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Groundwater: Pathogens, detection, and disinfection

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Forward

Misconceptions about what causes a disease and how it spreads have been very numerous. At times, so engrained in the scientific community, that to suggest anything quant air was branded as ludicrous. For instance, throughout history a prevailing idea behind disease transmission had been the miasma theory and "bad air" was thought to cause disease in areas that contained a bad stench or aura of sickness. John Snow was skeptical of this and when a cholera outbreak occurred in London in 1854, he had a chance to investigate. In his study, he was able to link the outbreak to contaminated well water. Yet, public health protection agencies continued to reject the possibility of groundwater containing pathogens until the introduction of the Ground Water Rule (GWR)[1] 152 years later in 2006. Today the agricultural industry may possess a similar level of hubris regarding groundwater and biosecurity.

A note of interest is in 1908 Public Water Systems (PWS) began disinfection and treatment of drinking water. Over the next decade, thousands of cities across the United States followed suit. As they did, the number of deaths from infectious diseases declined markedly.[2] This decline contributed to a sharp drop in infant and child mortality and a 29.2-year increase in life expectancy. Today, disinfection and treatment of public water supplies represents one of the greatest achievements for protection in public health of the 20th century.[3]

Primary and secondary disinfection

Throughout this period technologies, engineering design standards, and protocols written for performance validation/verification became well established to better ensure water entering a drinking water distribution system is microbiologically safe. This practice is referred to as Primary Disinfection and was addressed within Part 1 of this paper, published in the proceedings of American Association of Swine Veterinarians (AASV), Annual Conference, Atlanta, Georgia, March 9th, 2020 Groundwater & Livestock Production and Husbandry, Part 1, Biosecurity. Secondary disinfection attempts to ensure drinking water remains absent of pathogens as it travels through a water distribution system and will be addressed within the current paper.

A review of enteric viruses within water

Over 100 types of pathogenic viruses are excreted in human and animal wastes. These viruses can be transported in the environment through groundwater aquifers. Collectively known as enteric viruses, they are transmitted via the fecal-oral route and primarily infect and replicate in the gastrointestinal tract of the host. Viruses are shed in extremely high numbers in the feces of infected individuals and animals, typically between 10^5 and 10^{11} virus particles per gram of stool.[4]

Enteric viruses can be transmitted by food, water, fomites, and human contact. In addition to causing acute diseases, they are of public health concern due to their low infectious dose. For example, the probability of infection from exposure to one rotavirus is 31%, and no more than 1 plaque-forming unit (PFU) is required to cause infection in 1% of healthy adults. The risk of infection when consuming viruses in drinking water is 10- to 10,000-fold greater than for pathogenic bacteria at similar exposures. Because of the potential for contamination from a variety of sources, enteric viruses in water are of particular concern. Since the 1980s, with significant advancements in the area of environmental virology, enteric viruses have been recognized as the causative agent in many nonbacterial gastroenteritis cases and outbreaks.[4]

Two of the most studied groups of enteric viruses as potential water quality indicators are enteroviruses and adenoviruses. While the occurrence of human enteric viruses in the environment and their role in waterborne transmission have been studied extensively, little information is available on environmental transmission of enteric viruses in animals. While 70% (62 serotypes) of nonpoliovirus enteroviruses have been associated with human infections, 30% have been associated with animal infections. Animal-specific enterovirus infections in hosts such as cattle and pigs are often asymptomatic but may cause diseases ranging from diarrhea to reproductive failure and neurological disorders. Two bovine enteroviruses (BEV), three porcine enteroviruses (PEV), 11 porcine teschoviruses (PTV) (10 were formerly classified as porcine enteroviruses), and 1 ovine enterovirus have been identified.[4]

While most studies are specific to pathogens infectious to human vs. swine populations, the question of address in Part 1 of this paper was related to the survivability and transport of virus in general. Though, PEV have a prevalence of 65% in pigs and wild hogs. PEV and PTV have been identified as the etiologic agents of the neurological disorder known as Teschen-Talfan disease, polioencephalomyelitis, vesicular diseases, myocarditis, pneumonia, diarrhea, fertility disorders, and dermal lesions in swine. Five porcine adenoviruses (PAdV), five bovine adenoviruses (BAdV), and six ovine adenoviruses have been classified under the genus Mastadenovirus. PAdV also has been isolated from pigs with encephalitis and pneumoenteritis.[4]

Presence of enteric viruses in groundwater

A plethora of peer-reviewed research, conducted in advance of 2006, served to validate groundwater as a significant vector for disease transmission and subsequently served as the foundation from which the GWR was formulated and included within the US Safe Drinking Water Act (SDWA).[5] The GWR now represents an enforceable standard to protect the health of individuals connected to PWS's.

Since then, a plethora of studies have been published in Professional Journals and serve to describe processes from which viral pathogens traverse from surface soils to groundwater aquifers, their survivability within subterranean environments, dynamic mobility throughout aquifers and propensity for appearing and

disappearing from any given well from days to months.[6] A number of these findings were presented at the AASV conference in 2020. Wherein, a literature review of 57 published peer-reviewed studies provided the following findings:

A. Subterranean environments will support survival (remain infectious) of virus for 3+ years.
B. Viral input concentrations to soils can be as high as 10^{11} virions/gram of stool.
C. Virus from surface soils can reach groundwater aquifers 900+ feet deep within a matter of weeks and on the order of hours in fractured bedrock.
D. Up to 96% of virus from surface soils are transported into aquifers, through 0.32% of the soil volume via Preferential Pathways.
E. In water, virus present themselves as colloidal particulates and are essentially immune to natural Filtration/Inactivation mechanism(s) within soils and aggregate geologies.
F. Viral concentrations are typically higher within groundwater aquifers vs. surface water sources.
G. Virus will migrate within aquifers on a 3-dimensional axis over many miles.
H. Will appear and disappear from any given well system within days to months.
I. The risk of infection when consuming viruses in drinking water is 10- to 10,000-fold greater than pathogenic bacteria at similar exposures.
J. Total chloroform and *E coli* analyses cannot be used as indicators for the presence of virus.

As viral pathogens exist and can remain infectious (viable) within subterranean medias and ground water aquifers, factors influencing their survival, (sunlight, oxygen, temperature and moisture) are among the most important as shown in Figure 1.

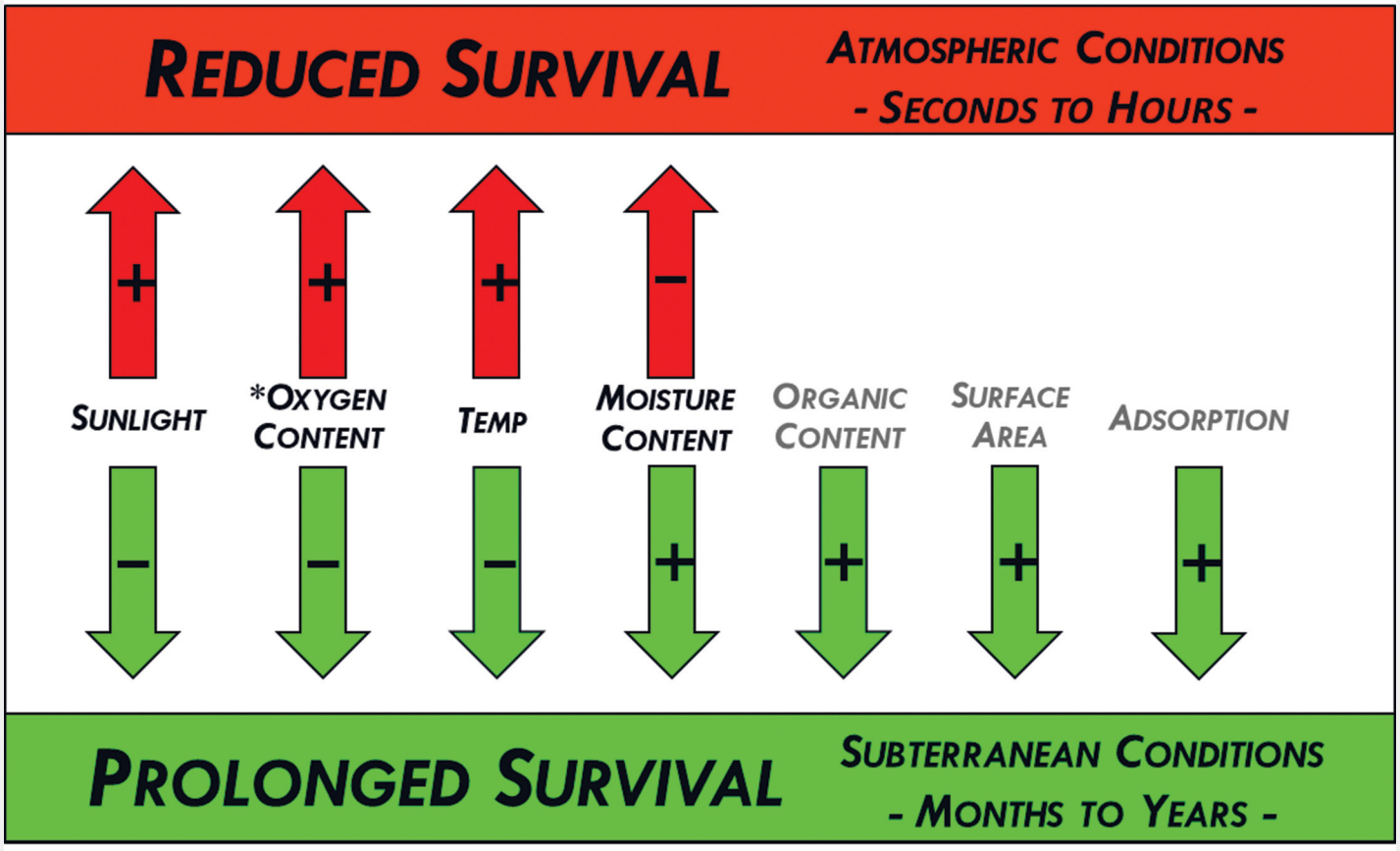
Since implementation of the GWR in 2006 and in part with the data it has produced, the presence of pathogens in groundwater still accounts for more than 50% of disease outbreaks related to public drinking water supplies within the United States[7] and the Center for Disease Control and Prevention (CDC) estimates up to 33 million cases of acute gastroenteritis illnesses occur each year due to the presence of pathogens within US PWS's. The following studies (by reference) provide further validation for the presence of infectious virus within groundwater aquifers.[8-23]

While the above information is specific to virus enteric to the human population it serves to supply insight for the transmission of viral disease via the groundwater vector within the swine industry. Studies characterizing the dynamic mechanisms encompassing viral transport, survival, accumulation, and release of viruses as they travel to and throughout aquifers may serve to be useful for diagnosticians within swine industry.

Comparisons for consideration: Human vs. swine populations

The first comparison worth noting between human vs. swine populations and risk associated with consuming groundwater containing pathogenic virus is related to differences between

Figure 1: Factors influencing survival of viral pathogens.



source inputs. Virtually all human waste from densely populated areas including rural cities, is processed through a wastewater treatment plant and undergoes disinfection in advance of being discharged. Accordingly, only a small fraction of viral pathogens enteric to the human population exiting a wastewater treatment plant remain infectious. On the other hand, all pig manure within geographic areas of high swine density, is collected and stored. Detection of virus enteric to swine, held in manure pits has been confirmed from 841 to 1,949 days after disease outbreaks[24] and then incorporated directly into subterranean soils, where given the survival factors illustrated in Figure 1, they may survive and remain infectious for 3+ years.

The second comparison relates to hydraulic movement of groundwater to and then throughout subterranean medias. While geological strata are by no means uniform and a multitude of other factors must be considered, though as greater volumes of water are drawn from one well as compared to others within the same geographic area, the greater the radius of landmass from which the aquifer is being recharged becomes. In turn, high production wells will draw greater amounts of water from localized surface water sources, in addition to water that has been held within the aquifer itself. Typically, this defines the daily service demand for water of a swine operation, and their wells serve as hydraulic funnels encompassing the radius of their location. Understanding most swine operations are surrounded by fields that may be regularly infiltrated with viral concentrations of up to 10^{11}/gram (10^{16}/ton). Analogy: If a virus represented 1 – inch, the number contained within 1 – ton would be equivalent to the distance of 57,499,478 times the circumference of earth.

The third comparison relates to vetting of water treatment/disinfection technologies. For instance, disinfection as it relates to public drinking water, refers to a measurable degree of assurance a water supply is absent of pathogens. This assurance is based upon efficacy of the technologies employed, the manner they are applied, installed, operated, and maintained. Wherein, continuing verification & documentation of performance with American Standard Test Method's (ASTM) are required. As a result, performance of water disinfection systems are measured based upon the level of protection for which they are designed to provide with ongoing verification for such.

For example, in advance of the installation of a disinfection process within a water treatment plant, or any improvement within an existing plant, a city is required submit an Engineering Plan for State (regulatory) review and acceptance. This plan must comply with all engineering design standards and guidance manuals that have been peer reviewed and published for this purpose. Plus, performance specifications for each step-process must include *validation that it has been, or otherwise can be *validated to achieve and sustain a quantifiable level of inactivation/removal (virus) over a defined range of water conditions, composition and flow rates. In addition, the water treatment plant must be operated, and ongoing performance monitored (via ASTM standards) by a licensed water treatment plant operator and a representative from a state regulatory authority will review the data collected by this operator on a regular basis and conduct a site inspection at least on an annual basis.

The absence of such a model within the swine industry has allowed the sale of pseudo water treatment and disinfection technologies and much continues to be invested for the purchase of products and services under the guise of unsubstantiated performance claims alone. The outcome of many evaluative "studies" either promote a technology and/or practice to a greater extent than adherence to criteria necessary to ensure validity, and the absence of cofounding/extraneous variable(s) within the design of the study employed as demonstrated in Figure 2.

Wherein, marketing initiatives are driven by sales goals, and less so upon the fundamental principles of experimental design and performance validation.

As a result, while water disinfection products have been marketed, bought, and deployed throughout the swine production arena over the course of nearly the past three decades, the vast majority will not eradicate any pathogen to a known and/or prescribed level, as would be required to sustain a credible biosecurity program. An example of such an installation is depicted in Figure 3.

Currently most installations for water disinfection within the livestock production industry simply use a chemical metering pump to inject a disinfectant directly into a water main serving a drinking water distribution system. Absent the address of concentration (mg/L), retention time, temperature, pH, and source water composition, as would be required to substantiate disinfection performance. While such installations offer no relevance as a measure of primary and/or secondary disinfection, they may provide for a water distribution system that is less septic than it had been and may provide a beneficial outcome. In addition, sodium chlorite has been labeled and marketed as aqueous chlorine dioxide throughout the agricultural industry. While chlorine dioxide (ClO_2) may be generated when sodium chlorite is mixed directly with chlorine and/or an acid, unless a generator, designed exclusively for this purpose is employed, the agricultural industry must purchase up to 10 - times the amount of sodium chlorite to obtain the same result.

The expected outcome of the scenarios described above is water treatment and disinfection have been left undervalued as a critical component within production metrics and/or biosecurity systems within the swine industry. This has left one of the most recognized means for disease transmission on a global basis[6] continue throughout the agricultural industry as being unaddressed, underestimated, and out-of-mind.

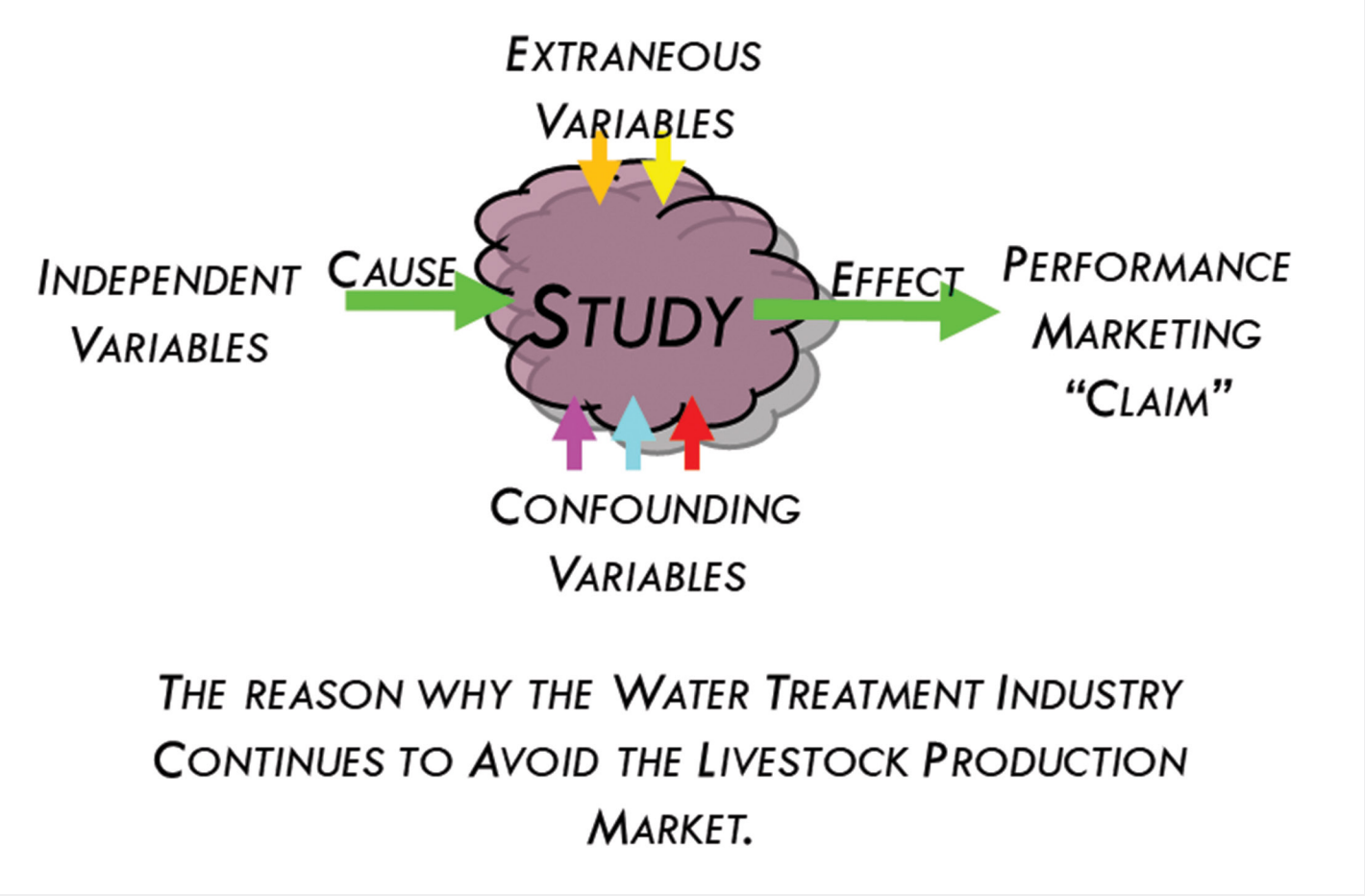
Notwithstanding a multitude of other potential vectors for transmission of PEDV and PRRS have been studied and addressed with the implementation of various measures of biosecurity, the comparative factors described above would suggest validation of disinfection for drinking water would serve a value if employed within the swine industry.

Disinfection

While the emphasis of this paper is specific to viral pathogens, studies have demonstrated pathogenic bacterium and protozoan parasites also reside in groundwater aquifers. However, viruses are more virulent and resistant to disinfection technologies. Likewise, if a disinfection system is designed to eradicate virus to a prescribed level, it will do so for bacterium to a much greater

* Via third-party testing under test protocols, methodologies, and standards accepted specifically for this purpose.

Figure 2: Visual characterization of how many products are currently marketed throughout livestock production industry.



extent. Protozoan parasites such as Giardia lamblia and Cryptosporidium parvum, require additional treatment processes and will be addressed upon request, as Part 3 of this paper.

Is there a viable model employed for drinking water biosecurity elsewhere?

Within the public drinking water industry, disinfection of drinking water serving a municipal water distribution system must include a means to: a.) Inactivate or otherwise remove bacteria, viruses, and other potentially harmful organisms from entering a public drinking water distribution system; is referred to as Primary Disinfection; b.) Maintain water quality by inactivation of pathogens which may enter a drinking water distribution system and inhibiting the formation of biofilms, which collect and harbor pathogens and their promulgation upon the interior of water lines; this is referred to as Secondary Disinfection.

Most water disinfection installations within the livestock production industry today simply use a chemical metering pump to inject a disinfectant directly into a water main serving a plumbing distribution system. This practice would lend itself to secondary disinfection and serves limited relevance as a measure of biosecurity in preventing pathogens from entering a farms' drinking water supply.

Furthermore, the term "disinfection" itself does not translate to a water supply as being free of pathogens. Rather, disinfection, at least as it relates to public drinking water systems, refers to the probability a water supply is absent of pathogens based upon efficacy of the technologies employed, the manner they are applied, installed, and a verification program to ensure its performance on a continuing basis.

Accordingly, in the event various drinking water disinfection systems are being considered for purchase within the swine industry, vendors whom suggest their technology disinfects water, and omits qualification (eg, $\geqq$ x $\log_{10}$ inactivation/removal virus at $\leqq$ x flow rate, X → Y temperature and X → Y pH) nor the means to verify disinfection performance (via ASTM standards) on-site, on a daily basis should be avoided.

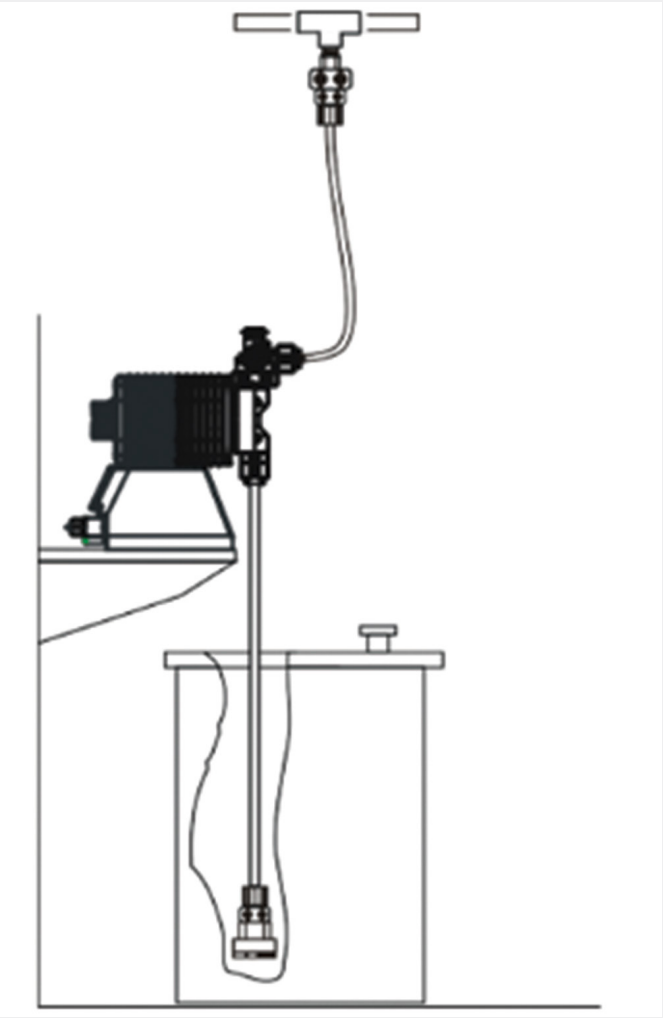
Disinfection (Primary) system design and performance validation

Refer to: American Association of Swine Veterinarians, Proceedings, Annual Conference, Atlanta, Georgia, March 9th, 2020 Groundwater & Livestock Production and Husbandry, Part 1, Biosecurity.[6]

Updated overview of Part 1:

Today it is accepted virus are present within groundwater aquifers in varying concentrations, may remain infectious from days to years, travel at various speeds and direction in advance of

Figure 3: Example of typical installations for water disinfection throughout the livestock production industry.



being detected and then subsequently disappear (from a groundwater well system). In other words, to prevent waterborne pathogens from entering a livestock operation, water tests would need to be performed on a continuous basis. Plus, analytical methods capable of providing results in real-time become a necessity. In addition, due to the implications of a false-positive result, analytical methods employed must be reliable, and Quality Assurance/Quality Control procedures vigorously implemented.

Alternatively, the installation of a robust pathogen barrier represents a more economical and practical approach. PWS's have been charged with the production and distribution of microbiologically safe drinking water for many years. Technology selection, engineering design and ongoing operation of such disinfection systems are well known and have proven to be effective and reliable over the course of the past 100+ years. Utilizing such a tried and proven model may serve as a practical approach the livestock industry should consider.

CT concept

The efficacy of chemical disinfectants can be predicted based on knowledge of the residual concentration of a disinfectant and factors that influence its performance, mainly water composition, suspended solids, temperature, pH, contact time and the level of disinfection required.[25] This relationship is commonly referred to as the CT concept, where CT is the product of "C" (the residual concentration of disinfectant, measured in mg/L) and "T" (the disinfectant contact time, measured in minutes) for a specific microorganism under defined conditions (eg, temperature and pH). To account for disinfectant decay, the residual concentration is measured at the exit of the hydraulic contacting system.

Contact time T is calculated using a T_{10} value (minutes), defined as the detention time at which 90% of the water meets or exceeds the required contact time. The T_{10} value can be estimated by multiplying the theoretical hydraulic detention time by a baffling factor, dictated by the design of the retention system. Otherwise, a hydraulic tracer test may be performed to determine the actual contact time under expected maximum flow (rate) conditions. The T_{10} value is dependent on retention volume and the hydraulics related to the design of the retention system. Improving flow hydraulics to achieve CT requirements serves greater utility than increasing the disinfectant concentration and managed with physical modifications (such as to achieve laminar flow and/or increasing the distance of flow paths) within the contacting system. CT tables for 2 log, 3 log and 4 log inactivation of viruses can be found in an Environmental Protection Agency (EPA) Guidance Manual.[26]

Determination of disinfection dose:

Log_{10} inactivation is based on the Delivered Dose, "CT"
Source Water Turbidity ≦ .5 Nephelometric Turbidity Units (NTU)
"C" is the disinfectant residual (mg/L)
"T_{10}" is the exposure or contact time (minutes)
Multiply them:
$C \cdot T_{10}$ = mg/L • min = (delivered dose)
CT values can be found in US EPA tables to determine log inactivation based on specific monitored parameters (pH, disinfectant residual and/or temperature):

Example

Design a 20 gallon per minute (gpm) water disinfection system capable of inactivating up to 99.99% of all viruses on a continuous basis within the water supply for a livestock operation. The source water has a pH ≦ 7 and temperature is 10°C.

A CT value of 6 is identified within the Guidance Manual to achieve 4 log_{10} inactivation of virus with Free Chlorine

The design of a disinfection system for this water is described below:

Hydraulic Factor (by Tracer Study @ 20 gpm) = 0.5
Net Hydraulic Retention Time (T_{10}) = (114 gallons/20 gpm) × .5 = 2.85 minutes
Calculation of Free Chlorine Concentration to achieve 4 log_{10} inactivation:
(CT Value/T) = mg/L
6/2.85 = 2.11 mg/L

With the performance parameters described above, when a chlorine solution enters the inlet of the first tank at a rate that will provide a concentration of free chlorine of ≧ 2.11 mg/L in the water exiting the last tank, performance is validated with an inactivation rate of 99.99% against viral pathogens.

The same model applied for ClO_2 follows:

CT value (mg/L*min) to achieve 4 $\log_{10}$ inactivation of virus with Chlorine Dioxide = 25.1. With a water temperature of 10°C and a pH ≦ 7 = 25.1 Net Hydraulic Retention Time (T) = (114 gallons/20 gpm) × .5 = 2.85 minutes.

Calculation of free chlorine concentration to achieve 4 $\log_{10}$ Inactivation:

(CT Value/T) = mg/L
25.1/2.85 = 8.81 mg/L

With the performance parameters described above, when a ClO_2 solution enters the inlet of the first tank at a rate that will provide a concentration of 8.81 mg/L in the water exiting the last tank, the level of protection is validated with an inactivation rate of 99.99% against viral pathogens.

Disinfectants

Currently disinfectants acknowledged by the EPA for disinfection of public water supplies include chlorine, chloramine, chlorine dioxide, and ozone. CT values for chloramine illustrate it is a much weaker disinfectant and recommended for secondary vs. primary disinfection or otherwise, if the formation of disinfection byproducts present an issue which unlikely presents a problem for livestock production. Alternative disinfectants can be used based upon proof of validity, safety, and ability to verify applied dose(s) for inactivation of select pathogens.

Ultraviolet Irradiation is accepted. Although performance (Ultraviolet [UV] dose) is dependent upon factors, including hydraulic profile within the reactor, flow rate, UV transmittance of the water, UV intensity, lamp output, lamp placement, lamp aging, fouling and microbe inactivation kinetics.[27,28] Extensive pretreatment technologies are typically required to ensure consistent performance and is generally considered impractical for livestock applications.

Ozone must be generated on-site and adds to complexity, extensive site-specific engineering and sophisticated operation and maintenance procedures. In addition, in atmospheric conditions ozone is gaseous, hazardous and only partially soluble in water. Its use is not recommended in the absence of a professional water treatment plant operator.

Hydrogen peroxide (H_2O_2) is currently employed within the livestock industry as a disinfectant. Unfortunately, the EPA, the governing body assigned to enforce public drinking water quality standards, does not consider H_2O_2 a viable disinfectant and finds no reason to assign CT values for its use. Nor is the author aware if CT values have been developed by an independent 3rd party on behalf of a H_2O_2 manufacturer and/or distributor.

ClO_2 studies were reviewed to compare the inactivation provided by free chlorine and ClO_2 on specific microorganisms. Overall, these studies, which were conducted in laboratory conditions and on bulk water samples, demonstrated that only free chlorine was able to provide 99.99 percent (4-log) inactivation of viruses.[29] ClO_2 can be explosive. This presents significant issues regarding shipping and storage. Similarly, the solid residue left from evaporated ClO_2 solutions presents a concern. ClO_2 must be generated on-site via mixing undiluted concentrations of Sodium Chlorite and Chlorine and/or a Strong Acid in a controlled and safe manner. While there may have been an advancement in ClO_2 chemistry and it is now being shipped, stored, injected into a water line safely and measured thereafter at sufficient concentrations, this advancement remains unknown to this author. Although, independent studies have suggested what has been labeled and marketed within the livestock production industry as ClO_2 is actually sodium chlorite. Accordingly, in the event you have, or currently use such a transportable ClO_2 product, the use of an ASTM standard or EPA approved method (**www.hach.com**) is recommended to verify measurable concentrations of ClO_2 are achieved and maintained throughout your retention and plumbing distribution systems.

Chlorination has proven to serve as an effective primary disinfectant for the inactivation of pathogens and for secondary disinfection as well. Further: A.) It is the easiest and least expensive disinfection method, regardless of distribution system size; B.) In a liquid state, such as sodium hypochlorous acid and preferred over a gaseous state within the agricultural industry, can easily be injected into water in advance of retention, measured and controlled; C.) The technologies used for chlorination are well developed as it is the most widely used and understood disinfection method throughout the world; D.) The pH of sodium hypochlorous acid solutions (vs gaseous) are elevated to 12 – 14 in address of restrictions related to shipping and storage. This becomes problematic as Cl solutions enter a water supply with elevated concentrations of hardness (calcium and magnesium ions). The higher pH will precipitate these ions and generate scale within chemical injection quills and several feet within the pipe downstream of the point of injection, as shown in Figure 4. This begins to limit the amount of Cl entering the waterline and disinfection is reduced, in addition to restricting the flow of water through the water pipe.

To resolve this issue, the pH of chlorine solutions must be neutralized in advance of being injected into a pressurized water supply. This cannot be accomplished at atmospheric pressure with the addition of an acid to a concentrated chlorine solution, as this will transfer chlorine into a gaseous state and must be avoided in address of safety considerations. A method to safely neutralize Cl includes utilization of a mixing chamber constructed with polyvinylidene fluoride, located within a pressurized water bath, wherein chlorine and a strong acid are introduced via metering pumps only utilizing valves, fittings, tubing that are compatible with the chemicals used and subsequently generated. The outlet of the mixing chamber delivers the reacted solution within the source water as it flows through a pressurized column and hardness ions remain soluble as they enter and traverse through a retention system. Which is designed to enhance contact time and facilitate disinfection within a minimal footprint as illustrated in Figure 5. ClO_2 may be generated (efficiently) in the same manner with the introduction of a concentrated sodium chlorite solution.

Overview:

A. A chlorine generator is used to create a low pH chlorine solution in a controlled and safe manner.
B. To prevent the possibility of chemical leakage and creating a hazardous work environment, a chemical safety storage/supply cabinet is utilized and contiguous lengths of thick-walled tubing comprised of polyvinylidene fluoride (PVDF) if used from the outlet of each chemical metering pump to the generator, contained within a water bath in a sealed and pressurized vessel.
C. Retention tanks contain hydraulic baffles to ensure laminar flow throughout their vertical length, extending retention time and enhancing disinfection performance.

Figure 4: Scale formation associated with injection of sodium hypochlorite.

Section of pipe removed from swine nursery with water <u>containing 19 grains/gallon of hardness</u>

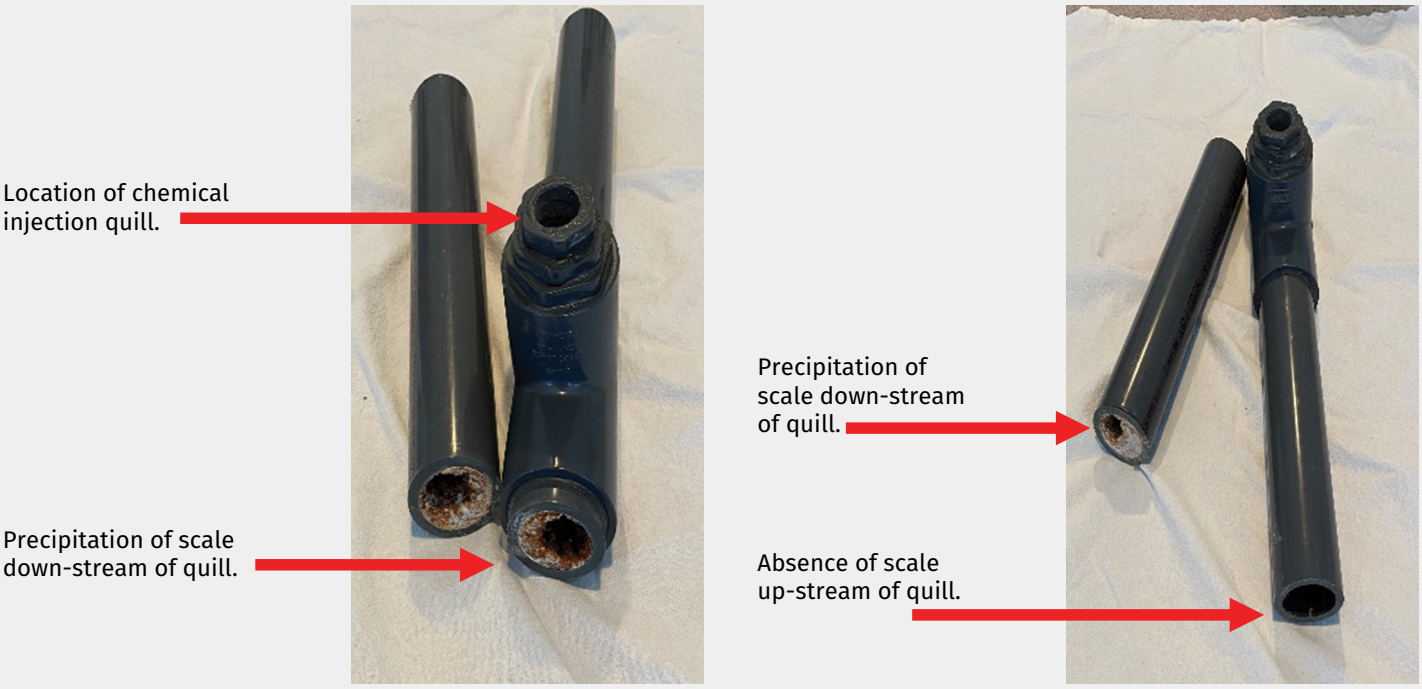
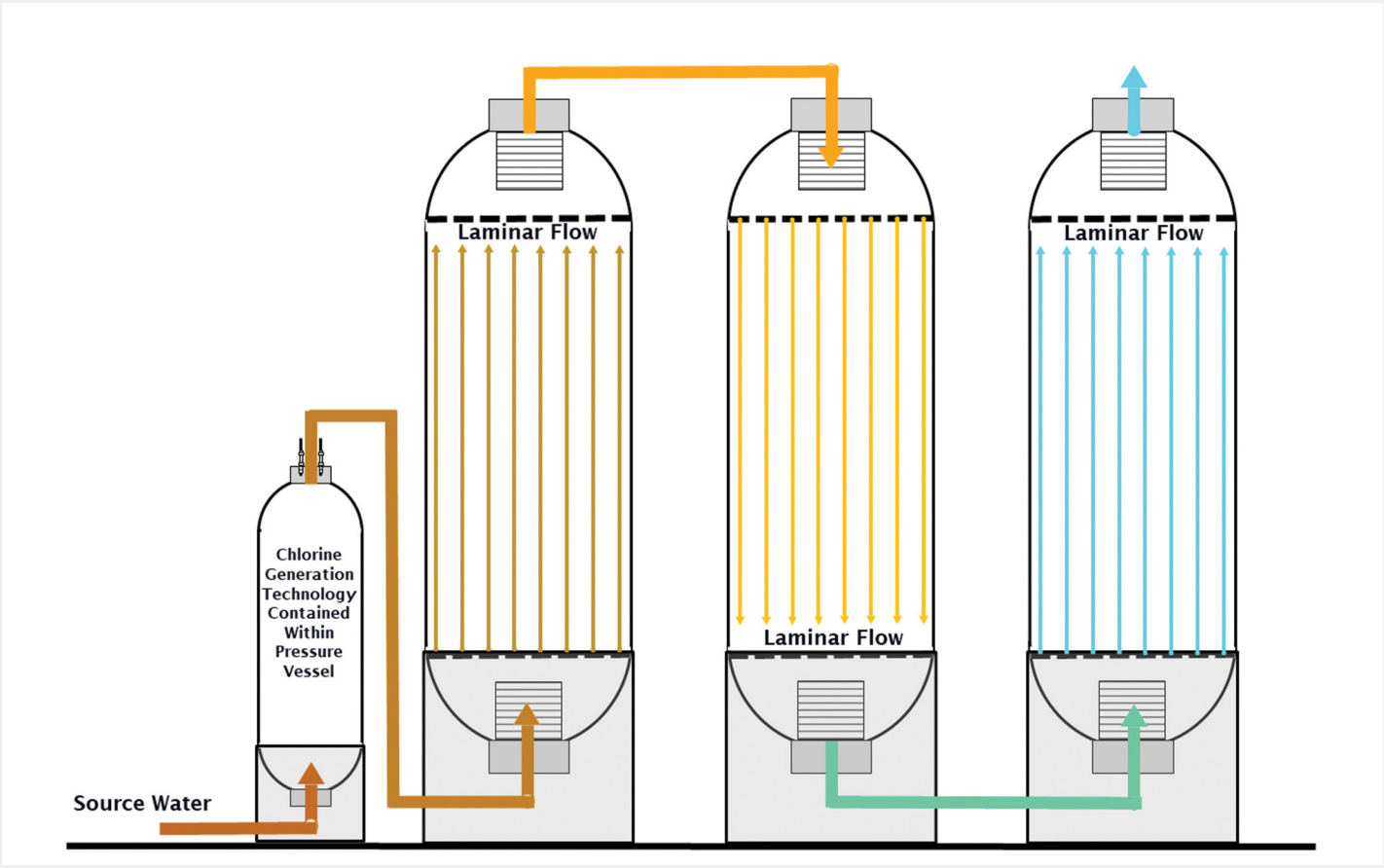


Figure 5: Efficient and safe in situ chlorine (or chlorine dioxide) generation with contacting (retention) system.

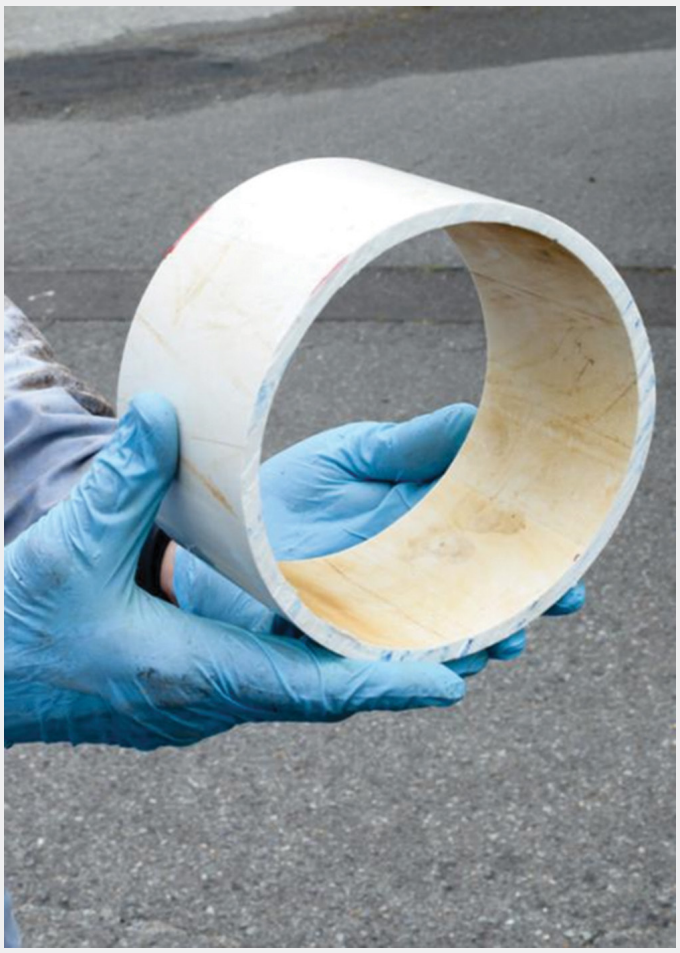


Disinfection (Secondary) system design and performance validation

As Primary Disinfection relates to the inactivation or physical removal of bacteria, viruses, and other potentially harmful organisms and preventing them from entering a drinking water distribution system. Secondary Disinfection relates to pathogens entering drinking water distribution systems via various means and their promulgation and protection within biofilms affixed to the interior of pipes (Figure 6).

Microbes exist in every water system in the world. They are perfectly adapted to their environmental conditions, making the disinfection process a challenging task. As microbes grow, they attach themselves to wetted surfaces in a water system. They protect themselves from disinfecting agents by forming biofilms. A biofilm contains a group of bacteria enveloped within a polymeric slime that ensures adhesion to the surface as seen in Figures 6 and 7. Among many other bacteria, biofilm will attract, harbor, accumulate and protect viral pathogens as well. One of the most compelling attributes, representing the persistence of biofilms is they're virtually impossible to prevent and/or fully eradicate. In fact, biofilms remain problematic for manufacturing facilities within the micro-electronics, pharmaceutical, and medical device industries, wherein water representing the closest to pure H_2O may be found; biofilm formations have been detected within 20 minutes of water-contact in pre-sterilized ultra-pure PTFE plumbing systems. In addition, biofilms are several hundred times more resistant to the action of various disinfectants and antibiotics than the same microorganisms within in a water suspension Each number in Figure 7 corresponds with a stage in biofilm development. The photomicrographs are of an actual Pseudomonas aeruginosa biofilm.

Figure 6: Example of biofilm formations within piping.



The best we can do within an agricultural setting is to create an environment for which their promulgation is limited and inactivate pathogens as they are released from biofilm matrices and distributed throughout a livestock's drinking water system.

Preferred disinfectants for biofilm control

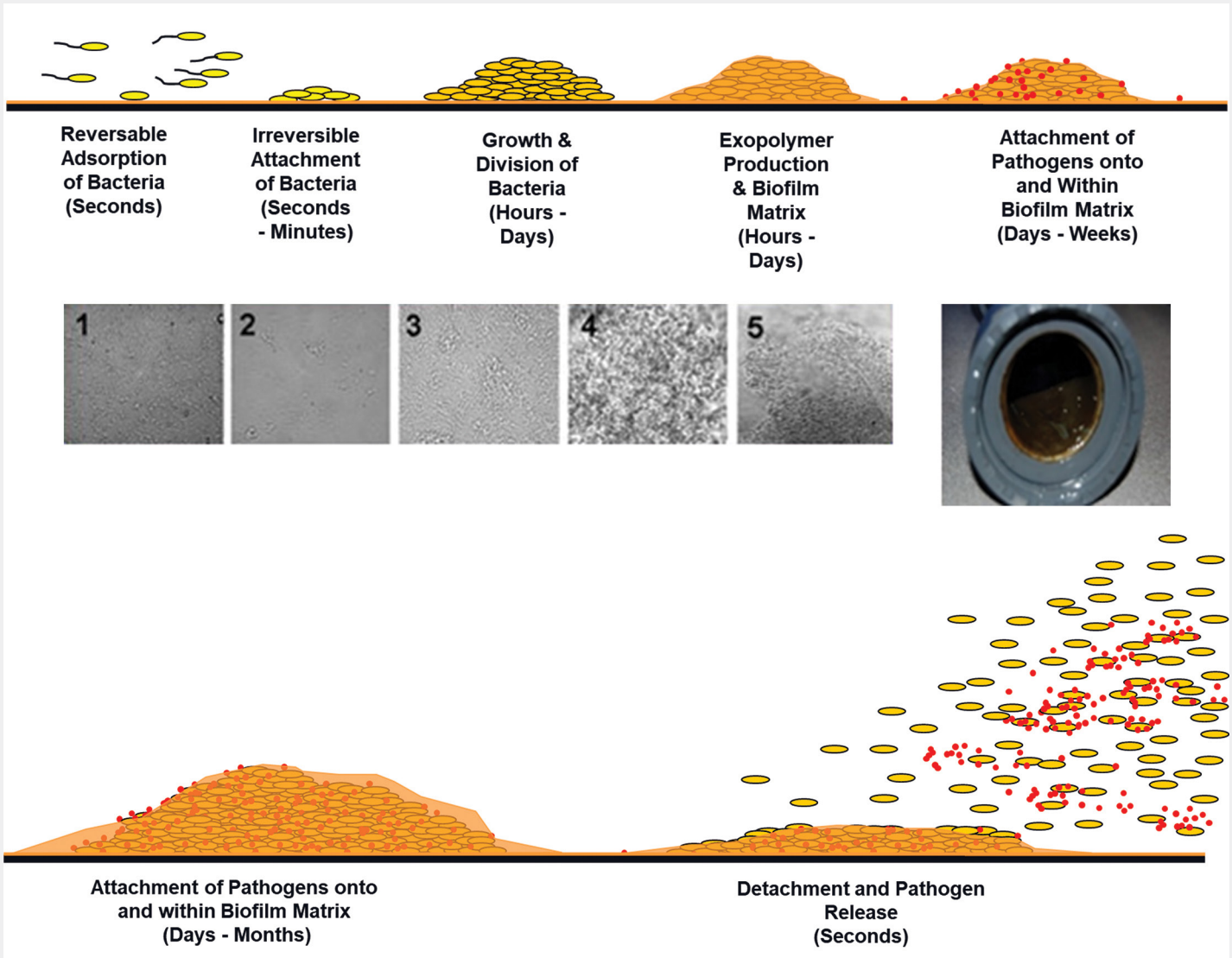
While ClO_2 has been marketed based upon its' superiority as a disinfectant throughout the livestock production industry, it is not frequently used within public water distribution systems for two reasons: 1) its residual concentration will not last as long as that of other disinfectants, and 2) it breaks down into chlorite (predominantly), a regulated disinfection byproduct (DBP) with an maximum concentration level (MCL).[29] (Water exceeding an MCL contained within plumbing distribution systems connected to fixtures that may be used to supply drinking water for employees is unlawful.) Although, as pH increases, disinfection efficacy for ClO_2 remains stable and this serves a measurable advantage when applied in source waters of higher pH values.

There is little information available about the effectiveness of ClO_2 at controlling biofilms. Since biofilm biocides appear to favor more specific reactants that can diffuse more readily into the biofilm, chlorine dioxide's high level of specificity suggests that it could be very effective at inactivating biofilm bacteria. Some studies suggest ClO_2 is effective at inactivating biofilm bacteria, but only when the concentrations are held at $\leqq$ 1.5 mg/L. This concentration exceeds the maximum residual disinfectant level (MRDL) for ClO_2 of 0.8 mg/L in drinking water. Other studies suggest it was effective at inducing biofilm sloughing as well as bacterial inactivation.[29] Efficacy of ClO_2, Cl, and chloramine as disinfectants for biofilm control are represented in Table 1.

In reference to monochloramine, commonly referred to as chloramine, has been used as a secondary disinfectant within drinking water mains for nearly 100 years. It is used in combination with Cl as part of drinking water treatment processes. Monochloramine can be an effective secondary disinfectant and is less susceptible to creating harmful disinfection byproducts.

There are some alternative secondary disinfectants being investigated by researchers (eg, potassium permanganate and ozone combined with hydrogen peroxide, copper combined with hydrogen peroxide, silver combined with hydrogen peroxide, and anodic oxidation) but currently there are no indications of their effectiveness within the distribution system.[29] It is also noted hydrogen peroxide is commonly used for primary and secondary disinfection within the Livestock Production Market and typically marketed as a Stabilized form of H_2O_2. While it is doubtful branding will enhance performance as a disinfectant, it is important to note in the presence of total organic carbon (TOC) within a source water supply or biofilm matrix, H_2O_2 will react with TOC and generate assimilable organic carbon (AOC), which in turn greatly accelerates the growth of biofilms.[30]

Figure 7: Pictographic illustration of biofilm formation, accumulation, and release with timeline.



An ongoing misconception

Measurement of residual concentrations of secondary disinfectants may become misleading. In comparison to primary disinfection, where such measurements reflect the concentration of disinfectant residuals as water traverses through retention vessels. The retention system is designed to provide the correct amount of contact time (minutes) with a disinfectant concentration (mg/L) prescribed to achieve a targeted level of inactivation (log_{10} value) of pathogens of concern as water flows through it at a known rate (gpm). In this case degradation of disinfectant residual is primarily influenced by source water composition, time and temperature and comparatively very little upon interaction with biofilms held upon the interior surfaces of the retention system. As such, measurement of disinfectant concentrations accurately reflects disinfection performance.

On the other hand, performance of secondary disinfectants must be evaluated based upon their ability of disrupting biofilm formations and inactivation of pathogens as they become suspended thereafter within the water traversing throughout a plumbing system. Accordingly, when disinfectants, representing the most virulent for biofilms and inactivation of pathogens, residual concentrations decline rapidly. Over time, as populations of biofilm decrease, demand of the disinfectant lessens, and residual concentrations eventually increase throughout the distribution system. Conversely, if a disinfectant, representing the least virulent for biofilms and inactivation of pathogens is introduced to the same plumbing system, residual concentrations will remain stable throughout its entirety while biofilms remain in place may continue to thrive.

These scenarios provide for a popular misconception. That being, disinfectants have been marketed based upon their ability of being impervious to such concentration decay and capable of maintaining a stable concentration throughout drinking water distribution systems. When in fact, this may represent a serious detriment vs. advantage. That is, unless the market application of such disinfectants includes pre-sanitized drinking water distribution systems, for which, do not exist.

Table 1: Comparison of disinfectant effectiveness for biofilm heterotrophic bacteria inactivation

Disinfectant	Residual (mg/L)	CT (min•mg/L)	Log inactivation
Chlorine dioxide	0.23 Low 0.45 High	14 27	0.3 2.17
Free chlorine	0.47 Low 0.95 High	28 57	1.6 2.44
Monochloramine (chloramine)	0.79 Low 1.85 High	58 111	0.86 2.15

US EPA (2007). The effectiveness of disinfectant residuals in the distribution system. US Environmental Protection Agency, Washington, DC.

Pipe material vs. biofilm

Pipe material plays an important role in biofilm growth and disinfectant effectiveness. In some instances, pipe material may be more influential than the level of organic matter in the system.[31] Some materials provide microbes a protective niche where growth can occur, while some provide nutrients to support microbial growth. Chlorine's ability to control biofilm depends on the pipe material, because different pipe materials demonstrate different levels of chlorine demand. Studies found that free chlorine residuals achieved greater biofilm inactivation compared to chloramine for PVC.[32] Iron pipes seem to exert the greatest disinfectant demand. In the same study, the disinfectant demand of biofilm on iron pipes was as much as ten times greater than for biofilms grown on other pipe materials.[29]

With consideration of the expense of materials such as polytetrafluoroethylene (PTFE), polyvinylidene fluoride (PVDF), and polyvinylidene fluoride (PVDF), polyvinyl chloride (PVC), chlorinated polyvinyl chloride (CPVD) and cross-linked polyethylene (PEX) demonstrate a lower disinfectant demand and fewer water chemistry interactions,[33] are recommended for livestock production operations.

Consideration of recirculation loops for plumbing distribution systems

Various industries incorporate recirculation loops within their plumbing systems to help manage the formation of biofilms and microbiological quality of water within their facility on an ongoing basis. Within a livestock production facility water from the endpoint of each branch of the plumbing distribution system would need to be piped back into the main water supply line between the point of the water meter which controls the metering pump used for the injection of the secondary disinfectant is controlled and the point this solution is injected into the water main. This provides the ability to ensure all water held within the drinking water distribution system maintains a consistent concentration of this solution regardless of degradation of disinfectant residuals based upon variations in residence time in response to differing levels of throughput volumes, and varying interaction with biofilms between each branch of the plumbing distribution system.

Such a recirculation loop would be especially helpful in preventing the influx of biofilm formations that serve to be problematic between livestock rotations wherein water/moisture remains stagnated and subject to higher temperatures provide an ideal environment for proliferation of biofilms throughout the entirety of drinking water distribution systems. This can be prevented with the use of recirculation loops, installation of an inline sensor for continuous measurement for residual disinfectant concentration, and electrically connected to an auxiliary metering pump. Individual drinking receptacles and faucet connections would still need to be flushed and sanitized in advance of delivery of weaned or feeder pigs, which are far less resistant to infectious diseases than older pigs.

In the absence of the methodologies described above, the entire plumbing distribution system must be stripped of as much biofilm as possible and sanitized as close to the arrival of replacement livestock as possible. Subjecting a drinking water distribution system to the promulgation of microbiological contamination especially biofilms, which are very difficult to eradicate via the application of any intermittent technique, is a formidable task.

Water composition and pretreatment

The type of treatment prior to primary disinfection, and the manner it is prescribed, designed, and operated, will have a significant influence on the performance of each water disinfection system. Due to the vast array of groundwater chemistries and density of micro-particulates/microorganisms, to ensure expected performance outcomes are achieved and to avoid the promulgation biofilms within piping and fixtures, filtration is considered synonymous with disinfection. Further, reducing pH to < 7.5 becomes an important consideration with the use of a sodium hypochlorous acid solution as a disinfectant.

It has also been established in research that iron effects the efficacy of antibiotics. Some antibiotics benefit from more available iron and some see their efficacy diminished. Either way, the presence of iron significantly contributes to biofilm issues within drinking water distribution systems and must be removed from source water chemistries. The effect upon antibiotic efficacies are shown in Tables 2 and 3.[34]

Information from which a water treatment/disinfection system is designed

The following parameters must be known in advance of the design of each water treatment system:

1. Allowable concentration of disinfectant within the drinking water.
2. Lethality of the disinfectant in relation to targeted pathogens.
3. The amount of retention (contact) time (T_{10}) required to achieve 90%, 99%, 99.9%, 99.99% …. etc inactivation/removal

Table 2: Relationships between iron and antibiotic resistance. Different bacterial species (vertical) were treated with various antibiotics (horizontal) in the presence of various levels of iron. A two-letter code is used to summarize the results reported. D indicates 'decreased', I 'induced by iron' and O 'no effect, with first antibiotic and resistance with the second'. DI indicates 'decreasing iron level increases antibiotic resistance', DD indicates 'decreasing iron level decreases antibiotic resistance', DO indicates 'decreasing iron level has no effect on the level of antibiotic resistance'. Multiple results within the same box indicate discrepancies between published findings.

antibiotic / bacteria	Aminoglycoside	Quinolone	ß-lactam	Tetracycline	Macrolide	Thiopurine	Rifampin	Isoniazid	Ethambutol	Cephalosporin	Glycylcycline	Lincosamide
M. avium	DI	DI			DI	DI	DD	DD	DD			
M. tuberculosis	DI	DI						DI	DI			
P. aeruginosa	DI ; DD	DI ; DD	DI ; DD							DI	DD	
E. coli	DI	DI ; DD	DI ; DD	DD	DD							DD
S. pneumoniae		DD										
K. pneumoniae	DD		DO ; DI	DD	DO		DO					
A. actinomycetemcomitans				DD								

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per measured concentration of disinfectant.

4. The means to validate T_{10}.
5. The means to validate an installations' expected performance in advance (of purchase), vs. what is claimed by the manufacturer, distributor, or vendor.
6. The means to accurately verify disinfection performance (ASTM test standards) in real-time and/or daily basis.
7. The means to accurately forecast an installations performance for the specific application(s) and site-specific conditions, including:
   a. A water analysis including all parameters that would serve to influence performance of the technologies to be installed.
   b. An understanding of the degree the source water's composition may change beyond the first year of the well systems installation and thereafter.
8. The maximum flow rate available from the well system accounting for:
   a. Static water level.
   b. Flow capacity of the well (gpm).
   c. Flow capacity of the well's pump.
   d. Total (actual) flow capacity available at the installation site based upon the following parameters:
      i. Calculated friction loss based upon static water level and piping (to installation site).
      ii. Specified performance curve (gpm vs. total friction loss + required operating pressure (water treatment plant and livestock operation).
9. With this information, a water treatment plant may be designed to ensure initial and ongoing performance of the disinfection system (primary) will be achieved with continued disinfection throughout the water distribution system (secondary).
10. Equally important is the availability of on-site personal that can be assigned responsibility to perform water analysis, record visual operational parameters of the water treatment plant, and monitor feed supply of chemical metering pump(s).

Economic considerations

The benefit of nearly all biosecurity systems is realized in response to events that occur on a periodic and unpredictable basis. Accordingly, the economic value they provide is based upon factors such as: a) How well they perform during these events; b) The probability such an event will occur; c) Potential of financial loss if it does; d) Virulence and lethality of the pathogen it removes.

Table 3: Effects of fur mutation on the resistance/sensitivity of *E coli* to antibiotics. The fur mutant accumulates high levels of intracellular iron and can be used to investigate the influence, if any, of increasing intracellular iron levels on antibiotic susceptibility. This table summarizes the results of the studies R indicates 'resistant'. S indicates 'sensitivity'.

		***fur* mutant phenotype**	
antibiotic family	**antibiotics**	Nichols *et al.* 2011	Liu *et al.* 2010
quinolone	ciprofloxacin	R	R
	norfloxacin	R	
aminoglycoside	gentamicin	R	
	tobramycin	R	
	streptomycin	R	
	spectinomycin	R	
tetracycline	tetracycline	R	
	doxycycline	R	
	minocycline	R	
rifampin	rifampicin	R	S
tunicamin	tunicamycin	R	
phenylpropanoid	chloramphenicol	R	
penicillin	amoxicillin	R	
	mecllinam	R	
	ampicillin	S	
aminoquinone	streptonigrin	S	
macrolide	clarythromycin	S	
	azithromycin	S	
	erythromycin	S	S
	spiramycin	S	
coumarin	novobiocin	S	
nucleoside	puromycin	S	
glycopeptide	vancomycin	S	S
nitrofuran	nitrofurantoin		S
nitroimidazole	metronidazole		S
sulfonamide	sulfamethoxazole		S
steroid	fusidic acid		S
chlorophenol	triclosan		S

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Therefore, the value of any biosecurity system is based on assessment of risk and cost. For water disinfection systems both capital and operating costs are directly dependent upon:

1. Process flow rate (gpm)
2. Water composition
3. Reliability of equipment components
4. Selection of disinfectant
5. Desired level of
   a. Level of assurance (probability) water will be free of pathogens
      i. ie: 90% - > 99.99% inactivation
      ii. System efficacy and reliability.
   b. Automation and instrumentation
   c. Fail-safe options
6. Pretreatment requirements
7. If and to what degree, the installation may (+/-) affect:
   a. Livestock performance metrics
   b. Operating cost(s)

As described above, while most, if not all current installations within the livestock industry may not provide much in the order of primary disinfection, it is evident many have served to provide other favorable outcomes in regard to livestock performance metrics and operating costs (ie, cleaning or replacement of plugged water fixtures, evaporative cool cells, etc). Meaning, drinking water "disinfection" even with a minimal degree of efficacy has proven to be a component of a viable economic model beyond serving as a measure of biosecurity.

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