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Routine Decontamination of Working Canines: A Study on the Removal of Superficial Gross Contamination

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Odor detection canines are a valuable resource used by multiple agencies for the sensitive detection of explosives, narcotics, firearms, agricultural products, and even human bodies. These canines and their handlers are frequently deployed to pathogen-contaminated environments or to work in close proximity with potentially sick individuals. Appropriate decontamination protocols must be established to mitigate both canine and handler exposure in these scenarios. Despite this potential risk, extremely limited guidance is available on routine canine decontamination from pathogenic biological materials. In this article, we evaluate the ability of several commercial off-the-shelf cleansing products, used in wipe form, to remove superficial contamination from fur, canine equipment, and toys. Using Glo Germ MIST as a proxy for biological contamination, our analysis demonstrated more than a 90% average reduction in contamination after wiping with a Nolvasan scrub solution, 0.5% chlorhexidine solution, or 70% isopropyl alcohol. Wiping with nondisinfectant baby wipes or water yielded an almost 80% average removal of contaminant from all surfaces. Additionally, researchers used Gwet's AC2 measurement to assess interrater reliability, which demonstrated substantial agreement ($P < .001$). These data provide key insights toward the development of a rapid, convenient, and fieldable alternative to traditional water-intensive bathing of working canines.

Keywords: Working canines, Decontamination, First responders, Veterinary medicine

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Introduction

For many years, odor detection canines have been used to identify a wide variety of scents, including those related to explosives, narcotics, agricultural products, and human bodies.[1-3] In many of these scenarios, no single piece of equipment has proven as precise and/or as mobile as a canine. More recently, a subset of odor detection canines has also been trained to rapidly detect the odor signatures of diseases like diabetes, bacterial infection, and certain types of cancer.[4-6] As a result of these successful forays into the medical field, the use of odor detection canines as a potential screening tool during the SARS-CoV-2 pandemic has garnered substantial attention. Deploying canines to potentially contaminated environments, however, increases the risk of pathogen exposure to both the canine and handler.[7,8] While the US Centers for Disease Control and Prevention currently considers canines to be at low risk of dog-to-human transmission of SARS-CoV-2, there remains a concern that contaminated surfaces, equipment, or fur may still act as fomites to transfer other bacterial, fungal, or viral pathogens.[9-12] To decrease the risk of secondary infection for both canines and handlers across all canine disciplines, appropriate decontamination protocols for the animal and its associated operational equipment must be established.

At this time, information about routine canine decontamination is extremely limited. Existing guidance in this space predominantly focuses on "one-off" cleaning procedures following exposures in emergency environments.[13-15] As full bathing of a canine after every shift is logistically burdensome, can lead to damage of fur and skin and could cause further handler exposure to pathogenic material, other approaches need to be considered. Soldiers commonly use wipe-down strategies to remove gross contamination in the field, which represents an appealing and practical alternative to full bathing for canines.[16] Here, we aim to identify an effective wipe-based protocol for routine decontamination of canines and their equipment using commercial off-the-shelf cleansing products. This protocol should decrease the risk of disease transmission and/or adverse health effects to canine–handler teams while maintaining operational deployment capabilities. We envision that this effort will inform the development of an effective canine decontamination methodology that can be applied across multiple odor detection canine fields, improving responses to current and emerging threats.

Materials and Methods

Tested Surfaces

For the purposes of this work, the term "coupon" is used to refer to small sections of each surface material cut from the respective full-sized items. To minimize risk to living animals, maintain sterile conditions, and improve standardization across trials, full-sized coyote pelts (Paulette Fur Company) served as a proxy for domestic canine fur. As canine dorsal, flank, and ventral fur differs in texture and thickness, 6.4 cm × 15.2 cm coupons were cut so as to include all 3 regions (see Figure 1).

TSA Handler Kits (Ray Allen Manufacturing, SKU RAM-K-TSA-2H) included 3 main components: dog harness, nylon collar, and leather leash. Harnesses were cut into 5.1 cm × 10.2 cm coupons that included a section of Velcro, military standard woven nylon webbing, and nylon straps. Leashes were cut into 10.2 cm coupons and the "smooth" and "rough" sides were tested separately. Collars were cut into 10.2 cm coupons with no alterations to width. Tennis balls (Quiet Glides, precut, gray, model T34GRY20) and KONG Classic dog toys (The KONG Company, item 53352) were cut in half lengthwise. Dummy bumpers (SportDOG, model SAC00-11672) were cut in half lengthwise and the white ends were removed to reduce autofluorescence during imaging. Examples of each toy and equipment coupon type are included in Figure 2. All coupons were used once and then discarded.

Cleansers and Wipes

Candidate cleansers were selected from a large number of candidates for testing based on safety, accessibility,

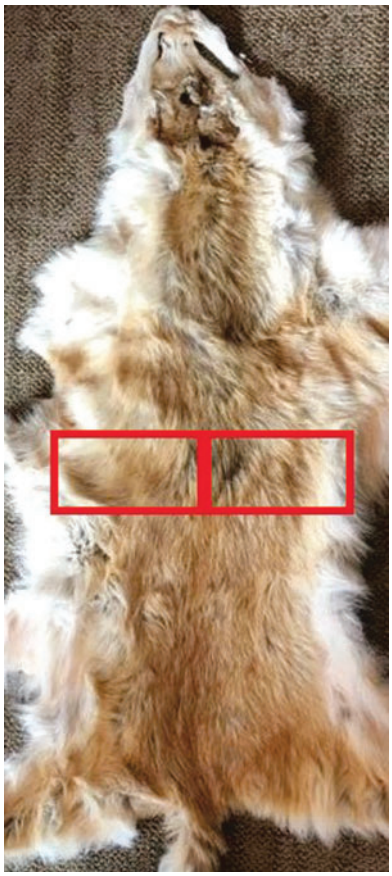

Figure 1. Representation of coyote pelt.

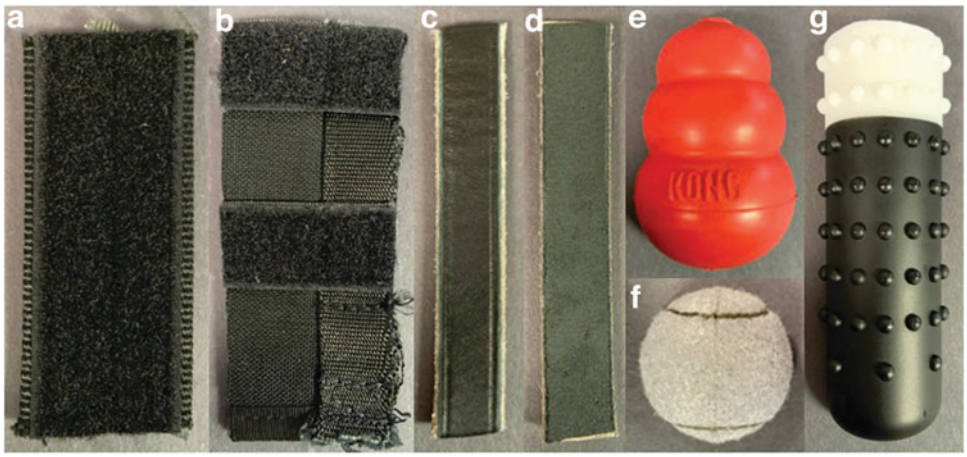

Figure 2. Representation of tested materials: (a) collar, (b) harness, (c) smooth side of the leash, (d) rough side of the leash, (e) KONG, (f) tennis ball, and (g) bumper.

antimicrobial/antiviral activity, and previous success in canine decontamination experiments.[14] Cleansers were included in the study only if they were labeled as safe for topical use on canines or commonly recognized as safe across the veterinary community. Deionized water (Milli-Q Water System) and perfume-free, water-based sensitive baby wipes (Pampers) were included as nondisinfectant solutions. Disinfectant solutions selected were: 70% isopropyl alcohol (Equate, model FG003032), Nolvasan 2% chlorhexidine acetate surgical scrub (Zoetis, product 1NOL411, diluted 1:4 in deionized water), 2% chlorhexidine gluconate solution (Durvet, NDC 30798-624-35, diluted 1:4 in deionized water), and Betadine 7.5% povidone-iodine surgical scrub (Purdue Frederick, item 25452, diluted 1:4 in deionized water). Diluted 6.15% sodium hypochlorite solution (household bleach) is an effective pathogen decontaminant and has previously been shown to be safe for topical use on canines, but can cause potential canine nose blindness.[17,18] For this reason, it was not considered in this study. After initial trials, Betadine was removed from the study because (1) the dark brown color of the solution masked the fluorescence emitted by the contaminant, and (2) it left a tacky brown residue on all surfaces, making it an impractical option for daily use in the field. All other disinfectant solutions were shown to not attenuate fluorescence at a 1:1 ratio of Glo Germ, used to simulate the spread of bacteria, to disinfectant.

Several strategies were considered for application of cleansing solutions. For fur, options included full bathing, low water bathing with a scrub brush, wet vacuum grooming, and general wiping. For equipment and toy cleaning, options included soaking in disinfectant solution, dishwasher or washing machine treatment, spraying surfaces with disinfectant solution, and general wiping. After evaluating all current recommendations, researchers selected wiping as the most promising strategy to pursue because of the low logistical burden and the ability of cleansing wipes to be used on all surfaces. Disposable soft-spun dry fabric wipes (Medline, Ultrasoft, model ULTRASOFT1013) were used for all experiments.

Chambers

Circular, 7.6 cm diameter holes were cut into the bottom of black plastic buckets (US Plastic Corporation, 3.5-gallon volume, item 1934) to construct contamination and imaging chambers. The inner edges of imaging chambers were lined with flexible UV Black Light Strips (Onforu, model ON-DT46-UV-US-NF) and laboratory tape marked the position of a cell phone camera. Black matte construction paper was placed under the imaging chamber to serve as a dark background for photos and to prevent UV light reflection. A second, shorter contamination chamber (Rubbermaid 9-cup food storage, ASIN B008HP7L1G) was created for the fur coupons to enable more concentrated application of contaminant. Representative images of the chambers are shown in Figure 3.

Contamination Simulant

Glo Germ MIST (Glo Germ Company, item RFMST) was selected due to its previous use as a superficial pathogen simulant in aerosol studies.[21-27] Fine mist spray bottles (Cosywell, 100 mL, ASIN B07R6KGRSW) filled with Glo Germ MIST solution were used to mimic aerosols produced by a cough or sneeze.[19,20] The proprietary Glo Germ solution does not provide specific information on all the constituents of the product, nor the specific fluorophore. For all canine toy and equipment coupons, the bottle was vigorously shaken and 2 sprays were applied from the hole at the top of a 30.5 cm tall contamination chamber (Figure 3c). After a 5-minute settling and 10-minute drying time, coupons were removed from the contamination chamber, transferred to the imaging chamber, and imaged before wipe decontamination.

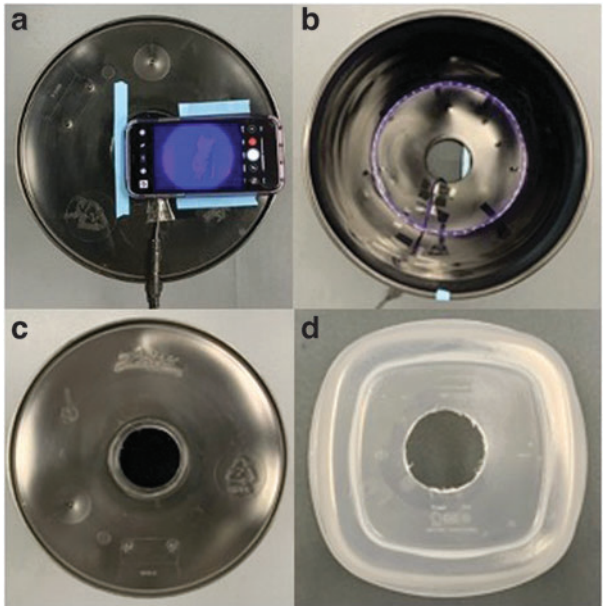

Figure 3. Imaging and contamination chambers: (a) external view of imaging chamber with phone in position, (b) interior of imaging chamber, (c) 30.5 cm height contamination chamber, and (d) 8.1 cm height contamination chamber.

Initial trials indicated that 2 sprays of Glo Germ MIST were not sufficient for fur coupon experiments, as color variation on the pelts caused issues during image analysis. Subsequently, 9 sprays were used on all fur coupons and applied using a shorter, 8.1 cm tall contamination chamber (Figure 3d). After a 5-minute settling period, the fur coupons were removed from the contamination chamber, set on a flat table, and dried for 1.5 hours with a small desk fan positioned across the table approximately 60.0 cm away from the coupons.

Droplet Size Distribution

The spray released from the fine mist spray bottle was characterized using the Spraytec particle and droplet sizer (Malvern Panalytical Ltd, model STP5342). The spray bottle was placed either 5.0 cm or 30.5 cm from the beam path and sprayed 4 to 5 times in quick succession. Data were reported as the fraction of total spray volume falling within each of 60 droplet size bins across a range of 0.1 µm through 900 µm at a time interval of 0.4 milliseconds (Figure 4). The droplet volume for each bin was divided by bin width, and droplet size distributions were generated for each spray event. Additionally, the volume produced by each spray from the bottle was measured volumetrically by spraying the bottle 10 times and measuring the volume discharged after each spray.

Decontamination

A 500 mL volume of each cleanser was placed in a plastic container and 25 wipes were added to the same container immediately before each trial, allowing for complete absorption of the liquid. Coupons contaminated with Glo Germ were placed face up on a clean absorbent pad and wiped, using moderate pressure, with 1 side of a clean wipe. The same researcher wiped all coupons to maintain consistency in pressure and approach. A standardized protocol was developed for each coupon type: (1) fur – a single downward wipe in the direction of the hair; (2) harness, leash, and collar – a single wipe, left to right, from one short edge of the coupon to the other; (3) tennis ball – a single wipe around the halved ball; (4) KONG toy – a single wipe from the smaller to larger diameter portion of the halved toy; and (5) bumper – a single wipe down the length of the halved toy, with special attention paid to the spaces between knobby protrusions. Following the initial wipe of all

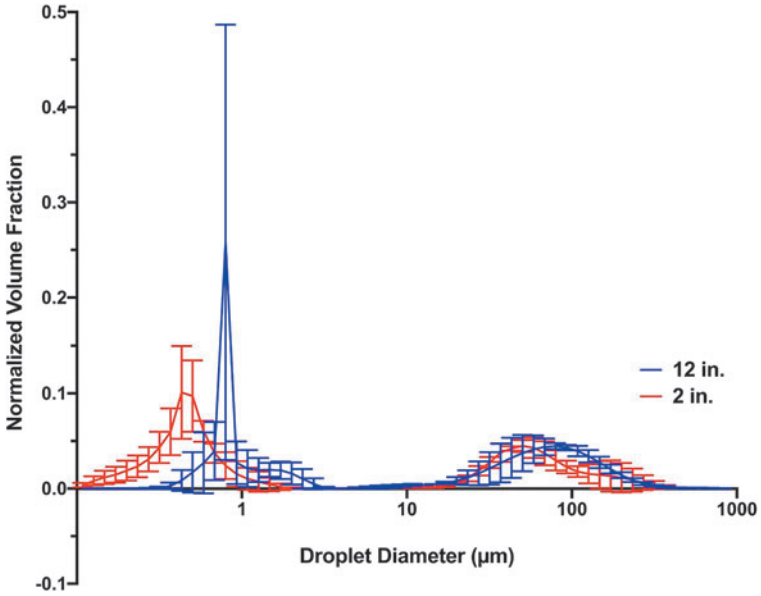

Figure 4. Size distribution of Glo Germ droplets produced by the Cosywell fine mist spray bottle. Droplets were sprayed at a distance of 5.0 cm or 30.5 cm from the Malvern Spraytec detector beam. Error bars represent standard deviation following 4 to 5 sprays at each distance.

coupon types, the dirty side of each wipe was folded in on itself and the coupons were wiped once more using the clean side.

Imaging

Researchers used the ProShot application on an iPhone XR to photograph a series of 3 images of each coupon. Images were captured before and after contamination, and after decontamination. The same focal plane, ISO, aperture, and shutter speed were used across all trials of the same coupon type. Images were imported and analyzed in Fiji, a biological image analysis platform based on ImageJ, originally developed by the National Institutes of Health.[28,29] The 3 images for each coupon were cropped to include only the region that underwent wiping and then compiled into a single stack. Analysis was performed using previously described methods and from ImageJ documentation.[30,31] Each image within a stack was converted from color to 8-bit to enable threshold selection. Thresholds were selected to minimize the background fluorescence of precontamination images while maximizing the Glo Germ-based fluorescence in postcontamination and postdecontamination images. The same threshold was applied to all images in a stack. Images were then converted to binary black and white images, and fluorescent area (in cm^2) and fluorescent particle counts were calculated using Fiji's Analyze Particles feature.

For each stack, postcontamination and postdecontamination values were normalized to the precontamination values. The reduction in fluorescence was reported as percentage of fluorescent area removed, calculated with the following equation:

$$Fluorescence\ Reduction = 1 - \left(\frac{A_{decontaminated} - A_{control}}{A_{contaminated} - A_{control}}\right)$$

For all trials, 2 team members independently selected thresholds and analyzed all images. The final reported results represent the average across these analyses. Fluorescence reduction ratings were divided into 5 quantiles: 0 to 20%, 21 to 40%, 41 to 60%, 61 to 80%, and 81 to 100%. Interrater agreement of category assignment was assessed using a linearly weighted Gwet's AC2 coefficient.[32] Fluorescence reduction ratings were imported into R version 4.0.5 (R Foundation for Statistical Computing, Vienna, Austria), and the Gwet's AC2 coefficient was evaluated using the irrCAC package.[33]

Results

Droplet Size Distributions

To determine whether Glo Germ MIST dispersal from an off-the-shelf spray bottle accurately recapitulated droplet sizes from human sneezes and coughs, a Malvern Panalytical Spraytec was used to measure droplet size distribution.[34] The spray bottle consistently produced a bimodal distribution of droplets, although actual droplet volumes varied based on the distance from the instrument. The smaller droplet mode peaked at diameters of 0.4 μm and 0.8 μm when sprayed 5.0 cm and 30.5 cm from the detector, respectively. The smaller diameter droplet mode did not appear in every spray from 30.5 cm, likely due to evaporation before contact with the detector. The larger droplet modes showed wider peaks, ranging from 40 μm to 70 μm at the closer distance and 70 μm to 200 μm at the farther distance. Previous Spraytec data has shown that 97% of the droplets produced by a human cough were smaller than 1 μm in diameter, with a mode of 0.3 μm, when initiated 17 cm from the detector.[35] This submicron droplet population is similar to the smaller mode produced by the fine mist spray bottle. Analysis of droplet size in human sneezes, also using the Spraytec, found both unimodal and bimodal volume distributions.[36] In bimodal sneezes initiated 5 cm from the detector, the most frequently observed droplets had diameters between 73.6 μm and 85.8 μm. This population overlaps with the larger diameter mode produced by the spray bottle. Together, these data suggested that the Glo Germ droplets produced by the spray bottle in these experiments closely represent the droplets produced by human coughs and sneezes in size and distribution.

Each spray released an average volume of 208.4 μL with a standard deviation of 0.516. The droplet size distribution applied to this spray volume produces approximately 3.0×10^5 droplets per spray at both distances with a mass median diameter of 0.637 μm for the 2-inch distance and 8.5 μm for the 12-inch distance.

Reduction in Fluorescence

To determine how best to remove gross superficial contamination from surfaces, coupons were sprayed twice with Glo Germ from a distance of 30.5 cm. After a 10-minute drying period, coupons were wiped twice with 1 of 5 solutions, as detailed in the Materials and Methods section. Special considerations were necessary for fur coupons, as individual pelts had differences in background fluorescence due to color variation. To mitigate these issues, researchers: (1) selected pelts based on consistent coloring, (2) increased the density of Glo Germ by applying 9 sprays from a distance of 8.1 cm, and (3) air-dried the wiped pelts before postdecontamination imaging. We found no difference in removal across the different fur samples based on region of the pelt used.

Each coupon was documented with a photo before and after contamination, and after decontamination. Background fluorescence thresholds were maintained across each coupon, and black and white images were used to determine the reduction in fluorescence following wiping. Figure 5 shows sample images of a leash coupon following treatment with a chlorhexidine wipe and Figure 6 shows sample images of a

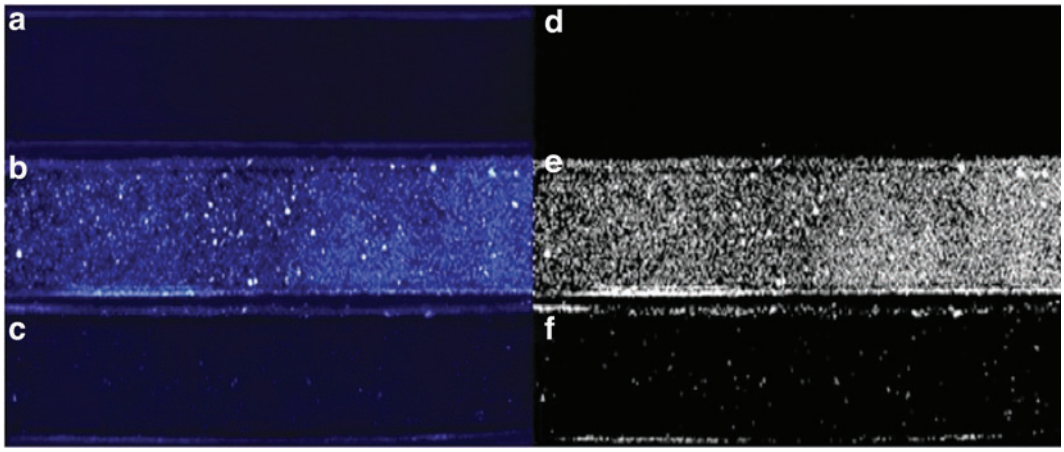

Figure 5. Contamination and decontamination of the smooth side of the leash with a chlorhexidine wipe. Images a, b, and c are before black and white conversion. Boxes a and d represent precontamination control images, b and e represent postcontamination images, and c and f represent postdecontamination images.

fur coupon following treatment with a water wipe. For all trials, 2 team members blinded to the contamination procedure individually processed the images.

The performance of all wipe solutions on all surfaces, as measured by percentage reduction in fluorescence, is shown in Figure 7. An average 86.1% of fluorescence was removed from surfaces following any wiping action, suggesting that this general strategy is an effective option for removal of gross surface contamination. Unsurprisingly, actual Glo Germ removal efficiencies varied between surfaces, with the more porous surfaces (ie, fur, tennis balls, and the soft side of the leash) proving more difficult to decontaminate. When all surfaces were considered, wiping with the 0.5% chlorhexidine solution provided the best option for gross decontamination, yielding 91.4% removal efficiency.

Interrater agreement was assessed using Gwet's AC2 coefficient using linear weights. This analysis was performed separately for fur samples and the equipment samples due to the high variability associated with the fur trials. For fur, 87.0% agreement was achieved (95% CI, 77.4% to

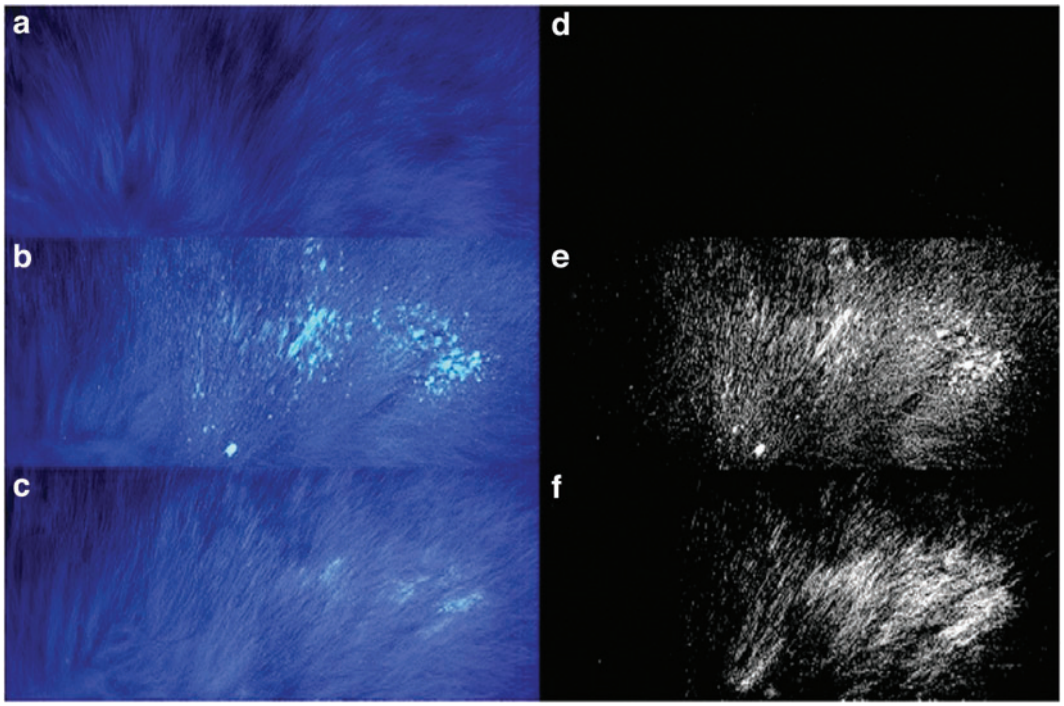

Figure 6. Fur contamination and decontamination with a water wipe. Images a, b, and c are before black and white conversion. Boxes a and d represent precontamination control images, b and e represent postcontamination images, and c and f represent postdecontamination images.

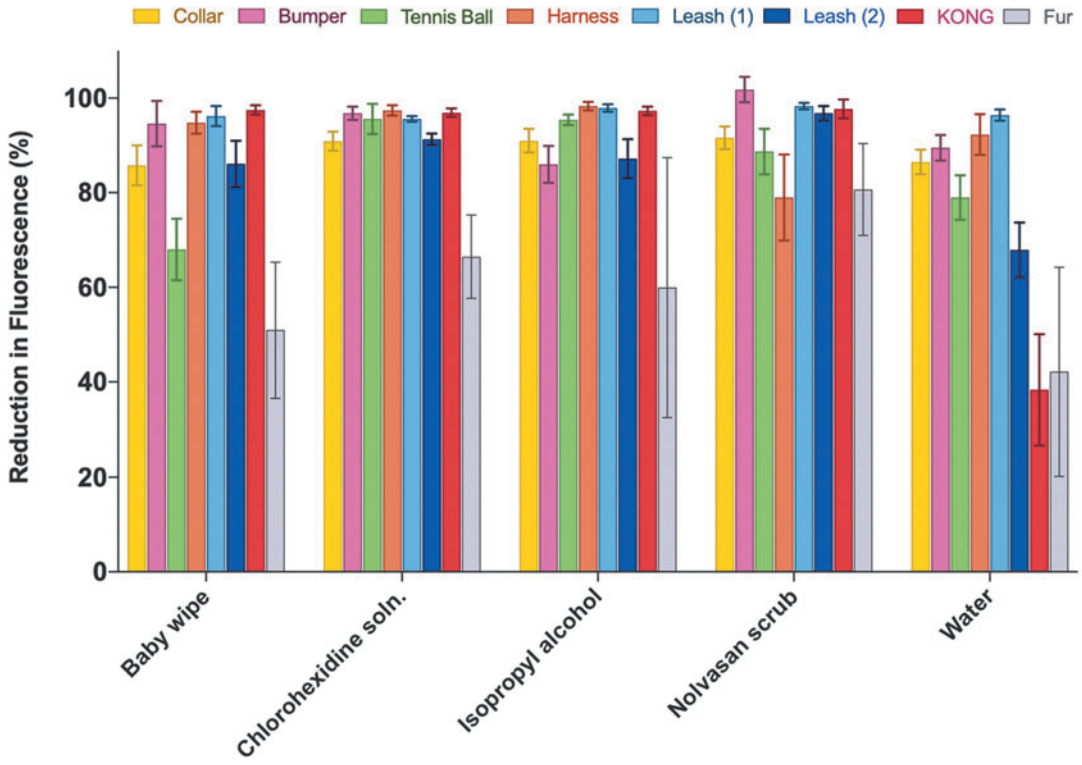

Figure 7. Efficacy of wiping across all coupon types, measured as a percent reduction in fluorescence. N = 3 for collar, bumper, tennis ball, harness, leash (side 1 and 2), KONG, and N = 5 for fur. Error bars represent standard deviation.

96.5%; $P < .001$). For the equipment samples, 95.1% agreement was observed (95% CI, 89.8% to 100.0%; $P < .001$). This demonstrates a high level of agreement between raters and provides additional confidence in the image analysis approach.

Discussion

The increased use of odor detection canines in environments with potential biological contamination necessitates the development of effective decontamination protocols compatible with routine use in the field. While previous work in this arena has shown that methods like high-pressure washing can be effective at cleaning even difficult-to-decontaminate porous surfaces, it is limited in its fieldability, and has the potential for unintentional aerosolization of contaminated particulates.[12,13] For these reasons, this study focuses on a flexible and logistically feasible wipe-based protocol.

Results show that this strategy is indeed effective, as an average of 86% of the Glo Germ proxy contaminant was removed from all tested surfaces, regardless of the solution used. The disinfectant solutions, which included a 0.5% chlorhexidine solution, 70% isopropyl alcohol, and Nolvasan scrub, were most effective, reducing Glo Germ fluorescence by approximately 91%. Due to the sticky residue left by the Nolvasan scrub, however, the chlorhexidine and isopropyl solutions appear to be the most practical candidates for routine decontamination. While the 70% isopropyl alcohol used in this study is designated for human use only, veterinary options are available and would be preferred.[37]

During testing, researchers observed that certain porous surfaces, including tennis balls, leather leashes, and fur, were more challenging to decontaminate. This finding is consistent with other studies and emphasizes the need for operators to carefully consider whether such materials should be decontaminated after working in potentially contaminating environments, or if they should be simply discarded and replaced.[12,13,38] In the case of tennis balls, for example, the risk of incomplete decontamination may outweigh the cost of replacing the item at the beginning of each working shift. For items like the currently fielded leather leashes, where financial constraints make daily replacement impractical, it may be prudent to consider alternate nonporous materials that are more easily decontaminated.

Conclusion

While findings from this study provide preliminary data to inform updated decontamination protocols, follow-on studies are necessary to determine if the reduction of superficial contamination observed here correlates with the removal and/or inactivation of actual infectious agents. The authors are currently completing experiments with live virus to directly address this question. Additional research

must also be performed using various working canine breeds to assure these protocols yield similar results on fur from living animals. Taken together, this body of work will help define the decontamination frequency and strategy (or strategies) best suited for work in the field, ensuring health security for working canines, their handlers, and the populations they serve to protect.

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