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Water line biofilm regrowth dynamics in six wean-to-finish farms post peracetic acid water line cleaning and disinfection

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Abstract

Water quality and water line management play a critical role in swine health; however, they are often overlooked aspects of swine production. Best practices for water line cleaning and disinfection in pig barns are limited, and a one-time (terminal) water line cleaning with peracetic acid (PAA) may reduce mineral scale and biofilm presence. The objective of this study was to evaluate swine water line biofilm regrowth dynamics in six commercial wean-to-finish farms on well water

following application of 0.78% PAA. Water line samples were collected aseptically pre-treatment (0), after water lines had been flushed 24 hours after PAA had been applied (1), and 3, 5, 7, 14, 21, 42, 56, and 77 days post-treatment. Biofilm was quantified via aerobic and anaerobic standard plate counts. Results demonstrate a significant reduction in biofilm quantities pre- (0) and post-treatment (1), with over a three-log reduction in log 10 colony forming units (CFU) per mL (adjusted p-value = 0.0000). Biofilms regrew within three days and were not significantly different than pre-treatment (0) biofilm quantities. This demonstrates that administration of 0.78% PAA is effective at reducing biofilm quantities, however, long-term impacts are limited. Following labeled dosages and continuous water disinfectants should be considered for long term management.

Keywords: biofilm, standard plate counts, water lines, peracetic acid, water quality

Introduction:

Water is an essential nutrient for swine, playing vital roles in maintaining health and welfare [1-4]. Despite its importance in maintaining daily operations on farms, it is an often-overlooked aspect of swine management with most efforts focused on providing drinking water access and only addressing water quality when clear health deficits appear. Water sources for farms primarily include private well water or surface water sources, with treated rural water occurring less commonly [5, 6]. In the United States, private wells are not covered under the Environmental Protection Agency’s Safe Drinking Water Act, making testing and treating for contaminants the responsibility of the well owner [7]. Swine farms can routinely experience water quality challenges regarding hard water, elevations in minerals, and microbial contamination [8]. Additionally, they often have conditions that favor

mineral deposit formations in water lines which can reduce pipe diameter, restrict water flow, and create a rough surface for biofilm colonization [9].

Biofilms can create challenges in swine water distribution systems (WDS) by potentially harboring pathogens, occluding drinking water access, and contributing to decreased microbial water quality [10, 11]. Biofilm-forming organisms can be introduced into WDS through the water source (i.e., well, surface water, or municipal water), or through access points into the WDS via the farm water medication dosing system, medication stock bucket, and water access points (i.e., nipple drinkers, cup waterers, and wet-to-dry feeders) [12]. Once introduced into the WDS, bacteria colonize and form an extracellular polymeric substance (EPS) matrix to create a biofilm [13]. Swine WDS design further contributes to biofilm development as they are open, branched systems with terminal ends and may include in-line water dosing systems for treatments [14, 15]. This design allows for water to move more slowly during times of low water consumption, provides areas of stagnation at the ends of the water lines, and additional nutrients (via oral administered medications, such as electrolytes and acids) to biofilm organisms [14, 16].

Farms with water quality and biofilm challenges could benefit from routine water line cleaning and disinfection, particularly between groups of pigs at wean-to-finish farms. There are many biocides available for water line cleaning and disinfection for livestock, however best practices for water line management, including cleaning and maintenance, have yet to be established for swine WDS.

Peracetic acid (PAA), also known as peroxyacetic acid, may be a solution to aid in water line cleaning and disinfection, but the residual antimicrobial effects of a one-time administration (terminal line cleaning and disinfection) of PAA in swine

water lines is unknown. PAA is a commercially available disinfection agent with broad-spectrum antimicrobial activity, has been reported to be effective against biofilms, and its acidity and oxidation potential allow the breakdown of mineral scale in water lines [17-20]. Depending on formulation and label claims, PAA can be used for water line cleaning and disinfection and water treatment in livestock operations. PAA slowly acts against organic matter and biofilms, suggesting a potential residual disinfection effect, but the regrowth dynamics after PAA disinfection are poorly understood [21].

The objective of the study was to obtain baseline levels of biofilm and investigate biofilm regrowth dynamics after a single application of 0.78% PAA in water lines on wean-to-finish swine farms between groups of pigs. To the authors knowledge, this is the first study to evaluate biofilm regrowth dynamics after terminal line cleaning with PAA in swine water lines in commercial conditions.

Materials and Methods:

1. *Site criteria*

Commercial, wean-to-finish swine farm sites (n=6) containing two rooms with private, untreated, well water sources in west-central Iowa were enrolled into the study. Sites enrolled were over three years of age, contained main water lines composed of polyvinyl chloride (PVC), and had at least one water dosing system that could be applied to both rooms. It was required that the site had not previously received a water line cleaner or disinfectant within the past six months prior to the study.

2. *Study design*

This observational, longitudinal study assessed water line biofilm regrowth dynamics after water line administration of CID 2000 Pro (CID LINES, an Ecolab Company, Ghent, Belgium), a commercially available hydrogen peroxide peroxyacetic acid (PAA). This study involves a subset of samples from one central sampling population outlined by Doughan, 2025 [11]. Replaceable sections of PVC pipe were installed into the main water line ("coupon side streams") an average of seven months prior to the trial. Pre-treatment (0) samples were collected prior to administering CID 2000 Pro (PAA) at 1:128 oz (0.78%). An off-label dosage of 0.78% PAA was chosen to be readily applicable to United States swine industry applications. Most swine farms in the United States have a fixed 1:128 oz (0.78%) ratio water dosing system readily available. The farm's water lines and coupon side streams were filled with 0.78% PAA and allowed a contact time of at least 24 hours. This occurred when the farm was empty between groups of pigs. Water line samples were collected at the following time points: Pre-treatment (0), 24 hours after PAA water line treatment and water line flushing (1), then 3, 5, 7, 14, 21, 42, 56, 77 days post-treatment. Samples were collected during May – November 2023.

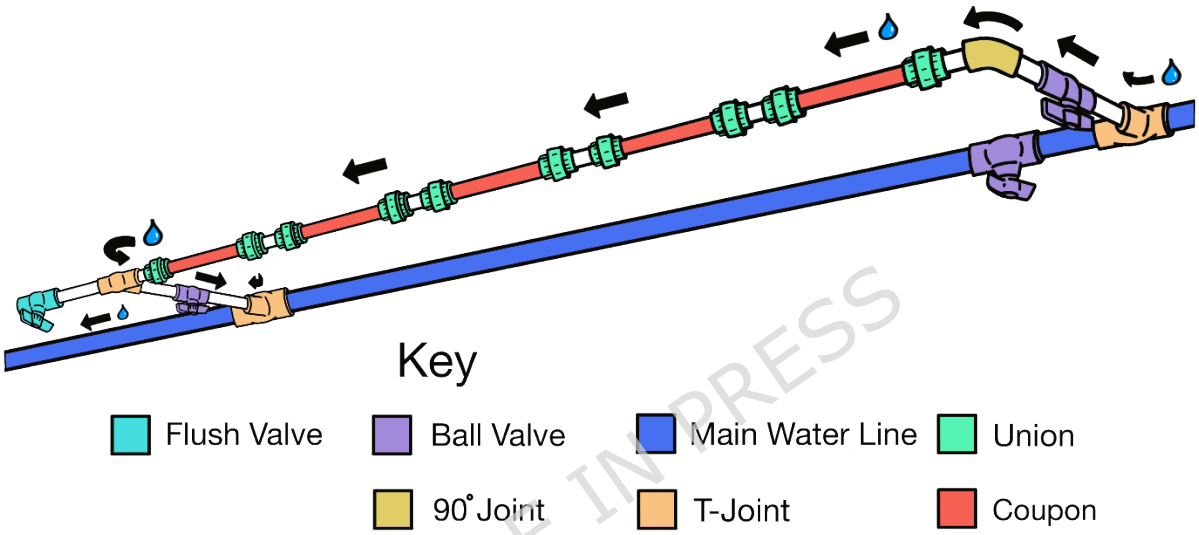
3. Coupon side streams

To assess biofilm regrowth rate, coupon side streams were installed into the six sites. This avoided main water line disruption and did not compromise water flow to pigs during sampling events. Two coupon side streams were installed near the middle of each room (Figure 1), four per farm site. Coupon side streams consisted of new PVC pipe, which matched existing pipe material and pipe diameters for all six sites, with an internal diameter of 1.905 cm, "T" and 90° joints, and PVC ball valves which diverted water flow from the original main water line into the new coupon side stream. The water would flow through the coupon side stream and then enter

back into the original main water line to supply the end of the water line (Figure 2). Each coupon side stream contained five 26.67 cm long (10.5”) PVC pipes which were connected via PVC unions, allowing for easy removal and replacement of PVC water line sections for sampling by unscrewing and re-screwing each union. Each coupon side stream had a “flush” ball valve on the distal (farthest away from the water source) end of the coupon side stream. Conduit brackets were installed and zip ties were used to support the coupon side stream to the ceiling at the same level as the existing plumbing. Coupon side streams were identified as “proximal” and “distal” side streams based on their proximity to the source water, with proximal being closest to the water source and distal being further from the water source.

Coupon side streams were installed and naturally developed biofilm for an average of 215 days (~ seven months) and ranged from 132-266 days prior to the start of the trial. Coupon side streams were exposed to farm water quality conditions, biofilm and biofilm dispersal events from existing main water line pipe, farm flow rates, and on-farm temperatures for the seven months. Therapeutic water line treatments such as vaccines, electrolytes and antimicrobial medications were permitted and recorded. Water line cleaning or continuous water disinfection treatments were prohibited before and after the start of the trial. No water line treatments except 0.78% PAA were permitted during terminal water line cleaning. Figure 1 demonstrates coupon side stream assembly and water flow dynamics. Relative location of their installation in each room of the barn on the farm site and a real photograph of the coupon side stream can be found in the Supplementary Information (Figure S1-S2).

Figure 1. Visual of a coupon side stream containing five removable "coupons" of 1.905 cm PVC pipe joined via threaded unions. Water flow (arrows) was directed away from the main water line pipe into the coupon side stream before and during the study. Water flowed back into the main water line at the distal end of the coupon assembly to supply the rest of the farm.



4. *Peracetic acid preparation, administration, and validation*

Methods regarding peracetic acid preparation, administration, and validation of appropriate PAA dose (total oxidizing oxygen (TOO) test) processes are detailed in the Supplementary Information (Supplementary sections 1-4).

5. Coupon sampling regimen

One coupon was designated to be collected for each of the 10 collection time points in two rooms in six sites (n= 120 coupons). Coupon side streams were collected from distal to proximal coupons based on sample collection time point. Pre-treatment (0) being the most distally collected and sample collection time point 77 being the most proximally collected coupon on the proximal coupon side stream.

Coupons were further subdivided and designated for further testing as reported in Doughan, 2025 [11]. Coupons were subdivided into aerobic standard plate counts (ASPC)(n=120) and anaerobic standard plate counts (ANSPC) (n=120) in this study.

6. External coupon decontamination

At each sample collection time point, the water supply was turned off to the coupon side stream being collected. A 5-gallon bucket filled with water was obtained and dish soap (Dawn™ Proctor and Gamble (P&G), Cincinnati, OH, USA) was added to create a diluted soap solution. Protective eyewear and gloves were donned, and the exterior surface of the water line pipe was washed to remove any external debris until the exterior of the water line pipe was visually clean. Excess soap or water was removed with clean, dry, paper towels. The exterior of the pipe was marked with a permanent marker at 0 cm, 7.62 cm, 10.16 cm, 12.7 cm, 15.24 cm, and 17.78 cm length of the coupon to indicate PVC sections to be collected for the study. The ball valve on the coupon side stream was opened to remove any residual water from the coupon side stream. Gauze sheets were soaked in a solution of 2% diluted sodium hypochlorite (bleach) and utilized to scrub the exterior of the coupon plus 15.24 cm on either side of the pipe and the ceiling near the coupon to reduce potential for deoxyribonucleic acid (DNA) or ribonucleic acid (RNA) contamination [22]. The 2% bleach was applied to the pipe cutting tools (blades, handles, and the entirety of the surface) and the surface where the pipe cutter would rest during the two-minute contact time. A new pair of clean gloves were donned during the contact time for the 2% bleach dilution. Clean paper towels were utilized to dry off any residual bleach after this contact time.

7. Coupon collection for ASPC and ANSPC

Once the external surface of the coupon was decontaminated, coupon sections of PVC pipe were cut on the marked lines from most proximal to distal. The final two most distal sections (each section measured 2.54 cm) were collected and tested for ASPC, and ANSPC, respectively. Samples were collected and placed into Whirl-Pak® bags with 10 mL of 0.9% saline. Once the coupon sections were extracted, the two ends of the remaining coupon were unscrewed from the union and a new, intact, PVC coupon was screwed into the space to restore water access. The main water supply was then turned back on to fill the coupon side stream and the process was repeated for the second room. At the end of each sample collection time point, each site had two ASPC and two ANSPC samples.

8. Sample preparation and processing

Water line samples were stored on ice until processing. One person was designated to handle the water line sample with new gloves, while another person was designated to handle the exterior of the Whirl-Pak® bag to avoid contamination. Biofilm and mineral deposits were removed from the interior of the 2.54 cm pipe section with the plastic end of a puritan swab while holding it over the open end of the Whirl-Pak® bag. The circumference of the interior pipe was scraped two times, then the water line section was dropped back into the Whirl-Pak® bag and rinsed with the existing 10 mL of saline. This process was repeated until all or the majority of the visible biofilm on the inside of the pipe section was removed from the interior pipe wall. The saline, biofilm and mineral deposits in the Whirl-Pak® bag were then collected via a pipette and put into a 15 mL conical tube. The last ~1 mL of the liquid in the Whirl-Pak® bag was repeatedly aspirated to obtain any settled biofilm or mineral debris in the crease of the Whirl-Pak® bag. The samples were then centrifuged at 1300 rpm for five minutes in a LW scientific C3-

Select centrifuge to obtain a pellet. The samples were submitted to the Iowa State University Veterinary Diagnostic Laboratory (ISU VDL) within three days of sample collection for ASPC and ANSPC analysis.

Sixteen ASPC and ANSPC samples could not be submitted to the ISU VDL within three days of sample collection. Samples included ASPC and ANSPC from site 4 on sample collection time points 3 and 5 post-treatment, as well as ASPC and ANSPC from site 3 and 5 on sample collection time point 3. These samples were frozen at -70 °C and thawed prior to biofilm removal, centrifugation and submission to the ISU VDL.

9. Biofilm enumeration via standard plate counts

For each sample collection time point, four samples (two from each room) containing a biofilm pellet and 0.9% saline supernatant held in 15 mL conical tubes were submitted to the ISU VDL for ASPC or ANSPC analysis. Details on the methods for aerobic and anaerobic standard plate count preparation and testing processes can be found in the Supplementary Information (Supplementary section 5).

10.Statistical analysis

Microbial standard plate count data was transformed into a log base 10 scale. A linear mixed-effects model was implemented in R v.4.3.3 using the “lme4” package v.1.1-35.1 to assess the effect of sample collection time points on the log transformed count across all sites, with the site as the random effect. The experimental unit was the site. Post-hoc pairwise comparisons between pre-treatment (0) and all post-treatment time points were conducted using estimated marginal means via the “emmeans” package v.1.10.0, with Dunnett’s method to control a familywise error rate. A Welch two sample t-test was also performed for each site separately to compare the difference between collection time point 0 and

1 within site. Furthermore, room, standard plate count type (ASPC or ANSPC), and their interaction terms with sample collection time point were added in the linear mixed-effects model as fixed effect, with the site as the random effect. And a type III ANOVA was performed on the model to detect significant effects on the log transformed count across all sites. p-values of <0.05 were considered significant.

Results:

In this study, impact of terminal water line cleaning with 0.78% PAA on water line biofilms and biofilm regrowth dynamics in six wean-to-finish swine farms was evaluated. Over the six sites, pre-treatment ASPC and ANSPC ranged from 0 CFU/mL to 7.00×10^6 and 6.20×10^6, respectively. The highest ASPC and ANSPC reported over all collection time points were 3.30×10^9 and 1.80×10^9 respectively with both samples reported on post-treatment collection time point 7.

The type III ANOVA revealed there were no significant effects of room, standard plate count type, and interaction terms across all sites on CFU/mLs (p-value = >0.05). Therefore, biofilm quantities were assessed by combined ASPC and ANSPC results in the subsequent analysis.

Impact of 0.78% PAA on biofilm between pre-treatment (0) and post-treatment (1)

A significant difference in the log base 10 transformed CFUs/mL between pre-treatment (0) and post-treatment (1) was detected (adjusted p-value 0.0000) and the estimated reduction in log 10 CFU/mL was 3.3874 (Figure 2). Figure 2 provides visual representation in violin plots of the distribution of pre-treatment (0) and post-treatment (1) biofilm counts reported in log base 10 CFU/mL. Five out of six sites demonstrated significant reductions between pre-treatment (0) and post-treatment (1) sample collection time points. Table 1 displays the Welch t-test results from site

specific pre-treatment (0) and post-treatment (1) comparative analysis. These findings suggest that a one-time water line cleaning and disinfection with PAA is a viable method for reducing initial biofilm quantities in swine water lines, however site-specific considerations may impact results.

Figure 2. Violin plots demonstrate the distribution of log base10 CFU/ mL values for all sites and standard plate count types between pre-treatment (0) and post-treatment (1) sample collection time points.

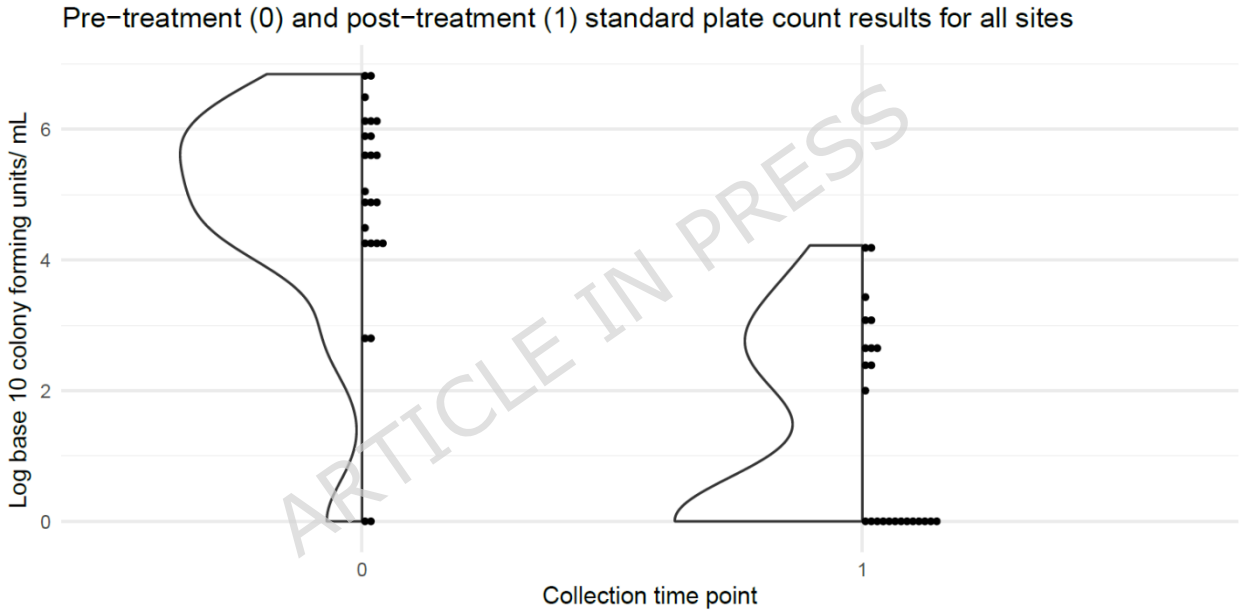


Table 1. Welch two sample t-test results of the impact of a one-time disinfection of 0.78% PAA on pre-treatment (0) and post-treatment (1) biofilm quantities. Five out of six sites demonstrated significant differences (p-value = <0.05) between pre- (0) and post-treatment (1) CFUs/mL. Sample differences demonstrate mean log base10 reductions between pre- (0) and post-treatment (1).

Site Number	t-test	Degrees of freedom	p-value	95% confidence interval	Sample mean difference (pre-treatment (0) – post-treatment (1))
Site 1	-0.0897	5.9506	0.9314	-3.0526, 2.8370	0.1078
Site 2	5.3758	5.0091	0.0030	1.2058, 3.4136	-2.3097
Site 3	7.15	5.2023	0.0007	3.1787, 6.6832	-4.9310
Site 4	33.377	3	5.912e-05	5.8648, 7.1012	-6.4830
Site 5	3.1666	3.9647	0.0344	0.3544, 5.5475	-2.9510
Site 6	7.497	3.0294	0.0048	2.1712, 5.3440	-3.7577

Biofilm regrowth dynamics

Pairwise comparisons between pre-treatment (0) and all post-treatment time points were conducted using Dunnett's method for p-value adjustment. Across all sites, pre-treatment (0) CFU's/mL estimates compared to 3- and 5-days post-treatment were not significantly different than one another (adjusted p-values= 0.9425 and 0.9742, respectively), indicating that biofilm accumulations were indistinguishable from pre-treatment biofilm quantities. However, pre-treatment (0) CFU/mL estimates compared to 7,14, 21, 42, and 56 post-treatment estimates increased above original biofilm quantities and were significantly different or approaching significance (Table 2). Biofilm quantities on sample collection time point 77 remained elevated over pre-treatment (0) quantities and the comparison was nearly statistically significant (adjusted p-value = 0.0513). Biofilm quantity

distributions across all sites and combined plate count type at each collection time point can be seen in Figure 3.

Figure 3. Violin plots demonstrate the distribution of biofilm log base 10 CFU/ mL values for all sites and combined plate count types at each sample collection each time point for the entire study.

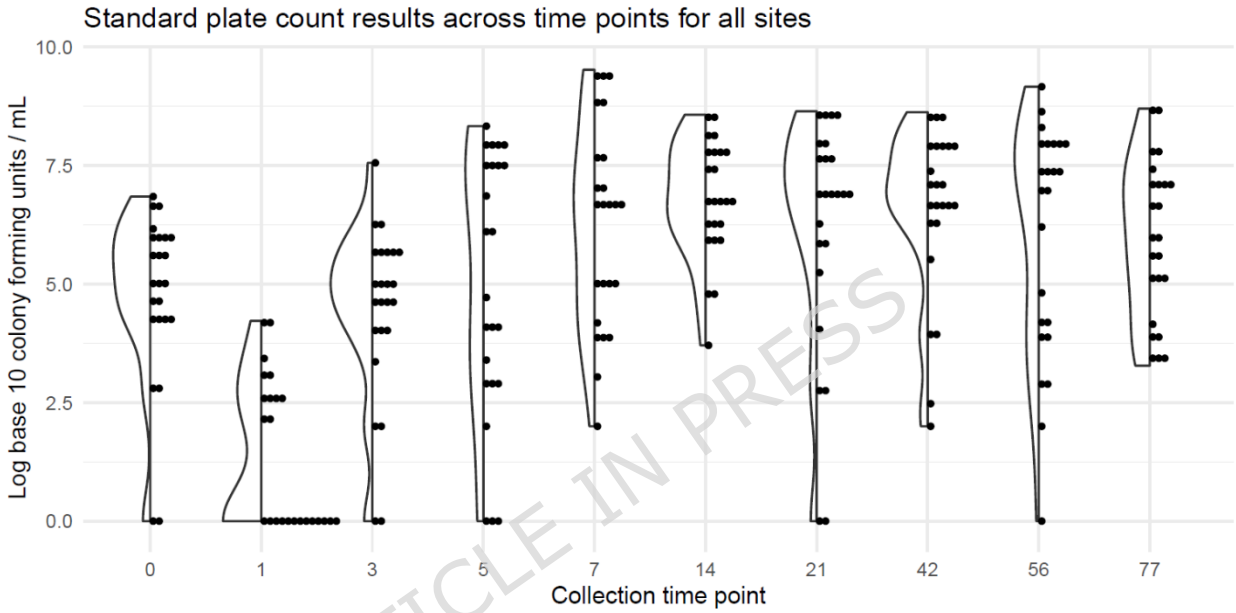


Table 2. Post-hoc pairwise comparisons between baseline and remaining collection time points using Dunnett's method for p-value adjustment. Negative estimates demonstrate increases from baseline biofilm quantities, as most post-treatment values were increased compared to pre-treatment quantities. p-values of <0.05 are considered significant.

Sample collection time point pairwise comparison	Estimate	Adjusted p-value

0-1	3.3874	0.0000
0-3	0.3270	0.9425
0-5	-0.2584	0.9742
0-7	-1.4672	0.0084
0-14	-2.0351	0.0001
0-21	-1.3905	0.0146
0-42	-1.8173	0.0005
0-56	-1.2660	0.0339
0-77	-1.1997	0.0513

Biofilm quantities on collection time points 7, 14, 21, 42, and 56 post-treatment were significantly different and had increased compared to pre-treatment (0) log 10 CFU/mL counts. Biofilm quantities on sample collection time point 77 were approaching significance. This finding is consistent with known biofilm colonization dynamics and that the biofilm is at its stationary phase in its growth, indicating maturation. Selective pressures such as inadequate growing conditions, limited nutrients, or competition over resources within high cell density areas within the water line biofilms can have had a self-limiting effect on biofilm numbers [9, 23].

Discussion

Over the six sites, water line cleaning and disinfection with 0.78% PAA demonstrated greater than a three-log reduction in biofilm quantities (CFUs/mL) with statistically significant results between pre- and post-treatment (p-value= 0.0000). However, the absence of significant differences between pre-treatment

biofilm quantities taken on days 3- and 5-post-treatment indicates that biofilm regrew to quantities indistinguishable from pre-treatment. These findings suggest that a one-time water line cleaning and disinfection with PAA is a viable method for reducing initial biofilm quantities in water lines, but rapid regrowth occurs under field conditions. Rapid regrowth likely reflects incomplete removal of established biofilm, potential site-specific water chemistry interactions with 0.78% PAA, original organic load in water lines, and protection provided by the biofilm's EPS.

To the authors' knowledge, this is the only evaluation of swine water line biofilm regrowth dynamics after water line cleaning and disinfection with PAA. One similar swine water line biofilm study evaluated water quality, biofilm, and endotoxin concentrations in sodium hypochlorite treated water versus untreated water pipes [12]. Biofilm quantities in untreated water lines were comparable between studies, supporting the present study's findings [12]. While both studies focused on aspects of biofilm evaluation in swine water systems, they differed in scope and methodology providing complementary insights [12]. One poultry study evaluated biofilm regrowth pre-flush and post-flush, and 43 days into the grow out period. Day 43 biofilm quantities were not statistically different than pre-flush quantities [24]. This contrasts the present study's results as non-significant biofilm quantities from pre-treatment (0) were achieved at 3 and 5 days post-treatment, however, were not achieved again until collection time point 77, which only approached significance. Further investigation into factors influencing biofilm regrowth dynamics in poultry versus swine farms should be investigated.

In this study biofilm regrowth was evaluated immediately post-treatment (1), and three days post-treatment. Similarly, in vitro studies for poultry water line biofilms demonstrated biofilm regrowth in potable, clean water by day 3 and 7 on

PVC coupons [25]. Other studies have reported either planktonic or biofilm growth within a few hours after PAA disinfection [21], while other researchers generally estimate biofilms could develop within two-to-three days [26, 27]. Future studies could investigate if biofilms return even faster than three days post-treatment with PAA with shorter evaluation intervals.

PAA may have greater efficacy from pre-treatment (0) to post-treatment (1) reductions with on-label administrations of 2% application concentrations. A previous study assessed 2% PAA concentrations in vitro and found drastic reductions in CFUs/mL after administration of PAA at 4 hours, and even further reductions at 24 hours [27]. The present study was designed for United States industry applications, where most swine producers have a fixed 1:128 oz (0.78%) ratio water dosing system readily available. Therefore, off-label administration of the PAA was utilized to replicate applicable field conditions. Practically, this provides further guidance that terminal line cleaning can reduce biofilm quantities, but continuous management and control is needed.

Although PAA dose (0.78%) and application time remained the same over all sites, individual site differences in the level of reduction were noted. Five out of six sites had significant biofilm reductions between pre- (0) and post-treatment (1). Although, some sites had greater reductions than others. Many variables could have interfered with the efficacy of the PAA during terminal line cleaning [28]. Any present organic matter in the water lines, thickness of the pre-treatment biofilm, temperature, water pH, residual EPS matrix, and mineral and chemical profiles of the farm’s well water could have impacted the PAA efficacy [17, 21, 23, 29-31]. Inadequate removal of biofilm subsequent to 0.78% PAA administration post-treatment could have contributed to biofilm regrowth dynamics. The medication

dosing system in which the PAA was applied could have influenced the efficacy of the biofilm reduction. For the present study, existing on-farm water medicator dosing systems were used. Age of medicators, maintenance history of medicators, or calibration were not recorded or performed at application. Quality control mechanisms were performed to ensure accurate dosing of the 0.78% PAA (methods described in the Supplementary Information sections 1-4) and were considered within target. Future research is needed to determine dosing efficiency variations between medicator types and over the lifetime of the medicator.

While this study provides many insights into water line biofilms, water line cleaning and disinfection, and their implications for swine water line management, several limitations must be considered when interpreting the results from this work. This study included a limited sample size, as the experimental unit is the site, and a lack of a control group for comparison.

This study presented a novel, complementary methodology for routine investigations of in situ water line biofilm conditions in livestock operations [12, 32-35]. New PVC pipe was utilized for the installation of the coupon side streams and was assumed to be clean and microbial free. It is possible that contaminants from the new PVC pipe or installation could have impacted biofilm regrowth dynamics. The coupon side stream may not be representative of original biofilm from the main water line pipe in regards to biofilm maturity and internal pipe surface roughness, impacting biofilm regrowth dynamics [36]. Despite these limitations, the methodology was designed to account for the use of new PVC pipe. Coupon side streams were allowed to naturally develop biofilm over a period of time (~ seven months) similar to methods completed in the literature [37, 38]. Future

investigations into the comparison of biofilm maturity and surface roughness between coupon side streams and existing main water lines should be pursued.

Despite careful sample collection and processing, field conditions could have allowed for a small risk of contamination from direct contact or environmental exposure of a contaminant. Samples were removed and divided into subsections for further testing in the field rather than removed as one coupon and further subdivided in laboratory conditions. This was performed to reduce the number of individuals handling the samples and potential introduction of contaminants. It is important to note that environmental contamination from the room that housed the water lines would not necessarily equate to the introduction of novel contributions to the biofilm. Swine water systems are considered open to the environment at several points.

During sample collection, the coupon side stream was drained of water to avoid shearing stress to any neighboring biofilm in nearby coupons [39]. Draining the coupon side stream could have affected the composition or accurate reporting of aerobic to anaerobic organisms due to air exposure [9, 39]. This phenomenon could have impacted the types of organisms grown in our SPCs. Anaerobic and aerobic organisms enumerated in SPCs could also represent facultative organisms that could grow in both conditions.

One of the greatest limitations of the study is that SPC results are limited to identifying only culturable bacteria. In this study, we only evaluated ASPC and ANSPCs, which include general classes of bacterial organisms. SPC results are limited to a small fraction of bacteria that are culturable and viable, including those not in a viable but non culturable state (VBNC) state [40-42]. Biofilms are complex, multi-species structures that could include bacteria, viruses, and protozoa and the

mechanisms available to assess and quantify it have benefits and limitations [9, 43-46]. Enumeration through standard plate counts is a routine and repeatable mechanism for quantifying the number of viable cells within a sample and is reported in colony forming units (CFU) per mL. This technique for enumeration of biofilm microorganisms likely grossly underestimates the true enumeration in the water distribution system at the farm level. SPCs were chosen due to the accessibility, reliability, and commonality with reported measures in the previous literature, and feasibility to repeat the process for swine veterinarians and producers. Sixteen of 240 biofilm samples could not be tested via ASPC or ANSPC within three days of sample collection and were frozen at -70°C and thawed prior to sample submission. Freeze-thaw cycles may have reduced bacterial viability, disproportionately impacted certain bacterial taxa, and lead to an underestimation of CFU/mL results in these samples [47].

It is worth noting that additional investigations into visual and genetic evaluations of biofilm regrowth and colonization, such as confocal laser scanning microscopy, scanning electron microscopy, and 16S rRNA sequencing should be performed in future research [46]. Identifying dominant bacterial taxa and functional groups could provide additional insights into targeted control strategies.

Conclusions

Determining biofilm regrowth dynamics can inform decisions on best practices for interventions against challenges of water line biofilms in swine farms. This study demonstrated that while 0.78% PAA significantly reduced biofilm quantities immediately after application, biofilm regrowth occurred rapidly, accentuating the need for continuous water line biofilm management. Future

assessments could include evaluating the impact of varying dose, biocide product, and visual and genetic evaluations of biofilm regrowth dynamics. This present work addresses a critical knowledge gap in microbial colonization of swine water line biofilms, introduces a practical approach for routine, on-farm biofilm sampling, and provides a foundation for the development of comprehensive water management strategies in swine production systems.

List of abbreviations:

PAA: peracetic acid

CFU/ mL: colony forming units per mL

PVC: polyvinyl chloride

WDS: water distribution system

EPS: extracellular polymeric substance

TOO: total oxidizing oxygen

SPC: standard plate count

ASPC: aerobic standard plate count

ANSPC: anaerobic standard plate count

ISU VDL: Iowa State University Veterinary Diagnostic Laboratory

VBNC: viable but non-culturable

Declarations:

- **Ethics approval and consent to participate**

Oral consent to perform the project was obtained from veterinarians representing farm 1-6's owners to perform the project. No procedures on swine were performed and the study was IACUC exempt.

Consent for publication

Not applicable

Availability of data and materials

The dataset generated during this study is available in the Iowa State University Open Data repository "DataShare" at the following link: https://doi.org/10.25380/iastate.29175365.v1.

Competing interests

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Authors' contributions

GD and LK contributed equally to the study design, project execution, data analysis and manuscript preparation. BK, MP, KS, JTB contributed equally to this work through study design review, project execution, and manuscript review. NM contributed to the study design and directed sample processing at the Clinical Microbiology laboratory and aided in preparation of the manuscript. CS contributed

significantly to sample collection, sample processing, and editing of the manuscript. JLB contributed to study design and contributed to the preparation of the manuscript. DZ performed statistical analysis. All authors have read, revised, and approved the final version of the manuscript.

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