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Article

How CAFOs affect *Escherichia coli* contents in surrounding water sources

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SUMMARY

Commercial Concentrated Animal Feeding Operations (CAFOs) produce large quantities of waste material from the animals being housed in them. These feedlots found across the United States contain livestock that produce waste that results in hazardous runoff. Water sources surrounding CAFOs become contaminated making the water unusable for humans, livestock, and uninhabitable for aquatic life. This study examines how CAFOs affect water sources by testing for *Escherichia coli* (*E. coli*) content in bodies of water near CAFOs. We hypothesized that the level of *E. coli* content in water samples near CAFO sites will be higher than in water samples not near CAFOs sites. To test our hypothesis, private drinking water bacteriological tests were performed on water samples sourced within one mile of a CAFO and then compared to water samples sourced more than five miles from the nearest CAFO. We found an increase in the *E. coli* concentration in water source samples closer to CAFOs. This research is crucial to understanding the impact that CAFOs have on the environment. It also provides important information for those living near CAFOs so that they can make informed decisions regarding their use of nearby water sources.

INTRODUCTION

Large-scale industrial agricultural facilities are common in the United States. These facilities, known as Concentrated Animal Feeding Operations (CAFOs), usually house animals in high-density to produce goods for public consumption of meat, eggs, or milk. The United States Department of Agriculture (USDA) considers an agricultural facility a CAFO if it confines over 1000 animals for over 45 days a year with no grass or other vegetation present during confinement of the normal growing season (1, 2). In animal husbandry, CAFOs differ significantly from traditional farming and have historically been considered the cause of major problems in their surrounding communities due to offensive odors and as producers of runoff waste (3). CAFOs affect surrounding water sources when the waste runoff from rain carries feces and other pollutants into nearby creeks, streams, and rivers (1). Additionally, liquid manure from CAFOs is used in crop fields to increase crop yield. While this helps with the growth of crops, the runoff can be detrimental to surrounding water sources (1).

Runoff waste impairs water sources with many kinds of bacteria, but one of particular concern is pathogenic *Escherichia Coli* (*E. coli*). Extraintestinal strains of pathogenic *E. coli* is harmful to animals, including humans, when contaminated food or water is consumed. For example, pathogenic *E. coli* is often responsible for huge losses within broiler chickens (2). *E. coli* is a bacterium that is commensal in the intestines or other digestive organs of animals. Most types of *E. coli* are harmless, but some strains, like Shiga toxin-producing *E. coli* (STEC) can cause infection even if ingested in small amounts. An STEC infection in livestock usually ends up being life-threatening due to complications involving hemolytic uremic syndrome, a type of kidney failure, and dehydration in the animal (4).

Infectious bacteria are commonly transmitted through oral-fecal contamination where an animal's food or water becomes contaminated with infected feces and then is introduced to the animal via the oral cavity (5). If the fecal matter of an animal with pathogenic *E. coli* gets into the drinking water, the animals that drink from the contaminated water are exposed to potentially life-threatening *E. coli* infections. This makes *E. coli* highly dangerous in concentrated animal feeding operations since the animals are close in proximity and share watering troughs or water sources near areas where they also defecate. *E. coli* can live in the intestines of cattle and other livestock, which is also dangerous for consumers (6). CAFOs are an ideal environment for *E. coli* growth and transmission. All residences and wildlife near CAFOs are at risk of having surface and wastewater contaminated by pathogenic *E. coli*.

The main goal of our research was to determine if water sources near CAFO sites across mid-Missouri are contaminated by pathogenic *E. coli*. In 2005, the USDA passed a bill allowing the department to enter any CAFO facility at any time to test water for unwanted bacteria (3). Even though this bill was passed and the USDA, through the federal Environmental Quality Incentives Program, indirectly subsidizes CAFOs to pay for manure lagoons and their management, contaminated water at CAFO facilities remains an ongoing problem and sites go untested (3, 7). Additionally, Missouri's Environmental Service Program which samples ground, surface, storm, and drinking waters for pathogens, does not specifically monitor or inspect *E. coli* content in water sources surrounding CAFOs. To date, no data has been recorded in Missouri for *E. coli* content in water sources surrounding CAFOs. Further, there are no published findings for how these large farming operations affect surrounding water sources that are both consumed by livestock and used for their cleaning.

The Missouri Department of Natural Resources reports that as of 2019, there were 113 lakes in Missouri that are impaired for aquatic life and designated for recreational use according to the United States Environmental Protection Agency (EPA) standards (8). State lakes, streams, and wetlands in Missouri

are assessed using a regional Numeric Nutrient Criteria Rule. The Missouri Clean Water Commission adopted this rule on April 20, 2018, after Missouri was unable to meet EPA Clean Water Act requirements (8). The rule evaluates and predicts the degree of eutrophication in these bodies of water by measuring variables such as nutrient concentrations of nitrogen and phosphorus, algal biomass (measured as chlorophyll), as well as turbidity (8). Eutrophication, the presence of excessive nutrients in water, is usually due to land runoff, which increases plant productivity thereby decreasing oxygen levels for aquatic life (8). Proliferative toxigenic cyanobacteria are shown to positively influence the growth and survival of *E. coli* (9). For example, algae are known to shelter *E. coli* from solar radiation; thereby affecting survivorship (9). It has been demonstrated that these species-specific interactions result in synergistic relationships (10). *E. coli* has long been known as an indicator for the potential presence of pathogens in natural waters (10).

CAFOs are an ideal environment for *E. coli* to grow because of manure production as well as from abundant respiration and flatulence that occurs in the CAFOs. Fecal matter found in water run-off could contaminate nearby water sources and streams. It can be assumed that contamination of *E. coli* in CAFO water occurs at greater rates than in non-CAFO water. To test this assumption, we sent ten water samples to The Missouri State Public Health Laboratory (MSPHL) for a specific count bacterial analysis. Samples were analyzed for the enumeration of *E. coli* and total coliform bacteria. We found moderate evidence that supports significant differences in *E. coli* content among our experimental and control samples. Information regarding *E. coli* content in water sources surrounding CAFOs is an ecological and public health concern. Our findings may alert people who traditionally farm or ranch near CAFOs of the health dangers they pose to their animals.

RESULTS

To evaluate our hypothesis that water sources near CAFO sites across mid-Missouri have higher concentrations of *E. coli*, the water of multiple creeks and streams within one mile of a CAFO was collected and tested for *E. coli* content. These water samples were then compared to the *E. coli* content of waters that are not within one mile of a CAFO. In total, we tested *E. coli* levels at ten locations across mid-Missouri (**Table 1**). Five of the locations were from water sources near known CAFOs, and five from water sources not near CAFOs. The samples were then compared using descriptive statistics and nonparametric statistics (**Table 2**).

The null hypothesis (H_O) is that there will be no difference in *E. coli* content in water source samples across mid-Missouri: H_O = r = 0. *E. coli* count measured in most probable number (MPN) per 100 ml was compared using Pearson's correlation coefficient (r = 0.5776). The Pearson's correlation is modest; thereby, suggesting moderate evidence against the null hypothesis in favor of the alternative; that *E. coli* count varies among experimental and control samples. The average *E. coli* content from sites within one mile of CAFOs is nearly ten times greater than the average of the control samples. On average, samples within one mile of CAFOs had 1177.88 MPN of *E. coli* while control samples averaged 106.82 MPN (**Table 2**). One statistical limitation of this study is the small sample size and the inability to use parametric statistics because of the non-randomness of the samples. Only statistical measures that make no assumptions about the population distributions from which the samples are drawn are used.

Experimental (Near CAFOs sites)			Control (Non-CAFOs sites)		
Location	**Date**	***E. coli***	**Location**	**Date**	***E. coli***
Campbell Bridge	12/20/2020	83.9	Drew's	10/25/2020	19.7
Bear Branch Rd 1	03/12/2021	2419.6	E Splice Creek	10/25/2020	45.5
Bear Branch Rd 2	03/12/2021	920.8	Echo Valley	10/25/2020	387.3
Prairie Rd	12/20/2020	45.5	Happy Hollow	10/25/2020	45.7
Strawberry Rd	03/12/2021	2419.6	Kodi Rd	10/25/2020	35.9

Table 1: *E. coli* Specific Count Content (MPN) by Sample and Collection Dates. MPN results for *E. coli* using the IDEXX Quanti-Tray/2000 System with Colilert reagent. Counts reported from 1-2,419.6 with anything greater than 1 MPN considered undrinkable by MSPHL.

	Experimental (Near CAFOs sites)	Control (Non-CAFOs sites)
Mean	1177.88	106.82
Median	920.8	45.5
Mode	2419.6	set
Standard Deviation	1186.27	157.15
Range	2,374.1	367.6
Minimum	45.5	19.7
Maximum	2419.6	387.3
Sum of Data	5,889.4	534.1
Count	5	5

Table 2: Descriptive Statistics for Experimental and Control Samples. Descriptive statistics for MPN results for *E. coli* from five water sources near known CAFOs, and five from water sources not near CAFOs. The average *E. coli* content from near CAFOs sites is nearly ten times greater than the average from non-CAFOs sites. The Pearson's correlation is modest (r = 0.5776).

DISCUSSION

The results of this study provide moderate evidence that *E. coli* counts vary between control and experimental samples with higher levels of *E. coli* recorded closer to CAFO sites. While there are numerous reasons that could explain increased levels of *E. coli* in these natural water sources, we hypothesize that the most likely explanation is their proximity to CAFOs. Since there is such a high concentration of animals within a CAFO, the feces produced by the livestock can build up rapidly inside the building, under the outdoor shelters, or in the outdoor feedlots. Additionally, liquid manure from CAFOs is often spread on surrounding crop fields (1). If waste from CAFO shelters and outdoor spaces gets carried away in water runoff during rainstorms, it will likely result in increased *E. coli* content in nearby water sources.

One factor concerning water runoff as a source of sample contamination is stormwater management in the area surrounding our collection site. Stormwater can carry several pollutants including dirt, vehicle oils, toxic chemicals like fertilizers, as well as bacteria and waste from pets, livestock, and failed septic systems (11). In suburban areas, runoff is diverted into untreated storm drains, which during heavy rains can combine with sewer overflow and into natural water sources (11). It is unlikely that contamination from suburban runoff is a factor in our study, given that our sample sites in Moniteau and Cooper counties are in rural areas removed from stormwater systems.

Another explanation for findings of increased levels of *E. coli* in natural water sources is anthropogenic activities. For example, there is always the possibility that recreational use

of natural water sources could result in increased levels of *E. coli*. Again, this explanation is unlikely in our study, given that none of the sites tested are public areas designated for recreational use and given the low human population density in these rural areas. At present, manure waste produced by CAFOs is the best explanation for our findings.

One limitation of our study is that the water sources sampled from the collection area vary in size. It could be argued that *E. coli* content would be concentrated in smaller water sources. Similarly, not all the CAFOs in the study were of the same size. It would be helpful to know how many animals are housed at each operation since the size of the CAFO will also likely influence the quantity of waste. Though there is no way to control for the size of the CAFO or the size of water sources available for sampling, it is recognized these variables would affect our statistical analysis.

Another limitation of our study is that the water samples were taken at different times of year. Ideally, all the water samples would be taken at the same time of year to control for seasonal variations, such as temperature, humidity, and precipitation. This was challenging to accomplish given the mileage between each collection site. Temperature fluctuations were not considered extreme between dates of collection. Temperature readings at the times of collection ranged from 45-52 degrees Fahrenheit. There was also little deviation between water levels for each site considering there had not been a substantial amount of rainfall directly prior to any of the tests. Only two sites, both in our experimental sample (Strawberry Road and Bear Branch 2) received rainfall prior to collection. Both sites received ¾ inch of rain 30 hours prior to collection.

All samples were taken from grassland, prairie streams of Missouri. This area known as the Prairie Aquatic Faunal Region of Missouri is dominated by cropland and typically occupies broad, flat valleys that slope gradually into the surrounding uplands (12). The stream bottoms in the Prairie Region are generally turbid with fine substrate. Stream flow and other water conditions can vary over the course of the year (12); thereby, making it difficult to determine if collections were up or downstream from CAFO sites. An additional investigation that takes into consideration the limitations of our current research design would likely reveal statistically significant findings.

Further investigation requires more samples to improve statistical analysis and significance. A larger, nonrandom sample could allow for more powerful inferences to be made from water sources throughout the state. The non-randomness of the current samples limits the findings to the use of descriptive statistics (13). All the data collected for this study was from a relatively small area of central Missouri, but it would be interesting to expand the testing area to all of mid-Missouri, or even to the entire state of Missouri.

Our study examines how waste from CAFOs affects surrounding water sources by measuring *E. coli* content in water sources at varying proximity to these facilities. This study and additional research is important because it is the first of its kind to document *E. coli* levels relative to proximity of CAFOs. Pathogenic *E. coli* at CAFOs facilities can end up in the ground water and water sources near these facilities, as well as in livestock sold for consumption. *E. coli* outbreaks pose a health risk to the community at large and cause illnesses in humans, such as meningitis, septicemia, kidney infections, urinary tract infections, and intestinal infections (14).

MATERIALS AND METHODS

This study examined impaired waters in Missouri in connection to the CAFOs surrounding them. During this study, the water of multiple creeks and streams within one mile of a CAFO was collected and tested for *E. coli* content. These water samples were then compared to the *E. coli* content of waters that are not within one mile of a CAFO. The water sources for the control sample were taken within 15 miles of Jamestown, Missouri (Moniteau County) and at least five miles from the nearest CAFO. The water sources for the experimental sample were selected by first locating permitted CAFOs within about 40 miles of Jamestown, Missouri. The landscape in this region was then surveyed to determine the nearest water source within one mile of these CAFOs facilities. Samples were collected from sites located in Cooper, Moniteau, and Boone Counties in Missouri. Once located, accessing some of the water sources required permission of local landowners while others were considered public property. The water sources on public property were accessed from low water crossings, bridges, or county installed culverts.

Water samples were collected in 120 ml shrink-banded, sterile bottles. The bottles were placed approximately one inch below the surface of the water. We were careful not to disturb sediment on the bottom of the streams or to touch the bottle mouth or inside of the cap. The date, time, and temperature were recorded at each collection, as well as rain accumulation totals if rain occurred within 30 hours of collection. None of the water sources had been frozen at the time of collection.

Samples were sent directly to MSPHL operated by Missouri Department of Health and Senior Services in Jefferson City, Missouri for specific count bacterial analysis. The MSPHL can test for three different types of bacteria (coliform, *E. coli*, and iron bacteria) in private water samples using presence/absence tests. Tests that provide specific count for waterborne pathogens are available by special request. MSPHL uses an EPA approved IDEXX Colilert Quanti-Tray/2000 test to quantify *E. coli* by most probable number (MPN) per 100 ml.

Like a presence/absence test, the Colilert test adds a reagent which provides nutrients to grow coliform bacteria in the sample. Coliform bacteria occurs naturally in soil and in surface waters. It can be found in the intestines of humans or in other animals, but most coliform bacteria are not harmful and are used as “indicator bacteria” in drinking water. After the Colilert® reagent is added and dissolved in the undiluted sample, the sample is transferred to Quanti- Trays®/2000 and sealed using the Quanti-Tray sealer. Samples are incubated at 35.0°C ± 0.5°C for 24 hours. The presence of coliform bacteria will make the wells in the trays appear yellow. If coliform is present, the wells will then be placed under ultraviolet light. Both yellow and fluorescent wells indicate *E. coli*. The number of positive wells are then converted to MPN with upper and lower 95% confidence limits.

MPN measures of the two samples, near CAFOs and not near CAFOs, were then compared using descriptive and nonparametric statistics. Descriptive statistics were calculated using Microsoft Excel version 16.67 (**Table 2**). Pearson’s

Product Moment Correlation was used to determine the linear association between the two variables. Pearson's correlation coefficient (r) was calculated by hand.

Pearson's Product Moment Correlation Equation

$$r_{xy} = \frac{n\sum x_i y_i - \sum x_i y_i}{\sqrt{n\sum x_i^2 - (\sum x_i)^2}\sqrt{n\sum y_1^2 - (\sum y_1)^2}}$$

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