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Antibiotic resistance associated with small-scale poultry farming in rural Ecuador

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By

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A.B.
Princeton University
2006

Thesis Committee Chair: Karen Levy, PhD, MPH

An abstract of
A thesis submitted to the Faculty of the
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Abstract

Antibiotic resistance associated with small-scale poultry farming in rural Ecuador

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Background: Antibiotic resistance poses a significant health threat worldwide. Many studies have investigated the impacts of animal antibiotic use in large industrial settings, yet very few have looked that the effects in small-scale farms. It is important to understand the dynamics of resistance spread in small-scale settings, especially in developing countries where the risk of bacterial transmission is higher due to inadequate sanitation and hygiene practices. The main goal of this study is to understand the impact of small-scale poultry farming on antibiotic resistant bacteria in the environment to determine potential mechanisms of resistance transmission between chickens and humans. **Methods:** Environmental samples (soil, drinking water, and kitchen surfaces) were collected from houses in rural Ecuador that either actively raised chickens in their backyard, previously raised chickens, or never raised chickens. If the household raised chickens, chicken coop soil and surfaces were also collected. Samples were also taken from one village that had a collective community farm located away from the houses. This village switched to backyard farming during the study, providing a useful comparison of farming operations. *E. coli* was isolated from all samples and tested for resistance against a panel of 12 antibiotics. The outcomes considered for analysis were resistance to fluoroquinolones, antibiotics used in chickens in the study area, and multidrug resistance. **Results:** There were extremely high levels of resistance in the coop area of the community farm. Approximately 50% were resistant to fluoroquinolones and 67% were resistant to chicken antibiotics and multidrug resistant, which is roughly equivalent to estimates from chickens in industrial broiler facilities. In addition, there were higher levels of resistance in the indoor environment in houses that had backyard farms than those with the community farm. This suggests the proximity of the farm to the household is a factor in potential spread of resistant bacteria. **Conclusion:** Intensive use of antibiotics in small-scale operations is associated with high levels of resistant bacteria, and the potential for transmission of resistant bacteria is heightened when the farm is in the backyard.

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Introduction

Antibiotic resistance is a growing public health problem worldwide, fueled by the overuse or misuse of antibiotics (Sosa 2010). Many resistant strains of common infections such as pneumonia and tuberculosis are common throughout the world, causing high rates of morbidity and mortality. In addition, there are economic costs associated with the development of antibiotic resistance. As organisms become resistant to current treatments, new therapies will have to be developed and administered. It is estimated that these new treatments will cost the United States approximately $4-5 billion annually (McGowan 2001). However, for countries that do not have the money to employ these new treatments, it will be the patients that have to bear the cost of non-treatment (Okeke 2010).

One of the leading users of antibiotics is the agriculture sector: according to a recent report by the Food and Drug Administration, over 80% of antibiotics produced are used in animal agriculture for non-therapeutic purposes (Food and Drug Administration 2009). Antibiotics in the agriculture sector are largely used as growth promoters or prophylactics, and this overuse of antibiotics may heighten the risk of resistance to humans (Olivier, Williams-Jones et al. 2010) . This is because many of the same antibiotics that are used for human treatment are also used in farming. Regulations on antibiotic use, particularly in the agriculture sector, have been established and surveillance systems are used to track antimicrobial use in animals in developed countries. Fluoroquinolones have been banned for animal use

in the United States since 2005 (Nelson, Chiller et al. 2007), and the FDA just recently approved new cephalosporin regulations in January 2012 (Harris 2012).

Fluoroquinolones in particular are important to focus on because of their utility in treating human infections. Fluoroquinolones are a class of synthetic antibiotics that include ciprofloxacin, enrofloxacin, norfloxacin, and levoflaxacin, among others. For many years, resistance to fluoroquinolones was only believed to be developed by point mutations (Hernandez, Sanchez et al. 2011). This was because they do not exist in the environment (they are synthetic), so there is no natural selection pressure for resistance and the only way for it to develop was through the use of fluoroquinolones specifically. However, it has been recently found that fluoroquinolone resistance can be transferred via plasmids (Hernandez, Sanchez et al. 2011), which leads to the possibility of cross-resistance developing because many resistance genes can be carried on the same plasmid.

The environment is a known reservoir for antibiotic resistant bacteria (Allen, Donato et al. 2010) because most antibiotics originate from bacteria and fungi in the soil. The same antibiotic-producing strains of bacteria and fungi also contain genes for resistance (Allen, Donato et al. 2010). In addition, some bacteria use antibiotics as a nutrient source, so resistance genes have developed to many classes of clinically relevant antibiotics (Dantas, Sommer et al. 2008). While there is natural resistance in the environment, the use of antibiotics in agriculture can create selective pressure for increased resistance (Allen, Donato et al. 2010). Antibiotics given to farm animals enter the environment largely through animal waste. In industrial settings, it is through the application of manure for fertilizer or through wastewater

runoff; in rural settings, it is from the defecation of farm animals in the outdoor environment. There is evidence that antibiotic use in animals has led to resistance in bacteria that cause human infection, but the mechanism is not well understood (Smith, Dushoff et al. 2005) and could be mediated through the environment.

Only a few studies have suggested that the environment is an important reservoir for antibiotic resistant bacteria from animal agriculture. Esiobu et al. (2002) compared soil and water samples taken from dairy farms to those from non-agricultural sources. They found significantly higher amounts of resistant bacteria in dairy farm manure and soil from a residential garden that was fertilized with farm manure than at other sites, suggesting that dairy farming was contributing to resistance in the environment. In another study, da Costa et al. (2008) tested water from poultry slaughterhouse wastewater treatment plants. More resistant strains were present in water samples from slaughterhouses with conventionally raised poultry than those with free-range poultry, suggesting that the use of antibiotics in conventional poultry contributed to resistance. More importantly, there was no difference in the amount of resistant bacteria in water from the inflow, the outflow, and the sludge from the treatment plant, indicating that treatment did not affect the levels of resistant bacteria that could pose a public health threat (da Costa et al. 2008).

However, other studies have found that the environment is not an important reservoir for resistant bacteria from animal agriculture. Ghosh et al. (2007) found no significant differences in resistance levels between soil from land with swine manure application, from a dairy farm with limited antibiotic use, and from a non-

agricultural site. They therefore concluded that antibiotic use in farm animals was not contributing to resistance in the environment. In addition, Brooks et al. (2010) noted much greater resistance levels in biological aerosols and poultry litter inside the poultry house than out, suggesting that resistant bacteria were not escaping into the outdoor environment. It is clear that more studies of environmental reservoirs are needed to determine the extent to which animal agriculture is contributing to environmental resistance.

Small-scale poultry farming operations at the household level in developing countries could present new challenges in controlling antibiotic resistance development and spread in the environment. In many developing countries, sanitation and hygiene practices are poor, and this could facilitate the spread of bacteria. In addition, there is little control of antimicrobial use in animals in developing countries and little data collected on the farming practices because they tend to be small and at the household level (Olivier, Williams-Jones et al. 2010).

It is important to study small-scale operations for these reasons and also because poultry farming is being widely encouraged as a development strategy throughout the world. It has become popular because of the many advantages: poultry are inexpensive, an efficient source of protein, can be farmed in any country, have little cultural or religious beliefs attached to them that would limit consumption, and they grow quickly compared to other livestock (National Research Council 1991). Small-scale operations also comprise the majority of production in many countries; for example, 70-99% of all poultry production in many African countries is from family farms (Ahuja, Sen et al. 2007). Initiatives that

support the creation of small-scale farms for sustainable development purposes include the Global Agenda of Action, which began in 2011 by The Food and Agriculture Organization in partnership with the Ministry of Economic Affairs, Agriculture, and Innovation of the Netherlands (Food and Agriculture Organization 2011). There are also programs such as "Fowls for Africa," supporting poultry farming throughout the country by providing resources and knowledge (Agricultural Research Council 2010). While poultry farming is a promising strategy, the potential health risks of small-scale farms need to be addressed, including the transmission of antibiotic resistant bacteria.

Only one study has examined the potential transmission of antibiotic resistance from chickens to humans in a rural household-level operation. Riccobono et al. (2011) compared resistant *E. coli* strains from children and chickens in twelve households with backyard farming operations in Bolivia and Peru. While they found similar levels of resistant bacteria to ten different antibiotics in children and chickens, they did not find the same resistant strains. The lack of similarity between the strains suggests that resistance was not transferring between chickens and humans; however, it is possible that resistant bacteria were persisting in the environment with the potential for transmission. Therefore it is also necessary to test environmental samples.

The EcoDess research group is conducting a study that aims to understand the development and transmission of antibiotic resistant bacteria associated with small-scale poultry farming. Chicken and human fecal samples were collected from households in rural Ecuador that raise backyard chickens to determine if poultry

farming is contributing to resistance in children. However, it is also important to understand the potential transmission mechanisms, including the environment as a reservoir. This study aims to fill that gap and examine the environmental sources of antibiotic resistant bacteria in this study system. It is the first study to examine environmental resistance in small-scale poultry farming operations in rural communities. The main questions of this study are:

1. Is small-scale poultry farming associated with antibiotic resistant bacteria in the environment?
2. Does small-scale poultry farming contribute to household exposure to antibiotic resistant bacteria?
3. If poultry farming contributes to household exposure of resistant bacteria, then where are people most likely to be exposed?

The study focuses on three main outcomes of antibiotic resistance: fluoroquinolones, antibiotics used in chickens, and multidrug resistance. Understanding the environment's role as a reservoir for resistant bacteria is critical in determining transmission mechanisms and developing public health interventions.

Methods

Study Area

Data were collected between June and October of 2011 from eight villages in the Esmeraldas Region of northern Ecuador that lie between the Onzole and Santiago Rivers (Figure 1). The construction of a new highway through the region, which was completed in 2003, connected remote villages to the region's main city

center, Borbón. This increased access to resources, including antibiotics, and has led to an increase of household-level poultry farming as a way to generate income. Study villages lie along a secondary dirt road that connects to the two-lane highway.

Village and Household selection

Villages were selected based on data that was previously collected by village health promoters from July 2009-August 2010. The data included the poultry farming activities of each house in the village. Villages with the greatest intensity of chicken farming, judged by the number houses with farms, were selected for the study. These villages were Punta de Piedra, San Augustín, Timbire, Colon Eloy, Valdez, Las Cruces, San Francisco, and Yalares (Figure 1). All villages except Punta de Piedra had backyard farms, meaning that the chicken coop was located in the back of the house and the farms were run individually by each household. Punta de Piedra had a community-level operation in which 16 houses farmed their chickens collectively in one coop, located about a quarter of a mile from the nearest house. However, during the course of the study, Punta de Piedra changed their farming

Figure 1: Study area in northern, coastal Ecuador. All villages on the map were studied, except for Borbón, the city center.

operation from the community operation to backyard farms in individual houses. They underwent this change as an attempt to control a disease that infected the chicken population. This provided a unique opportunity for a natural experiment to compare backyard farms to a community farm in the same village because we were able to take samples from Punta de Piedra both before and after the change.

Seventy-two houses were sampled from the eight villages listed above. Houses in Punta de Piedra, San Augustín, Timbire, Colon Eloy, and Valdez were chosen based on the same farming data that was used to determine village participation. All houses that had raised chickens within the past two years were selected to be sampled to ensure a sufficient sample size of houses with farms. For every five houses that raised chickens, one control house that did not raise chickens was chosen in each village. However, if there were only five houses that raised chickens in a village, then two control houses were chosen. Only three households were sampled from Las Cruces, San Francisco, and Yalares because they were visited during the regular research cycle of the EcoDess project. Table 1 provides a summary of the number of houses sampled from each village.

Control houses were selected based on distance from chicken farms. Zones were mapped to determine groups of houses farthest from houses with chicken farms, and control houses were selected at random within each zone. If a house could not be sampled because the head of the household was not present or the house had been destroyed, then another one was chosen at random from within the zone. Houses where there were no inhabitants or that refused to participate were omitted from the study.

At each house, we collected two samples of both household drinking water and surfaces (one used for cooking and the other for eating) because those are the likely routes of human exposure. Two samples of soil surrounding the house were also collected to characterize the immediate outdoor environment. If the house was actively raising chickens, we also collected two samples of both soil and surfaces from chicken coops to determine resistance levels in the chicken environment.

Sample Collection & Laboratory Analysis

Household Survey. The head of each household was identified upon arrival, and they were asked a series of questions regarding their farming practices and their household water storage practices. In addition, if the house currently raised chickens, a more thorough questionnaire was given about their farming practices, including the use of antibiotics to ensure we had the most recent data. These surveys can be found in the Appendix. The Institutional Review Boards of Emory University, the University of Michigan, and the Universidad San Francisco de Quito approved all interaction with human subjects.

Household water. Two different containers of stored drinking water were identified and 50mL samples were collected from each container in Whirlpak® Bags (Nasco) in the same manner that water was dispensed for drinking. If there was only one container for drinking water, then the second sample was taken from water used to wash dishes or bathe because those are other likely routes of exposure. Data were collected on the water source, the type of container the water was stored in, and if the

water had been treated in the past 24 hours because these are all factors that can influence the presence of *E. coli* in the sample.

Water samples were processed using membrane filtration. Two quantities varying between 3-50mL of water were filtered depending on the expected amount of *E. coli* that was determined from the water source. For example, if the water was from the tap, then we expected less *E. coli* and processed a higher quantity of water. Because the goal was to recover and isolate *E. coli*, it was not necessary to keep the quantities uniform. Membranes were plated onto Chromocult© agar (Merck) and incubated at ambient temperature (32-35^{o}C) for 48 hours.

Soil. Two pooled soil samples were taken from around the house and two were taken from around the chicken coop. Each soil sample consisted of approximately 5cm^3 of soil from three locations: in front of the house/coop, one side of the house/coop, and the back of the house/coop, totaling 15cm^3. We sampled from all three locations to better characterize each house and coop. Samples were collected by placing soil from just below the surface into a conical tube using a sterile plastic spoon that was discarded after each use. The presence of other animals in the backyard was recorded. Samples were stored on ice until processing in the lab, approximately 4-6 hours later.

Each soil sample was diluted with 30mL of deionized water and mixed thoroughly with a cotton tip applicator to obtain a dilution of 2:1, ensuring that we could isolate bacterial colonies. The same cotton tip applicator was then placed in 10mL of deionized water and mixed to obtain a second dilution. The supernatant of the

original sample was streaked onto Chromocult Agar for isolation, and the same was done for the second dilution.

Surfaces. Household surface samples were taken from two locations: where food is prepared (e.g. a cutting board) and where food is eaten (e.g. a table). A 28x30cm plastic stencil was used to define a consistent sampling area. If the area was dry, a cotton tip applicator that had been submerged in 1mL 0.9% NaCl solution was passed over the area without rotating the cotton tip. It was then placed back into the test tube with the NaCl solution for temporary storage until samples were processed in the lab. If the area was wet, a dry swab was used and placed in Cary Blair transport medium. Data was collected on whether the households had washed the surfaces before and after preparing and eating their meals. Two surface samples from the inside or outside of the chicken coop were also taken using the same procedures. Surface samples were placed directly on Chromocult Agar and streaked for isolation.

Human samples. Human fecal samples were collected in conjunction with the EcoDess research project. Samples were collected from both cases and controls of diarrhea. See Eisenberg et al. (2006) for details of the sampling and laboratory analysis.

Sample processing. All samples were incubated at ambient temperature, ranging from 32-35^{o}C. After approximately 24-48 hours, up to four MUG+ *E. coli* colonies from each sample on Chromocult were randomly selected and transferred onto MacConkey Lactose agar (MKL) to confirm the presence of *E. coli*. If *E. coli* was not isolated in

Chromocult due to its abundance or contamination with other bacteria, it was re-isolated onto Chromocult before transferring it onto MKL. After a 24-hour incubation at 37°C, *E. coli* colonies that were Lac+ (lactose-fermenting), were transferred from MKL back onto Chromocult to ensure pure isolates and incubated for another 24 hours.

Out of the four isolated *E. coli* colonies, three from soil samples and two from water and surface samples that were MUG+ on Chromocult were randomly selected for antibiotic resistance testing. Multiple isolates were used from the sample because many different strains of *E. coli* existed in each sample and testing multiple ones would increase the probability of detecting resistance in a given sample. In addition, more isolates from soil samples were used than from water or surfaces because we expected higher microbial diversity in soil. The selected colonies were placed into nutrient agar slants and incubated for 24 hours at 37°C. Small amounts of bacteria from the colonies were then placed into a 0.9% NaCl solution to obtain a turbidity matching a 0.5% McFarland Standard. Using this solution, bacteria were plated onto Mueller-Hinton Agar, creating a lawn for resistance testing.

Antibiotic sensitivity was assessed using the Kirby-Baur disc diffusion method with 12 antibiotics: Ampicillin, Amoxicillin, Cefotaxime, Cephalothin, Chloramphenicol, Ciprofloxacin, Trimethoprim, Gentamicin, Streptomycin, Enrofloxacin, Sulfasoxazole, and Tetracycline. Zones of inhibition were measured after a 24-hour incubation period and then compared to the Performance Standards for Antimicrobial Disk Susceptibility Tests (Clinical and Laboratory Standards Institute 2009) to determine if each isolate was resistant, susceptible, or intermediate for each antibiotic. DNA was also extracted from each isolate for genetic testing, and samples were stored in 10% glycerol at -80°C.

Statistical Analysis

Resistance data was collapsed by sample, meaning that if any isolate in a sample was resistant to a certain antibiotic, then that sample was considered resistant to that antibiotic. Samples that showed intermediate resistance based on the standards were considered susceptible. Estimates of resistance prevalence are calculated as percentages of samples resistant out of total samples taken, including those samples where *E. coli* was not recovered. This is because we wanted to best approximate prevalence of resistant *E. coli* in the environment, so if there was no *E. coli* in a sample, then there was no resistant *E. coli* in that sample either.

Three outcomes were considered for analysis:

1. Resistance to fluoroquinolones, defined as samples that were resistant to ciprofloxacin and/or enrofloxacin
2. Resistance to antibiotics used in chickens in the study area, defined as resistance to streptomycin, chloramphenicol, ciprofloxacin, or enrofloxacin.
3. Multidrug resistance, defined as any sample resistant to more than five antibiotics. This cut-point was chosen based on the distribution of multi-drug resistance; eighty percent of all samples were resistant to less than five.

These outcomes were chosen because of their importance to human health and direct relationship to chicken farming in the study area.

It is important to note that previous reported usage of antibiotics in poultry farming in the study area included ciprofloxacin, enrofloxacin, streptomycin, and chloramphenicol. However, upon arrival in the villages, we found that people were only using nutritional supplements in households with backyard farms, and in Punta de

Piedra (the community level farm), they were using Zinaprim and oxytetracyline. Zinaprim contains trimethoprim and sulfamethazine, an antibiotic similar to sulfisoxazole. The combination of tetracycline, trimethoprim, and sulfisoxazole (tet-sulf-trim) is one of the most common combinations of antibiotics, and therefore the outcome of tet-sulf-trim not analyzed because resistance to those antibiotics is so prevalent in the environment that it would not provide enough discriminatory power. For example, preliminary analysis showed that the three highest resistance levels overall in the study are to these antibiotics (which are about seven times higher than fluoroquinolone prevalence).

Sample types were categorized as indoor, outdoor, and coop samples. Indoor samples are household water and surfaces, outdoor samples are only household soil, and coops samples are coop soil and coop surfaces.

Separate analyses were performed for the one village with a community-level farming operation, Punta de Piedra. All other villages were combined and defined as those with backyard farms. Chi-square tests were performed to determine the difference in antibiotic resistance prevalence between household farming status, type of village (backyard farms or community farm), and type of sample (indoor, outdoor, or coop). In addition, chi-square tests were used to compare the prevalence of antibiotic resistance in Punta de Piedra before and after the change in farming operation, which was from the community farm to backyard farms.

Multi-level correlated logistic regression models were created to assess the factors associated with resistance to each outcome. The predictors included in the model were location of the sample (indoor, outdoor, or coop), the household farming

status (currently farmed, farmed within the past two years, or never farmed), and the village type (backyard farms or community farm). A random intercept was included to account for potential differences between villages. All statistical analyses were run in SAS 9.3 (Cary, NC).

Results

Seventy-two houses were sampled from eight different villages in the study area. Twenty-six of those houses had never farmed chickens, 31 had farmed within the past two years, and 15 had a poultry farming operation at the time of sample collection (Table 1). Overall *E.coli* recovery rates ranged from 34.8% for household water to 91.1% for chicken coop surfaces (Table 2). It was difficult to recover isolates from household water samples due to overgrowth of bacteria on the plates, not because there was no *E. coli* growth. However, there was little *E. coli* growth on household surfaces, which is why those recovery rates are so low.

Antibiotic usage data, which was recorded as part of previous work in the region prior to the study period, revealed the following usage categories in the study area:

1. Antibiotics used in humans: ampicillin and amoxicillin
2. Antibiotics used in chickens: streptomycin, chloramphenicol, ciprofloxacin, or enrofloxacin
3. Antibiotics used in both: trimethoprim, gentamicin, sulfisoxazole, tetracycline
4. Antibiotics used in neither: cefotaxime, cephalothin.

Table 1: Number of houses sampled per village based on farming status

Village	Never farmed	Farmed within past 2 years	Currently farming	Total
Punta de Piedra	0	9	6	14
Colon Eloy	5	5	7	18*
Valdez	4	8	1	13
San Augustín	2	3	1	6
Timbire	10	2	0	12
Las Cruces	0	3	0	3
San Francisco	3	0	0	3
Yalares	2	1	0	3
Total	26	31	15	72

*There was no farming information available for one house in Colon Eloy

Table 2: Sample size and *E. coli* recovery rates

	N	Backyard farms	Community farm	Punta de Piedra backyard farms	Samples recovered	Recovery Rate (%)
HH water	148	116	26	6	84	56.8
HH surfaces	155	124	25	6	54	34.8
HH soil	156	124	26	6	142	91.0
Coop soil	37	27	6	4	33	89.2
Coop surfaces	34	24	6	4	31	91.1
Total	530	415	89	26	344	64.9

Overall prevalence of antibiotic resistance in environmental samples

The highest level of resistance was to tetracycline (38.1% of all samples, n=530), followed by sulfisoxazole (29.1%) and trimethoprim (26.8%). Resistance to cefotaxime, an antibiotic with no reported use in the study area, was only found in 0.8% of all samples (Figure 2). Soil and surface samples from the coop showed the highest resistance levels to fluoroquinolones, antibiotics used in chickens, and multidrug resistance (Figure 3). Household water and surfaces showed the lowest

resistance levels to all three outcomes. Resistance to all three outcomes increases along a gradient from the household environment to the outdoor environment to the coop environment. This effect is stronger in the community with a collective farm. Comparing resistance between the different outcomes, there are much higher levels of multidrug resistance than resistance to fluoroquinolones or antibiotics used in chickens. For example, in the household water, the prevalence of MDR was 14.6%, yet it was only 2.6% for fluoroquinolones and 7.8% for chicken antibiotics (Figure 3).

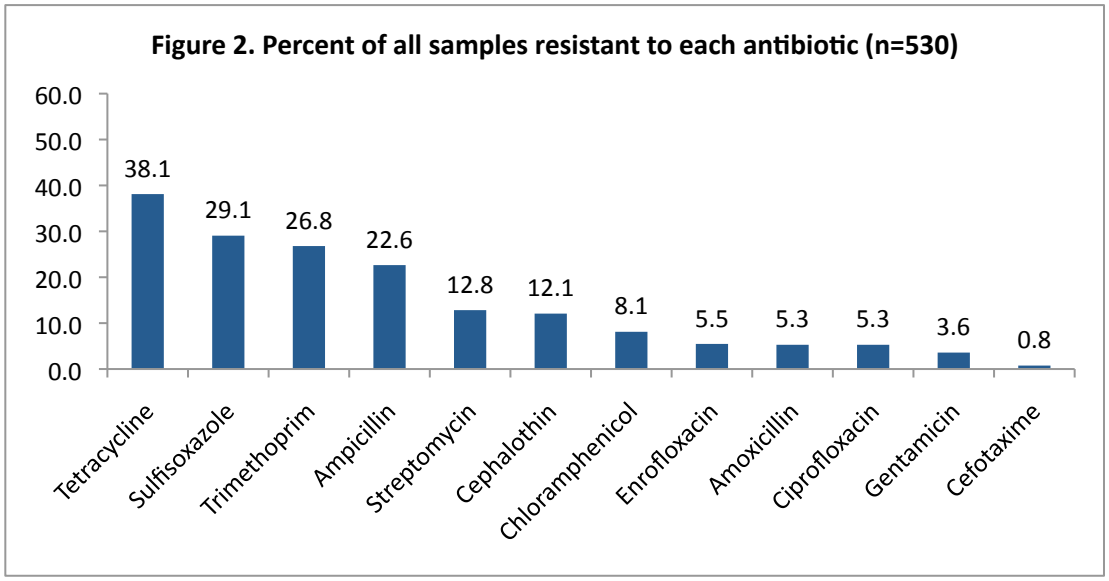


Figure 2: Overall prevalence of antibiotic resistance in environmental samples.

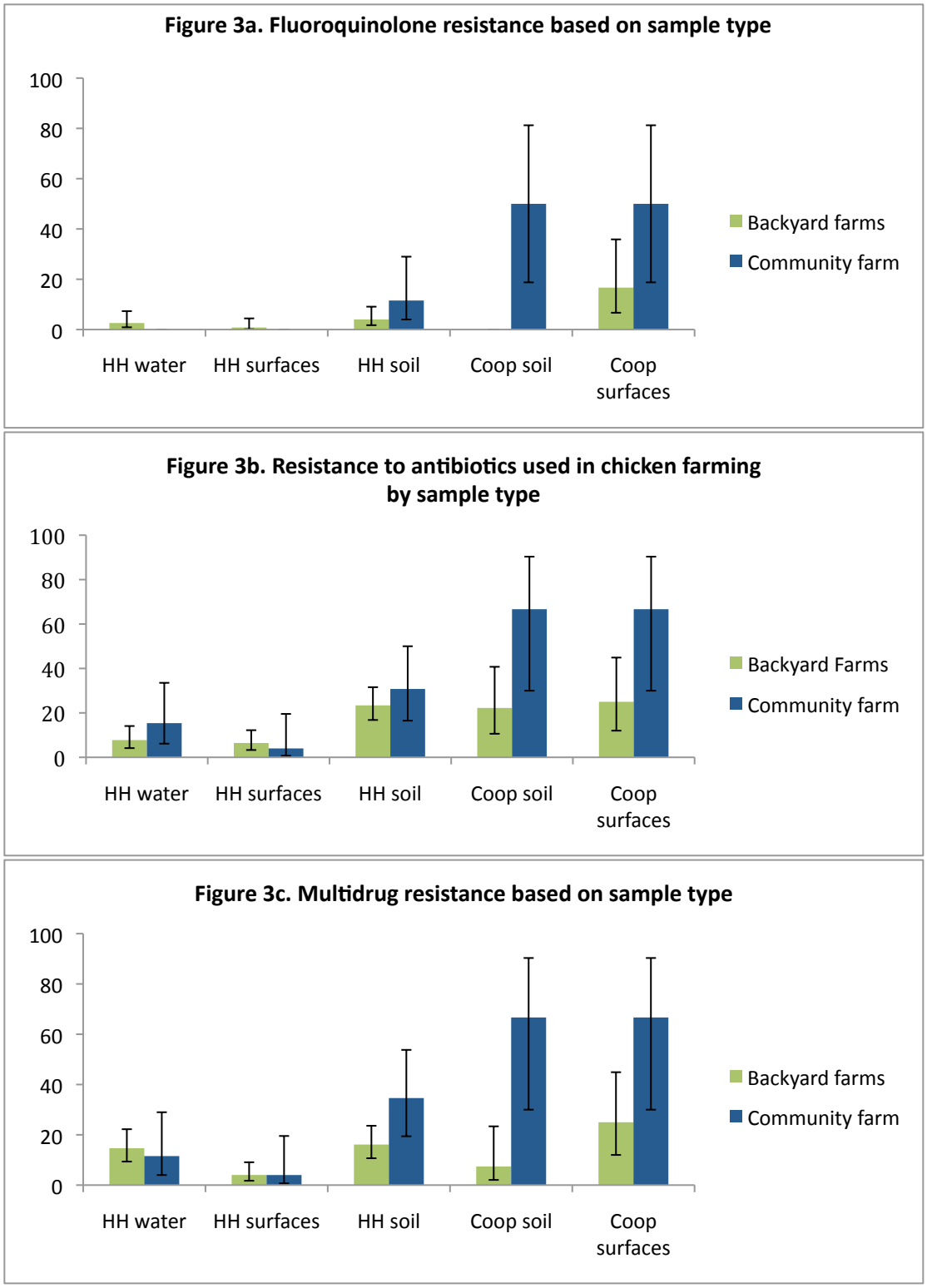


Figure 3. Prevalence of resistance to a) fluoroquinolones, b) chicken antibiotics, and c) multidrug resistance for each sample type in villages with backyard farms and community farms. For backyard farms, sample sizes are: 116 for HH water, 124 for HH surfaces, 124 for HH soil, 27 for coop soil, and 24 for coop surfaces. For the community farm, sample sizes are 26 for HH water, 25 for HH surfaces, 26 for HH soil, 6 for coop soil, and 6 for coop surfaces.

Comparison of antibiotic resistance prevalence based on household farming status

Among households located in villages with backyard farming operations, the highest resistance levels are seen in outdoor samples from households that are actively farming (Figure 4). There are increased levels of indoor fluoroquinolone resistance in households that are actively farming compared to those that have never farmed or previously farmed, but that trend is not significant (Fisher's exact test, $p = 0.6705$). There are also no differences in indoor or outdoor resistance levels to chicken antibiotics among houses based on farming status (indoor: Fisher's exact test, $p=0.9445$; outdoor: Chi-square=2.70, $p=0.2591$). In addition, there are no differences in multidrug resistance levels among households based on farming status in indoor or outdoor samples; all levels are around 8-18% (indoor: Fisher's exact test, $p=0.8724$; outdoor: Fisher's exact test, $p=0.7568$). Surprisingly, for all outcomes, there is antibiotic resistance seen in indoor samples for households who have never farmed (1% fluoroquinolone resistance, 8% resistance to antibiotics used in chickens, and 10% MDR). The community farm was excluded from this analysis because there were no houses sampled that had never farmed.

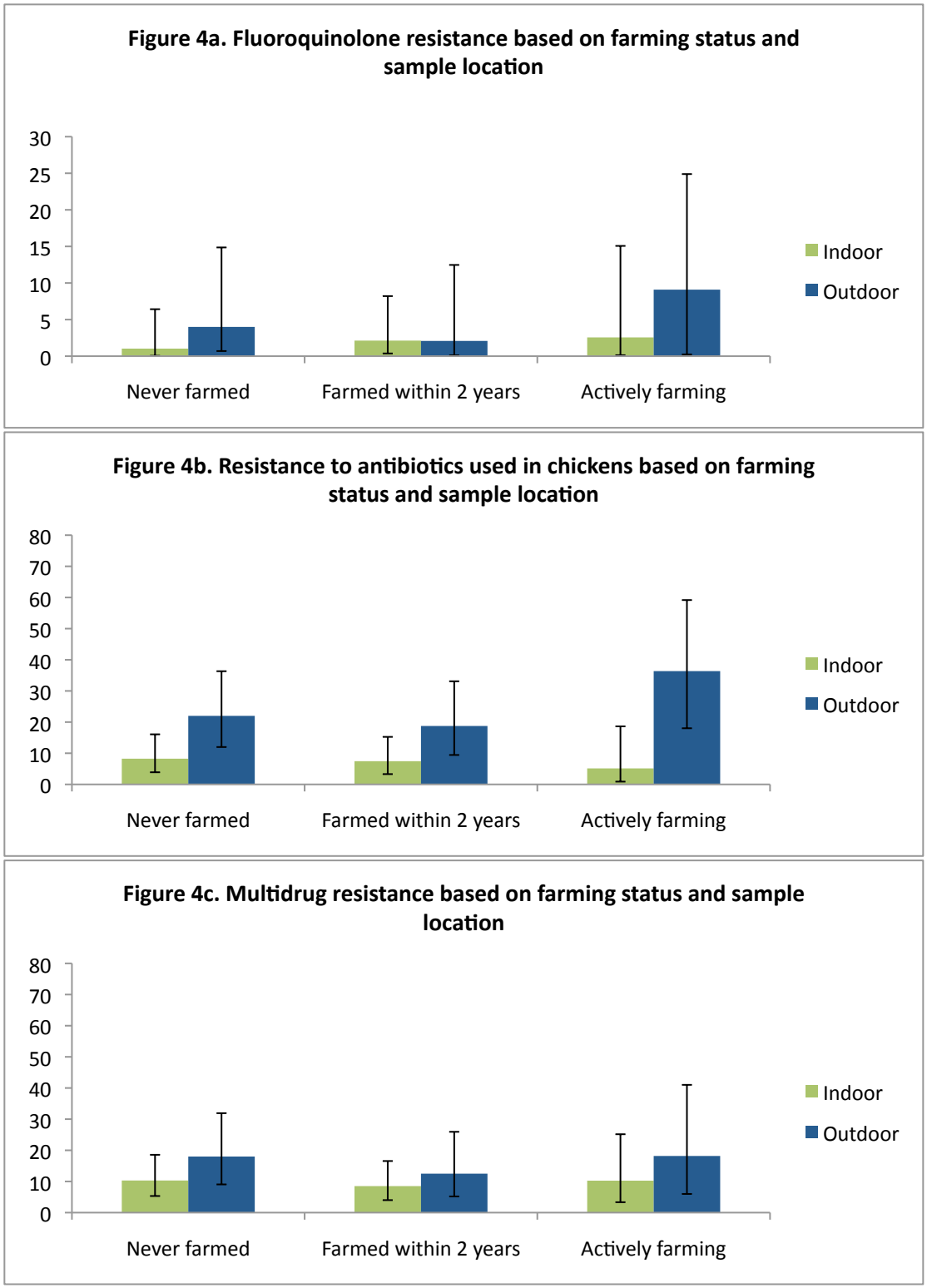


Figure 4. Antibiotic resistance in the indoor and outdoor environment in households that have never farmed chickens, those that have farmed in the past two years, and those that are actively farming. Indoor samples include household water and surfaces; outdoor samples include soil around the household. Coop samples were excluded from this analysis because households that have never farmed do not have a chicken coop. In addition, the

only households included in this analysis are those with backyard farming operations. Note that the scale on the y-axis is smaller for graph 6a then for 6b or c. Sample size for houses that have never farmed are 97 for indoor and 50 for outdoor; for houses that farmed within 2 years, they are 94 for indoor and 48 for outdoor, and those that are actively farming, they are 39 for indoor and 22 for outdoor.

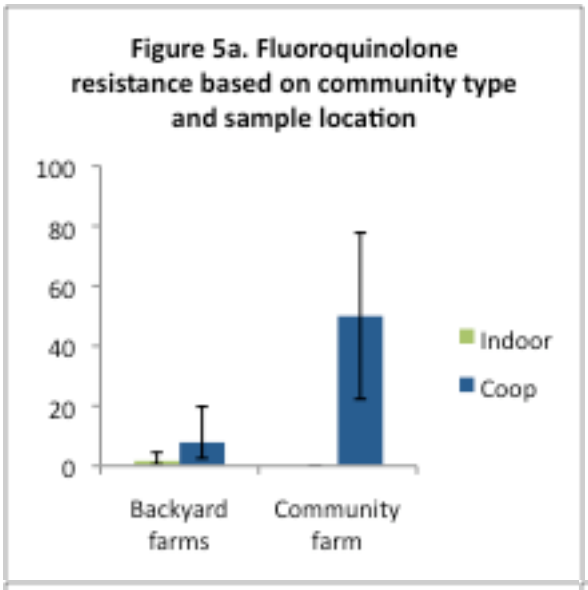
Comparison of antibiotic resistance prevalence between backyard farms and a community farm

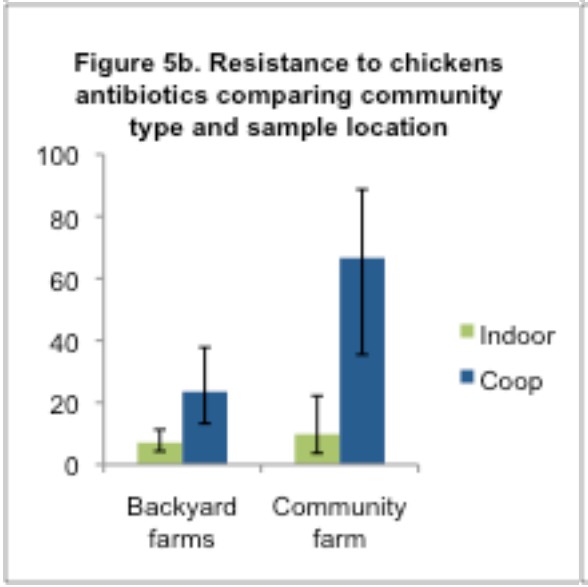
To better understand the relationship between resistance in the coop and resistance in the indoor environment, outdoor samples were eliminated from the following analyses. Community-level farming was associated with significantly higher levels of fluoroquinolone resistance in the coop than backyard farming (Fisher's exact test, $p=0.0019$), with 50% of coop samples resistant in the community-level farm. However, there was fluoroquinolone resistance present in the indoor environment in houses with backyard farms, while there was none in houses with the community-level farm (Figure 5a).

Resistance levels to antibiotics used in chickens were significantly greater in the coop than indoors for houses with backyard farms (Chi-square =10.91, $p<.001$) and houses with the community farm (Fisher's exact test, $p<0.001$). There was also significantly higher resistance in the coop for community farm than backyard farm (Figure 5b; Fisher's exact test, $p=0.012$). However, there was no difference in indoor resistance between backyard farms and community farms (Fisher's exact test, $p=0.558$).

Multidrug resistance (MDR) was significantly greater in the coop than in indoor samples in houses with the community farm (Figure 5c; Fisher's exact test, $p<0.0001$), though this same relationship was not significant for backyard farms

(Chi-square=1.29, p=0.256). In addition, there were higher levels of MDR in the coop in houses with the community farm than in houses with backyard farms (Fisher's exact test=0.0009), though there were no significant differences in MDR between indoor samples in backyard farms and indoor samples from houses with the community farm (Fisher's exact test, p=0.98).





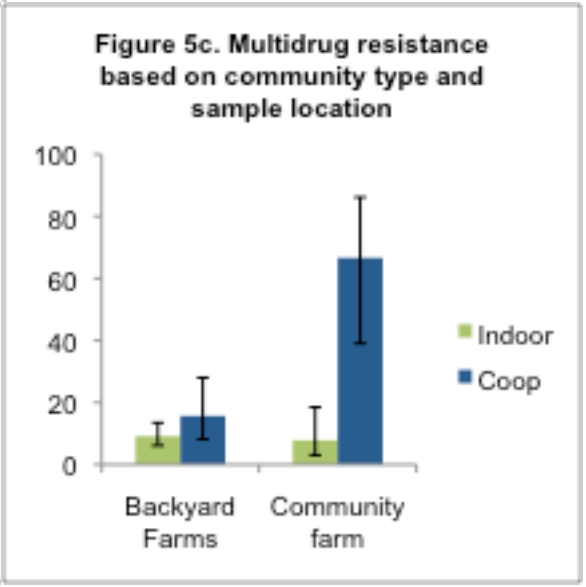


Figure 5: Antibiotic resistance levels in backyard farms and the community farm. Backyard farms are household-level operations with approximately 2-50 chickens and minimal use of antibiotics, while the community farm includes over 600 chickens and intensive use of antibiotics. Sample size for backyard farms is 240 for indoor and 51 for coop; sample size for the community farm is 51 for indoor and 12 for coop.

Antibiotic resistance prevalence in Punta de Piedra

With the change in farming operation from the community level farm to backyard farms, the levels of resistance increase in the indoor environment in houses with backyard farms in Punta de Piedra for all three outcomes, but not significantly (Figure 6). Indoor resistance to fluoroquinolones was absent when there was the community farm, yet with the change to backyard farms, 8.3% of indoor samples were resistant. Multidrug resistance indoors increases just slightly (from 7.8% to 8.4%) with the transfer to backyard farms, and the difference is not significant (Fisher's exact test, p=0.98). Indoor resistance to antibiotics used in chicken farming also increases with the transfer to backyard farms, though the difference is not significant (Fisher's exact test, p=0.17).

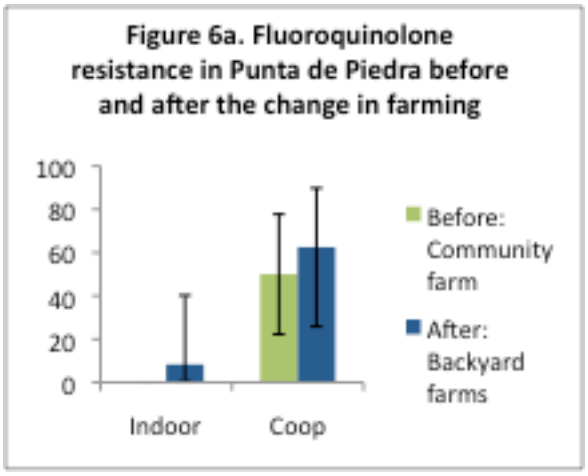
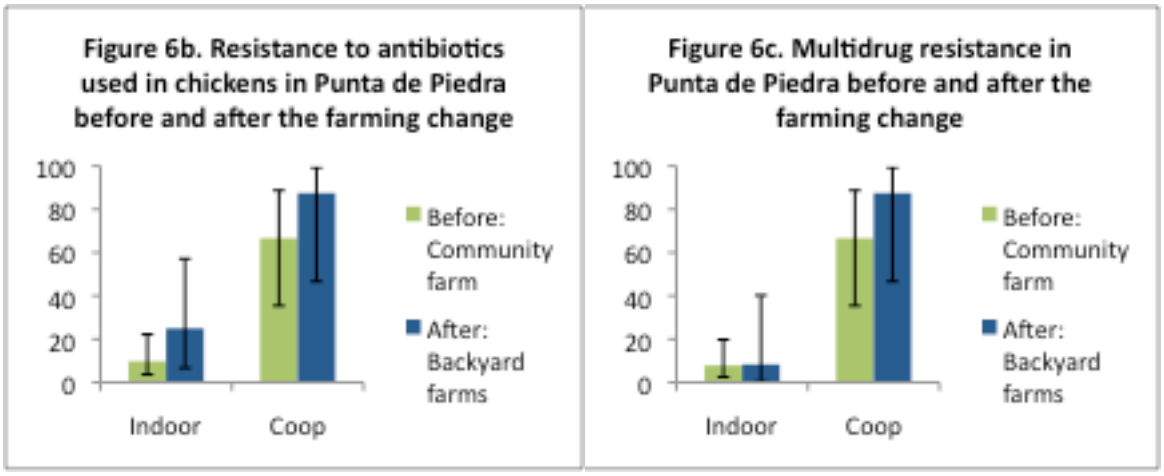


Figure 6: Antibiotic resistance levels in Punta de Piedra before and after the change in farming operation. Before the change, the village had a community farm with over 600 chickens in a coop located away from the households. Afterwards, they had backyard farms at each household with significantly less chickens at each house. Sample size for "before" is 51 for indoor and 12 for coop; sample size for "after" is 12 for indoor and 8 for coop.



Resistance to all outcomes in human samples significantly increased with the change from the community farm to backyard farms (Figure 7). Fluoroquinolone resistance in humans follows the same pattern as indoor environmental samples. When Punta de Piedra had the community-level farm, there was no resistance in human samples to fluoroquinolones. However, once they changed to backyard farming, 3% of the human samples (representing one individual) were resistant to fluoroquinolones. Resistance to antibiotics used in chickens and MDR increased dramatically in humans after the change in farming operation; nearly 94% of all samples were resistant when the community had backyard farms. This was significantly higher than the 38% and 28% of human samples resistant to chicken antibiotics and MDR respectively during the period of the community farming operation (For chicken antibiotics: Chi-square=22.30, $p<0.0001$; MDR: Chi-square=29.66, $p<0.0001$).

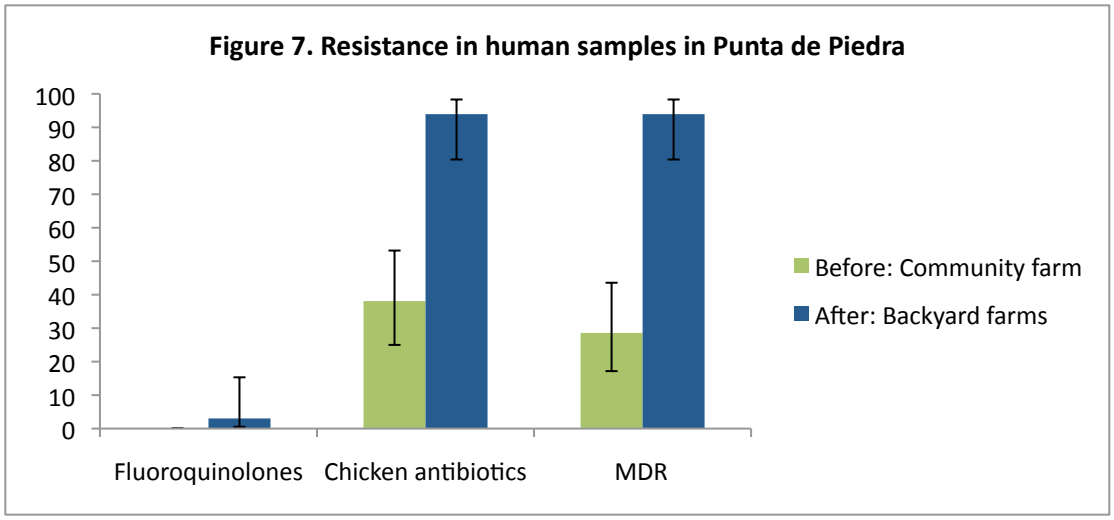


Figure 7. Resistance in human samples in Punta de Piedra before and after the change in farming operation (n=42 before; n=33 after).

Factors associated with resistance in environmental samples

The strongest factor associated with resistance to any of the three outcomes is the type of village. Punta de Piedra, the community-level farm that switched to the backyard farm showed the greatest effect after the change compared to communities with backyard farms; the odds of finding any resistant environmental sample in Punta de Piedra after the change in farming compared to backyard farms was 9.3 (1.94, 45.17) times as great for fluoroquinolones, 6.4 (1.67, 24.53) for antibiotics used in chickens, and 5.8 (1.84, 18.25) for multidrug resistance. These odds ratios were calculated controlling for the location of the sample (indoor, outdoor, or coop) and farming status of the house. In addition, the coop samples had elevated resistance to fluoroquinolones. The odds of finding a fluoroquinolone resistant sample in the coop area is 5.46 (1.33, 22.36) times that then an indoor sample. Model results are in Table 3 below.

Table 3: Multilevel correlated logistic regression models assessing factors associated with antibiotic resistance. Samples were correlated at the household and village level. A random intercept was added for each village to account for potential village-level effects. Reference levels include "never farmed" for farming status, "indoor" for location of sample, and "backyard farms" for village type.

	Parameter	Estimate	Std Error	p-value
Model 1: outcome is resistance to fluoroquinolones	Intercept	-3.6581	0.7219	0.0023
	Location of sample: coop	1.6977	0.7164	0.0184
	Location of sample: outdoor	0.4951	0.5966	0.4072
	Village type: Community farm switched to backyard farm (Punta de Piedra after)	2.2371	0.7996	0.0055
	Village type: Community farm (Punta de Piedra before)	0.4718	0.8488	0.5787
	Farming status: within 2 years	0.3535	0.7431	0.6346
	Farming status: current	-0.1736	0.8915	0.8457
Model 2: outcome is resistance to antibiotics used in chickens	Intercept	-1.5967	0.3440	0.0035
	Location of sample: coop	0.7840	0.4296	0.0690
	Location of sample: outdoor	0.4931	0.3004	0.1017
	Village type: Community farm switched to backyard farm (Punta de Piedra after)	1.8533	0.6844	0.0071
	Village type: Community farm	0.2801	0.5247	0.5939
	Farming status: within 2 years	0.02688	0.3667	0.9416
	Farming status: current	0.06145	0.4330	0.8872
Model 3: outcome is multi-drug resistance	Intercept	-1.3982	0.3125	0.0042
	Location of sample: coop	0.5931	0.4463	0.1848
	Location of sample: outdoor	0.06531	0.3054	0.8308

	Village type: Community farm switched to backyard farm (Punta de Piedra after)	1.7562	0.5835	0.0028
	Village type: Community farm	0.6036	0.4318	0.1632
	Farming status: within 2 years	-0.1922	0.3647	0.5985
	Farming status: current	-0.4468	0.4455	0.3167

Discussion

Antibiotic resistance associated with poultry farming has been well studied on the large scale in industrialized nations, yet very few studies have researched the impacts of poultry farming in developing countries. The present study is the first to investigate environmental reservoirs of antibiotic resistant bacteria in rural communities that have small-scale poultry operations.

There was high prevalence of antibiotic resistance in the study communities, especially in the coop area. Between 50-88% of samples taken from the coop in the community level operation were resistant to each of the outcomes, which were fluoroquinolones, antibiotics used in chickens in the study area, and multidrug resistance. The levels of resistance to fluoroquinolones observed in this coop are roughly equivalent to levels observed in chickens and litter in industrial broiler facilities (Table 4). These numbers are alarmingly high, especially because communities in the study area lack adequate sanitation, so there is a higher risk of transmission of the resistant bacteria from the environment to humans.

Table 4: Review of fluoroquinolone resistance rates found in chickens and environmental samples from current literature.

Reference	**Sample type**	**% Fluoroquinolone resistance**
Sapkota, Hulet et al. 2011	Poultry litter	Cipro = 5%
Simjee, McDermott et al. 2007	Poultry litter	Cipro= 21-54% for enterococci
Norstrom, Johnsen et al. 2007	Broiler environment (Inside & outside, Inside included feces)	Quinolones = 0%; note: low use of these in Norway, the study area
Kalter, Gilman et al. 2010	Humans in rural community, HH chickens	Cipro in HH chickens =0.0%-3.8%; cipro in market chickens = 9.5%-32.7%
Riccobono, Pallecchi et al. 2011	Humans in rural community, HH chickens	Cipro = 43% chicken, 10% human
Thorsteinsdottir, Haraldsson et al. 2010	Humans, broiler chickens	2005-7 Cipro =18.2%; 2008= 42.5%. 2005-7 Enro =14.5%; 2005-7 Nalidixic acid = 18.2%; 2008-42.5%
van den Bogaard, London et al. 2001	Farmers, broiler chickens	Cipro: 50% broilers, 0% laying hens, 8% broiler farmers, 0% laying hen farmers, 7% broiler slaughterers
Lee, Cho et al. 2005	Broiler chickens	Cipro = 60.2%; Enro=73.4%
Bartoloni, Bartalesi et al. 2004	Humans in rural community	Nalidixic acid = 0%
Taylor, Davies et al. 2008	Chickens (broiler), swine manure	Cipro 0-37% for poultry; 0-53% for swine manure
Alali, Scott et al. 2008	Workers, swine	Cipro = 0% in all categories of swine (breeding, slaughter, etc). Cipro = 0-1.9% in humans
Sabate, Prats et al. 2008	Wastewater from humans, poultry, swine farms	Cipro: 20% human, 56% chicken, 21% swine
da Costa, Vaz-Pires et al. 2008	Wastewater from poultry slaughterhouses	From conventional farm, Enro = 20%; from free-range farm, Enro= 11.5%
Schwartz, Kohnen et al. 2003	Drinking water biofilms	Cipro= 2.5%
Roe, Vega et al. 2003	Irrigation water	Cipro <1%

Abbreviations: Cipro= ciprofloxacin, enro=enrofloxacin, HH=household

There were no differences in resistance prevalence among houses with backyard farms that are actively farming, had previously farmed, or had never farmed. This was likely due to the low sample size of houses and the fact that those with backyard farms

were not intensively using antibiotics. However, there was resistance present in the indoor environment (for all outcomes) in households that have never farmed, which is surprising. The most likely explanation is that human consumption of antibiotics is driving resistance, though it could also be because of the proximity of households to others that have a farm. In addition, many households share the same water source or prepare meals together, so that could be another source of resistance in the indoor environment for those who do not have a farm.

The most salient differences in this study are the levels of resistance between backyard farms and the community-level operation, which was located farther away from homes. There were significantly higher rates of resistance in the coop area for the community-level operation compared to backyard farms, likely due to the extensive use of antibiotics in the community operation. However, there were *lower* resistance levels in indoor samples with the community operation. There was less multidrug resistance in indoor samples for the community-level operation than the backyard operation, and more importantly, there was no indoor fluoroquinolone resistance in the community-level operation. This suggests that the proximity of the farming operation to the household is important for the transmission of resistance from the coop area to the indoor environment.

To test this hypothesis, we were provided with a unique natural experiment in which the village with the community operation, Punta de Piedra, switched to backyard farms during the study period. With this change, fluoroquinolone resistance was observed in the indoor environment where it previously had not been, although sample sizes were too small to consider this a statistically significant effect. In addition, the

levels of multidrug resistance and resistance to antibiotics used in chickens were also elevated in the indoor environment in Punta de Piedra after the change to backyard farms. This is highly suggestive that the distance to the farming operation is an important factor in the potential spread of resistant bacteria into the indoor environment. Potential confounding factors that could explain the difference in indoor resistance levels, such as human use of antibiotics, were minimized in this natural experiment because the same community was tested each time.

Increased resistance in the indoor environment with backyard level poultry operations naturally raises the question of human exposure. Are humans more likely to carry resistant bacteria if they raise chickens in their backyard than if they are part of the community operation or do not raise chickens at all?

These study results suggest that backyard farming is contributing to the transmission of resistant bacteria to humans. In Punta de Piedra, the community that switched operations, there no fluoroquinolone resistance was observed in human samples when they had the community-level farm. However, after they changed to backyard farming operations, fluoroquinolone resistance was found in at least one human sample. This pattern was the same for indoor environmental samples, suggesting that the indoor environment could be a mechanism for transmission. This is certainly feasible given that indoor samples included drinking water and kitchen surfaces where food was prepared or eaten. What is more concerning is that with the switch to backyard farming in Punta de Piedra, the prevalence of MDR and resistance to chicken antibiotics in human samples was near 100%. This is more than twice the level it was when there was the community farm.

There were several limitations of this study that could have influenced the resistance levels determined. First, there was a small sample size of houses that currently farm because a chicken epidemic hit the study area three months before the study period, killing the majority of the chickens in the villages. Only two villages out of the eight study villages had more than one household that currently farmed chickens. We were expecting to sample 33 households currently raising chickens, yet only 15 actually had chickens when we arrived, so our sample size and power decreased dramatically. In addition, there were lower sample sizes for household water and surfaces than expected because it was difficult to recover *E. coli* from these samples. Water samples had far too many bacteria to isolate *E. coli* even at small dilutions, and surface samples did not have enough *E. coli* to isolate. This could mean that the prevalence of resistant bacteria in water was actually higher than we found, though we could not test for it because of bacterial overgrowth.

Another major limitation was that human antibiotic usage data was not taken during the present study, so the human use of antibiotics could be influencing resistance in human samples and in the environment, especially indoors. However, the natural experiment in Punta de Piedra provided a control for human antibiotic usage because the same village was re-sampled in different conditions. Lastly, this study only assessed *E. coli* resistance, and it has been shown that fluoroquinolone resistance develops faster in other bacteria associated with chicken farming, namely *Campylobacter* spp. (van Boven, Veldman et al. 2003). Therefore, it is possible that there are actually higher levels of resistance than we have observed in a more dangerous species of bacteria.

To further investigate the impacts of antibiotic use in this study area, we plan to conduct a follow up study during the summer of 2012 that focuses on fluoroquinolone resistance. We selected two communities to compare—one with backyard farm and the other with the community farm (Punta de Piedra)—and will intensively sample from both the domestic and chicken environment using the same methods as the present study. In addition, we will be able to collect more accurate data on antibiotic usage in the following study because we have placed trash bins in the villages for people to use when disposing of antibiotic packets. We will also collect human fecal samples from those who are currently raising chickens and those who have never raised chickens to better understand the relationship between chicken farming and occupational exposure. Apart from the phenotypic analysis of resistance, we will genetically compare strains isolated from environmental samples and humans to examine resistance transfer.

Conclusion

The extensive use of antibiotics in small-scale farming operations is associated with high levels of antibiotic resistance in the environment, especially in the coop area. In addition, the proximity of the farm to the household is associated with resistance found in the indoor environment, providing the potential for human exposure to resistant bacteria. This suggests that small-scale operations should be located further from houses to minimize risk of human exposure to resistant bacteria, and also suggests that antibiotic usage should be minimized. However, more studies are needed to better understand the human risk associated with poultry farming. This can be done

by comparing the strains of *E. coli* that are present in indoor environmental samples, chicken samples, and humans to determine if transmission is being mediated through the environment.

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Appendix A: Household Survey

Casa #:	**Comunidad:**
Fecha:	**Informante Clave:**

Formulario 7d: Muestras Medioambientales

POLLOS

1. ¿La familia ha criado pollos de incubadora alguna vez?

☐ **Sí [1]** ☐ **No [0]** ☐ **No sabe/ no responde [9]**

⬇

1a. ¿Hace cuanto tiempo?

☐ **Hace más que un año [1]** ☐ **Durante el último año pero ahorita no [2]**

☐ **Ahorita está criando [3]** ☐ **No sabe/no recuerda/no responde [9]**

⬇

Si están criando pollos de incubadora ahorita, llenar Formulario 7b

2. ¿La familia ha criado gallinas ponedoras que comen balanceado alguna vez?

☐ **Sí [1]** ☐ **No [0]** ☐ **No sabe/no responde [9]**

⬇

2a. ¿Hace cuanto tiempo?

☐ **Hace más que un año [1]** ☐ **Durante el último año pero ahorita no [2]**

☐ **Ahorita está criando [3]** ☐ **No sabe/no recuerda/no responde [4]**

⬇

Si están criando gallinas ponedoras ahorita, llenar Formulario 7b

AGUA

Pídale al informante clave mostrarle los recipientes de agua para tomar. Escoja dos recipientes. Si hay mas de dos recipientes, escoja dos recipientes de fuentes diferentes. Si todos son de la misma fuente, escoja dos tipos de recipientes diferentes. Si todos son iguales, escoja dos para tomar la muestra. Tome la muestra de 100mL de agua de cada uno de los dos con una funda whirlpak, en la misma manera que el informante toma el agua del recipiente.

Recipiente CA1:
Muestra: _____-_____-_ D _- _CA1_
[#Pueblo]-[#Casa] – [Agua de casa] – [#muestra de casa]

D1.1. ¿Cuál fue la fuente de agua para llenar este recipiente? [fuente]

☐ **Pozo [1]** ☐ **Río [2]** ☐ **Lluvia [3]**

☐ **Llave del vecino [4]** ☐ **Llave dentro de la casa [5]**

☐ **Llave fuera de la casa [6]** ☐ **Agua comprada [7]**

☐ **Otra:_____[8]** ☐ **No sabe [9]**

D1.2. ¿En qué tipo de recipiente está el agua almacenada?

☐ **Balde [1]** ☐ **Galón [2]** ☐ **Tanque [3]** ☐ **Olla [4]**

☐ **Poma [5]** ☐ **Tambucho [6]** ☐ **Bidón [7]**

☐ **Botella [8]** ☐ **No hay recipiente [10]** ☐ **Otro _____ [11]**

☐ **N/R [9]**

D1.3.¿En las últimas 24 horas, ha tratado esta agua?

☐ **Sí [1]** ☐ **No [0]** ☐ **N/R [9]**

🡻

A3a. Si es que sí, ¿Cómo trató el agua?

☐ **Cloro [1]** ☐ **Hirviendo el agua [2]** ☐ **Abate [3]**

☐ **Espera que la suciedad se hunda al fondo del recipiente [4]**

☐ **Otro _____ [5]** ☐ **N/R [9]**

D1.4. ¿Lavó el recipiente antes de llenarlo?

☐ **Si [1]** ☐ **No [0]** ☐ **N/R [9]**

🡻

A4a. Si es que sí, ¿Como lavo el recipiente? (selecciona todas las opciones que apliquen)

☐ **Con agua del fuente [1]** ☐ **Con cloro [2]**

☐ **Con jabón [3]** ☐ **Otro _____ [4]** ☐ **N/R [9]**

D1.5. ¿Qué es el uso del agua en este recipiente o fuente?

☐**Para tomar [1]** ☐**Para cocinar [2]**

☐**Para lavar los platos [3]** ☐ **Para bañarse** [4]

☐**Otro_____[5]** ☐**N/R [9]**

OBSERVACIONES:

D1.6. ¿Existe contaminación visible dentro del recipiente o fuente? (selecciona todas las opciones que apliquen)

☐ **No, esta limpio [1]** ☐ **Un poco de suciedad [2]**

☐ **Amarilla/verde algas [3]** ☐ **Espuma negra [4]**

☐ **Insectos [5]** ☐ **Otro _____ [6]** ☐ **N/R [9]**

Recipiente B:
Muestra : _____-_____- D - CA2
[#Pueblo]-[#Casa] – [Agua de la casa] – [#muestra de casa]

D2.1. ¿Cuál fue la fuente de agua para llenar este recipiente? [fuente]

☐ **Pozo [1]** ☐ **Río [2]** ☐ **Lluvia [3]**

☐ **Llave del vecino [4]** ☐ **Llave dentro de la casa [5]**

☐ **Llave fuera de la casa [6]** ☐ **Agua comprada [7]**

☐ **Otra:_____[8]** ☐ **No sabe [9]**

D2.2. ¿En qué tipo de recipiente está el agua almacenada?

☐ **Balde [1]** ☐ **Galón [2]** ☐ **Tanque [3]** ☐ **Olla [4]**

☐ **Poma [5]** ☐ **Tambucho [6]** ☐ **Bidón [7]**

☐ **Botella [8]** ☐ **No hay recipiente [10]** ☐ **Otro _____ [11]**

☐ **N/R [9]**

D2.3.¿En las últimas 24 horas, ha tratado esta agua?

☐ **Sí [1]** ☐ **No [0]** ☐ **N/R [9]**

🡻

D2.3a. Si es que sí, ¿Cómo trató el agua?

☐ **Cloro [1]** ☐ **Hirviendo el agua [2]** ☐ **Abate [3]**

☐ **Espera que la suciedad se hunda al fondo del recipiente [4]**

☐ **Otro _____ [5]** ☐ **N/R [9]**

D2.4. ¿Lavó el recipiente antes de llenarlo?

☐ **Si [1]** ☐ **No [0]** ☐ **N/R [9]**

🡻

D2.4a. Si es que sí, ¿Como lavo el recipiente? (selecciona todas las opciones que apliquen)

☐ **Con agua del fuente [1]** ☐ **Con cloro [2]**

☐ **Con jabón [3]** ☐ **Otro _____ [4]** ☐ **N/R [9]**

D1.5. ¿Qué es el uso del agua en este recipiente o fuente?

☐**Para tomar [1]** ☐**Para cocinar [2]**

☐**Para lavar los platos [3]** ☐ **Para bañarse** [4]

☐**Otro_____[5]** ☐**N/R [9]**

OBSERVACIONES:

D2.6. ¿Existe contaminación visible dentro del recipiente? (selecciona todas las opciones que apliquen)

☐ **No, esta limpio [1]** ☐ **Un poco suciedad [2]**

☐ **Amarilla/verde algas [3]** ☐ **Espuma negra [4]**

☐ **Insectos [5]** ☐ **Otro _____ [6]** ☐ **N/R [9]**

<u>SUPERFICIES</u>

Superficie X1 (donde se preparó la comida):

Pídale al informante clave mostrarle el superficie en donde preparó su última comida y use la plantilla para tomar la muestra del un lugar en este superficie.

Muestra # _____- _____-X - CA1
[# Pueblo] [#Casa] – [Superficie] – [#Muestra de casa]

X1.1 ¿De qué material es la superficie?

☐**Madera [1]** ☐**Cemento [2]**

☐**Plástico [3]** ☐**Pambil [4]**

☐**Vidrio [5]** ☐**Ceramico [6]**

☐**Otro: _____ [7]** ☐**NR [9]**

X1.2 ¿Limpió Ud. este superficie después de cocinar la última comida?

☐**Sí [1]** ☐**No [0]**

🡻

X1.2a. Si es que sí, ¿Qué usó Ud. para limpiar?

☐**trapo húmedo [1]** ☐**creso [2]**

☐**trapo seco [3]** ☐**jabon o detergente [4]**

☐**Otro: _____[5]** ☐**NR [9]**

OBSERVACIONES:

X1.3. ¿Qué tipo de suciedad hay en el superficie? (selecciona todas las opciones que apliquen):

☐**restos de comida [1]** ☐**polvo [2]**

☐**humedad [3]** ☐**crecimiento biológico [4]**

☐**excremento de animales [5]** ☐ **Insectos [6]**

☐**Otro: _____[7]**

Superficie X2 (donde se comió la comida):

Pídale al informante clave mostrarle el superficie en donde se comió su última comida y use la plantilla para tomar la muestra del un lugar en este superficie.

Muestra _____- _____-_ X - CA2
[# Pueblo] [#Casa] – [Superficie] – [#Muestra de casa]

X2.1 ¿De qué material es el superficie?

☐**Madera [1]** ☐**Cemento [2]**

☐**Plástico [3]** ☐**Pambil [4]**

☐**Vidrio [5]** ☐**Ceramico [6]**

☐**Otro: _____ [7]** ☐**NR [9]**

X2.2 ¿Limpió Ud. este superficie después de cocinar la última comida?
☐**Sí [1]** ☐**No [0]**
🡻

X2.2a. Si es que sí, ¿Qué usó Ud. para limpiar?
☐**trapo húmedo [1]** ☐**creso [2]**
☐**trapo seco [3]** ☐**jabon o detergente [4]**
☐**Otro: _____[5]** ☐**NR [9]**

OBSERVACIONES:

X2.3. ¿Qué tipo de suciedad hay en el superficie? (selecciona todas las opciones que apliquen):

☐**restos de comida [1]** ☐**polvo [2]**
☐**humedad [3]** ☐**crecimiento biológico [4]**
☐**excremento de animales [5]** ☐ **Insectos [6]**
☐**Otro: _____[7]**

SUELO

Pídale al informante permiso para tomar muestras del suelo afuera de la casa.

Muestra S1:

Muestra _____- _____-_ S - CA1
[# Pueblo] [#Casa] – [Suelo] – [#Muestra de casa]

S1.1 Lugar de muestreo:
☐ **En frente de la casa [1]**
☐ **A lado de la casa—zona de declive hacia la casa [2]**
☐ **Tras de la casa [3]**

OBSERVACIONES (selecciona todas las opciones que apliquen)

S1.2.
☐ **Contaminación fecal [1]** ☐ **Seco [2]**
☐ **Húmedo [3]** ☐ **Otro_____[4]**

Muestra S2:
Muestra _____- _____-_ S _- _CA2

[# Pueblo] [#Casa] – [Suelo] – [#Muestra de casa]

S2.1. Lugar de muestreo (selecciona 5 gramos de todos los 3, y meterlos en una funda ziplock):

☐ **En frente de la casa [1]**

☐ **A lado de la casa—zona de declive hacia la casa [2]**

☐ **Tras de la casa [3]**

OBSERVACIONES (selecciona todas las opciones que apliquen)

S2.2.

☐ **Contaminación fecal [1]** ☐ **Seco [2]**

☐ **Húmedo [3]** ☐ **Otro_____[4]**

OBSERVACIONES EN GENERAL:

1. Hay… (selecciona todas las opciones que apliquen):

☐**Pollos criollos [1]** ☐**Vacas [2]** ☐**Cerdos [3]**

☐**Patos [4]** ☐**Otros animales [5]** ☐**Otro_____[6]**

Appendix B: Survey for households actively raising chickens

Criadero # _____ **Entrevistador _____**
Casa # o
Grupo (nombre) _____ **Comunidad _____**

Fecha _____ **Informante Clave _____**

Crianza de Pollos
Encuesta 7b

Por cada casa o grupo que crian pollos de incubadora o gallinas ponedoras que comen balanceado, pregunte al informante clave las siguientes preguntas.

I. EL CRIADERO

1. ¿Dónde está el criadero?

☐ **En el mismo sitio de la casa (debajo o detrás de la casa)** [1]

☐ **En otro sitio fuera de la casa (lejos de la casa)** [2]

☐ **No sabe/no recuerda** [9]

2. ¿Qué aserrín hay en el piso del criadero?

☐ **Aserrín de madera [1]** ☐ **Aserrín de arroz [2]**

☐ **Noy hay aserrín [3]** ☐ **Otro: _____[0]** ☐**N/R [9]**

3. ¿Hace cuánto tiempo pusó este aserrín?

☐ **Hace menos que una semana [1]**

☐ **Hace _____ semanas [2]**

☐ **Hace _____ meses [3]** ☐ **N/R [9]**

4. ¿Qué hizo Ud. para limpiar el criadero ántes de meter estos pollos?

(Se puede escoger más de una opción.)

☐ **Nada [1]** ☐**Cambió el aserrín[2]** ☐ **No sabe porque es el primer lote [3]**

☐ **No sabe/no recuerda [9]** ☐ **Otro: _____ [0]**

☐ **Desinfectar [5]**

⬇

Si dijo “desinfectar”, ¿con qué?

☐ **Cloro [1]** ☐ **Yodo [2]** ☐**Gasolina [3]**

☐ **Creso [4]** ☐ **Tips [5]**

☐ **Otro: _____ [6]**

☐ **Dejó vacío por un tiempo [6]**

⬇

Si dijo dejó vacío, ¿por cuánto tiempo?

____ días

____ semanas

____ meses

5. ¿Qué hizó con el aserrín después del último lote de pollos?

☐ **Lo botó al río [1]** ☐**Lo botó a campo abierto [2]**

☐ **Lo enterró [3]** ☐ **Lo usó como fertilizante para las siembras [4]**

☐ **Otro: _____ [0]** ☐ **N/R [9]**

II. LOS ANIMALES:

II.1. POLLOS DE INCUBADORA

1. ¿Crian pollos de incubadora con balanceado ahorita?

☐ **No →** siga a la sección II.2. GALLINAS PONEDORAS

☐ **Sí →**Conteste las preguntas abajo

2. ¿Cuantos pollos están criando de cada edad? **Escribe los números**.

1 semana o menos _____ **2 semanas_____**

3 semanas_____ **4 semanas_____**

5 semanas_____ **6 semanas o más_____**

3. ¿En cuántos corrales están los pollos? **Escribe el número_____**

4. ¿De dónde vienen estos animales? (Se puede escoger una o más respuestas)

☐**Esmeraldas [1]** ☐**San Lorenzo [2]** ☐**Borbón[3]**

☐**Ibarra [4]** ☐**Guayaquil [5]** ☐**N/R [9]**

☐**Otro_____[0]**

5. ¿Cómo financió la compra? (Se puede escoger una o más respuestas)

☐**Dinero particular [1]** ☐**Dinero prestado [2]**

☐**Fue un regalo [3]** ☐**Otro_____[0]** ☐**N/R [9]**

6. ¿Qué marca de balanceado usa Ud? (Se puede escoger una o más respuestas)

☐**Nutril [1]** ☐ **Pronaca [2]** ☐**Avisol [3]**

☐**N/R [9]** ☐**Otro_____[0]**

7. ¿Qué tipo de balanceado está comiendo los pollitos ahorita? (Se puede escoger una o más respuestas)

☐**Pre-Inicial [1]** ☐**Inicial/crecimiento [2]** ☐**Engorde/Final [3]**

☐**Postura [4]** ☐**NR [9]** ☐**Otro_____[0]**

8. ¿De dónde viene el balanceado? (Se puede escoger una o más respuestas)

☐**Esmeraldas [1]** ☐**San Lorenzo [2]** ☐**Borbón [3]**

☐**Ibarra [4]** ☐**Guayaquil [5]** ☐**NR [9]** ☐**Otro_____[0]**

9. ¿Ud usa medicamentos en la cianza de estos pollos?

☐**Sí [1]** ☐**No [2]**

🡻

9a. Si dice que sí usa medicamentos, ¿está el paquete?

☐ **Sí [1]** ☐**No [2]**

9b. ¿Qué tipo de medicamentos usan? (si paquete) o ¿Qué tipo de medicamentos dicen que usan (no paquete)? (Se puede escoger una o más respuestas)

☐**Baitril [1]** ☐**Beta-strep [2]** ☐**Ciproflox [3]**

☐**Clortetraciclina [4]**

☐**Doxiciclina [5]** ☐**Enroflox [6]** ☐**Enrofloxacina [7]** ☐**Enrovet [8]**

☐**Enrox [9]** ☐**Fullxacina [10]** ☐**Gadexil [11]** ☐**Gallimicina [12]**

☐**Ganadecir [13]** ☐**Kao-Peg [14]** ☐**Lincomicina [15]** ☐**Neumosol [16]**

☐**Oxitetraciclina [17]** ☐**Oxitra [18]** ☐**Oxitrax [19]** ☐**Pembex [20]**

☐**Penicilina [21]** ☐**Pensibec [22]** ☐**Petercilin [23]** ☐**Piperazina [24]**

☐**Piperex [25]** ☐**Spirimicina [26]** ☐**Sulfatex [27]** ☐**Sulfavit [28]**
☐**Sulobactone [29]** ☐**Supervitex [30]** ☐**Terramicina [31]** ☐**Tetramax [32]**
☐**Tifocol [33]** ☐**Tilotex [34]** ☐**Unimast [35]** ☐**Vxitamax [36]**
☐**Vitamix-13 [37]** ☐**Zetapen [38]** ☐**Zinaprim [39]**
☐**Otro antibiotico [40]_____**
☐**Antibiotico (no se sabe el nombre) [41]** ☐**Vitaminas [42]** ☐**Vacuna [43]**
☐**N/R [999]**

10.¿Para qué son estos pollos de incubadora?
☐**Principalmente para consumir en la casa** [1] ☐**N/R** [9]
☐ **Principalmente para vender** [1]
🡻 🡻
10a. Si se vende los pollos, ¿A quién los vende? (se puede escoger más que una opción)
☐**A personas particulares [1]** ☐ **A otros pueblos [2]**
☐ **A una tiende en el pueblo [3]** ☐ **No sabe/no recuerda [4]**
☐ **Otro: _____ [0]**

II.2 GALLINAS PONEDORAS

12. ¿Crian gallinas ponedoras con balanceado ahorita?
☐ **No →** siga a la sección II.3 POLLOS CRIOLLOS
☐ **Sí →**Conteste las preguntas abajo

13. ¿Cuantos gallinas ponedoras están criando de cada edad? **Escribe los números**.
1 semana o menos _____ **2 semanas_____**
3 semanas_____ **4 semanas_____**
5 semanas_____ **6 semanas o más_____**

14. ¿En cuántos corrales están las gallinas? **Escribe el número_____**

16. ¿De dónde vienen estas gallinas? (Se puede escoger una o más respuestas)
☐**Esmeraldas [1]** ☐**San Lorenzo [2]** ☐**Borbón[3]**

☐Ibarra [4] **☐Guayaquil [5]** **☐N/R [9]**
☐Otro_____[0]

17. ¿Cómo financió la compra? (Se puede escoger una o más respuestas)
☐Dinero particular [1] **☐Dinero prestado [2]**
☐Fue un regalo [3] **☐Otro_____[0]** **☐N/R [9]**

18. ¿Qué marca de balanceado usa Ud? (Se puede escoger una o más respuestas)
☐Nutril [1] **☐ Pronaca [2]** **☐Avisol [3]**
☐N/R [9] **☐Otro_____[0]**

19. ¿Qué tipo de balanceado está comiendo las gallinas ahorita? (Se puede escoger una o más respuestas)
☐Pre-Inicial [1] **☐Inicial/crecimiento [2]** **☐Engorde/Final [3]**
☐Postura [4] **☐NR [9]** **☐Otro_____[0]**

20. ¿De dónde viene el balanceado? (Se puede escoger una o más respuestas)
☐Esmeraldas [1] **☐San Lorenzo [2]** **☐Borbón [3]**
☐Ibarra [4] **☐Guayaquil [5]** **☐NR [9]** **☐Otro_____[0]**

21. ¿Ud usa medicamentos en la cianza de estas gallinas?
☐Sí [1] **☐No [2]**
🡻
22a. Si dice que sí usa medicamentos, ¿está el paquete?
☐ Sí [1] **☐No [2]**

22b. ¿Qué tipo de medicamentos usan? (si paquete) o ¿Qué tipo de medicamentos dicen que usan (no paquete)? (Se puede escoger una o más respuestas)

☐Baitril [1] **☐Beta-strep [2]** **☐Ciproflox [3]**
☐Clortetraciclina [4]
☐Doxiciclina [5] **☐Enroflox [6]** **☐Enrofloxacina [7]** **☐Enrovet [8]**
☐Enrox [9] **☐Fullxacina [10]** **☐Gadexil [11]** **☐Gallimicina [12]**

☐Ganadecir [13] **☐Kao-Peg [14]** **☐Lincomicina [15]** **☐Neumosol [16]**

☐Oxitetraciclina [17] **☐Oxitra [18]** **☐Oxitrax [19]** **☐Pembex [20]**

☐Penicilina [21] **☐Pensibec [22]** **☐Petercilin [23]** **☐Piperazina [24]**

☐Piperex [25] **☐Spirimicina [26]** **☐Sulfatex [27]** **☐Sulfavit [28]**

☐Sulobactone [29] **☐Supervitex [30]** **☐Terramicina [31]** **☐Tetramax [32]**

☐Tifocol [33] **☐Tilotex [34]** **☐Unimast [35]** **☐Vxitamax [36]**

☐Vitamix-13 [37] **☐Zetapen [38]** **☐Zinaprim [39]**

☐Otro antibiotico [40]_____

☐Antibiotico (no se sabe el nombre) [41] **☐Vitaminas [42]** **☐Vacuna [43]**

☐N/R [999]

23.¿Para qué son los huevos de las gallinas?

☐Principalmente para consumir en la casa [1] **☐N/R [9]**

☐ Principalmente para vender [2]

⬇ ⬇

23a. Si se vende los pollos, ¿A quién los vende? (se puede escoger más que una opción)

☐A personas particulares [1] **☐ A otros pueblos [2]**

☐ A una tiende en el pueblo [3] **☐ No sabe/no recuerda [4]**

☐ Otro: _____ [0]

II.3. POLLOS CRIOLLOS

24. ¿Cuántos pollos criollos tienen? _____

III. PERSONAS QUE CRIAN

1. ¿Desde cuándo Ud (o el grupo) cria pollos de incubadora o gallinas ponedoras?

☐ **menos de un més**

☐ **______ meses**

☐ **______ años**

2. En la última semana, ¿Quién tuvo contacto con estos animales?

ID	**Casa #**	**Nombre**	**¿Qué hizo?** (puede escoger más que una opción)
			☐ Da agua a los animales ☐ Limpia el criadero ☐ Da comida a los animales ☐ N/R ☐ Otra actividad: ________________
			☐ Da agua a los animales ☐ Limpia el criadero ☐ Da comida a los animales ☐ N/R ☐ Otra actividad: ________________
			☐ Da agua a los animales ☐ Limpia el criadero ☐ Da comida a los animales ☐ N/R ☐ Otra actividad: ________________
			☐ Da agua a los animales ☐ Limpia el criadero ☐ Da comida a los animales ☐ N/R ☐ Otra actividad: ________________
			☐ Da agua a los animales ☐ Limpia el criadero ☐ Da comida a los animales ☐ N/R ☐ Otra actividad: ________________
			☐ Da agua a los animales ☐ Limpia el criadero ☐ Da comida a los animales ☐ N/R ☐ Otra actividad: ________________
			☐ Da agua a los animales ☐ Limpia el criadero ☐ Da comida a los animales ☐ N/R ☐ Otra actividad: ________________
			☐ Da agua a los animales ☐ Limpia el criadero ☐ Da comida a los animales ☐ N/R ☐ Otra actividad: ________________