (Fagerlund et al., 2017; Srey et al., 2013). This helps explain why treatments that were highly effective against aggregate cells did not necessarily produce the same reductions against biofilms. Similar trends have been reported in previous studies where biofilms showed lower susceptibility to antimicrobial treatments than aggregates or less attached cells (Nett et al., 2008; Shi et al., 2024; Smith & Hunter, 2008). This difference between cell states is

important because it changes how treatment performance should be interpreted. CO₂ and CDG were among the strongest treatments for aggregate cells, whereas HP and CO₂ became more prominent for biofilms, particularly when combined with scraping or vacuum cleaning. This shift suggests that biofilm control depended not only on the antimicrobial strength of the disinfectant but also on whether the cleaning step improved access to attached cells.

Organic matter played a major role in these treatment differences. Organic residues can reduce disinfectant efficacy by reacting with active ingredients, increasing disinfectant demand, shielding cells, and preventing direct contact between disinfectant and the target organism (Corcoran et al., 2014; Gómez-López, 2012; Maillard, 2018). This is especially relevant for livestock trailers, where manure, moisture, and surface roughness can create protected areas that are difficult to disinfect (Parker et al., 2025). Scraping and vacuum cleaning generally supported higher reductions than air cleaning. These methods likely removed more organic material and exposed cells that were otherwise protected within residues or biofilm material. Air cleaning, in contrast, may remove loose debris but is less effective at removing adherent material and may redistribute particles across the surface rather than fully removing them. However, enhanced cleaning did not improve every disinfectant equally. Treatments such as Quat, CDG, SH, and steam appeared more sensitive to cleaning method, whereas HP and the CO₂ treatment maintained higher reductions across cleaning conditions. Chlorine-based compounds, chlorine dioxide, and quaternary ammonium compounds can lose activity through reaction with or binding to organic material, reducing the amount of active compound available to inactivate cell (Maillard, 2018; Virto et al., 2005). In contrast, HP and the CO₂ formulation may have retained more activity after limited cleaning, although their strongest biofilm reductions still occurred when paired with scraping or vacuuming.

Temperature modified treatment performance, but it did not produce a single response pattern across disinfectants. This was expected because temperature can affect disinfection through several mechanisms, including reaction rate, chemical stability, volatility, drying time and surface wetting (Bancroft & Richter, 1931; Beltrame et al., 2012; Cheng et al., 2025; Gómez-López et al., 2009). A warmer surface can increase chemical reaction rates for some products, but it can also accelerate evaporation or reduce the time that a disinfectant remains wet on the surface (CDC, 2023). In this study, CDG performed best at 10 °C, steam improved at 30 °C against aggregate cells, HP remained comparatively stable, and CO₂ decreased under some 30 °C conditions unless paired with vacuum cleaning. These results suggest that temperature acted as a modifier of disinfectant behavior rather than as a direct predictor of higher or lower microbial reduction.

3.4.2 Disinfectants performance

The CO₂ treatment produced some of the strongest and most consistent reductions, especially against aggregate cells and in several biofilm combinations. In this study, CO₂ was used as a carrier for an alcohol-based disinfectant containing isopropyl alcohol and quaternary ammonium compounds. Therefore, its efficacy should be interpreted as the effect of a CO₂-assisted alcohol-quat application rather than CO₂ alone. The alcohol component may have provided rapid antimicrobial activity through membrane disruption and protein denaturation, while the quaternary ammonium component may have contributed additional membrane damage in exposed cells (Boyce, 2023; McDonnell & Russell, 1999). Similar reductions have been reported for alcohol-quat formulations against bacterial contaminants (Kenters et al., 2017). The CO₂ carrier may also have improved surface distribution of the disinfectant, allowing the formulation to reach areas that would otherwise receive less uniform liquid coverage (Tarafa et

al., 2010). However, the decrease in log reductions observed for the CO₂ treatment under some 30 °C conditions may be related to the volatility of the alcohol component. Isopropyl alcohol evaporates readily at ambient temperatures, and evaporation increases as temperature rises (Boyce, 2018).

HP also showed strong performance, particularly when combined with scraping or vacuum cleaning. Reductions reached up to 4.57 log CFU/cm² for aggregate cells and approximately 3.9 log CFU/cm² for biofilms. HP acts as an oxidizing agent that damages membranes, proteins, and nucleic acids (McLaren et al., 2011). Its relatively stable performance across temperatures suggests that it maintained antimicrobial activity within the evaluated range. However, its best biofilm response occurred with vacuuming at 30 °C, supporting the idea that improved surface exposure was still needed for attached cells. These findings are consistent with previous studies reporting reductions of approximately 2.8 to 3 log CFU for static biofilms of *Pseudomonas aeruginosa* and *Staphylococcus aureus*, and reductions approaching 4.8 log CFU for aggregate cells treated with hydrogen peroxide (Fedorova & Ilyakova, 2023; Raval et al., 2021).

CDG showed a different behavior. It was highly effective against aggregate cells at 10 °C, with all cleaning combinations exceeding 4 log CFU/cm², and it also performed well against biofilms at this temperature. At 18 and 30 °C, CDG reductions decreased, especially for biofilms. This response may be related to chlorine dioxide stability and residual concentration. Lower temperatures may help retain more available CDG during exposure, whereas warmer temperatures may increase decomposition or reaction with organic matter before sufficient contact with cells occurs (Gómez-López et al., 2009; S.-H. Park & Kang, 2018; Trinetta et al., 2011). In biofilms, this limitation may be amplified because the EPS matrix and remaining

organic material increase disinfectant demand and slow penetration into the attached cell structure (Flemming et al., 2016).

SH and Quat showed more condition-dependent responses. SH produced useful reductions in selected aggregate and biofilm combinations, but its performance shifted with cleaning method and temperature. This variability may be related to the sensitivity of SH to organic matter, which can rapidly consume active chlorine and reduce the concentration available for microbial inactivation (Maillard, 2018; Virto et al., 2005). Quat showed an intermediate response and appeared to benefit from scraping or vacuuming in specific combinations. Quaternary ammonium compounds are membrane-active disinfectants, but their efficacy can be reduced by organic material, surface residues, and biofilm matrices that limit access to embedded cells (Boyce, 2023; Saverina et al., 2023). This helps explain why Quat improved with enhanced cleaning in some conditions but did not reach the highest reductions observed with CO₂, CDG, or HP.

Steam was the most unexpectedly limited treatment. Steam is often considered an effective non-chemical intervention because microbial inactivation can occur when sufficient heat reaches the contaminated surface for an adequate exposure time (Park & Kang, 2018; Rana et al., 2022). However, in this study, steam produced lower reductions against both aggregate and biofilm cells compared to the other disinfectants. Although steam improved against aggregate cells at 30 °C when combined with scraping, this improvement was not enough to approach the reductions achieved by the strongest chemical treatments. For biofilms, steam remained below 3 log CFU/cm² reductions across all evaluated conditions. The steam response may have resulted from limited heat transfer. Steam efficacy depends not only on the temperature generated by the equipment, but also on surface temperature, distance from the

nozzle, exposure time, and whether heat reaches protected cells before the surface cools (Bhana et al., 2013; Park & Balasubramaniam, 2025). Therefore, the limited steam performance observed in this study reflects insufficient delivered heat on the contaminated surface rather than a complete lack of antimicrobial potential of steam. Translating these findings into practical settings, trailer surfaces, organic residues, and biofilm material may function as insulating barriers, preventing cells from reaching lethal temperatures during short exposures.

3.4.3 Practical implications for trailer C&D

These findings have practical implications for commercial trailer C&D. Shared livestock transportation creates repeated opportunities for microbial transfer between animal groups (Cook & Lee, 2023). Some production systems attempt to reduce this risk by separating trailers according to production stage or animal destination, but these practices do not eliminate the need for effective C&D (Mannion et al., 2008). The experimental conditions used in this study were selected to reflect challenges encountered in live-haul environments, including pig feces, biofilms, variable temperatures and limited contact time. Even when C&D procedures are standardized, variation in cleaning thoroughness, disinfectant coverage, and contact time can occur in practice (Alarcón et al., 2021; Parker et al., 2025). Therefore, disinfection success depends on cleaning steps to remove enough organic material for the disinfectant to reach the microbial target.

The results also suggest that biofilm evaluations provide a more conservative assessment of disinfection efficacy than aggregate cell models alone. Aggregate cells were more susceptible and often reached reductions that met the reduction target used in this study. Biofilms required more specific combinations of C&D, and most treatments did not reach 4 log CFU/cm². This agrees with previous reports showing that mature biofilms can persist after exposure to sodium

hypochlorite, sodium hydroxide, benzalkonium chloride, and other disinfectants, even with longer contact times than those used in this study (Bridier et al., 2011; Corcoran et al., 2014; Koti et al., 2024; Van Houdt & Michiels, 2010). For trailer biosecurity programs, this distinction is important because cells protected in biofilms or residual organic matter may survive routine C&D and contribute to recontamination.

3.4.4 Study limitations

This study has limitations that should be considered when interpreting the results. First, the contact time of the liquid disinfectants was less than 1 min. This short exposure reflects the practical difficulty of maintaining wet contact on trailer surfaces, but it may have limited the full antimicrobial potential of some products. Longer exposure could produce greater reductions under controlled conditions, although maintaining extended contact in trailers may be difficult because of drainage, surface angle, airflow, and rapid drying (Cheng et al., 2025; Parker et al., 2025; Rutala & Weber, 2014). Second, this study used single-species *E. coli* biofilms. Multispecies biofilms may differ in matrix composition, microbial interactions, and susceptibility to disinfectants (Van Houdt & Michiels, 2010). Third, biofilm development occurred under controlled laboratory conditions, which may not fully reproduce the environmental variability of commercial trailers. In practice, biofilms may develop under fluctuating temperature, moisture and nutrient availability, which could alter their structure and resistance (Flemming et al., 2016).

3.5 Conclusions

The results showed that treatment efficacy depended on cell state, disinfectant, cleaning method, and temperature. Aggregate cells were more susceptible than biofilm cells, reaching reductions up to 5.06 log CFU/cm², whereas biofilm reductions remained below 4 log CFU/cm². CO₂ and CDG produced the strongest reductions for aggregate cells, while HP and CO₂ were the

most effective options for biofilms when combined with vacuuming or scraping. Temperature modified treatment performance differently depending on the disinfectant, with CDG performing better at 10 °C and steam showing limited efficacy, especially against biofilms. These findings suggest that trailer C&D protocols should pair effective disinfectants with cleaning methods that reduce organic material and improve access to contaminated surfaces.

Figures and tables

Figure 4. Aluminum coupons (10 × 10 cm). The left coupon was used for L3, and the right coupon was used for L1 and L2.

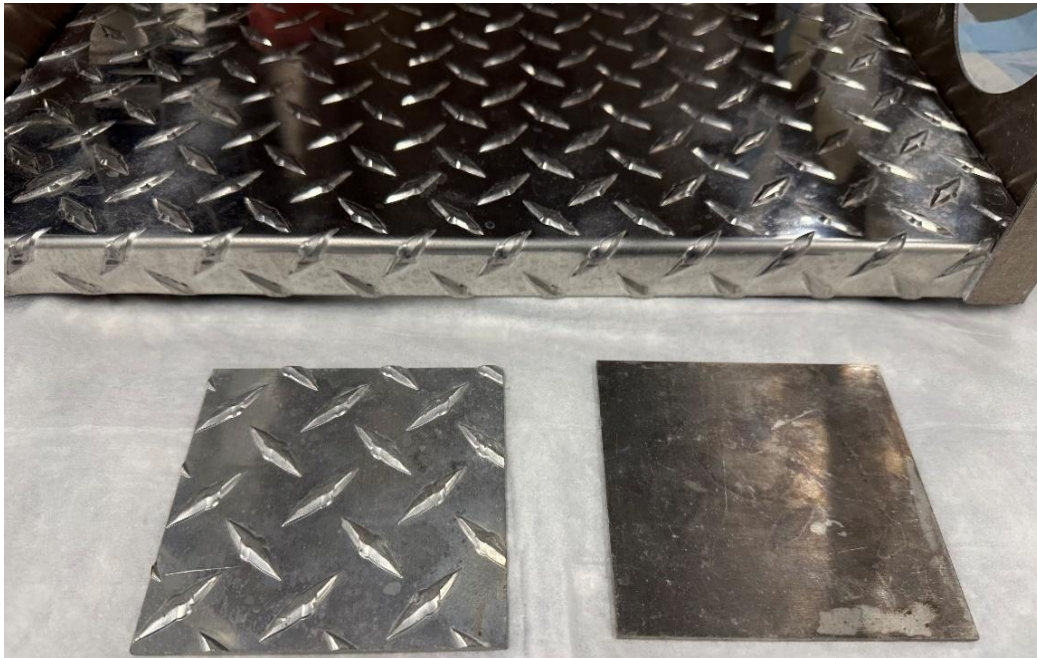


Figure 5. Lab trailer model, made of aluminum. L1 and L2 coupons clipped onto the wall. L3 coupon placed on the floor.

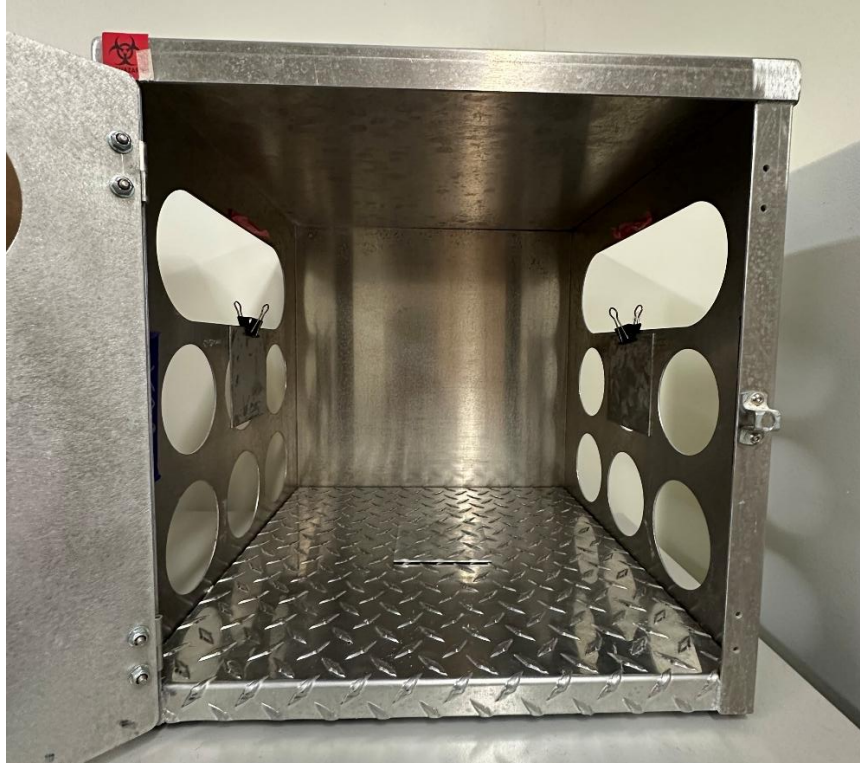


Figure 6. Experimental design overview.

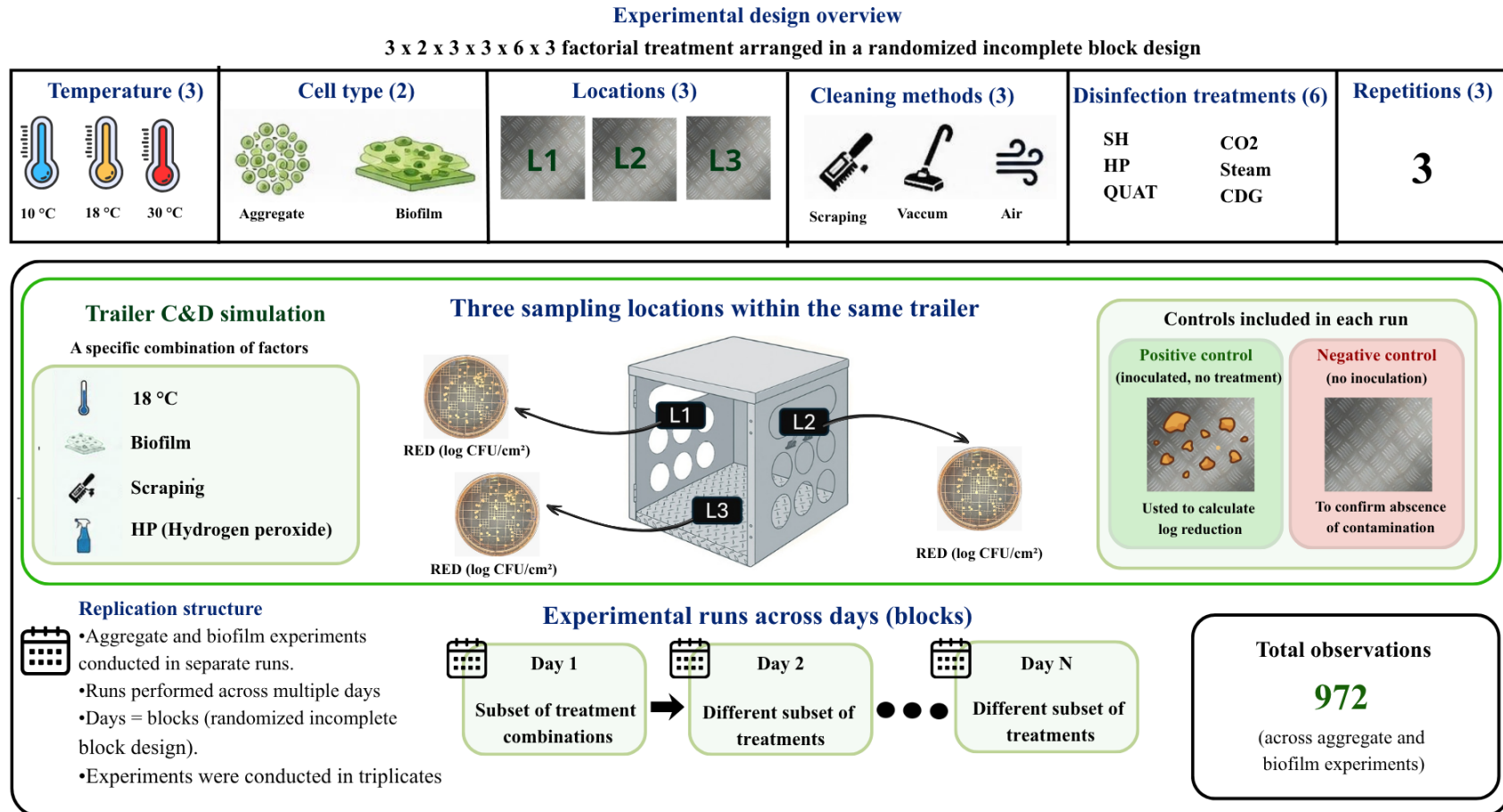
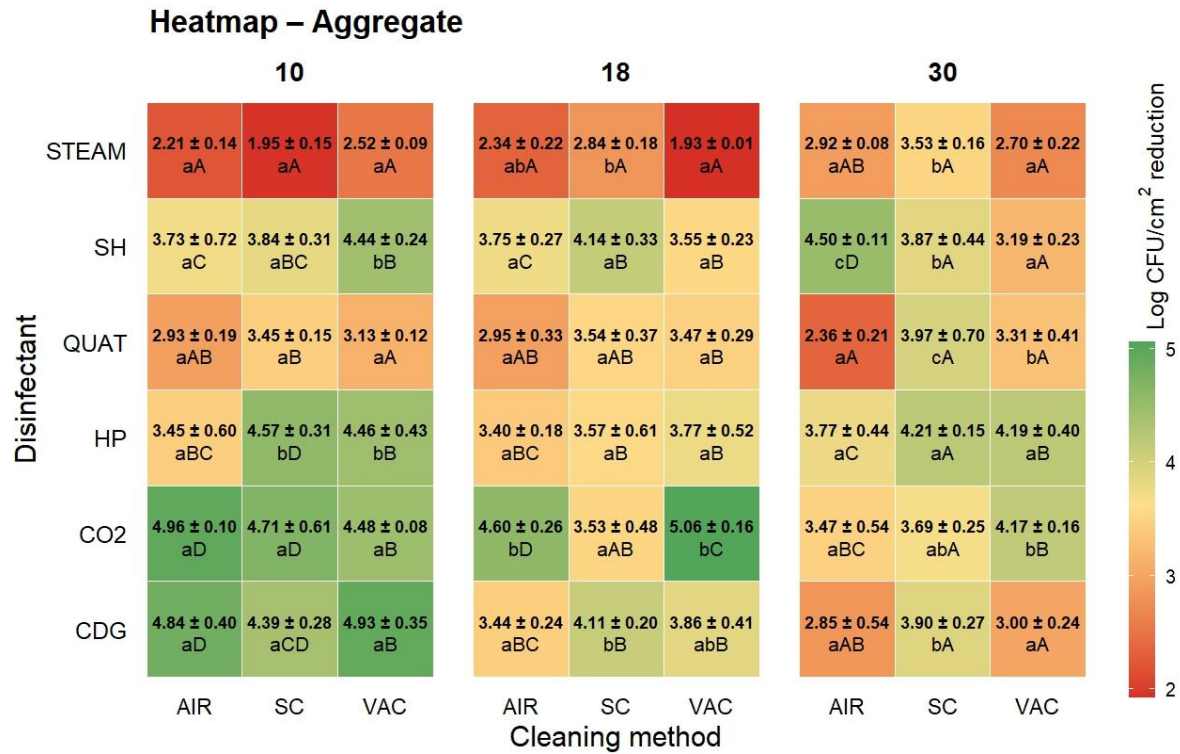
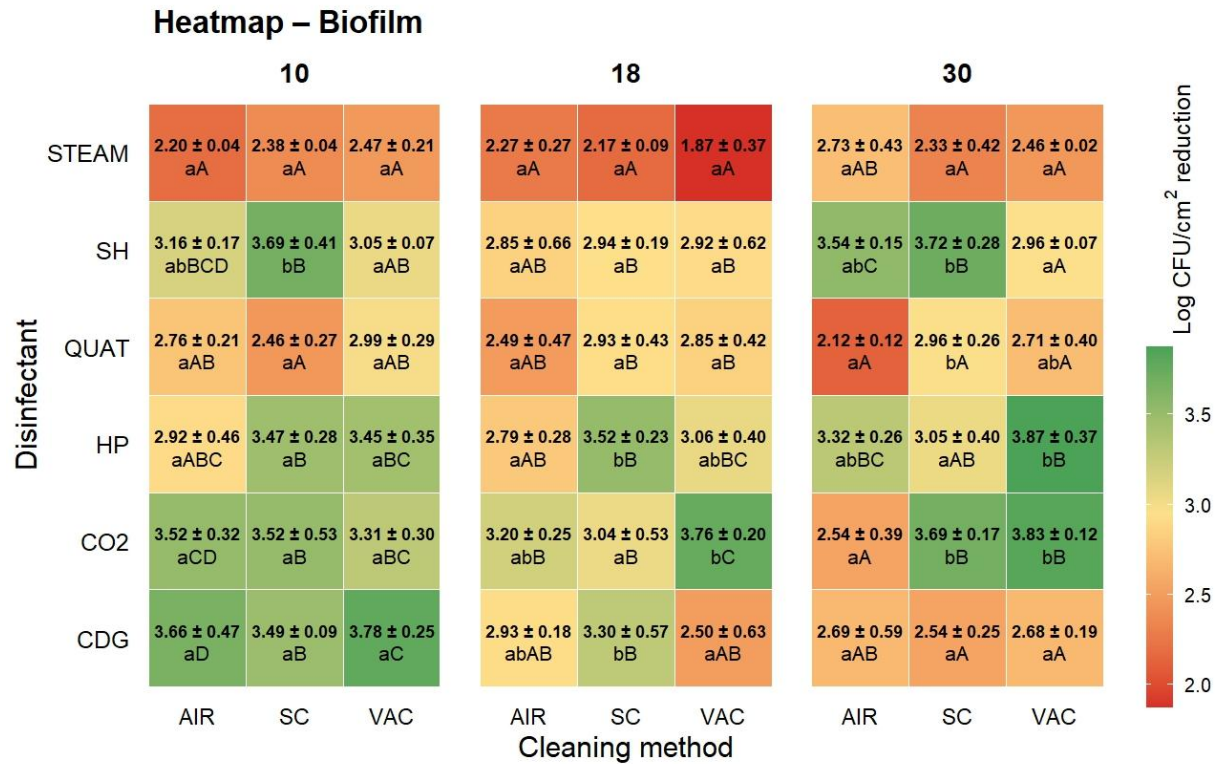


Figure 7. Heatmap showing log CFU/cm² reduction of aggregate *E. coli* cells across cleaning methods, disinfectants, and temperatures.



Note. Values represent least squares mean reduction values and standard deviations of the reductions calculated across the three sampling locations. Lower SD values indicate more consistent results across locations in the trailer. Cleaning methods: air, SC = scraping; VAC = vacuum. Disinfectants: CO₂ = carbon dioxide; CDG = chlorine dioxide gas; HP = hydrogen peroxide; QUAT = quaternary ammonium; SH = sodium hypochlorite; STEAM = steam. Lowercase letters indicate differences between cleaning methods within the same temperature, while uppercase letters indicate significant differences between disinfectants within the same cleaning method ($P < 0.05$). Experiments were conducted in triplicate.

Figure 8. Heatmap showing log CFU/cm² reduction of *E. coli* biofilms across cleaning methods, disinfectants, and temperatures



Note. Values represent least squares mean reduction values and standard deviations of the reductions calculated across the three sampling locations. Lower SD values indicate more consistent results across locations in the trailer. Cleaning methods: air, SC = scraping; VAC = vacuum. Disinfectants: CO₂ = carbon dioxide; CDG = chlorine dioxide gas; HP = hydrogen peroxide; QUAT = quaternary ammonium; SH = sodium hypochlorite; STEAM = steam. Lowercase letters indicate differences between cleaning methods within the same temperature, while uppercase letters indicate significant differences between disinfectants within the same cleaning method ($P < 0.05$). Experiments were conducted in triplicate.

Table 2. ANOVA of fixed effects for log reduction of *E. coli*

Effect	P-value
Temperature × disinfection	<0.0001
Temperature × location	0.28954
Temperature × cleaning	0.14030
Location × cleaning	0.96205
Location × disinfection	0.20389
Cleaning × disinfection	<0.0001
Temperature × location × cleaning	0.57508
Temperature × location × disinfection	0.08095
Temperature × cleaning × disinfection	<0.0001
Location × cleaning × disinfection	0.76511
Location × cleaning × disinfection × temperature	0.77670

Note. P-values were obtained from the analysis of variance. Effects were considered statistically significant when $P < 0.05$.

References

- Alarcón, L. V., Allepuz, A., & Mateu, E. (2021). Biosecurity in pig farms: A review. *Porcine Health Management*, 7(1), 5. <https://doi.org/10.1186/s40813-020-00181-z>
- Amaeze, N. J., Akinbobola, A. B., Kean, R., Ramage, G., Williams, C., & Mackay, W. (2024). Transfer of micro-organisms from dry surface biofilms and the influence of long survival under conditions of poor nutrition and moisture on the virulence of *Staphylococcus aureus*. *Journal of Hospital Infection*, 150, 34–39. <https://doi.org/10.1016/j.jhin.2024.03.023>
- Arthur, M., Afari, E. L., Alexa, E., Zhu, M., Gaffney, M. T., Celayeta, J. M. F., & Burgess, C. M. (2025). Recent advances in examining the factors influencing the efficacy of biocides against *Listeria monocytogenes* biofilms in the food industry: A systematic review. *Comprehensive Reviews in Food Science and Food Safety*, 24(1), e70083. <https://doi.org/10.1111/1541-4337.70083>
- Bancroft, W. D., & Richter, G. H. (1931). The Chemistry of Disinfection. *The Journal of Physical Chemistry*, 35(2), 511–530. <https://doi.org/10.1021/j150320a009>
- Barros, M. M., Castro, J., Araújo, D., Campos, A. M., Oliveira, R., Silva, S., Outor-Monteiro, D., & Almeida, C. (2023). Swine Colibacillosis: Global Epidemiologic and Antimicrobial Scenario. *Antibiotics*, 12(4), 682. <https://doi.org/10.3390/antibiotics12040682>
- Beloin, C., Roux, A., & Ghigo, J.-M. (2008). *Escherichia coli* Biofilms. In T. Romeo (Ed.), *Bacterial Biofilms* (Vol. 322, pp. 249–289). Springer Berlin Heidelberg. https://doi.org/10.1007/978-3-540-75418-3_12

Beltrame, C. A., Kubiak, G. B., Lerin, L. A., Rottava, I., Mossi, A. J., Oliveira, D. D., Cansian, R. L., Treichel, H., & Toniazzo, G. (2012). Influence of different sanitizers on food contaminant bacteria: Effect of exposure temperature, contact time, and product concentration. *Food Science and Technology*, 32(2), 228–232. <https://doi.org/10.1590/S0101-20612012005000046>

Bhana, N., Zanwar, A. S., Trivedi, V., & Jain, D. (2013). A Review: Steam Sterilization a Method of Sterilization. *Journal of Biological & Scientific Opinion*, 1(2), 138–141. <https://doi.org/10.7897/2321-6328.01222>

Blanco-Lizarazo, C. M., & Sierra-Cadavid, A. (2023). Prevalence of *Escherichia coli* generic and pathogenic in pork meat: Systematic review and meta-analysis. *World Journal of Microbiology and Biotechnology*, 39(9), 247. <https://doi.org/10.1007/s11274-023-03687-0>

Böhm, R. (1998). Disinfection and hygiene in the veterinary field and disinfection of animal houses and transport vehicles. *International Biodeterioration & Biodegradation*, 41(3–4), 217–224. [https://doi.org/10.1016/S0964-8305\(98\)00030-4](https://doi.org/10.1016/S0964-8305(98)00030-4)

Boyce, J. M. (2018). Alcohols as Surface Disinfectants in Healthcare Settings. *Infection Control & Hospital Epidemiology*, 39(3), 323–328. <https://doi.org/10.1017/ice.2017.301>

Boyce, J. M. (2023). Quaternary ammonium disinfectants and antiseptics: Tolerance, resistance and potential impact on antibiotic resistance. *Antimicrobial Resistance & Infection Control*, 12(1), 32. <https://doi.org/10.1186/s13756-023-01241-z>

Bridier, A., Briandet, R., Thomas, V., & Dubois-Brissonnet, F. (2011). Resistance of bacterial biofilms to disinfectants: A review. *Biofouling*, 27(9), 1017–1032. <https://doi.org/10.1080/08927014.2011.626899>

- CDC. (2023). *Factors Affecting the Efficacy of Disinfection and Sterilization*.
<https://www.cdc.gov/infection-control/hcp/disinfection-sterilization/efficacy-factors.html>
- CDC. (2025, July). *About One Health*. About One Health. <https://www.cdc.gov/one-health/about/index.html>
- Cheng, H., Chen, J., Yu, H., Sun, B., Zhou, J., & Wu, G. (2025). Environmental factors and antimicrobial efficacy: The impact of temperature and humidity on material surfaces. *Microbiology Spectrum*, 13(11), e00960-25. <https://doi.org/10.1128/spectrum.00960-25>
- Chowdhury, D., Rahman, A., Hu, H., Jensen, S. O., Deva, A. K., & Vickery, K. (2019). Effect of disinfectant formulation and organic soil on the efficacy of oxidizing disinfectants against biofilms. *Journal of Hospital Infection*, 103(1), e33–e41.
<https://doi.org/10.1016/j.jhin.2018.10.019>
- Ciuderis-Aponte, K., Diaz, A., Muskus, C., Peña, M., Hernández-Ortiz, J., & Osorio, J. (2022). Farm management practices, biosecurity and influenza a virus detection in swine farms: A comprehensive study in Colombia. *Porcine Health Management*, 8(1), 42.
<https://doi.org/10.1186/s40813-022-00287-6>
- Clayton, G. E., Thorn, R. M. S., & Reynolds, D. M. (2021). The efficacy of chlorine-based disinfectants against planktonic and biofilm bacteria for decentralised point-of-use drinking water. *Npj Clean Water*, 4(1), 48. <https://doi.org/10.1038/s41545-021-00139-w>
- Codex Alimentarius. (2023). *General principles of food hygiene* (pp. 6–20). FAO; WHO;
<https://doi.org/10.4060/cc6125en>

Cook, H., & Lee, S. (2023). *The United States Pork Industry 2023*. <https://nppc.org/wp-content/uploads/2024/06/U.S.-Economic-Contribution-Report-6.28.24.pdf>

Corcoran, M., Morris, D., De Lappe, N., O'Connor, J., Lalor, P., Dockery, P., & Cormican, M. (2014). Commonly Used Disinfectants Fail To Eradicate *Salmonella enterica* Biofilms from Food Contact Surface Materials. *Applied and Environmental Microbiology*, 80(4), 1507–1514. <https://doi.org/10.1128/AEM.03109-13>

Dee, S. A., Niederwerder, M. C., Patterson, G., Cochrane, R., Jones, C., Diel, D., Brockhoff, E., Nelson, E., Spronk, G., & Sundberg, P. (2020). The risk of viral transmission in feed: What do we know, what do we do? *Transboundary and Emerging Diseases*, 67(6), 2365–2371. <https://doi.org/10.1111/tbed.13606>

Dhiman, S., Kumar, A., Kaur, G., Mukherjee, G., Rustagi, S., Shreaz, S., Negi, R., & Yadav, A. N. (2024). Bacterial biofilms: Pathogenesis, monitoring, treatment approaches and associated challenges. *Biologia*, 79(10), 3161–3181. <https://doi.org/10.1007/s11756-024-01767-6>

Fagerlund, A., Møretrø, T., Heir, E., Briandet, R., & Langsrud, S. (2017). Cleaning and Disinfection of Biofilms Composed of *Listeria monocytogenes* and Background Microbiota from Meat Processing Surfaces. *Applied and Environmental Microbiology*, 83(17), e01046-17. <https://doi.org/10.1128/AEM.01046-17>

Fedorova, L. S., & Ilyakova, A. V. (2023). Comparative evaluation of disinfectant efficacy against biofilm-residing microorganisms. *Journal of Microbiology, Epidemiology and Immunobiology*, 100(5), 302–309. <https://doi.org/10.36233/0372-9311-422>

Flemming, H.-C., Wingender, J., Szewzyk, U., Steinberg, P., Rice, S. A., & Kjelleberg, S. (2016). Biofilms: An emergent form of bacterial life. *Nature Reviews Microbiology*, 14(9), 563–575. <https://doi.org/10.1038/nrmicro.2016.94>

Fratamico, P. M., DebRoy, C., Liu, Y., Needleman, D. S., Baranzoni, G. M., & Feng, P. (2016). Advances in Molecular Serotyping and Subtyping of *Escherichia coli*. *Frontiers in Microbiology*, 7. <https://doi.org/10.3389/fmicb.2016.00644>

Galié, S., García-Gutiérrez, C., Miguélez, E. M., Villar, C. J., & Lombó, F. (2018). Biofilms in the Food Industry: Health Aspects and Control Methods. *Frontiers in Microbiology*, 9, 898. <https://doi.org/10.3389/fmicb.2018.00898>

Galvis, J. A., Corzo, C. A., & Machado, G. (2022). Modelling and assessing additional transmission routes for porcine reproductive and respiratory syndrome virus: Vehicle movements and feed ingredients. *Transboundary and Emerging Diseases*, 69(5). <https://doi.org/10.1111/tbed.14488>

Gómez-López, V. M. (Ed.). (2012). *Decontamination of Fresh and Minimally Processed Produce* (1st ed.). Wiley. <https://doi.org/10.1002/9781118229187>

Gómez-López, V. M., Rajkovic, A., Ragaert, P., Smigic, N., & Devlieghere, F. (2009). Chlorine dioxide for minimally processed produce preservation: A review. *Trends in Food Science & Technology*, 20(1), 17–26. <https://doi.org/10.1016/j.tifs.2008.09.005>

HIPRA. (2025). *Transmission within herds*. <https://www.hipra.com/en/animal-health/knowledge/1-transmission-within-herds>

- Kenters, N., Huijskens, E. G. W., De Wit, S. C. J., Van Rosmalen, J., & Voss, A. (2017). Effectiveness of cleaning-disinfection wipes and sprays against multidrug-resistant outbreak strains. *American Journal of Infection Control*, 45(8), e69–e73. <https://doi.org/10.1016/j.ajic.2017.04.290>
- Koti, K., Rodas-Gonzalez, A., Nadon, C., McAllister, T., Yang, X., & Narváez-Bravo, C. (2024). Evaluating disinfectant efficacy on mixed biofilms comprising Shiga toxigenic *Escherichia coli*, lactic acid bacteria, and spoilage microorganisms. *Frontiers in Microbiology*, 15, 1360645. <https://doi.org/10.3389/fmicb.2024.1360645>
- Lim, S.-M., Lim, E. S., Kim, J.-S., Paik, H.-D., & Koo, O. K. (2020). Survival of foodborne pathogens on stainless steel soiled with different food residues. *Food Science and Biotechnology*, 29(5), 729–737. <https://doi.org/10.1007/s10068-019-00705-6>
- Liu, H. Y., Prentice, E. L., & Webber, M. A. (2024). Mechanisms of antimicrobial resistance in biofilms. *Npj Antimicrobials and Resistance*, 2(1), 27. <https://doi.org/10.1038/s44259-024-00046-3>
- Lowe, J., Gauger, P., Harmon, K., Zhang, J., Connor, J., Yeske, P., Loula, T., Levis, I., Dufresne, L., & Main, R. (2014). *Role of Transportation in Spread of Porcine Epidemic Diarrhea Virus Infection, United States—Volume 20, Number 5—May 2014—Emerging Infectious Diseases journal—CDC*. <https://doi.org/10.3201/eid2005.131628>
- Luppi, A. (2017). Swine enteric colibacillosis: Diagnosis, therapy and antimicrobial resistance. *Porcine Health Management*, 3(1), 16. <https://doi.org/10.1186/s40813-017-0063-4>
- Maillard, J.-Y. (2018). Resistance of Bacteria to Biocides. *Microbiology Spectrum*, 6(2), 6.2.19. <https://doi.org/10.1128/microbiolspec.ARBA-0006-2017>

Mannion, C., Egan, J., Lynch, B. P., Fanning, S., & Leonard, N. (2008). An Investigation into the Efficacy of Washing Trucks Following the Transportation of Pigs—A *Salmonella* Perspective. *Foodborne Pathogens and Disease*, 5(3), 261–271.

<https://doi.org/10.1089/fpd.2007.0069>

Martelli, F., Lambert, M., Butt, P., Cheney, T., Tatone, F. A., Callaby, R., Rabie, A., Gosling, R. J., Fordon, S., Crocker, G., Davies, R. H., & Smith, R. P. (2017). Evaluation of an enhanced cleaning and disinfection protocol in *Salmonella* contaminated pig holdings in the United Kingdom. *PLOS ONE*, 12(6), e0178897. <https://doi.org/10.1371/journal.pone.0178897>

Martinez, N., Corzo, C. A., Machado, G., Ekiri, A. B., Deza-Cruz, I., & Prada, J. M. (2025). Truck cleaning and disinfection, and the risk of PRRSV dissemination in multi-site pig production systems in the United States: A network-epidemiological model approach. *Preventive Veterinary Medicine*, 240, 106539. <https://doi.org/10.1016/j.prevetmed.2025.106539>

Masserdotti, M., Formenti, N., Donneschi, A., Guarneri, F., Scali, F., Romeo, C., Giacomini, E., Bertasio, C., Boniotti, M. B., Alborali, G. L., & Luzzago, C. (2024). *The role of short journey transportation in the spreading of swine pathogens and antimicrobial-resistant bacteria*. In Review. <https://doi.org/10.21203/rs.3.rs-4251132/v1>

McDonnell, G., & Russell, A. D. (1999). Antiseptics and Disinfectants: Activity, Action, and Resistance. *Clinical Microbiology Reviews*, 12(1), 147–179.

<https://doi.org/10.1128/CMR.12.1.147>

McKenzie-Reynolds, P., Millner, P., Hashem, F., Marsh, L., Smith, B., Kenney, A., Allen, A., Parveen, S., & Collick, A. S. (2025). Survival and transfer of *Escherichia coli* to fresh

produce from organically managed soils amended with poultry litter. *Frontiers in Sustainable Food Systems*, 9, 1502841. <https://doi.org/10.3389/fsufs.2025.1502841>

McLaren, I., Wales, A., Breslin, M., & Davies, R. (2011). Evaluation of commonly-used farm disinfectants in wet and dry models of *Salmonella* farm contamination. *Avian Pathology*, 40(1), 33–42. <https://doi.org/10.1080/03079457.2010.537303>

Nett, J. E., Guite, K. M., Ringeisen, A., Holoyda, K. A., & Andes, D. R. (2008). Reduced Biocide Susceptibility in *Candida albicans* Biofilms. *Antimicrobial Agents and Chemotherapy*, 52(9), 3411–3413. <https://doi.org/10.1128/AAC.01656-07>

Park & Balasubramaniam (2025). Infrared Thermal Image Processing Technique for Evaluating Superheated Steam as a Dry Sanitation Method. *Food and Bioprocess Technology*, 18(2), 1417–1430. <https://doi.org/10.1007/s11947-024-03529-3>

Park, & Kang (2018). Effect of temperature on chlorine dioxide inactivation of *Escherichia coli* O157:H7, *Salmonella typhimurium*, and *Listeria monocytogenes* on spinach, tomatoes, stainless steel, and glass surfaces. *International Journal of Food Microbiology*, 275, 39–45. <https://doi.org/10.1016/j.ijfoodmicro.2018.03.015>

Parker, T. B., Meiklejohn, K. A., Machado, G., Rahe, M., Darrow, B. S., & Ferreira, J. B. (2025). Evaluation of porcine epidemic diarrhea virus RNA contamination on swine industry transportation vehicles. *Preventive Veterinary Medicine*, 237, 106447. <https://doi.org/10.1016/j.prevetmed.2025.106447>

Perrucci, A., Magri, A., Cardana, V., Zoppi, S., Cossettini, C., & Scollo, A. (2026). Hygiene procedures of trucks transporting live pigs: Multi-assessment validation of a

standardized C&D protocol. *Frontiers in Veterinary Science*, 13, 1789484.

<https://doi.org/10.3389/fvets.2026.1789484>

Rajkowski, K. T., Eblen, S., & Laubauch, C. (1998). Efficacy of Washing and Sanitizing Trailers Used for Swine Transport in Reduction of *Salmonella* and *Escherichia coli*. *Journal of Food Protection*, 61(1), 31–35. <https://doi.org/10.4315/0362-028X-61.1.31>

Rana, Y. S., Chen, L., Balasubramaniam, V. M., & Snyder, A. B. (2022). Superheated steam effectively inactivates diverse microbial targets despite mediating effects from food matrices in bench-scale assessments. *International Journal of Food Microbiology*, 378, 109838. <https://doi.org/10.1016/j.ijfoodmicro.2022.109838>

Raval, Y. S., Flurin, L., Mohamed, A., Greenwood-Quaintance, K. E., Beyenal, H., & Patel, R. (2021). *In Vitro* Antibacterial Activity of Hydrogen Peroxide and Hypochlorous Acid, Including That Generated by Electrochemical Scaffolds. *Antimicrobial Agents and Chemotherapy*, 65(5), e01966-20. <https://doi.org/10.1128/AAC.01966-20>

Rutala, W. A., & Weber, D. J. (2014). Selection of the Ideal Disinfectant. *Infection Control & Hospital Epidemiology*, 35(7), 855–865. <https://doi.org/10.1086/676877>

Saverina, E. A., Frolov, N. A., Kamanina, O. A., Arlyapov, V. A., Vereshchagin, A. N., & Ananikov, V. P. (2023). From Antibacterial to Antibiofilm Targeting: An Emerging Paradigm Shift in the Development of Quaternary Ammonium Compounds (QACs). *ACS Infectious Diseases*, 9(3), 394–422. <https://doi.org/10.1021/acsinfecdis.2c00469>

Shi, Y., Wen, T., Zhao, F., & Hu, J. (2024). Bacteriostasis of nisin against planktonic and biofilm bacteria: Its mechanism and application. *Journal of Food Science*, 89(4), 1894–1916. <https://doi.org/10.1111/1750-3841.17001>

Smith, K., & Hunter, I. S. (2008). Efficacy of common hospital biocides with biofilms of multi-drug resistant clinical isolates. *Journal of Medical Microbiology*, 57(8), 966–973.

<https://doi.org/10.1099/jmm.0.47668-0>

Srey, S., Jahid, I. K., & Ha, S.-D. (2013). Biofilm formation in food industries: A food safety concern. *Food Control*, 31(2), 572–585. <https://doi.org/10.1016/j.foodcont.2012.12.001>

Tarafa, P. J., Jiménez, A., Zhang, J., & Matthews, M. A. (2010). Compressed carbon dioxide (CO₂) for decontamination of biomaterials and tissue scaffolds. *The Journal of Supercritical Fluids*, 53(1–3), 192–199. <https://doi.org/10.1016/j.supflu.2010.02.006>

Trinetta, V., Vaidya, N., Linton, R., & Morgan, M. (2011). Evaluation of Chlorine Dioxide Gas Residues on Selected Food Produce. *Journal of Food Science*, 76(1).

<https://doi.org/10.1111/j.1750-3841.2010.01911.x>

Urbaniec, J. W., Davies, P. L., & Neary, J. M. (2025). Disinfection reduces but does not eliminate drug resistant *Escherichia coli* from livestock trailers following transport of calves.

Journal of Dairy Research, 1–6. <https://doi.org/10.1017/S0022029925101581>

USDA. (2018). *Standard operating procedures: cleaning and disinfection*.

https://www.aphis.usda.gov/sites/default/files/sop_cd.pdf

Van Houdt, R., & Michiels, C. W. (2010). Biofilm formation and the food industry, a focus on the bacterial outer surface. *Journal of Applied Microbiology*, 109(4), 1117–1131.

<https://doi.org/10.1111/j.1365-2672.2010.04756.x>

Virto, R., Mañas, P., Álvarez, I., Condon, S., & Raso, J. (2005). Membrane Damage and Microbial Inactivation by Chlorine in the Absence and Presence of a Chlorine-Demanding

Substrate. *Applied and Environmental Microbiology*, 71(9), 5022–5028.

<https://doi.org/10.1128/AEM.71.9.5022-5028.2005>

Yang, Q., Martínez-López, B., Moon, S. A., Gomez-Vazquez, J. P., & Scoglio, C. (2025).

Animal movement estimation and network-based epidemic modeling: Illustration for the swine industry in Iowa (US). *PLOS One*, 20(6), e0326234.

<https://doi.org/10.1371/journal.pone.0326234>

Chapter 4 - Evaluating hydrogen peroxide disinfection in live haul trailers under field conditions

Abstract

Live haul trailers can retain organic material, moisture, and microorganisms after animal transport, which may limit the performance of cleaning and disinfection. Although hydrogen peroxide has shown antimicrobial activity under laboratory conditions, its field performance in naturally contaminated trailers may be affected by surface characteristics. The objective of this study was to evaluate 3 % hydrogen peroxide applied for 30 s after pressure washing in a naturally contaminated swine trailer using aerobic counts as an indicator of overall hygiene. Field evaluations were conducted on three separate days at ambient temperatures of 3, 18, and 26 °C. Samples were collected from the front, middle, and back sections of the trailer and from floor and wall surfaces before cleaning, after power washing, and after hydrogen peroxide application. Aerobic counts were affected by intervention step ($P < 0.001$), surface type ($P < 0.001$), and their interaction ($P = 0.004$). Hydrogen peroxide produced significant additional reductions of 1.36 to 1.80 log CFU/cm² across trailer sections and 1.13 to 1.95 log CFU/cm² across surface types after pressure washing. Total reductions ranged from 1.66 to 2.52 log CFU/cm² across trailer sections and 1.76 to 2.27 log CFU/cm² across surface types. Aerobic counts did not differ among trailer sections at any intervention step. However, counts were higher on the floor surface than on the wall surface initially and after hydrogen peroxide application. Overall, hydrogen peroxide contributed to aerobic count reduction when applied after pressure washing.

4.1 Introduction

The transportation of live animals is an essential component of meat production (Miranda et al., 2014). However, it also represents an important point for the introduction and spread of

microorganisms throughout the production chain (Parker et al., 2025). During transport, animals are exposed to environments where fecal material, moisture, and microorganisms accumulate, creating conditions that favor microbial survival and transfer between animal groups (Barham et al., 2002; Berry & Wells, 2016). Transportation-related stress has also been associated with increased pathogen shedding and greater susceptibility to disease in animals (Rostagno, 2009). In some cases, animals may shed high concentrations of pathogens through fecal material, further increasing the microbial load within transport environments (Robinson et al., 2004). As a result, live haul trailers can become contaminated environments that may contribute to cross-contamination between production cycles if not properly C&D.

Microorganisms can survive for extended periods on surfaces commonly found in agricultural environments, particularly under conditions involving fluctuating temperatures and high organic loads (Bonardi et al., 2015; Dos Santos et al., 2023). In live haul trailers, these conditions are common after animal transport because fecal material, moisture, and organic residues can remain on floors, walls, and joints even after visible debris is removed (Urbaniec et al., 2025). In these environments, microorganisms may persist as individual cells, aggregates, or biofilms (Flemming et al., 2016). Biofilms are especially difficult to control because cells are attached to surfaces and embedded within an extracellular matrix that can reduce the penetration and activity of disinfectants (Simões et al., 2010). Moreover, organic material left after transport can also protect microorganisms and interfere with disinfection (Bridier et al., 2011). Together, these factors can reduce disinfection effectiveness and make field performance more variable than what is typically observed under controlled laboratory conditions.

In commercial settings, power washing (PW) is one of the most commonly used methods for cleaning live haul trailers due to its practicality, speed, and ability to remove visible

contamination (USDA APHIS, 2025). Disinfection, however, can be less consistently applied because it adds a step that requires product preparation, adequate application, and contact time before the trailer can be returned to service (Parker et al., 2025). In addition, disinfectant applications may vary under commercial conditions depending on product mixing and the amount of residual water left on trailer surfaces after PW. When disinfectants are applied to wet surfaces, residual moisture may dilute the active concentration and reduce the effectiveness of the product, particularly on porous surfaces (USDA APHIS, 2025). Although allowing surfaces to drain or dry before disinfection would be preferable, this step is often limited by time constraints in routine trailer cleaning. Still, visible cleanliness does not necessarily indicate microbial reduction. Studies evaluating trailer C&D practices have shown substantial variability in procedures across the livestock industry (Galvis et al., 2026; Magalhaes et al., 2026; Parker et al., 2025). Therefore, trailers that appear clean may still retain microbial contamination, highlighting the need to evaluate disinfection performance under practical operating conditions. Aerobic counts are commonly used as a general indicator of surface hygiene. It provides an estimate of the overall viable bacterial population present in an environment. This approach has been used in previous studies to evaluate the effectiveness of C&D in animal production facilities and was therefore selected in the present study to evaluate C&D performance (Luyckx et al., 2015; Makovska et al., 2025).

Before being approved for commercial use, disinfectants are evaluated under standardized laboratory protocols that require substantial microbial reductions, often including a 5-log reduction of selected reference microorganisms (EPA, 2025). However, reductions observed under controlled laboratory settings are not always achieved in the field, even when products are applied according to manufacturer guidelines. For example, Luyckx et al. (2015)

showed that C&D under commercial conditions can result in variable microbial reductions, reflecting the difficulty of achieving consistent performance outside controlled laboratory settings. In this study, field evaluation refers to the assessment of hydrogen peroxide disinfection under naturally contaminated trailers to determine how its performance differs from previous laboratory findings. This distinction is important because field conditions introduce factors that are difficult to reproduce in the laboratory, including organic matter, biofilm formation, surface variability, temperature changes, and differences in C&D application. These factors can influence both microbial survival and the effectiveness of disinfection (Arthur et al., 2025; Simões et al., 2010).

Hydrogen peroxide (HP) has been widely used as a disinfectant because of its broad antimicrobial activity and its ability to oxidize cellular components (Abdelshafy et al., 2024; Tao et al., 2024). In previous work, HP showed promising reductions against bacterial biofilms and aggregate cells under different temperature conditions. Although that study was designed to reproduce some of the challenges expected under field conditions, laboratory experiments cannot fully capture the variability present in live animal transport environments (Makovska et al., 2025; Wales et al., 2021). Therefore, field evaluation is needed to determine how HP performs under practical conditions, where contamination is naturally occurring, and environmental and operational factors may influence disinfection performance. Despite the use of disinfectants in live animal transport systems, limited information is available regarding their validation under commercial field conditions. It was hypothesized that HP disinfection would provide an additional reduction in aerobic counts after PW, but that reductions under field conditions would be lower and more variable than those observed in the laboratory. Therefore, the objective of this study was to evaluate the field performance of HP disinfection in a live haul trailer under

different ambient temperatures using aerobic counts as an indicator of overall hygiene and discuss field reductions in relation to previous laboratory findings.

4.2 Materials and methods

The trailer used for this experiment was a Cimarron (Oklahoma, USA) livestock trailer, approximately 4 years old, with a tandem axle and slat-side ventilation design constructed of aluminum. The trailer belongs to the Kansas State University Department of Animal Science and Industry and is used every 3 weeks to transport approximately 12-15 pigs per load, with pigs averaging 2 years of age. Under routine conditions, the trailer is cleaned in the afternoon after pigs are unloaded, using pressurized water at ambient temperature only. The trailer measured 5.49 m in length, 2.13 m in width, and 2.13 m in height (Figure 9). The interior walls were constructed of smooth aluminum panels reinforced with vertical aluminum supports. Horizontal support bars were located along the midline of the walls. The trailer floor consisted of a porous rubber material (Figure 10). Although the construction materials were similar to those used in commercial livestock trailers in the United States, the trailer evaluated in this study was smaller. A typical commercial pig transport trailer measures approximately 14.7 m in length, 2.6 m in width, and 4.11 m in height, and can transport 170-175 market-weight pigs at full capacity (Sutherland et al., 2009; Xiong et al., 2018).

4.2.1 Cleaning and disinfection

C&D were performed at the Kansas State University Farm Shop (2900 College Avenue, Manhattan, KS 66506). The experiments were conducted on three different days at temperatures of 3, 18, and 26 °C. Each experiment began at 2:30 pm, and the ambient temperature at that time was recorded from a local weather station (Weather Underground, USA). For the cleaning step, PW was performed using a gas-powered pressure washer (RYOBI, 3000 PSI, China). Cleaning

was carried out by facility personnel who routinely perform trailer cleaning as part of their normal job responsibilities. Operators were instructed to clean from the back to the front of the trailer until no visible fecal material remained (Figures 11 & 12). The trailer was positioned on a slight incline to allow water and organic residues to drain toward the front. The cleaning process took approximately 45 minutes on average. Immediately after PW, without a drying period, disinfection (DIS) was performed using 3% hydrogen peroxide (HP; Aqua Solutions, Deer Park, TX) with a contact time of 30 s. This contact time was selected to reflect a practical field scenario, where trailer turnaround time is limited and prolonged disinfectant contact periods may not be feasible under routine commercial challenges. The disinfectant was applied using a handheld sprayer (Chapin 2000, China) at a reported flow rate of 1.51 L/min. Based on this flow rate, approximately 0.76 L of disinfectant was delivered during the 30-s application period. The nozzle was held 30 cm from the surface, resulting in an estimated spray dispersal area of 40 × 40 cm. The sprayer was repositioned every 2 s to promote uniform coverage across both wall and floor surfaces, and the ceiling was not treated. The estimated operating pressure ranged from 40 to 60 PSI. The disinfectant was applied by the same individual across all experimental runs.

4.2.2 Treatment and sampling areas

To better characterize contamination within the trailer, it was divided into three sections: front, middle, and back. This approach allowed microbial levels to be evaluated across different areas of the trailer, since fecal material accumulation, drainage, and animal movement during transport may vary by location. The front section was defined as the area where pigs are loaded and unloaded. Within each section, two sampling surfaces were selected: floor (L1 and L3) and wall (L2). The sampling area for each location was 40 × 40 cm. Samples were collected as controls prior to C&D, representing the condition in which pigs left the trailer. Additional

samples were collected after PW and after DIS. Neutralizing sponges (Neogen, Lansing, MI) containing 20 mL of neutralizing solution were used for sample collection. After sampling, sponges were processed individually in a stomacher (400 Lab Blender, West Sussex, United Kingdom) for 30 s. Following homogenization, serial dilutions were prepared in 0.1% peptone water (Bacto, Sparks, MD) and plated onto aerobic count Petrifilm (Neogen, Lansing, MI). Petrifilm plates were incubated at 35 °C for 48 h. Aerobic count (AC) colonies were enumerated using an automated colony counter (Interscience, Woburn, MA), and results were reported as log CFU/cm². The LOD was 20 CFU/cm², and the LOQ was 300 CFU/cm². All observed values were above these limits.

4.2.3 Experimental design and statistical analysis

The field evaluation was conducted on three experimental days using the same pig transport trailer. The ambient temperatures recorded during the trials were 3, 18 and 26 °C. Each trial corresponded to one ambient-temperature condition and one pig load. Samples were collected from three trailer sections (front, middle and back), two surface type (floor and wall) and three intervention stages: before cleaning (Control), after PW, and after HP disinfection.

A total of 81 samples were collected, with 27 samples collected per trailer. For each trailer section and intervention step, two floor samples and one wall sample were collected. The floor samples were collected in duplicate from the same 40 × 40 cm area to better represent the porous floor surface and averaged for the analysis. Wall samples were collected from a corresponding vertical surface. There were 54 observations for analysis, because the two floor samples were averaged.

Aerobic counts were expressed as log CFU/cm² and analyzed using a linear mixed-effect model. Experimental day, trailer section, surface type, intervention, and the interaction between

surface type and intervention stage were included as fixed effects. The experimental day $\times$ intervention combination was included as the random effect. Estimated marginal means were calculated using the emmeans package. Reductions associated with PW, HP, and the complete C&D were estimated using contrasts between intervention stages. All statistical analyses were performed in R, and statistical significance was defined at $P < 0.05$.

4.3 Results

The analysis of variance showed significant effects of intervention step ($P < 0.001$), surface type ($P < 0.001$), and their interaction ($P = 0.004$) on aerobic counts. Trailer section was not significant ($P = 0.972$), and none of the interactions involving trailer section were significant. The intervention step and surface interaction indicates that changes in aerobic counts across intervention steps depend on the type of surface.

4.3.1 Overall intervention steps

Overall estimated reductions in aerobic counts, averaged across trailer sections and surface types, are presented in Appendix B Table 5. Compared with initial counts, PW resulted in an estimated reduction of 0.48 log CFU/cm² (95% CI: -0.007 to 0.969); however, this reduction was not statistically significant. Following HP application, aerobic counts decreased by an additional 1.54 log CFU/cm² (95% CI: 1.047 to 2.024) relative to counts after PW. Together, PW and HP resulted in a total estimated reduction of 2.02 log CFU/cm² (95% CI: 1.528 to 2.505).

4.3.2 Trailer section and intervention step

Estimated aerobic counts for each trailer section and intervention step are presented in Table 3, and pairwise comparisons among trailer sections at each intervention step are presented in Appendix C Table 6. Aerobic counts did not differ among the front, middle and back sections at any intervention step ($P > 0.05$). Initial counts ranged from 3.74 to 4.18 log CFU/cm². The

estimated count in the front section was 0.45 log CFU/cm² lower than the back section, although the difference was not significant ($P = 0.078$). After PW, estimated counts were similar across sections, ranging from 3.43 to 3.46 log CFU/cm². After HP, estimated counts ranged from 1.67 to 2.07 log CFU/cm². The front section was 0.41 log CFU/cm² higher than the back section, but this difference was not statistically significant ($P = 0.116$). The interaction between section and intervention step was not significant ($P = 0.061$), indicating insufficient evidence that changes across intervention steps differed among trailer sections.

Within each trailer section, the contrasts were used to evaluate changes in aerobic counts across intervention steps. Estimated PW, HP, and total reductions by trailer section are presented in Appendix B Table 5. After PW, estimated counts were 0.30 and 0.42 log CFU/cm² lower than initial counts in the front and middle sections; however, neither reduction was statistically significant ($P = 0.440$ and $P = 0.220$). In the back section, the estimated count after PW was 0.72 log CFU/cm² lower than the initial counts ($P = 0.026$). After HP, estimated counts were significantly lower than initial counts in all three sections, with reductions of 1.67, 1.87 and 2.52 log CFU/cm² in the front, middle and back sections ($P < 0.001$). Compared with after PW, counts after HP were 1.36 log CFU/cm² lower in the front, 1.45 log CFU/cm² lower in the middle, and 1.80 log CFU/cm² lower in the back. These reductions were significant in all three sections.

4.3.3 Surface type and intervention step

The intervention step by surface type interaction was significant ($P = 0.004$), indicating that the difference between floor and wall surfaces changed across intervention steps. Pairwise comparisons between floor and wall surfaces at each intervention step are presented in Appendix C Table 6. Initially, estimated aerobic counts were higher on the floor than in the walls (4.13 vs. 3.73 log CFU/cm²), with an estimated difference of 0.40 log CFU/cm² ($P = 0.020$). After PW,

estimated counts were similar between the floor and wall (3.49 vs. 3.41 log CFU/cm²; P = 0.610). After HP, estimated aerobic counts were higher on the floor than on the wall (2.36 vs. 1.46 log CFU/cm²), with an estimated difference of 0.91 log CFU/cm² (P < 0.001).

Estimated PW, HP and total reductions by surface type are presented in Appendix B Table 5. On the floor surface, estimated aerobic counts decreased by 0.64 log CFU/cm² after PW compared with initial counts (P = 0.039). Counts after HP were 1.76 log CFU/cm² lower than initial counts (P < 0.001) and 1.13 log CFU/cm² lower than after PW (P = 0.002). On the wall, counts after PW were 0.33 log CFU/cm² lower than initial counts, but this difference was not significant (P = 0.321). Counts after HP were 2.27 log CFU/cm² lower than initial counts and 1.95 log CFU/cm² lower than after PW (both P < 0.001). The interaction between section and surface type was not significant (P = 0.193). The triple interaction between section, intervention step and surface type was also not significant (P = 0.300).

4.4 Discussion

In this field evaluation, PW followed by HP disinfection reduced aerobic counts by approximately 2 log CFU/cm². This corresponds to a 98-99% reduction in the initial microbial load. Aerobic counts were reduced but not eliminated. This difference between reduction and elimination is important because field conditions introduce challenges that are difficult to reproduce in the laboratory, including residual organic matter, surface moisture, surface material, drainage, and trailer geometry, all of which can affect C&D performance (Stallings et al., 2025).

4.4.1 Field effectiveness of PW and HP

In commercial swine production, trailer C&D protocols may include washing, disinfection, and thermo-assisted drying before trailers return to the farm. However, the use of heated drying systems can increase operating costs, particularly because of propane use. As a

result, some farms may rely mainly on visual inspection when resources for more intensive C&D procedures are limited (Letsch et al., 2024). Visual cleanliness, however, does not necessarily reflect the microbial condition of trailer surfaces (Alvarado et al., 2022). In the present study, PW was intended to physically remove visible organic material and prepare trailer surfaces for HP disinfection. According to the National Animal Health Emergency Management System guidelines (USDA-NAHEMS, 2014), cleaning can remove 90% of microorganisms from contaminated surfaces, which corresponds to about 1 log reduction. In this field evaluation, however, PW achieved an overall reduction of 0.48 log CFU/cm². One possible explanation is that visible organic material removal alone was not enough to achieve a substantial microbial reduction. Residual organic material was not measured after PW, so this interpretation remains a possible explanation rather than a demonstrated conclusion. Future field studies could evaluate surface cleanliness by combining ATP, as a rapid indicator of residual biological material, with aerobic counts to quantify viable bacterial contamination, as described by Letsch et al. (2024).

The complete field treatment, consisting of PW followed by HP disinfection, resulted in total overall reduction of 2.02 log CFU/cm². This reduction was lower than those observed in previous work. In that laboratory study, reductions obtained with HP ranged from 2.79 to 3.87 log CFU/cm² for *E. coli* biofilms and from 3.40 to 4.57 log CFU/cm² for *E. coli* aggregates, depending on temperature and cleaning method. However, this comparison is not direct because the field evaluation used naturally occurring aerobic counts, whereas the laboratory study evaluated inoculated *E. coli* aggregates and biofilm. Despite these differences, HP produced a significant additional overall reduction of 1.54 log CFU/cm² after PW. In the trailer sections counts decreased by 1.36 to 1.80 log CFU/cm². This additional reduction is relevant because HP was applied immediately after PW, without a drying period, and with a short contact time of 30

seconds. HP is classified as an oxidizing agent with broad-spectrum antimicrobial activity. Its antimicrobial effect is associated with oxidation of cellular components, including proteins and lipids, which can lead to membrane disruption and loss of cell integrity (Linley et al., 2012). However, HP solutions can degrade over time, so fresh solutions are recommended for effective disinfection (USDA-NAHEMS, 2014). A previous field study in pig transport trucks reported aerobic count reductions close to 2 log after C&D using a non-corrosive 2% glutaraldehyde disinfectant applied without a prior drying step (Perrucci et al., 2026). Although the disinfectant differed from the present study, the similar reduction range provides useful context for interpreting HP performance when disinfection is applied immediately after washing.

4.4.2 C&D response by trailer section and surface type

Trailer section did not significantly affect aerobic counts, and the interaction of section by intervention step was also not significant. Initial aerobic counts were similar among the front, middle, and back sections. This indicates no evidence of differences among sections before C&D. Although the total reduction was numerically greater in the back section (2.52 log CFU/cm²) than in the middle (1.87 log CFU/cm²) and the front section (1.67 log CFU/cm²), there was insufficient statistical evidence that aerobic counts differed among trailer sections. Nevertheless, previous research has found that residual contamination can vary among sampling locations within livestock trailers (Alvarado & Predicala, 2022; Letsch et al., 2024). The absence of detectable differences in the present study may be related to the smaller size of the experimental trailer compared with commercial trailers. Commercial swine trailers can transport more than 180 pigs per load, resulting in greater trailer contamination (Sutherland et al., 2009). The possible effects of washing direction, drainage, and residual moisture could not be

determined in the present study because these factors were not measured. Future field studies could evaluate whether these operational conditions contribute to differences in C&D outcomes.

Surface type influenced both initial aerobic counts and the response to C&D. The significant interaction between intervention step and surface type indicated that the difference between floor and wall surfaces changed across intervention steps. Initially, floor surfaces had higher initial aerobic counts than wall surfaces, suggesting that the floor retained more contamination after pig transport. After PW, aerobic counts decreased on the floor but did not significantly change on the wall, resulting in similar counts between these two surfaces. A possible explanation for the limited reduction on the wall is that PW dispersed contaminated particles within the trailer, which may have limited the reduction observed on this surface. PW has been shown to generate aerosols and transfer bacteria from contaminated sites to other surfaces in a commercial meat processing environment (Yadav et al., 2025). After HP, counts remained higher on the floor than on the wall surfaces. Following the complete treatment, aerobic counts decreased by 2.27 log CFU/cm² on the wall and 1.76 log CFU/cm² on the floor. These findings suggest that the floor was more difficult to clean and disinfect under the conditions of this field evaluation. This difference may be related to the surface materials because the trailer walls were made of smooth aluminum, whereas the floor was composed of porous rubber. Previous research has shown that surface material can affect bacterial attachment and the cleaning process (Waldhans et al., 2023; Zheng et al., 2021). From a practical standpoint, the surface-dependent response after HP suggests that surface type should be considered when evaluating C&D performance and selecting locations for microbiological verification.

4.5 Conclusion

In conclusion, this field evaluation supports the use of HP as a component of C&D protocols for pig transport trailers. Its application after PW improved the reduction of aerobic bacterial contamination, even without a drying period. The surface-dependent response indicates that field performance depends not only on the disinfectant but also on the characteristics of the surface being treated. These findings highlight the importance of evaluating disinfectants under field conditions and considering surface type when developing C&D protocols. Future studies should include additional field replications and the evaluation of other disinfectants that performed well under laboratory conditions, such as CO₂ alcohol-quat-based disinfectant.

Figures and tables

Table 3. Aerobic counts before, after PW and HP disinfection.

Factor	Level	Initial	After PW	After HP
Section	Front	3.74 ± 0.27 aA	3.43 ± 0.31 aA	2.07 ± 0.57 bA
	Middle	3.87 ± 0.22 aA	3.45 ± 0.50 aA	2.00 ± 0.61 bA
	Back	4.18 ± 0.40 aA	3.46 ± 0.49 bA	1.67 ± 0.75 cA
Surface	Floor	4.13 ± 0.32 aA	3.49 ± 0.40 bA	2.36 ± 0.41 cA
	Wall	3.73 ± 0.25 aB	3.41 ± 0.45 aA	1.46 ± 0.47 bB

Note. Values are estimated marginal means ± descriptive SD (log CFU/cm²). PW = power wash, HP = hydrogen peroxide. Different lowercase letters within a row indicate significant differences among interventions. Different uppercase letters within a column indicate significant differences among levels of the corresponding factor (P < 0.05). Letters should be interpreted separately for section and surface

Figure 9. Cimarron livestock trailer with a tandem axle and slat-side ventilation design constructed of aluminum.



Figure 10. Floor of the trailer (porous rubber).

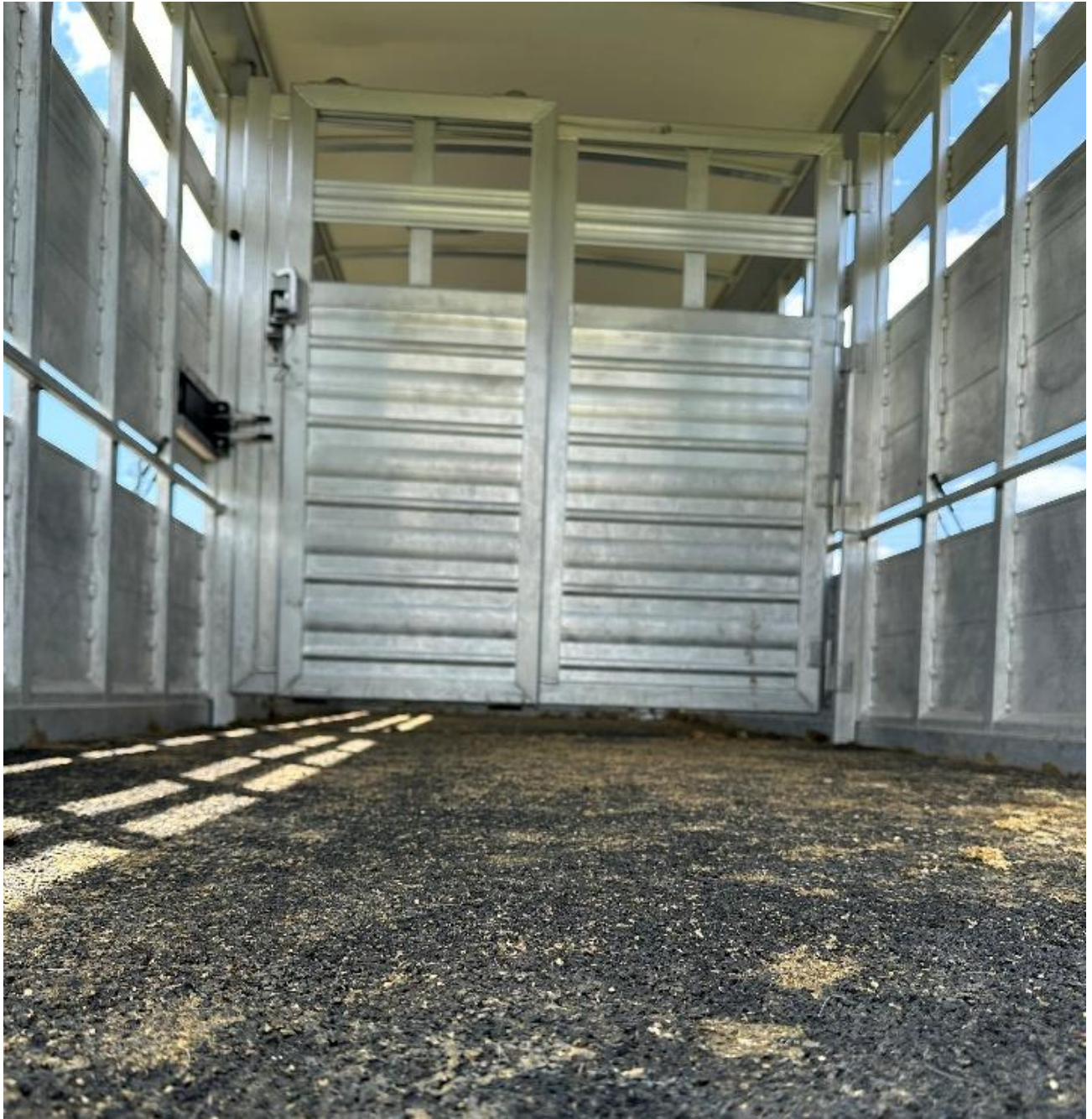


Figure 11. Operator cleaning the trailer



Figure 12. Trailer after cleaning



References

Abdelshafy, A. M., Neetoo, H., & Al-Asmari, F. (2024). Antimicrobial Activity of Hydrogen Peroxide for Application in Food Safety and COVID-19 Mitigation: An Updated Review. *Journal of Food Protection*, 87(7), 100306. <https://doi.org/10.1016/j.jfp.2024.100306>

Alvarado, A., Cabahug, J., & Predicala, B. (2022). ATP bioluminescence method as a rapid tool for assessment of cleanliness of commercial animal transport trailers. *Canadian Biosystems Engineering*, 62(1), 5.1-5.7. <https://doi.org/10.7451/CBE.2020.62.5.1>

Alvarado, A., & Predicala, B. (2022). ATP bioluminescence method as a rapid tool for assessment of cleanliness of commercial animal transport trailers. *Canadian Biosystems Engineering*, 62(1), 5.1-5.7. <https://doi.org/10.7451/CBE.2020.62.5.1>

Arthur, M., Afari, E. L., Alexa, E., Zhu, M., Gaffney, M. T., Celayeta, J. M. F., & Burgess, C. M. (2025). Recent advances in examining the factors influencing the efficacy of biocides against *Listeria monocytogenes* biofilms in the food industry: A systematic review. *Comprehensive Reviews in Food Science and Food Safety*, 24(1), e70083. <https://doi.org/10.1111/1541-4337.70083>

Barham, A. R., Barham, B. L., Johnson, A. K., Allen, D. M., Blanton, J. R., & Miller, M. F. (2002). Effects of the Transportation of Beef Cattle from the Feedyard to the Packing Plant on Prevalence Levels of *Escherichia coli* O157 and *Salmonella* spp. *Journal of Food Protection*, 65(2), 280–283. <https://doi.org/10.4315/0362-028X-65.2.280>

Berry, E. D., & Wells, J. E. (2016). Reducing Foodborne Pathogen Persistence and Transmission in Animal Production Environments: Challenges and Opportunities. *Microbiology Spectrum*, 4(4), 4.4.56. <https://doi.org/10.1128/microbiolspec.PFS-0006-2014>

Bonardi, S., Alpigiani, I., Tozzoli, R., Vismarra, A., Zecca, V., Greppi, C., Bacci, C., Bruini, I., & Brindani, F. (2015). Shiga toxin-producing *Escherichia coli* O157, O26 and O111 in cattle faeces and hides in Italy. *Veterinary Record Open*, 2(1), e000061. <https://doi.org/10.1136/vetreco-2014-000061>

Bridier, A., Briandet, R., Thomas, V., & Dubois-Brissonnet, F. (2011). Resistance of bacterial biofilms to disinfectants: A review. *Biofouling*, 27(9), 1017–1032. <https://doi.org/10.1080/08927014.2011.626899>

CDC. (2025). *Estimates: Burden of Foodborne Illness in the United States*. <https://www.cdc.gov/food-safety/php/data-research/foodborne-illness-burden/index.html>

Dos Santos, G. F., De Sousa, F. G., Beier, S. L., Mendes, A. C. R., & Leão, A. M. G. E. S. (2023). *Escherichia coli* O157:H7 strains in bovine carcasses and the impact on the animal production chain. *Brazilian Journal of Microbiology*, 54(3), 2243–2251. <https://doi.org/10.1007/s42770-023-01034-x>

EPA. (2025). *Guidance for Products Adding Residual Efficacy Claims*. <https://www.epa.gov/pesticide-registration/guidance-products-adding-residual-efficacy-claims>

Flemming, H.-C., Wingender, J., Szewzyk, U., Steinberg, P., Rice, S. A., & Kjelleberg, S. (2016). Biofilms: An emergent form of bacterial life. *Nature Reviews Microbiology*, 14(9), 563–575. <https://doi.org/10.1038/nrmicro.2016.94>

Galvis, J. A., Parker, T. B., Corzo, C. A., Ferreira, J. B., Meiklejohn, K. A., & Machado, G. (2026). *Descriptive and risk analysis of vehicle movements linked to porcine reproductive and respiratory syndrome and porcine epidemic diarrhea transmission in US commercial swine farms* (Version 1). arXiv. <https://doi.org/10.48550/ARXIV.2601.18819>

Letsch, F. G., Welch, M. W., Meyer, M., Hedblom, G. A., Parr, E., Classen, D. M., Dillard, M., & Boler, D. D. (2024). Evaluation of ATP bioluminescence for rapid determination of cleanliness of livestock trailers after a commercial wash. *Translational Animal Science*, 8, txae052. <https://doi.org/10.1093/tas/txae052>

Linley, E., Denyer, S. P., McDonnell, G., Simons, C., & Maillard, J.-Y. (2012). Use of hydrogen peroxide as a biocide: New consideration of its mechanisms of biocidal action. *Journal of Antimicrobial Chemotherapy*, 67(7), 1589–1596. <https://doi.org/10.1093/jac/dks129>

Luyckx, K., Dewulf, J., Van Weyenberg, S., Herman, L., Zoons, J., Vervae, E., Heyndrickx, M., & De Reu, K. (2015). Comparison of sampling procedures and microbiological and non-microbiological parameters to evaluate cleaning and disinfection in broiler houses. *Poultry Science*, 94(4), 740–749. <https://doi.org/10.3382/ps/pev019>

Magalhaes, E., Aljets, E., Mil-Homens, M., Gauger, P., Almeida, M., Guo, B., Zhang, J., Silva, G., Linhares, D., & Main, R. (2026). Effectiveness of alternative livestock trailer cleaning methods on porcine epidemic diarrhea virus inactivation and risk of disease introduction to farms during pig load out. *Journal of Swine Health and Production*, 34(1), 12–22. <https://doi.org/10.54846/jshap/1440>

Makovska, I., Biebaut, E., Dhaka, P., Korniienko, L., Jerab, J. G., Courtens, L., Chantziaras, I., & Dewulf, J. (2025). Methods for assessing efficacy of cleaning and disinfection

in livestock farms: A narrative review. *Frontiers in Veterinary Science*, 12, 1581217.
<https://doi.org/10.3389/fvets.2025.1581217>

Miranda, G. C., Villarroel, M., & María, G. A. (2014). Livestock transport from the perspective of the pre-slaughter logistic chain: A review. *Meat Science*, 98(1), 9–20.
<https://doi.org/10.1016/j.meatsci.2014.04.005>

Parker, T. B., Meiklejohn, K. A., Machado, G., Rahe, M., Darrow, B. S., & Ferreira, J. B. (2025). Evaluation of porcine epidemic diarrhea virus RNA contamination on swine industry transportation vehicles. *Preventive Veterinary Medicine*, 237, 106447.
<https://doi.org/10.1016/j.prevetmed.2025.106447>

Perrucci, A., Magri, A., Cardana, V., Zoppi, S., Cossettini, C., & Scollo, A. (2026). Hygiene procedures of trucks transporting live pigs: Multi-assessment validation of a standardized C&D protocol. *Frontiers in Veterinary Science*, 13, 1789484.
<https://doi.org/10.3389/fvets.2026.1789484>

Pork Checkoff. (2013). *SHIC Wean-to-Harvest Biosecurity: Transport Trailer Cleanliness Study Using Bioluminescence Final Report*. <https://www.swinehealth.org/shic-wean-to-harvest-biosecurity-transport-trailer-cleanliness-study-using-bioluminescence-final-report/>

Robinson, S. E., Wright, E. J., Hart, C. A., Bennett, M., & French, N. P. (2004). Intermittent and persistent shedding of *E. coli* O157 in cohorts of naturally infected calves. *Journal of Applied Microbiology*, 97(5), 1045–1053. <https://doi.org/10.1111/j.1365-2672.2004.02390.x>

Rostagno, M. H. (2009). Can Stress in Farm Animals Increase Food Safety Risk? *Foodborne Pathogens and Disease*, 6(7), 767–776. <https://doi.org/10.1089/fpd.2009.0315>

Simões, M., Simões, L. C., & Vieira, M. J. (2010). A review of current and emergent biofilm control strategies. *LWT - Food Science and Technology*, 43(4), 573–583.

<https://doi.org/10.1016/j.lwt.2009.12.008>

Stallings, K., Buckley, D., Teska, P., & Jiang, X. (2025). Impact of surface roughness on the effectiveness of commercial disinfectants against wet biofilms. *Journal of Applied Microbiology*, 136(11), lxaf263. <https://doi.org/10.1093/jambio/lxaf263>

Sutherland, M. A., McDonald, A., & McGlone, J. J. (2009). Effects of variations in the environment, length of journey and type of trailer on the mortality and morbidity of pigs being transported to slaughter. *Veterinary Record*, 165(1), 13–18.

<https://doi.org/10.1136/vetrec.165.1.13>

Tao, C., Tang, X., Gan, Y., Qin, Y., Yang, S., & Huang, F. (2024). Investigation of the disinfection efficiency of commercial hydrogen peroxide, chlorine dioxide, and chlorine disinfectant on different surfaces. *American Journal of Veterinary Research*, 85(8), ajvr.24.03.0079. <https://doi.org/10.2460/ajvr.24.03.0079>

Urbaniec, J. W., Davies, P. L., & Neary, J. M. (2025). Disinfection reduces but does not eliminate drug resistant *Escherichia coli* from livestock trailers following transport of calves. *Journal of Dairy Research*, 1–6. <https://doi.org/10.1017/S0022029925101581>

USDA. (2025). *Cost Estimates of Foodborne Illnesses*. Economic Research Service. <https://www.ers.usda.gov/data-products/cost-estimates-of-foodborne-illnesses>

USDA APHIS. (2025). *NVAP Reference Guide: Cleaning and Disinfection*. <https://www.aphis.usda.gov/nvap/reference-guide/cleaning-disinfection>

- USDA-NAHEMS. (2014). *Guidelines: Cleaning & Disinfection* [Computer software].
https://www.aphis.usda.gov/sites/default/files/cleaning_disfection.pdf
- Xiong, Y., Gates, R. S., & Green-Miller, A. R. (2018). Factors Affecting Trailer Thermal Environment Experienced by Market Pigs Transported in the US. *Animals*, 8(11), 203.
<https://doi.org/10.3390/ani8110203>
- Waldhans, C., Hebel, M., Herbert, U., Spoelstra, P., Barbut, S., & Kreyenschmidt, J. (2023). Microbial investigation of cleanability of different plastic and metal surfaces used by the food industry. *Journal of Food Science and Technology*, 60(10), 2581–2590.
<https://doi.org/10.1007/s13197-023-05778-0>
- Wales, A. D., Gosling, R. J., Bare, H. L., & Davies, R. H. (2021). Disinfectant testing for veterinary and agricultural applications: A review. *Zoonoses and Public Health*, 68(5), 361–375.
<https://doi.org/10.1111/zph.12830>
- Yadav, B., Fan, Y., Hrycauk, S., McAllister, T., Narvaez-Bravo, C., Brown, T., & Yang, X. (2025). Effects of Sanitation Practices on Microbial Dynamics in Meat Processing Environment. *Journal of Food Protection*, 88(12), 100647.
<https://doi.org/10.1016/j.jfp.2025.100647>
- Zheng, S., Bawazir, M., Dhall, A., Kim, H.-E., He, L., Heo, J., & Hwang, G. (2021). Implication of Surface Properties, Bacterial Motility, and Hydrodynamic Conditions on Bacterial Surface Sensing and Their Initial Adhesion. *Frontiers in Bioengineering and Biotechnology*, 9, 643722. <https://doi.org/10.3389/fbioe.2021.643722>

Chapter 5 - Conclusions

Transportation is an important stage in livestock biosecurity because contaminated vehicles can contribute to the transfer of microorganisms between animal groups and farms. Live haul trailers are challenging environments to clean and disinfect because organic material can accumulate in hard-to-clean areas and on different surface types. Materials that improve animal safety, such as porous flooring, can also retain moisture, organic material, and microorganisms, limiting C&D efficacy.

The laboratory experiments showed that C&D outcomes were influenced by temperature, cleaning method, disinfectant, and cell state. Biofilm *E. coli* cells were more resistant than aggregate cells. Temperature affected treatment performance, but its effect depended on the disinfectant and cleaning method used. Scraping and vacuuming generally improved treatment performance compared with air. Among the disinfectants evaluated, HP and CO₂ alcohol-quat-based disinfectant showed some of the most consistent reductions across temperatures and cleaning methods. Steam, compared with the other evaluated treatments, showed the poorest performance, especially against biofilms.

The field study evaluated HP in a naturally contaminated trailer after pig transportation. When applied after PW, HP further reduced aerobic counts, although the reduction pattern differed between floor and wall surfaces. This difference shows how difficult it can be to transfer results from controlled laboratory experiments to live haul trailers, where surfaces and contamination are more variable. Together, the two studies show that laboratory and field evaluations are both needed: laboratory experiments help identify promising treatments and the factors that affect them, while field studies help determine how those treatments perform on naturally contaminated trailers.

Improving trailer C&D requires attention to the entire process. Cleaning must remove enough organic material to allow disinfectant to reach the contaminated surface, and the protocol must account for the type of material being treated. Future studies should evaluate multiple full-size trailers in different seasons. This work would provide a stronger basis for developing practical and reliable C&D protocols for swine transportation

Appendix A- ANOVA for the main effects only.

Appendix A Table 4

Effect	P-value
Temperature	< 0.001
Location	0.395
Cleaning	< 0.001
Disinfection	< 0.001

Note. P-values were obtained from the analysis of variance. Effects were considered statistically significant when $P < 0.05$.

Appendix B - Estimated reductions in aerobic counts after PW, after HP, and total reduction

Appendix B Table 5.

Factor	Level	Reduction	Estimate	SE	95% CI	P-value
Intervention effect	Overall	PW reduction	0.481	0.176	−0.007, 0.969	0.0522
		HP reduction	1.536	0.176	1.047, 2.024	0.0009
		Total reduction	2.016	0.176	1.528, 2.505	0.0003
Trailer section	Front	PW reduction	0.302	0.239	−0.333, 0.936	0.4402
		HP reduction	1.360	0.239	0.726, 1.994	0.0002
		Total reduction	1.662	0.239	1.027, 2.296	<0.0001
	Middle	PW reduction	0.422	0.239	−0.212, 1.057	0.2206
		HP reduction	1.449	0.239	0.814, 2.083	0.0001
		Total reduction	1.871	0.239	1.237, 2.505	<0.0001
	Back	PW reduction	0.718	0.239	0.084, 1.352	0.0266
		HP reduction	1.799	0.239	1.164, 2.433	<0.0001
		Total reduction	2.517	0.239	1.882, 3.151	<0.0001
Surface type	Floor	PW reduction	0.637	0.210	0.036, 1.237	0.0390
		HP reduction	1.125	0.210	0.524, 1.726	0.0018

	Total reduction	1.761	0.210	1.161, 2.362	<0.0001
	PW reduction	0.325	0.210	-0.276, 0.926	0.3211
Wall	HP reduction	1.947	0.210	1.346, 2.548	<0.0001
	Total reduction	2.272	0.210	1.671, 2.872	<0.0001

Note. Estimated reductions are expressed as log CFU/cm² and were obtained from pairwise contrasts of estimated marginal means. PW reduction was calculated as Initial – After PW, HP reduction as After PW – After HP, and total reduction as Initial – After HP. PW = pressure washing; HP = hydrogen peroxide.

Appendix C- Pairwise comparisons of aerobic counts between surface types and trailer sections at each intervention step.

Appendix C Table 6.

Factor	Intervention step	Contrast	Estimate (log CFU/cm ²)	SE	95% CI	P-value
Surface type	Initial	Floor vs. Wall	0.395	0.162	0.065, 0.726	0.0207
	After PW	Floor vs. Wall	0.084	0.162	-0.247, 0.414	0.6096
	After HP	Floor vs. Wall	0.906	0.162	0.575, 1.236	<0.0001
Trailer section	Initial	Front vs. Middle	-0.133	0.198	-0.622, 0.356	0.7814
		Front vs. Back	-0.447	0.198	-0.936, 0.041	0.0780
		Middle vs. Back	-0.314	0.198	-0.803, 0.175	0.2678
	After PW	Front vs. Middle	-0.013	0.198	-0.502, 0.476	0.9976
		Front vs. Back	-0.031	0.198	-0.520, 0.458	0.9865
		Middle vs. Back	-0.018	0.198	-0.507, 0.470	0.9954
	After HP	Front vs. Middle	0.076	0.198	-0.413, 0.565	0.9226
		Front vs. Back	0.408	0.198	-0.081, 0.896	0.1164
		Middle vs. Back	0.332	0.198	-0.157, 0.820	0.2319

Note. Estimates were calculated as the first level listed minus the second and reported in log CFU/cm². Positive values indicate higher aerobic counts for the first level, whereas negative values indicate higher counts for the second. SE = standard error; CI = 95% confidence interval; PW = pressure washing; HP = hydrogen peroxide.