A Pilot Study of Potential Public Health Hazards in the Animal Hoarding Environment

THESIS

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Abstract

Animal hoarding is an occurrence of abuse found in most communities, with an estimate of 700 to 2000 new cases reported annually in the US. Environmental insults, in the animal hoarding environment, include air quality, biological disease agents, and physical hazards associated with the animals or structural integrity of the home. This study evaluated the potential public health hazards posed to home occupants and investigational workers at two animal hoarding sites (AH) and compared the environmental conditions with two non-hoarding sites (NAH).

To assess air quality, indoor and outdoor sample measurements for variables such as, concentrations of ammonia, particulate matter, and bacteria and fungi, along with sound pressure levels were conducted. Pathogenic microbiological agents were assessed with a standardized set of surface samples and bulk stool collection. The potential physical and structural hazards were cataloged using a modified HOMES survey which recorded the presence or absence of issues such as, unstable flooring, exposed electrical wires, blocked exits, and animal waste.

As a pilot study, the sample size was too small to establish statistical significance; however, comparison of AH and NAH environments demonstrated some noteworthy differences. The results for biological hazards, show an increase in the diversity in the
AH versus NAH sites, with six types found at the largest AH site (41 animals) and
*Staphylococci aureus* the only type at NAH site AH02, while *S. aureus* and *S.
pseudointermedius* species were found at NAH site AH01. Several of these pathogens are
zoonotic, primarily causing illness in humans through accidental ingestion. Salmonella
and campylobacter were detected on the dining room table and kitchen at AH sites AH03
and AH04, respectively. Both of these areas are associated with food preparation,
consumption and storage. This finding suggests a potential site for cross contamination
that could result in transmission to AH occupants.

After responder activity began, measurable concentrations of ammonia were detected at
both AH environments. The AH site AH04 had an average sound pressure level of
92.45dBA, which would exceed actionable levels if this remained constant over an eight
hour day. At AH03, with 9 cats and 2 dogs, the indoor particulate matter levels exceeded
the National Ambient Air Quality Standards, if this were a continual exposure over a 24
hour averaging time. This study found multiple significant public health hazards and
supports the need for further research to provide prevention and mitigation
recommendations for responders; as well as recognition and response advice for those
involved with intervention of individuals suffering from animal hoarding disorder.
Dedication

This document is dedicated to my family, Robert, Elizabeth, James, and Levi.
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I would like to thank the many individuals that have provided guidance and expertise during the planning and execution of this project. As a pilot study with multiple areas of interest, the interdisciplinary approach provided immense support in achieving the objectives.

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Fields of Study

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Introduction

I. Animal Hoarding History: Defining the Abuse

Animal hoarding is a public health concern that crosses the boundaries of animal health and welfare, child and elder neglect, environmental health and safety and is complicated by the documented relationship to mental health. The Humane Society of United States reports that almost 250,000 animals are victims of animal hoarding environments annually, with reports occurring in communities across the nation. (102) Global occurrence is also prevalent, from the United Kingdom to Australia, comprising of hundreds of animals. (103, 104, 105) Concentrated large populations of animals, when not properly managed, can result in multiple public health concerns. Through activities such as, in-home social services, family visits, and emergency response activities (fire and medical response), as well as animal hoarding response, recovery, and care of rescued animals; these public health impacts are expanded beyond the confines of the animal hoarding environment and the animal hoarder. This study is being undertaken to characterize specific hazards in the animal hoarding environment so that those who may be exposed can be better informed and prepared.
Within the United States 56% of households own pets, on average those that own cats have 2.1 cats; while those owning dogs have 1.6 dogs. (27) Animal hoarding is defined by several factors, one being the acquisition of more than the average number of animals. While the animals are in overabundance the level of sanitation within the home, the delivery of adequate nutritional requirements and the provision of veterinary care, are typically lacking. The Hoarding of Animals Research Consortium (HARC) has a well-established definition of animal hoarding:

- Having more than the typical number of companion animals
- Failing to provide even minimal standards of nutrition, sanitation, shelter, and veterinary care, with this neglect often resulting in illness and death from starvation, spread of infectious disease, and untreated injury or medical condition
- Denial of the inability to provide this minimum care and the impact of that failure on the animals, the household, and human occupants of the dwelling
- Persistence, despite this failure, in accumulating and controlling animals(28)

In 1979 the Minister of Agriculture, Fisheries, and Food developed what is now referred to as, “The Five Freedoms”. (29) These guidelines have been incorporated into the “Guidelines for Standards of Care in Animal Shelters”, and illustrate a societal drive to prevent situations as described in the animal hoarding definition. (75) These five freedoms—freedom from hunger and thirst; from discomfort; from pain, injury, or disease; to express normal behavior; from fear and distress—are rarely afforded to the
animals in animal hoarding environments. These animals are victims of animal cruelty and are the focus of much of the literature evaluating the impact of animal hoarding. (28, 30, 31, 36, 75)

As an animal welfare issue it cannot be ignored that the animals within these homes are in a grave state of neglect and abuse. Animals suffering debilitating injuries and even death, from neglect in these situations, are the expectation rather than an anomaly. The cases of Barbara Erickson and Vikki Kittles illustrate this, both of whom hoarded over a hundred animals in squalid conditions. In the case of Barbara Erickson who hoarded 552 dogs, authors Arnold Arluke and Celeste Killeen report that 134 dogs were euthanized while over 400 were saved. Amongst those euthanized were animals with old injuries, swelling on the extremities, severe dehydration, emaciation, meaning they were thin and weak, typically resulting from illness or lack of food, and characteristics commonly associated with inbreeding. (38) In the case of Vickie Kittles there were 116 dogs, four cats, and two chickens kept inside of a school bus. When responders entered the bus they found not only dehydrated, emaciated, parasite ridden animals covered in urine and feces, but they also discovered several bodies of deceased animals. (39)

In 1999, a prevalence range of 700 to 2000 new animal hoarding cases reported annually in the United States was extrapolated, by Dr. Gary Patronek. (30) Many consider this to be an underestimate due to the nature of reporting; only the most severe come to the attention of the authorities. (44) Animals are left to suffer in a state of squalor sometimes
leading to death or euthanasia after having endured environmental insults ranging from ammonia and particulate matter to biological disease agents, as well as stress from isolation, noise, and overcrowding. (62)

Dr. Gary Patronek published several sentinel studies that described the problems that exist in the animal hoarding environment. The cases of Erickson and Kittles mentioned above are not unique. One study found that 80% of 54 cases reported the presence of dead, malnourished, injured, or ill animals. (30) In this same report Dr. Patronek discussed the reasons that led to a case being reported to the authorities. The top five complaints were: unsanitary conditions (75.9%), excessive numbers of animals (61.1%), animals in need of medical attention (59.3%), odor (50.0%), and malnourished animals (40.7%).(30) Of these 54 cases, 58% of the hoarders did not recognize that there was a problem; as reported to the investigator during the investigation. (30) This introduced the immense mental disconnect between the hoarder and the conditions in the animal hoarding residence.

In a study of 71 professionally acquired case reports of animal hoarding, Arluke et al. found that 80.3% of hoarders said they were accumulating animals because they loved them, 67.6% said they were accumulating the animals in order to save them. (31) This is in direct contrast to the care actually provided to these animals. When we consider the extent of illnesses and injuries suffered by the victims and the perceptions held by the perpetrators, we understand that there is much more to animal hoarding than animal
abuse. This is clearly illustrated in a case in Pennsylvania where an individual resided in a home with a broken sewer pipe resulting in pools of human waste in the basement, there was cat urine and feces spread throughout the rest of the house, a freezer in the basement with the frozen remains of cats, and the resident continued to proclaim the love they had for the cats stating, “I fed those cats. They were treated like queens”. (87) Studies have been conducted to evaluate the characteristics of animal hoarders, in order to better understand this complex issue; these have sparked much discussion over whether or not mental illness plays a role. (30, 36, 38)

Simply removing the animals from the residence is not enough to resolve the issue. There is almost 100% recidivism among animal hoarders, meaning that the collection of animals is almost certain to resume. (71) This was the case in a New York town where two sisters were discovered hoarding 150 cats, after having 77 cats removed from their premises just four years prior. (84)

II. Root Causes of Hoarding Disorder: Who is at risk of being an animal hoarder?

Multiple studies present characteristics of the animal hoarder, indicating that women are more often the perpetrator than men, with the greatest occurrence in the 40- 60 year old range. (30, 31, 83) It has also been shown that approximately 72% of animal hoarders are single, divorced, or widowed. (30, 31) However, it is imperative to the understanding of this issue, that we acknowledge that this situation occurs across gender lines, age groups,
and socioeconomic levels. (62) Arluke, et al. reports that of 71 cases studied, where employment was recorded, 54.9% were unemployed, retired, or disabled. (31) Although, it is noted that, “hoarders who were employed held a variety of jobs, including those considered white-collar or professional positions, such as teacher, mortgage broker, realtor, or marketer.” (31) Professionals in animal health and welfare and those in social services need to recognize that animal hoarding can occur in any setting, regardless of community or individual characteristics. Considering the behavior of animal hoarders, Patronek, et al. classified animal hoarders into three general categories: the overwhelmed caregiver, the rescue hoarder, and the exploiter hoarder. (40)

The Humane Society of the United States and the National Center on Elder Abuse conducted a survey of Adult Protective Service supervisors and frontline case workers and found that, “More than 92% said that adult protective service (APS) workers encountered animal neglect coexisting with a client's inability to care for him or herself. This indicates that reports of animal neglect may be an important warning sign for the presence of self-neglect by vulnerable adults.” (47, 62) Dr. Patronek remarks that in the animal hoarding environment, “Serious unmet health needs are commonly observed, and the conditions often meet the criteria for adult self-neglect, child neglect, or elder abuse.” (62) Coinciding with the HARC report of 10-15% of cases including the presence dependent individuals (children, elderly, and disabled). (48)
Unfortunately, societies’ image of an animal hoarder as the “cat lady” has conjured sympathy for hoarders; many times resulting in people becoming “enablers” for the animal hoarder. (38, 40, 67, 80, 81) The perception society holds, in regard to an issue, can impact how the situation is handled. Arluke, et al. conducted a media review and illustrated the emotional categories with which animal hoarding is presented to the public. The review found that presentation of this complex issue left people confused about whether animal hoarders should be regarded as criminals, if hoarding is a pattern of animal abuse or a stand-alone oddity, whether or not hoarding is bad for the animals, and how the mental status of the hoarder affected the situation. (82) This review illustrates a divergence between public perception of animal hoarding and the true nature of this situation, therefore impacting mitigation efforts. This same lack of understanding has been historically observed among public health and social service providers who have viewed living in these conditions as a lifestyle choice rather than evidence of a mental illness. More recent emphasis has been placed on the fact that animal hoarders are in need of treatment and surveillance to reduce recidivism and the potential public health hazards within these environments. (36, 72)

Animal hoarding has previously been associated with Diogenes Syndrome and multiple mental health disorders such as Obsessive Compulsive Disorder (OCD). (40, 64) Diogenes Syndrome is the existence of self- neglect, living in squalid conditions, social withdrawal and hoarding. (64) Squalor as defined by Snowden, et al. as the,
“Accumulation of dirt, refuse, degraded material, and vermin that can result in living conditions within a dwelling being unhealthy, unsafe, and potentially harmful.”(37) If the filth and degradation result in adverse effects on the occupants and nearby residents, Snowden believes that “Severe Domestic Squalor”, defines this situation. (37)

Recently the Diagnostic and Statistical Manual of Mental Disorders (DSM – 5) has added hoarding as a mental health disorder. Hoarding has been grouped with obsessive – compulsive disorder, body dysmorphic disorder, trichotillomania, and excoriation; due to similar characteristics. However, the American Psychiatric Association states that, “there are enough important differences between them to exist as distinct orders.” (35)

The DSM-5 defines hoarding disorder as, “Persistent difficulty in discarding or parting with possession, regardless of the value others may attribute to these possessions. The behavior usually has harmful effects – emotional, social, financial, and even legal – for the person suffering from the disorder and family members.” (35) In a study comparing object and animal hoarding, Frost, et al. reports that the diagnostic features of hoarding characterizes those that hoard animals. (36) However, he acknowledges that the environment is distinctly different between object hoarders and animal hoarders; explaining that animal hoarders tend to live in squalid conditions whereas only a small portion of object hoarders live in such a state. (36)

As research continues to develop around animal hoarding, certain aspects of the hoarder and type of animals have been recognized. Table 1 shows a comparison of the type of
animals hoarded from two different studies, one consisted of a review of 54 cases while the other consisted of 71 cases; conducted by Patronek et al. and Arluke et al. respectively. (31, 30) From these studies, we can conclude that cats are the top hoarded animal followed by dogs, however the range of animal species is broad and this can impact what biological hazards are potentially present in this environment.

III. Hazards Associated with Animal Hoarding

The environment within animal hoarding homes is in a continued state of neglect. Many studies have been undertaken to decipher the mental state of the hoarder themselves, but research is lacking in regards to the environmental hazards that may exist within these homes. (30, 31, 36, 47, 62, 64) The interiors are often reported as unsanitary, with feces, urine, garbage, and even decomposing bodies of deceased animals on the floors, in freezers, and even in walls. (30, 31, 38, 40, 58, 64) The situation that results from animal hoarding is an abuse upon the animals kept in such a manner, as well as an abuse on any other individuals living within an animal hoarder’s home; primarily dependent children, elderly, and the disabled.

The current literature recognizes that there exists a zoonotic transmission potential, potential illness from ammonia concentration, and potential hazards from particulate matter (specifically respirable particulate matter (< 10µm diameter) and the noise level rising from the large number of animals housed within this environment. (31, 40, 57, 58,
However, research is largely lacking for these potential hazards, which have ramifications that could extend well into the community.

**Biological Hazards**

Zoonotic pathogens are defined as disease agents that can be transmitted from an animal to a human. (42) The higher concentration of animals and the lack of basic veterinary care can lead to the presence of these pathogens in the hoarding environment. (57, 75)

There also exists the potential for reverse zoonosis which is a zoonotic disease, “maintained primarily in nature by human to human transmission that can be transmitted from humans to animals.”(43) In these cases the animals could play a role in the household maintenance of the pathogen, potentially increasing the risk of exposure to the occupants. One such disease agent would be Methicillin Resistant Staphylococcus aureus (MRSA). (118, 119)

In the first volume of, “Zoonoses and Communicable Diseases Common to Man and Animals”, the authors state that, “It must be remembered that parasites, viruses, bacteria, and other agents of zoonotic infection can take up residence in any territory where they find suitable ecologic conditions. Ignorance, economic or personal interests, and human customs and needs also favor the spread of these diseases.” (41) In the animal hoarding environment there persists individual interests and mental health disorders that contribute to the “ignorance” of the situation and impacts the conditions that support bacterial, viral, and parasitic proliferations and sustainability. Ignorance is placed in quotation to indicate
that there exist serious mental health conditions that have a major impact on the animal hoarding environment. As indicated by researcher Dr. Gary Patroneks statement that, “Hoarders are often not lying; they lack the insight to appreciate the true conditions present.” (62) Increasing the knowledge concerning the animal hoarding environment, in regards to zoonotic disease agents, is crucial for responders and shelter staff to safely mitigate these situations.

Biological organisms including bacteria, virus, protozoans, parasites, and fungi, can exist in the conditions found in the animal hoarding environment. (Table 2) Some of them, particularly those producing oocysts and spores, can remain in an environment from months to years. In a recent study it was discovered that responders, the animal hoarder, shelter workers, and the animals, within an animal hoarding environment, had been exposed to Extraintestinal Pathogenic *Escherichia coli*. (38) It was believed that many of the dead animals found at the scene may have succumbed to this pathogen.

Extraintestinal Pathogenic *Escherichia coli* are known to be zoonotic and this event reiterates the need to evaluate this environment, in order to be fully prepared for mitigation of the scene. (38)

There are several factors reported in animal hoarding environments which are linked to maintenance of pathogens in the environment or impaired removal practices. Arluke et al. researched 71 cases and looked at several aspects of the environment and the functionality within the home. Laundry facilities, shower or bath, hot water, and stove
were among the top non-functional appliances among the cases studied. (31) In 1999 a study of 54 animal hoarding cases categorized sanitary conditions through the use of a rating table, ranking sanitation on a 1 to 5 scale with 1 being relatively clean and 5 having a filthy environment with profuse feces, urine and garbage in the environment. (30)

Of the 54 cases it was reported that 38 had a rating between 3 and 5, with 22.2% of the 54 cases receiving the highest rating of 5. (30) The combination of squalid conditions that impedes the normal functioning of household appliances and daily activities, creates an environment ideal for growth, persistence, and transmission of pathogens. (40) Transmission between the inhabitants of the home, new animal arrivals, or any person coming in contact with the environment may potentially carry the pathogen away from the home to other locations within the community. (57, 58)

A study, undertaken at a Florida animal shelter, reported diarrhea prevalence among shelter cats; attributing the prevalence to many factors including enteropathogenic viruses, parasites, protozoa, and bacteria. (46) In the shelter environment Sabshin et al. states that, “In addition to stress, impounded cats face crowding, mixing of animals from various environments, and exposure to other species. These factors facilitate the transmission of infectious diseases among animals in shelters and potentially to their human caregivers and shelter visitors.” (46) Variation in geographical range of pathogens and the characteristics of the particular pathogen must also be considered, as this can dictate which pathogens have the greatest potential to be present. (60)
The presence of zoonotic pathogens within the home environment can be detrimental and it is reported that removal of canine or feline feces from the home environment will help reduce potential transmission of zoonotic agents. (14, 15, 60, 61) In the Feline zoonoses guidelines from the American Association of Feline Practitioners, transmission of zoonotic pathogens is stated to, “potentially occur by direct contact with the animal, indirect contact with secretions or excretions from the animal, and contact with vehicles like water, food or fomites that were contaminated by the animal. For many agents, infection of the animal and human occurs from a shared vector or environmental exposure.” (61)

Considering the impacts of vulnerable populations living within squalid conditions, ripe for pathogen growth and transmission, it is clear that we need to understand what pathogens are likely to be present and at what concentrations. Table 2 outlines common pathogens that have zoonotic potential, the primary (1°) species affected, and the prevalence of the pathogen within the United States. These pathogens were selected based on the literature review and the interdisciplinary team discussions. While some may have been previously reported in animals from hoarding environments, studies have not yet been done to identify the presence of these pathogens in hoarding homes or examine their distribution within the home.
Structural Hazards

Structural hazards are another issue that may result in serious injury to individuals entering the animal hoarding environment. The characteristics such as, damp and weakened floorboards and stairs, floors soaked with urine and feces, exposed electrical wires, and flammable objects near heat sources, complicates safe entry and remediation of these sites. (31, 40, 57, 58, 62, 64, 86) Accumulated urine can flow between floorboards and seep down to the subflooring where it will warp and degrade building materials, if not properly cleaned and removed in a timely manner. (115) Studies have shown that animal hoarding homes are often layered in animal waste and floorboards are softened and falling apart. These potential hazards can lead to multiple opportunities for injury. Understanding all of the potential structural hazards requires knowledge of building materials and design, underscoring the benefit of having a community task force with members from multiple disciplines.

In, “The Hoarding Handbook: A Guide for Human Service Professionals,” by Christina Bratiotis, et al. the use of community task forces is described. These task forces can be designed to suit the needs of each community and the agencies available to handle animal hoarding. Animal shelters are generally the agency that deals with the removal and the aftermath from these situations, but with the complexity of this issue more agencies need to follow up with the individual hoarder and site. (30, 31) Other agencies often involved in hoarding task forces include public health, code enforcement, social services and legal
aid. The task force model allows for implementation of a plan that can be adapted to each hoarding event. To assist in the development of these groups the HOMES Survey was designed (28, 72). This survey provides a method for responders to collect site information pertaining to specific issues in the categories of Health, Obstacles, Mental Health, Endangerment, and Structure and Safety. This information can be utilized as the animal hoarding environment is remediated, especially when multiple trips back to the site are necessary.

Air Quality

Indoor air quality (IAQ) is defined by the Environmental Protection Agency as, “the air quality within and around buildings and structures, especially as it relates to the health and comfort of building occupants,” pollutants affecting IAQ include particles and dust, fibers, mists, bioaerosols and gases or vapors. (113) Noise also impacts the indoor environment, is a portion of air pollution, and is defined as unwanted sounds. (106) Noise can result in serious health effects and is an important public health concern. Sources of noise are numerous, persistent, and pervasive, affecting all aspects of society from residential to occupational settings, resulting in direct and cumulative adverse health effects. (106) The Occupational Safety and Health Administration (OSHA) set the permissible exposure limit (PEL) for an eight hour time weighted average (TWAₘ₈) at 90dBA. (96) While the National Institute for Occupational Safety and Health has a recommended exposure limit (REL) of 85 dBA TWAₘ₈; which is also the action level
(50% dose) and the level in which employers are required to have a hearing conservation program in place for employees.

Through the use of the allowable time calculation, exposure durations can be calculated that reflect how long employees can be exposed to different levels of sound pressure. The United States Department of Health and Human Services, Criteria for a Recommended Standard reports that, “NIOSH previously recommended an exchange rate of 5 dB for the calculation of time-weighted average (TWA) exposures to noise. However, NIOSH now recommends a 3-dB exchange rate, which is more firmly supported by scientific evidence. The 5-dB exchange rate is still used by OSHA and MSHA, but the 3-dB exchange rate has been increasingly supported by national and international consensus.” (111, 112) This illustrates the need to be aware of new scientific evidence because standards may change and public health professionals that are aware of levels that approach permissible exposure limits (PEL), and recommended exposure limits (REL) can provide the most efficient and conservative recommendations. Other factors of air quality include contaminants in solid, liquid, or gaseous form.

Zhao et al. states, “Air quality is the concentration of air pollutants in the ambient air and directly affects people and animal health”. (49) Air contaminants are defined as, “a substance (solid, liquid, gaseous) not found in the normal composition of the atmosphere.” (65) The presence of feces, urine, and even dead animals in the hoarding
environment create toxic gases including ammonia, hydrogen sulfide, carbon dioxide, and volatile organic compounds. (30, 40, 49, 57)

Ammonia is an irritant that present in the air of many animal hoarding environments. It has been reported that one hoarding environment had an ammonia concentration of 152 ppm (parts per million) after being partially ventilated, exceeding the Occupational Safety and Health Administration (OSHA) permissible exposure limit (PEL) time weighted average (TWA) of 50 ppm (35 mg/m³). (55, 62) The National Institute of Occupational Safety and Health (NIOSH) has a recommended exposure level (REL), time weighted average (TWA) of 25 ppm (18mg/m³) and a short term exposure limit (STEL) of 35 ppm (27 mg/m³). (62) This means that an individual should not be exposed to more than 50 ppm of ammonia throughout an eight hour workday, although it is recommended, and good management practices would dictate, not to exceed the REL of 25. Most animal shelters and kennels, that are routinely sanitized and that appropriately manage animal waste, have ammonia levels below the detection rate of common handheld meters (<1-2 ppm). (107) Even when levels are below the PEL and REL it does not indicate that chronic exposure to low levels of ammonia are normal or harmless.

Repeated or chronic exposure to ammonia can cause irritation to the respiratory tract, resulting in chronic cough, asthma, lung fibrosis, as well as irritation of the eye membranes. (108) Other health effects from ammonia exposure range from eye, nose, and throat irritation to death at extremely high levels (5000 ppm for 30 minutes). (49, 69)
An immediate danger to life and health limit (IDLH) of 300 ppm has also been set by NIOSH, in the previous example of the home with an ammonia level of 152 ppm, the PEL and REL were both exceeded and the level was over halfway to the IDLH limit, representing a serious health hazard to individuals exposed to this environment. (55) It is important to note that the animal hoarder is residing in these conditions exposing themselves, and the captive animals and any dependents present, to these high levels that have the potential to exceed OSHA and NIOSH limits.

Air contaminants are reported to have serious public health impacts. (53) Anderson et al. reports that, “air pollution contributes to 800,000 premature deaths each year, ranking it the 13th leading cause of mortality worldwide,” and indicates that particulate matter (PM) is the, “fraction of air pollution that is most reliably associated with human disease.” (52) Particulate matter includes, “a broad range of substances that are small enough to remain suspended in the air,” which can include allergens, liquid aerosols, hair, feathers, dander, and bioaerosols. (49, 65) Bioaerosols originate from biological organisms such as: viruses, bacteria, parasites, fungi, and mold. (51) This can consist of “fragments of cell walls, fungal spores, hyphae, endotoxins, plant cell debris, animal cell debris, and whole cells,” as reported by the EPA. (68)

More animals lead to increased production of hair, feathers, dander, and liquid aerosols, that become a portion of particulate matter, suspended in the air; while activities of animal movement and breathing can increase suspension of these materials. (49) There
are no published articles that have measured particulate matter in the air of the animal hoarding environment. However, data acquired from concentrated animal feeding operations (CAFO), where large numbers of livestock are housed, share much in common with the animal hoarding environment. (57) It is reported that there is a presence of dust (particulate matter), ammonia, hydrogen sulfide emissions, volatile organic compounds, and endotoxins in the air at CAFO locations. Of these dust (particulate matter), ammonia, and hydrogen sulfide emissions have the greatest potential for adverse health effects. (49, 51) The Environmental Protection Agency (EPA) documents that particulate matter on CAFO’s consist of soil particles, bedding material, fecal matter, litter, feed, bacteria, viruses, and fungi. (68, 51) Zhao states that, “particulate matter can absorb odors and gases and carry bacteria; potentially transmitting odors and disease.” (49) This nature of particulate matter adds to the dangers that may reside within an animal hoarders’ home and the potential impact on public health and animal welfare.

Particulate matter is categorized by size and respirable has been defined as particles with a diameter equal to or less than 10.0µm. These fine dust particles can have serious health effects due the ability for them to, “easily penetrate into the human respiratory system and decrease lung function and increase the rate of cardiovascular disease.” (49, 52, 65) Studies indicate that health effects can consist of allergic reactions (70), cardiovascular and cerebrovascular disease, infectious disease, acute toxic effects, and cancer. (51, 52, 53, 70) These findings have created an increase interest in the occupational and
residential indoor environments (53). The animal hoarding environment is a residential indoor environment, as well as a non-traditional occupational setting, that may impose serious health hazards to the residents, captive animals, and responding agencies.

“The Clean Air Act, which was last amended in 1990, requires EPA to set National Ambient Air Quality Standards (40 CFR part 50) for pollutants considered harmful to public health and the environment.” (56) Within this act there are two categories: 1) Primary standards which, “provide public health protection, including protecting the health of susceptible populations such as asthmatics, children, and the elderly,” (56) and 2) Secondary standards that “provide public welfare protection, including protection against decreased visibility and damage to animals, crops, vegetation, and buildings.” (56) Through the Environmental Protection Agency (EPA) principle pollutants referred to as “criteria pollutants” were developed and particulate matter is among these pollutants. Primary and Secondary PM$_{2.5}$ has a 24-hour averaging time level of 35 $\mu$g/m$^3$ and PM$_{10.0}$ has a 24-hour averaging time level of 150 $\mu$g/m$^3$. (56)

These indices are referenced for outdoor air rather than indoor air, but since data is lacking for concentrations within animal hoarding environments, it is useful to know what levels, in ambient air, indicate a serious public health risk. The Factors that increase these pollutants are temperature, humidity, activities around buildings, renovation, and poor ventilation. (74, 109) If we refer back to the characteristics of the animal hoarding environment and consider the dampness, overcrowded areas, lack of functioning
household appliances, widespread urine, feces, and garbage, it can be reasonable to conclude that these factors will impact the IAQ of such an environment.

Research indicates that, “air within homes and other buildings can be more seriously polluted than the outdoor air in even the largest and most industrialized cities.” (109) While also showing that people spend approximately 90% of their time indoors. This can greatly increase the adverse health effects resulting from poor IAQ. (109). IAQ is a source of adverse health effects and the EPA has connected this with the following health symptoms, “headaches, fatigue, trouble concentrating, and irritation of the eyes, nose, throat and lungs.” (110), Specific diseases have also been linked to specific air contaminants or indoor environments, such as asthma associated with damp indoor environments.” (74)

Utilizing information from similar scenarios, such as the absence of filtration and associated particulate matter concentration in residential environments as well as evaluations of indoor particulate matter concentrations, provides a comparison to the animal hoarding environment. In a study that considered filter effectiveness in indoor residential environments, the concentration of particulate matter was found to be $29 \pm 23 \mu g/m^3$ averaged over the study homes as a baseline before intervention with filters. (78) These measurements can be taken into consideration when reviewing the animal hoarding environment, where deterioration and lack of proper ventilation or maintenance of the home is common. In an evaluation of ambient, personal, residential indoor air, and
residential outdoor air, the Detroit Exposure and Aerosol Research Study assessed 136 residents in Michigan for a three year period. (114) This study investigated environmental pollutants such as particulate matter, criteria gases, and semi-volatile and volatile organic compounds. (114) This provides another source for comparison of indoor particulate matter concentrations. Since this was a large collaborative effort, several smaller study reports were generated. One in particular was designed to characterize factors impacting healthy adult exposure to particulate matter from multiple sources. The study found that personal exposure concentrations were greater than indoor concentration exposures which were greater than outdoor concentration exposures. (79) Meaning that individual’s personal exposures had the highest concentration, but indoor exposures were more significant than the outdoor exposures. The unadjusted average of particulate matter concentration for indoor exposure was 18.7µg/m³. (79) This supports the public health concern that the animal hoarding environment may present exposures of particulate matter for susceptible populations and occupational exposures for responders and social service employees.

IV. Populations at Risk

As a public health issue, the animal hoarding environment harbors many serious health and safety issues such as, unsafe housing structures (fire and collapse hazards), intense noise levels, potential hazards from airborne particulate matter and concentrations of ammonia, as well as the potential transmission of zoonotic disease. When these situations
are discovered we have to be prepared for the multitude of potential hazards. Rescue responders must enter these homes to remove animals, law enforcement must be present to issue warrants, and social service agencies must enter to care for residents; all of which lead to the potential exposure to particulate matter within the air, high noise levels, high concentration levels of ammonia, and zoonotic disease.

Susceptible populations such as immunocompromised, elderly, and young children are among the populations potentially exposed to the animal hoarding environment. The HARC website reports that “All forms of hoarding carry the risk of elder neglect, child neglect, and self-neglect. Dependent/vulnerable adults or children are found in 10-15% of hoarding cases.”(48) Accounts of child endangerment and elder abuse are easily found through media reports; one such case involved the removal of 23 chihuahuas, 26 cats, two chinchillas, and one ferret and resulted in the sentencing of the offender for animal and child abuse. The offender had a 10 year old child living within the residence along with the 52 animals, in a state of filth. (85)

Multiple adverse health effects exist in this environment and impact many populations of individuals. Noise can result in hearing impairment, interference with spoken communication, sleep disturbances, cardiovascular disturbances, mental health disturbances, impaired task performance, negative social behavior and annoyance reactions. (106) Groups particularly vulnerable to noise include, neonates, infants, children, those with mental or physical illnesses, and the elderly. (106)
Zoonotic pathogens are also indicated as a public health concern and we have established the potential presence of both zoonotic pathogen and susceptible populations in the animal hoarding environment. Although, anyone can be infected by a zoonotic agent regardless of immune status, individuals that are immunocompromised may acquire an infection that has the potential to be life-threatening. Tuzio et al. explained this with the following example, “primary *Toxoplasma gondii* infection of an immunocompetent person is usually unapparent whereas infection in an immunosuppressed person can cause life-threatening disease.” (61) Understanding that immunocompromised individuals are at a heighten threat from many of the zoonotic pathogens potentially present in the animal hoarding environment supports public health action. (2, 16, 19, 22, 60)

Particulate matter and ammonia levels, which are a portion of indoor air quality also impacts residents, who may be considered susceptible. The EPA reports that, “people who may be exposed to indoor air pollutants for the longest periods of time are often those most susceptible to the effects of indoor air pollution. Such groups include the young, the elderly, and the chronically ill, especially those suffering from respiratory or cardiovascular disease.” (109) The residents, however, are not the only individuals exposed to these environments.

It is reasonable to consider this environment as a non-traditional occupational environment, resulting in the potential hazards impacting community responders. Emergency responders for fire, medical, and animal rescue must enter these homes and
often conduct work over an extended period of time. Commercial services are also marketing themselves to conduct cleaning within these environments, resulting in another occupational population that may be exposed to any hazards that are present. (34)
Without understanding, in depth, the potential hazards associated with the animal hoarding environment, we cannot be suitably prepared to enter this work site, remediate the conditions, and create desired outcomes.

Consideration of personnel exposed to the animals removed from the site is another population with potential exposure, mainly to zoonotic pathogens. Sokolow, et al. evaluated diarrhea occurrence in dogs at an animal shelter and reported that; “approximately 33% of the case dogs and 16% of the control dogs had evidence of fecal parasites on fecal flotation.” Those of importance to public health included: Cryptospridium spp, *Giardia lamblia*, *Toxocara canis*, and *Ancylostoma caninum*; all of which have zoonotic potential. (45) The authors suggest that routine monitoring for all suspected pathogens is unattainable and the identification of causal and predisposing factors would be an effective management tool for shelters; thus, decreasing the risk of exposure for shelter animals, personnel and the public coming in to adopt pets. (45) The causal and predisposing factors can result from animal hoarding victims being brought into the shelter, thus supporting the need for hazard evaluation of the animal hoarding environment.
V. Research Focus: Animal Hoarding Environment

The HARC website has a wealth of resources pertaining to animal hoarding and delineates this issue as not only an animal welfare issue but a serious public health concern. (28) The previous pages have outlined many issues that exist in the animal hoarding environment and the complexity of the animal hoarding issue. Research is needed to understand the impact that this environment has on animal hoarders health, the animals they imprison, rescue responders, and community members and organizations such as animal shelters. In one media report the added dangers for firefighters in hoarding situations discussed issues of maneuverability, rapidity of the fire to spread, and potential transmission of disease. (86) It is apparent that the animal hoarding environment may present a source of multiple hazards that can affect not only the animals and individuals residing in the home, but any social service, community agency, rescue responder, or visitors that may frequent the home. Currently, there is a research gap in regard to the impacts these hazards present from an occupational viewpoint. Responders are not monitored for exposures through medical surveillance and personal protective equipment is not standardized for work conducted in the animal hoarding environment. Knowledge is also lacking in relation to the occupants and the role of social services. Considering hoarding as a mental health condition, that prevents individuals from fully understanding the adverse outcomes, social service intervention may need to play a larger role in mitigation and follow up activities.
Assessing qualitatively and quantitatively, the role that the environment within these homes has on the outcomes for the resident animals, humans, and community responders, will provide information for appropriate mitigation and monitoring activities. This also provides a starting point to assess biological hazard potential within the animal hoarding environment, to be used by responders, shelters, and the community to plan remediation activities. Planning can include anticipation of appropriate personal protective equipment (PPE), therapeutics for animals and responders, disinfection for PPE and the hoarding site, as well as isolation of the site and/or the animals.

Along with harboring these potential hazards, the cleanup can leave a heavy burden on property owners (many times these individuals rent a home), community agencies (animal shelters), and the increase to community social service burdens. (38) Utilizing multiple agencies to mitigate these situations is a huge undertaking that can be approached through the use of community task forces. These task forces can provide effective and efficient application of knowledge to each specific hoarding case; be it material possessions or animals. Increasing the knowledge base through use of the modified HOMES© survey will provide communities access to information necessary to develop a community task force. However, the fact remains that there is a knowledge gap as to what hazards are present and what the magnitude of these hazards are within the animal hoarding environment.
Based on current research gaps this pilot study was designed to address three main objectives:

- Characterize the potential environmental public health hazards present in the animal hoarding environment.
- Provide screening results for future risk assessment research relative to investigational responders and occupants of the animal hoarding environment.
- Characterize the hazards that could adversely impact investigational responders, so that appropriate respiratory, dermal, ocular and hand Personal Protective Equipment (PPE) can be selected.

Future research activities will be able to use this information to guide efforts in relation to the hazards that have the greatest impact on occupants and occupational responders.

<table>
<thead>
<tr>
<th>Patronek et al: 54 Case Review</th>
<th>Arluke et al: 71 Case Review</th>
</tr>
</thead>
<tbody>
<tr>
<td>Animal Type</td>
<td>Percent of 54 cases</td>
</tr>
<tr>
<td>Cats</td>
<td>65</td>
</tr>
<tr>
<td>Dogs</td>
<td>60</td>
</tr>
<tr>
<td>Farm Animals</td>
<td>11</td>
</tr>
<tr>
<td>Birds</td>
<td>11</td>
</tr>
<tr>
<td>Reptiles</td>
<td>5.6</td>
</tr>
<tr>
<td>Horses</td>
<td>5.6</td>
</tr>
<tr>
<td>Cattle, sheep, or goats</td>
<td>5.6</td>
</tr>
</tbody>
</table>

*Median number of animals/case = 39, although four were >100 animals*  

*Median number of animals for those employed = 56; the median number of animals for those unemployed = 42*

Table 1. Comparison of animal species hoarded from two independent studies (30, 31)
<table>
<thead>
<tr>
<th>Pathogen</th>
<th>1º Species Affected</th>
<th>Prevalence</th>
<th>Zoonotic Potential</th>
<th>Persistence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Campylobacter</td>
<td>Dogs, Cats, and Birds (24)</td>
<td>30% in healthy adult dogs and cats (26)</td>
<td>Yes</td>
<td>Long periods in urine, feces, milk, at (~4°C)</td>
</tr>
<tr>
<td><em>Salmonella enterica</em></td>
<td>Mammals, poultry, reptiles (21,23)</td>
<td>1-36% healthy dogs; 1-18% healthy cats (23)</td>
<td>Yes</td>
<td>Up to 15 months, condition dependent (22, 116)</td>
</tr>
<tr>
<td><em>Listeria monocytogenes</em></td>
<td>Ruminants: cattle, sheep, and goats. Many avian and mammal species (76)</td>
<td>Undetermined</td>
<td>Yes</td>
<td>Persists in soil, water, and animals. (19)</td>
</tr>
<tr>
<td>Staphylococcus spp (MSSA, MRSA)</td>
<td>Human, cat, dog, pet birds, horses, pigs, and cattle</td>
<td>MSSA: 7.85% dogs &amp; cats, (77) MRSA: 3.41% dogs &amp; cats(77) 42% Horses, 50% Pigs in another study (18)</td>
<td>Yes</td>
<td>Months on dry surfaces (17)</td>
</tr>
<tr>
<td><em>Trichuris vulpis</em></td>
<td>Dog Cats (rare)</td>
<td>19.4% in U.S. dogs (9)</td>
<td>No</td>
<td>Persists for years (15)</td>
</tr>
<tr>
<td><em>Toxocara canis</em></td>
<td>Toxocara canis: Dog, Toxocara cati: Cat,</td>
<td>T. canis: 13.2% (9) T. cati: 21.6 % (9)</td>
<td>Yes</td>
<td>persists for years (14)</td>
</tr>
<tr>
<td><em>Hookworm:</em> Anscylostoma, Uniceria</td>
<td>Dogs and Cats</td>
<td>33.3% Canine A. caninum in US (9) Ancylostoma tubaeforme in Felines 8.6% (9)</td>
<td>Yes</td>
<td>3- 4 weeks, in favorable conditions (7)</td>
</tr>
<tr>
<td><em>Tapeworm:</em> Diplydium (dog), Taenia (cat)</td>
<td>Dog, Cat,</td>
<td>4- 60% in dogs 1.8 – 52.7% in cats (5)</td>
<td>Yes</td>
<td>Maintained in intermediate hosts - fleas (9)</td>
</tr>
<tr>
<td>Cryptosporidia</td>
<td>Species specific, (4)</td>
<td>52.7% dogs (11) 7% of feral cats and 6% of pet cats (10)</td>
<td>Yes, C. parvum - potentially</td>
<td>fecal contaminated water. Oocyst resist chlorine. (60)</td>
</tr>
<tr>
<td>Giardia</td>
<td>Dog, Cat,</td>
<td>Dogs: 15.6% Cats: 10.3% (3)</td>
<td>Potential-immuno-compromised (3,60)</td>
<td>Persists in soil, water, and on inanimate objects. (73)</td>
</tr>
<tr>
<td><em>Toxoplasma gondii</em></td>
<td>All warm blooded animal – although outcomes from infection vary. (59)</td>
<td>63% feral cats, 34% pet cats (10)</td>
<td>Yes</td>
<td>Oocyst can persist for years. (2)</td>
</tr>
<tr>
<td>Coccidia</td>
<td>Dog and Cat specie specific (1)</td>
<td>Dogs3.1%, Cats: 4.2% (8)</td>
<td>No, Species specific (1)</td>
<td>Oocysts can persist up to a year, conditions permitting (1)</td>
</tr>
</tbody>
</table>

Table 2. Etiology of Potential Pathogens in the Animal Hoarding Environment
Materials and Methods

This was a pilot study conducted to explore public health parameters within animal hoarding residential environments. The study team was established between The Ohio State University College of Public Health, The Ohio State University College of Veterinary Medicine Department of Veterinary Preventive Medicine faculty and an animal shelter in Ohio.

A literature review was conducted using a combination of the following keywords: animal hoarding, zoonotic disease, ammonia concentration, environmental sampling. All of the articles that contained information pertaining to animal hoarding and associated environmental issues were filed into either an animal hoarding folder, zoonotic disease folder, or an environmental folder in Endnote software (Version X7, Thomas Reuters). These articles were then located through OhioLink and, if appropriate, used as part of my literature review. The 130 articles initially gathered led to other reference materials such as animal shelter guidelines, national standard references, and books such as, “Stuff: Compulsive Hoarding and the Meaning of Things,” “The Hoarding Handbook,” and “Inside Animal Hoarding The Case of Barbara Erickson and Her 552 Dogs.” Throughout
the literature review multiple areas of concern were recognized, including the need to evaluate ammonia and particulate matter concentrations, presence of zoonotic disease agents, deterioration of the residence and associated structural and fire hazards, and the level of noise generated within the animal hoarding environment. A more focused literature search was then conducted to find specific information regarding the prevalence of zoonotic agents to determine those that would be more likely to occur and create a health concern in this environment, as well as specific regulations and safety guidelines regarding ammonia and particulate matter concentration, and sound pressure levels. Additionally, articles concerning health outcomes such as those pertaining to ammonia and particulate matter concentration in animal shelters and concentrated animal feedlots and the prevalence of bacteria and parasite infections in animal shelters were identified using Endnote, Google, OhioLink, and the Hoarding of Animal Research Consortium (HARC) website. (28)

The HARC website was a valuable resource due to the multiple links to relevant articles, books, and specific information regarding the animal hoarding phenomenon. One particular resource on this site was the HOMES® Multi-disciplinary Hoarding Risk Assessment. (72) This survey tool was designed for collecting information that characterizes the animal hoarding environment in regards to the specific categories of Health, Obstacles, Mental Health, Endangerment, and Structure and Safety. The HOMES® survey was adapted for use in this study as described later.

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A list of pathogens that may be associated with animal hoarding environment was compiled from the literature review. Through discussions with the research team, pathogens of interest were selected for this study. These included listeria, salmonella, *Escherichia coli*, campylobacter, staphylococci to include Methicillin-resistant *Staphylococcus aureus* (MRSA), and parasites *Trichuris vulpis* (whipworm), Ancylostoma spp and Uncinaria spp (hookworm), *Diplydium caninum* and *Taenia pisiformes* (tapeworm), *Toxocara canis* and *Toxocara cati* (roundworm), cryptosporidia, giardia, coccidia, and *Toxoplasma gondii*. The basis for pathogen selection relied largely on the prevalence of these pathogens within animal populations, the ability to isolate them through laboratory technique, and the potential public health impacts that they represent.

Based on previous findings in the animal hoarding environment and similar environments such as concentrated animal feeding operations, the team also determined that potential health hazards included air quality, ammonia concentration, sound pressure level, and structural concerns (fire hazards, avalanche, and decreased structural integrity). Once the list of pathogens and other potential hazards was delineated, the team decided how best to sample them. It was decided that a diverse sampling protocol would be needed and included, surface sampling for bacterial presence, collection of bulk feces for parasite evaluation, air sampling for bacteria, fungi/mold, particulate matter and ammonia concentration, measuring sound pressure level, temperature, and relative humidity, and utilizing the HOMES® survey.
A convenience sample of two control homes were evaluated as a baseline for the study: one with pets and one without. One home was occupied by a single individual with no pets, while the other was inhabited by a family of five with two adults, three children, two dogs and one cat. The other residential environments in the study were determined to be animal hoarding environments by the animal shelter’s cruelty investigation team. They based this determination on meeting the criteria established by the Hoarding of Animal Research Consortium and the legal definition of animal cruelty in Ohio. During the study period from May 2014 until January 2015 there were three animal hoarding cases investigated and two of these cases were sampled for this study. One case was not sampled due to short notification (<24 hours), which made it too difficult to get enough team members together to accomplish the sampling. In each sampling site, sound pressure level, temperature, relative humidity, survey using the modified HOMES© assessment tool, air sampling for bacteria, fungi/mold, ammonia concentration, and particulate matter, surface sampling, and collection of bulk feces were conducted, as further described below.

Building Characterization

*Modified HOMES® Survey*

General observations of residence characteristics were evaluated using a modified HOMES® Multi-disciplinary Hoarding Risk Assessment. The modified HOMES Survey,
was intended as an initial assessment, to measure the level of risk in a hoarded environment. It is formatted as a checklist so that the administrator can easily select whether or not a specific condition is present. The survey contains five categories containing related issues. For example, the Health category records issues like whether or not residents can sleep in a bed, have access to a working toilet, or have spoiled food present. The Structure and Safety category notes flammable items beside a heat source, blocked exits, and a lack of running water. Because this study focused on the environment the Mental Health category of the survey was eliminated. The other category that was adjusted was the Endangerment category, which was altered to add the issue of the hoarding environment being a threat to the community and/or a threat to the responders. The modified survey can be found in Appendix B.

**Air Sampling for Physical, Chemical and Biological Agents**

*Sound Pressure Level*

To evaluate sound pressure level in the residence a 3M™ 2200 Integrating- Averaging Sound Level Meter (SKC, Eighty Four, PA) was used. Calibration and measurement were conducted according to manufacturer instructions. Readings were conducted inside the residence during responder activity as well as outside the residence at a distance of 15 feet. The distance of 15 feet was selected for all outdoor readings for sampling consistency. At each site we took one reading indoor and one outdoor, we allowed the approximately 1 minute for the reading to allow us to evaluate any large variations. At
site AH04 the reading did vary and we decided to record the lowest reading and the highest. This site had the largest population of animals that were reacting to the cruelty investigation teams’ activities.

*Relative Humidity and Temperature*

Relative humidity and temperature were collected with a Barometer (H-B Instrument Company, Collegeville, PA). Calibration was conducted, prior to use, according to the manufacturer’s instructions. The barometer was set up inside the home, in the same location as the Biostage Impacter and TSI Dust Trak, during air sample collection and read at the end of the collection time frame. The outside sample was collected similarly during the outside air sample collection.

*Ammonia*

Ammonia Concentration was evaluated using a GasTec Piston Pump (GasTec GV- 110, GAsTec Corporation, Kanagawa, Japan)) with GasTec Ammonia Detector Tube (No.3La, GasTec Ayase, Japan) which had a scale range of 5-100 ppm. Collection was conducted immediately upon entry of the sampling team to the residence. Activities that occurred prior to measurement included local law enforcement issuing the warrant and the cruelty investigation team making an initial assessment and removal of the human occupants. This activity was unavoidable and may have impacted the ammonia concentration measurements. Another sample was conducted outside the home at a distance of 15 feet from the exterior of the residence. The detector tube was inserted into the piston pump and set to 100ppm pump stroke. The piston was then pulled out fully and
locked in position for 45 seconds. If there was a reading after 45 seconds the measurement was complete. If there was no measurable reading the procedure was repeated up to five times, in the same location. If after five pump strokes there was still no measurable reading, a reading of No Significant Finding (NSF) was recorded. This sampling procedure was conducted according to manual instructions for ammonia detector tube, with the exception of position lock time, which was extended to 45 seconds from 30 seconds in order to ensure enough time for low readings to be recorded. Measurements were then adjusted based on the indoor temperature using a correction factor and an equation provided from the manufacturer, this can be found in the results.

Particulate Matter

Particulate Matter was assessed using a DustTrak Aerosol Monitor (DustTrak DRX 8533, TSI Incorporated, Shoreview, MN). The DustTrak was run inside the home during animal rescue activities in the same location as the Biostage Impactor. The outdoor sample was also at a distance of fifteen feet from the residence and in the same location as the Biostage Impactor. The measurements were used to help decipher the source of the particulate matter by comparing the indoor and outdoor measurements in the same manner as the bacteria and fungi/mold sample measurements. The DustTrak was factory calibrated. Prior to and following completion of sampling the machine was zeroed according to manufacturer instructions. The DustTrak is a direct read instrument, meaning that it provides an instantaneous reading. Sampling was conducted for a five
minute interval and data was recorded for particulate matter sizes, 2.5µm, 10.0µm, and Total Particulate Matter.

**Bacteria and Mold**

To assess bacterial and fungal/mold factors in air quality, a Quick Take 30 with single-stage BioStage Impactor (SKC, Eighty Four, PA) was used to sample the air inside the residence and outside the residence. Indoor air samples were taken during responder activity and in a location of close proximity to the activity of the responders, without being in the way. This allowed for measurements that would reflect the exposure of the responders during recovery activities. The precise location was dependent on the layout of the home, but generally was located near the point of entry into the animal hoarding environment. Air sampling was repeated outside the home at a distance of 15 feet from the home’s exterior.

The impactor was pre-calibrated to 28.4L/min by running the impactor for five minutes, then connecting a rotameter and adjusting the flow rate until 28.4L/min was reached. The air was then sampled for five minutes for each of three types of media (Potato Dextrose Agar (PDA), Mannitol, and MacConkey media). Potato Dextrose Agar was used for detection of fungal and mold growth. MacConkey media is selective for Gram (-) bacteria and differential for lactose fermentation, which is used to detect coliforms and enteric pathogens based on their ability to ferment lactose. (93, 94) Mannitol media is selective for Gram (+) bacteria and is differential for mannitol fermentation which, is used to
detect staphylococci species (93, 94). Each media type was accompanied by a field plate to ensure quality control. After sampling was completed each of the six samples, plus six field plates, were sealed with paraffin paper, and placed in the transport kit. The Ultra High Volume Biostage Impactor was post-calibrated by connecting the rotameter and recording the measurement in L/min, this was done three times to ensure that the impactor had run at the pre-calibrated measurement of 28.4L/min.

Surface Sampling for Biological Agents

In order to collect samples, that would be comparable across sampling sites, a universal set of surfaces and locations were pre-determined. General locations were based on the typical usage patterns of various areas in a residence, such as an entryway, a sleeping area, bathroom facilities, food preparation, and a main sitting area of the residence. Judgment areas were reserved with the expectation that there would be some variability between sites as to the locations where there may be high animal density or animal use. Categorizing the samples this way allowed the team the ability to plan and group samples for ease of comparison. See Table 8 for sampling locations.

Specific surfaces in each location were also pre-determined and designates as either human touch surfaces or common touch surfaces. Human touch surfaces were defined as surfaces that are typically exclusive to human use, such as doorknobs, faucets, and handheld devices while common touch surfaces were defined as surfaces that both
humans and animals come in contact with, including floors, animal food dishes, and litter pans (Table 8). The delineation between human touch and common touch surfaces was based on previous environmental sampling studies, which looked at the risk of contamination and potential transmission between human and animal inhabitants. (88, 89, 91) These designations allowed for a comparison between animal hoarding sites and non-animal hoarding sites regarding the presence and distribution of bacteria while ensuring a standard set of samples retrieved from each hoarding site. Most surfaces were the same in each home; however, “handheld devices” in the main living area and in the main bedroom could include items such as remote controls or phones.

During the sampling process surface samples were collected based on the point of entry to the animal hoarding environment. For each site the entryway doorknob was the first sample collected. Once inside the site, designated areas of the universal sample set were sampled in the order they were entered in the residence. In this way, sampling occurred without the research team passing through an area multiple times prior to sampling. Within a room, samples with similar usage patterns were pooled to maximize detection while minimizing the number of samples, as seen in Table 8.

The total universal sample count was 22 at each site, including four bulk fecal samples and approximately 19 surfaces (Table 8). There was also a judgement category which could include food dishes and litter pans to account for the variation in animal type and presence of these surfaces. Finally, bulk feces was collected, however these samples were
not included in analysis of human touch surfaces versus common touch surfaces but were used to evaluate parasitic presence within the animal hoarding environment.

All surface samples were collected by one of two methods as described below. Larger surfaces such as counters, floors, doorknobs, and tabletops were sampled with a sterile electrostatic cloth (Swiffer Dry Cloth, Proctor and Gamble, Cincinnati, Ohio) and placed in sterile Whirlpak® Sample collection bags, for transportation. Swiffers® were prepared in advance using the clean hands- dirty hands technique (Appendix 2) and pre-labelled according to the universal sampling set. Sterile swabs were used for smaller surfaces such as handheld devices – remotes, telephones, and computer keyboards and were placed in transport tubes containing 2.5% NaCl Trypticase Soy Broth (TSB). All samples were acquired using the clean hands – dirty hands technique.

Bulk feces, for parasitology evaluation, was collected in designated areas of the universal sample set (kitchen, main living room, main bedroom, and main bathroom) If there were other locations such as litter pans or an area of high animal density such as a basement, collection of bulk feces would be added to the category designated judgment areas. Each sample was placed in a sterile plastic container and sealed with its lid.

Following collection, each sample was place in a transport kit and held at room temperature, for transport to the designated laboratory immediately following completion of site evaluation. Upon arrival at the lab bacterial samples were prepared in media and stored in a specified incubator appropriate for each type of analysis. In the laboratory, the
Swiffer® samples were separated into two halves, using sterile surgical blades size 22 and the clean hands - dirty hands technique. Once the Swiffers® were separated, one half was prepared for MRSA analysis and the other half was prepared for Salmonella, E.coli, Campylobacter, and Listeria analysis. Bulk fecal samples were placed in the refrigerator for fecal centrifugation within 24 hours. Additional details on the analysis can be found below.

Laboratory Analysis of Surface Samples

*Methicillin-Resistant Staphylococcus aureus (MRSA)*

MRSA analysis was conducted at the Diagnostic and Research Laboratory for Infectious Diseases at OSU, College of Veterinary Medicine, using methods validated and published by Hoet et al. 2011, van Balen et al. 2013. (88, 89) Positive and negative controls were prepared for both swab and Swiffer® samples, in a sterile area. The swab positive controls had 2.5% NaCl Trypticase Soy Broth (TSB) placed in laboratory tube which was then inoculated with Control Strain MRSA 433000. The Swiffer® positive controls had 45 mL of TSB placed in a Whirlpak®, which was then inoculated with Control Strain MRSA 433000. The negative controls contained TSB in a laboratory tubes and 45mL of TSB in a Whirlpak® bag. Swab samples remained in the laboratory transport tubes, which already contained TSB, and were placed in an incubator set to 35°C for MRSA analysis. In each Whirlpak® bag, containing a sample, 45mL of TSB
was added and worked through the Swiffer® by squeezing the Swiffer® until all of the air was released. Once preparations were completed, all of the samples (Swiffers®, swabs, and controls) were placed in the 35°C incubator for 24 hours.

After the 24 hour incubation the broth was streaked onto mannitol salt agar plates (BD BBL™ Mannitol Salt Agar, Dickinson and Company) with 2µm/mL of oxacillin and incubated for another 24 hour. (88, 89) Afterwards 1 to 3 colonies were selected from each plate and streaked onto trypticase soy agar with 5% sheep blood agar plates (Remel®, Blood Agar [trypticase soy agar, TSA, with 5% sheep blood], Lenexa, KS) (88) Staphylococcus aureus was identified using standard morphology and biochemical reaction testing. This included size, pigmentation, hemolysis pattern, mannitol fermentation, gram stain, catalase, tube coagulase, anillin fermentation, Polymixin B susceptibility, acetoin production (Vogues-Proskauer test) and latex agglutination (Sure-Vue® ColorStaph ID, Biokit USA, Inc, Lexington, MA) (88, 89) In order to phenotypically classify isolates as methicillin – susceptible Staphylococcus aureus (MSSA) or as methicillin – resistant Staphylococcus aureus (MRSA), they were grown on Oxacillin Screen Agar (OSA) plates containing 6 µg/mL of Oxacillin supplemented with NaCl (BD BBL™, Beton Dickinson and Company, Maryland, USA), according to Clinical Laboratory Standards Institute protocols. (95)
Listeria

Listeria analysis was conducted at Dr. Wittum’s Research Laboratory, at the OSU College of Veterinary Medicine. Samples AH-06 through AH-09 were used to assess presence of listeria at each site. AH-06 and AH-08 were Swiffers® and AH-07 and AH-09 were sponges. Two different collection materials were used in order to compare their effectiveness at sampling for listeria. Samples were collected from the kitchen sink and the refrigerator. These two locations were divided by left and right side, for the sink, and by top and bottom portion of each handle on the refrigerator. The interior shelf sample of the refrigerator was also divided by left and right sides. This allowed sampling of one side with a Swiffer while on the other side a sponge was used to collect the sample. Each of these samples, contained in Whirlpak® bags, received 45mL of selective Listeria Enrichment broth, were mixed well, and then incubated at 30°C for 24 hours. The incubated broth was aseptically inoculated to ½ Modified Oxford agar plate and streaked for isolation. After 18 to 48 hours incubation at 35°C, resulting black colonies were stored on nutrient slants for further characterization. (92) Isolates were sent to the OSU College of Veterinary Medicine Microbiology Laboratory for MALDI-TOF identification, using the Bruker Biotyper (Bruker Corporation, Billerica, MA).

Campylobacter

Campylobacter analysis was conducted at Dr. Wittum’s Research Laboratory at the OSU College of Veterinary Medicine. Double Strength Bolton Broth was prepared by mixing 50mL Laked Horse Blood with 2 vials of selective supplement. The selective supplement
was first reconstituted with 5mL of Ethanol per vial. The Swiffer® samples were combined with 45mL of buffered peptone water (BPW) and mixed well, then 20mL of the rinsate was pipetted from the Whirlpak® and added to 20mL of double strength Bolton broth in a 50mL conical tube. These were incubated at 42°C in a ‘Campy Chamber’ with three GasPak sachets for 48h under microaerophilic conditions. After the initial 48h incubation the broth was inoculated to Campy-Cefex agar and incubated for 48h at 42°C under microaerophilic conditions. (90)

**Salmonella**

Salmonella analysis was conducted at Dr. Wittum’s Research Laboratory at the OSU College of Veterinary Medicine. The whirlpak® bag containing the Swiffer® sample and the remaining 25mL BPW (from the preparation for campylobacter analysis) was put into the incubator at 37°C. After the initial 24 hour incubation at 37°C, an aliquot of rinsate was transferred to Rappaport – Vassiliadis R10 broth and incubated overnight at 42°C and differentially selected on xylose-lysine-Tergitol 4 agar (XLT-4) with overnight incubation (90). A single black colony was selected on XLT-4 for isolation on MacConkey agar and Salmonella was identified using standard biochemical reactions: triple sugar iron agar, urea broth, and polyvalent antisera (90).

**Escherichia coli**

E. coli analysis was conducted at Dr. Wittum’s Research Laboratory at the OSU College of Veterinary Medicine. The 25mL rinsate in buffered peptone water (BPW) used for Salmonella analysis was used for E. coli analysis as well. After the initial 24 hour
incubation in 37°C the rinsate was aseptically inoculated to MacConkey agar, streaked for isolation and incubated for 24 hour at 37°C. The following day E. coli isolates were confirmed indole positive and stored for further analysis. (90)

Parasitology
Parasitology samples were evaluated at the Department of Veterinary Preventive Medicine’s Parasitology Laboratory at the OSU College of Veterinary Medicine. Fecal Evaluation was conducted using a centrifugation technique for roundworm, whipworm, hookworm, toxoplasma, coccidia, and tapeworm. For this technique approximately 1-2 grams of the fecal sample was placed in a plastic cup and mixed with water. This was then strained through a nylon screen into a centrifuge tube and centrifuged for 5 minutes. The supernatant was decanted into a discard container, float sulfate solution was added and the pellet mixed in this thoroughly. More float solution was added incrementally. Once the tube was almost full it was then centrifuged again for 5 minutes at 1000rpm. At the end of the 5 minutes the tube was removed from the centrifuge and placed in a test tube rack. More float solution was added to the tube until there was a bulging meniscus, at which point a coverslip was placed on top of the tube. The tube was left for another 10 minutes of flotation. After 10 minutes the coverslip was placed on a microscope slide and examined under a microscope at 10X and 40X power. Positives were confirmed with staff in the parasitology department. Refer to Appendix C for a more detailed description of the centrifugation technique.
Laboratory Analysis of Air Samples

*Mannitol*

Mannitol and associated field plates were analyzed at the Diagnostic and Research Laboratory for Infectious Diseases at the OSU College of Veterinary Medicine. Plates were incubated at 35°C upon arrival from the collection site. The numbers of colony forming units were counted at 24, 48, and 72 hours. The counts at 72 hours were done to ensure the accurate count of colonies that were present but too small to identify at 48 hours. Pictures of the plates were also taken at 24, 48, and 72 hours.

*MacConkey*

MacConkey and associated field plates were analyzed at Dr. Wittum’s Laboratory at the OSU College of Veterinary Medicine. Plates were incubated at 37°C upon arrival from the collection site. The colony forming units were counted at 24, 48, and 72 hours. The counts at 72 hours were done to ensure the accurate count of colonies that were present but too small to identify at 48 hours. Pictures of the plates were also taken at 24, 48, and 72 hours.

*PDA*

PDA and Associated field plates were analyzed at Dr. Bisesi’s Laboratory at the OSU College of Public Health. Plates were incubated at room temperature upon arrival from the collection site. The colony forming units were counted at 24, 48, and 72. Pictures of the plates were also taken at 24, 48, 72, and 168 hours. The 168 hour mark was the 7th
day, which is when these plates were taped, labelled, sealed in a freezer bag, and archived in the freezer.

Colony forming units were counted by marking the media plate with a permanent marker for each colony present on the plate. Fungal/mold colonies were distinguished from bacteria by morphology, such as the presence of branching growth versus a smooth appearance of bacterial colonies.

Archives

Isolates from each laboratory and bulk feces were archived for further evaluation.

» Subsets of fecal samples were placed in French squares with a 1:2 ratio of water to fecal material within 24 hours of site evaluation. The French Square was filled with 5mL of distilled water and raised to 10mL with addition of fecal material. These were labeled with date and sample ID and archived for possible giardia testing.

» Bacterial isolates were frozen upon identification of isolates.

» Fungi/Mold plates (PDA) were sealed with tape, placed in freezer bags, and frozen on the seventh day after initial collection.
<table>
<thead>
<tr>
<th>Sample</th>
<th>Location</th>
<th>Surface Category</th>
</tr>
</thead>
<tbody>
<tr>
<td>AH#-01</td>
<td>Entry Doorknob (Ent drknb)</td>
<td>Human</td>
</tr>
<tr>
<td>AH#-02</td>
<td>Dining(D.) room table</td>
<td>Human</td>
</tr>
<tr>
<td>AH#-03</td>
<td>D. room table chairs</td>
<td>Human</td>
</tr>
<tr>
<td>AH#-04</td>
<td>Kitchen(K.) floor</td>
<td>Common</td>
</tr>
<tr>
<td>AH#-05</td>
<td>K. faucets/cabinets (fau/cab)</td>
<td>Human</td>
</tr>
<tr>
<td>AH#-06</td>
<td>K. fridge handle/inside (_hdl)</td>
<td>Human</td>
</tr>
<tr>
<td>AH#-07</td>
<td>K. fridge handle/inside (sponge)</td>
<td>Human</td>
</tr>
<tr>
<td>AH#-08</td>
<td>Sink walls/drain(left)</td>
<td>Human</td>
</tr>
<tr>
<td>AH#-09</td>
<td>Sink walls/drain (right) (sponge)</td>
<td>Human</td>
</tr>
<tr>
<td>AH#-10</td>
<td>K. doorknob/light switch</td>
<td>Human</td>
</tr>
<tr>
<td>AH#-11</td>
<td>K. counters/table</td>
<td>Human</td>
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<td>AH#-12</td>
<td>K. bulk feces</td>
<td>N/A</td>
</tr>
<tr>
<td>AH#-13</td>
<td>Main Living(ML) doorknob/light switches</td>
<td>Human</td>
</tr>
<tr>
<td>AH#-14</td>
<td>ML handheld devices (hndhld)</td>
<td>Human</td>
</tr>
<tr>
<td>AH#-15</td>
<td>ML bulk feces</td>
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</tr>
<tr>
<td>AH#-16</td>
<td>Bath (Ba) doorknob/counters (drknb/cnt)</td>
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</tr>
<tr>
<td>AH#-17</td>
<td>Ba faucets/light switch</td>
<td>Human</td>
</tr>
<tr>
<td>AH#-18</td>
<td>Ba bulk feces</td>
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<td>AH#-19</td>
<td>Main Bedroom (MB) doorknob/light switch</td>
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</tr>
<tr>
<td>AH#-20</td>
<td>MB bedside table/headboard</td>
<td>Human</td>
</tr>
<tr>
<td>AH#-21</td>
<td>MB bulk feces</td>
<td>N/A</td>
</tr>
<tr>
<td>AH#-22</td>
<td>MB handheld devices</td>
<td>Human</td>
</tr>
<tr>
<td>AH#-XX</td>
<td>Judgment food dish/litter pans (Jdgmt)</td>
<td>Common</td>
</tr>
</tbody>
</table>

Table 3. Universal Sample Locations Including Delineation of Surface Category
Results

Building Characteristics

Two sites were sampled as non-hoarding environments: AH01 had one cat and two dogs while AH02 had no pets. The two hoarding sites that were sampled were characterized by an overabundance of animals: AH03 had 11 cats and dogs while AH04 had 41 dogs (Table 4). Each residence had one person living in the household except AH01 which had five people, two adults and three children. All sampling occurred between May and August, 2014.

The indoor and outdoor temperatures for the non-hoarding sites were relatively similar, while the hoarding sites were both 26°C indoors, which was two to five degrees cooler than outside. In both non-hoarding homes the relative humidity was similar too but a few percent lower than the outdoor humidity. However, both hoarding environments had indoor humidity levels that were at least 20 percent higher than the outdoor measurements. Additionally, the indoor humidity increased from 60 in the house with 11 animals to 80 in the house with 41 animals. This indicates a source of additional humidity within the hoarding environment that is not able to be remediated by the air handling capacity available in these single family homes.
Modified HOMES® Survey

The modified HOMES© survey tool was utilized to gather observational data found at each site, which is summarized in Table 7. The results ranged from site AH01, which had 0 issues in the home with three animals, to site AH04, which had 10 issues in the home with 41 animals (Figure 1). The most commonly found issue was the presence of insects, these included ants in one of the non-hoarding homes, flies and cockroaches in AH03, and flies and fleas at site AH04. The only other issue found in a non-hoarding home was a blocked egress; this issue was also present in one of the hoarding environments. Issues that were common to both hoarding homes included the presence of unstable floor boards or stairs, exposed electrical wires, mold or chronic dampness, insects, feces or urine on the floors and feces on the counters.

Air Sampling for Physical, Chemical and Biological Agents

Sound Pressure Level

The sound pressure level was recorded at each site and can be viewed in Table 6. In both of the controls and one of the hoarding homes the indoor SPL was lower than the outdoor SPL. The indoor levels, for these three sites, ranged from 32 to 54 dbA. In AH04, which had 41 dogs, the indoor SPL ranged from 90 to 95 dBA, which was higher than the outdoor SPL. This home was located on a busy city street and as we approached the residence the barking of dogs could be heard over the sound of the passing traffic.
Using the OSHA PEL of a time weighted average (TWA) of 90 dBA over an 8 hour period and the average indoor dBA of 92.45 an allowable time can be calculated.

Allowable Time = \(\frac{480 \text{ min}}{2^{(L_a - 90)/5}}\), where \(L_a\) is the value of the exposure in dBA

\[
\text{Allowable Time} (T) = \frac{480 \text{ min}}{2^{\frac{2.45}{3}}} = \frac{480 \text{ min}}{1.40445} = 341.77 \text{ minutes} \left(\frac{1 \text{ hr}}{60 \text{ minutes}}\right) = 5.70 \text{ hrs}
\]

The measurement indicates, that at the average SPL of 92.45 dBA, responders could be exposed for 5.70 hours, and remain in compliance with OSHA permissible exposure limits.

The time frame of work conducted was between 2 and 3 hours, and the peak SPL measurement was 95 dBA inside the animal hoarding environment. (96) Based on the Allowable Time estimated for site AH04, it can be assumed that the responders for this site did not exceed the PEL. Although, more data would broaden the scope of analysis, since this site had variable SPLs, due to changes in activity and animal density. It is relevant to note that employers are required to provide effective hearing conservation programs for employees exposed to an 8 hour time weighted average of 85 dBA, which is the action level for hearing conservation (50% dose). (96)

Ammonia

No measurable ammonia levels were detected in the control homes, while both hoarding homes had detectable levels (Table 8). For both sites, AH03 and AH04, the correction factor was 0.95, based on inside temperatures of 26° C at both sites, resulting in the equation: AH04 [AMM]ppm = 12 ppm(0.95) = 11.4 ppm; AH03 [Amm]ppm = 51
3ppm(0.95) = 2.85ppm. Site AH03 had an ammonia concentration of 2.8 ppm while AH04 had the highest ammonia concentration measurement of 11.4 ppm (Figure 2). Ammonia was not detected outside of the hoarding homes indicating an indoor source. This is further supported by the accumulations of animal waste seen in both hoarding homes (Table 7).

Particulate Matter

PM$_{2.5}$

The next factor evaluated was the level of airborne particulate matter (PM) in the indoor and outdoor environments. For PM$_{2.5}$ the two non-hoarding homes had moderately low levels both indoors and outdoors (range 6 to 15 µg/m$^3$) as shown in Table 9. One of the hoarding homes (AH03) also had low levels outside while AH04 had the highest outdoor level of 41 µg/m$^3$. Both hoarding homes had indoor levels that were higher than the outdoor levels at that time and location (Figure 3). AH04 was 46 µg/m$^3$ and AH03 was 99 µg/m$^3$. The Environmental Protection Agency calculates an Air Quality Index based on the levels of five major pollutants regulated by the Clean Air Act, including particulate matter. Using the online calculator at the AirNow website (http://airnow.gov/index.cfm?action=resources.conc_aqi_calc), a concentration of 46 µg/m$^3$, if this were the only pollutant present, converts to an air quality index (AQI) that is in the “unhealthy for sensitive groups” range with sensitive groups listed as those with respiratory or cardiovascular illness, the elderly, and children. The 99µg/m$^3$ concentration
converts to the “unhealthy” range which cautions that the general population could experience respiratory signs and should limit prolonged exertion.

$PM_{10}$

As shown in Table 10 and Figure 4, the concentrations of $PM_{10}$ followed a pattern similar to $PM_{2.5}$ with respect to indoor levels being higher than outdoors at three of the sites and with the indoor levels of both hoarding homes ($49 \, \mu g/m^3$, $105 \, \mu g/m^3$) being much higher than the non-hoarding homes ($9 \, \mu g/m^3$, $21 \, \mu g/m^3$). When the concentrations were converted to AQI at the AirWeb website all values were in the “good” range except the indoor $105 \, \mu g/m^3$ concentration which was in the “moderate range” with a caution for unusually sensitive people to reduce prolonged exertion. The tolerances for $PM_{2.5}$ are lower than for $PM_{10}$ because the smaller sized particles can be deposited deeper in the airways of the lungs.

$PM_{Total}$

Readings for total particulate matter were recorded for each site as seen in Table 11; however in all but one they match the values produced for $PM_{10}$, suggesting that most of the particulates measured in the respirable range ($\leq 10 \mu m$)

$Bacteria and Mold$

$PDA$

For the PDA media, which evaluated fungi/mold growth, the number of colony forming units per cubic meter of air (cfu/m³) both indoors and outdoors increased with the increasing number of animals at each site (Table 12). As these measurements are greatly
impacted by the season and local weather, it was decided to examine the indoor cfu count as a percentage of the outdoor cfu count. Three sites had indoor concentrations that were lower than those measured outdoors. Though not the only factor involved, the percentages increased as the number of the animals in the home increased: 22% (0 animals), 46% (3 animals), and 66% (11 animals). Site AH04 with 41 animals exceeded the outdoor concentration by 114% suggesting an additional source from the indoor environment.

*MacConkey*

Bacterial growth on MacConkey media is predominantly Gram (-) with a preference for coliforms and enteric organisms. For three of the four locations at the control sites, no growth occurred (Table 13). The indoor plates at AH01, a non-hoarding home with three animals, 14 cfu/m$^3$, were found. This was the same amount found inside the hoarding home with 11 animals. The hoarding site AH04 had an indoor concentration of 21 cfu/m$^3$, 1.5 times greater than the other two indoor levels (Table 13).

*Mannitol*

The evaluation of bacteria using Mannitol media (Table 14 and Figure 5) show concentrations from indoor air samples to be higher than outdoor air samples, at all four sites. These plates select for gram (+) bacteria, especially staphylococci and the four indoor measurements ranged from 78 to 2028 cfu/m$^3$. At site AH04 the indoor level was 2028 while the outdoor level was 35 cfu/m$^3$; clearly suggesting an indoor source for these bacteria in the air.
Surface Sampling for Biological Agents

The overall prevalence of positive results out of 77 samples from the four locations was 33.7%. The prevalence on common touch surfaces was 41.6% of the 12 samples while the prevalence on human touch surfaces was 30.5% of the 72 samples. The surface sampling results are suggestive of increasing diversity in both the type of pathogen and location where these pathogens were found (Table 15, 16, and 17). In the non-animal hoarding environments (AH01 and AH02) one pathogen type, a staphylococci, was reported whereas in the animal hoarding environments, AH03 and AH04, there were three and six types reported, respectively (Table 15 and Figure 8). Staphylococci spp was also found in one hoarding site, AH04. The positive samples for Staphylococci spp in AH01 came from two locations, while AH02 had the largest distribution of all the sample sites with nine different locations testing positive. All but one of those nine locations was human touch surfaces.

Only one sample was positive for Salmonella; it was collected from a dining table in AH03. For campylobacter, one sample was positive; it was collected from the kitchen floor in AH04. *Escherichia coli*, a marker for fecal contamination, were found in eight samples at AH04 from five different rooms. Unfortunately, due to a communication error, samples from the other three sites were not cultured for E. coli. Table 15 suggests that areas such as the kitchen, bathroom, and bedroom had more positive results, as the number of animals increased. A portion of these results were for *Escherichia coli*, which,
as previously mentioned, was only analyzed in AH04. However, positive results for parasites were more widespread at site AH04.

Upon MALDI-TOF identification, Listeria was ruled out but *Enterococcus faecalis*, *Enterococcus faecium*, and *Pseudomonas aeruginosa* were found. These are all common pathogens that are found in the environment. While there is some concern for immunocompromised individuals, these pathogens do not present an increased public health concern.

No bulk stool was present in the control sites so, no parasite testing was done at AH01 and AH02. Whipworms were detected in bulk stools from both animal hoarding sites. Additionally AH04, with 41 dogs, had positive samples for roundworms and hookworms. AH03 was predominantly cats with a few dogs. It should be noted that the shelter reported that they had been working with AH03 to help reduce the number of animals and provide preventive medical care to those remaining. This included the provision of antiparasitic treatments which would help control fleas and intestinal parasites.

As seen in Table 15 and Figure 6, areas such as the kitchen, bathroom, and bedroom had more positive results as the number of animals increased. Site AH03 had positive samples in the kitchen, dining room, judgement, and main living area. Though this is fewer locations compared to AH02 which had five positive rooms, AH03 had positive results for three different pathogens compared to one in AH02. Site AH04 had 18
positive samples, representing six different pathogens in five different rooms, excluding E. coli positives.

Overall, the kitchen had the highest occurrence of positive results from bacteria and parasite analysis. As this also represents a greater opportunity for contamination of food during preparation and consumption, the types of pathogens found in the kitchen at each site were graphed in Figure 7. There were no positive kitchen samples from site AH01. The number of pathogen types was higher in the hoarding sites (3 and 4) compared to the non-hoarding sites (0 and 1). With the exception of parasites which were only tested from bulk stool found on the floor or in litter boxes, the majority of positive samples in the hoarding environments were found on human touch surfaces.

Statistical Analysis

Surface samples were designated to one of two categories for this study: common touch surfaces, those surfaces considered common between animal and human contact, and human touch surfaces, areas generally considered to be touched only by the human occupants. The number of positive samples in hoarding versus non-hoarding sites was compared for both common touch and human touch surfaces. A Fisher’s Exact test was utilized for this comparison due to the small sample size. Though not statistically
significant, positive samples on common touch surfaces were twice as likely in hoarding homes compared to non-hoarding homes (Tables 18).

The distribution of concentrations (cfu/m³) of fungi/mold and bacteria in indoor air samples and outdoor air samples in all four sites, was analyzed (Table 20). Since the sample sizes were small nonparametric statistical analysis, using Wilcoxon Rank Sum and Kruskal-Wallis methods were used. The same methods were used to compare the indoor and outdoor particulate matter concentrations across all four sites (Table 21). The statistical analysis suggested that there was insufficient data to reject the null hypotheses, for each of the comparisons, with no p – values <0.05. There was not enough evidence of a significant difference in the distribution of concentrations of colony forming units or particulate matter across sites for indoor and outdoor air samples. Ideally, statistical analysis comparing the hoarding sites to the non-hoarding sites could have been conducted; however, the small sample size negated the value of these efforts. For the purposes of this case study, descriptive data provides the best depiction of the conditions. While definitive conclusions cannot be drawn, these findings can be used to generate more hypotheses and direct further research objectives.
<table>
<thead>
<tr>
<th>Sites</th>
<th># Human</th>
<th># Dog</th>
<th># Cat</th>
<th>Total Animals</th>
<th>Total Inhabitants</th>
</tr>
</thead>
<tbody>
<tr>
<td>AH01</td>
<td>5</td>
<td>2</td>
<td>1</td>
<td>3</td>
<td>8</td>
</tr>
<tr>
<td>AH02</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
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<td>2</td>
<td>9</td>
<td>11</td>
<td>12</td>
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<td>AH04</td>
<td>1</td>
<td>41</td>
<td>0</td>
<td>41</td>
<td>42</td>
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</table>

Table 4. Number of inhabitants by species

<table>
<thead>
<tr>
<th>Sites</th>
<th>Temperature Indoor (°C)</th>
<th>Temperature Outdoor (°C)</th>
<th>Relative Humidity Indoor (%)</th>
<th>Relative Humidity Outdoor (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AH01</td>
<td>21</td>
<td>21</td>
<td>64</td>
<td>68</td>
</tr>
<tr>
<td>AH02</td>
<td>22</td>
<td>20</td>
<td>60</td>
<td>66</td>
</tr>
<tr>
<td>AH03</td>
<td>26</td>
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<tr>
<td>AH04</td>
<td>26</td>
<td>28</td>
<td>80</td>
<td>61</td>
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</tbody>
</table>

Table 5. Temperature and Relative Humidity measurements of the indoor and outdoor environment

<table>
<thead>
<tr>
<th>Sites</th>
<th>SPL Indoor (dBA)</th>
<th>SPL Outdoor (dBA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AH01</td>
<td>32.1</td>
<td>46.7</td>
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<td>AH02</td>
<td>53.8</td>
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</tr>
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<td>AH03</td>
<td>51.8</td>
<td>53.2</td>
</tr>
<tr>
<td>AH04</td>
<td>89.9 - 95 *</td>
<td>65 – 80 *</td>
</tr>
</tbody>
</table>

Table 6. Sound Pressure Level measurements in dBA scale
*Due to variation in activity level the highest and lowest readings measured, were recorded at this site.
<table>
<thead>
<tr>
<th>Structure and Safety</th>
<th>AH01</th>
<th>AH02</th>
<th>Notes</th>
<th>AH03</th>
<th>Notes</th>
<th>AH04</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unstable floorboards, stairs, porches</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td></td>
<td>Main living area</td>
<td>1</td>
<td>Through out</td>
</tr>
<tr>
<td>Leaking roof</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td></td>
<td>Dining room</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Flammable items beside heat source</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Presence of air purifiers</td>
<td>0</td>
<td>1*</td>
<td>Bedroom</td>
<td>1*</td>
<td>Bedroom</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Electrical wires/cords exposed</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td></td>
<td>Bedroom</td>
<td>1</td>
<td>Main Living</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Health</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Cannot use bathtub or shower</td>
<td>0</td>
<td>0</td>
<td>0^</td>
<td></td>
<td>Litter pans &amp; personal hygiene products in tub</td>
<td>0^</td>
<td>Dog dishes &amp; personal hygiene products in tub</td>
</tr>
<tr>
<td>Presence of mold or chronic dampness</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td></td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cannot sleep in bed</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Presence of insects or rodents</td>
<td>0</td>
<td>1</td>
<td>ants</td>
<td>1</td>
<td>Cockroaches, flies</td>
<td>1</td>
<td>Flies, fleas</td>
</tr>
<tr>
<td>Presence of spoiled food</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
<td>1</td>
<td></td>
<td>Strong odor from fridge</td>
</tr>
</tbody>
</table>

Table 7. Results of the modified HOMES© Survey risk assessment tool showing factors present in at least one site
Table 7 continued

<table>
<thead>
<tr>
<th>AH01</th>
<th>AH02</th>
<th>Notes</th>
<th>AH03</th>
<th>Notes</th>
<th>AH04</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Presence of feces or urine (human or animal origin)</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>Throughout -out on floor</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
<td>Throughout -out</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Feces on counter</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>Dried amounts</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

**Obstacles**

<table>
<thead>
<tr>
<th></th>
<th>AH01</th>
<th>AH02</th>
<th>Notes</th>
<th>AH03</th>
<th>Notes</th>
<th>AH04</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Egresses or exits blocked</td>
<td>0</td>
<td>1</td>
<td></td>
<td>0</td>
<td></td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Unstable piles/avalanche risk</td>
<td>0</td>
<td>0</td>
<td></td>
<td>0</td>
<td></td>
<td>1</td>
<td>Storage room</td>
</tr>
</tbody>
</table>

*air purifiers were not included in figure, impact on the environment and working condition were not assessed; ^Labelled 0 because there was evidence that the resident was utilizing the bathtub or shower; presence of hygiene products
Figure 1. The modified HOMES© Survey results illustrating the number of negative* issues that were present for each category of the survey by site.
*the presence air purifiers were not reported here because the impact on the environment and working condition were not assessed

<table>
<thead>
<tr>
<th>Sites</th>
<th>Indoor Ammonia [ppm]</th>
<th>[Indoor] relative to [Outdoor]</th>
<th>Outdoor Ammonia [ppm]</th>
</tr>
</thead>
<tbody>
<tr>
<td>AH01</td>
<td>NSF, n=5</td>
<td>NA</td>
<td>NSF, n=5</td>
</tr>
<tr>
<td>AH02</td>
<td>NSF, n=5</td>
<td>NA</td>
<td>NSF, n=5</td>
</tr>
<tr>
<td>AH03</td>
<td>2.85ppm, n=1</td>
<td>&gt;</td>
<td>NSF, n=5</td>
</tr>
<tr>
<td>AH04</td>
<td>11.4ppm, n=1</td>
<td>&gt;</td>
<td>NSF, n=5</td>
</tr>
</tbody>
</table>

Table 8. Ammonia concentration from indoor and outdoor samples
Figure 2. Concentration of ammonia from indoor and outdoor samples

<table>
<thead>
<tr>
<th>Sites</th>
<th>Indoor [PM$_{2.5}$] $\mu$g/m$^3$</th>
<th>[Indoor] relative to [Outdoor]</th>
<th>Outdoor [PM$_{2.5}$] $\mu$g/m$^3$</th>
</tr>
</thead>
<tbody>
<tr>
<td>AH01</td>
<td>6.00</td>
<td>&lt;</td>
<td>13.00</td>
</tr>
<tr>
<td>AH02</td>
<td>15.00</td>
<td>&gt;</td>
<td>7.00</td>
</tr>
<tr>
<td>AH03</td>
<td>99.00</td>
<td>&gt;</td>
<td>11.00</td>
</tr>
<tr>
<td>AH04</td>
<td>46.00</td>
<td>&gt;</td>
<td>41.00</td>
</tr>
</tbody>
</table>

Table 9. The concentration of PM2.5 from indoor and outdoor samples
Figure 3. The concentration of PM$_{2.5}$ from indoor and outdoor samples

Table 10. The concentration of PM$_{10.0}$ from indoor and outdoor samples

<table>
<thead>
<tr>
<th>Sites</th>
<th>Indoor [PM$_{10.0}$] $\mu$g/m$^3$</th>
<th>[Indoor] relative to [Outdoor]</th>
<th>Outdoor [PM$_{10.0}$] $\mu$g/m$^3$</th>
</tr>
</thead>
<tbody>
<tr>
<td>AH01</td>
<td>9.00</td>
<td>&lt;</td>
<td>13.00</td>
</tr>
<tr>
<td>AH02</td>
<td>21.00</td>
<td>&gt;</td>
<td>12.00</td>
</tr>
<tr>
<td>AH03</td>
<td>105.00</td>
<td>&gt;</td>
<td>11.00</td>
</tr>
<tr>
<td>AH04</td>
<td>49.00</td>
<td>&gt;</td>
<td>43.00</td>
</tr>
</tbody>
</table>
Figure 4. The concentration of PM$_{10.0}$ from indoor and outdoor samples

Table 11. The concentration of PM$_{Total}$ from indoor and outdoor samples

<table>
<thead>
<tr>
<th>Sites</th>
<th>Indoor [PM$_{Total}$] $\mu g/m^3$</th>
<th>[Indoor] relative to [Outdoor]</th>
<th>Outdoor [PM$_{Total}$] $\mu g/m^3$</th>
</tr>
</thead>
<tbody>
<tr>
<td>AH01</td>
<td>9.00</td>
<td>&lt;</td>
<td>13.00</td>
</tr>
<tr>
<td>AH02</td>
<td>25.00</td>
<td>&gt;</td>
<td>12.00</td>
</tr>
<tr>
<td>AH03</td>
<td>105.00</td>
<td>&gt;</td>
<td>11.00</td>
</tr>
<tr>
<td>AH04</td>
<td>49.00</td>
<td>&gt;</td>
<td>43.00</td>
</tr>
<tr>
<td>Site</td>
<td>Indoor [fungi/mold] cfu/m³</td>
<td>[Indoor] relative to [Outdoor]</td>
<td>Outdoor [fungi/mold] cfu/m³</td>
</tr>
<tr>
<td>-------</td>
<td>---------------------------</td>
<td>-------------------------------</td>
<td>-----------------------------</td>
</tr>
<tr>
<td>AH01</td>
<td>471.83</td>
<td>&lt;</td>
<td>1035.21</td>
</tr>
<tr>
<td>AH02</td>
<td>147.88</td>
<td>&lt;</td>
<td>683.09</td>
</tr>
<tr>
<td>AH03</td>
<td>985.91</td>
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<tr>
<td>AH04</td>
<td>1739.43</td>
<td>&gt;</td>
<td>1521.12</td>
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</tbody>
</table>

Table 12. The concentration of fungi/mold after 48h incubation at room temperature

<table>
<thead>
<tr>
<th>Site</th>
<th>Indoor [bacteria] cfu/m³</th>
<th>[Indoor] relative to [Outdoor]</th>
<th>Outdoor [bacteria] cfu/m³</th>
</tr>
</thead>
<tbody>
<tr>
<td>AH01</td>
<td>14.08</td>
<td>&gt;</td>
<td>ND*</td>
</tr>
<tr>
<td>AH02</td>
<td>ND*</td>
<td>NA</td>
<td>ND*</td>
</tr>
<tr>
<td>AH03</td>
<td>14.08</td>
<td>&lt;</td>
<td>21.13</td>
</tr>
<tr>
<td>AH04</td>
<td>21.13</td>
<td>&lt;</td>
<td>77.46</td>
</tr>
</tbody>
</table>

Table 13. The concentration of bacteria on MacConkey plates after 48h incubation at 37°C
*None detected (ND); `Not Applicable (NA)

<table>
<thead>
<tr>
<th>Site</th>
<th>Indoor [bacteria] cfu/m³</th>
<th>[Indoor] relative to [Outdoor]</th>
<th>Outdoor [bacteria] cfu/m³</th>
</tr>
</thead>
<tbody>
<tr>
<td>AH01</td>
<td>77.46</td>
<td>&gt;</td>
<td>ND</td>
</tr>
<tr>
<td>AH02</td>
<td>253.52</td>
<td>&gt;</td>
<td>ND</td>
</tr>
<tr>
<td>AH03</td>
<td>218.31</td>
<td>&gt;</td>
<td>91.55</td>
</tr>
<tr>
<td>AH04</td>
<td>2028.17</td>
<td>&gt;</td>
<td>35.21</td>
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</tbody>
</table>

Table 14. The concentration of bacteria on Mannitol plates after 48h of incubation at 35°C
Figure 5. The concentration of bacteria on Mannitol plates after 48h incubation at 35° C
<table>
<thead>
<tr>
<th>Site</th>
<th>Salmonella</th>
<th>Campylobacter</th>
<th>Environ mental Species</th>
<th>*E. coli</th>
<th>Staphylococci</th>
<th>Roundworm</th>
<th>Hookworm</th>
<th>Whipworm</th>
</tr>
</thead>
<tbody>
<tr>
<td>AH01</td>
<td>MLdrknb</td>
<td>1</td>
<td></td>
<td>1</td>
<td>1</td>
<td>1</td>
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<tr>
<td>Food dish</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AH02</td>
<td>Dngtbltp</td>
<td>1</td>
<td></td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Dng Chr</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Kfloor</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kfauct/cab</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kcounter</td>
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<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MLdrknb</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ba drknb</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
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</tr>
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<td>1</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
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<td>Frdghand</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Top/Left side</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
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<td>Frdghnd</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Btm/Rgt side</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>K sink</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Left</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>K sink Rgt</td>
<td>NRI</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D room</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Bulk feces</td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>Litter box</td>
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<td>bulk feces</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>K bulk</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>feces</td>
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<td></td>
<td></td>
</tr>
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<td></td>
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</tr>
</tbody>
</table>

Continued

Table 15. Type of pathogens and locations where they were found at the site
Table 15 continued

<table>
<thead>
<tr>
<th>Site</th>
<th>Salmonella</th>
<th>Campylobacter</th>
<th>Environmental Species</th>
<th>*E. coli</th>
<th>Staphylococci</th>
<th>Roundworm</th>
<th>Hookworm</th>
<th>Whipworm</th>
</tr>
</thead>
<tbody>
<tr>
<td>AH04</td>
<td>K floor</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>K sink left</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>K sink right</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Entdrknb</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>K floor</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>K fauc/cab</td>
<td>1 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>K drknb/lgt</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
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<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ba drknb/nt</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>MB drknb/lgt</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Food dish</td>
<td>1 1</td>
<td></td>
<td></td>
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<td></td>
<td></td>
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</tr>
<tr>
<td></td>
<td>ML Hndhld</td>
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<td></td>
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<tr>
<td></td>
<td>Ba fauc/cab</td>
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<td>Jdgmt drknb</td>
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<td>MB handheld</td>
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</tr>
<tr>
<td></td>
<td>MB drknb</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ba bulk feces</td>
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<td>1 1 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>MB bulk feces</td>
<td>1</td>
<td>1 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>K bulk feces</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Jdgmt bulk feces</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
<td></td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td></td>
<td>1 1 6 8 18 2 2 8</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*E. coli was only tested at site AH04; NRI – not reliable identification
### Table 16. Culture positive results for bacterial pathogens on surface samples

<table>
<thead>
<tr>
<th>Sites</th>
<th>Salmonella</th>
<th>Campylobacter</th>
<th>Listeria Spp</th>
<th>Escherichia coli</th>
<th>Staphylococci</th>
<th>NRI*</th>
<th>Total</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>AH01</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>NA</td>
<td>2</td>
<td>NA</td>
<td>2</td>
<td>20</td>
</tr>
<tr>
<td>AH02</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>NA</td>
<td>9</td>
<td>NA</td>
<td>9</td>
<td>18</td>
</tr>
<tr>
<td>AH03</td>
<td>1</td>
<td>ND</td>
<td>0</td>
<td>NA</td>
<td>ND</td>
<td>1</td>
<td>2</td>
<td>20</td>
</tr>
<tr>
<td>AH04</td>
<td>ND</td>
<td>1</td>
<td>0</td>
<td>8</td>
<td>7</td>
<td>NA</td>
<td>16</td>
<td>19</td>
</tr>
</tbody>
</table>

*Humane society was providing some preventive medicine; NA indicate Not Applicable, and ND indicates None Detected.

### Table 17. Laboratory analyzed positive results for parasites.

<table>
<thead>
<tr>
<th>Sites</th>
<th>Roundworm</th>
<th>Hookworm</th>
<th>Whipworm</th>
<th>Total</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>AH01</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>0</td>
<td>NA</td>
</tr>
<tr>
<td>AH02</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>0</td>
<td>NA</td>
</tr>
<tr>
<td>AH03</td>
<td>ND</td>
<td>ND</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>AH04</td>
<td>2</td>
<td>2</td>
<td>4</td>
<td>8</td>
<td>5</td>
</tr>
</tbody>
</table>

*Humane society was providing some preventive medicine; NA indicate Not Applicable, and ND indicates None Detected.
Figure 6. The total positive samples for bacteria* and parasites by location within the sites
*excluding E. coli, which was not sampled at each site
Figure 7. Bacterial* and parasitic diversity in kitchen samples by site
*Site AH04 was positive for *Escherichia coli* however; this was not included in the figure because the other sites were not evaluated for this pathogen. ^Site AHO3 had one more positive result but MALDI-TOF resulting in - not reliable identification (NRI)
Figure 8. The total positive samples of bacteria* and parasites by pathogen.
^Site AH03 had 1 positive result that was not a reliable identification (NRI). *E. coli is included in the graph for site AH04, but is not considered in the interpretation, since it was not evaluated in the other sites.

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Exposure</th>
<th>N</th>
<th>Positive Prevalence (%)</th>
<th>Overall Positive Prevalence</th>
<th>Odds Ratio</th>
<th>95% Confidence Interval</th>
<th>Fisher Exact p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Common Touch Surfaces</td>
<td>Animal Hoarding</td>
<td>6</td>
<td>50.0</td>
<td>41.6</td>
<td>2.0</td>
<td>0.1941, 20.61</td>
<td>&gt;0.999</td>
</tr>
<tr>
<td></td>
<td>Non-Animal Hoarding</td>
<td>6</td>
<td>33.3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 18. Comparison of positive sample results, for bacterial pathogens, on common touch surfaces within the animal hoarding and the non-animal hoarding environment.

*Significant at p< 0.05
<table>
<thead>
<tr>
<th>Outcome</th>
<th>Exposure</th>
<th>N</th>
<th>Positive Prevalence (%)</th>
<th>Overall Positive Prevalence</th>
<th>Odds Ratio</th>
<th>95% Confidence Interval</th>
<th>Fisher Exact p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human Touch Surfaces</td>
<td>Animal Hoarding</td>
<td>36</td>
<td>19.4</td>
<td>22.2</td>
<td>0.7241</td>
<td>0.2367, 2.215</td>
<td>0.778</td>
</tr>
<tr>
<td></td>
<td>Non-Animal Hoarding</td>
<td>36</td>
<td>25.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 19. Comparison of positive sample results, for bacterial pathogens, on human touch surfaces within the animal hoarding and the non-animal hoarding environment

| Media  | Site Location | Mean CFU/m³ | SD   | Z     | P>|Z| |
|--------|---------------|-------------|------|-------|------|
|        | N = 4         |             |      |       |      |
| PDA    | In            | 836.27      | 694.0| 0.72  | 0.4705 |
|        | Out           | 1181.34     | 399.1|       |       |
| MAC    | In            | 12.32       | 8.86 | 0.0   | 1.0   |
|        | Out           | 24.65       | 36.6 |       |       |
| MS     | In            | 644.36      | 925.67| -1.89 | 0.0591 |
|        | Out           | 31.69       | 43.22|       |       |

Table 20. Comparison of the distribution of indoor and outdoor air sample concentrations across sampling sites
*Significant p –values are highlighted; p < 0.05
Ho: The distribution of indoor and outdoor air sample concentrations, of fungi/mold and bacteria across sampling sites, is the same.
| Particulate Matter | Site Location | Mean µg/m³ | SD   | Z     | P>|Z| |
|-------------------|---------------|------------|------|-------|------|
|                   | N = 4         |            |      |       |      |
| PM$_{2.5}$        | In            | 41.5       | 41.98| -0.72 | 0.4705 |
|                   | Out           | 18.0       | 15.53|       |       |
| PM$_{10.0}$       | In            | 46.0       | 42.75| -0.72 | 0.4705 |
|                   | Out           | 19.75      | 15.52|       |       |
| PM$_{Total}$      | In            | 47.0       | 42.02| -0.72 | 0.4705 |
|                   | Out           | 19.75      | 15.52|       |       |

Table 21. Comparison of the distribution of indoor and outdoor air sample concentrations across sampling sites
Ho: The distribution of indoor and outdoor air sample concentrations of particulate matter (2.5, 10.0, and Total) across sampling sites, is the same.
Discussion

Many studies have been conducted to examine the epidemiology of animal hoarding and describe the living conditions within the residence. (30, 31, 36, 47, 62, 62) However, relatively few address public health concerns and no other studies have detailed a comprehensive evaluation of the hazards found in the animal hoarding environment. In these situations the potential hazards that may be present within and around the home, can impact law enforcement and rescue responders that enter the residence to remove animals, health and social service agencies entering to care for residents, and residents either willfully living in the environment or those that are dependent on the primary resident (children, elderly, and disabled individuals). All of these individuals are potentially faced with elevated risks associated with structural issues, poor air quality, and the presence of infectious agents in the hoarding environment.

In our study, four sampling sites were evaluated, which consisted of one apartment with one individual and no animals, one home with five individuals and three animals present, one home with one individual and eleven animals present, and the last home with one individual and 41 animals present. Physical and structural hazards were evaluated using the modified HOMES© survey (Appendix B).
For the categories of structure and safety, health, and obstacles there was an upward trend in the occurrence of issues with increasing animal population. In the animal hoarding sites there were six total structure and safety issues while there were zero in the non-animal hoarding sites. These included exposed electrical wires at both animal hoarding residences, leading to a potential hazard of electrical shock, electrocution, or fire injuries to both human and animal occupants. Unstable floors, stairs, and porches were also reported, which could impede rescue responders for emergencies of various causes, such as medical, fire, or issues with animal welfare. There are many reports of animal hoarding from firefighters responding to house fires, so it was not surprising that our study found flammable items near heat sources, in addition to the exposed electrical wires; representing real public health concerns for residents and emergency and social service responders. Increased awareness of the environmental characteristics would be beneficial for emergency and social service training, since they have a higher likelihood of entering these types of premises.

The greatest number of issues present in this environment came from the Health category. These included the presence of feces or urine on the floors and counters, chronic dampness, and insect infestation. In the non-animal hoarding environment there was one issue recorded, the presence of ants. Presence of organic matter (feces and urine), chronic dampness, and insect infestation, found at both animal hoarding sites, can provide an environment perfect for the growth of mold and bacteria, as well as increased
concentrations of ammonia. These issues can lead to severe health concerns related to respiratory illness and bacterial infection. Individuals entering this environment should be aware of the potential for exposure to these hazards in the air and on the surfaces in which they may come in contact.

Overall, the character of the animal hoarding residence suggests deterioration, disrepair, and lack of hygiene. These findings are consistent with what has been reported in the literature.(30, 31, 40, 57, 58) Individuals entering this environment must be prepared for not only handling the animals present, but also for any structural issues that may impose health and safety issues for their team. Appropriate evaluation of the structure is advisable before a recovery team enters the premises.

Excessive noise is another potential hazard recognized in facilities where animals are housed in large numbers, especially dogs and pigs; however, this has not been evaluated in the animal hoarding environment. The sound pressure level (SPL) during responder and sampling team activity was measured in this study. In site AH04 where 41 dogs were found, a sound pressure level (SPL) peak of 95 dBA and a lull of 89.9 dBA were recorded. Taking the average of these measurements (92.45 dBA) an allowable time estimate suggests that responders could spend 5.70 hours in this environment without an expectation of hearing impairment. This is based on the OSHA and MSHA supported 5dB exchange rate however, NIOSH currently supports use of a 3dB exchange rate which would lower the allowable time. (111) The Occupational Safety and Health
Administration (OSHA) set the permissible exposure level (PEL) for an eight hour time
weighted average (TWA₈) at 90dBA. (96) While the National Institute for Occupational
Safety and Health has a recommended exposure level (REL) of 85 dBA TWA₈; which is
also the action level and the level in which employers are required to have a hearing
conservation program in place for employees.

It should be taken into consideration that at the sampling site the level of noise varied
during responder activity, decreasing over the few hours it took to remove the 41 dogs.
Sound pressure levels were in the low 50’s at site AH03, where nine cats and two dogs
were housed. The sound pressure levels would be expected to vary dramatically based on
the animal number, species, reaction to responders, and location within the animal
hoarding environment, be it indoor or outdoor. Given these results it would be advisable
for agencies responding to animal hoarding to consider noise as a potential health hazard
and measure levels when indicated, such as when large numbers of dogs are involved.
Hearing protection for responders may be an advisable action for the prevention of
hearing loss or damage.

In the animal hoarding environment animal waste (feces and urine) is a common reported
aspect of the environment. This study reported presence of feces and urine in both animal
hoarding residences, with the sheer number of animals present and the profusion of waste
through the residence, a potential health hazard from increased ammonia levels could
occur. The health effects from ammonia exposure range from eye, nose, and throat
irritation to death at extremely high levels (5000ppm for 30 minutes). (49, 69) An immediate danger to life and health limit (IDLH) of 300ppm has also been set by NIOSH. In exposure durations as short as 10 minutes and ammonia concentrations ranging from 30 – 50 ppm, mild eye and throat irritation have been recognized, while more severe respiratory effects occur at exposure durations of 30 minutes and ammonia concentration at 500 ppm. Acute exposure can cause a variety of effects such as, irritation of the eyes, respiratory tract, and cause violent coughing and painful breathing. Whereas chronic exposure can result in irritation of the eyes, nose, and cause coughing and difficulty breathing. (101)

In this study we used a piston pump and detector tube to detect measurable concentrations of ammonia in the environment. In the non-animal hoarding sites there was no significant finding for ammonia present inside or outside of the sampling site. However, at the animal hoarding sites ammonia was detectable and was highest in the site with an animal population of 41, with a measured reading of 11.4 parts of ammonia per one million parts of air (ppm) just inside the entryway of the home. While at site AH03, with an animal population of 11, the measured ammonia reading was 2.85 ppm, just inside the entryway (Table 13). Ammonia could also be detected by smell upon immediate entry into the animal hoarding environment and at site AH04 we could smell the ammonia on the front porch.
Upon arrival to site AH03, the solid front door was open leaving just a screen door present, prior to taking the ammonia measurement. Upon entry to the residence the smell of ammonia could be sensed but seemed to increase as we moved deeper into the home, where several litter pans were located and there were no windows or doors open. The ventilation at the entryway may have impacted that measurement, since ammonia readily dissipates with ventilation. Neither of the control residences had discernible scent of ammonia. OSHA reports that the odor threshold, the lowest concentration that 50% of test animals respond to, for ammonia is between 5 – 50 ppm. (98, 100) In another study it was reported that the odor detection threshold, which is the lowest concentration of a chemical compound that can be perceived by the human sense of smell, was as low as 2.6 ppm. (99) Ammonia has been shown in other studies to be present in the animal hoarding environment with a concentration as high as 152 ppm, which exceeds the OSHA PEL of 50 ppm (55, 62). A measurement this high was not detected during the sampling for this study and responders were able to work in this environment without respiratory protection for ammonia. Responders are strongly encouraged to measure ammonia upon entry, in order to take necessary precautions, in regard to PPE and ventilation of the site. When evaluating ammonia concentration it must be remembered that the animal hoarder is residing in these conditions exposing themselves as well as exposing the captive animals and any dependent individuals (children, elderly, or disabled) present, to these high levels that may exceed OSHA and NIOSH limits.
Another area of concern for indoor air quality is particulate matter and its association with areas of high animal density. An environment with concentrated animals can increase the amount of dust, hair, feathers, dander and liquid aerosols that are circulating in the air. Particulates can carry mold spores, bacteria, allergens, and irritants potentially causing illness in humans. In addition to generating particulates, animals can disturb settled particles moving them back into the air, especially if they are trying to evade capture. Particulate matter is categorized by size and has been recently designated as “Inhalable” – less than 10 micrometers (µm)(PM10.0) and “respirable” – particles equal or less than 2.5µm (PM2.5). These inhalable and respirable size particles can result in serious health effects since they can penetrate the human respiratory tract. This penetrating function of PM2.5 can decrease lung function and increase cardiovascular disease. (49, 52, 65) Other effects of particulate matter include allergic reactions, cerebrovascular disease, infectious disease, and acute toxic effects. Particulates have not been examined in an animal hoarding environment. The Environmental Protection Agency (EPA) documents that particulate matter on Concentrated Animal Feeding Operations, where large numbers of livestock are housed in large barns, consist of soil particles, bedding material, fecal matter, litter, feed, bacteria, viruses, and fungi. (68, 51)

Although this study did not detect a significant association in increased particulate matter within the animal hoarding environment, Figure 5 shows an increasing trend in particulate matter with increasing animal density. The levels found in these environments
did not exceed the OSHA PEL of 15mg/m³ for Total PNOC (particulates not otherwise classified) or the ACGIH TLV of 10mg/m³. However, both animal hoarding sites, AH03 and AH04, had measurements exceeding levels set by National Ambient Air Quality Standards of 35.0 micrograms per cubic meter of air (µg/m³) for PM$_{2.5}$, for a 24 hour averaging time. (56); with measurements of 99.0 µg/m³ and 46.0 µg/m³, respectively (Table 14). In order to determine the impacts of these measurements, the concentrations were input into an Air Quality Index (AQI) calculator on the AirNow website. The AQI is used to report daily air quality, providing information about the level of pollution in the air, focusing on health effects that may result within hours to days after exposure to polluted air; with focus on the five major regulated pollutants: “ground-level ozone, particle pollution (also known as particulate matter), carbon monoxide, sulfur dioxide, and nitrogen dioxide.” (117)

Inputting the measurement from AH03 of 99.0 µg/m³ of PM$_{2.5}$, the following information was generated. An AQI score of 173, resulted in a rating of unhealthy, indicating that people with respiratory or heart disease, the elderly and children are at the highest risk. The health effects could consist of, “increased heart or lung disease aggravation, premature mortality for individuals with cardiopulmonary disease and the elderly, as well as increased respiratory effects for the general population.” A cautionary statement was also provided, recommending that, “people with respiratory or heart disease, the elderly and children should avoid prolonged exertion and everyone else should limit prolonged
exertion. At site AH04 the category was reduced to unhealthy for susceptible populations, which also provided strong recommendations for limiting activity and maintaining awareness of the situation. At both non-animal hoarding sites the AQI was much lower, 25 for site AH01 and 57 for site AH02, these were categorized as good and moderate, respectively. Therefore, suggesting that constant exposure to the indoor environments at the animal hoarding environments could result in adverse health outcomes for individuals occupying the site. Although, more integrated sampling would be necessary to make further determinations.

Site AHO1 was the only site with indoor PM$_{2.5}$ measurements lower than the outdoor measurements and can be reviewed in Table 9 of the results. It was undetermined why the particulate level in AH03 appeared to be much higher than AH04, although this site housed mainly cats, which may produce more dander and the ceiling consisted of open rafters where several cats were observed to be climbing during sampling. There was also several litter pans throughout the residence and large bowls of dry cat and dog food present on floors and tables. These factors may have added to airborne particulates in this residence compared to site AH04. At site AH04 the flooring appeared damper and looked as if the resident had been attempting to remove debris from the floor, evident by smeared wet food and animal waste across the floorboards and linoleum.

Although, these indices (OSHA PEL and NAAQS) are referenced for industrial settings and outdoor air, respectively, rather than residential indoor air, it is useful to know what
levels in the occupational setting and the ambient air indicate a serious public health risk. Indoor air quality (IAQ) is a source of adverse health effects and the EPA has connected IAQ with the following health symptoms, “headaches, fatigue, trouble concentrating, and irritation of the eyes, nose, throat, and lungs.

Also, some specific diseases have been linked to specific air contaminants for indoor environments, like asthma with damp indoor environments.” (74) The EPA relates many factors to IAQ such as temperature, humidity (high or low), activities around buildings, renovation, and poor ventilation. (74) Considering the sampling sites, AH02 was an apartment complex, which could have multiple factors affecting the PM measurements, such as ventilation, heating and cooling system mechanics, carpeting, and the number of inhabitants in close proximity. The characteristics known about the animal hoarding environment such as, the dampness, overcrowded areas, lack of functioning household appliances, widespread urine, feces, and garbage, it can be reasonable to conclude that these factors will impact the IAQ of such an environment, as were found at sampling sites AH03 and AH04. These potential hazards can have severe health impacts for susceptible groups, such as the elderly, the young, and the immunocompromised including those with pre-existing conditions like asthma.

Findings related to bioaerosols, from this study, suggest an upward trend in bacterial and fungi/mold particles in the air as was shown in Tables 17 and 19 and Figure 5, of the results. These samples were collected using an ultra-high volume impactor that drew air...
into the biostage chamber where particulates were intercepted by a media plate. In the animal hoarding environment the concentration of fungi/mold at site AH04, with 41 animals present of 1739.43 cfu/m³, was nearly 12 times the concentration at site AH02, which had zero animals, of 147.88 cfu/m³. The concentration of bacteria, grown on mannitol plates, at site AH04 of 2028.17 was 8 times the concentration at site AH02 of 253.52. Factors that can aid bacterial or fungi/mold growth such as chronic moisture and presence of organic matter were present in both of the animal hoarding residences (Table 4).

Evidence of chronic dampness in the animal hoarding environments found in this study could be either from animal excrement or failing ventilation and plumbing systems or a combination of both; whereas the evidence of organic matter was directly related to the presence of animals, such as fecal matter, urine, and animal food throughout the environment. Infestations of pests, such as cockroaches, fleas, and flies were also evident in the two animal hoarding sites. The combination of these factors promotes the growth of bacteria and fungi/mold and this can in turn impact the level of particulate matter available to become airborne.

As important as air sampling and associated respiratory and auditory issues are, there still remains the potential hazard that may exist, in regard to zoonotic pathogens. These pathogens may be in or on the animals as well as in the environment, and have the potential to cause illness in the human residents, responders, shelter staff, and the shelter
animal population. Animal hoarding is typically associated with inadequate veterinary
care in both preventive and medical treatment situations. This study focused on
pathogens associated with animal hoarding and their presence in the environment, which
would allow for transmission between the animal populations as well as to the human
residents and visitors. Transmission from the environment can occur directly, through
contact with the animals, indirectly, through contact of contaminated surfaces, or carriage
on a vehicle such as water, food, or fomite (inanimate object). To evaluate pathogen
presence, collection of surface samples from the environment on surfaces such as
counters, doorknobs, cabinet handles, pet food dishes, etc., provided data for a
comparison of human touch surfaces and common touch surfaces. Using the fisher’s
exact test, two - by - two tables was constructed for a comparison of common touch
surfaces in animal hoarding versus non-animal hoarding environments, as well as a
comparison of human touch surfaces in these same environments.

For both comparisons there was not enough evidence of a significant difference in
bacterial presence on either human touch or common touch surfaces. In the animal
hoarding environment there was a 50% positive prevalence on common touch surfaces,
whereas there was a positive prevalence of 33.3% in the non-animal hoarding
environments, with an overall positive prevalence of 41.6%. For the human touch
surfaces there was a 36.1% positive prevalence in the animal hoarding environments
compared to a 25% positive prevalence in non-animal hoarding environments, with an
overall positive prevalence of 30.5%. This is an important finding because surfaces contaminated with bacteria present an opportunity for transmission to residents and responders. If residents of animal hoarding environments do not properly wash their hands, cross-contamination during food preparation and consumption is a potential source of pathogen transmission. Responders should be aware of the potential for surfaces to be contaminated and protect themselves by appropriately covering skin and cleaning any injuries, promptly, with clean water and disinfectant.

Another portion of the descriptive data (Figure 8) suggests an increase in the diversity of pathogens present from the non-animal hoarding to the animal hoarding sites. With the largest animal population site having six species of bacteria and parasites, where both of the non-animal hoarding sites had only one type of bacteria present, this was a staphylococci spp. This is of interest because staphylococci species of bacteria are a common type of bacteria normally found on human skin and certain species would be expected to be found within human residences. In the animal hoarding sites the bacterial species included campylobacter, salmonella, and staphylococci, along with whipworm, roundworm and hookworm parasites. These positive samples were found in four of the five universal sampling sections plus a judgment area, at site AH04 with 41 animals present. Enteric pathogens were found in both animal hoarding environments and present a potential for foodborne transmission from cross contamination for occupants. Salmonella was found on the dining room table at site AH03, while campylobacter was
found in the kitchen at site AH04 along with *Escherichia coli* on several human touch surfaces (doorknobs and faucets).

The International Scientific Forum on Home Hygiene reports that, “in the USA up to 39% of dogs may carry Campylobacter, and 10–27% may carry Salmonella,” (97) both of which have zoonotic potential. If we apply this prevalence to an animal hoarding home with dozens of dogs, we can conceive of a situation where even one infected dog could perpetuate the spread of either of these bacteria through the environment infecting the dozens of dogs present, resulting in a contamination of the environment with a zoonotic bacteria.

Though no *listeria* spp were detected, both animal hoarding homes had isolates of *Enterococcus faecalis* in both animal hoarding refrigerators. *Pseudomonas aeruginosa* was identified in the kitchen sink at site AH03 and *Enterococcus faecium, Enterococcus faecalis*, and *Vagococcus fluvialis* were identified in the kitchen sink at site AH04. These species are common environmental species and may increase risk to those individuals that are immunocompromised. Within the animal hoarding environment individuals that have a suppressed immune system should be aware of the potential for transmission of these species of bacteria. (120, 121)

In determining the level of potential hazards, the population directly impacted should also be considered; this would be the population living or forced to live in these conditions. Studies have suggested that the average hoarder is an older individual and often times
there are dependent individuals present. Susceptible populations require special attention as many zoonotic diseases impact them more acutely. Salmonella, for instance, is the number one cause of foodborne illness in the U.S., resulting in infections that can lead to diarrhea, fever, and abdominal cramps. In susceptible populations this can become severe enough to require hospitalization. (20) While campylobacter is the number one bacterial cause of diarrhea in humans and some patients have severe disease resulting in Guillain-Barre Syndrome. In immunocompromised patients infections from campylobacter spp can potentially spread to the bloodstream, resulting in a life threatening situation. (25)

The impact these pathogens have on the animal populations, forced to live in unsanitary conditions and potentially in a continuous disease state, is not to be overlooked. Although, we look to the public health impact on humans, these animals are being forced to inhabit an environment that has the potential to exert multiple hazards and reduce their existence to a continual battle between their immune defenses and environmental assaults. Understanding the pathogens present in the animal hoarding environment provides an opportunity to bolster cruelty investigation and improve regulations governing the abuse imparted on the animal victims and the dependent individuals forced into this situation. Discerning the potential hazards also impacts the ability of shelter managers to prevent transmission to shelter populations and home environments, where these rescued victims may eventually reside.
This study looked at a multitude of issues within the animal hoarding environment and found several concerning public health and safety issues. However, there were limitations to the study. The most notable limitation was the small sample size. The study evaluated two animal hoarding sites and two non-animal hoarding, one with and one without pets. During the planning stages of this study it had been expected that a larger sample size would be acquired based on the number of hoarding cases in the previous year. However, animal hoarding events are difficult to predict and we could therefore address this issue by increasing the number of shelters involved and the geographical range in the study design. However, increases in shelter involvement and geographical range of the study would also incur economical increases for the study design.

The small sample size did not provide enough sampling to demonstrate statistical significance or to identify other hazards that may be associated with animal hoarding environments. Although, the descriptive data collected suggested trends that can be used to direct further evaluations; such as using integrated sampling methods as opposed to instantaneous and short duration measurements. The lack of evaluation for Escherichia coli in the first three sampling sites was another shortcoming. Had these sites been evaluated for E.coli, more data to determine presence of fecal material in various locations would have been added to the descriptive statistics of the study results.
Conclusion

Although, this study was limited by its small sample size it was the first comprehensive study to examine the multifaceted issues that exist in the animal hoarding environment; from particulate matter, sound level pressure, and ammonia concentrations to presence of bacteria and fungi/mold. It was also the first to examine environmental surfaces for bacterial presence, concentrations of particulate matter, and airborne bacteria and fungi/mold presence in the animal hoarding environment. As well as document the presence of campylobacter and salmonella in the hoarding environment. Though fecal borne pathogens are known to be present in animal stool, this was the first study to show that the pathogens are not only in the home but present on human touch surfaces such as doorknobs, faucets, dining room tables, and light switches. The presence of pathogens on human touch surfaces can more easily result in transmission to residents and responders.

The recognition of elevated particulate matter, ammonia, and sound pressure levels, in one or both animal hoarding homes, demonstrates the presence of these hazards. Evaluation of more animal hoarding environments could provide detailed characterization and allow for identification of specific risk factors which could be used as an indicator of risk. The results of this study suggest several areas, within
the hoarding environment, that need further evaluation in order to better understand the hazards present and the impact these hazards may present to residents and responders.

With this knowledge, human healthcare providers managing the healthcare of residents from the hoarding environment will have a better understanding of potential exposures their patients may have had, aiding in the selection of diagnostics and treatment. Responders will gain knowledge useful for the selection of appropriate personal protective equipment. While multi-disciplinary animal hoarding task forces will have more information on which to base prevention, mitigation, and intervention efforts.
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Appendix A: Characteristics of Biological Agents
<table>
<thead>
<tr>
<th>Pathogen</th>
<th>1° Species Affected</th>
<th>Prevalence</th>
<th>Zoonotic Potential</th>
<th>Persistence in Environment</th>
<th>Transmission</th>
</tr>
</thead>
<tbody>
<tr>
<td>Campylobacter</td>
<td>Dogs and Cats Birds carry the bacterium due to ideal body temperature. (24)</td>
<td>30% in healthy normal adult dogs and cats (26)</td>
<td>Yes</td>
<td>Long periods in urine, feces, milk, at temperatures~4°C (26)</td>
<td>Foodborne (Meat, poultry, and dairy products) waterborne (surface water and mountain streams) Fecal – oral route (25)</td>
</tr>
<tr>
<td>Salmonella enterica</td>
<td>Mammals, poultry, reptiles (21,23)</td>
<td>1-36% healthy dogs; 1-18% healthy cats (23)</td>
<td>Yes</td>
<td>Persist in moist environments, common disinfectants aid in removal of pathogen. Keeping environment dry, clean will help prevent persistence. (22)</td>
<td>Foodborne, waterborne</td>
</tr>
<tr>
<td>Listeria monocytogenes</td>
<td>Cattle, sheep, and goats. Also many avian and mammal species (Rabbits, Chinchillas, rodents); dogs and cats are rare (76)</td>
<td>Undetermined in the pet population (dogs and cats) Although, Laikko reported 6 cases in dogs from 1947 – 1999. (76)</td>
<td>Yes</td>
<td>Persists in soil, water, and within animals asymptomatically. Keeping the environment clean, washing hands, and cooking meats thoroughly can reduce the potential risk of becoming infected. (19)</td>
<td>Foodborne, environmental Ingestion of the bacterium</td>
</tr>
</tbody>
</table>

Table 22. Characteristics of Biological Agents
<table>
<thead>
<tr>
<th>Pathogen</th>
<th>1º Species Affected</th>
<th>Prevalence</th>
<th>Zoonotic Potential</th>
<th>Persistence in Environment</th>
<th>Transmission</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staphylococcus spp (MRSA)</td>
<td>Human, cat, dog, pet birds, horses, pigs, and cattle</td>
<td>S. aureus: 6.6% Dogs, 39.6% Cats, 83.3% (18); 7.85% in dogs and cats (77) MRSA: 2.5% Dogs, 12.5% Cats, (18); 3.41% dogs and Cats (77)</td>
<td>Yes</td>
<td>Months on dry surfaces (17)</td>
<td>Skin – skin contact, contact with an infected wound, sharing personal items (razors, towels, etc) (16)</td>
</tr>
<tr>
<td>Trichuris vulpis</td>
<td>Dog Cats (rare)</td>
<td>19.4% in U.S. (9)</td>
<td>No</td>
<td>Can persist in the environment for years (15) Prompt removal of feces from the environment is essential in preventing infections. (15)</td>
<td>Ingestion of eggs from the environment that contain infective larvae. (15)</td>
</tr>
</tbody>
</table>
Table 22 continued

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>1° Species Affected</th>
<th>Prevalence</th>
<th>Zoonotic Potential</th>
<th>Persistence in Environment</th>
<th>Transmission</th>
</tr>
</thead>
<tbody>
<tr>
<td>Toxocara canis</td>
<td>Toxocara canis: Dog, human; Toxocara cati: Cat, Human</td>
<td>Toxocara canis: 13.2% in dogs (9)</td>
<td>Yes</td>
<td>Ingestive for several years. Disinfectants are not reliable. Removal of feces and routine deworming is essential to preventing contamination. (14)</td>
<td></td>
</tr>
<tr>
<td>Toxocara cati</td>
<td></td>
<td>Toxocara cati: 21.6% (9)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hookworm: Anscylostoma, Uniceria</td>
<td>Dog: A. caninum Uncinaria stenocephala Cat: A. tubaeforme, Unicaria stenocephala</td>
<td>33.3% Dog: A. caninum (9) 8.6% Cat: Ancylostoma tubaeforme (9)</td>
<td>Yes</td>
<td>Eggs passed in the stool, to the environment, hatch within 1-2 days, if conditions permit; This stage last from 5-10 days and then the larvae enter the third stage which is the infective form and can remain in the environment, for 3-4 weeks. (7)</td>
<td></td>
</tr>
<tr>
<td>Tapeworm</td>
<td>Diplydium caninum: Dog, cat, human; Taenia pisiformes: Dog, intermediate host Rabbit</td>
<td>4-60% in dogs, 1.8 – 52.7% in cats (5) dependent geographic location, and opportunity to ingest an intermediate host. (5)</td>
<td>Yes</td>
<td>Intermediate hosts in environment – fleas. Proglottids released from infected animal contain D. caninum eggs. (9)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Dogs and cats must ingest the intermediate host. (5) . Flea Prevention is to prevent tapeworm infection. (12)</td>
</tr>
</tbody>
</table>

Continued
<table>
<thead>
<tr>
<th>Pathogen</th>
<th>1° Species Affected</th>
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<th>Transmission</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cryptosporidia</td>
<td>C. canis – dogs, rarely humans; C. cati – cats, rarely humans; C. parvum – many species excluding humans and thus creates a zoonotic potential. (4)</td>
<td>Dogs: Seroprevalence of Cryptosporidium species was 52.7% (11) (Romania study &gt;300 dogs 2007-09) 7% of feral cats and 6% of pet cats (10)</td>
<td>Yes</td>
<td>Persists in the environment, especially water contaminated with feces. (60) The oocyst is resistant to chlorination and can be a concern for public water sources and recreational sites.</td>
<td>Ingestion of oocyst from contaminated environment.</td>
</tr>
<tr>
<td>Giardia</td>
<td>Giardia duodenalis: Dogs and Cats Giardia lamblia: humans</td>
<td>Dogs: 15.6% Cats: 10.3% (3)</td>
<td></td>
<td>Persists in soil, water, and on inanimate objects. (73)</td>
<td>Ingestion of cyst from soil, water, or fomites. Prompt removal of feces from the environment and proper disposal is effective in reducing environmental contamination.</td>
</tr>
<tr>
<td>Toxoplasma gondii</td>
<td>All warm blooded animal – although outcomes from infection vary. (59)</td>
<td>34% Owned cats 63% Feral Cats (10)* study in a rural county of N. Carolina.</td>
<td>Yes</td>
<td>Oocyst can persist in the environment for years. (2)</td>
<td>Prompt removal of feces from the environment – oocysts require 24 hours to become infective.</td>
</tr>
<tr>
<td>Coccidia</td>
<td>Canine: C. canis C. ohiensis C. neorivolta C. burrowsi Feline Species: Cystoisospora felis Cystoisospora rivolta (1)</td>
<td>Dogs 3.1% Cats: 4.2% (8)</td>
<td>No</td>
<td>Oocysts can persist in environment for up to a year, barring extreme weather events (freezing or excessive heat &gt;40°C) (1)</td>
<td>Ingestion of oocyst from contaminated environment. Prompt removal of feces from the environment will reduce exposure. Oocysts are resistant to common disinfectants. (1).</td>
</tr>
</tbody>
</table>
Appendix B: HOMES© Survey Risk Assessment Tool
HOMES® Multi-disciplinary Hoarding Risk Assessment (Homes Scale adjusted to necessary information required by project)

☐ **Structure & Safety**

- Unstable floorboards(1)/stairs(2)/porch(3)
- Leaking roof
- Blocked vents/heaters
- Caving walls
- Flammable items by heat source
- Presence air purifiers
- Electrical wires/cords exposed
- No running water/plumbing issues
- No heat/electricity/AC

Location ______________________
Notes _________________________

☐ **Health**

- Cannot use bathtub/shower
- Cannot prepare food
- Presence of spoiled food
- Cannot access toilet
- Cannot sleep in bed
- Presence of feces/urine (human or animal)
- Presence of mold or chronic dampness
- Garbage/trash overflow
- Presence of insects/rodents
- Cannot use stove/fridge/sink
- Type: _____________
- Presence of feces on floor; amount _____
- Presence of feces on counters; amount _____
- Presence of feces on furniture/tables; amount _____
- Cannot locate medication/equipment
- Presence dead animals
- **Obstacles**
  - Cannot move freely/safely in home
  - Unstable piles/avalanche risk
  - Inability for EMT to enter
  - Egresses, exits, or vents blocked or unusable

- **Endangerment**
  - Threat to community
  - Threat to agency responders

**NOTES:**

______

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Household Composition

# of Adults: ____________ ________________    # of Children: ______________________________

Primary Occupant Gender: Male/ Female    # and kinds of Pets: ___________________

Ages of individuals (Senior, Adult, or Child): ______________________________

Person who smokes in home: ☐ Yes ☐ No

Person(s) with physical disability______________________________________________________________

Notes:_____________________________________________________________________________________

___________________________________________________________________________________________

___________________________________________________________________________________________

_____________________________________________

Date: ____________ Client Name: ____________________________

Assessor: ________________________________________________________________________________

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Appendix C: Collection and Analysis Techniques

Sterile Collection

This is a two person sample collection technique, in which one person is designated as clean hands and collects the environmental samples, while the other is designated as dirty hands and prepares sample materials along with providing assistance to clean hands; in order to maintain sample integrity during the collection phase.

Clean Hands (CH) and Dirty Hands (DH)

1. Clean Hands(CH):

   Puts on gloves without touching the fingers of the gloves and receives Swiffer®, swab, media plates, or bulk collection container from dirty hands team member. The sample is designated for a specific location from the onsite sample list. CH team member returns sample material to DH

   i. Returning Swiffer® - first fold the Swiffer® in thirds then again in thirds with sample surface facing out, do not touch the inside of sample bag. Slide the Swiffer® into the same Whirlpak® bag that DH removed Swiffer from.

   ii. Returning swabs - returned to DH, who has removed the cap, by placing into the sample tube labelled with location number and container TSB 2.5%NaCl. DH will replace the cap and place in transport container.
iii. Return bulk collection - Once sample is collected in the container and sealed with accompanying lid, give to DH, who will place this in transport container. CHANGE GLOVES Dirty Hands touches

iv. *everything that clean hands cannot- wait for next sample media from Dirty Hands.*

2. Dirty Hands(DH):

Put on gloves without touching the fingers of the gloves.

Prep Swiffer® for CH by:

i. Open the sample bag using the side tabs and push/slide Swiffer® to the top - Do not touch the inside of the sample bag, maneuver Swiffer up the bag from the outside.

ii. Note the location on data sheet, mark off sample, and note any pertinent information about the sample.

iii. Assist CH as necessary for moving around sampling location; holding or opening doors and items, ONLY touching surfaces that have already been sampled or that are not going to be sampled.

Prep Swab for CH by:

i. DH opens swab package, be careful not to open too quickly, they may fall out of packaging and then be unusable.
ii. CH takes a swab and only touches the top 1/5 of the stick.

iii. DH opens the tube with containing the media

iv. CH dips swab into media and presses excess media out by rubbing swab against the inside of the tube

v. CH samples the area, remember to rotate swab and press hard enough to collect sample

vi. DH marks off the sample on data log

vii. DH has the collection tube available for CH to place swab back inside

viii. DH caps the collection tube and places in transport rack

ix. DH wipe off any residue left behind on sampled areas/items

Returning samples to collection bags, tubes, or containers:

i. Whirlpak® bag - use side tabs to open the bag and CH will place Swiffer into the bag. Then close the top of the bag, press the air, out and roll the top down 3-4 turns. Close the Whirlpak® with the metal tabs located on the side of the bag.

ii. Swab Tube – DH removes the cap from the media tube and allows CH to place the swab back in. DH team member then replaces the cap, checks the sample ID and puts it in the transport rack.

iii. Bulk Collection Container – CH will seal the container and give it to DH, who will check the sample ID and place it in transport kit.
iv. If DH feels they have contaminated their gloves too heavily, by touching surfaces within the environment, then change the gloves; otherwise, prep the next sample.

Tool Kit: One side of kit is for clean materials the other side is for dirty materials (samples and any garbage). When clean-hands brings the sample, DH will place them in the dirty side of transport kit and change gloves as needed.

Communication between clean hands and dirty hands will be essential to organizing collection of samples, confirm sample identification and location to be sampled.

On-Site:

- The team will determine movement through site, will we have a central spot for supplies and travel with sampling materials or we will move together through the site. This will be dependent on the hoarding environment.

- Start sampling from the point of entry, door and door knob; upon entry determine movement plan – as a group or from a central location. From a central location – place a garbage bag on the floor to place equipment. Then move sample team through environment and collect samples.
Fecal Centrifugation
Source: Parasitology Department: The Ohio State University

**Basic Centrifugation Technique:**

1) Place about 1-2 grams of fecal sample in a disposable plastic cup. Mix with water, strain through the nylon screen into a centrifuge tube

Alternatively, you *could* mix the fecal sample directly with zinc sulfate solution, but I recommend you begin with the water wash to improve the final results for microscopy

2) Centrifuge for 3-5 minutes as your your first wash step. You can repeat this step if necessary

3) Decant the supernatant into a discard vessel of some type for appropriate disposal.

4) Add a small amount of float sulfate solution and thoroughly mix into the pellet before adding more solution in incremental steps. If you don't mix this pellet well, you diminish the sensitivity.

5) Continue to add solution, mixing until the tube is almost full. You want the level to be just a millimeter or so below the rim of the tube. You will be able to see that the surface is concave based on light reflection. If too full, you will spray the top liquid around inside the centrifuge. If not high enough, you reduce sensitivity.

6) Centrifuge for 5 min at 1000 rpm or about 400 – 1000X g. The actual g force will depend on the rotor size of your centrifuge

7) After 5 minutes, remove the tube from the centrifuge and place in a test tube rack where you will add more solution to produce a bulging meniscus. Place a coverslip on the meniscus and allow 10 more minutes of flotation.
Note: Get yourself some dropper bottles or Coplin jars with bulb pipettes to facilitate putting just a drop or 2 onto the top of the tube. Squeeze bottles don’t work well enough. You will make a real mess and at this stage, if you overflow the tube, you just lost all the parasitic structures.

8) After 10 minutes is up, take the coverslip from atop the tube and place on a microscope slide to examine at 10X and 40X as described in the routine examination.

The biggest difference you will find between my technique and that reported in other manuals is that I don’t put the coverslip on top of the tube during centrifugation. The reason is that you will lose an occasional coverslip during the process and it makes a real mess in the centrifuge. I did compare results and this method works great.

**Zinc sulfate centrifugation:** A couple significant differences exist between zinc and sugar solutions of the same specific gravity. Following centrifugations, zinc will have a cleaner supernatant than sugar solutions, thus there may be less debris recovered that can obscure microscopic exams. Zinc sulfate is superior if you are looking for Giardia. On the down side, zinc can form a lot of microscopic bubbles that obscure microscopy, and zinc sulfate has several crystal formations that can be confusing the first few times you see them. Some could be mistaken for Giardia.

**Zinc sulfate and iodine:** You can add some Lugol’s iodine and mix into the pellet before adding the zinc sulfate solution. This will give you the very best recovery and visualization of Giardia cysts. You can train your eye to see Giardia cysts without iodine staining, but the iodine gives the cysts a golden to brown color that makes them much easier to see. Keep in mind that many complex carbohydrates will absorb the iodine, not just Giardia cysts, and these are found in other parasite eggs or oocysts, pollen grains, fungal spores, so many objects look different following the addition of iodine. If you are looking for
Giardia and forgot to add Lugol's iodine to the pellet before centrifugation, you can place a small drop to the edge of the coverslip on the microscope slide so that the iodine diffuses into the solution. I do this routinely if I find something on a fecal that has the ghost-like appearance of an unstained Giardia cyst. You can add Lugol's iodine to any fecal sample, whether doing a passive float, or centrifugations independent of your choice of float medium.

**Sugar centrifugations:** The only difference that I practice when using sugar is to wash the pellet more thoroughly than I do when I want to use zinc. Sugar solutions are more viscous and keep things in solution that would pellet in zinc, so the microscopy can be harder if the pellet isn't cleaner. Sugar solutions range in specific gravity from 1.20 up to what's called Sheather's sugar, which runs from 1.27 – 1.30 and is horribly viscous. Granted, a higher specific gravity can recover heavier eggs, but Sheather's sugar brings up too much debris for my tastes. If I need a very heavy solution, I make it using a base of zinc sulfate solution and add undissolved sugar into that until the specific gravity rises to 1.25+ and that reduces the viscosity and recovery of debris.
"Washing' a Fecal Sample:"

Centrifugation steps can be used to 'wash' a fecal sample. When centrifuged in water, the parasitic structures sink to the bottom allowing you to discard the supernatant without losing them. This washing step will remove many of the water-soluble bile pigments and much of the free lipid. Washing a fecal sample always aids in your microscopic examination and this makes the step worth the small time investment. If you are familiar with looking at fecal samples, you'll know how much debris can obscure your view of real parasitic structures, and some of the debris can look like small protozoal structures.

Washing' is useful with all fecal samples but particularly effective for cases of watery diarrhea or steatorrhea. Lipids are less dense than water, thus lipids will float above water or saline. Additionally, much of the smaller, water-soluble debris can be removed in this fashion as well.

With a case of watery diarrhea, a large volume can be placed in a tube and centrifuged for 10 minutes to pellet any of the solid material in the sample. I recommend a wash step for 3-5 minutes using water, but go 10 minutes when centrifuging a lot of diarrhea because this is more viscous and eggs or oocysts require more time to pellet through the thicker material.

After a wash step, the supernatant can be discarded because it will be free of any parasitic structures (they are in the pellet). If the supernatant is still very opaque such that you can't...
tell where the pellet begins and the supernatant ends, the pellet could be washed again. Any practice that objects to the extra time this requires is more interested in money than a correct diagnosis. Any improvement of the microscopy will aid in the diagnosis. Clinics need to consider the wasted time of their clients returning for an accurate diagnosis rather than the small investment of time that it takes to do the technique correctly the first time.

The tube to the left has a supernatant where I can see the division between the pellet and the supernatant, so I would proceed. Any darker than this I would consider doing a second wash step.

Once a fecal pellet has been washed, you could move directly to adding the float solution and doing the centrifugation, or, for special cases, you can make a smear onto a microscope slide to air dry, then fix and stain looking for bacterial pathogens, or to look at the bacterial flora that is present. Normal feces should have a wide distribution of bacteria, including Gram positive and negatives, rods, cocci, some filamentous bacteria, a few yeast, etc. When you see a stain that isn't normal, that can indicate that something has disrupted the normal flora, such as antibiotic treatments, so you should consider a regime of probiotics instead of antibiotics. Practitioners usually forget that antibiotics will have a by-stander effect on the normal bacterial populations. Disrupting those can lead to an overgrowth of opportunistic organisms, such as yeast or Clostridium. This situation can lead to a self-perpetuating diarrhea, or, in the case of Clostridium overgrowth, recurring bouts of loose stool or explosive diarrhea.