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Comparisons of Selected Household and Commercial Disinfectants Against Poultry Salmonella Isolates and a Survey of Internal Parasites in Backyard Poultry in Arkansas

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Comparisons of Selected Household and Commercial Disinfectants Against Poultry *Salmonella* Isolates and a Survey of Internal Parasites in Backyard Poultry in Arkansas

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Abstract

Backyard and exhibition poultry have been gaining in popularity and as such there has been a large increase in the number of small flocks. As the interaction with poultry has increased, so has the opportunity for diseases and parasites, for both birds and people. One of the major zoonotic illnesses is caused by the bacteria *Salmonella*, which can be found in commercial and small flocks. *Salmonella* is the number 2 contributor of foodborne illnesses so its prevalence in commercial flocks is of high concern. Despite improved cleaning, disinfection, and biosecurity practices, there is still potential for disease outbreaks and infections in the industry. This thesis concentrates on helping small flock holders fight diseases. In a two pronged approach I looked at preventing Salmonellosis by evaluating the efficacy of 6 disinfectants, then took the opportunity to sample and evaluate the prevalence of internal parasites in small flocks in Arkansas. Results found that household bleach is an effective disinfectant, whereas lemon juice and vinegar are not as effective as commercial disinfectants. Additionally, internal parasites are common in backyard flocks in Arkansas.

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CHAPTER 1

Literature Review

INTRODUCTION

Commercial poultry production is the fastest growing sector of the animal agricultural industries. Per capita consumption of chicken in the United States has risen from 36.9 pounds in 1965, to an estimated 92.1 pounds in 2016 [1]; as such, poultry production has dramatically risen to meet demand.

Chickens that are raised specifically for meat are called broilers. As of 2015, broilers reached an average market weight of 6.24 pounds in 48 days [2]. In 2014 there were 8.54 billion broilers produced in the United States alone, which amounted to 51.4 billion pounds, live weight [3]. In order to meet this demand and create supply, intensive production methods have been developed. However, despite large improvements in health and management, there is still potential for disease outbreaks and infections in flocks.

Keeping poultry as a hobby is one of the fastest growing hobbies in the United States. Reasons for keeping small flocks include: egg production; meat; exhibition; insect control; family tradition; teaching children responsibility and animal care; and companionship. Many owners like knowing and controlling exactly what their birds eat, enabling owners to keep certain lifestyle choices such as eating only organic food [4-5].

One of the most important bacteria to control in poultry is *Salmonella*, which can also affect humans and poses a threat to public health. *Salmonella* is the number 2 contributor of foodborne illnesses, second only to norovirus. *Salmonella* causes an estimated 11% of all foodborne illness; additionally, an estimated 34% of foodborne illnesses resulting in hospitalizations are caused by *Salmonella*. Furthermore, *Salmonella* has the highest number of deaths due to foodborne illnesses, around 400 people per year [6].

Although *Salmonella* are a major concern in commercial poultry, they are also of concern in small hobby flocks. Recently, backyard flocks have been blamed for 8 ongoing outbreaks in

the year 2016. Thus far, 611 people in 45 states have been infected; out of 493 patients interviewed, 88% had contact with live poultry within a week before developing symptoms [7].

Salmonella are members of the Enterobacteriaceae family, and are named after USDA veterinary bacteriologist Daniel E. Salmon. *Salmonella* are rod-shaped, gram-negative, non-spore forming, predominantly motile bacteria with a diameter of 0.3-1.5 microns and a length of two to five microns. The optimum temperature for growth is 37 C, but growth is possible in a range of 5 to 45 C. *Salmonella* cannot survive above 70 C. The optimum pH is around 7, but growth can occur anywhere between 4.0 to 9.0. They can survive in soil, litter, and water for long periods of time, and are resistant to dehydration. Medias that supply nitrogen and carbon can be used to culture *Salmonella* [8-10].

There are almost 2,500 serotypes of *Salmonella* identified so far, which are divided into two species; *Salmonella enterica* and *Salmonella bongori. S. enterica* is further divided into six subspecies: enterica (I), salamae (II), arizonae (IIIA), diarizonae (III B), houtenae (IV) and indica (VI), which all differ biochemically. *S. bongori* and *S. enterica* subspecies II-VI are all found in cold blooded animals and the environment. This paper will focus on *S. enterica* subsp. Enterica, which is found in warm blooded animals. Out of the 2,463 *Salmonella* serotypes, 1,454 of them belong to *S. enterica* subsp. Enterica. Serotypes are differentiated based on three antigens: the flagellar "H" antigen, the oligosaccharide "O" antigen of the outer bacterial membrane, and the polysaccharide "Vi" virulence antigen [8, 10-11].

Salmonella serotypes can be further divided based on host specificity: host restricted/adapted, or unrestricted. Host restricted serotypes are generally defined as being almost exclusively associated with one particular host species. Examples of this include *Salmonella* serotype Gallinarum, and *Salmonella* ser. Pullorum, which cause disease only in poultry. Host

adapted serotypes affect mainly one species but are capable of causing disease in other species, especially those that are closely related [12]. An example of this is *Salmonella* ser. Choleraesuis, which causes swine paratyphoid but is also highly pathogenic to humans [12-13]. Unrestricted *Salmonella* serotypes cause disease in a wide range of unrelated hosts. Unrestricted serotypes include *Salmonella* ser. Typhimurium and *Salmonella* ser. Enteritidis [12].

Salmonellosis in humans is acquired by consuming bacteria, usually through contaminated foods such as poultry, eggs, beef, pork, unpasteurized milk or juice, and fresh produce. Foods can become contaminated by contact with animal feces, by mixing raw and uncooked meat, mixed utensils in the kitchen at home, or by cross contamination during processing. Food may also become contaminated after coming into contact with infected animals and not properly washing before handling food. Transmission from person to person is also possible, through the fecal-oral route [6, 10].

Salmonellosis in humans can be divided into 3 categories: gastroenteritis (normal food poisoning), enteric fever (typhoid and paratyphoid fever), and septicemia [10]. *S.* Typhimurium and *S.* Enteritidis are the most common serotypes that cause gastroenteritis in the United States. The incubation period is normally 12-72 hours, and symptoms include diarrhea, abdominal cramps, fever, nausea, vomiting, and headache. Illness may last for 4-7 days, and most people recover without treatment. *Salmonella* ser. Typhi and *S.* ser. Paratyphi cause typhoid and paratyphoid fever, respectively. *Salmonella* Typhi and *S.* Paratyphi are found in and transmitted only by humans. Septicemia occurs when *Salmonella* pass through the intestine and into the blood stream. This is most often caused by invasive types of *Salmonella*. Young children, the elderly, and people with compromised immune systems are more likely to have septicemic infections than the normal populace [6, 10].

Diseases can be transferred two ways: from organism to organism (horizontal), or directly from mother to embryo/fetus during pregnancy (vertical). *Salmonella* can be spread both ways. Vertical transmission is especially important because *Salmonella* that is passed into the eggs by layers makes its way into the human food supply, creating the potential for foodborne illness.

There are two serovars that affect only poultry: *S.* Pullorum and *S.* Gallinarum. *Salmonella* Pullorum causes Pullorum disease (PD), previously known as Bacillary White Diarrhea (BWD). PD is found mostly in young chicks and mortality peaks during the second or third week after hatch. Symptoms include: weakness, depressed appetite, poor growth, white diarrhea, labored breathing, and swollen joints. Lesions in young birds usually include unabsorbed yolk sacs and nodules in the liver, spleen, lungs, heart, gizzard, and intestine. Pericarditis is frequently observed. If adult birds get infected with PD, there is a decrease in egg production, egg fertility, and hatchability. Signs in adults also include anorexia, diarrhea, depression, and dehydration [5, 8].

Salmonella Gallinarum causes fowl typhoid (FT). Fowl typhoid symptoms are similar to those of PD, without the characteristic white diarrhea. FT also tends to infect older chicks and adults. Sudden outbreaks may begin by a sudden drop in feed consumption; birds will be droopy, have ruffled feathers, and the combs of the birds become pale and shrunken. Morbidity and mortality for both PD and FT can range anywhere between 0 to 100%, depending on age, strain of bird, nutrition, flock management, concurrent diseases, and exposure to the environment. Both PD and FT can be transferred vertically and horizontally [5, 8].

PD was first described in 1899 and FT was recognized in 1888. These two diseases quickly became prevalent and caused high mortality in early chicken production and flocks. In

1935 the National Poultry Improvement Plan (NPIP) was created in part to control PD and eventually FT. Due to the control measures taken by the NPIP, FT and PD have been almost completely eradicated from the United States. They are still found and cause problems in other countries, especially those that do not have a poultry industry developed, are not developed as a country, or are in the process of developing [8, 14].

As *S.* Gallinarum and *S.* Pullorum were eradicated, *S.* Enteritidis began to emerge as the predominate *Salmonella* problem faced by producers, filling the niche left behind [15]. As *S.* Enteritidis also causes salmonellosis in humans, it replaced *S.* Typhimurium as the primary cause of salmonellosis in humans. The NPIP began to target *S.* Enteritidis in 1989, and along with the use of vaccines and natural immunity development, prevalence has decreased. *S.*ser. Heidelberg was the most commonly isolated serovar in poultry until 2007, when it was surpassed by *S.*ser. Kentucky [15-16].

Paratyphoid infections (PT) in chickens are caused by non-host adapted *Salmonella* such as *S.* Enteritidis (SE), *S.* Typhimurium and *S.* Heidelberg. The ability to cause disease and death varies widely, dependent on strain, age of the bird, and amount of inoculation. PT mostly affects newly hatched birds and can sometimes lead to illness and death at high frequencies. Typical symptoms include: somnolence, droopiness, ruffled feathers, anorexia, diarrhea, and occasionally blindness and lameness. Mortality reaches peak levels between 3-7 days of age. Susceptibility to infection decreases with age. When there is infection of adult birds, most often the only symptom is brief, mild diarrhea. Although the birds most often do not show signs of disease, *Salmonella* colonize the intestine and then systemically disseminate to the internal organs throughout the body. As such, *Salmonella* can be found and spread to the environment via the feces; *Salmonella* that infect the ovary and oviduct may contaminate the eggs before they are laid. Both *S.*

Enteritidis and *S.* Heidelberg are known for infecting the reproductive tract and being found in eggs. Eggs that have been contaminated with *Salmonella* may have a higher level of embryo mortality and death of newly hatched chicks [8].

Because birds can be infected with *Salmonella* without showing any signs, poultry products have been identified as important sources of human foodborne illness. As of 2011 there were an estimated 1.028 million cases of non-typhoidal salmonellosis with just over 19,000 hospitalizations and 452 deaths [17]. As of 2014, the top 6 serotypes were: Enteritidis, Typhimurium, Newport, Javiana, I 4,[5],12:i:-, and Infantis [6]. In a report released by the CDC [6], the top serovars for the year 2012 that were important for both human and poultry infection were Enteritidis, Typhimurium, Heidelberg, Infantis, Montevideo, and I 4,[5],12:i:-.

Salmonella can be transmitted to birds through fomites such as people, water, feed, litter, insects, rodents, wild birds, and many other sources. Fomites are any objects or substances that can carry and transmit infectious organisms, such as vehicles, clothing, footwear, and equipment. Insects and rodents can serve as reservoirs and vectors, spreading the disease from one flock to another even when houses are cleaned out between flocks [10-21]; contaminated feed and water are also important sources of infection [22]. *Salmonella* contamination can be introduced anywhere along the production chain: in the hatchery, during transport, on the farm, in the processing plant, or at home.

CONTROL OF *SALMONELLA*

There are many measures that can be taken to prevent *Salmonella* infection. These include: maintaining *Salmonella*-free breeding stock; proper cleaning and disinfection of the farm, hatchery, transportation crates, and equipment; strict biosecurity measures; the all in all out

system for flocks; feed additives and antibiotics; competitive exclusion; water sanitation; vector and reservoir control such as insects and rodent control programs; and vaccination.

Vaccination is one biological measure that has been researched lately as a way to decrease the potential of vertical transmission of *Salmonella*. Vaccination has been shown to be effective in decreasing prevalence of *S.* Gallinarum, Entertidis, and Typhimurium. Vaccination of hens may decrease the level of contamination, decreasing both vertical transmission as well as egg shell contamination. This prevents colonization of chicks during hatching, and decreases the prevalence of foodborne illness resulting from consumption of contaminated eggs. Additionally, vaccination causes the formation of antibodies that can be passed from mother to egg, which can also provide some protection against infection in newly hatched chicks [23-24]. Research has shown that breeder flocks that had been vaccinated had progeny with lower prevalences and loads of *Salmonella* than progeny from unvaccinated flocks [24-25]. Vaccination is more effective for broiler breeders and for layers rather than broilers, because vaccinating broilers is neither cost nor time effective. Vaccination can be used as part of a comprehensive prevention program for controlling *Salmonella* in poultry. However, vaccines do not provide complete protection or cross-protection against all serotypes [15].

Chicks do not have a fully developed immune system or intestinal microflora when they first hatch; as such, they are highly susceptible to *Salmonella*. A developed intestinal microflora makes it difficult for *Salmonella* to colonize and invade the intestinal tract, therefore limiting pathogenicity. This can be accomplished in several ways: production of antimicrobial substances; fewer niches for *Salmonella* colonization; stimulation of the immune system, and competition for nutrients and other resources [15]. When one species outcompetes another for the same resource, it is called competitive exclusion [26]. In an attempt to build a healthy

intestinal microflora in chicks, growers use feed additives, such as probiotics and prebiotics, so as to increase resistance to *Salmonella* [22].

Preventive doses of antibiotics can also be given to prevent *Salmonella* infections but antibiotic therapy is discouraged in food production animals because of the increase in antibiotic resistance, including those serotypes that can cause human disease [27-28]. Giving antibiotics prophylactically or as growth promoters may select for bacteria that are resistant, including bacteria other than *Salmonella*, such as *E. coli* [29]. When those bacteria are transferred to humans, such as through consumption, the genes for resistance to antibiotics can be transferred to the normal microflora already present [28]. This poses a potential public health risk, as new ways to treat antibiotic resistant bacteria and their diseases must be developed. Additionally, there is public concern about residual antibiotic residues in meat, and most antibiotics are unable to completely eliminate *Salmonella*, creating carrier birds that appear to be *Salmonella* negative but are not [22]. Finally, there is a high cost to administer antibiotics, which gets passed on to the customer in the form of more expensive products.

Cleaning followed by disinfection is one of the best ways to control the spread and prevalence of *Salmonella.* Everything needs to be cleaned: the poultry houses between flocks and all equipment inside, hatcheries and all equipment, processing plants and equipment, feed mills, clothing, vehicles, and transport crates. Cleaning is defined as a mechanical process in which organic matter is removed. Disinfection is defined as the process or agent used on inanimate objects/surfaces that either destroys or renders inactive most microorganisms (but not always spores) [30-31]. In order for disinfection to be effective cleaning must be done first.

Cleaning should include brushing, scraping, scrubbing, and dusting all of the litter, debris, feces, feed, trash, debris, feathers, and removing any other organic matter from the

surfaces that will be disinfected. Equipment may need to be dismantled and cleaned separately; if it can be cleaned well and efficiently inside the house, however, this is not necessary. The entire house should then be pressure washed from ceiling to floor with hot water and a soap or detergent. Rinsing after will remove any remaining matter and detergents, and decrease the chances of animals coming into contact with residual detergents. After cleaning, there should not be any visible organic matter left [8, 32]. Cleaning and disinfecting poultry houses between flocks has been shown to reduce or eliminate infection [18, 33-35]. When cleaning and disinfection is not done to a high standard, however, carry-over of *Salmonella* between consecutive flocks is common [21, [33-35].

Maintaining cleanliness in transit as well as within the processing plant is also very important. Efforts to reduce *Salmonella* in poultry carcasses during processing have an impact on reducing human illness [36]. Heyndrickx et al. [37] found a correlation between *Salmonella* positive fecal material in transport crates and contamination of carcasses. Transportation crates are often contaminated with *Salmonella* [19, 38-39] and can therefore serve to spread the bacteria.

DISINFECTION

After cleaning the house, all equipment should be disinfected. This can be done by spraying or foaming all surfaces with a disinfectant, or by fogging. Concentrations and contact times should be used according to the manufacturer recommendations. The type of disinfectant selected depends on several factors: type of contaminating organism; degree of contamination; presence of organic matter; contact time required for efficacy; toxicity to the environment; application method; corrosiveness; type of surface to be treated; environmental factors such as

temperature, humidity, pH, and water hardness; stability during storage; and finally, cost [8, 40-41]. Efficacy of disinfectants within a disinfectant group can also vary as a result of formulation [20].

The efficacy of most disinfectants increases with temperature, and using higher concentrations or longer contact times may compensate for temperatures that are lower than recommended [41]. The temperature range in which a disinfectant is most effective varies. For example glutaraldehyde based disinfectants are effective down to about 5 °C while formaldehyde needs at least 16 °C.

Disinfectant manufacturers do research in order to determine the most effective concentrations and contact times for their product, so anything less than recommended will not have the desired effects and may even contribute to an increase in resistance by the microorganisms [41]. Concentration also impacts the effectiveness of disinfectants. For example, alcohol is most effective in the 60-90% range; peracetic acid, however, is effective at <0.3% [31].

Another factor that affects disinfectant activity and efficacy is pH. The stability and susceptibility of a bacterial membrane is partly due to electrical charges within its structure, which can be altered by pH. Some types of disinfectants, such as oxidizing agents, formaldehyde, and QACs, act by chemical reactions to molecules, compounds, structures, or interrupting processes within the cell [42]. pH can impact the state of a disinfectant influencing the ionization or by increasing/decreasing the concentration of the active compound. For example, sodium hypochlorite ionizes to produce Na^+ and the hypochlorite ion, OCl^-. The OCl^- then establishes a pH dependent equilibrium with hypochlorous acid, HOCl. Phenols,

hypochlorites, and iodine may show decreased activity as pH increases; quaternary ammonium compounds (QAC) and glutaraldehyde may show an increased activity [31].

One of the biggest factors that interferes with efficacy is the presence of organic matter (OM). Organic matter can be in the form of feathers, litter, feces, carcasses, blood, eggs and egg residues, chick fluff, etc. OM provides a protective physical barrier between disinfectants and microorganisms, as well as providing habitat for bacteria to grow. This is why cleaning of a high quality needs to be done before disinfection. The level of impact from OM depends on the disinfectant. In a study by Stringfellow et al. [43] decreases in disinfectant efficacy were observed in response to increasing levels of OM, dependent on dose. Phenolic disinfectants were the least impacted and still reduced *Staphylococcus aureus* populations with the inclusion of 3% OM, whereas a chlorhexidine compound was ineffective. OM may react with oxidative disinfectants and neutralize others [41].

The efficacy of disinfectants is also dependent on the type, number and physiological state of microorganisms. *Salmonella* are less tolerant to hypochlorite than *Staphylococcus aureus*; *S.* Kentucky, *S*. ser. Montevideo, and *S*. ser. Senftenberg have been shown to be more resistant to disinfectants than *S.* Typhimurium. If there is a high initial number of bacteria left from cleaning, disinfection may not be completely effective and some cells may survive. Bacteria that are actively growing are more susceptible to disinfectants than bacteria that are not [41]. Bacteria that are in biofilms are more resistant [44-45]. We cannot completely control which serotype may be present in the poultry house, and we can't control what stage of life the bacteria may be in but we can at least partly control the number of bacteria through a high quality cleaning before disinfection.

Bacterial resistance is becoming a bigger health issue every year; as *Salmonella* and other microorganisms become resistant to disinfectants and antibiotics, the severity of illness increases and the probability of full recovery decreases because there are fewer options for prevention and treatment. Bacterial resistance varies between different types and species of microorganisms; within *Salmonella*, resistance also varies between serotypes. For example; *Salmonella* strains Agona, Kentucky, Montevideo and Senftenberg are more resistant to disinfectants as compared to *S.* Typhimurium ATCC 13311 [41]. One of the biggest drivers of bacterial resistance is using biocides, antibiotics, and disinfectants at sub-lethal levels. A study looked at susceptibility to chlorhexidine in *S*. Typhimurium and found that sub-lethal concentrations of the chemical was capable of inducing physiological changes that decreased susceptibility [46].

There is evidence that building material may have an effect on efficacy of disinfectants [44-45, 47]. Berchieri and Barrow [47] found that wood shavings neutralized some of the sanitizers used, whereas metal foil and polythene strips did not have an effect. Wood is hard to clean due to its structure and nature. The variable surface is conducive for biofilm formation while cracks and crevices provide protection for bacteria from cleaning and disinfectants. Using metal, plastics, or other materials to either replace or cover wood surfaces in poultry housing is likely to improve both ease and efficacy of cleaning and disinfection.

In broiler houses the most commonly used classes of disinfectants are: peroxides and oxidizing agents, alcohols, halogens, QACs, aldehydes, phenolic compounds, and biguanides[22, 43]

CLASSES OF DISINFECTANTS

Alcohols

Alcohols are effective against vegetative bacteria, viruses, and fungi, but are not sporicidal. They have rapid broad-spectrum antimicrobial activity and are often used for disinfecting hard surfaces and skin as they leave no residue. They are often used in formulations combined with other disinfectants [48]. Ethyl alcohol and isopropyl alcohol are the most widely used alcohols, and isopropyl alcohol is considered slightly more effective against bacteria. Alcohols are most effective in concentrations between 60-90%, and efficacy significantly drops when concentration is below 50%. An ethanol concentration of 70% is effective against *Salmonella* on surfaces, including *Salmonella* in biofilms found in the feed industry [49]. Efficacy is increased in the presence of water, and many alcohol products have low levels of other biocides mixed in. These other products can increase product efficacy by decreasing the evaporation time of the alcohol or remain on the skin following the evaporation and have their own biocidal activity [31]. However, alcohols are good for situations where low residual water after use is necessary because of their quick evaporation time [49].

Alcohols are not effective in the presence of organic matter, and are expensive so use is mainly restricted to hand sanitizers and equipment that cannot be cleaned with water. They are also very flammable and can pose a safety and health risk [40-41].

Aldehydes

Aldehydes are organic compounds that include formaldehyde and glutaraldehyde. Aldehydes are potentially carcinogenic and are irritating and toxic to humans. They are effective in the presence of organic matter, act rather slowly, reactive with other chemicals, and are potentially dangerous to respiratory systems [48].

Formaldehyde exists naturally as a gas but is used as an aqueous solution with 34-38% formaldehyde with methanol called formalin. Formaldehyde is bactericidal, sporicidal, and virucidal. It is very reactive, interacting with protein, DNA, and RNA [31]. Formaldehyde is very potent but is also highly toxic to humans and animals so must be used with caution in a well ventilated area [40]. Because formaldehyde is so toxic and needs to be handled carefully it is best applied by specialist contractors, which may incur extra costs [34]. Formaldehyde has shown efficacy against *Salmonella* in the presence of organic matter [50].

Glutaraldehyde also has a broad spectrum of activity against bacteria, spores, fungi, and viruses. It works more quickly than formaldehyde and is also effective in the presence of OM [47]. Glutaraldehyde associates strongly with the outer layers of bacteria, inhibits transport and enzyme systems, and inhibits RNA, DNA, and protein synthesis. It is more active at alkaline pHs [31].

Oxidizing Agents

Oxidizing agents produce free radicals that act by disrupting lipid membranes, proteins, and nucleic acids. The most common oxidizing agents are hydrogen peroxide and peracetic acid [31, 50]. Oxidizing agents have moderate to wide germicidal activity, are moderately corrosive, and not very toxic. They have little residual activity and limited shelf life [40]. They have some activity in the presence of OM, although this seems to be debated[51-52].

Hydrogen peroxide (H2O2) has broad-spectrum efficacy against viruses, bacteria, yeasts, and bacterial spores. It tends to have better activity against gram-positive bacteria than gram-negative bacteria. Hydrogen peroxide can be bought in concentrations ranging from 3-90%. and

is considered environmentally friendly because it breaks down into water and oxygen [31]. H2O2 is reactive and is not very stable [48].

Peracetic acid is a mix of hydrogen peroxide and acetic acid. It also decomposes into safe by-products, acetic acid and oxygen, but is considered more potent than H2O2. It is also more likely to remain active in OM than H2O2 [48, 51].

Acetic acid, more commonly known as vinegar, can inhibit carbohydrate metabolism and can induce apoptosis, resulting in the death of an organism. Acetic acid up to 2.5% is allowed as a processing aid on carcass surfaces in the US [53]. A study by Nei et. al [54] found that a gaseous acetic acid treatment on black pepper and fenugreek seeds produced an approximate 5.0 log CFU/g reduction in *Salmonella* Enteritidis after 3 hours. Reduction of *S.* Enteritidis in that study after only one hour was still significant, showing that a gaseous application of acetic acid can be one option for disinfection. A study by Chang and Fang [55] found a 3 log reduction of *E.coli* on inoculated lettuce that had been exposed to commercial vinegar containing 5% acetic acid for 5 minutes. Although *E. coli* and *Salmonella* are not the same, they are similar and it is reasonable to believe that they have similar susceptibilities to disinfectants.

Citric acid is naturally found in citrus fruits and is another organic acid that can potentially be used as a disinfectant. A study by Sengun and Karapinar [56] using fresh lemon juice to disinfect carrots found log reductions of *S.* Typhimurium ranging from 0.079-3.95, depending on time and rate of bacterial inoculation. The times tested were 0 minutes, 15, 30, and 60 minutes. The study also tested the efficacy of mixing acetic and citric acid together and found the mixture to be more effective than either acid alone.

Quaternary Ammonium Compounds

Quaternary ammonium compounds (QAC) are surfactant compounds made of a hydrophobic group and a cationic group. QACs are excellent for hard-surface cleaning and deodorization. They work by interacting with phospholipid components and causing generalized damage to the membrane [31]. QACs are not effective against non-enveloped viruses, fungi, and bacterial spores, but are effective against gram positive and negative bacteria and enveloped viruses. QACs are more effective in preventing bacterial growth, rather than killing them, and they tend to be more active against Gram-positive bacteria rather than Gram-negative [48]. Hard water, other soaps/detergents, and OM limits efficacy; however, QACs are reasonably quick acting, have a low cost, and are relatively noncorrosive and nontoxic [40, 48, 57].

Phenols

Phenols are substances derived from coal tar and have been used for many years [48]. They act by inducing progressive leakage of intracellular components. Phenolic disinfectants are still effective in OM [43, 47-48]. They have a broad spectrum of activity, are effective in hard water, are of low to moderate cost, and have little residual activity unless mixed with other compounds [40]. However, efficacy, toxicity, and corrosiveness greatly varies between formulations [43, 48, 50, 58].

Biguanides

Biguanides are organic compounds that feature a specific formula and functional group. The most commonly used biguanide is chlorhexidine. Chlorhexidine is bactericidal but is not sporicidal and has limited antiviral properties. Its activity is pH dependent and can be greatly

reduced when there is OM present [43]. Chlorhexidine has a two-phase effect: in the first, it works by damaging the cell membrane. If concentrations of chlorhexidine are high, after the membrane is damaged the intracellular components become coagulated [31]. Biguanides have good residual activity and are relatively nonirritating to tissues, nontoxic, and cheap [5, 40].

Halogens

Halogens are derived from the halogen group of the periodic table. Chlorine and iodine based compounds are the most common halogens used as disinfectants.

Chlorine Releasing Compounds. Chlorine disinfectants are active against viruses, fungi, algae, and bacteria. They are not effective against spores. They corrode metals, deteriorate fabrics, and can cause irritation in high concentrations. They are cheap and active in low concentrations but inactivated by OM [40]. Although the mechanism of action for chlorine disinfectants is not known, it is known that they are oxidizing agents. Therefore, chlorine disinfectants are most active between pH 4 and 7 when there is the most of HClO, the compound that actively oxidizes. Chlorine disinfectants disrupt protein activity as well as inhibit DNA synthesis [31].

Bleach, or sodium hypochlorite, is a common example. Bleach is commonly used in the food industry, especially for washing produce. Sodium hypochlorite is used for more than washing produce, however, as it has also been to treat water, deodorize, stain removal, and disinfect equipment for various uses. Bleach is very effective against all types of microorganisms, is easy to use, and relatively cheap. However, concentrated solutions are corrosive to skin, metals, and other materials, and sodium hypochlorite tends to break down over time [48].

Another common and important chlorine releasing agent is chlorine dioxide, which is also used for food processing as well as routinely utilized in the process for disinfection of drinking water.

Iodine and Iodophors. Iodine based disinfectants have been used for many years and are less reactive than chlorine but rapidly bactericidal, fungicidal, virucidal, and sporicidal at both low and high temperatures. Because aqueous and alcoholic solutions of iodine cause staining and are generally unstable, iodophors, complexes of iodine and a solubilizing agent or carrier, have been developed [31, 48]. Some of the iodophors have been developed to minimize staining and irritancy. Iodine based disinfectants act upon proteins, nucleotides, and fatty acids. Iodine and iodophors become inactivated in the presence of OM [47].

INTERNAL PARASITES

Despite being a very important disease, *Salmonella* is not the only organism that causes infection and disease in poultry. Both commercial and backyard flocks of all sizes can be plagued by internal parasites; some of the most important include members of the genera *Ascaridia, Eimeria, Capillaria*, and *Heterakis*.

Ascaridia

Members of the genus *Ascaridia* are round worms that are commonly found in poultry. They are usually in the small intestine but birds with heavy infestations can have worms in other parts of the digestive system, as well as in the reproductive system. If the round worms become established in the reproductive system, occasionally worms may be found in eggs laid by the hens. Adult worms range from 2 -4.5 inches, with the females being larger than the males. They are thick and yellow-white worms.

The life cycle of round worms is direct – there is no intermediate host. Eggs are passed in the feces of the host and develop outside of the bird in about 10-12 days under ideal conditions; longer if conditions are less favorable. Eggs are resistant to low temperatures and can remain viable for over 3 months. However, eggs are easily killed by hot, dry weather. Poultry become infected when they swallow the eggs with food or water, or ingest earthworms that swallow the eggs. Once swallowed by the host, the eggs will hatch in the intestine. The larva will go through several stages before maturing in 28-30 days after hatch.

Infection by *Ascaridia* can cause weight depression, weight loss, and decrease in egg production. Heavy infections of round worms can actually obstruct the intestine. Other symptoms include anemia, diarrhea, retarded growth, and lesions in the intestine. Birds younger than 3 months are more susceptible to infection, as well as birds with dietary deficiencies such as protein or vitamins A and B [8].

In addition to the symptoms of ascaridiasis, a correlation with *Salmonella* infection has been found. The eggs of *A. galli* can transfer *Salmonella* and therefore increase the risk of persistence in the environment[59]. Additionally, a study by Eigaard et al. [60] found that infection with *A. galli* may increase the colonization rate of *S*. Enteritidis and prolong the shedding of bacteria in the feces, leading to increased risk of infection in other birds.

Birds that are infected with ascarids can be treated with peperazine products, which are relatively nontoxic to poultry.

Heterakis gallinarum

These are worms that are found in the ceca of poultry. These are small worms, ranging in size from 0.25-0.6 in long. The females are larger than the males.

Cecal worm eggs are passed out in the feces of the host and develop outside the body. Under optimal conditions the eggs become infective after 2 weeks. The eggs are very resistant and may remain viable for years. Once ingested, infective eggs hatch in the intestine and within 24 hours may be found in the ceca, where they go through several stages and molts. Cecal worms mature 24-30 days after hatch [8].

Even chickens with heavy infections show very few signs of infection, other than inflammation and thickening of the ceca walls in necropsy. The importance of *H. gallinarum* lies in its role as a carrier for the disease in turkeys known as blackhead, caused by *Histomonas meleagridis*. *H. meleagridis* can be found in both the eggs and the bodies of cecal worms.

Medications that can be used to treat against cecal worms include fenbendazole, Hygromycin B, and ivermectin. Ivermectin is not approved for use in poultry, however.

Capillaria

Species of the genus *Capillaria* are known as hair, crop, and/or thread worms. There are 6 species that are commonly found in poultry, are small and thin, resembling hair or thread, and range in size from 0.20-3.1 inches long, depending on species.

Two species*, C. annulata* and *C. contorta,* are found in the crop and esophagus. Three species are found in the small intestine, and the final species, *C. anatis,* occurs in the ceca. For some species, the life cycle is direct; eggs shed in the feces become infective, are consumed, and cause infection. Eggs voided in the feces usually take 9-15 days to become infective, then mature

after 18-60 days after hatching in the host, depending on species and conditions. In the other species the life cycle requires an earthworm as an intermediate host. The eggs are shed from the host and must be swallowed by earthworms before becoming infective. Birds then consume the earthworms to become infected. The eggs become infective 22-25 days after leaving the host and hatched worms mature after 20-26 days.

Crop worms cause inflammation and thickening of the crop and esophagus. In heavy infections, the crop may become dysfunctional. Hair worms can cause diarrhea and inflammation and thickening of the intestine. Other symptoms from infection by any of the species include depression, malnutrition, emaciation, anemia, and death [8].

Medications that can be used to treat *Capillaria* infections include methyridine and fenbendazole. Other compounds that are effective but not approved for use in poultry include ivermectin, tetramisole, flubendazole, phenothiazine, and haloxon.

Eimeria

Coccidiosis is caused by a protozoan parasite of the genus *Eimeria*. There are 7 species that cause disease in chickens and they live and multiply in the cells of the intestinal tract, including the ceca.

Birds become infected after consuming infective oocysts, thick-walled structures containing the parasite. Once ingested oocysts break down and release the sporozoites, a stage of the parasite, that then enter the cells lining the intestines. The sporozoites multiply and rupture the cells in order to escape. Sporozoites may go through multiple stages and cycles of entering and rupturing cells before forming oocysts that are released in the feces. Oocysts require 12-48 hours to become infective, depending on species and conditions such as temperature and

humidity. The minimum time between ingestion and new oocysts being released, the prepatent period, ranges between 4-6 days.

Signs of coccidiosis may include decreased feed and water consumption, decreased egg production, pigmentation loss, weight loss, slow growth and poor feed conversion, bloody diarrhea, and high mortality. Coccidiosis affects younger birds usually 3-6 weeks of age before they develop immunity; however, it can affect older birds. The severity of infection depends on the health of the bird and the number of oocysts ingested. Chickens will usually develop immunity quickly, thus self-limiting the infection. Chickens may be infected with multiple species of coccidia at the same time, and immunity to one species will not prevent infection with another species [8].

Eimeria has additional importance because infection with coccidia has been shown to enhance establishment and persistence of S. Typhimurium infection [61-64]. It has also been shown that coccidial infection can induce a recrudescence of previous *Salmonella* infection [65].

Coccidia are commonly found everywhere, so it is not possible to completely eliminate or prevent infection through quarantine, disinfection or sanitation. Oocysts are extremely resistant to common disinfectants, and it is not possible to completely sterilize a chicken house. There are numerous anticoccidials available on the market, and few are effective against all species of coccidia. Medications that can be used to treat a coccidiosis infection include quinolones, sulfonamides, and ionophores.

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CHAPTER 2

Comparisons of selected household and commercial disinfectants against poultry *Salmonella* isolates

Comparisons of selected household and commercial disinfectants against poultry *Salmonella* isolates

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Primary Audience: Small and Medium-Scale Poultry Producers, Organic and Sustainable Agricultural Practitioners, International Poultry Producers

SUMMARY

Salmonella is one of the most important bacteria to control in poultry as it not only impacts poultry production but also poses a threat to public health. Disinfection of housing, materials, equipment, etc., after cleaning is one of the best ways to protect against infection. There are several different classes of disinfectants and within those classes, numerous formulations. Additionally, there is a 'green' or 'organic' movement in the general populace away from conventional chemicals towards items that are seen as more natural. The preparation, use, and efficacy of each disinfectant vary, so the objective of this study was to determine the efficacy of 3 commercial disinfectants at various dilutions over the course of 4 time periods. Six serotypes of *Salmonella* were tested. In addition to the commercial disinfectants, lemon juice, bleach, and vinegar were also tested for efficacy at various dilutions over the course of 4 time points. Results showed bleach to be very effective, followed by the commercial disinfectants. Efficacy is dependent on serotype, concentration of disinfectant, and contact time.

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Key words: *Salmonella*, disinfectant, poultry

DESCRIPTION OF PROBLEM

Salmonella are a gram-negative bacterium that can be found all over the world. They can cause disease in animals and humans, and is naturally zoonotic[1-2]; therefore, control is essential. Because of this demand, many varieties of chemical disinfectants have been developed, each with varying properties and abilities[3-5]. Recent outbreaks of Salmonellosis caused by interaction with backyard flocks[6] have shown that health of small flocks is as important as the health of commercial flocks. Although there are various disinfectants available in developed areas for control of *Salmonella*, people in rural and developing areas may not always have access to such. There has also been a movement in developed areas away from chemical disinfectants, towards what is seen by the general populace as more natural or organic compounds and methods of disinfection. Due to interest in alternatives and unavailability for others, this project looks at the efficacy of three commercial disinfectants (Virkon-S, Virocid, and Pheno-Tek) and three common household compounds (lemon juice, bleach, and vinegar) that have some antibacterial properties and are often readily available, even in rural and developing areas. All six disinfectants were tested at varying dilutions against six serotypes of *Salmonella* over the course of four time points. Bleach was found to be very effective, with rapid action and maintaining effectiveness at low concentrations. The commercial disinfectants were more effective than lemon juice and vinegar.

MATERIALS AND METHODS

Salmonella Isolates

Six *Salmonella* isolates were obtained from Cobb Vantress Laboratory, isolated from breeder flocks. The six isolates were: *Salmonella* Enteritidis, Kentucky, Senftenberg, Montevideo, Typhimurium, and Heidelberg.

Each isolate was maintained in a frozen glycerol stock. The glycerol stock was made by growing isolates in Brain Heart Infusion (BHI) broth for 24 hours. An aliquot of the broth was then centrifuged; the supernatant was discarded. The pellet was reconstituted with a 20% glycerol and BHI mix. An aliquot was then dispensed in cryovials and stored in a -70 degree ultralow.

A loop full of glycerol stock was mixed with 5 ml of BHI and incubated for 24 hours before testing with disinfectants.

Agar Plate Preparation

Nutrient agar plates were prepared by mixing 23 grams of powdered DIFCO nutrient agar with one liter of distilled water in a glass beaker. The solution was mixed on a heated stirring plate for 25-30 minutes then boiled for one minute to completely dissolve the powder. After autoclaving for 15 minutes, fifteen ml of the mixture was pipetted into a 100 x 15mm plastic petri dish. Two to three prepared petri dishes were placed in a bacteriological incubator and incubated at 37°C for 24 hours. The plates were checked after 24 hours of incubation for contaminants.

Disinfectants

Disinfectant solutions were prepared at 4 concentrations. Common household items were lemon juice (ReaLemon 100% lemon juice), bleach (Great Value), and distilled white vinegar (Great Value). Each were tested at full strength (straight from the bottle), half strength, quarter strength, and 1/10 strength. Bleach was additionally serially diluted to 1/100, 1/500, 1/1000, and 1/5000 concentrations.

Commercial disinfectants were used at standard concentration, half concentration, 1/10 concentration, and 1/100 concentration. The directions for making the standard concentration of the commercial disinfectants were on the label of each disinfectant.

The standard concentration of Virkon-S was mixed in a 500 ml Pyrex bottle. One tablet (0.18 oz.) was mixed with one pint of distilled water, according to directions on the label.

The standard concentration of Virocid was prepared in a 50 ml centrifuge tube by mixing 0.1 ml of disinfectant with 40 ml of distilled water.

The standard concentration of Pheno-Tek was prepared in a 50 ml centrifuge tube by mixing 0.1 ml with 25.6 ml of distilled water.

The 0.5X concentrations of all disinfectants used was created by mixing 2 ml of standard concentration with 2 ml of PBS. The 0.1X concentration of all disinfectants was created by adding .5 ml of standard concentration to 4.5 ml of PBS. The 0.25X concentration of the household items was created by mixing 1 ml of the disinfectant with 3 ml of PBS. The 0.01 concentration of the commercial disinfectants was created by mixing 0.05 ml of standard disinfectant with 4.95 ml of PBS. All concentrations were created in 14 ml culture tubes.

Preparation of Bacterial Solutions

To prepare the bacterial solution, a loopful of the frozen bacteria glycerol stock was mixed with 5 ml of BHI in a 14 ml tube then placed in the incubator for 24 hours. The next day, the solution was vortexed and the optical density was determined.

The optical density of the bacterial solution was determined using a spectrophotometer at 625nm wavelength by mixing BHI and bacterial solution in a cuvette tube. The target Optical Density (OD) value was between 0.6 and 0.8. If an OD reading was outside of the range then BHI was added to the cuvette to dilute the solution and another reading was taken. This is the bacterial solution that was used to test the efficacy of disinfectants. A tube containing only BHI was used as a blank for a control.

The optical densities of the *Salmonella* solutions were determined to estimate the concentration of bacterial cells in suspension. The target estimation of cells was 4×10^8 to 6×10^8 colony forming units (CFU)/ml, which is equivalent to an optical density of 0.6-0.8, based on a growth curve for S. Enteritidis [7]. However, since this was only an estimate a plate count was performed for each isolate to determine the actual number of bacterial cells in the solution at the time of testing.

Standard Plate Count

A standard plate count was performed after obtaining the optical density range for each *Salmonella* isolate. The standard plate count method was used to determine the number of viable bacterial cells per unit volume of a sample using nutrient agar plates.

In a culture tube (12 x 75mm 5 ml), 0.1 ml of bacterial solution was added to 0.9 ml PBS and vortexed to make 1/10 dilution. A 0.1 ml aliquot from this tube was transferred to another

tube also containing 0.9 ml of PBS and vortexed to make the 1/100 dilution. This procedure was repeated until the 10^{-10} dilution was achieved. The tubes having serial solutions of 10^{-5} to 10^{-10} were plated in triplicate on nutrient agar plates to count the bacteria. A 0.1 ml aliquot from each tube was plated onto nutrient agar plates and evenly distributed on the plate with the help of an L shaped glass rod. The plates were placed in an incubator at 37 °C for 24 hours. After 24 hours, the colonies on the plates were counted and recorded.

Efficacy of Disinfectant Against Salmonella Isolates

After the optical density has been determined and the standard plate count started, 0.1 ml aliquots of a bacterial solution were placed in 1.7 ml microcentrifuge tubes and centrifuged at 13,000 rpm for 5 minutes to pelletize the bacteria. The supernatant was discarded and the pellet was resuspended with 1.5 ml of a disinfectant solution. Timing began as soon as a disinfectant solution was added to the tube. After 5, 15, 30, and 60 minutes 0.1 ml from the tube was plated on nutrient agar plates with the help of an L-shaped glass rod. Three replicate plates were prepared for each time. After inoculating the solution on each plate, the L shaped glass rod was dipped in 70% alcohol and heated with a flame to sterilize it. After incubation for 24 hours, the colonies on the plates were counted and recorded.

RESULTS AND DISCUSSION

Efficacy of Disinfectants Against S. Enteritidis

Full strength lemon juice reduced the bacterial concentration by 5.84 logs after only 5 minutes, and after 15 minutes no colonies were isolated. Half strength lemon juice reduced bacterial counts by 7.03 logs after 15 minutes and killed all by 30 minutes. Quarter strength juice

also killed after 30 minutes, but 0.1 strength lemon juice did not reduce bacterial counts until an hour after contact.

Full strength and half strength vinegar were able to completely kill *S.* Enteritidis after only 5 minutes, and colonies were never isolated from any following time point. Quarter strength vinegar reduced bacterial concentration by 4.97 logs after 15 and 30 minutes, and killed by an hour after contact. 0.1 strength vinegar was not effective at any tested time point.

Virkon-S performed similarly to vinegar in this instance; no bacteria were isolated from the X and 0.5X concentrations of Virkon at any time point. The 0.1X concentration reduced bacterial counts by 4.88 logs after 15 minutes and 6.83 logs after 30 minutes. There were no colonies observed after one hour. The 0.01X concentration was not effective.

Virocid was effective at the X and 0.5X concentrations; no bacteria were observed at any time. However, the 0.1X concentration was only effective after one hour and the 0.01 concentration was not effective at any time.

Pheno-Tek killed at all time points with the X and 0.5X concentrations. It did not show evidence of effectively reducing or killing at any time when diluted to 0.1X or 0.01X.

Bleach was effective at killing at all time points at all concentrations measuring between X to 0.01X. The 0.002X concentration was not effective within 5 minutes of contact but killed at all later time points. The 0.001X dilution was not effective within 5 minutes either, but reduced by 6.28 logs after 15 minutes and 6.34 after 30 minutes. No colonies were observed after one hour. The 0.0002X concentration was never effective (Table 2).

Efficacy of Disinfectants Against S. Heidelberg

Full strength lemon juice was not effective after 5 minutes, but after 15 minutes no colonies were observed. The 0.5X concentration reduced bacterial count by 6.76 logs after 30 minutes and killed within 60 minutes. The 0.25X concentration didn't reduce bacterial count until 60 minutes, and the 0.1X concentration was not effective at any time.

Vinegar reduced bacterial counts by 6.63 logs after 15 minutes with the full strength concentration, and killed after 30 minutes. The 0.5X concentration reduced bacteria by 6.98 logs after 30 minutes and killed after 60. The 0.25X and 0.1X concentrations were not effective at any time.

Virkon-S and Virocid both performed similarly with this strain of bacteria. The X and 0.5X concentrations killed at all time points. Bacterial counts were reduced by 6.16 logs after 15 minutes with the 0.1X concentration of Virocid and killed thereafter. 0.1 Virkon-S reduced counts by 5.39 logs. The 0.01X concentration of either disinfectant was not effective at any time.

No colonies were observed with the X and 0.5X concentrations of Pheno-Tek at any time; however, neither the 0.1X nor the 0.01X concentrations were effective at any time.

Bleach was effective at killing at all time points at all concentrations measuring between X and 0.002X. S. Heidelberg was affected differently by the 0.001X concentration. After 5 minutes, no bacteria were observed; however, too many colonies to count were observed after 15 minutes. There was a reduction in colonies again after 30 minutes, and after an hour no colonies were isolated. The 0.0002X concentration of bleach was not effective (Table 3).

Efficacy of Disinfectants Against S. Kentucky

Lemon juice at full strength was efficacious after 15 minutes, killing all bacteria. No bacterial colonies were observed after 30 minutes with the 0.5X solution. The 0.25X concentration reduced bacterial counts by 6.33 logs after 30 minutes and killed after one hour, while the 0.1X concentration was not effective at any time.

Full strength vinegar was effective at all times; half strength vinegar reduced counts 5.98 logs after 5 minutes and killed at the following time points. The 0.25X concentration didn't reduce counts until 30 minutes (7.17 logs) but killed after 1 hour, and the 0.1X concentration was not effective at any time.

Virkon-S was effective at killing *S.* Kentucky at all time points at both full strength and half strength. The 0.1X concentration reduced bacterial counts 5.45 log after 30 minutes and killed after one hour. The 0.01X concentration did not reduce bacterial counts for any time point tested.

Virocid was also effective at killing *S.* Kentucky at all time points at both full strength and half strength. After 15 minutes there were no colonies observed with the 0.1X concentration; however, there were colonies observed after 30 minutes but not after one hour. It is possible that there was contamination somehow. The 0.01X concentration did not reduce or kill the bacteria present.

Pheno-Tek killed all bacteria present at all times with both the X and 0.5X concentrations. The 0.1X concentration did not reduce the bacterial count until the hour time

point (5 log reduction), and the 0.01X concentration never effectively reduced the bacteria present.

Bleach was effective at killing at all time points at all concentrations measuring between X to 0.001X. The 0.0002X dilution was not effective after 5 minutes but did decrease the bacteria by 5.21 logs after 15 minutes. No bacteria were recovered at 30 or 60 minutes (Table 4).

Efficacy of Disinfectants Against S. Typhimurium

Lemon juice at full strength was not effective at 5 minutes, but at 15 minutes and after no colonies were observed. Colonies were reduced by 7.72 logs after 15 minutes with the .5X juice and killed after 30. The 0.25X were not effective at 5 or 15 minutes but killed at 30 and one hour. The 0.1X lemon juice reduced bacterial counts 8.28 log after one hour but were ineffective until then.

Full strength vinegar was efficacious in killing at all times, and 0.5X was able to reduce the bacteria count 7.61 log after 5 minutes and kill it thereafter. The 0.25X vinegar reduced counts 8.16 log after 15 minutes and killed at the following time points, whereas the 0.1X vinegar was never effective.

Virkon-S was effective at killing at all measured times at both the X and 0.5X concentrations. The 0.1X concentration did not reduce counts until 30 minutes (7.2 log), killing by one hour. The 0.01X solution was never effective.

There were no bacterial colonies observed at any time for both the X and 0.5X concentrations of Virocid. The 0.1X concentration reduced counts 6.37 log after 5 minutes, reduced by 7.35 after 15 minutes, and killed after 30; however, the 0.01X solution never reduced counts successfully.

Pheno-Tek was completely effective with both the X and 0.5X solutions; it was completely ineffective with both the 0.1X and 0.01X concentrations.

Bleach was effective at killing at all time points at all concentrations measuring between X to 0.002X. The .0.001X concentration caused a 6.76 log reduction and killed thereafter. The 0.0002X dilution was not effective at 5, 15, or 30 minutes, but no bacteria were recovered after one hour (Table 5).

Efficacy of Disinfectants Against S. Senftenberg

No colonies were ever observed for full strength lemon juice. Bacterial counts for the half strength juice were reduced 7.35 logs after 15 minutes, and none were observed for time points after. The 0.25X solution was not effective at 5 or 15 minutes, but it killed at 30 and 60 minutes. The 0.1X concentration was able to reduce the bacterial count by 6.18 logs only after an hour had passed.

Vinegar was effective at killing at both full strength and half strength. However, the quarter strength solution didn't reduce counts (6.17 logs) until 15 minutes, and killed all at 30 and 60 minutes. The 0.1X concentration was never effective.

Virkon-S and Virocid performed similarly when tested against *S.* Senftenberg. Both the X and 0.5X solutions killed the bacteria at all time points, and the 0.1X Virkon-S reduced count 4.89 logs after 15 minutes. 0.1X Virocid reduced by 6.6 logs. No colonies were found after 30 and 60 minutes, and the 0.01X solution was completely ineffective for all times.

Pheno-Tek was effective at full strength and half strength at all times, and ineffective at 0.1X and 0.01X concentrations for all times.

Bleach was effective at killing at all time points at all concentrations measuring between X and 0.002X. The 0.001X solution reduced bacterial counts after 5 minutes and killed after; the 0.0002X concentration was ineffective (Table 6).

Efficacy of Disinfectants Against S. Montevideo

Lemon juice at full strength was not effective at 5 minutes, but killed *S.* Montevideo at the later time points. The 0.5X solution reduced by 5.49 logs after 15 minutes and killed at 30 and 60 minutes, while the 0.25X solution reduced by 6.9 logs at 30 minutes and killed after one hour. The 0.1X solution was ineffective until it reduced bacterial counts 5.82 logs after an hour.

Vinegar was slightly more effective against *S.* Montevideo than was lemon juice. Both the X and 0.5X solutions killed all bacteria for all times, while the 0.25X solution killed all bacteria at 30 and 60 minutes. Unlike lemon juice, however, the 0.1X concentration of vinegar was not effective at reducing or killing the bacteria at any time.

Virkon-S killed at all times with the full and half strength concentrations. The 0.1X concentration was able to reduce the bacterial count 7.6 log and 8.77 log after 30 and 60 minutes, respectively, while the 0.01 concentration was completely ineffective.

Once again, there were no colonies found for any time point with the full and half strength solutions of Virocid. The 0.1 concentration was ineffective until it reduced counts 7.82 log after one hour, and the 0.01 concentration never reduced counts.

Pheno-Tek was effective at full strength and half strength at all times, and ineffective at 0.1X and 0.01X concentrations for all times.

Bleach was effective at killing at all time points at all concentrations measuring between X to 0.01X. There were reductions in the number of colonies observed after 5 and 15 minutes with the 0.002X strength solution, and no colonies recovered at later times. *S*. Montevideo also had an interesting response to the 0.001X concentration; colony counts were reduced after 5 minutes, but were too numerous to count after 15 minutes. At 30 and 60 minutes, however, no colonies were isolated. As similar to the other serotypes, the 0.0002X solution was not efficacious (Table 7).

Number of Salmonella Strains Each Disinfectant Reduced/Killed at Each Time

Out of all the disinfectants, bleach was by far the most effective since it reduced/killed all six *Salmonella* serotypes within 5 minutes at the 0.01X concentration. The three commercial disinfectants (Virkon-S, Virocid, Pheno-Tek) were completely ineffective at that low of a concentration and the two common household disinfectants (vinegar, lemon juice) were not effective at the 0.1X.

The commercial disinfectants were equal at the full and half strength concentrations; variation was seen at the 0.1X solution. Virkon-S killed fewer serotypes than Virocid after 15 minutes, but then killed more after 30 minutes. Pheno-Tek was ineffective at the 0.1X and 0.01X concentrations.

Full strength vinegar affected more serotypes after 5 minutes than lemon juice; they were equal after 15 minutes. At the 0.5X and 0.25X concentrations vinegar was more effective than lemon juice at all times. However, 0.1X lemon juice retained some efficacy after an hour whereas vinegar was not effective at that concentration, regardless of time (Table 8).

Discussion

One important lesson demonstrated in this experiment is that serotype matters when attempting to control *Salmonella*. Even though the various strains all belong to the same species, they react differently to various disinfectants. For example, lemon juice and vinegar are much more effective against *S.* Senftenberg than against *S.* Heidelberg; however, they both react similarly to Pheno-Tek. *S.* Kentucky and *S.* Montevideo were still present after 30 minutes of exposure to 0.1X Virkon-S, while *S.* Heidelberg and Senftenberg were not. If farmers know what serotype of *Salmonella* they have present, they can choose a disinfectant that will be more efficient and effective.

A comparison to similar research done by Aslam [7] indicates that bacteria can become resistant to disinfectants within a few years. Some of the strains of *Salmonella* used in this experiment were also used in the previous research, and results suggest that within that time frame the bacteria adapted. In the research by Aslam, *S.* Kentucky was killed by 0.1X concentration of Virkon-S by 30 minutes, whereas present data indicates the same concentration didn't reduce bacterial counts until 30 minutes had passed and did not kill all bacteria present until an hour after contact. Virocid at 0.1X concentration killed all *S.* Enteritidis by his 10 min time point, whereas current data indicates Virocid didn't kill until 1 hour after contact. One plausible explanation for this discrepancy between studies would be that the bacteria have evolved and developed a resistance to certain disinfectants in the time period between experiments.

Disinfectants can be effective at lower concentrations when contact time is increased. For example, Virocid at 0.1X was not effective at 5 minutes but grew increasingly more so at later time points. All strains but *S*. Montevideo were killed with 0.1X by one hour after contact.

It is reasonable to assume that some people using disinfectants will dilute products in order to save costs; this can be effective but has limitations. In this study, some disinfectants perform just as well at half the recommended concentration as at the recommended concentration. However, performance at further dilutions decreases and eventually becomes completely ineffective, regardless of contact time. Rather than diluting commercial disinfectants to cut costs, it is possible to consider an alternative such as bleach, which is low cost and highly effective.

Many hobby farmers routinely use apple cider vinegar in the water of the birds to help maintain health and fight disease (F. D. Clark, personal communication, 2016). This supports the idea of using vinegar as a disinfectant in small flocks and shows that it is an easy and inexpensive way to do so. Additionally, farmers harbor less inhibitions or qualms with putting vinegar or lemon juice in animal water than using bleach, or using chemical disinfectants for cleaning. This data has shown that organic acids may be used as disinfectants which would allow individuals who are raising organic poultry for market or personal use to improve and maintain bird health.

Personal experience in rural areas of Guatemala and Mozambique emphasized the lack of resources and the need for effective disinfectants. One local described a textbook case of Pullorum Disease when discussing problems with her flock. Any animal medications or treatments for disease were unavailable to most of the populace and farm disinfectants were hard

to find. However, bleach was available and used by many people in their households for various purposes. Furthermore, citrus fruits such as lemons and limes grow naturally in many of the areas visited; it would be possible for the juice to be harvested and used as a potential natural disinfectant.

Undeveloped areas that do not have access to our commercial disinfectants can use one of the household alternatives tested in order to decrease *Salmonella* contamination. As demonstrated, efficacy is dependent on time and serotype but any decrease in bacterial count is preferable and these alternatives are often available to even the most rural areas. The data collected in this experiment should be considered general guidelines, rather than hard and fast rules.

CONCLUSIONS AND APPLICATIONS

1. Serotypes may respond to disinfectants differently.
2. *Salmonella* are constantly evolving, so disinfectants may become less effective over time.
3. Disinfectants should be periodically tested for efficacy against intended bacteria.
4. Bacterial load has an impact on disinfectant efficacy.
5. Lower concentrations of disinfectant may or may not be as efficacious as the solution suggested on disinfectant labels.
6. Longer contact time increases efficacy and in some cases can make up for a lower strength dilution.
7. When commercial disinfectants are unavailable, alternatives that are often easily found around the household may be substituted. However, efficacy varies.

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Table 1. Disinfectants used and their active ingredients.

Disinfectant	Active Ingredients
Virkon-S	Potassium peroxymonosulfate…….21.41% Sodium chloride….1.50%
Virocid	Alkyl dimethyl benzyl ammonium chloride…17.060% Didecyl dimethyl ammonium chloride…7.8% Glutaraldehyde…10.725%
Pheno-Tek	Para tertiary amylphenol…8.24% Ortho benzyl para chlorophenol…5.3% Ortho phenylphenol…4.96%
Bleach	Sodium hypochlorite…8.5%
Lemon Juice	Citric acid…3.41%
Vinegar	Acetic acid…5%

Table 2. Plate count values for S. Enteritidis at different time intervals and concentrations of disinfectant

		MINUTES			
		5	**15**	**30**	**60**
LEMON	X*	26	0	0	0
	.5X*	T	1	0	0
	.25X*	T	T	0	0
	.1X*	T	T	T	7
VINEGAR	X*	0	0	0	0
	.5X*	0	0	0	0
	.25X*	T	260	1	0
	.1X*	T	T	T	T
BLEACH	X*	0	0	0	0
	.5X*	0	0	0	0
	.25X*	0	0	0	0
	.1X*	0	0	0	0
	.01X†	0	0	0	0
	.002X†	T	0	0	0
	.001X†	T	13	5	0
	.0002X†	T	T	T	T

		MINUTES			
		5	**15**	**30**	**60**
VIRKON-S	X‡	0	0	0	0
	.5X‡	0	0	0	0
	.1X‡	T	245	1	0
	.01X‡	T	T	T	T
VIROCID	X‡	0	0	0	0
	.5X‡	0	0	0	0
	.1X‡	T	T	T	0
	.01X‡	T	T	T	T
PHENO-TEK	X‡	0	0	0	0
	.5X‡	0	0	0	0
	.1X‡	T	T	T	T
	.01X‡	T	T	T	T

* Starting isolate concentration was 9.35 log CFU ‡ Starting isolate concentration was 9.2 log CFU

† Starting isolate concentration was 9.13 log CFU T: too numerous to count

Table 3. Plate count values for S. Heidelberg at different time intervals and concentrations of disinfectant

		MINUTES			
		5	**15**	**30**	**60**
LEMON	X*	T	0	0	0
	.5X*	T	T	4	0
	.25X*	T	T	T	37
	.1X*	T	T	T	T
VINEGAR	X*	T	3	0	0
	.5X*	T	T	1	0
	.25X*	T	T	T	37
	.1X*	T	T	T	T
BLEACH	X*	0	0	0	0
	.5X*	0	0	0	0
	.25X*	0	0	0	0
	.1X*	0	0	0	0
	.01X†	0	0	0	0
	.002X†	0	0	0	0
	.001X†	0	T	31	0
	.0002X†	T	T	T	T

		MINUTES			
		5	**15**	**30**	**60**
VIRKON-S	X‡	0	0	0	0
	.5X‡	0	0	0	0
	.1X‡	T	110	0	0
	.01X‡	T	T	T	T
VIROCID	X‡	0	0	0	0
	.5X‡	0	0	0	0
	.1X‡	T	24	0	0
	.01X‡	T	T	T	T
PHENO-TEK	X‡	0	0	0	0
	.5X‡	0	0	0	0
	.1X‡	T	T	T	T
	.01X‡	T	T	T	T

* Starting isolate concentration was 9.19 log CFU ‡ Starting isolate concentration was 9.6 log CFU

† Starting isolate concentration was 9.2 log CFU T: too numerous to count

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Table 4. Plate count values for S. Kentucky at different time intervals and concentrations of disinfectant

		MINUTES			
		5	**15**	**30**	**60**
LEMON	X*	T	0	0	0
	.5X*	T	T	0	0
	.25X*	T	T	12	0
	.1X*	T	T	T	T
VINEGAR	X*	0	0	0	0
	.5X*	29	0	0	0
	.25X*	T	T	1	0
	.1X*	T	T	T	T
BLEACH	X*	0	0	0	0
	.5X*	0	0	0	0
	.25X*	0	0	0	0
	.1X*	0	0	0	0
	.01X†	0	0	0	0
	.002X†	0	0	0	0
	.001X†	0	0	0	0
	.0002X†	T	46	0	0

		MINUTES			
		5	**15**	**30**	**60**
VIRKON-S	X‡	0	0	0	0
	.5X‡	0	0	0	0
	.1X‡	T	T	104	0
	.01X‡	T	T	T	T
VIROCID	X‡	0	0	0	0
	.5X‡	0	0	0	0
	.1X‡	T	0	5	0
	.01X‡	T	T	T	T
PHENO-TEK	X‡	0	0	0	0
	.5X‡	0	0	0	0
	.1X‡	T	T	T	270
	.01X‡	T	T	T	T

***** Starting isolate concentration was 9.4 log CFU ‡ Starting isolate concentration was 9.4 log CFU

† Starting isolate concentration was 9.1 log CFU T: too numerous to count

Table 5. Plate count values for S. Typhimurium at different time intervals and concentrations of disinfectant

		MINUTES			
		5	**15**	**30**	**60**
LEMON	X*	T	0	0	0
	.5X*	T	19	0	0
	.25X*	T	T	0	0
	.1X*	T	T	T	3
VINEGAR	X*	0	0	0	0
	.5X*	13	0	0	0
	.25X*	T	3	0	0
	.1X*	T	T	T	T
BLEACH	X*	0	0	0	0
	.5X*	0	0	0	0
	.25X*	0	0	0	0
	.1X*	0	0	0	0
	.01X†	0	0	0	0
	.002X†	0	0	0	0
	.001X†	4	0	0	0
	.0002X†	T	T	T	0

		MINUTES			
		5	**15**	**30**	**60**
VIRKON-S	X‡	0	0	0	0
	.5X‡	0	0	0	0
	.1X‡	T	T	2	0
	.01X‡	T	T	T	T
VIROCID	X‡	0	0	0	0
	.5X‡	0	0	0	0
	.1X‡	T	1	0	0
	.01X‡	T	T	T	T
PHENO-TEK	X‡	0	0	0	0
	.5X‡	0	0	0	0
	.1X‡	T	T	T	T
	.01X‡	T	T	T	T

* Starting isolate concentration was 10.92 log CFU ‡ Starting isolate concentration was 9.83 log CFU

† Starting isolate concentration was 9.31 log CFU T: too numerous to count

Table 6. Plate count values for S. Senftenberg at different time intervals and concentrations of disinfectant

		MINUTES			
		5	**15**	**30**	**60**
LEMON	X*	0	0	0	0
	.5X*	T	1	0	0
	.25X*	T	T	0	0
	.1X*	T	T	T	27
VINEGAR	X*	0	0	0	0
	.5X*	0	0	0	0
	.25X*	T	28	0	0
	.1X*	T	T	T	T
BLEACH	X*	0	0	0	0
	.5X*	0	0	0	0
	.25X*	0	0	0	0
	.1X*	0	0	0	0
	.01X†	0	0	0	0
	.002X†	0	0	0	0
	.001X†	9	0	0	0
	.0002X†	T	T	T	T

		MINUTES			
		5	**15**	**30**	**60**
VIRKON-S	X‡	0	0	0	0
	.5X‡	0	0	0	0
	.1X‡	T	207	0	0
	.01X‡	T	T	T	T
VIROCID	X‡	0	0	0	0
	.5X‡	0	0	0	0
	.1X‡	T	5	0	0
	.01X‡	T	T	T	T
PHENO-TEK	X‡	0	0	0	0
	.5X‡	0	0	0	0
	.1X‡	T	T	T	T
	.01X‡	T	T	T	T

***** Starting isolate concentration was 9.67 log CFU ‡ Starting isolate concentration was 9.15 log CFU

† Starting isolate concentration was 9.33 log CFU T: too numerous to count

Table 7. Plate count values for S. Montevideo at different time intervals and concentrations of disinfectant

		MINUTES			
		5	**15**	**30**	**60**
LEMON	X*	T	0	0	0
	.5X*	T	62	0	0
	.25X*	T	T	2	0
	.1X*	T	T	T	31
VINEGAR	X*	0	0	0	0
	.5X*	0	0	0	0
	.25X*	T	T	0	0
	.1X*	T	T	T	T
BLEACH	X*	0	0	0	0
	.5X*	0	0	0	0
	.25X*	0	0	0	0
	.1X*	0	0	0	0
	.01X†	0	0	0	0
	.002X†	5	1	0	0
	.001X†	133	T	0	0
	.0002X†	T	T	T	0

		MINUTES			
		5	**15**	**30**	**60**
VIRKON-S	X‡	0	0	0	0
	.5X‡	0	0	0	0
	.1X‡	T	T	27	1
	.01X‡	T	T	T	T
VIROCID	X‡	0	0	0	0
	.5X‡	0	0	0	0
	.1X‡	T	T	T	27
	.01X‡	T	T	T	T
PHENO-TEK	X‡	0	0	0	0
	.5X‡	0	0	0	0
	.1X‡	T	T	T	T
	.01X‡	T	T	T	T

* Starting isolate concentration was 9.19 log CFU ‡ Starting isolate concentration was 11.13 log CFU

† Starting isolate concentration was 9.16 log CFU T: too numerous to count

Table 8. Number of *Salmonella* strains each disinfectant reduced and/or killed at each time

		MINUTES			
		5	**15**	**30**	**60**
LEMON	X	2	6	6	6
	.5X	0	4	6	6
	.25X	0	0	5	6
	.1X	0	0	0	4
VINEGAR	X	5	6	6	6
	.5X	5	5	6	6
	.25X	0	3	5	6
	.1X	0	0	0	0
BLEACH	X	6	6	6	6
	.5X	6	6	6	6
	.25X	6	6	6	6
	.1X	6	6	6	6
	.01X	6	6	6	6
	.002X	5	6	6	6
	.001X	5	4	6	6
	.0002X	0	1	1	3

		MINUTES			
		5	**15**	**30**	**60**
VIRKON-S	X	6	6	6	6
	.5X	6	6	6	6
	.1X	0	3	6	6
	.01X	0	0	0	0
VIROCID	X	6	6	6	6
	.5X	6	4	6	6
	.1X	0	4	4	6
	.01X	0	0	0	0
PHENO-TEK	X	6	6	6	6
	.5X	6	6	6	6
	.1X	0	0	0	1
	.01X	0	0	0	0

CHAPTER 3

A survey of the prevalence of internal parasites in chickens shown at Arkansas Fairs

A survey of the prevalence of internal parasites in chickens shown at Arkansas Fairs

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Primary Audience: Small and Medium Scale Poultry Producers, Hobby Flock Owners, Veterinarians, Extension Agents

SUMMARY

Backyard and exhibition poultry have been gaining in popularity and as such there has been a large increase in the number of small flocks. Typically most backyard flocks are allowed to roam freely for part or all of the day. As these free-range birds interact with the environment there is an increased risk of exposure to parasitic infection due to factors such as exposure to wild birds, intermediate hosts such as earthworms, and lack of knowledge. Quite often the owners are new to poultry and don't recognize the potential for parasite infection, nor how to identify and treat it if infection does occur. Four of the most common internal parasites found in backyard poultry are *Eimeria, Capillaria, Heterakis*, and *Ascaridia*. Some research has been done on the prevalence of parasites in small flocks internationally but very little in the United States. Coccidia were found in more than half of flocks for two years, and roughly a quarter of flocks are infected with ascarids. At least a quarter of flocks showed evidence of *Capillaria*, and almost a third of flocks were infected with 2 or more species of parasites. This study indicates that there is a high prevalence of parasite infection in backyard flocks, especially with coccidia.

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Key words: poultry, hobby flock, parasites, ascarids, coccidia

DESCRIPTION OF PROBLEM

Research and surveys have been done on the prevalence of internal parasites in flocks internationally [1-2], especially in Africa [3–6], but very little has been done in the United States [7]. The purpose of this study was to determine the prevalence of common internal parasites in small poultry flocks in Arkansas.

MATERIALS AND METHODS

Samples were randomly collected every year from the Arkansas-Oklahoma State Fair held in Ft. Smith, Arkansas, and the Arkansas State Fair in Little Rock for the years 2013-2015. In 2015 samples were also collected from the Washington County Fair in Northwest Arkansas. Fresh fecal samples were individually collected from cages then transported to the University Of Arkansas Center Of Excellence for Poultry Science and analyzed for parasites by performing fecal flotation per Soulsby [8]. Fecal flotation was performed by mixing 1 gram of fecal material with 10 ml of saturated sodium chloride solution. A glass coverslip was placed on top of the test tube and left for at least 20 minutes. The coverslip was placed on a slide and examined under microscope for presence or absence of parasites.

RESULTS AND DISCUSSION

Over the course of three years, a total of 909 samples from different birds were analyzed, collected from 279 entrants; each entrant is considered an individual flock. In an effort to understand the dynamics of parasites in small flocks we analyzed the data for the years individually in order to track variation in infection. The study found that in backyard flocks

shown in Arkansas, the percent of all flocks infected with *Ascaridia* ranged between 24-28% over the course of 3 years. Infection with *Eimeria* ranged between 44-71%; infection with *Capillaria* was between 25-34%, and incidence of *Heterakis* varied between 2-10%. Flocks that had evidence of more than one parasite present started at 34% but then dropped down to 27% by 2015. (Graph 1)

When the data for the Little Rock State Fair was analyzed, a drop (from 55% to 38% then back to 66%) was observed in the number of flocks infected with coccidia during the year 2014. Flock infection with *Ascaridia* ranged between 24% and 29%, whereas presence of *Capillaria* ranged between 27% and 38%. Infection with *Heterakis* varied between 3-11%. (Graph 2)

Data for the AR/OK State Fair showed an increase in the amount of coccidial infection, rising from 61% in 2013 to 85% in 2015. No evidence of *Heterakis* was found in 2014 or 2015. In 2013 9% of flocks had evidence of *Heterakis*. Infection with *Capillaria* jumped from 20% in 2014 to 54% in 2015. (Graph 3)

There are numerous variables that could have influenced the rate of infection for any of the species surveyed, including sampling error, Extension hobby flock outreach programs, management and husbandry, bird housing, bird health, etc. Another major variable is weather, which influences the quality of the bedding, be it litter or the ground outside. 2014 was a record cool year with drier than normal conditions during the latter half of the year [9], when samples were collected. In contrast, the year 2013 was average for temperatures and rainfall [10] while 2015 was warmer and wetter than the 2 years previous [11]. A study done by Permin et al. [3] examined rural scavenging poultry in Tanzania to see if there was a difference in prevalence of parasites during the dry season versus the wet season. *Ascaridia galli*, *Heterakis* species, and

Capillaria species were all identified in the flocks. Although all birds examined were infected by at least one species of parasite, the study did not find a correlation between season and mean worm burdens, or season and prevalence. In contrast, a study of organic free range layer systems in Germany [2] found that risk of infection with *H. gallinarum*, *A. galli,* and *Capillaria* was 50% higher in the summer. A study of scavenging poultry in two rural areas with different climates in Zimbabwe [4] found a lower prevalence of *Ascaridia* in the warm humid area than in the dry, hot area. However, the study also found a slightly higher prevalence of *Capillaria* in the warm humid area than in the dry, hot area. The prevalence of *Heterakis* was similar for both locations. Although weather does seem to have an effect on parasite prevalence, the influence it has seems to fluctuate, most likely depending on various other factors.

Although it is commonly thought that round worms are a bigger issue than thread worms (F. D. Clark, personal communication, 2016), this data indicates that thread worms are slightly more prevalent in small backyard flocks in the state of Arkansas.

The low incidence of *Heterakis* found in this study is similar to data found by Wilson et al. [7] in their study of commercial broiler chickens from 2 companies. In that study, 7.15% of farms from company A were infected with *H. gallinarum* and 1.9% of farms from company B were infected. The study also found 37.3% of farms from company A to be infected with *A. galli,* whereas farms from company B only showed 3.9% of farms being infected. These surveys of poultry in Arkansas show that *A. galli* is a much bigger problem than *H. gallinarum*.

This study found there is a high prevalence of parasite infection in backyard and urban poultry flocks. The data shows coccidia are especially widespread in Arkansas.

CONCLUSIONS AND APPLICATIONS

1. *Ascaridia, Eimeria*, and *Capillaria* are common in backyard flocks in Arkansas.

2. Evidence of multiple parasites present in a flock is also relatively common.

3. Extension programs to educate small flock owners about internal parasites and how to treat and prevent them could prove beneficial.

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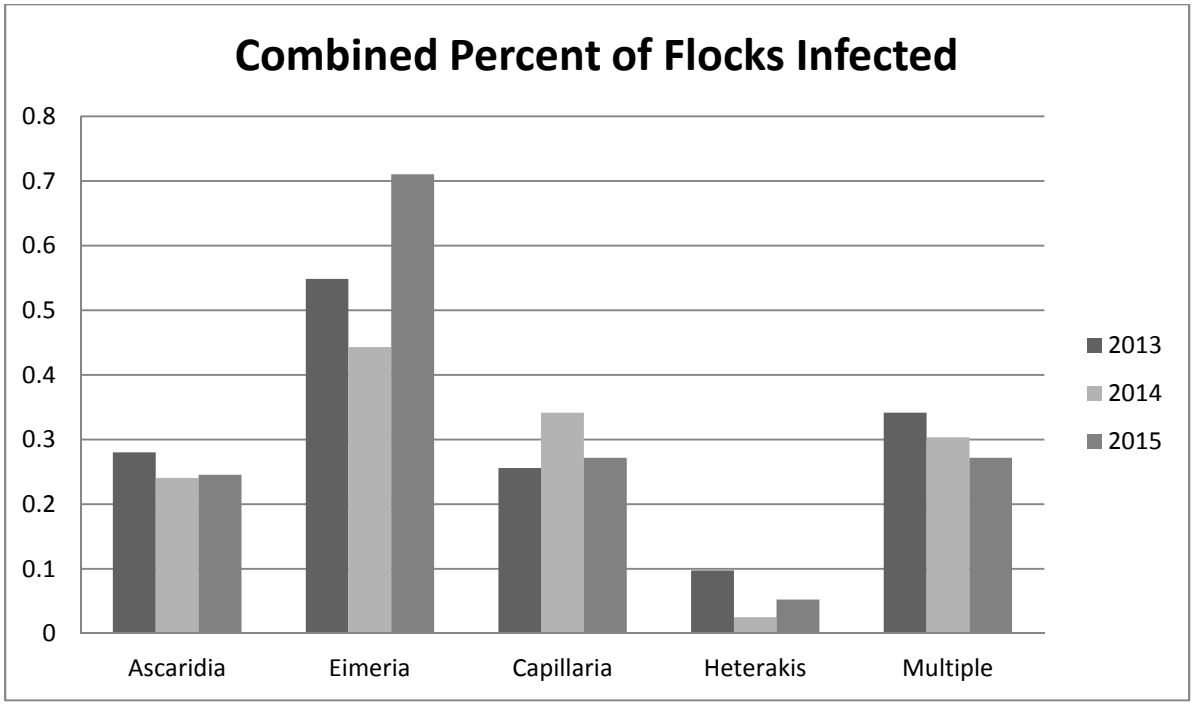
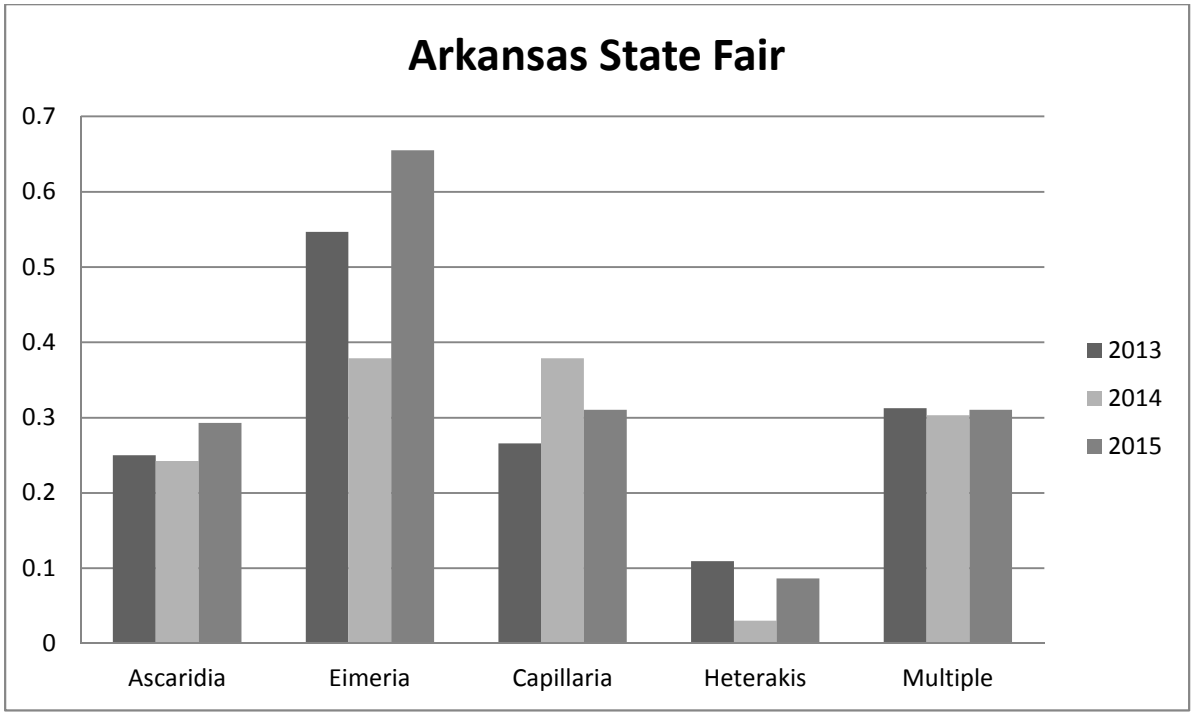
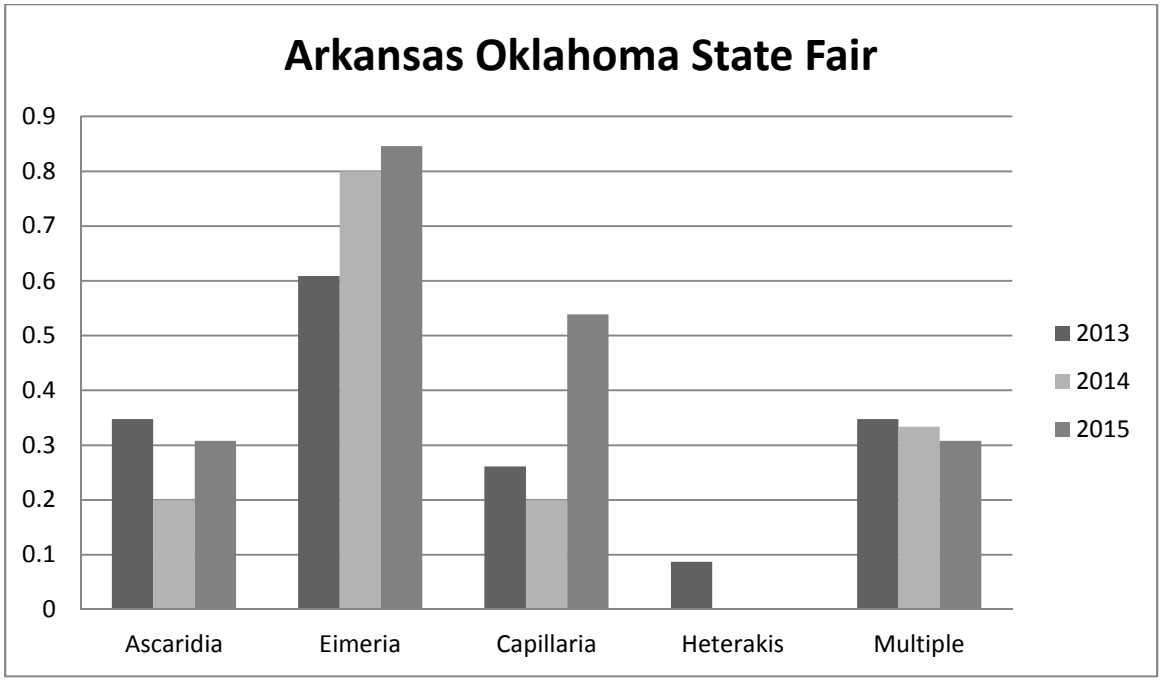
Graph 1. Percent of flocks infected with parasites from data collected for the years 2013-2015 at the AR-OK Fair, Arkansas State Fair, and Washington County Fair.



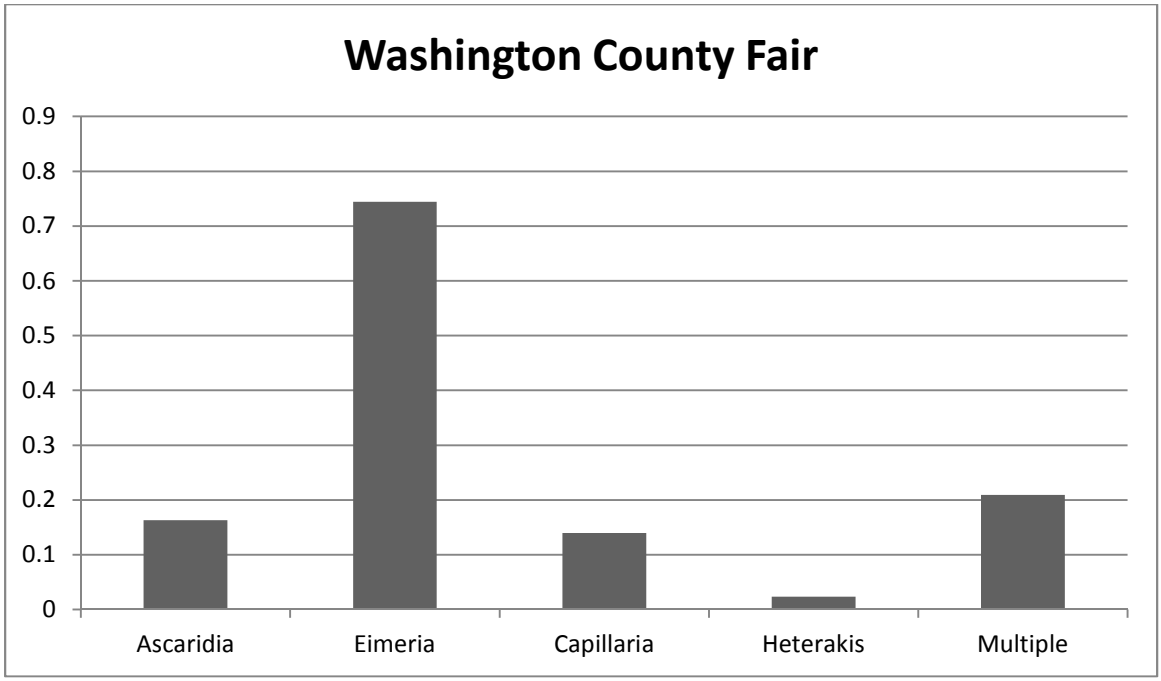
Graph 2. Percent of flocks infected with parasites from data collected at the Arkansas State Fair in Little Rock during the years 2013-2015.



Graph 3. Percent of flocks infected with parasites from data collected at the Arkansas Oklahoma State Fair during the years 2013-2015.



Graph 4. Percent of flocks infected with parasites from data collected at the Washington County Fair in 2015.



CHAPTER 4

Conclusion

CONCLUSION

Salmonella and internal parasites have a major impact on people and poultry worldwide. Knowledge as to species or serotype, prevalence, prevention, and removal are important in order to maintain health and productivity.

The first section of this thesis explored the efficacy of 3 commercial disinfectants and 3 common household compounds against various serotypes of *Salmonella*. The research showed that knowledge of serotype present in a location or flock is important to determine which disinfectant would be best, as serotypes respond differently. To make choosing a disinfectant more difficult, however, *Salmonella* are evolving over time and resistance to chemicals that were previously effective is possible. Even the best disinfectant may be ineffective when the bacterial load is too high or if organic matter is present, so cleaning before disinfection is advised. The data showed that lower dilutions of disinfectant solutions can maintain efficacy if contact time is increased; however, there are limitations for how much each disinfectant can be diluted before it becomes completely ineffective. For farmers in rural or international locations that are unable to access commercial disinfectants, or for those people who want to avoid chemicals that they view as unnatural, bleach, lemon juice, and vinegar are possible alternatives.

Bleach is highly effective even at low concentrations, works rapidly, is inexpensive, and readily available. Because of these factors, it can be reasonably presumed that the average farmer would not scientifically measure out amounts of bleach; they would most likely measure by using the cap from the bottle, or by the ‘glug’ method. A capful of bleach from the bottle used in this experiment contained 12 ml of fluid, and although the size of a glug is variable, it is safe to presume that most would contain at least 24 ml of liquid, equivalent to 2 capfuls. The data demonstrates that bleach will kill all the bacteria tested after 15 minutes at a solution of one part

bleach, 500 parts water (0.002X). Therefore, one capful of bleach in 1.5 gallons of water would be enough to completely kill after 15 minutes, and it would kill most of the bacteria present after only 5 minutes. One capful of bleach and 3.2 gallons of water (0.001X) would reduce bacteria after 5 minutes, and completely kill after 30. However, one cap of bleach for 3 gallons often does not seem like much, so most farmers would use the glug method. Just 1 glug for 3 gallons would give the 0.002X concentration, killing after only 15 minutes. More glugs would continue to increase the concentration, improving efficacy and reducing kill time.

Lemon juice and vinegar are not as effective as commercial disinfectants or bleach and are highly influenced by the serotype, but are still capable of reducing bacterial counts under certain conditions. For those that do not have better resources, lemon juice and vinegar are possible alternatives. They should be used at higher concentrations. Further research can look into the efficacy of combining these two acids to determine if there is a synergistic effect.

There are various studies that can be performed following this data, looking further into possible alternatives that could be used in developing areas. Examples of possible items to try include various sodas, such as Sprite and Coca-Cola, and slaked lime. An additional real world application to examine would be the antibacterial effect of leaving items out in the sun for various periods of time.

The second portion of this thesis discusses a survey of backyard flocks in Arkansas and the prevalence of four common internal parasites. Data revealed that over half of flocks were positive for coccidia. Over the course of 3 years, a quarter of flocks showed evidence of *Ascaridia* and *Capillaria.* Prevalence of *Heterakis* remained below 10%. Between a quarter and a third of flocks have concurrent infection with multiple parasites. This study revealed that

internal parasites in Arkansas are more common than previously thought, and that coccidia are especially prevalent.

Future research can look at the impact that length of time has on bird health. Some farmers have been exhibiting birds at fairs for years, whereas some are kids just beginning their journey. A survey conducted in conjunction with the internal parasite data could give more information as to how knowledgeable farmers are about the health of their birds and what they have or have not done in order to maintain their flocks.

While these studies did not examine prevalence of *Salmonella* in flocks, the increased incidence of health problems in small flocks, including internal parasites, does necessitate further study. A preliminary study looking at *Mycoplasma synoviae, M. gallisepticum*, and *Salmonella* incidence in backyard flocks has been started by Extension but the data is not yet complete. Thus far the incidence of *Salmonella* has been very low; however, the birds sampled have all been adults while the majority of outbreaks have been linked to very young chicks. Additional studies may be of benefit after the conclusion of the preliminary survey.

This data should allow anyone who keeps poultry or works in the poultry industry to have a better understanding of how to maintain the health of their birds. Additional knowledge of disinfectant efficacy allows farmers and others to choose a compound that will fit their situation. In decreasing the prevalence of *Salmonella* on their farm, they should be able to improve not only the health of their flocks but the health of themselves and their families, due to the zoonotic nature of the bacteria. Familiarity with the frequency of internal parasite infection should allow farmers to be proactive, such as decreasing the conditions required for contact and spread. Additionally, farmers will be able to medicate their birds for a specific condition rather than

using a broad spectrum treatment for diseases the birds do not have. By becoming aware of the common diseases in an area, being proactive, and knowing how to maintain clean premises, farmers will be better able to maintain their health and the health of their flocks.