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The effect of Virkon®S fogging on survival of *Salmonella enterica* and *Staphylococcus aureus* on surfaces in a veterinary teaching hospital

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Abstract

The objective of the study was to determine the disinfection efficacy of aerosolizing (cold fogging) Virkon®S on survival of *Stahpylococcus aureus* and *Salmonella enterica* on different surfaces. Two experiments were conducted in different locations. *Salmonella enterica* and *S. aureus* were grown in broth culture and then seeded into pre-marked areas in each location and allowed to dry. Virkon®S (1%) was aerosolized into the rooms (approximately 1 L of per 30 m^3). Samples were collected pre- and post-fogging for quantitative cultures to evaluate the efficacy of aerial disinfection. The reduction of *S. enterica* or *S. aureus* counts ranged from 3.40 to 0.95 $\log_{10}$ (*Salmonella*) or 4.92 to 0.02 $\log_{10}$ (*Staphylococcus*). The greatest reduction was evident in samples collected from non-porous horizontal surfaces, which were not obstructed from the air flow. These results indicate that fogging with Virkon®S could be beneficial in routine disinfection of pre-cleaned surfaces. The benefits of routine use of cold fogging with Virkon®S in veterinary hospital settings would include its wide-range antimicrobial action and minimal working-men power required to disinfect large areas. Also, fogging would potentially minimize microbial contamination in the hard to reach areas.

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1. Introduction

Several different bacteria including *Salmonella* and *Staphylococcus* species have been associated with outbreaks of nosocomial infections among patients in veterinary hospitals (Castor et al., 1989; Tillotson et al., 1997; Seguin et al., 1999; Anon., 2001; Schott et al., 2001). Nosocomial infections with *Salmonella* and *Staphylococcus* species have also been reported in human health care settings (Cetinkaya et al., 2000; Spearing et al., 2000; Fung et al., 2001; Olsen et al., 2001; Bornemann et al., 2002). Increasing numbers of outbreaks of methicillin (oxacillin) resistant *S. aureus*

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(MRSA) in both human and veterinary health care settings are of particular concern (Hartmann et al., 1997; Seguin et al., 1999; Cetinkaya et al., 2000; Fung et al., 2001). The Large Animal Hospital at the James L. Voss Veterinary Teaching Hospital at Colorado State University (JLV-VTH) was closed in 1996 and 2001 in order to mitigate against nosocomial spread of *Salmonella* among hospitalized animals. The 1996 outbreak resulted in partial or total hospital closure of large animal facility over a 3-month period and cost more than an estimated $500,000. Total losses were greater than this because this estimate only included expenses related to mitigation, and did not include opportunity losses such as lost revenues. The results of a survey conducted in 1997 among veterinary teaching hospitals showed that 12 of 18 respondents reported 18 outbreaks of nosocomial disease in the period between 1985 and 1996. Seventy-eight percent of outbreaks were contributed to *Salmonella* infection and six of these outbreaks resulted in hospital closure (Morley, 2002).

Several protective measures can be implemented in order to control or minimize risks of nosocomial spread of infectious agents between veterinary patients, but adequate cleaning and disinfection are of utmost importance (Morley, 2002). Contamination with environmentally adapted organisms can be difficult to eliminate using routine cleaning and disinfection procedures. For example, a single strain of *Salmonella Heidelberg* was isolated from horses admitted to one veterinary hospital in Australia over a period of 6 years (Amavisit et al., 2001). From authors' personal experience at JLV-VTH, it has been necessary at times to ''deep clean'' the facility which involved completely emptying rooms including removal of all furniture, equipment and materials and thoroughly scrubbing all the surfaces before applying disinfectant. This is very disruptive to normal operations and almost always requires reduction in number of patients admitted to the hospital. Therefore, it would be useful to identify a method of disseminating disinfectant that would be less disruptive to normal functioning of the hospital and would not require complete removal of all equipment from an area to be disinfected.

Virkon®S (Antec International) is a broad spectrum disinfectant containing peroxygen compounds (peroxymonosulfate). It is marketed as a bactericidal, virucidal, fungicidal and sporicidal agent for use in human and veterinary health settings. Recently, cold fogging (low temperature aerosolization) has been described as a method for disinfection of airborne infectious agents such as foot-and-mouth disease virus or influenza (Antec International, http://www.antecint.co.uk/main/virkons.htm). The generation of small particle size (5–50 μm) aerosols has several attractive properties including dissemination of disinfectant into hard-to-reach areas (such as around pipes, lighting fixtures, or on elevated surfaces), reduction of the airborne infectious agents, and reduction of dust particles (which can carry infectious agents or chemical irritants/toxins). Additionally, cold fogging has a potential to deliver disinfectant to an enclosed area with minimal disruption to daily routines and operation in that area. Therefore, the purpose of this study was to evaluate the efficacy of cold fogging with Virkon®S on survival of *S. areus* and *S. enterica* on different types of surfaces at JLV-VTH.

2. Materials and methods

Two separate experiments were performed testing for survival of *Salmonella* and *Staphylococcus*. The design of both trials was similar and included inoculation of a predetermined number of bacteria onto a 25 cm^2 square marked with a white type on various types of surfaces in various locations, followed by recovery by swabbing and quantitative bacteriological cultures. The trial evaluating efficacy against *S. aureus* was performed in the small animal critical care unit (CCU) of the JLV-VTH, while a trial evaluating efficacy against *S. enterica* was performed in a stall used for isolation of large animal (LA) patients.

2.1. Stock cultures

S. aureus (ATCC strain 29213) or *S. enterica* serotype Typhimurium (ATCC strain 43971) were grown overnight in 5 mL of tryptone soya broth at 37 °C. A 100 μL aliquot ($\approx 10^8$–10^9 colony forming units (cfu) of *S. enterica* or 4.5×10^7 cfu of *S. aureus*) of bacterial culture was dispersed onto each of pre-selected and pre-cleaned areas in several locations (see details below) and allowed to dry before collection and fogging began.

2.2. Fogging

Fogging was performed using a Dyna-Fog Cyclone® fogger (Curtis) with 1% Virkon®S (approximately 1 L per 30 m^3) at the maximum volume rate setting (approximately 4 L/h). All doors, windows and ventilation outlets in an area to be fogged were closed for the time of fogging and for at least 2 h following fogging. Access to fogged areas was restricted during and following fogging. Protective equipment such as nitrile gloves or NIOSH N95 disposable particulate respirators (Moldex-Metric Inc., Culver City, CA) was available to anybody who was required to access the fogged areas either during or immediately after fogging. This included personnel refilling and replacing the fogger and persons collecting swab samples.

Fogging in CCU was performed from two different positions. To facilitate air flow, three box fans were placed in the CCU during fogging. Fogging in LA isolation stall was performed from one position only without utilization of box fans (Fig. 1).

2.3. Recovery of bacteria

Bacteria were recovered using pre-moistened sterile cotton swabs into 2 mL of neutralizing broth (NB, Beckton Dickinson) which contained neutralizers for common disinfectants. After collection, serial 10-fold dilutions of NB were prepared, spread onto tryptic soy agar with 5% sheep blood (TSA, BBL) and Hektoen enteric agar (HE, BBL) plates (*Salmonella*) or just TSA plates (*Staphylococcus*). Plates were incubated overnight at 37 °C. The estimated concentration of recovered bacteria was calculated based on colony counts read the following day.

2.4. Locations in which Salmonella and Staphylococcus were seeded

2.4.1. Staphylococcus trial

Six different locations in a CCU area were chosen and labeled A through F (Fig. 1a). The locations consisted of a varnished wooden bench (A), painted wooden door (B), vinyl floor (C), laminate floor of one of small animal cages (D), laminate wall of the same cage (E) and an untreated wooden computer desk in an adjacent office (F). In each location, three 25 cm^2 square areas were marked, labeled #1 through #3, and pre-cleaned with 70% ethanol. One area (#3) in each location was covered with sterile foil after *S. aureus* had been seeded.

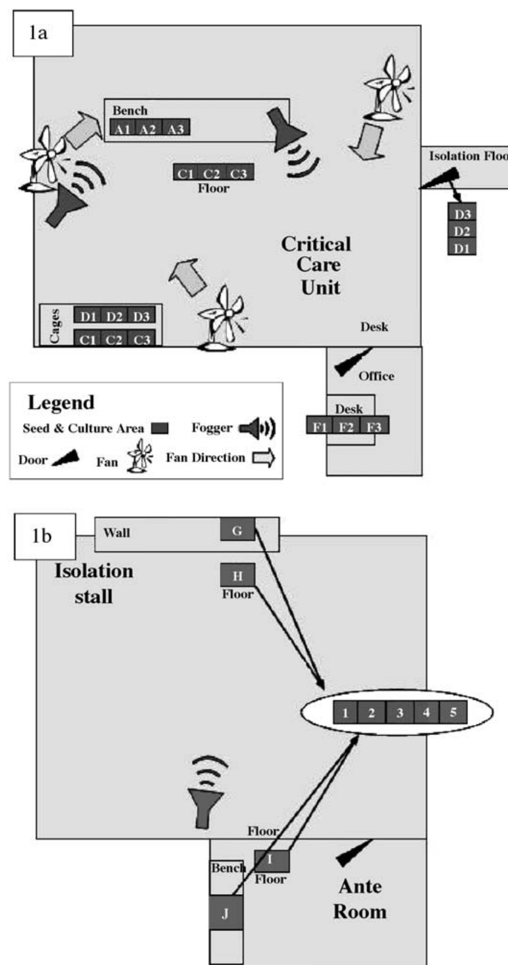

Fig. 1. Schematic diagram of locations where *Staphylococcus aureus* or *Salmonella enterica* were seeded in the critical care unit (CCU, a) or large animal isolation (b), respectively. Positions of fans and a fogger are shown. The position of a fogger was changed midway during fogging in CCU—both positions are shown. Locations A through J are depicted as gray rectangles. Locations A–F consisted of three areas (#1–3) each, locations G–J consisted of five areas (#1–5) each, which is shown in an oval insert.

2.4.2. Salmonella trial

Four different locations in a LA isolation stall were chosen and labeled G through J (Fig. 1b). Location G was a painted brick wall, locations H and I were concrete floors, and location J was a stainless steel

bench. Locations G and H were in the isolation stall, while locations I and J were in the anteroom. In each location, five 25 cm^2 square areas were marked, labeled #1 through #5, and pre-cleaned with 70% ethanol. No *Salmonella* was seeded in areas #5 in any of the locations (negative control).

2.5. Experimental timeline

2.5.1. Staphylococcus trial

The entire CCU area was fogged with 8 L of 1% Virkon®S (approximately 1 L/m^3). The placement of the fogger was changed after the first 4 L of Virkon®S were fogged into a CCU area (Fig. 1a). The following samples were collected from each location: prior to fogging (area #1), 2 h after finish of fogging (area #2) and 2 h after finish of fogging from the area covered with foil (area #3).

2.5.2. Salmonella trial

The entire area was fogged with 4 L of 1% Virkon®S (approximately 1 L/m^3). The following samples were collected from each location: prior to fogging (area #1), 0.5 h after start of fogging (area #2), 2 h after finish of fogging (areas #3 and #4), and 2 h after finish of fogging from the area that had not been seeded with *Salmonella* (area #5). A separate experiment was set up to serve as a positive control. *S. enterica* culture was deposited in two areas only in each location, equivalent to areas #1 and #3 above. *Salmonella* was collected from these areas directly after drying (area #1) and three hours later (area #3) to evaluate the possibility that reduction in bacterial counts observed after fogging was attributable entirely to the environmental conditions.

3. Results

The results are presented as $\log_{10}$ reduction in bacteria counts before and after fogging (Tables 1–4). In all areas and locations a reduction of bacterial counts was observed after fogging. The results from TSA plates were comparable with those from HE plates for the *Salmonella* trial. Bacteria were not isolated from clean areas (#5 in *Salmonella* trial) indicating that pre-cleaning with ethanol was successful in eliminating background contamination. Bacterial counts obtained from areas #1 (pre-fogging) were comparable with the estimated numbers of deposited bacteria indicating that methods employed were effective for recovery of seeded bacteria.

For the *Staphylococcus* trial, the greatest reduction was observed in location A (4.9 logs), followed by locations C (2.5 logs), B (2.1 logs), D (2.0 logs), E (1.5 logs) and F (0.02 logs). For the *Salmonella* trial, the greatest reduction was evident in samples collected from location H (3.23 logs), followed by the samples from locations I (2.13 logs), J (1.71 logs), and G (0.96 logs). With the exception of the untreated wooden desk in CCU (location F), reduction in bacterial counts was greater on horizontal surfaces in comparison with vertical surfaces in both CCU and LA isolation.

4. Discussion

The purpose of this study was to evaluate efficacy of cold fogging with Virkon®S on survival of *S. enterica* and *S. aureus* on various surfaces in JLV-VTH. The results indicated that fogging with Virkon®S was effective in reducing bacterial load

Table 1
Recovery of *Staphylococcus aureus* on TSA plates from locations A through F at critical care unit (CCU). At each location 4.5×10^7 cfu of *S. aureus* was deposited in areas #1–3

Area	Location					
	A[a]	B[a]	C[a]	D[a]	E[a]	F[a]
#1 (before fogging)	2.0×10^8	8.2×10^6	7.2×10^5	1.0×10^8	9.2×10^5	4.2×10^7
#2 (after fogging)	2.4×10^3	7.2×10^4	2.4×10^3	9.2×10^5	2.8×10^4	4.0×10^7
#3 (after fogging - foil)	3.2×10^7	4.0×10^6	2.4×10^6	3.6×10^7	1.4×10^6	1.6×10^6
log reduction #1/#3	0.79	0.31	−0.52	0.44	−0.18	1.42
$\log_{10}$ reduction #1/#2	4.92	2.06	2.48	2.04	1.52	0.02

[a] Results are presented as colony forming units (cfu) recovered per sample.

Table 2
Recovery of *Salmonella enterica* on TSA plates from locations G (painted wall in main isolation), H (floor in main isolation), I (floor in ante room) and J (bench in ante room)

Area/time[a]	Location			
	G[b]	H[b]	I[b]	J[b]
#1/0 h	1.7×10^8	4.3×10^9	1.7×10^9	1.1×10^9
#2/0.5 h	$>6 \times 10^7$	$>6 \times 10^7$	5.0×10^7	3.3×10^7
#3/2 h	8.3×10^6	6.4×10^6	5.3×10^6	1.5×10^7
#4/2 h	3.0×10^7	3.0×10^6	1.3×10^7	1.8×10^7
#3/4 h	1.9×10^7	4.7×10^6	8.9×10^6	1.6×10^7
#5/2 h[c]	$<2 \times 10^2$	$<2 \times 10^2$	$<2 \times 10^2$	$<2 \times 10^2$
$\log_{10}$ reduction[d]	0.95	2.96	2.28	1.83
Adjusted[e]	0.76	2.68	2.24	1.40

At each location 2.7×10^8 cfu of *S. enterica* was deposited in each of the areas labeled #1 through #5.

[a] Time of sample collection after start of fogging. Time 0 denotes collection before the start of fogging.

[b] Results are presented as colony forming units (cfu) recovered per sample.

[c] Area #5 was not seeded with *Salmonella*.

[d] $\log_{10}$ reduction between recovery of *S. enterica* before fogging (0 h) and 2 h after fogging.

[e] $\log_{10}$ reduction between recovery of *S. enterica* before fogging (0 h) and 2 h after fogging adjusted by the amount of reduction in recovery after 3 h without any treatment (Table 4).

Table 3
Recovery of *Salmonella enterica* on hektoen enteric plates from areas G (painted wall in main isolation), H (floor in main isolation), I (floor in ante room) and J (bench in ante room)

Area/time[a]	Location			
	G[b]	H[b]	I[b]	J[b]
#1/0 h	7.4×10^7	2.0×10^9	1.9×10^8	1.2×10^8
#2/0.5 h	5.0×10^7	$\geq 6.0 \times 10^7$	2.3×10^7	1.5×10^7
#3/2 h	2.6×10^6	1.0×10^6	2.1×10^6	2.7×10^6
#4/2 h	1.3×10^7	6.0×10^5	2.8×10^6	4.4×10^6
#3/4 h	7.7×10^6	8.2×10^5	2.4×10^6	3.5×10^6
#5/2 h[c]	$<2 \times 10^2$	$<2 \times 10^2$	$<2 \times 10^2$	$<2 \times 10^2$
$\log_{10}$ reduction[d]	0.98	3.40	1.90	1.53
Adjusted[e]	0.70	2.80	1.57	0.97

At each location 2.4×10^8 cfu of *S. enterica* was deposited in each of the areas labeled #1 through #5.

[a] Time of sample collection after start of fogging. Time 0 denotes collection before the start of fogging.

[b] Results are presented as colony forming units (cfu) recovered per sample.

[c] Area #5 was not seeded with *Salmonella*.

[d] $\log_{10}$ reduction between recovery of *S. enterica* before fogging (0 h) and 2 h after fogging.

[e] $\log_{10}$ reduction between recovery of *S. enterica* before fogging (0 h) and 2 h after fogging adjusted by the amount of reduction in recovery after 2 h without any treatment (Table 4).

on the surfaces tested. However, the level of reduction differed among sites. The lowest reduction in *Salmonella* counts was observed in location G, which was a painted wall surface (0.95 and 0.98 logs for TSA and HE plates, respectively), whereas the highest reduction occurred in location H—floor in main isolation (2.96 and 3.40 logs for TSA and HE plates, respectively). This suggested that prolonged contact between Virkon®S and *S. enterica* that was provided by droplets accumulating on the horizontal surfaces was important in inactivating *Salmonella*. This relationship was less obvious in results from the *Staphylococcus* trial in the CCU. While the highest reduction in *S. aureus* counts was observed on a horizontal surface in location A (varnished wooden bench—4.92 logs difference), the lowest reduction in *S. aureus* counts was observed on another horizontal surface in location F (unpainted wooden desk—0.02 logs difference). Thus, it appeared that for *S. aureus*, other factors, apart from the position of the surface (horizontal versus vertical) were more important for the efficacy of fogging.

Such factors may have included a type of material from which the surface was made (stainless steel versus wood) and the dynamics of air flow in the fogged room, which would have influenced the dispersal of Virkon®S to various areas. It may be important to take these factors into consideration when contemplating using fogging for routine disinfection. In CCU, three fans were used in order to facilitate air flow and thus dispersal of Virkon®S. The highest reduction in bacterial counts was observed in areas positioned closest to the fogger in the direction of the airflow. Additionally, the lowest reduction in bacterial counts was observed on a wooden desk, emphasizing the importance of avoiding porous surfaces in clinical settings. Such surfaces are more difficult to disinfect than non-porous surfaces such as stainless steel (Mafu et al., 1990).

Although positive control for the *Salmonella* trial was performed separately to the main experiment, spontaneous dying of *S. enterica* within time of fogging is unlikely, and this was confirmed further by harvesting *S. enterica* 3 h after seeding without any treatment from

Table 4
Recovery of *Salmonella enterica* on hektoen enteric (HE) and tryptic soy agar (TSA) plates (positive control) from areas G (painted wall in main isolation), H (floor in main isolation), I (floor in ante room) and J (bench in ante room)

Area	Location			
	G[a]	H[a]	I[a]	J[a]
TSA plates				
#1	2.5×10^7	2.7×10^7	2.9×10^7	9.8×10^7
#3	1.6×10^7	1.4×10^7	2.6×10^7	3.6×10^7
$\log_{10}$ reduction	0.19	0.28	0.04	0.43
HE plates				
#1	1.1×10^7	2.0×10^7	1.4×10^7	4.8×10^7
#3	5.8×10^6	5×10^6	6.6×10^6	1.3×10^7
$\log_{10}$ reduction	0.28	0.60	0.33	0.56

[a] Results are presented as colony forming units (cfu) recovered per sample. At each location 1.3×10^9 (TSA plates) or 6.9×10^8 (HE plates) cfu of *S. enterica* was deposited in areas #1 and #3.

the same surfaces as those used for the main trial (Table 4). Reduction in *S. enterica* counts observed on both TSA and HE plates in these settings was less than 0.6 logs in all locations and on all surfaces tested. This reduction in bacterial counts was accounted for in estimated adjusted reduction levels for areas fogged with Virkon®S (Tables 2 and 3). However, since the two experiments were performed at different times, the numbers in Table 4 should serve only as an indication that the reduction in bacterial counts of *S. enterica* was negligible without any intervention.

The choice of using Virkon®S (Antec International) as a disinfectant in this study was dictated by its reportedly high disinfection efficacy, broad spectrum of action and good safety characteristics. Virkon®S is a stabilized blend of peroxygen compounds, surfactant, organic acids, and inorganic buffering compounds; the primary active ingredient is potassium peroxymonosulphate. It is sold as a powdered concentrate and is generally used as a 1% aqueous solution. Virkon®S is sold as a disinfectant in about 100 countries for use in animal handling/housing facilities, veterinary hospitals, human health facilities, and research laboratories. Virkon®S acts as a disinfectant through oxidation effects, similar to hydrogen peroxide, resulting in loss of cell wall integrity and inhibition of enzyme systems. It is generally believed that micro-organisms are less likely to develop resistance mechanisms to oxidation or drying effects compared to other disinfectants such as the quaternary ammonium compounds, for which genetic mechanisms for resistance are fairly common and widely disseminated. Results of numerous laboratory trials published on the manufacturer's web page showed that Virkon®S has a wide spectrum bactericidal, virucidal, sporicidal and fungicidal activity including important respiratory and enteric agents that are of great concern to the JLV-VTH and other veterinary hospitals (including *Salmonella*, *E. coli*, *Pseudomonas*, *Klebsiella*, *Enterococcus*, *Streptococcus*, *Staphyloccus*, *Bordetella*, *Pasteurella*, West Nile virus, rhabdoviruses, lentiviruses, pestiviruses (bovine viral diarrhea virus, swine fever, equine infectious anemia virus), and many of the animal and human agents of concern from the bioterrorism/biowarfare point of view) (Antec International web page). The few studies published in peer-reviewed journals confirmed bactericidal and virucidal activities of Virkon®S (Walker et al., 1992; Gasparini et al., 1995; Herruzo-Cabrera et al., 1999; Hernandez et al., 2000). The efficacy studies of Virkon®S against mycobacteria, spores and fungi produced more variable results (Broadley et al., 1993; Herruzo-Cabrera et al., 1999; Hernandez et al., 2000). The discrepancies between different investigations most probably reflect different experimental conditions including hardness of water used for re-suspension of Virkon®S, types of surfaces, strains of microorganisms tested or differences in testing conditions including temperature, time of exposure, methods of recovery and others. Since "real life" conditions are even more variable, field efficacy of Virkon®S, or any other disinfectant, would be expected to vary between establishments and may not show the same effectiveness as demonstrated in in vitro experiments.

While "field testing" can theoretically provide valuable information regarding efficacy of a given method of disinfection under condition specific to a given environment, this kind of disinfectant testing carries inherent problems related to repeatability of results. The accuracy of testing is affected by many variables including environmental conditions such as temperature or humidity or ability to recover organisms from surfaces by swabbing. Nonetheless field testing provides important verification of the more standardized methods such as suspension tests or

carrier tests. In our study, the numbers of bacteria recovered from the testing areas before fogging were within 1 $\log_{10}$ of the estimated numbers of bacteria that had been deposited in each area on 11 out of 14 occasions (counting *Salmonella* results on TSA plates separately from the results on HE plates). This indicates that our swabbing technique allowed for consistent recovery of live bacteria from the testing areas.

One of the major problems associated with both in vitro and in vivo testing of efficacy of disinfection against selected microorganisms is difficulty in experimentally reproduce real life conditions. In our experiments we used commercially available strains of *S. aureus* and *S. enterica*. These strains are culture adapted and possibly behave differently than some of the pathogenic strains recovered from the JLV-VTH environment in the past. On the other hand, the use of commercially available strains make our study more reproducible. For example, it would be possible for others to compare our results with the results they may observe using the same methods in a different environment. Also, use of commercially available strains enabled us to optimize the recovery of bacteria, as some of the ''field'' strains may be less culture adapted, and therefore more difficult to quantitatively recover from the environment (Cremieux et al., 2001).

In addition to the possibility that bacterial strains recovered from the environment may have different characteristics to the culture adapted strains, bacteria in the natural environment often survive by forming biofilms. Biofilms are organized bacterial communities that are attached to a surface and produce an extracellular matrix, which enhances their survival (Hall-Stoodley et al., 2004; Joseph et al., 2001; Fux et al., 2003; Parsek and Singh, 2003). Bacterial biofilms are more resistant to antimicrobial treatement than their planktonic equivalents (Joseph et al., 2001). Although bacterial biofilms likely play an important role in bacterial survival, persistence and pathogenicity, the study of biofilms is limited by lack of appropriate experimental models. This is mostly due to a very complex nature of biofilms. It has been shown that the characteristics of a biofilm are dependent on many factors including, but probably not limited to, the type of surface, type of bacteria and environmental conditions such as availability of nutrients and moisture (Parsek and Singh, 2003; Prouty and Gunn, 2003). While several in vitro systems of biofilm production have been developed, including culture on disks in microtitre plates, the complex nature of bacterial populations in biofilms makes every biofilm likely to have its own characteristics that are nearly impossible to fully reproduce in the laboratory. Thus, the efficacy of fogging may be different when applied to bacterial population organized in a biofilm to that observed in our study. In a recent paper (Ramesh et al., 2002) the authors investigated susceptibility of *Salmonella* contaminated stainless steel discs to various disinfectant. The steel surfaces were contaminated with *Salmonella* by either deposition of broth-grown bacteria mixed with organic matter or by establishment of *Salmonella* biofilms in a microtitre plate. Two out of 12 disinfectant tested were found to be highly effective in eliminating *Salmonella* biofilms with the reduction of more than 7 $\log_{10}$. Interestingly, the same disinfectants showed only 1–3 $\log_{10}$ reduction when applied to the same surfaces contaminated with the bacterial broth/organic matter mixture, further confirming that the interactions between disinfectant and bacterial populations are complex.

The benefits of routine using of cold fogging with Virkon®S in veterinary hospital settings would include its wide-range antimicrobial action and minimal number of people required to disinfect large areas. Also, fogging would potentially minimize microbial contamination in the hard to reach areas. Virkon®S has very low toxicity and is biodegradable and thus environmentally friendly (Antec International web page). It has been reportedly used in the presence of animals without adverse effects (Sainsbury, Antec International web page). However, there are no reports in peer-reviewed journals regarding Virkon®S safety when used in this manner over a period of time. Until such data become available it would be prudent to remove animals from fogged buildings whenever possible. Also, although 1% Virkon®S solution is classified as non-irritant and non-toxic, proper protective clothing including masks and eye protection is recommended by the manufacturer.

A number of safety precautions can be implemented in order to minimize any potentially hazardous effects of exposure to aerosolized Virkon®S. These

include closing off and clearly marking the areas to be fogged, fogging at night when there is minimal numbers of personnel present, advance notice to all personnel that will be present during fogging in order to facilitate possible personnel changes (to enable individuals allergic to some of the compounds avoid working in the proximity of the fogged area), removal of patients from the fogged areas in order to minimize any risks associated with exposure to Virkon®S and also to alleviate potential need for the VTH personnel to work with them during fogging, availability of approved respiratory protective devices to all personnel involved with fogging or personnel required to enter fogged areas before they have been completely ventilated, availability of ear plugs, and allowing enough time for ventilation before opening the areas for unrestricted traffic.

While two to three-fold reduction in bacterial counts may not seem very efficient, this reduction was obtained in experimental conditions, where *Salmonella* spotted onto surfaces was at very high concentration, optimal viability, and supplied with protective and nourishing microenvironment of tryptone soya broth. These will not be the conditions encountered in the ''real world'', where the numbers and viability of *Salmonella* should be minimized by routine cleaning and disinfection procedures. Also, it may be useful to remember that 2–3 $\log_{10}$ reduction corresponds to 99.0–99.9% reduction in bacterial counts. Thus, fogging of an area with a high initial load would still result in detectable contamination; lighter loads may be reduced to undetectable (and possibly unimportant) levels of contamination. In addition, bacterial concentrations greater than 10^7 cfu/mL can induce ''inoculum effect'' where efficacy of the tested disinfectant is reduced (Gilbert et al., 1987). Since more than 10^7 cfu/mL of both *S. enterica* or *S. aureus* was used to seed the bacteria in the testing areas, it is possible that larger reduction would occur during routine in-hospital applications when fogging is applied to clean surfaces. Also, even two to three-fold reduction may be very beneficial, especially if fogging was to be applied several times to the same area, for example, in the situation when increased biosecurity efforts are needed due to an outbreak of an infectious disease. On the other hand, bacteria in the environment are often organized in biofilms, which may be potentially less susceptible to the action of Virkon®S. The susceptibility of bacterial biofilms to fogging has not been addressed in this study and warrants further investigation. Taken together, the results of this study indicate that fogging alone should not be used as a sole means of cleaning and disinfection, but rather as a useful adjunct to further minimize bacterial contamination on pre-cleaned surfaces. The manufacturer of Virkon®S recommends fogging as a way of controlling respiratory infections in veterinary settings. While this aspect of cold fogging was not evaluated in this study, it would seem to be a potential additional benefit of routine fogging with Virkon®S, particularly in areas where animals showing signs of infectious respiratory disease are stabled.

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