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THE EFFICACY OF CLEANING BIRD FEEDERS WITH 10 % BLEACH WIPES TO REDUCE BACTERIA[1]

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ABSTRACT

Many authorities recommend cleaning bird feeders regularly to prevent the transmission of disease at feeders, but the effectiveness of cleaning methods has not been studied. We tested the effectiveness of cleaning bird feeders with 10 % bleach wipes to reduce bacteria immediately after cleaning and over ten weeks when cleaning feeders every two weeks. Aerobic bacteria were significantly reduced on feeders immediately following cleaning with bleach wipes. Over ten weeks, aerobic bacteria on feeders were significantly reduced by cleaning with bleach wipes, but aerobic bacteria increased over time. There also was a significant interaction between cleaning and time on aerobic bacterial counts, which suggests that bleach wipes were effective in reducing aerobic bacteria in the first weeks of the study but became less effective over the long term. We suspect the loss of effectiveness was due to a buildup of organic matter on feeders over time. We found no significant relationship between animal activity (as indicated by seed consumption) and aerobic bacteria on cleaned and non-cleaned feeders, which indicates that aerobic bacteria were influenced by multiple environmental sources. By contrast, neither cleaning nor time significantly influenced Gram-negative bacteria on feeders. The lack of a significant influence on Gram-negative bacteria may have been due to the high variability in the numbers of Gram-negative bacteria on feeders and in the environment. However, we found that animal activity was significantly related to Gram-negative bacteria at non-cleaned feeders but not at cleaned feeders. The lack of a relationship at cleaned feeders suggests that cleaning with bleach wipes helped reduce Gram-negative bacteria on very active feeders. Overall, our results suggest that bleach wipes may be a simple and useful sanitization method for bird feeders if organic matter can be removed first using another cleaning technique. [J PA Acad Sci 88(4): 220-226, 2014]

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INTRODUCTION

Feeding wild birds is a popular activity throughout the western world (Jones and Reynolds 2008) and has become an important part of the economy. For example, in 2011, 50 million people maintained bird feeders in the United States and spent $4 billion on food alone (U.S Fish and Wildlife Service 2012). Individuals that feed birds gain pleasure from watching birds in their yards (Jones and Reynolds 2008) and wild birds benefit from this supplemental food source as well. Bird feeders provide food for birds when natural food sources are limited and can increase winter survival rates (Brittingham and Temple 1988, 1992). More recently, supplemental feeding has been shown to provide birds with more energy during the breeding season, which leads to increased reproductive success (Robb *et al.* 2008). Despite these benefits, most people do not realize that bird feeders may act as intermediates in the transfer of diseases (Luttrell 1997; Hess and Groskin 2006; Jones and Reynolds 2008). Bird feeders are very active locations where birds of many species are concentrated and have the potential to spread disease (Brittingham and Temple 1986).

A number of avian diseases may be transmitted among birds at feeders (Luttrell and Mead 2005). For example, major outbreaks of salmonellosis and mycoplasmosis in songbirds have been linked to feeders (Hartup *et al.* 1998; Tizard 2004). Because *Salmonella* is shed in feces, bird feeders contaminated with feces are a likely site of transmission to other birds (Friend and Franson 1999; Daoust and Prescott 2007). Although mycoplasmosis appears to be primarily spread through direct contact between infected birds (Friend and Franson 1999; Luttrell and Mead 2005), infected birds are known to linger at bird feeders where other birds are concentrated thereby increasing the likelihood of transmission (Fischer *et al.* 1997; Hartup *et al.* 1998). More recently, Dhondt *et al.* (2007) confirmed that transmission of mycoplasmosis can also occur via the physical surface of bird feeders. These major disease risks have to led some debate over whether or not the public should feed wild birds (Hess and Groskin 2006; Prescott 2002; Schreiber 2010).

To keep bird feeders clean and potentially decrease the spread of disease, many authorities recommend routine cleaning of feeders (Brittingham and Temple 1986;

Luttrell 1997; Friend and Franson 1999). These cleaning recommendations are disseminated to the public through popular bird watching organizations. For example, the Audubon Society recommends soaking bird feeders in 10 % bleach at least once or twice a month (Audubon Society 2004). Similarly, the Cornell Lab of Ornithology recommends cleaning feeders with hot soapy water every two weeks or with 10 % bleach if sick birds have been observed nearby (Cornell University 2011). Both organizations recommend removing seed hull debris on the ground below feeders as well. However, recommended cleaning intervals and methods vary and, more importantly, they are not based on any published studies of feeder hygiene. Clearly, research is needed to determine whether various cleaning methods are effective at reducing the risk of disease transmission.

In this study, our objective was to determine the effectiveness of a cleaning method and frequency at reducing bacterial populations on bird feeders over time in winter. We tested a modified version of the Audubon Society's recommended cleaning method of using 10 % bleach to clean feeders every two weeks. Instead of soaking feeders in bleach, we cleaned feeders with commercially available sanitizing wipes that contain a 10 % bleach solution. Wipes were used because these products are much easier to use than immersing a feeder in a large volume of bleach solution and may be a more feasible option for use on a regular basis. We determined whether cleaning hopper-style bird feeders every two weeks with 10 % bleach wipes reduced the counts of total aerobic bacteria and Gram-negative bacteria on feeders over time in the winter. Gram-negative bacteria were used as a sampling measure because they are good indicators of potential pathogenic bacteria that are commonly used in food and water testing (Toranzos *et al.* 2007) and because avian pathogens can be difficult to detect in the environment (e.g., Prescott *et al.* 2000). Total aerobic bacteria were also used as a sampling measure because this provided a more general measure of bacteria present to test cleaning effectiveness, which may be particularly important if potential pathogens were not present in any abundance in our study area.

MATERIALS AND METHODS

Immediate Effectiveness of Bleach Wipes at Reducing Bacteria on Feeders

We tested whether Sani-cloth 10 % bleach wipes (H24795, Professional Disposables Inc., Orangeburg, NY) were effective in cleaning feeders in the short term by measuring the reduction in counts of total aerobic bacteria before and after cleaning. Eleven hopper-style feeders (Perky-Pet No. 316, Woodstream Corp., Lititz, PA) made of plastic were placed out in Berks County, PA for six weeks from March to April 2013. When returned to the lab, the entire surfaces of the four perches on each feeder were swabbed using Q-swabs™ sample collection devices (Weber Scientific, Hamilton, NJ). The swabs were mixed by vortex for 30 seconds. The rinsate was then serial diluted, and the dilutions were plated on Luria Bertani agar (LB; Neogen Corp., Lansing, MI). LB agar was used to culture the total aerobic bacteria present per ml of rinsate. After swabbing, the four perches of each feeder were "cleaned" with Sani-Cloth bleach wipes. One to two wipes were used to remove any debris present on the surface. Another wipe was used to sanitize the perches of the feeder. The feeders were then allowed to air dry. After the feeders were completely dry, the entire perch area was swabbed using Q-swabs™ The swabs were mixed by vortex for 30 seconds. The rinsate was serial diluted and plated on LB agar. Both sets of plates were incubated at 37 °C for 24 hours. Colony counts were performed to determine whether the Sani-cloth wipes reduced the level of total aerobic bacteria present on the surface of the feeders.

Feeder Sanitization prior to Ten Week Cleaning Study

Prior to our ten-week experiment, forty hopper-style feeders (Perky-Pet No. 316, Woodstream Corp., Lititz, PA) were scrubbed using a sponge with soap and water to remove any debris. We then sprayed feeders with a 10 % bleach solution and allowed them to air dry to sanitize microorganisms present on the feeders. After cleaning, the entire perch surfaces of the feeders were swabbed with Q-swabs™ sample collection devices to ensure bacteria and fungi were not present after cleaning. The swabs were mixed by vortex for 30 seconds. The swabs were then spread across the surface of Potato Dextrose Agar (PDA; Neogen Corp., Lansing, MI) and LB agar. PDA was used to culture fungi and LB agar was used to culture total aerobic bacteria present on the feeders. Plates were incubated at 25 °C for 48 hours to ensure growth and detection of fungal colonies. The presence or absence of fungi and bacteria was determined by growth on the plates.

Feeder Placement and Maintenance

The feeders were placed in pairs at locations throughout Berks, Bucks, Lehigh and Carbon counties in Pennsylvania in late January 2012 and maintained until April by volunteers. Each pair of feeders was hung from a double-sided bird feeder post (i.e., a shepherd's hook) or from a tree. A paired design was used to ensure that the cleaned and non-cleaned feeders were exposed to the same environmental conditions. Although a paired design might facilitate the transfer of bacteria between cleaned and non-cleaned feeders, this proximity of feeders reflects actual feeder use and the environmental conditions expected in suburban and urban areas. For example, Fuller *et al.* (2008) estimated

feeder density at 925 feeders/km^2 in Sheffield, UK, which is the equivalent of one feeder per 0.27 acres. Furthermore, the average birdwatcher maintains multiple feeders in their yards, e.g., average of 5.7 feeders per household in Britain (Schreiber 2010). The feeders were filled with Feathered Friend premium black oil sunflower seeds (CHS Sunflower, Grandin, ND).

The feeders were refilled periodically as needed. The approximate volume of seed added to the feeders each time they were refilled was recorded. To measure this, four evenly spaced lines were drawn on the feeders. Volunteers noted the seed volume before and after seeds were added to each feeder. The mass of seeds within the feeders at each given increment was pre-determined and used to determine seed consumption by animals, i.e., birds and gray squirrels (*Sciurus carolinensis*), at each feeder over time. gray squirrels are regular visitors to bird feeders and are a likely source of bacteria in addition to birds. This project was ethically reviewed and approved by Kutztown University's Institutional Animal Care and Use Committee.

Feeder Cleaning Protocol

One feeder in each pair served as a control and was not cleaned throughout the experiment (hereafter non-cleaned feeders). The other feeder was cleaned once every two weeks (the weeks alternate to swabbing) using a Sani-cloth Bleach wipe that contained 10 % bleach across the entire surface and interior of the feeder (hereafter cleaned feeders). Seed was removed from each feeder prior to cleaning. One to two wipes were first used to remove dirt or debris. A final bleach wipe was then used to sanitize the surface by wiping it across surface of the feeder and allowing it to air dry. After the feeder was dry, the seed was returned to the feeder. However, we did not clean up the seed hull debris on the ground under the feeder.

Surface Sampling

Every two weeks (the weeks alternate to cleaning), the surfaces of the four perches and seed wells of each feeder were swabbed using Q-swabs™ sampling devices. The Q-swabs™ were mixed by vortex for 30 seconds and then were serial diluted in sterile buffered peptone water. Dilutions were plated on LB agar and Eosin Methylene Blue agar (EMB; Neogen, Lansing, MI). EMB agar was used to culture Gram-negative bacteria present on the feeders. The plates were incubated at 37 °C for 24 hours. Colony counts were performed on each type of media.

Statistical Analysis

To evaluate the immediate effectiveness of bleach wipes, a paired t-test was used to determine whether the wipes reduced the counts of total aerobic bacteria after cleaning a sample of feeders. Experimental cleaning data were analyzed using a two-way repeated measures ANOVA to determine whether the counts of total aerobic bacteria (hereafter aerobic bacteria) and counts of Gram-negative bacteria were significantly influenced by experimental cleaning and by time in weeks. Finally, linear regression analysis was used to determine whether there was a relationship between the mass of seed consumed at each feeder (as an indicator of animal activity) in the two weeks prior to each sampling period and the counts of bacteria on the feeders. All statistical analyses were conducted using SPSS version 19.

RESULTS

Immediate Effectiveness of Bleach Wipes at Reducing Bacteria on Feeders

The Sani-cloth bleach wipes significantly reduced aerobic bacteria from the surface of the feeders ($t = 10.91$, $n = 11$ feeders, $p < 0.001$). Before cleaning, the count of aerobic bacteria on feeders was 3.4 log CFU/ml of rinsate (± 0.24 SE), and after cleaning, the count of aerobic bacteria on feeders was 1.7 log CFU/ml of rinsate (± 0.19 SE). Thus, the bleach wipes effectively reduced the aerobic bacteria count by 1.7 log CFU/ml of rinsate.

Feeder Sanitization prior to Ten Week Cleaning Study

After the preliminary cleaning of feeders with soap and water followed by a 10 % bleach solution, no colonies were present in feeder samples on PDA or LB agar plates, which indicated that the bleach sanitization process was effective at killing microbes and that all feeders began our ten-week experiment without microorganisms present. Over the ten-week experiment, one pair of feeders was removed from the study due to squirrel damage and the week two swabbing samples were lost due to logistical problems, so 19 feeders were used and the swab sampling began at week four.

Effectiveness of Cleaning Feeders with Bleach Wipes Over Ten Weeks

Cleaning with bleach wipes significantly reduced aerobic bacteria on feeders ($F_{1, 18} = 7.52$, $p = 0.013$; Figure 1A), but aerobic bacteria significantly increased on feeders over time ($F_{3, 16} = 8.16$, $p = 0.002$; Figure 1A). In addition, there was

a significant interaction between cleaning and time that influenced aerobic bacteria on feeders ($F_{3, 16} = 3.47$, $p = 0.041$; Figure 1A). This interaction suggests that cleaning became less effective over time because counts of aerobic bacteria on cleaned feeders increased from weeks 4 and 6 to weeks 8 and 10 and these counts were similar to counts on non-cleaned feeders at the end of our experiment (Figure 1A). By comparison, cleaning with bleach wipes did not significantly reduce Gram-negative bacteria on feeders ($F_{1, 18} = 1.88$, $p = 0.187$; Figure 1B) and time in weeks did not significantly influence Gram-negative bacteria on feeders ($F_{3, 16} = 2.09$, $p = 0.141$; Figure 1B). In addition, there was no significant interaction between cleaning and time that influenced Gram-negative bacteria on feeders ($F_{3, 16} = 0.61$, $p = 0.619$; Figure 1B).

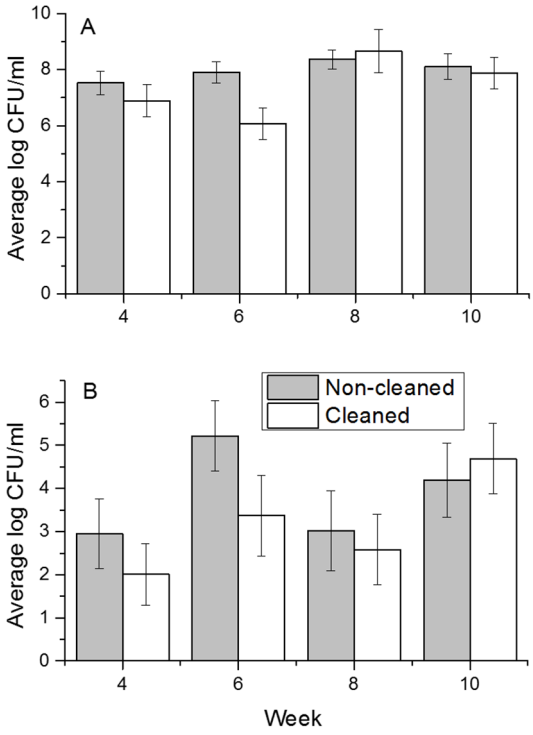

Figure 1. Average (± SE) aerobic (A) and Gram-negative (B) bacterial counts (log CFU/ml of rinsate) over ten weeks on bird feeders cleaned with 10 % bleach wipes compared with non-cleaned feeders. Bacterial counts occurred every two weeks beginning with week four.

Most of the bacteria counts on the cleaned and non-cleaned feeders were not related to the amount of seed consumed at those feeders. No relationship was found between aerobic bacteria and seed consumed at the cleaned feeders ($r^2 = 0.193$, $n = 19$, $p = 0.095$; Figure 2A) or the non-cleaned feeders ($r^2 = 0.155$, $n = 19$, $p = 0.181$; Figure 2B). There was no relationship between Gram-negative bacteria and seed consumed at the cleaned feeders ($r^2 = 0.151$, $n = 19$, $p = 0.194$; Figure 2C), but there was a significant positive relationship between Gram-negative bacteria and seed consumed at the non-cleaned feeders ($r^2 = 0.382$, $n = 19$, $p = 0.001$; Figure 2D).

DISCUSSION

We found that bleach wipes were effective at reducing aerobic bacteria on feeders, but the wipes did not completely sanitize the surface of feeders. In our ten-week cleaning experiment, we recorded high counts, i.e., $> 10^7$ CFU/ml, of total aerobic bacteria by week four (Figure 1A) despite beginning the experiment with no colonies detected in feeder surface samples. These levels suggest that there was a need to clean feeders after four weeks of use. Cleaning with bleach wipes every two weeks reduced aerobic bacteria on feeders, but aerobic bacteria increased over subsequent weeks, notably so after week six. The bleach wipes may have become less effective over time, i.e., by weeks eight and ten (see Figure 1A), as indicated by a significant interaction between time and cleaning on aerobic bacteria counts. By contrast, we found no significant trends between seed consumption and aerobic bacteria at cleaned and non-cleaned feeders. This suggests that counts of aerobic bacteria were influenced by multiple environmental sources and not solely by animal activity.

We suspect that the loss of cleaning efficacy for aerobic bacteria over time was due to the accumulation of organic material from soil, seed hull debris, and feces on the surface of feeders. Organic matter can potentially inactivate bleach (i.e., sodium hyperchlorite) or act as a barrier for bacteria against disinfectants (Russell 1999; Sharma *et al.* 2009). Although the Sani-Cloth wipes contain surfactants and other cleaners in addition to bleach, they are designed to disinfect surfaces and equipment for the healthcare industry and are not intended for removing large amounts of debris. Thus, our feeder cleaning protocol of using only bleach wipes likely left behind organic matter in the corners of the seed wells and in the fine grooves present on the perches of the feeders. This organic matter build up may have increased over time leading to the reduction in cleaning efficacy after several weeks.

By contrast, neither cleaning nor time significantly influenced Gram-negative bacteria on the surface of feeders despite an apparent trend toward lower counts on cleaned feeders in three of the four sampling periods (see Figure 1B). The lack of a significant effect of cleaning was likely due to the high variability in Gram-negative bacteria at both cleaned and non-cleaned feeders. This variability is not surprising because potential pathogens have been difficult to detect at bird feeders (e.g., Prescott *et al.* 2000) and the prevalence of

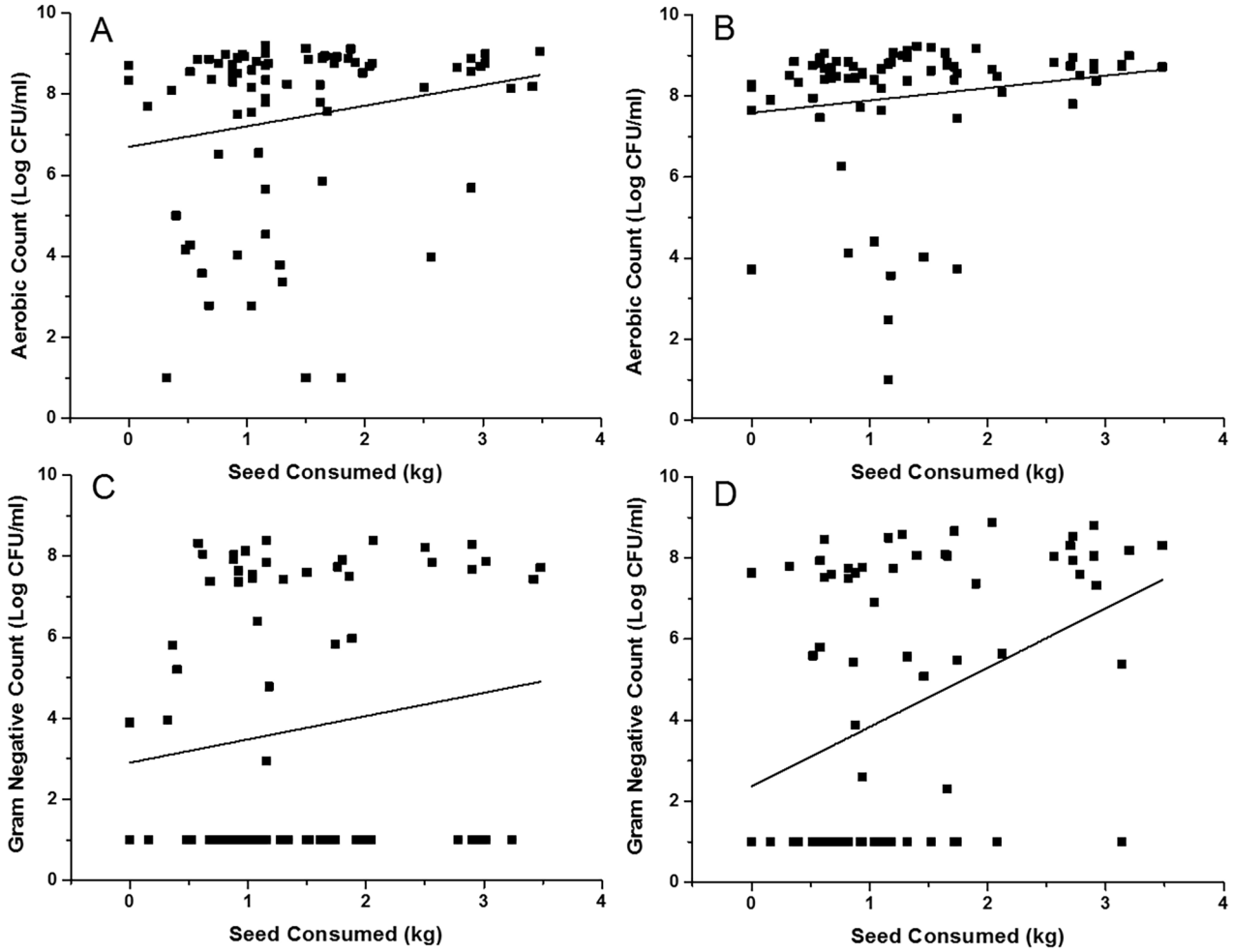

Figure 2. The influence of animal activity (as measured by seed consumption at each feeder two weeks prior to each bacterial sampling period) on bacterial counts (log CFU/ml of rinsate) on individual feeders for: total aerobic bacteria on cleaned feeders (A), total aerobic bacteria on non-cleaned feeders (B), Gram-negative bacteria on cleaned feeders (C), and Gram-negative bacteria on non-cleaned feeders (D). The trendlines represent linear regression best of fit lines.

potential pathogens is low among wild songbirds (Brittingham *et al.* 1988), but can be variable in some pathogens, such as *Salmonella* (Benskin *et al.* 2009). Interestingly, there was a significant positive relationship between seed consumption and Gram-negative bacteria at non-cleaned feeders and no significant relationship at cleaned feeders. The positive relationship for non-cleaned feeders suggests that birds and squirrels were a source of Gram-negative bacteria at feeders through either their physical contact or the deposition of feces on feeders. Although we did not measure the amount of fecal matter present on feeders, Prescott *et al.* (2000) found that fecal matter does accumulate at feeders, especially hopper-style and platform feeders. More importantly, the lack of a significant relationship for cleaned feeders also suggests that cleaning with bleach wipes helped to control Gram-negative bacteria on very active feeders.

Overall, we found that bleach wipes reduced aerobic bacteria on feeders and were able to control aerobic bacteria for a few weeks. However, this efficacy appeared to diminish after several weeks, which may have been due to the buildup of organic matter on feeders. Bleach wipes were not effective at controlling Gram-negative bacteria over several weeks but showed some ability to reduce Gram-negative bacteria on very active feeders. Our results suggest that bleach wipes might be a simple and useful sanitizing method for bird feeders if organic matter can be removed first through other means of cleaning or scrubbing. Future research should investigate the influence of organic matter on the efficacy of disinfectants to clean bird feeders and to determine whether the amount of feces or debris on feeders is related to levels of potential pathogenic bacteria.

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