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# Impacts from the use of antibiotics in livestock: methods of transmission of antibiotic resistance from livestock to humans

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from livestock to humans

## **Introduction**

Antibiotics first appeared in the late 1920s when Alexander Fleming discovered the first antibiotic, penicillin (Rosenblatt-Farrell 2009). Since the introduction of antibiotics in healthcare and animals Fleming immediately warned about the possibility of bacteria becoming resistant to the antibiotics' effects. As Fleming predicted, ten years after the invention of penicillin some bacteria had already become resistant to the antibiotic (Rosenblatt-Farrell 2009). Antibiotics have now revolutionized the healthcare industry and are even used in animals in veterinary medicine and livestock production as well as plants in agriculture. Currently the world is facing an epidemic of antibiotic resistant bacteria. This poses a threat public health as bacterial infections are becoming harder and more costly to treat. Antibiotic resistance has also become an economic burden as these illnesses are also lasting longer (Lessing 2010). The largest impact of antibiotic resistance comes from their misuse in the health fields. Many times physicians will prescribe antibiotics when a patient presents with certain symptoms such as cough, earache, and sore throat. Unfortunately many of these symptoms are linked to viral infections instead of bacterial infections. Antibiotics do not have any effect on viruses and should only be used to treat bacterial infections. The misuse of antibiotics from prescribing medications for viruses has contributed to antibiotic resistance in humans. Another factor impacting the increase of antibiotic resistance comes from patients not finishing their antibiotic therapy. In these instances, not all of the pathogenic bacteria is killed, and when bacterium are exposed to antibiotics in low doses, they become resistant to that antibiotic.

There is speculation that antibiotic resistance in humans also comes from the use of antibiotics in livestock. Some research suggests antibiotic use in livestock has no effect on resistance in humans while other studies link antibiotic resistance in humans directly to livestock. The use of antibiotics in livestock poses a threat to antibiotic resistance in humans because the resistant bacteria can be transmitted from animal to human in many different ways: direct transmission, and indirect transmission through plants, soil, water, and other animal vectors. If antibiotics continue to be used in livestock as much as they are being used today, we could face a major public health concern from the increase of antibiotic resistance in humans, ultimately causing once treatable illnesses to become untreatable. This literature review will cover the methods of transmission of antibiotics from livestock to humans and its potential impact on our health.

### **Antibiotic Resistance**

*What is antibiotic resistance?*

Antibiotic resistance occurs when an antibiotic loses its ability to control or kill bacterial growth (APUA 2014). When one is diagnosed with a bacterial illness it is common for a very small amount of the pathogenic bacteria to be resistant. The normal flora within our bodies have the ability to keep the resistant bacteria from growing out of control. When antibiotics are introduced they kill both the infectious and commensal bacteria within our system, allowing for resistant bacteria to divide and increase in numbers (CDC 2015). This is when antibiotic resistance becomes an issue to human health.

*How does antibiotic resistance spread?*

Antibiotic resistant genes can appear in bacteria through multiple processes: plasmids, transposons, naked DNA, and mutations. A plasmid is a circular, double-stranded DNA structure (Scitable 2014). Plasmids containing antibiotic resistance genes can be inserted into different bacteria via conjugation. During conjugation there is a donor cell and a receiver cell. If a cell contains an f-factor (fertility factor) then it is a donor cell (McGraw Hill 2016). The donor bacterium will connect to a recipient cell using a structure called a pilus. The plasmid in the donor cell will be cleaved in a specific region and one of the strands will be inserted into the recipient cell via the pilus. Both bacteria then have a single-stranded plasmid which will be replicated to reform its double-stranded circular structure (McGraw Hill 2016). Conjugation can occur between cells of the same species as well as differing species of bacteria (McGraw Hill 2016).

Transposons are a small segment of DNA that can be cut out of one site within a genome and can be inserted onto another site of a genome. Transposons can also replicate, leaving one copy in its original spot and allowing the new copy to insert itself onto a new site within the genome. They can also attach themselves to plasmids, then allowing for resistant genes to be transferred over to a new bacterium when conjugation occurs (McGraw Hill 2016).

Naked DNA is DNA that lacks proteins that normally maintain the DNA's coiled form (The Free Dictionary 2016). When a cell dies or lyses, naked DNA can be picked up and inserted within a recipient cell and inserted onto the new cell's genome (Levy 2002). Antibiotic resistant genes can be passed cell to cell via naked DNA. Mutations also allow for bacteria to gain antibiotic resistance, however this is the least occurring method. Mutations occur in only one of ten million cells (APUA 2014). When they do occur, antibiotic resistance can occur in a few

different ways. Some mutations create new enzymes which can combat against antibiotics (APUA 2014). Other mutations will insert pumping mechanisms to export antibiotics from a cell (APUA 2014). There are many other different mutations that can occur that will allow bacteria to protect themselves from antibiotic resistance, but it is more common for a bacterium to pick up a resistant gene from other bacteria. Finally antibiotic resistance can be acquired from viruses. Viruses can carry genes from one bacterium and insert them into another bacterium, allowing those genes to be passed on to other bacteria (APUA 2014).

### *Multidrug Resistance*

Multidrug resistance (MDR) occurs when a bacteria contains genes that code for the protection against more than one antibiotic. MDR can occur within bacteria in multiple ways. Some antibiotics work in similar ways with one another. For instance, tetracyclines, a common antibiotic used in livestock as growth promoters, inhibit bacteria from protein synthesis through binding to a specific ribosome (Zwenger et al 2008). Tetracycline is actually considered a class of antibiotics meaning there are many antibiotics that work in this same manner, such as doxycycline, minocycline, and oxytetracycline (List of Antibiotics 2016). Because those three antibiotics use the same mechanism to inhibit bacterial growth none of them would work against a bacteria that was resistant to just one of those drugs. Therefore a bacterium can be multidrug resistant in this manner.

MDR can occur from bacteria picking up more than one gene for antibacterial resistance. If the genes code for different resistance mechanisms (i.e. efflux pumps and enzymes) then the bacteria can be defensive against more than one class of antibiotics. Bacteria can pick up multiple genes through horizontal transfer, such as conjugation (Rosenblatt-Farrell 2009).

## **Antibiotics in Livestock**

### *History of antibiotic usage in agriculture*

Farming practices used to be small family-run businesses until the 1940s when farming became industrialized (Lessing 2010). Animal production turned into large business industries when the dependence on food began to rise due to rapidly increasing populations. Animal Feeding Operations (AFOs) and Concentrated Animal Feeding Operations (CAFOs) became the norm in animal husbandry (Lessing 2010). These operations could house 100,000 to 1,000,000 livestock in each facility. Animals were usually crammed into large indoor pastures, frequently unable to move because of the excessive amount of animals being housed. The tight quarters observed in the AFOs caused for bacterial illnesses to become an issue for livestock, allowing for more animals to get sick through direct contact or indirectly through fecal matter. Antibiotics started to appear as sub-therapeutic doses in animal feed in the 1950's (Hardy 2002).

Sub-therapeutic antibiotics differ than therapeutic doses as they are only given in 1/10-1/100 the normal concentration for an animal. Sub-therapeutic doses also differ from the normal therapeutic doses because they are fed to animals for the majority of their life. Normal therapeutic doses are only given for a short amount of time (a few days to a couple weeks) until the illness is fully treated (Johnston 2002).

### *The use of antibiotics in livestock*

Antibiotics are fed to livestock (including pigs, cows, chicken, turkey, and fish) for three reasons: treatment of illnesses, illness prevention, and growth promotion. As it was previously stated, the close living confinements of animals in AFOs and also the stress impacts on those animals provide a great reservoir for harmful bacteria, and it is not uncommon for animals to get

sick in these facilities (Lessing 2010). Treating animals with bacterial infections are the only instance in which therapeutic concentrations are given to one animal for a short period of time. However antibiotics are also given at sub-therapeutic doses in hopes of preventing illnesses in livestock.

Finally, antibiotics are fed to animals to promote their growth. Animals that are treated with sub-therapeutic levels of antibiotic will gain more weight in both muscle and fat due to the increased amount of nutrient availability (Hardy 2002). This occurs because the antibiotic will decrease the amount of commensal bacteria within the animal's gastrointestinal (GI) tract therefore allowing for more nutrients to be absorbed instead of those nutrients being absorbed by bacteria (Hardy 2002).

### *The Problem*

Antibiotic use in livestock poses a threat to both the environment and to human health as antibiotic resistance is becoming an increasing issue in the world. There are many studies showing the relationship between antibiotic resistance and the healthcare field, but little is known about the effects of antibiotic resistance in humans from the use of antibiotics in livestock (Johnston 2002). Antibiotic resistance in humans can be transmitted from livestock directly, through exposure of animals and their environment, and indirectly, through plants, water, and other animals (such as insects and birds).

## Methods of Transmission

### *Direct Transmission*

Possibly the most impactful study of direct transmission of antibiotic resistance comes from Stuart Levy in 1976. His study proposed that chicken fed with tetracycline (Tet) would induce antibiotic resistance in certain bacteria such as *E.coli* and will also spread those antibiotic resistant bacteria to those working on the farm (Levy 1976). In his experiment, Levy fed a sample of chickens with tetracycline and the other sample without. He collected fecal samples every two weeks from both groups of chickens, the family living on the farm, and five families that lived within the same neighborhood. In his results Levy found that after two weeks over 90 percent of chickens fed with tetracycline had bacterial resistance to tetracycline. Over 80 percent of the family showed antibiotic resistance to Tet while just under ten percent of neighbors also showed resistance to Tet (Levy 1976). Also, after four months the chickens without Tet feed also became resistant to the antibiotic.

Final results showed that 36 percent of the family became multidrug resistant, six percent of neighbors had MDR, and 20-25 percent of chickens (control and experimental) had MDR. Table 1 in the appendix summarizes the amount of antibiotic resistance found in family members and neighbors from October through February. In this data we see that both family members of the farm and neighbors living nearby had MDR or resistance to one antibiotic throughout the whole sampling period. This study shows that direct transmission of antibiotic resistance from livestock to human does occur, and during a short amount of time.

A very similar experiment was conducted in the 1980's. The study was conducted in Germany and it examined the transfer of antibiotic resistant bacteria from pigs to humans. Unlike

Levy's experiment, the entire surrounding neighborhood was tested for antibiotic resistant microbes (Levy 2002). The results showed that after two years the same resistance gene found in pigs were also found within 16-18 percent of all *E. coli* present in human fecal samples from the surrounding neighborhood (Levy 2002). Between these two studies we see clear evidence that antibiotic resistance can be directly transmitted through contact from animals and humans.

One major concern with direct transmission of antibiotic resistance from livestock to humans is MRSA. Methicillin-resistant *Staphylococcus aureus* (MRSA) is a concerning disease currently on the rise around most of the world. It begins as a skin infection and can spread rapidly to different body systems. The problem with MRSA is that it is resistant to methicillin, one common antibiotic used in treating staph infections. VRSA (vancomycin-resistant *Staphylococcus aureus*) is another staph infection that is resistant to the antibiotic vancomycin. What used to be a treatable condition is now considered a superbug and is very difficult to fight.

A potential issue with MRSA today is the transmission from livestock to humans. The first MRSA infection found in a human was actually contracted from a farm sometime in the 1990s (Mole 2013). Many studies have shown that livestock such as pigs, cows, turkeys, and chickens have been seen as carriers of MRSA and even been shown to transmit MRSA to farmers (Cuny Wieler & Wolfgang 2015). Studies have successfully traced MRSA strains from livestock to humans through sampling nasal swabs in both animals and humans (Wulf et al 2008). There are also studies comparing MRSA in conventional versus organic farms, and data shows that conventional farms hold more MRSA carriers in animals than in organic farms (Wulf et al 2008). Once MRSA infects humans it begins to cause an issue in the healthcare environment as well, as it is easily transmissible. This is just one example of the many issues caused from antibiotic resistance being transmitted from livestock.

The Alliance for the Prudent Use of Antibiotics (APUA) designed a figure (Figure 1 in the appendix) showing the route of transmission that antibiotic resistance has from livestock to the environment and also to humans. Most of the methods of transmission that come into contact with humans are indirect, coming from plants and water. Indirect methods of transmission of antibiotic resistance will be examined next, as these routes are more likely to affect those in suburban areas, not affiliated with farming or livestock.

#### *Indirect Transmission: Through Soil and Plants*

It is very common for animal manure to be used as fertilizers on crops. Livestock can produce 130 million tons of manure each year, and using their waste as fertilizer is a good method of recycling manure (Kumar et al 2005). There are many studies showing the consequences of using manure from livestock that are fed antibiotics as they produce antibiotic resistant bacteria within the soil and also in plants. 75 percent of antibiotics are excreted in animal waste products, such as manure and urine (Lessing 2010). The antibiotics being excreted are still in an active form, allowing it to have effects on bacteria in the soil. Antibiotics that are excreted by animals and humans are said to enter a “post-therapy” phase as the antibiotic concentration is lower than the normal therapeutic dosage (Levy 2002). Although livestock are fed sub-therapeutic levels already, this causes problems as lower doses of antibiotics are ideal for bacteria to select resistance (Levy 2002).

Antibiotics can remain active in soils anywhere from a few days to several hundred days (Kumar et al 2005). Manure is used as fertilizer for many crops because it provides the soil with nutrients such as nitrogenous compounds and it also improves the physical and chemical properties of the soil, making it hospitable for growing plants (Kumar et al 2005). One study examined the effect of antibiotics in manure-amended soil from swine feedlots. Researchers

sampled ten different feedlots in Kansas, taking soil samples that contained animal manure. Antibiotic resistance was tested using the minimum inhibitory concentration (MIC) assay (Zwenger et al 2008). Their results showed that nineteen bacterial isolates collected from the ten farms had shown bacterial resistance to at least one antibiotic (the antibiotics being tested were ciprofloxacin, chloramphenicol, and tetracycline) (Zwenger et al 2008). Researchers noted that antibiotic resistance to ciprofloxacin (Cipro) was not seen, however they added that fluoroquinolones, the class of antibiotic that Cipro places into, are a relatively new class that have only been used to treat animals since 1988 (Zwenger et al 2008).

Table 2 in the appendix shows the results gathered from each of the feedlots. Growth on an MIC assay (+) means that the bacteria was resistant to that specific antibiotic while no growth (-) shows that the antibiotic was effective against the bacteria. We can see that Cipro was effective against all bacterial samples, as was mentioned above.

In summary the researchers were able to show that antibiotics used in livestock are transmitted to soil through manure, allowing for antibiotic resistance to spread in soils. Not only is antibiotic resistance found in soils on farms containing livestock, but soils in locations further away from farms have also shown traces of antibiotic resistance bacteria.

One study sampled soil and water in seven areas ranging from farmland to a suburban area (Table 3). 47-89 percent of all bacteria collected from soil samples showed antibiotic resistance to tetracycline (Esiobu et al 2002). Resistance to other antibiotics including penicillin and ampicillin were also present in their samples. 82 percent of one bacterial species, *Pseudomonas*, showed multidrug resistance (Esiobu et al 2002). In one area of sampling the researchers noted that manure-amended soil was used for gardening, which contributed to the bacteria that showed antibiotic resistance (Esiobu et al 2002). Antibiotic resistance can also

occur in plants, as uptake of nutrients and bacteria from contaminated soil occur during the lifecycle of plants.

One study observed the impact of manure-amended soils on plants. Two antibiotics were observed during this study which tested three different plants: corn, cabbage, and green onions (Kumar et al 2005). The antibiotics being observed were tetracycline and tylosin. Researchers noted that tetracycline is more water soluble than tylosin and that tylosin was a much larger molecule than tetracycline. They compared these two antibiotics because of the differences in their physical and chemical properties (Kumar et al 2005). In their results they found that tetracycline was readily absorbed within the plants while tylosin was not. As the concentration of tetracycline within the soils increased so did the concentrations of tetracycline found within the plants (Kumar et al 2005). So how does the presence of antibiotics in our crops impact antibiotic resistance within humans?

As humans eat crops containing traces of antibiotics, it allows for selection of antibiotic resistance in our intestinal flora. This means that susceptible bacteria will most likely be killed while other bacteria have the ability to become resistant through the exposure of low dosage antibiotics (Levy 2002). Although the bacteria being affected are our own natural microbes, it can become a serious problem if a particular bacteria grows out of hand or if there is a shift in bacterial diversity.

For example, *Escherichia coli* is a normal bacterial colony present in our intestinal system. *E. coli* can become resistant through the digestion of plants containing trace amounts of antibiotics. If the resistant *E. coli* disrupts the normal microflora within our bodies we can become sick and the *E. coli* infection will become difficult to treat because of its antibiotic resistant properties.

There is now evidence verifying that antibiotics in plants can be traced back to soils and even further back to antibiotic usage in livestock. With the example stated above in regards to *E. coli*, we can see how antibiotic use in livestock can ultimately impact antibiotic resistance in humans. Another method of transfer from livestock to humans is through water. Similarly to soils we will see a trend in antibiotic usage in livestock contributing to water contamination and the impacts of human health.

#### *Indirect Transmission: Through Water*

Figure 2 in the appendix is a nice representation that shows where our drinking water comes from. Streams and lakes flow into estuaries which are ultimately filtered and end up in water bottles and as tap water. Looking closely it is noted that streams running through farms are also water sources. This poses a threat to humans as antibiotic residues have been traced in our water sources.

Wastewater storage ponds are another method for manure management from livestock. Like manure being used as dry fertilizer in soils, the wastewater is also irrigated onto crop fields (Zhang et al 2013). Zhang et al. made it apparent that there are two parts of a storage pond. The top layer consists of a liquid slurry while the bottom half consists of a more solid 'sludge'. The wastewater is used as irrigation on crops about once every year while the sludge is applied to crops every 5-20 years (Zhang et al 2013). The two separate compartments of the manure management systems are important, as antibiotics act differently in water than in solids. Both sections of the storage ponds were tested for antibiotic resistance. Zhang found that all sixteen antibiotics they tested for were apparent in at least one of the seven water sources in both cattle and swine operations. Since the wastewater is used to irrigate crops it is likely that low doses of

antibiotics are being absorbed into the crops as well as within the soil. Reversely, antibiotics found in soil from fertilization can also contaminate groundwater and surface water.

Many studies have found antibiotic residues in water varying in locations from farms to nearby streams to lakes miles away. Earlier I discussed a study that sampled soil and water in seven different areas all with varying locations. Table 3 in the appendix shows which areas samples were collected from as well as information of the composition of the soil and what types of water sources the water samples were connected to. As it was stated for the soil, antibiotic resistant bacteria were found in all samples (Esiobu et al 2002). Figure 3 shows results that the researchers obtained. Even the RLW (residential lake water) which was manmade showed antibiotic resistant bacteria within the samples. This experiment shows that bacteria have the ability to transmit resistance genes, and those genes can spread far and wide geographically.

There is another study showing bodies of water having antibiotic residues within them. The Po River in Italy and the Rio Grande in the United States were two water sources of many others tested showing antibiotic residues (Riley et al 2013). Along with this study, another study examined multiple locations following the Cache la Poudre River in Colorado. They found that no residues were found at the top of the river, where the location was away from any city or suburban area in the mountains, however as the researchers tested the same water downstream near local suburbs antibiotic residues became apparent in the water (Riley et al 2013).

Sixty five percent of our drinking water in the United States comes from rivers and streams (American Rivers 2016). The presence of antibiotic residues in those water sources play a large role in public health as we are now ingesting those antibiotics, allowing for selection of antibiotic resistance to occur within our own microbiota. Most of these antibiotics are contaminating our water through the use of antibiotics in livestock, as agriculture runoff plays a

role in contamination. We also get antibiotic residues and antibiotic resistant genes in our water from sewage runoffs; however this is more due to antibiotic treatment in humans.

There is evidence showing how antibiotic use in livestock production can contaminate our water, as it has been shown through multiple studies which were mentioned. Those antibiotics are entering our rivers and streams, which accounts for sixty five percent of our drinking water. This is just another method of transmission from livestock to human. Finally, one other method of indirect transmission of antibiotic resistance from livestock to humans is through insects and other animals that carry (but are not affected by) antibiotic resistance genes.

*Indirect Transmission: Through insects and other animals*

A study was conducted to follow one specific resistant *E. coli* strain from a calf into different animal and human sources. This was done by biochemically marking an *E. coli* bacterium, allowing for that one specific strain to be detected in other animals (Levy 2002). The findings of this study showed that the resistant *E. coli* strain was found in mice, pigs, chicken, and turkeys. They also found that the strain was apparent in flies surrounding the area as well as in humans also within the same location (Levy 2002). Another study also examined the transmission of antibiotic resistant genes from houseflies and cockroaches, which could ultimately be picked up by humans.

Growing up many of us were told that flies poop or vomit every time they land on something. This is somewhat true, as they use their mouth parts (a labella) to decipher what's edible and what isn't (About Education 2013). However the main concern about flies landing on us and our food comes from what other sources the fly has previously landed on, as they are often attracted to foul smelling sources.

One study determined the prevalence of virulent enterococci species in houseflies and German cockroaches. Fecal samples of pigs, cockroaches and houseflies were taken. Using PCR researchers were able to determine the presence of antibiotic resistant genes (Ahmad et al 2011). Four species of enterococci were detected within all three fecal samples, and they were tested against eight different antibiotics. Figure 4 shows the results of their study. Resistance was found against all antibiotics except for one, vancomycin. Ampicillin resistance was found in only two enterococci species, while there were resistant strains of the last six antibiotics in all four enterococci species (Ahmad et al 2011).

Flies and cockroaches are often vectors of certain types of illnesses in humans, such as cholera and forms of gastroenteritis. They are considered “vectors” for the viruses and bacteria that cause these illnesses as they are present in the insect and transmit the illness to humans while the insect is unaffected.

Other animals such as migratory birds have also been reported carrying antibiotic resistant genes. In one study, migratory birds in the arctic were found carrying antibiotic resistant *E. coli*. Other animals native to the arctic were also found with the same antibiotic resistant strain (Rosenblatt-Farrell 2009). Because those animals are nowhere near any farms, it was concluded that the migratory birds were spreading the resistant *E. coli* to other species of animals in the arctic (Rosenblatt-Farrell 2009).

In another study, animals living in coastal regions, including marine mammals, sharks, and birds, were tested for bacterial resistant strains. Resistant bacteria were present within 58 percent of the animals while 43 percent had shown multidrug resistance (Rosenblatt-Farrell 2009). As more and more studies are being conducted, more evidence is gathered showing the impact of antibiotic resistance in the environment and its abilities to travel far and wide.

## **Efforts to control antibiotic resistance**

Very little is being done to try to control the spread of antibiotic resistance. Most efforts to prevent resistance come only from healthcare (Rosenblatt-Farrell 2009). Many countries in Europe have banned certain antibiotics to be used in livestock production, while other countries such as Sweden banned the use of all antibiotics in animal feed (Hardy 2002).

In 1969 the Swann Report was a response to increasing concerns about antibiotic resistance. The Swann report highlighted concerns about antibiotic use in livestock as being a method of increasing antibiotic resistance in humans (Barton 2001). The report concluded that the use of antibiotics at sub-therapeutic levels in animals is a potential hazard to human health. They suggested little to no antibiotics be used in animal feed for growth production and the use of antibiotics in normal therapeutic doses be used consciously from vets and farmers (Barton 2001). With response to the report the UK, parts of Europe, and Australia banned the use of penicillins and tetracyclines, the most commonly used antibiotics in sub-therapeutic levels for livestock. The United States did not have a response to the Swann Report as the FDA was unable to relate antibiotic use in livestock to being a public health concern to humans (Barton 2001).

There is still debate whether it is too late to control antibiotic resistance or if there is still time to act. The following proposed experiment will provide a conclusion to that debate, as the study examines the effects of ceasing antibiotic use in livestock production.

## **Proposed Experiment**

### *Introduction*

In Stuart Levy's experiment showing antibiotic use in chicken feed spreading antibiotic resistance to family members living on the farm, he noted that as time passed after the removal

of antibiotics in chicken feed, the antibiotic resistant bacteria within fecal samples obtained from chickens and family members began to decrease (Levy 1976). This proposed experiment will examine the effects of removing antibiotic feed in one farm to see if antibiotic resistance in animals and humans will decrease. The purpose of this experiment is to predict if it is too late to reverse antibiotic resistance by ceasing the use in livestock production. The hypothesis is that if antibiotic use in animal feed is discontinued, over time antibiotic resistance will decrease in animals and humans.

### *Methods*

This experiment would be a longitudinal study, taking place over the course of one year. Two farms will be selected, both having used antibiotics in their animal feed for livestock production. One farm will serve as a control, continuing the use of antibiotics in their animal feed while the other farm will be the experimental group, as antibiotic feed will be discontinued. A baseline sample will be collected before the start of the experiment, then every two weeks fecal samples will be obtained from livestock and farmers. All samples will consist of the same number of humans and animals on each farm (ex. 5 samples from animals and 5 human samples). Samples will be obtained every two weeks.

Fecal samples will be collected using a sterile swab which will then be inserted into a tube of tryptic soy broth (TSB), a standard media used for bacterial growth. The broths will be incubated at 35 degrees Celsius (human body temperature) for 24 hours.

*E. coli* is a very common enteric bacterium found in both human and animal microflora, also commonly found to have antibiotic resistance. Therefore we will observe the effects of antibiotic feed on *E. coli* samples. In order to isolate *E. coli* from the fecal samples, the incubated

TSB will be streaked onto MUG agar using the quadrant streak method. Plates will be incubated for 24 hours at 35 degrees Celsius. MUG agar will allow for fluorescence under a UV light if *E. coli* is present (American Society for Microbiology 2013). Any isolated colonies that appear to fluoresce blue under UV light will then be streaked onto tryptic soy agar (TSA) and incubated at the same standards as before.

In order to determine whether or not the *E. coli* is resistant, minimum inhibitory concentration (MIC) assays will be performed. 96 well plates with MUG agar will be used to perform the assay. MUG agar can be helpful for the MIC assay to secondarily confirm that the isolated colonies being tested are *E. coli*, using UV exposure after growth. The first part of the procedure will be to add two-fold dilutions of antibiotic into each column (1-10). The antibiotic with the highest concentration will be put into column 1 while the antibiotic in its lowest concentration will be in well 10 (for example well 1 will have 500  $\mu$ L of antibiotic while well 2 will have 250  $\mu$ L of antibiotic, well 3 consisting of 125  $\mu$ L, until the concentration reaches 1 $\mu$ L). Wells 11 and 12 will act as positive and negative controls.

In this proposed experiment, Penicillin G (Pen G) and tetracycline (Tet) will be the two antibiotics used for the MIC assays. Pen G and Tet are the most commonly used antibiotics in animal feed.

The same concentration of *E. coli* will be added in liquid form (mixed with sterile water) into columns 1-11. The 96 well plates will be incubated under the same conditions. The appearance of colonies in the wells show that bacteria still have the ability to multiply and grow, while the absence of growth means that the antibiotic has effectively inhibited bacterial growth. Again, we can use UV light and the fluorescence that *E. coli* emits to determine the presence and

absence of bacteria. The plate with the lowest concentration of antibiotic that inhibits bacterial growth is the minimum inhibitory concentration.

### *Results*

Results will be recorded and tables will be presented as the example, Table 4, in the appendix. The MIC assays will be conducted every two weeks for a year in order to accurately track and observe trends in data.

In order to find significance, a one-way ANOVA will be conducted with the results. Significance will be calculated between every test conducted (1 to 2, 2 to 3, 3 to 4, etc.). Once the experiment is complete significance will be calculated between the first and last sample. Significance will have a p value of  $p < .05$ .

### *Limitations & Conclusion*

Some limitations with this study include that it is being performed on only two farms. This can cause for a larger standard deviation which could cause values in our data to be widely spread apart when observing on a bell curve. It is optimal to have a small standard deviation with the values being closer together, which helps support statistical significance. One other limitation is that MUG agar is costly, which may be unreasonable to use depending on the amount of funding that would be given for this experiment.

To conclude, Levy's experiment of the antibiotic use in chicken feed found that after a few months of normal conditions both chickens and humans showed decreased resistance to antibiotics in *E. coli*. In this proposed experiment we would expect to see a similar pattern, however the big question is whether or not antibiotic resistance in *E. coli* will disappear completely.

## Discussion

As antibiotic resistance is on the rise the world is coming to face new challenges in public health. Antibiotic use will no longer be as effective as it used to be, and humans will contract diseases that are no longer treatable due to antibiotic resistance. There is plenty of evidence linking antibiotic usage in livestock production and antibiotic resistance found in the environment and humans. The most important thing that can be done to stop the rise in antibiotic resistance would be to cut back on its uses. Unfortunately the use of antibiotics in livestock produces bigger animals, helping with increased food production and also allowing farmers to be paid more per animal. The FDA does not recognize the use of antibiotics in livestock production as being a threat to human health, which is the reason why no measures have been taken to decrease or ban the use of antibiotics in livestock. Other countries in Europe and Australia have made regulations on the use of antibiotics in livestock, some countries even banning the use completely. Those countries are now witnessing a decrease in antibiotic resistance in humans.

One article suggests that using antibiotics in livestock production only increased the price of a pig by 79 cents (Lessing 2010). So is the risk of increasing antibiotic resistance in humans really worth those extra three quarters? Unfortunately due to the lack of regulations of antibiotics in livestock, it is clear that antibiotic resistance will continue to be an increasing problem within public health. Until more countries including the U.S. and Canada join the regulation bandwagon, we will continue to see a rise in antibiotic resistance in the environment and in humans.

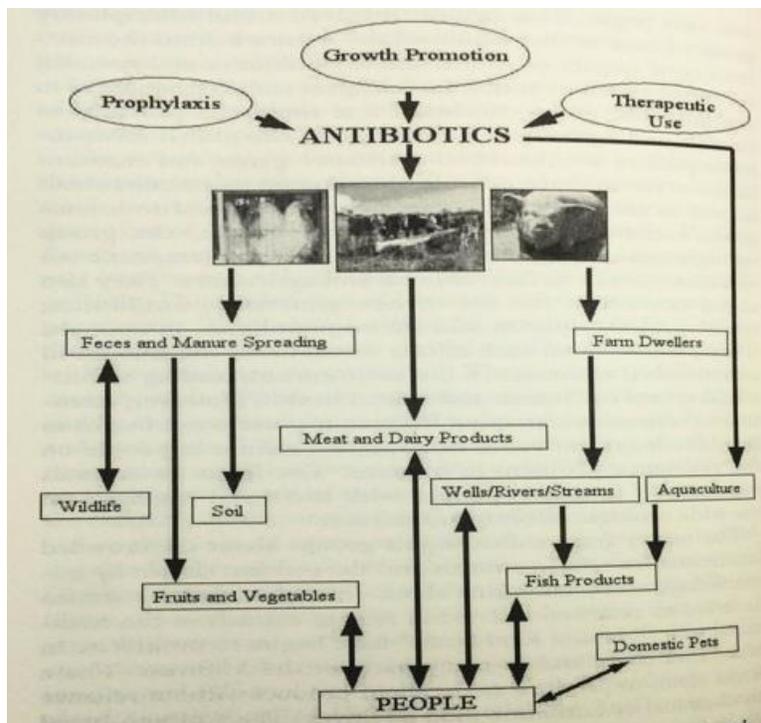
Appendix

**Table 1: Antibiotic-Resistance Pattern of *Esch. coli* Strains Cultured from Feces.\***

Mo.	NO. OF PEOPLE SAMPLED	TOTAL SAMPLES	SENSITIVE STRAINS	NO. OF RESISTANT STRAINS																	
				Te	N	Sm	Te	Te	Sm	Am	Te	Te	Sm	Te	Te	Am	Te	Am	Te	Am	Te
Farm Family:																					
Oct	6	6	2	2		1	1														
Nov	8	13	6	1	1	2															
Dec	9	17	5		1		2														2
Jan	10	20	9		2	1	1							1							
Feb	8	14	4	3		1								1							
Totals		70	26	6	4	5	4	0	0	0	0	0	1	1	1	0	1	10	7	3	2
Neighbors:																					
Oct	12	14	10				2		1												
Nov	3	4	2							2											
Dec	7	13	8				1			4											
Jan	15	26	22				1		1	1	1										
Feb	5	9	6							1	2										
Totals		66	48	0	0	0	3	1	2	8	3	0	0	1	0	0	0	0	0	0	0

\* Te denotes tetracycline, N neomycin, Sm streptomycin, Su sulfonamides, Am ampicillin, Cb carbenicillin, Cm chloramphenicol, & SXT trimethoprim + sulfamethoxazole.

**Table 1.** Number of family members and neighbors presenting with antibiotic resistance from October through February.



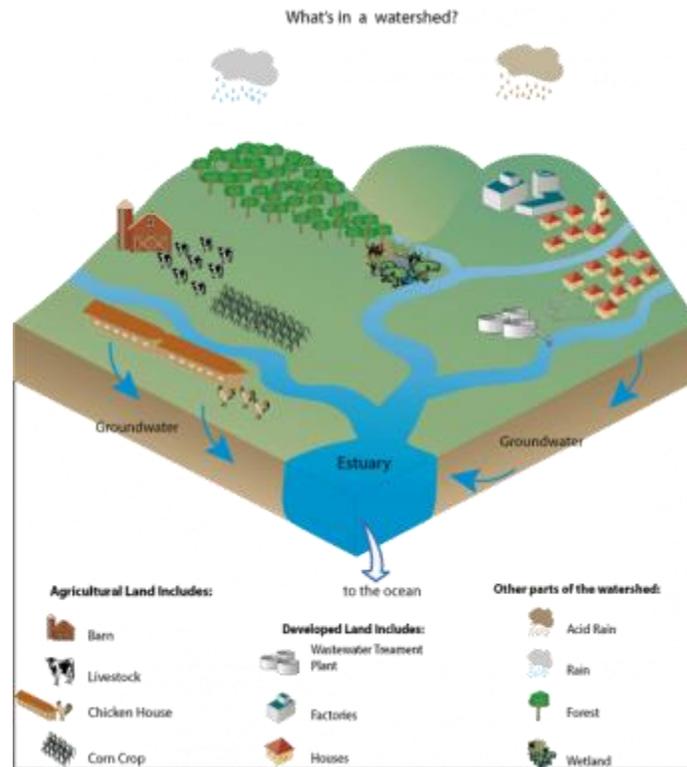
**Figure 1.** Distribution of antibiotic resistance in the environment from antibiotics in livestock feed.

Isolate	Ciprofloxacin		Chloramphenicol		Tetracycline	
	10	100	10	100	10	100
<b>Feedlot 1</b>						
<i>Citrobacter farmeri</i>	-	-	-	-	+	+
<i>Enterobacter cancerogenus</i>	-	-	X	-	+	+
<b>Feedlot 2</b>						
<i>Escherichia/Shigella</i> sp.	-	-	-	-	+	+
<b>Feedlot 3</b>						
<i>Acinetobacter Iwoffii</i>	-	-	-	-	X	X
<i>Brevibacterium (Cory) stationis</i>	-	-	+	+	X	-
<b>Feedlot 4</b>						
<i>Brevibacterium (Cory) stationis</i>	-	-	+	+	-	-
<i>Escherichia/Shigella</i> sp.	-	-	+	+	X	X
<i>Escherichia/Shigella</i> sp.	-	-	X	X	+	+
<b>Feedlot 5</b>	no growth					
<b>Feedlot 6</b>						
<i>Pseudomonas stutzeri</i> U65012	-	-	+	+	-	-
<i>Staphylococcus equorum</i>	-	-	+	+	-	-
<i>Enterococcus hirae</i>	-	-	-	-	+	+
<b>Feedlot 7</b>						
<i>Staphylococcus equorum</i>	-	-	X	X	+	+
<b>Feedlot 8</b>						
<i>Escherichia/Shigella</i> sp.	-	-	+	+	X	X
<i>Brevibacterium (Cory) stationis</i>	-	-	+	+	-	-
<i>Enterococcus hirae</i>	-	-	-	-	+	+
<i>Staphylococcus simulans</i>	-	-	+	+	X	X
<b>Feedlot 9</b>	no growth					
<b>Feedlot 10</b>						
<i>Pseudomonas putida</i> AY6622320	-	-	X	-	+	+
<i>Brevundimonas</i> sp.	+	+	-	-	-	-
<i>Pseudomonas stutzeri</i> U65012	-	-	+	+	-	-

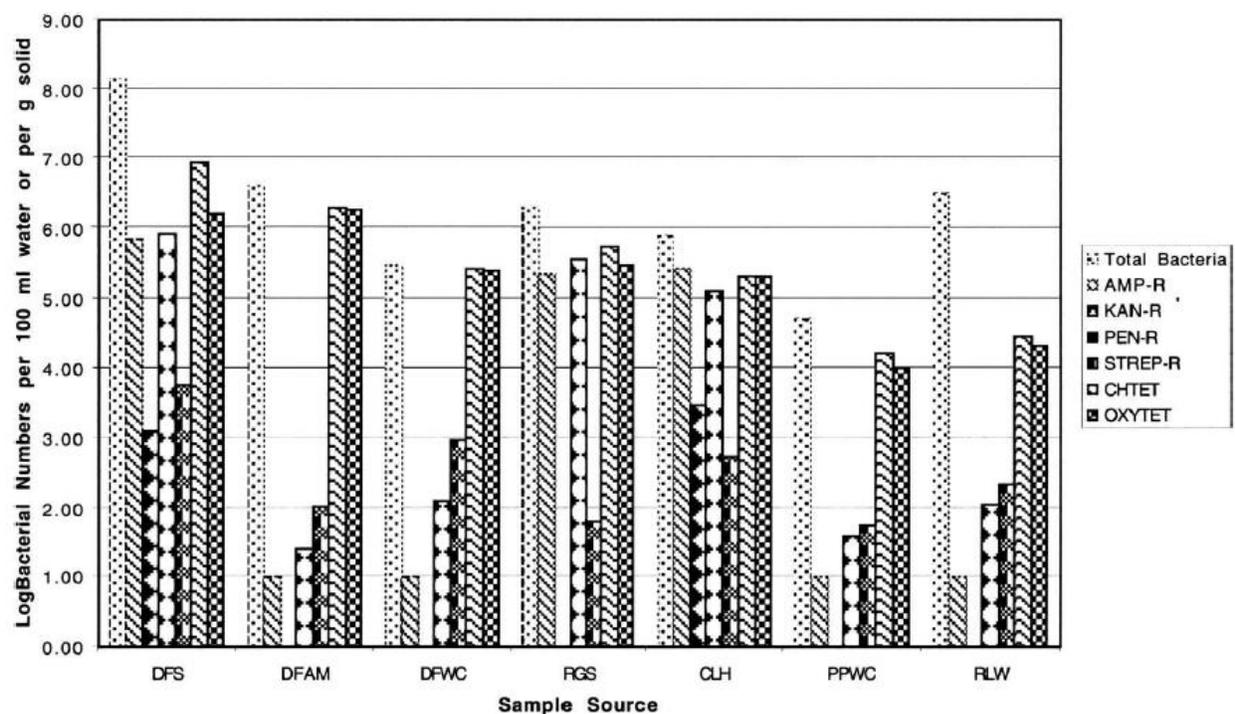
**Table 2.** Results of antibiotic resistance found in the soil of each feedlot sampled. Growth (+) and no growth (-) of MIC assays for each antibiotic are presented.

<i>Sample code</i>	<i>Description</i>	<i>Remark</i>
DFS	Dairy farm soil	Soil mixed with fresh cow manure
DFAM	Dairy farm animal manure	Composting animal manure
DFWC	Water canal by dairy farm	Receives farm run-off
RGS	Residential garden soil	Far from hospitals, no history of use of antibiotics
CLH	City lake by hospital	Recycles irrigation water; receives run-off from environment
PPWC	Public park water canal	Collects run-off from recreational park
RLW	Residential lake water	Man-made lake. No nearby hospital or farm.

**Table 3.** Samples of soil and water were taken in multiple areas. This table shows where the sample was taken.

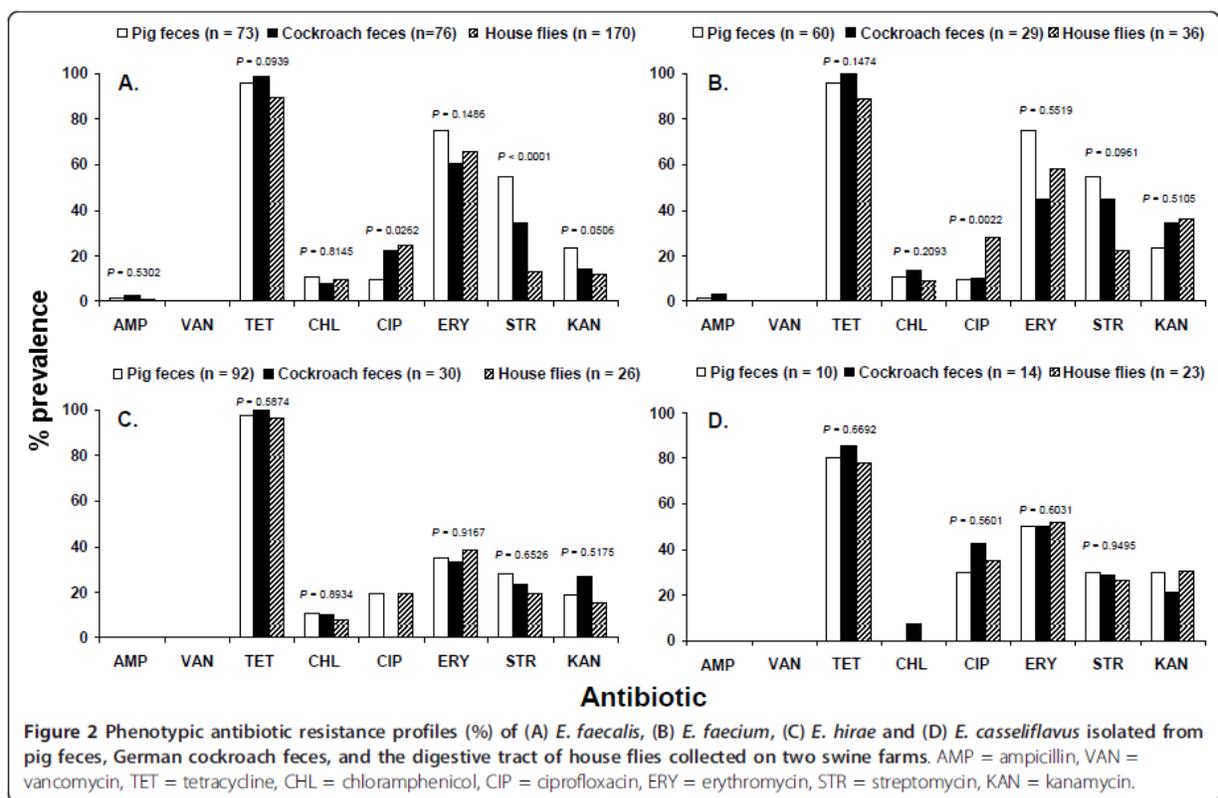


**Figure 2.** Shows where drinking water comes from in our environment.



**Fig. 1.** Distribution of bacterial resistance to various antibiotics (25 µg ml<sup>-1</sup>) in soil and water environments. DFS, dairy farm soil; DFAM, dairy farm animal manure; DFWC, water canal by dairy farm; RGS, residential garden soil; CLH, city lake by hospital; PPWC, public park water canal; RLW, residential lake water.

**Figure 3.** Amount of bacterial resistance of different antibiotics from each sample location.



**Figure 4.** Amount of antibiotic resistance of multiple antibiotics tested in each enteric species found in pig, cockroach, and fly feces.

Fecal samples	MIC for TET	MIC for PenG
Animal #1		
Animal #2		
Animal #3		
Animal #4		
Animal #5		
Human #1		
Human #2		
Human #3		
Human #4		
Human #5		

**Table 4.** A bi-weekly data collection table showing MIC concentrations for each fecal sample.

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