UNDERSTANDING THE USE OF ANTIBIOTICS ON SMALL DAIRY FARMS IN RURAL PERU

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ABSTRACT

UNDERSTANDING THE USE OF ANTIBIOTICS ON SMALL DAIRY FARMS IN RURAL PERU

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Dairy production is a rapidly expanding sector of animal agriculture in lower/middle income countries (LMICs) where small farms generally constitute the majority of producers. Antibiotics are commonly used in dairy medicine to treat sick animals and healthy animals during periods of stress. These uses can improve animal health and productivity but can also contribute to antibiotic resistance among bacteria isolated from animals and humans. Furthermore, when antibiotic residues remain in the final food product, consumers can be chronically exposed to low levels of antibiotics. Residues can also diminish the economic value of the food product through interference with cultures necessary for processed products.

Very little is known about antibiotic use on small dairy farms in LMICs. If the judicious use of antibiotics is to be promoted, it is essential to understand how and why antibiotics are currently used on these farms. This study aimed to understand how and why antibiotics are used on small dairy farms in Cajamarca, a major dairy-producing region of Peru, and to improve the methods used to collect this type of pharmacoepidemiologic data.

We found that antibiotics are infrequently used (0.48 episodes of treated disease per cow-year) and that few active ingredients are used. The use of antibiotics did not always appear to follow clinical guidelines, a finding that was confirmed by interviews with prescribers (veterinarians and feed-store vendors). The point prevalence of contamination of milk with antibiotic residues on a given day for a commercial milk route was low (0-4.2%), but 92% of farmers who were treating their cows with antibiotics sold contaminated milk. The farmer's knowledge of antibiotics and the purchaser of the milk were significantly associated with the self-reported sale of milk from treated cows. Finally, the use of self-report to collect data on antibiotic use on small farms is inadequate and could be improved by collecting discarded drug packaging from farmers.

These findings suggest that improved prescribing practices and management on the farms are needed to improve animal health and the judicious use of antibiotics. In addition, milk purchasers must implement measures to incentivize farmers to withhold milk contaminated with antibiotic residues.

TABLE OF CONTENTS

Contents

LIST OF TABLES

LIST OF ILLUSTRATIONS

CHAPTER 1: Introduction to antibiotic use in dairy farming on small farms in lower/middle-income countries

Food production and dairy farming in lower/middle-income countries

Producing enough food for the world's population is one of the most significant challenges of the future, given population growth and constraints on land resources. Under-nutrition kills five million children every year and costs households in lower/middle income countries (LMICs) world more than 220 million years of productive life[1]. Food production is under transition globally, with the most rapid change occurring in the developing world. The Green Revolution, scientific agricultural research and the development of new technologies have enabled mankind to experience an unprecedented growth in food production such that we are now able to produce more food on fewer acres of land and with fewer animals than previously thought possible[2]. However, the adoption of technology that improves plant and animal productivity remains elusive in LMICs, especially on small farms that do not have access to the capital needed to make the transition to technology-based farming [3,4].

Small farms constitute the majority of farms in LMICs[5] and are often a predominate supplier of animal products to their domestic markets. As population growth increases in these countries, food production will need to increase commensurately. While the number of large farms is increasing in LMICs[6], in many areas, small farms remain predominant and will increasingly be called upon to fill the gap in supply. However, despite the growing demand for food products, small farmers often belong to the poorest segments of the population[7,8]. Small farms often cannot compete with vertically integrated, transnational food-producing corporations and are becoming an increasingly marginalized minority, excluded from the food system as both producers and consumers[1]. To transition to sustainable, economically-viable farming systems, these farms will need to improve farming practices that can optimize animal health and productivity but do not impose an unmanageable economic burden on the farmer.

Dairy production is a rapidly expanding sector of animal agriculture in the developing world because of population growth, increases in per capita income, urbanization and the westernization of diets[6,9]. Smallholder dairies constitute the majority of producers in the many LMICs[7] but low levels of milk production, the need to sell milk daily and the high fixed costs of dairy production often leave these farmers in a vulnerable position[9]. In addition, because these farmers frequently have little available capital, few are able to invest in preventative care for their animals. As a result, disease (infectious, metabolic or management-related) represents a major challenge for dairy farmers that can compromise the health and limit the productivity of their animals. Drugs used to treat animal disease are therefore an important component of dairy farming.

Antibiotic use in animal agriculture and dairy farming

Antibiotics are commonly used in animal agriculture for a number of reasons, including growth promotion, treatment of sick animals and prophylactic treatment of healthy animals during periods of stress[10]. In the dairy industry, antibiotics are primarily used for therapeutic and prophylactic purposes but not for growth promotion[11]. Little is known about antibiotic use in animal agriculture in LMICs in general and on small farms in particular.

In the United States, approximately 28.7 million pounds of antimicrobials were sold for use in food-producing animals in 2009 [12], and studies using data from the United States Department of Agriculture (USDA) National Animal Health Monitoring System (NAHMS) have described the use of antibiotics in cattle[13] and swine[14,15]. There is very little comparable information on antibiotic use in LMICs where small farms predominate and treatment records are rarely kept. A study measuring national antibiotic use over a five-year period in Kenya found that 14,594 kg of antibiotics were used in animal agriculture, with cattle, sheep, pigs and goats using 10,989 kg [16]. Roderick et al. measured antibiotic use in cattle of Maasai pastoralists over a five-year period and found an annual treatment rate of 0.52 treatments per animal-year with almost exclusive use of oxytetracycline [17]. Sudershan and Bhat found that oxytetracycline was the most commonly used drug in cattle of farmers in Hyderabad, India and that the most common indication for its use was mastitis[18]. Luna-Tortos et al. surveyed 60 dairy farms in Santa Cruz, Costa Rica, evaluated patterns and determinants of antibiotic use and found that antibiotics were often used without objective diagnostic criteria or veterinary input, and that penicillins, tetracyclines and aminoglycosides were the most commonly used drugs[19]. The World Health Organization (WHO) indicated that certain antibiotics are licensed for use in food animals in Asia, Latin America and South Africa, however the magnitude of use in these regions was not specified[20].

Knowledge of patterns of antibiotic use is fundamental to understanding farming practices and animal health on small farms. A basic knowledge of how drugs are used can provide some measures of drug misuse, the magnitude of the risk of disease and the need to introduce other disease control methods. This knowledge is also necessary for designing, implementing and evaluating regional and local interventions directed at optimizing the use of veterinary drugs and improving farming practices. More detailed data on antibiotic use and determinants of use on small farms are needed.

Public health impact of antibiotic use in animal agriculture

The use of antibiotics in livestock has demonstrated benefits, including improved animal health, higher production levels and reduction of foodborne pathogens[10]. However, it has also been suggested that the use of antibiotics can result in a number of problems, including the emergence of antibiotic-resistant bacteria, human and animal illness, economic loss for farmers and dairy processors and environmental contamination[10,21-24]. Antibiotic resistance occurs in bacteria as an inevitable manifestation of bacterial evolutionary capabilities. The resistance of pathological bacteria to available treatments has arisen as a consequence of natural selection, environmental factors and the use and misuse of antibiotics.

Foodborne illnesses can arise as a consequence of the handling or consumption of food or water contaminated with bacterial pathogens. Meat can become cross-contaminated at slaughter and milk can become contaminated through contact with fecal material on milking equipment or cows' udders. The Centers for Disease Control (CDC) estimate that each year 48 million people get sick, 128,000 are hospitalized and 3000 die of foodborne diseases in the US alone[25]; the incidence is likely much higher in LMICs where more people live in close contact with livestock, where food hygiene is not well practiced[26] and where food safety surveillance systems and quality control are generally less developed[27]. Foodborne illnesses with antibiotic-resistant pathogens can lead to treatment failures, and studies have documented excess mortality associated with drug-resistant foodborne pathogens[28]. Foodborne illnesses are becoming more of a global public health threat with the emergence of "superbugs" and the rapid transport of perishable foods facilitated by the global economy[29].

It has been suggested that use of antibiotics in animals can contribute to resistance in a number of ways: the direct transfer of resistant pathogens from animals to human via the food supply or via the environment[30], the transfer of resistance genes to human bacteria[31] and chronic exposure to low levels of antibiotics in the form of food product drug residues[21]. There is conflicting evidence that antibiotic use in animals causes resistant infections in humans[32], however it has been clearly documented that foods of animal origin and direct contact with livestock are sources of antibiotic-resistant bacteria that cause infections in humans[33,34]. Furthermore, the occurrence of antibiotic residues in food products can result not only in acute reactions such as aplastic anemia caused by chloramphenicol or allergic reactions induced by beta-lactam antibiotics, but also chronic effects such as immunodepression, teratogenicity, mutagenicity and carcinogenicity[35].

The inappropriate use of antibiotics can enhance the likelihood of adverse events associated with antibiotics, including the promotion and dissemination of bacterial resistance and the adulteration of animal food products[36]. The inappropriate usage of antibiotics in human medicine in LMICs has been extensively documented[37-46]. The WHO reported that in developing countries throughout the world, the percentage of human patients treated according to clinical guidelines was below 50% regardless of income level of the country, and prescribing patterns were substandard regardless of the type of prescriber[45]. The inappropriate use of antibiotics in LMI countries appears to be due to a variety of factors: ineffective or inexistent regulation of drug distribution and drug quality; over-the-counter availability of antibiotics; unnecessary prescribing of antibiotics by medical professionals or pharmacists; consumer beliefs and perceptions of antibiotics, often reinforced by prescribers or dispensers; economic factors that lead to inappropriate dosing/underdosing[42].

Given that antibiotics are used inappropriately in human medicine in LMICs, it is highly likely that they are used inappropriately in animal agriculture. Furthermore, small farms tend to operate on a subsistence basis and therefore may be more likely to make treatment decisions based on economic factors, which can result in inappropriate dosing or the use of antibiotics of dubious quality. In addition, because animal agriculture tends to be more decentralized in LMICs than in developed countries and characterized more by smallholders farms,[47] a larger proportion of the population in LMI countries lives in close contact with animals, which may increase the likelihood of transmission of resistant microorganisms from animals to humans.

Antibiotic residues in animal food products

When antibiotics are used in animal agriculture, farmers must follow a drug-specific withdrawal time during which the drug is eliminated from the animal's body before its meat or milk can be sold [48,49]. These withdrawal times are established by pharmacokinetic trials conducted by the drug manufacturer. Maximum residue limits (MRL) in meat, milk and eggs are set by regulatory bodies such as the Food and Drug Administration (FDA) [32,48] in the United States or internationally by the Codex Alimentarius of the Food and Agriculture Organization (FAO) of the United Nations (UN). Residues are strictly enforced in developed countries by a system of penalties imposed upon producers if violative residues are detected. While LMICs are encouraged to adopt Codex MRLs by the FAO and WHO, not all national governments do[50]. Furthermore, even if de jure MRLs are adapted, they are rarely enforced, especially when food products are sold on the informal market [51,52].

High rates of contamination of animal food products with antibiotic residues have been documented in LMICs[18,51-58]. In Cajamarca, Peru, up to 71% of raw milk sold in the market has been found to contain antibiotic residues[59,60].

There are many possible reasons why antibiotics could be used inappropriately and residues could occur in food products. Figure 2 illustrates the conceptual framework for the emergence of antibiotic resistance and residues in the food product. A farmer may not be aware of the issue of residues or resistance, may administer antibiotics to cows improperly (in terms of dosage or route of administration), or may not want to lose income from milk which must be discarded if residues are to be avoided[61]. Friedman et al. found that limited finances and lack of time were the principal barriers to following proper antibiotic procedures on dairy farms in South Carolina[61]. Other studies have found management practices, economic factors and farmers' levels of awareness and education to be associated with producing adulterated milk[58,62,63]. In Chile, van Schaik et al. found a number of management factors associated with high somatic cell count in milk,[64] which in turn, may influence the use of antibiotics and the risk of residues; however, this association has yet to be confirmed.

Replicating previous findings and potentially identifying site-specific risk factors associated with the production of milk adulterated with antibiotic residues can aid policy makers, organizations and animal health-care professionals develop ways to improve milk quality and guarantee producers and consumers a high-quality safe food product

While farmers are often the final appliers of antibiotics to livestock, the inappropriate use of antibiotics can also result from the inappropriate prescription of antibiotics. Studies of antibiotic use in humans have found that prescribing practices of physicians and pharmacists, especially in LMICs, are often at the root of inappropriate antibiotic use[38,39,41]. Figure 3 shows a conceptual framework for the role and decision-making process of the provider in prescribing antibiotics. Briefly, provider knowledge, client expectations and the provider's perceptions of clients are considered to be the main drivers behind a prescriber's decision to prescribe an antibiotic[39]. No similar studies examining the role of providers in antibiotic use and misuse have been conducted in veterinary medicine. It is possible that veterinarians and feed-store vendors, who sell antibiotics over-the-counter (i.e., without a prescription), may similarly inappropriately prescribe antibiotics or may not provide adequate instructions to farmers to ensure proper use of antibiotics. The contribution of the veterinarian and feed-store vendor to the appropriate or inappropriate use of antibiotics in animal agriculture consumption warrants investigation to obtain as comprehensive a description of antibiotic use on farms as possible.

Methods of collecting antibiotic use data

As stated previously, very few quantitative data are available on antibiotic use in animal agriculture on small farms in LMICs, and it can be difficult to accurately collect this type of pharmacoepidemiologic data. Data can be collected at the national, regional, local and farm level, from a variety of sources, such as pharmaceutical companies, distributors, feed stores, pharmacies, over-the-counter retailers, veterinary clinics, or farmers directly[65]; however, each source of data can be more or less accessible, especially in LMICs where record-keeping and regulatory oversight may be limited. Data collected from the final consumer (or guardian or owner of consumers – i.e., the farmer) are ideally suited for investigations on patterns of drug use[65]. However, the ascertainment of drug use data from consumers is subject to misclassification of drug exposure due to recall bias, reporting bias or social desirability bias[66]. Using data from prescriptions or sales records can also be unreliable, as such data do not take into account the adherence of consumers or the possibility of obtaining drugs from other sources (over-the-counter drugs, drugs sold on the black market, etc.)[66]. Enhancing the validity of pharmacoepidemiologic data obtained from the final consumer is vital for using antibiotic use data to make inferences or design interventions aimed at improving use. If novel methods can be identified that prove to be more valid and reliable than simple self-report, they can be used in other situations that require the collection of this type of pharmacoepidemiologic data.

A number of methods listed in Table 1 have been used to improve upon self-report of drug use data in both human and veterinary medicine. In human medicine, methods have included the use of medical or pharmacy records [67,68], national health care databases[69,70], drug sales records[71], patient diaries, electronic medication event monitoring systems (MEMS) cap measurements[72] and drug levels in the body[73]; many of these methods have been compared to simple self-report, and varying levels of concordance were found between these methods[74-78]. In veterinary medicine, some of the same techniques have been used, including mailed questionnaires[15,63,79,80], surveying of on-farm treatment records[81,82], use of official state or national-level surveillance systems[15,62], use of sales records[82], tissue residue levels[83] and collection of discarded drug packaging[84]. However, many of these methods are not well adapted to LMICs where, on small farms in particular, treatment records are generally not kept, producers may be illiterate and surveillance systems tend to be rudimentary at best. More reliable and valid methods to ascertain antibiotic use at the farm level that can be specifically adapted to smallholder farms are needed. The collection of drug packaging in buckets was found to be “convenient for producers and useful for estimating or validating recorded treatment rates”[84] and could be useful on small farms where treatments are limited in number.

Study site

With a population of nearly 30 million people, 34.8% of whom live below the poverty line (defined as earning less than $1.25/day), Peru is a middle-income country with a large agricultural focus and a livestock industry worth over $2 billion annually[85]. The dairy industry in Peru has experienced significant growth at a rate of 4.5% per year since 1996; an estimated

108,000 farms with 690,000 cows produced 1.27 million tons of milk in 2005; in comparison, the United States produced 76 million tons of milk with slightly over 9 million cows in 2005[5]. Peru has three major dairy-producing centers: Lima, Arequipa and Cajamarca. Arequipa produces 26% of Peru's milk, Lima 17% and Cajamarca 25%. Dairy farms in Lima and Arequipa tend to be larger, more concentrated and more modernized, whereas Cajamarca is characterized mostly by small peri-urban and rural farms (<15 cows/farm)[86], with 30,000 registered milk producers producing an estimated 307,187 kg of milk a day[87] (Figure 1). The number of large dairy farms is growing in Peru; however, small farms remain the dominant suppliers of milk in Peru in general and in Cajamarca in particular. The major purchasers of milk are dairy processing companies Nestlé and Gloria and local cheesemakers. In Cajamarca, approximately 69% of milk goes to the dairy processing companies (48% to Nestle, 21% to Gloria) while 31% is sold on in the informal market.

Milk in Cajamarca has been found to contain high levels of antibiotic residues: a survey of fresh milk in the markets of Cajamarca in 2007 found that 60.3% of milk samples contained beta-lactam residues, 33.3% contained tetracycline residues and 52.4% contained aminoglycoside and macrolide residues[88]. Another study evaluating milk at the farm level found that 52.5% of farms produced milk with aminoglycoside or macrolide residues[59]. Llanos-Cortesana et al. tested 216 milk samples from the open-air market and farms in Cajamarca and found that 21.2% and 20.8% of these samples, respectively, tested positive[56]. Anecdotally, cheese-makers in Cajamarca have expressed great frustration with high levels of antibiotic residues in milk from their providers, as such residues impede the functioning of starter cultures necessary for making cheese.

To date, no studies have attempted to assess patterns and determinants of antibiotic use on dairy farms in Cajamarca. It is known, however, that 95% of surveyed producers in Cajamarca administer an average of three antiparasitic treatments per year, with 74% of those farmers treating according to clinical signs only[89]. Antibiotic use is thought to occur in a similar manner for similar indications (i.e., only when clinical signs appear in an animal), but this hypothesis remains to be tested. The first goal of this study was therefore to assess patterns of antibiotic use on small dairy farms in Cajamarca and to assess the appropriateness of antibiotic use. The second related goal of this study was to determine the proportion of farmers who sell milk contaminated with antibiotic residues and to identify factors associated with doing so.

As mentioned previously, prescribers of antibiotics play a significant role in promoting the appropriate use of antibiotics on small farms. However, very few studies have examined the role of prescribers (veterinarians and feed-store vendors) in the sale and dispensing of antibiotics in dairy medicine on small farms in LMI countries. The third aim of this study was therefore to obtain the perspectives of antibiotic providers on current antibiotic use and its appropriateness by dairy farmers in Cajamarca and to determine which factors influence a provider's prescribing practices.

Finally, because the methods used to collect antibiotic use data are subject to inaccuracies and biases, the two methods of collecting antibiotic use data best suited to small farms in LMI countries (self-report and the collection of discarded drug packaging) were compared to determine whether these methods performed similarly.

Detailed rationale, methods, and results of these four objectives are presented in Chapters 2, 3, 4 and 5. Results of these studies will shed light on an important topic on which little is known that could be relevant to small farms in other LMI countries. The results will be useful for animal-health professionals, policy makers, farmers and consumers of dairy products and can be used to design interventions to improve the use of antibiotics on farms and the quality of dairy products. These studies will also provide results that can be useful to other investigators seeking to collect pharmacoepidemiologic data on farms in similar settings and will inform future studies and funding proposals aimed at improving antibiotic use on small dairy farms in LMICs.

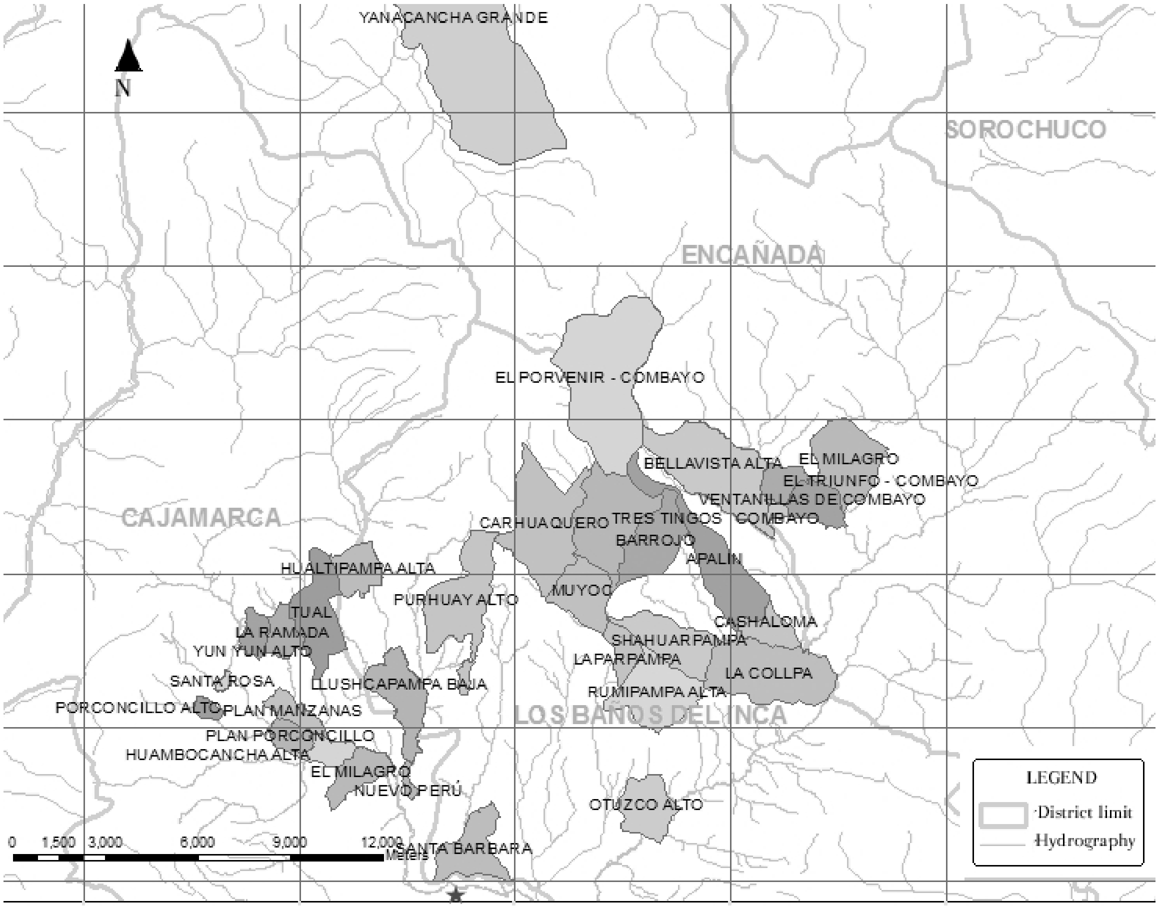

Figure 1 Map of the region of Cajamarca and the communities visited in this study.

Farmers from the shaded communities were interviewed in our first study (Aim 1). The star indicates the city of Cajamarca.

Figure 2 Conceptual framework for antibiotic use, antibiotic resistance and antibiotic residues on dairy farms

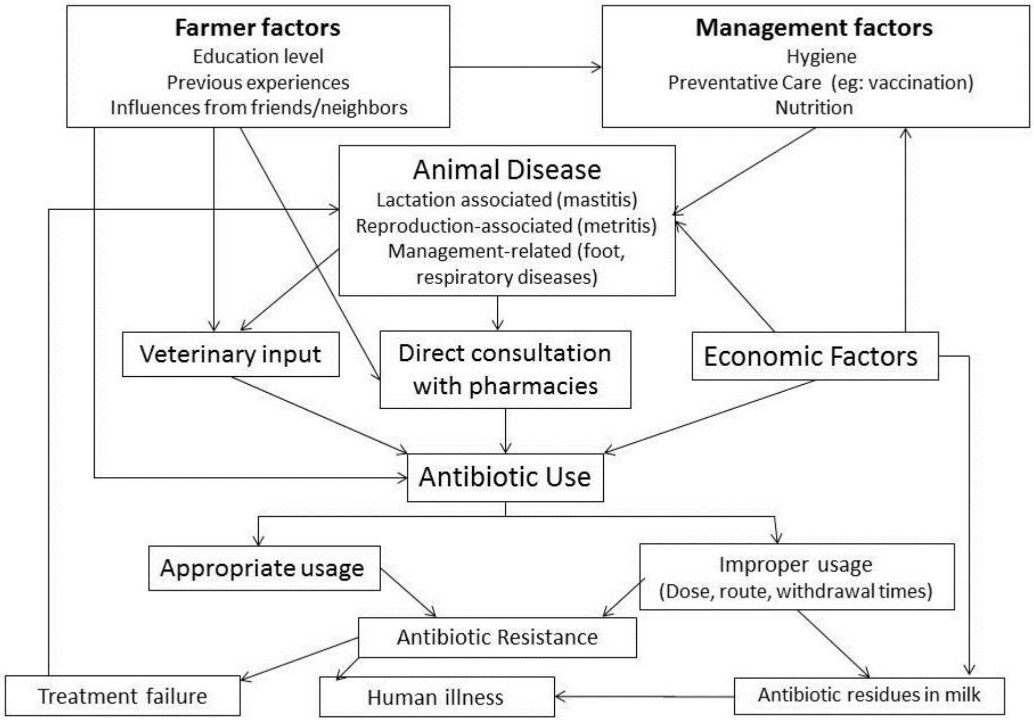

Figure 3 Conceptual Framework for provider antibiotic prescribing practices

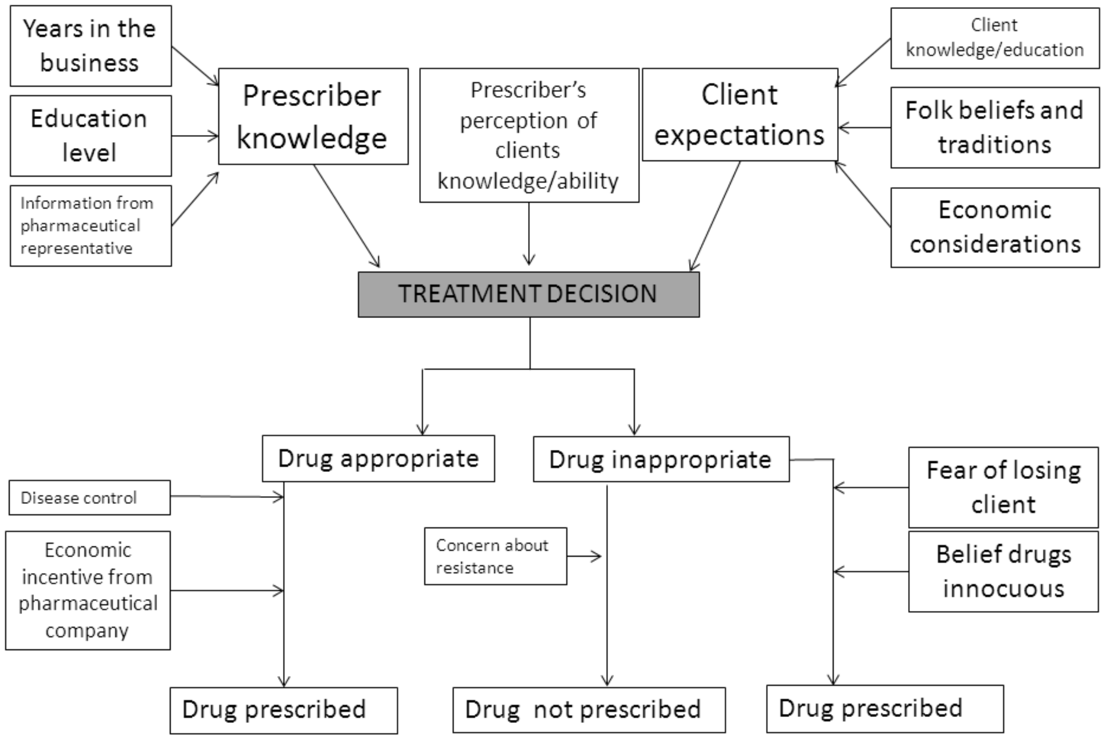

Table 1 Examples of methods used to acquire pharmacoepidemiological data in human and veterinary medicine and selected references of studies using the relevant methods.

Human Medicine	Veterinary Medicine
In-person interviews of patients[66,74]	Mailed/online questionnaires[15,63,79,80,90]
State or national-level health care databases[69,70]	State or national-level surveillance systems[15,62,91-93]
Drug sales records[71]	Drug sales records[16,82,94-96]
Medical records[67]	In-person interviews of farmers[19,97-100]
Pharmacy records[68]	On-farm treatment records[81,83,101-103]
Patient diaries[72]	Tissue drug levels[83]
MEMS cap measurements[72]	Collection of drug packaging[84,102,104]
Drug levels in the body[73]	

CHAPTER 2 : Patterns and determinants of antibiotic use on small dairy farms in Cajamarca

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Abstract

Very little is known about the use of antibiotics on small dairy farms in lower/middle-income countries. The use of these drugs can have profound impacts on animal health, farmer income and public health. A survey of 156 farmers was conducted in Cajamarca, a major dairy-producing center in the highlands of Peru characterized by small farms (<15 cows) to assess patterns and determinants of antibiotic use and farmers' knowledge of antibiotics. The reported incidence of disease on these farms was relatively low (0.571 episodes of disease per cow-year), but more than 83% of the reported episodes were treated with antibiotics. The most commonly used antibiotics were oxytetracycline, penicillin and trimethoprim-sulfamethoxazole drugs; antiparasitic drugs were also used to treat what were likely bacterial infections. An increased incidence of treated disease was significantly associated with smaller farm size, lower farmer income, the previous use of the Californian Mastitis test on the farm and antibiotic knowledge. Farmers' knowledge of antibiotics was assessed with a series of questions on antibiotics, resulting in a "knowledge score". Increased knowledge was significantly associated with the use of antibiotics for preventative reasons, the purchase of antibiotics from feed-stores, the experience of complications in animals after having administered antibiotics, the number of workers on the farm and the educational level of the farmer. Overall, antibiotics appeared to be used infrequently, most likely because therapeutic interventions were sought only when the animal had reached an advanced stage of clinical disease. Few farmers were able to define an antibiotic, but many farmers understood that the use of antibiotics carried inherent risks to their animals and potentially to the consumers of dairy products from treated animals. The results of this study are useful for understanding the patterns of antibiotic use and associated management, demographic and knowledge factors of farmers on small dairy farms in rural Peru.

1. Introduction

Small farms constitute the majority of farms in the developing world [5], and in many low/middle income (LMI) countries, they are still predominate suppliers of animal products to their domestic markets. Dairy production is a rapidly expanding sector of animal agriculture in the developing world because of population growth, increases in per capita income, urbanization and the westernization of diets [6,9]. However, few countries where dairy production is growing have adequate systems to ensure food animal product safety and quality. One of the areas where this is most evident is the use of antibiotics in animal agriculture. Very little is known about antibiotic use on small farms in LMI countries.

In the dairy industry, antibiotics are primarily used for therapeutic and prophylactic purposes[11]. These uses have demonstrated benefits, including improved animal health, higher production levels and the reduction of foodborne pathogens[10]; however, they can also result in a number of problems, including the emergence of antibiotic-resistant bacteria, human and animal illness, economic loss for farmers and dairy processors and environmental contamination[21,23,105]. The inappropriate use of antibiotics, defined by the World Health Organization as overprescription, underprescription, inappropriate dosing, an incorrect duration of treatment or the incorrect choice of drug for the relevant organism, can exacerbate these problems.

Knowledge of patterns of antibiotic use is fundamental to understanding farming practices and animal health on small farms; furthermore, a basic knowledge of how drugs are used can provide some measures of drug misuse, the magnitude of the risk of disease and an the need to introduce other disease control methods. This knowledge is also necessary for designing, implementing and evaluating regional and local interventions directed at optimizing the use of veterinary drugs and improving farming practices.

Very few attempts have been made to document antibiotic usage on smallholder dairy farms in either the developed or the developing worlds. The studies that have evaluated antibiotic use on farms have either only examined a small number of farms[17,19] or enrolled farms through mailed questionnaires, resulting in low response rates and potential selection bias[79,80,84]. The aim of this study was therefore to comprehensively assess patterns of antibiotic use on small dairy farms in Cajamarca, a major dairy-producing center in the northern highlands of Peru. Cajamarca is characterized mostly by small peri-urban and rural farms (<15 cows/farm) with 30,000 registered milk producers[86] producing an estimated 307,187 kg of milk per day [87]. The farms encountered in Cajamarca are typical of small dairy farms in many other LMI countries, especially in Latin America.

2. Materials and Methods

2.1 Participants

This cross-sectional study was conducted in the countryside surrounding the city of Cajamarca, the capital of the region of Cajamarca. A list of randomly selected farmers was generated from among the farmers who work with the non-profit organization Foncreagro using simple random sampling. Foncreagro is a non-profit organization that works with small farms to develop agricultural projects related to improved farming practices and sustainable development. Foncreagro works with approximately 6000 farmers in two provinces and five districts of the region of Cajamarca. All farmers who agreed to participate provided verbal consent, and approval for this study was granted by the Institutional Review Boards of the University of Pennsylvania and the Universidad Peruana Cayetano-Heredia in Lima.

2.2 Questionnaire

The questionnaire developed for this study was adapted from questionnaires used by Zwald et al. (2004), Sawant et al. (2005), Raymond et al. (2006) and Jimenez-Velasco (2002). The questionnaire was divided into four sections: 1) information on the farms and animals, 2) disease incidence, antibiotic use and knowledge of antibiotics, 3) farm management and 4) demographic and economic information pertaining to the farmer. A copy of the questionnaire is included in the appendix with indicators of the test-retest reliability of the survey in the sampled population for a subset of questions. Questionnaires were piloted on a convenience sample of ten dairy farms in two villages outside of Cajamarca and optimized before they were administered to the full sample of farms. All questionnaires were administered in Spanish by a Peruvian veterinary student and a veterinary student from the United States. After the questionnaire was administered, the California Mastitis Test (CMT) was performed on all lactating cows on the farm and an average CMT score was generated for each farm. The California Mastitis Test (CMT) measures the somatic cell count in milk, which reflects the degree of inflammation present in the udder. Ali and Shook (1980) showed that a log transformation of SCC to a somatic cell score of the type used in the CMT achieves nearly normal distribution, and a CMT score of zero (corresponding to 200,000 cells/ml) is a generally accepted cut off with high sensitivity and specificity for intra-mammary infection[106].

2.3 Disease incidence and drugs used

Farmers were asked about disease incidence and drug use on their farm in various ways. First, they were asked if they had used antibiotics to treat any diseases in the past year, and if so, to name the drugs used. Next, they were shown pictures of antibiotics available on the local market and asked if they had used any of those drugs in the past year. Finally, they were asked about specific categories of disease (mastitis, peri-parturient infections, respiratory infections, diarrhea, skin/foot infections and others (mostly non-specific symptoms such as febrile or off-feed)) in the past year. Farmers who reported that their cows experienced one or more episodes of disease were asked how many episodes they had observed, if they had treated any of the episodes, and, if so, with which antibiotic (using the illustrations to guide their choices).

2.4 Knowledge score

A knowledge score was generated to assess farmers' understanding of what antibiotics are and the risks associated with their use. First, farmers were asked if they knew what an antibiotic was and to define the term in their own words. Farmers were also asked if they knew what drug withdrawal times were and to define them in their own words. For each of these questions, zero points were awarded for not knowing and one point was awarded for knowing and providing an appropriate definition. Subsequently, farmers were presented with three questions: “Do you think that the use of antibiotics could produce allergic reactions in animals”, “Do you think that using the same product more than once can create resistance” and “Do you think milk from treated cows is good for human consumption”; farmers could answer “Yes”, “Don’t know” or “No”. Correct answers, “Don’t know” and incorrect answers were assigned scores of 2, 1 and 0 points, respectively. A “knowledge” score consisting of the sum of the points obtained for these five questions was generated. The score thus ranges from 0 to 8 points; a score of 3 could indicate no knowledge, a score of less than 3 indicates incorrect knowledge and a score of more than 3 indicates some knowledge.

2.5 Milking hygiene score

The milking hygiene score was similarly composed of points attributed to answers for the following questions: “Do you clean the udder before milking?” (Never=0 points, Sometimes=1, Always=2); “What do you clean the udder with?” (Water=0, Water and soap=1, Disinfectant=2); “Do you clean your hands before milking?” (Never=0, Sometimes=1, Always=2); “Do you clean your hands between milking different cows?” (Never=0, Sometimes=1, Always=2); and “Do you seal the teats with iodine after milking?” (Never=0, Sometimes=1, Always=2).

2.6 Statistical analysis

Data from questionnaires were examined for normality (using a skewness/kirtosis test) and missing values and described in terms of means, standard deviations and ranges or medians and interquartile ranges for continuous variables and frequencies for categorical variables. Drugs used for various indications were compiled for each farm, and the rates of each treatment (number of antibiotic treatments/cow-year) were calculated. Incidences of treatment per cow-year were determined for each farm with a Poisson model with the number of cows (dry and lactating) on the farm as an offset and no covariates. Associations between the treatments rates and demographic, management and knowledge-related factors were assessed with a negative binomial regression model, where cow-years was included as an offset for each farm. This model allows for an assessment of extra-variability (that is, variability beyond what would be expected in a Poisson distribution) to serve as a test of model fit (Horton et al., 2007). A p-value entry threshold of 0.2 was used for initial variable selection along with a backwards elimination strategy to develop multivariable models and assess confounding. Risk factors with a p-value of <0.05 and any confounders that altered other associations by 15% or more were retained in final models. An additional analysis using linear regression was conducted to determine factors associated with farmers' antibiotic-knowledge score. The selection of variables proceeded as described above. A sub-sample of 13 farmers were administered the survey twice two weeks apart to assess the test-retest reliability of the survey. Intraclass correlation coefficients and kappa coefficients were calculated to determine the reliability of continuous and categorical parameters, respectively. All analyses were conducted in Stata, v.11 (StataCorp. College Station, TX).

3. Results

3.1 Participants

A total of 168 farmers from 48 villages were invited to participate in the study (Table 2); 12 farmers declined to participate, resulting in a participation rate of 92.9%. Two farmers (one man and one women) tended to work on each farm, and 92 (59.0%) of the participants interviewed were women. The mean age of the farmers was 45 years, and more than half of them were analphabetic. The farms had a median size of seven cattle, including three lactating cows producing a median production of 5.63 L of milk per day each. For more than 90% of the farmers, the sale of milk was their sole of source of income, and farmers earned approximately 300

Peruvian Nuevo Soles ($115 according to the 2013 exchange rate) on a monthly basis. More than half (57.1%) of the farmers sold their milk to dairy processing companies (Nestlé or Gloria), while 35.3% and 7.7% of farmers sold their milk to cheese-makers and on the open market, respectively. The median CMT score on farms was 0.5 on a scale of 0-3 (0=negative, 1=weak positive, 2=distinct positive, 3=strong positive), which corresponds to a somatic cell count of approximately 300,000.

3.2 Disease incidence

Reported disease incidence was low, with an average incidence of 0.571 episodes of disease per cow-year, the majority (83.5%) of which were treated with antibiotics. Clinical mastitis occurred most frequently, followed by diarrhea, respiratory infections and peri-parturient infections (Table 3).

3.3 Antibiotic Use

Data were gathered on 216 reported treatments (Table 4). The most commonly used antibiotic was oxytetracycline, used in 107 of 216 (49.5%) reported treatments. Penicillin with or without streptomycin was used in 23 of 216 treatments (10.6%). Trimethoprim/sulfamethoxazole was used in 15 of 216 (6.5%) treatments. Cloxacilin intra-mammary injections were used in 25 of 59 (42.4%) reported cases of mastitis and in one case of metritis. Antiparasitic drugs, including fenbendazole, albendazole, levamisole and triclabendazole, were often used to treat diarrhea and infrequently used to treat respiratory disease.

Antibiotics were mostly used for therapeutic purposes; however, 27.2% of the farmers reported using antibiotics (either oxytetracycline or cloxacilin) for prophylactic purposes post-calving and/or for drying off.

3.4 Knowledge of antibiotics

When asked “Do you know what an antibiotic is?”, 35 of 156 (22.4%) farmers answered “Yes”. When asked to define the drug in their own words, answers ranged from “a medicine”, to “something for infections”, to “for fever or mastitis”; only one farmer knew that antibiotics specifically killed bacteria. Fifty-one of 156 (33.1%) farmers knew what drug withdrawal times were, and the majority defined this term as “when you are not supposed to send milk to the truck”.

The mean scores for the three questions pertaining to risk (risk of an allergic reaction, risk of producing resistance and risk for consumers) were 1.4, 1.5 and 1.7, respectively, of a maximal two points.

The mean knowledge score was 5.2 (SD=1.7) of a maximal 8 points, suggesting that, while farmers might not have been clear on the nature of antibiotics, they tended to understand that their use carried an inherent risk to their animals and potentially to the consumer.

The knowledge score was significantly associated with a number of factors (Table 5). Farmers who bought antibiotics from a feed-store themselves, farmers who used antibiotics for preventative reasons, farmers who administered more drugs when treatment failure occurred, farmers who observed complications in their animals after administering antibiotics, more highly educated farmers and farmers from farms with more workers had higher antibiotic-knowledge scores.

3.5 Acquisition of antibiotics

Antibiotics can be obtained from three sources in Cajamarca: a veterinarian, a feed-store or travelling drug distributors. Fifty-five (35.3%), 35 (22.4%) and eight (5.1%) farmers reported obtaining their drugs from a veterinarian only, a feed-store only and a distributor only, respectively; 47 (30.1%) farmers reported getting their drugs from a veterinarian and from the feed-store.

The farmers who reported buying drugs themselves at the feed-store were subsequently asked which factor was most important when buying antibiotics; 66 (80.5%) farmers reported that the recommendation of the veterinarian or feed-store vendor was the most important factor; 27 (32.9%) cited the quality of the product or the drug manufacturer as the determining factor, while eight (9.8%) stated that previous experience with the drug directed their choice. Seven farmers (8.5%) stated that the price of the drug drove their decision, acknowledging that the cheapest drug was often not the most effective choice.

3.6 Administration of antibiotics

Sixty-five (41.7%) farmers reported that they never treated their animals themselves and instead let the veterinarian or technician administer all treatments, while 91 (58.3%) farmers stated that they administered treatments to their animals themselves. Of the farmers who reported treating themselves, 73 (80.2%) reported always following the dosage recommended by the prescriber; of the 18 (19.9%) farmers who said they did not follow the recommended dosage, 15 (83.3%) and three (16.7%) farmers reported not doing so because the animal appeared better after the first dose and because the drug caused secondary effects, respectively.

Forty-five (29.0%) farmers reported having observed complications when antibiotics were administered to their animals, including weakness, vocalization, salivating, going off feed, inflammation at the site of injection, diarrhea, falling down and running around. Twenty-six (17.5%) farmers stated that they had never experienced treatment failure (i.e., treatments always cured the animal); of the farmers who had experienced treatment failure at some point (i.e., the animal did not improve after an initial treatment), 22 (16.9%) reported that they had increased the dose, 84 (64.6%) reported that they had administered a different drug, and 27 (20.8%) reported that they had sold the cow.

3.7 Milking hygiene score

The mean (SD) milking hygiene score was 8.78 (1.7) out of a maximal 13 points. One-hundred forty farmers (89.7%) reported always cleaning the udder (65.3% with water, 33.3% with water and soap and 1.4% with disinfectant), and 150 farmers (96.2%) reported always cleaning their hands before milking.

3.8 Factors associated with antibiotic treatments

In the regression, the incidence rate of treated episodes of disease was associated with a number of factors. Larger farms, defined as having more than seven cattle (the median number of cattle on farms in the sample), had fewer treatments per cow-year than smaller farms (incidence rate ratio (IRR)=0.72, 95% CI=0.54-0.97, p=0.016). Farmer income was negatively associated with treatment incidence: for every increase in 100 soles ($38.5) of income, the rate of treatments per cow-year decreased by 4.0% (IRR=0.96, 95% CI=0.934-0.998, p=0.015). The previous use of the California Mastitis Test on the farm (by either the farmer him/herself or by a veterinarian) was associated with a 59% increase in the incidence of treatments (IRR=1.59, 95% CI=1.24-2.04, p<0.0001). Finally, the knowledge score was also associated with treatment: for every one-point increase in knowledge score, an increase in the incidence rate of treatment of 8.2% was observed (IRR=1.08, 95% CI=1.01-1.17, p=0.033).

4. Discussion

Understanding antibiotic use on small farms in a region such as Cajamarca is important for a number of reasons. Small farms constitute the majority of providers in most of the developing world, and the use of antibiotics on these farms has implications for animal health, public health and farmer income (and therefore profitability and sustainability). We found that antibiotics were used relatively infrequently on surveyed farms (0.48 treated episodes of disease per cow-year) but that they were used more than 83% of the time when disease occurred.

The low incidence of disease was surprising, especially given that most of the animals are exclusively raised on low-quality pasture, frequently infested with parasites and provided with little preventive care (vaccination, concentrates, vitamins, etc.). A possible reason for this low reported incidence is that farmers may only notice (and remember) and seek veterinary care or treatment when the animal has reached an advanced stage of disease. Infections in chronically under-nourished animals may not initially appear dramatic to the farmer, and self-limiting diseases may not be noticed. Farmers may only decide to treat their animals when the animal has reached a clinical stage that can no longer be ignored. Training farmers to provide preventative care and recognize early signs of disease can be very useful in preventing animals from reaching the late stages of disease when treatments are less likely to be effective.

An increased incidence of treated disease was significantly associated with smaller-sized farms and lower incomes. This may seem counter-intuitive, as one would expect larger farms with more animals to be more likely to experience disease. However, farmers from larger farms and farmers with higher incomes had significantly higher levels of education (p=0.002 for trend) and therefore may have adopted better management practices that improved the general health of their animals. For example, education was significantly associated with the milking hygiene score (p=0.01 for trend), which represents one aspect of management that can significantly contribute to reducing the incidence of mastitis. Morrison et al. (1991) reported that the incidence of certain reproductive diseases was higher on small farms than on medium-sized farms in Columbia[107]; Hill et al. (2009) also reported that the within-herd prevalence of disease in the United States decreased with increasing herd size and attributed this finding to differential management strategies adopted within different-sized herds[97]. It is unclear why exposure to the CMT was associated with increased incidence of disease. It is possible that farms that experience higher rates of disease seek treatment from veterinarians who employ the CMT on their farm – in other words, disease may have preceded exposure to the CMT. Similarly, the association between increased incidence of disease and increased antibiotic knowledge score is likely due to the fact that farmers who experience disease and treatment more often are more knowledgeable about antibiotics (Table 4).

A limited number of active ingredients was used on the farms (oxytetracycline, cloxacilin, penicillin and trimethoprim-sulfamethoxazole), and antiparasitic drugs were sometimes used to treat what likely would have been bacterial infections. Studies of small farms conducted in other LMI countries have indicated that a similarly small number of active ingredients are used to treat cattle. In a survey of 60 small farms in Costa Rica, Luna-Tortos et al. (2006) reported that penicillins, tetracyclines and aminoglycosides were the most commonly used drugs and that cephalosporins and fluoroquinolones were used infrequently (10 and 15% of treatments, respectively)[19]. In a survey of 155 farmers in Hyderabad, India, Sudershan and Bhat (1995) reported that oxytetracycline was the most commonly used antibiotic in cattle and buffalo and was used by 55% and 38% of urban and rural farmers, respectively[18]. Three herds of cattle belonging to Maasai pastoralists in Kenya were followed for four years by Roderick et al. (2000) who reported that oxytetracycline was used at a rate of 0.20-1.00 treatments per animal-year[17]. In Sudan, tetracyclines, penicillins, quinolones and sulfonamide-trimethoprim antibiotics were used in 25, 18, 32 and 4% of treatments (n=73), respectively[108]. In contrast, a survey of large dairy and beef farms in Lima reported that 15 active ingredients were commonly used[109], while a survey of 113 dairy herds in Pennsylvania reported that 24 different types of antibiotics were used[79]. Pol and Ruegg (2007) reported that conventional dairy farms in the United States used an average of 5.43 defined daily doses of antibiotics per cow-year[110], which is significantly more than was reported to be used on the farms of the present study.

The use of a limited number of drugs on small farms can enhance antibiotic resistance to these drugs[111] and increase the likelihood of treatment failure. While it was unclear from these interviews why so few active ingredients were used, veterinarians interviewed on antibiotic use in Cajamarca in a previous study[112] mentioned that a limited number of active ingredients were available on the market and that farmers were often accustomed to using the same products repeatedly. Subsequent studies would need to be conducted to determine the degree of resistance to these antibiotics that exists on these farms. If the degree of resistance is high, animal health professionals could encourage the use of alternate active ingredients. The addition of new active ingredients to treatment regimens in Cajamarca would require the importation of new drugs, the promotion of these new ingredients by prescribers and the familiarization of farmers with these drugs. If, on the other hand, the degree of resistance in the field is low and if these treatments remain effective, then the use of a small number of active ingredients could actually be beneficial. It was interesting to note that no farmers claimed to have used drugs such as third or fourth generation cephalosporins (which are considered critically important antimicrobials by the WHO). Because a majority of dairy farmers in developed countries use these drugs on a regular basis for prophylactic purposes (e.g., drying off)[79], there is concern that bacterial resistance to these products may be developing, resulting in the potential diminished efficacy of these drugs.

The use of antiparasitics drugs to treat what are probably bacterial or viral infections is of concern as well. Not only will these treatments likely be ineffective, but they will also exacerbate the already-significant problem of drug-resistant fascioliasis that exists in Cajamarca, where prevalence in cattle has been reported to be 78%[113] and resistance to commonly used antiparasitic drugs can reach 87% (unpublished data). Indeed, 95% of surveyed producers in Cajamarca administered an average of three antiparasitic treatments per year to their livestock[89]; using antiparasitics even more frequently is likely to exacerbate the problem of resistance, which, in turn, will make parasitized animals even more predisposed to opportunistic infections.

Farmers appeared to have some knowledge of and familiarity with antibiotics. Although only 38% of farmers reported knowing what an antibiotic was, the majority of farmers thought that antibiotics could provoke an allergic reaction or generate resistance (i.e., that it was bad to use the same product repeatedly) and that milk with residues was bad for consumers (55.6, 58.6 and 75.0%, respectively). A study conducted in Chile reported that 60.4% of farmers (n=926) reported knowing what an antibiotic was and that 42.6% of these farmers specifically associated antibiotic with infectious disease[114]. Education, as expected, was positively associated with knowledge. Eltayb et al. (2012) also concluded that higher levels of education were significantly associated with higher levels of knowledge of antibiotic resistance and disease among farmers in Khartoum. Buying antibiotics from a feed-store was also associated with a higher knowledge score, most likely because a farmer who went to purchase antibiotics him/herself may have been more knowledgeable about animal health and confident in his/her ability to treat the disease. Similarly, a farmer who used antibiotics for preventative reasons most likely used antibiotics more often than a farmer who did not and therefore had more familiarity with these drugs. A farmer who had observed complications in his/her animals after drug administration was more likely to have a higher knowledge score, most likely because he/she was more likely to assign greater risk to antibiotic use. Finally, farmers from farms with more workers (family members) had higher knowledge scores, most likely due to the effect of pooled knowledge and greater interactions (and therefore communication) between farmers.

Overall, farmers in Cajamarca do not appear to be overusing antibiotics in their cattle. Antibiotic use was infrequent, and farmers' knowledge of antibiotics and the risks inherent in their use was adequate. The relatively high number of farmers (29%) who had observed complications/side-effects in their animals after administering antibiotics suggests that antibiotics may not have been administered correctly and that the farmer may not have received sufficient instructions on how to properly administer them. For example, some of the complications experienced by the animals being treated by the farmers included shock, vocalizing, falling down, salivating and "going crazy". These types of reactions suggest that the drug may have been administered incorrectly (e.g., incorrect dose, administering the drug into a vein instead of into a muscle).

Because we only asked farmers to enumerate episodes of treated disease, we were not able to assess whether appropriate drug doses were applied. While 80.4% of farmers reported always following the dosage recommended by the veterinarian or feed-store vendor, it is not clear whether the appropriate dosages were actually prescribed and followed. Indeed, veterinarians and feed-store vendors in Cajamarca cited underdosing as a serious problem and mentioned sometimes under-prescribing because of limited economic means of farmers[112].

Certain management factors that can influence the incidence of disease (such as the milking hygiene score) appeared adequate. The non-profit organization Foncreagro has conducted a number of training sessions on good milking hygiene, and milk companies often try to instruct their farmers on good milking hygiene, which may explain the relatively high hygiene scores. Other factors, however, appeared lacking; for example, only 17.9% of farmers reported vaccinating their animals, and 54.4% of farmers reported never giving their animals concentrates. Interventions to improve preventative care in animals could be of great importance to improving animal health.

Several limitations apply to this study. Recall bias among farmers may have resulted in the underreporting of drug treatments and the attenuation of associations between drug use and demographic/management factors. However, because the obtention of self-reported pharmacoepidemiological data can be enhanced by asking questions in a variety of ways (i.e., with visual prompts)[66], asking farmers to recall drug treatments in a variety of ways (directly, by association with disease entities and using pictures of antibiotics available on the market) likely mitigated issues of poor recall. In addition, answers related to the incidence of disease had high reliability (intraclass correlation coefficient of 0.80). It is also possible that social desirability may have influenced some of the answers provided by the farmers, especially those related to proper drug use (e.g., always following the dose prescribed) and management (e.g., milking hygiene). However, the purpose of the survey was explained to the farmers and it was made very clear that their participation and responses would have no impact on their interaction with Foncreagro or on the care administered to their animals. Because this was a cross-sectional study, it is also possible that some of our findings may be due to reverse causality. For example, the finding that farmers exposed to the CMT had higher rates of treated disease may be due to the fact that the CMT was used to diagnose the disease. Nevertheless, these associations are important to elucidate, as they point to the interconnected nature of farmer knowledge, farm management and disease incidence.

Finally, the results of the study may not be generalizable to other small dairy farms in other LMI countries. In particular, because these farmers were involved with Foncreagro, they may have received training and educational interventions that other farmers may not have received and therefore may have had improved knowledge and awareness of good farming practices. However, even if these farmers represent best-case scenarios, we were still able to glean an understanding of the patterns of and indications for antibiotic use that are likely generalizable to farms of this size and type in other countries.

Table 2 Characteristics of small farms and farmers (n=156) participating in a study on antibiotic use in Cajamarca, Peru.

Farm or Farmer characteristic	No. of respondents
Farmers interviewed - women, n(%)	92/156 (59)
Mean (SD, min, max) age (years)	45.0 (15.1, 17, 89)
Education level, n(%)	
None (analphabetic)	84/156 (53.6)
Some primary school	58/156 (37.2)
Some secondary school	14/156 (8.97)
Median (interquartile range) number of farmers on each farm	2 (1-3)
Men	1 (0-1)
Women	1 (1-2)
Median (interquartile range, min, max) number of animals on each farm	7 (5-9, 1, 27)
Cattle (cows, calves/heifers and bulls)	3 (2-4, 1, 14)
Lactating cows	1 (0-2, 0, 7)
Dry cows	
Median (interquartile range, min, max) amount of milk produced daily (L)	16 (10-25, 3, 120)
On-farm total	5.63 (4.58-8, 2, 18)
Per cow	
Median (interquartile range, min, max) monthly income from milk ($)	115 (67.3-173, 15.4, 1076)
Destination of milk, n(%)	
Nestlé	24/156 (15.4)
Gloria	65/156 (41.7)
Cheese-makers	55/156 (35.3)
Home consumption/open market	12/156 (7.69)
Median (interquartile range) mastitis score on a scale of 0 to 3	0.5 (0-1)

Table 3 Incidence of treated and untreated disease on small dairy farms in Cajamarca, Peru (n=156)

Type of disease		Number of episodes on all farms	Incidence rate (number of episodes/cow-year)	
			Rate	95% CI
Mastitis	Total	126	0.182	0.152-0.216
	Treated	102	0.147	0.121-0.178
Peri-parturtient infections	Total	68	0.100	0.080-0.126
	Treated	66	0.095	0.078-0.121
Respiratory infections	Total	82	0.119	0.096-0.148
	Treated	74	0.106	0.085-0.133
Diarrhea	Total	76	0.101	0.080-0.127
	Treated	70	0.082	0.063-0.106
Skin/Foot infections	Total	11	0.016	0.009-0.029
	Treated	8	0.011	0.006-0.023
All diseases	Total	363	0.571	0.512-0.630
	Treated	320	0.477	0.428-0.531

Table 4 Antibiotics used in reported treatments on small dairy farms in Cajamarca

Disease	Drug	Percent of reported treatments
Mastitis	Cloxacilin intra-mammary	42 (25/59)
	Penicillin±Streptomycin	27 (16/59)
	Oxytetracycline	22 (13/59)
	Cephalexin intra-mammary	6.8 (4/59)
	Other	1.7 (1/59)
Peri-parturient infections	Oxytetracycline	89 (42/47)
	Penicillin±Streptomycin	8.5 (4/47)
	Cloxacilin	2.1 (1/47)
Respiratory disease	Oxytetracycline	80 (45/56)
	Penicillin±Streptomycin	5.4 (3/56)
	Trimethoprim/sulfamethoxazole	5.4 (3/56)
	Tylosin+Gentamycin	3.6 (2/56)
	Antiparasitics	1.8 (1/56)
Diarrhea	Antiparasitics	54 (29/54)
	Trimethoprim/sulfamethoxazole	22 (12/54)
	Oxytetracycline	13 (7/54)
	Tylosin+Gentamycin	1.9 (1/54)

Table 5 Identification of factors associated with the antibiotic knowledge score by linear regression analysis in a sample of 156 dairy farmers in Cajamarca, Peru

	Univariate analysis			Multivariate analysis		
	Coefficient	p-value	95% CI	Coefficient	p-value	95% CI
Farmer buys antibiotics from feed-store	0.98	<0.001	0.440-1.52	0.65	0.009	0.165-1.13
Antibiotics used for preventative reasons	0.79	0.014	0.166-1.42	0.67	0.019	0.110-1.22
In response to treatment failure, the farmer increases the dose	0.86	0.026	0.103-1.61	0.77	0.027	0.087-1.45
Farmer has observed complications in animals administered antibiotics	1.29	<0.001	0.726-1.86	0.65	0.019	0.106-1.19
Education (compared to none)						
Some primary	0.84	0.003	0.281-1.40	0.61	0.017	0.109-1.10
Some secondary	1.50	0.002	0.563-2.45	1.07	0.011	0.248-1.90
Number of workers on the farm	0.46	<0.001	0.216-0.716	0.29	0.01	0.073-0.517

CHAPTER 3 Antibiotic residues in milk from small dairy farms in Cajamarca

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Abbreviations:

CC: Cheese-making company

MC1: Milk Company 1

MC2: Milk Company 2

NPV: Negative predictive value

PPV: Positive predictive value

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Abstract

The use of antibiotics in livestock can pose a public health threat, especially if antibiotic residues remain in the food product. Understanding how often and why farmers sell products with antibiotic residues is critical to improving the quality of these products.

The goal of this study was to understand how often milk with antibiotic residues is sold on small farms in a major dairy-producing region of Peru and identify factors associated with selling milk with antibiotic residues. First, we tested samples of milk from every provider on six routes of three commercial milk companies for antibiotic residues on a single day. Second, we tested milk samples from the bulk tanks of farmers who were currently treating cows with antibiotics one day after the last treatment was administered. Third, we asked farmers what they did with milk from treated cows and examined factors associated with the tendency to report withholding milk from treated cows. The point prevalence of milk contamination with antibiotic residues on commercial routes was low (0-4.2%); however, 33/36 farmers treating their animals with antibiotics sold milk that tested positive for antibiotic residues. The self-reported sale of milk from treated cows had a sensitivity, specificity and positive and negative predictive value of 75.8%, 100%, 100% and 27.2%, respectively (with testing of milk for residues as the gold standard). Finally, 69 of 156 randomly selected farmers reported selling milk from treated cows, and farmers' knowledge of antibiotics and the milk purchaser were significantly associated with a farmer's tendency to report doing so.

Educating farmers on risks associated with antibiotics and enforcements of penalties for selling contaminated milk by milk companies are needed to improve milk quality.

1. Introduction

The use of antibiotics in dairy cattle can improve animal health and productivity [10]; however, these drugs can represent a public health threat, especially if residues end up in the final food product[21]. The chronic exposure of consumers to antibiotics can result in allergic reactions, toxicity and antibiotic resistance[115]. Residues can also diminish the economic value of milk by interfering with bacterial cultures needed to make cheese and yoghurt.

When antibiotics are used in animal agriculture, farmers must follow a drug-specific withdrawal time established by pharmacokinetic trials during which the drug is eliminated from the animal's body before its meat or milk can be sold [48,49]. Maximum residue limits (MRL) in meat, milk and eggs are set by regulatory bodies such as the Food and Drug Administration (FDA) [48,63] in the United States or internationally by the Codex Alimentarius of the Food and Agriculture Organization (FAO) of the United Nations (UN). Residues are strictly enforced in developed countries by a system of penalties imposed upon producers if violative residues are detected. While lower/middle income (LMI) countries are encouraged to adopt Codex MRLs by the FAO and WHO, not all national governments do [50]. Furthermore, even if *de jure* MRLs are adapted, they are rarely enforced, especially when food products are sold on the informal market [48,52].

High rates of contamination of dairy products with antibiotic residues have been documented in many LMI countries [18,51-57,59] where quality assurance programs are ineffectual or inexistent. There are many possible reasons why antibiotics could be used inappropriately and residues could occur in food products. A farmer may not be aware of the issue of residues or resistance, may administer antibiotics to cows improperly (in terms of dosage or route of administration) or may not want to lose income from milk that must be discarded if residues are to be avoided. Studies have found management practices, economic factors and farmers' levels of awareness and education to be associated with producing milk contaminated with antibiotic residues [61-64].

Determining the proportion of farmers who sell milk with antibiotic residues and identifying site-specific risk factors associated with the production of milk with antibiotic residues can aid policy makers, organizations and animal health-care professionals develop ways to improve milk quality and guarantee producers and consumers a high quality, safe product.

However, the majority of studies that have documented the prevalence of antibiotic residues in animal food products sampled these products in the open market or on the farm and established a point prevalence of contamination. Because antibiotic use on small farms in LMI countries is sporadic and infrequent[116], this method may not capture the true extent of the problem. We sought to measure the level of contamination of milk with antibiotic residues in Cajamarca and to identify factors associated with the sale of milk with antibiotic residues.

2. Materials and Methods

Three approaches were used to understand how often milk with antibiotic residues is sold in Cajamarca and to identify factors associated with selling milk with antibiotic residues. All farmers who agreed to participate provided verbal consent, and approval for this study was granted by the Institutional Review Boards of the University of Pennsylvania and the Universidad Peruana Cayetano-Heredia in Lima.

2.1 Approach 1: sampling of commercial milk routes

We approached the three primary dairy processing companies of the region (Milk Company 1 [MC1], Milk Company 2 [MC2] and a cheesemaking company [CC]) to request permission to accompany milk trucks on their morning routes for one day. Samples were taken from the bulk tank of each farmer on the route and tested for antibiotic residues using Snap Duo™ Beta-Tetra test kits provided by Idexx. According to the manufacturer, these tests detect oxytetracycline and penicillin (the most commonly used antibiotics in Cajamarca[116]) at levels of ≤100 ppb and ≤4 ppb, respectively, in raw commingled milk.

The point prevalence of contamination of milk with antibiotic residues on a given route was calculated as the proportion of farmers on the route who sold milk that tested positive for residues out of the total number of farmers who sold milk on that route that day. Confidence intervals for these proportions were calculated using exact binomial distribution assumptions for the data.

2.2 Approach 2: interviewing farmers and sampling milk from farms where cows were being administered antibiotics

The goal of this approach was to test milk from farms where cows were being treated with antibiotics to determine the proportion of farmers who sold milk contaminated with antibiotic residues and to assess the validity of the self-reported selling of milk from treated cows. Local veterinarians were asked to notify us when they had sold or administered antibiotics to the cows of a farm and to provide us with the contact information of the farmer.

On the day after the last dose of antibiotics was administered, we invited farmers to participate in a survey and provide a milk sample from their bulk tank. Farmers who agreed to participate (Sample 1) were administered a questionnaire adapted from questionnaires used in other studies[80,60,79,117]. The questionnaire obtained information on the farmers, farms and animals, disease incidence, antibiotic use, farmers' knowledge of antibiotics, farm management and economic information. An antibiotic knowledge score measuring a farmer's knowledge of what an antibiotic is and the risks associated with the use of antibiotics was generated (with more points indicating better knowledge of antibiotics). Further details of this knowledge score have been described elsewhere[116].

The collected milk samples were tested for antibiotic residues with the test kits described previously.

One of the questions posed to farmers in this survey was "What do you do with milk from cows treated with antibiotics?". The possible answers to this question were: 1) feed it to calves, 2) sell it to the milk truck, 3) feed it to the dogs, 4) drink it or 5) discard it. A new dichotomous variable was created from the results of this question indicating whether the farmer reported continuing to sell milk from treated cows. The farmers who reported not selling the milk were asked for how long they withheld the milk.

The proportion of farmers who were treating their cows at the time (Sample 1) and who sold milk that tested positive for antibiotic residues was calculated. The sensitivity, specificity and positive (PPV) and negative predictive values (NPV) (along with 95% confidence intervals) of the self-reported selling of milk from treated cows was calculated using results from the milk residue tests as the gold standard.

2.3 Approach 3: interviewing randomly selected farmers in Cajamarca

Simple random sampling was used to select a sample of farmers (Sample 2) from 6000 farmers in two provinces and five districts of the region of Cajamarca who work with a local non-profit organization involved in improving farming practices and promoting sustainable development. These farmers had previously been approached to participate in a survey on patterns and determinants of antibiotic usage[116]. The questionnaire described in the previous section was used.

A logistic regression analysis was conducted to identify factors associated with a farmer's tendency to report selling milk from treated cows. A p-value entry threshold of 0.2 was used for initial variable selection along with a backwards elimination strategy to develop multivariable models and assess confounding. Risk factors with a p-value of <0.05 and any confounders that altered other associations by 15% or more were retained in final models.

Because the NPV of self-reported selling of milk from treated cows was low (i.e., farmers who reported withholding may have not been honest), we conducted sensitivity analyses to evaluate the robustness of the results of the logistic regression analysis if farmers were to self-report selling contaminated milk more truthfully (Figure 4). First, to generate a simulated sample of more "truthful" farmers, we changed the answers of randomly selected farmers in Sample 2 who originally responded that they withheld milk from treated cows. The proportions of "withholds" that were changed to "No withholds" corresponded to the sum of the abovementioned NPV and 1 and 2 standard deviations of this proportion. Second, we re-ran the logistic regression analysis.

These two steps were performed 1000 times, and summary odds ratios and confidence intervals were generated to reflect the significance of the factors associated with reporting to sell contaminated milk in the simulated samples.

A p-value<0.05 was considered statistically significant. All analyses were conducted in Stata, v.11 (StataCorp. College Station, TX).

3. Results

3.1 Approach 1: Milk routes

Two routes with MC1 yielded 0/89 and 0/31 positives, or prevalences of 0 (95% CI: 0.00-0.04) and 0 (95% CI: 0.0-0.11), respectively. Three routes with MC2 yielded 1/60, 2/60 and 2/48 positives, or prevalences of 0.017 (95% CI: 0.00-0.09), 0.033 (95% CI: 0.00-0.11) and 0.042 (95% CI: 0.01-0.14), respectively. One route with CC yielded 0/22 positives or a prevalence of 0 (95% CI: 0.00-0.15).

3.2 Approach 2: Testing of milk samples from and interviews with farmers treating their animals with antibiotics (Sample 1)

Four veterinarians agreed to help us recruit farmers for this study; 40 farmers from 16 villages (Sample 1) who had been visited by one of the veterinarians and had received antibiotic treatments for their animals on the previous day were invited to participate in the study. Four farmers declined to participate (participation rate of 90%). Demographic data pertaining to these farms and farmers are presented in Table 6. The mean age of the farmers was 46.9 years, and 69.5% of them were illiterate. The farms had a median of three lactating cows (range 2-6) each and produced a median of 16 L of milk per day (5.63 L/cow/day); farmers earned a median of 300 Peruvian Nuevo Soles ($115 according to the 2013 exchange rate) per month from the sale of milk.

Thirty-three (91.7%) of the samples from the bulk tanks of these 36 farmers tested positive for antibiotic residues.

When asked what they typically did with milk from their treated cows, 25/36 farmers reported that they continued selling milk to the milk truck (Table 7). The milk samples from all 25 of these farmers tested positive (PPV of 100%). Eleven farmers reported that they did not continue sending milk to the truck; 4 reported giving this milk to their calves, 4 reported giving this milk to their dogs and 3 farmers reported discarding the milk. The milk samples from 3 of these 11 farmers tested negative (NPV of 27.2%). Three of the farmers whose milk tested negative reported not selling milk from treated cows (specificity of 100%); 25 of the 33 farmers who tested positive reported selling milk from treated cows (sensitivity of 75.8%). The farmers who claimed not to sell milk from treated cows reported withholding the milk for a median period of two days (range 1-4).

3.3 Approach 3: Interviews with randomly selected farmers (Sample 2)

A total of 156 farmers from 48 villages were invited to participate in the study; 12 farmers declined to participate (participation rate of 92.9%). The demographic characteristics of this sample were very similar to those of Sample 1 (Table 6). Further information on disease and antibiotic use in this population have been described elsewhere[116].

A total of 156 farmers from 48 villages were invited to participate in the study; 12 farmers declined to participate (participation rate of 92.9%). The demographic characteristics of this sample were very similar to those of Sample 1 (Table 1). Further information on disease and antibiotic use in this population have been described elsewhere[116].

Of the 156 farmers, 69 (44.2%) reported continuing to sell milk from treated cows. Thirty-three (21.1%), 34 (21.8%), 14 (8.97%) and 4 (2.56%) farmers reported giving the milk to calves, giving the milk to dogs, discarding the milk and drinking it, respectively. The farmers who reported withholding milk from treated cows reported doing so for a median period of 2 days (range 1-5).

In a multivariable analysis, the antibiotic knowledge score (OR=0.64, p<0.0001, 95% CI=0.528-0.843) and the purchaser of the milk (OR=2.25 for MC2, p=0.175, 95% CI=0.712-7.26; OR=6.11 for CC, p=0.003, 95% CI=1.50-15.1, with MC1 as the reference group) were found to be significantly associated with the farmer's tendency to report selling milk from treated cows (Table 8). The education level of the farmer was significantly associated with a farmer's response in a univariable analysis; however, this association did not persist in the multivariable analysis.

Because the PPV of the self-reported selling of milk from treated cows was 100% but the NPV was 27.3%, we assumed that not all farmers who reported withholding milk were honest. To examine the impact of these potential inaccuracies, we conducted a sensitivity analysis to evaluate the impact on the associations of interest: we changed the answers of 46% and 67% (NPV+1SD and NPV+2SD, respectively) of randomly selected farmers who originally reported withholding milk and re-ran the multivariable logistic regression analysis (Figure 4). This process was repeated 1000 times and summary odds ratios and confidence intervals were generated. The odds ratios for the antibiotic knowledge score and for the different purchasers were slightly attenuated but still statistically significant when 46 and 67% of the answers were "corrected" (Table 9).

4. Discussion

The goal of this study was to determine the extent of contamination of commercially sold milk with antibiotic residues. Three different approaches were used to obtain 1) a point prevalence of contamination for a given milk route on a given day, 2) the proportion of farmers who treat their cows with antibiotics and continue selling milk and 3) the factors associated with a farmer's tendency to report selling milk from treated cows. The point prevalence of contamination of milk for a given day and route was very low: 0% for MC1 and CC and 4.2% for MC2 (with upper limits of 11%, 15% and 14%, respectively). In agreement with these proportions, a study sampling milk from the plant bulk tanks of MC2 for a month in Arequipa found that 16.1% of samples were positive[118].

Milk from the bulk tanks of 33 of the 36 (91.7%) farmers who were treating their cows with antibiotics tested positive for penicillin and/or oxytetracycline residues. Finally, 44% of a population of randomly selected farmers reported selling milk from treated cows.

Because the PPV of the self-reported selling of milk from treated cows was 100%, we can conclude that *at least* 44% of farmers in Cajamarca continue to sell milk from treated cows. Because the NPV of this question was so low (27.3%), it is likely that more farmers do so. Furthermore, farmers who reported withholding milk from treated cows withheld for a median period of only 2 days (maximum 5). Given that the withdrawal times of oxytetracycline and penicillin are 7 and 5 days, respectively, it would appear that close to 100% of farmers sell milk with antibiotic residues at some point. However, because there are 30,000 registered milk producers[86] in Cajamarca and because antibiotic use is relatively infrequent in Cajamarca (we previously reported an incidence rate of 0.477 episodes of treated disease per cow-year[116]), the occurrence of antibiotic residues in milk appears to be relatively infrequent. Results from the sampling of providers on milk routes confirm this finding.

Previous studies have documented high levels of contaminated milk in Cajamarca. Forty-five of 63 (71%) samples of milk sold on the open-air market in Cajamarca tested positive [119] and 21of 40 (52.5%) milk samples from farms in the countryside tested positive[59]. Llanos-Cortesana et al. tested 216 milk samples from the open-air market and farms in Cajamarca and found that 21.2% and 20.8% of these samples, respectively, tested positive[56]. As these studies show, rates of contamination are highest in the open-air markets where no quality control is imposed and where farmers anecdotally sell milk they knew would be unacceptable to the milk truck. While we did not test milk from the open-air market in this study, we did find that the highest levels of contamination were found along the routes of MC2.

A farmer's antibiotic knowledge score and the purchaser of the milk were found to be associated with reporting to sell milk from treated cows. These associations remained significant, even when the responses of 46% and 67% of the farmers who originally reported withholding milk were changed, suggesting that these associations are relatively robust despite the low negative predictive value of the self-reported selling of contaminated milk.

Both findings were expected, as farmers who are aware of the risks associated with antibiotic use would know that milk *should* be withheld and because tolerance of contaminated milk varied by company (MC2 appeared to be the most tolerant). Furthermore, the bulk tanks at the plants with commingled milk from all routes of the day are tested for residues; therefore, companies that receive larger volumes of milk may dilute the contaminated milk to levels where the residues are no longer detectable. MC1 and MC2 receive more milk than CC, which would explain the higher association found for CC (OR=6.11, p=0.003, 95% CI=1.50-15.1).

Surprisingly, even though many farmers stated that they did not withhold milk because they could not afford to lose the income from that day's milk, income was not associated with reporting to sell milk from treated cows. However, because the median income of farmers who did and did not withhold milk was similar ($110 and $115, respectively; p=0.84), farmers in both groups were equally poor and therefore equally likely to be affected by the loss of income from withholding milk.

These findings have two important implications for interventions to improve milk quality. First, educating farmers about the risks associated with antibiotic use and the consumption of milk from treated cows is essential. Even if farmers do not withhold milk for the entire withdrawal period, withholding milk for a short-term period (when the concentration of residues in the milk is highest) can mitigate the problem of contaminated milk. Second, milk purchasers have the ultimate responsibility to enforce penalties for selling milk with residues. Anecdotally, MC2 reported docking the price of milk with residues 10%; while this measure acknowledges that milk with residues is problematic, a farmer will likely choose to receive 90% of his/her pay for that day instead of 0% (i.e., withholding the milk).

The issue of antibiotic residues is complicated, and neither farmers nor milk companies may have sufficient motivation to address the problem. Farmers in Cajamarca are very poor and milk is the sole source of income for more than 90% of farmers. In addition, these farmers generally have few producing cows (median of three); withholding milk from one of their cows entails a loss of a significant portion of their income for the day. At the same time, competition for milk among the milk companies makes them unlikely to enforce policies that will cost them providers; if one milk company institutes penalties for selling contaminated milk, the providers of that company may switch to another purchaser. In addition, because many of these farmers live in rural locations far from the city and milk plants, the milk trucks must compensate for the distance by collecting milk from as many farmers as possible. Governmental regulation and enforcement of milk quality may be required to consistently improve milk quality on a large scale.

This study showed that the majority of farmers in Cajamarca sell milk contaminated with antibiotics residues but that the point prevalence of contamination for a given day and route is quite low. However, this study also had certain limitations.

First, because the test kits used to detect antibiotic residues only detected penicillin and oxytetracycline, we may have underestimated the prevalence of contamination of milk. However, because oxytetracycline and penicillin are the two most commonly used antibiotics in Cajamarca (accounting for 79.8% of antibiotics used[116]), it is likely that the majority of instances were detected.

Second, the point prevalence of contamination of milk was only ascertained for six routes on a single morning. It is unclear how representative these days were of the rest of the year. Because samples were collected during the wet season when the incidence of disease tends to be higher [120], the point prevalence we detected may have been on the high end.

Third, our analyses examined the factors associated with *reporting* to continue to sell milk from treated cows, and not the actual selling of milk. Because the PPV of the question was 100%, we can assume that all farmers who reported selling milk from treated cows did so; therefore, the factors associated with *reporting* to sell milk were also associated with actually selling milk from treated cows. However, we cannot be sure that farmers who reported withholding milk actually did so; therefore, the absence of factors associated with reporting to sell milk may not have been associated with withholding milk from treated cows. However, sensitivity analyses showed that these associations remained significant even if up to 67% of farmers were not truthful about withholding milk.

Fourth, because the survey relied on self-report, reporting and social desirability biases may have affected our findings. In particular, farmers who were more knowledgeable about antibiotic residues may have been more likely to report withholding milk from treated cows or withholding milk for longer periods of time than they actually did.

Finally, the results of the study may not be generalizable to other small dairy farms in other LMI countries. In particular, these farmers were involved with a non-profit organization and may have received training and educational interventions that improved their knowledge and awareness of good farming practices.

The strengths of this study included the multiple approaches used to evaluate the issue of antibiotic residues, the high participation rates and the validation sub-study (Sample 1) that allowed us to measure the proportion of farmers who sold milk from treated cows and determine the validity of the self-reported selling of milk from treated cows. The results of this study suggest that certain approaches – especially educational approaches - may be useful for improving milk quality on small dairy farms in Cajamarca and other similar sites.

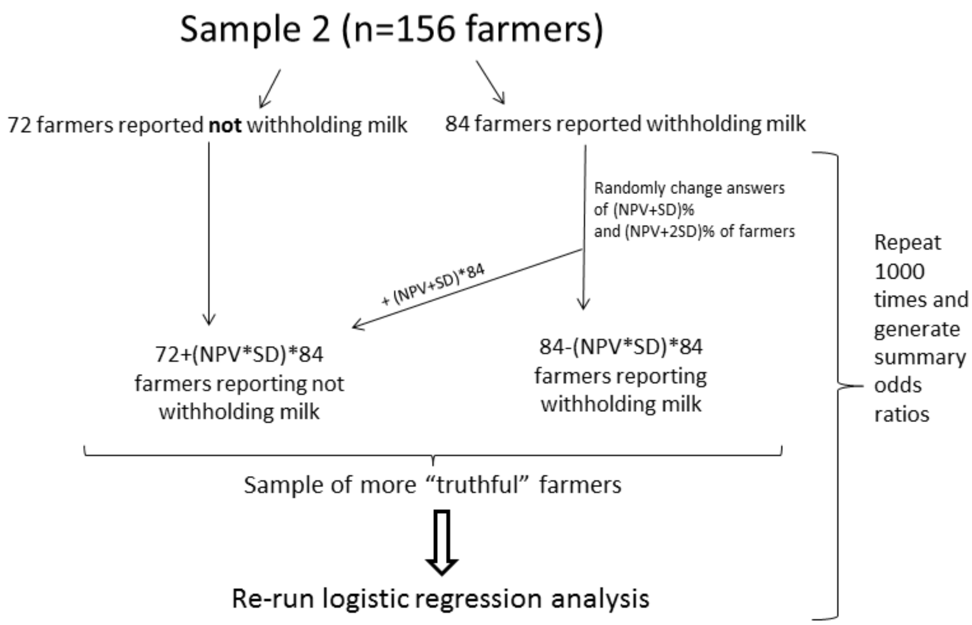

Figure 4 Procedure used to conduct sensitivity analyses for the logistic regression analysis examining factors associated with reporting to sell milk from cows treated with antibiotics in a sample of 156 farmers in Cajamarca, Peru

Table 6 Characteristics of small farms and farmers participating in a study on antibiotic use in Cajamarca

Farm or farmer characteristic	Population 1 (n=36)	Population 2 (n=156)
Mean (SD) age (years)	46.9 (13.4)	45.0 (15.1)
Education level, n(%)		
None (analphabetic)	25 (69.5)	84 (53.6)
Some primary school	5 (13.8)	58 (37.2)
Some secondary school	6 (16.7)	14 (8.97)
Median (interquartile range) number of	2 (2-3)	2 (1-3)
farmers on each farm	1 (0-1)	1 (0-1)
Men	1 (1-2)	1 (1-2)
Women		
Median (interquartile range, min, max) number of animals on each farm		
Cattle (cows, calves/heifers and	7 (6-8, 5, 18)	7 (5-9, 1, 27)
bulls)	3 (3-4, 2, 6)	3 (2-4, 1, 14)
Lactating cows	1 (0-2, 0, 7)	1 (0-2, 0, 7)
Dry cows		
Median (interquartile range, min, max) amount of milk produced daily (L)		
On-farm total	18 (14-23, 8, 60)	16 (10-25, 3, 120)
Per cow	5 (4.7-5.8, 4, 17.5)	5.63 (4.6-8, 2, 18)
Median (interquartile range, min, max) monthly income from milk ($)	125 (86.5-192, 38.5, 385)	115 (67.3-173, 15.4, 1076)
Destination of milk, n(%)		
Milk Company 1	8 (22.2)	24 (15.4)
Milk Company 2	12 (33.3)	65 (41.7)
Cheese-makers	15 (41.2)	55 (35.3)
Home consumption/open market	1 (2.8)	12 (7.69)

Table 7 Responses of farmers currently treating cows with antibiotics to the question on the fate of milk from treated cows in Cajamarca, Peru

Did the farmer report selling milk from treated cows?	Testing of milk from bulk tank for antibiotic residues (gold standard)	
	Tested positive	Tested negative
Yes	25	0
No	8	3

Measures of the validity of the self-reported sale of milk:

Sensitivity 0.76, Specificity 1.00, PPV 1.00, NPV 0.27

Table 8 Factors associated with a farmer's odds of responding that he/she sells milk from cows treated with antibiotics in a sample of farmers (n=156) in Cajamarca, Peru

Factor	Univariable analysis			Multivariable analysis			Interpretation
	OR	P-value	95% CI	OR	P-value	95% CI	
Antibiotic knowledge score	0.620	<0.0001	0.496-0.778	0.640	<0.0001	0.528-0.843	For every 1-pt increase in knowledge score, the log-odds of reporting selling milk from treated cows decreased 38%
Education level							
(compared to illiterate)		0.040					More highly educated farmers are less likely to report selling milk from treated cows than uneducated farmers
Some primary							
Some secondary	0.456	0.026	0.229-0.910	0.557			
	0.347	0.093	0.100-1.19	0.543			
Milk purchaser		0.005			0.020		Farmers selling milk to MC2 or CC are more likely to report selling milk from treated cows than farmers selling to MC1
(compared to MC1)							
MC2	2.38	0.125	0.786-7.17	2.25	0.175	0.712-7.26	
CC	6.15	0.002	1.76-15.8	6.11	0.003	1.50-15.1	

Table 9 Changes in odds ratios for factors associated with the tendency to report selling milk from cows treated with antibiotics when changing the responses of some farmers who originally reported withholding milk from treated cows

Factor	Original sample			46% of "Withhold" changed to "Do not withhold"			67% of "Withhold" changed to "Do not withhold"		
	OR	p-value	95% CI	OR	p-value	95% CI	OR	p-value	95% CI
Antibiotic knowledge	0.640	<0.0001	0.509-0.802	0.770	0.047	0.596-0.997	0.692	0.013	0.518-0.926
Milk purchaser (compared to MC1)									
MC2	2.23	0.175	0.701-7.08	2.78	0.058	0.964-8.06	2.36	0.178	0.677-8.28
CC	4.51	0.010	1.43-14.2	3.95	0.016	1.29-12.2	3.34	0.069	0.909-12.3

CHAPTER 4 The role of veterinarians and feed-store vendors in the prescription and use of antibiotics on small dairy farms in Cajamarca

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ABSTRACT

This study aimed to describe and compare the role of veterinarians and feed-store vendors in the use of antibiotics on small dairy farms in Cajamarca, Peru, a major dairy-producing center characterized by small, rural farms with poor, mostly uneducated farmers. We used a purposive sampling strategy to recruit 12 veterinarians into two focus group discussions and supplemented these data with eight semi-structured interviews with feed-store vendors. Participants reported that inappropriate antibiotic usage was widespread among their clients, which may prevent the efficient use of drugs on farms where animal disease can be devastating to the livelihood of the farmer. Participants also identified many barriers to appropriate prescribing and use, including availability of drugs, competition from other prescribers, economic constraints and habits of farmers, and limited farmer knowledge of drugs and disease. Veterinarians expressed mistrust towards non-professional prescribers, while vendors felt that veterinarians were important partners in promoting the health of their clients' animals.

1. Introduction

Dairy production is a rapidly expanding sector of animal agriculture in lower/middle income (LMI) countries, and small farms generally constitute the majority of producers in these countries. These farmers typically have very low educational levels, and their knowledge of appropriate drug use is often limited; they therefore often rely on prescribers to provide them with instructions for the proper use of veterinary drugs.

The use of antibiotics in livestock has demonstrated benefits, including improved animal health, higher production levels and the reduction of foodborne pathogens [10]. However, the use of antibiotics can result in a number of problems, including the emergence of antibiotic-resistant bacteria, human and animal illness, economic loss for farmers and dairy processors and environmental contamination [10,21-23,105].

The inappropriate use of antibiotics has been defined by the World Health Organization (WHO) as including any of the following: overprescription or underprescription, inappropriate dosing, incorrect duration of treatment, incorrect choice of drug for the relevant organism, and the unnecessary use of an expensive drug when older, cheaper drugs are available and clinically adequate [121]. The inappropriate use of antibiotics in human medicine in lower/middle income (LMI) countries has been extensively documented [37-46,122]. However, very little is known of the use of antibiotics in food animals on small farms in LMI countries.

2. Materials and Methods

2.1 Participants

In qualitative studies, participants are purposefully sampled to include “informants with a broad general knowledge of the topic or those who have undergone the experience and whose experience is considered typical”[123]. Veterinarians from a non-profit organization (Foncreagro) dedicated to improving farming practices in Cajamarca were recruited to participate in a series of focus group discussions (FGD), a study technique in which a group of relevant participants is convened and asked “focused” questions about a particular topic [124]. Because it would have been difficult to convene a FGD of FSVs, FSVs were individually approached and asked the same “focused” questions in the form of semi-structured questionnaires. Qualitative data were generated that can be useful for hypothesis generation on a topic where little is known about the key variables involved.

The FGD participants work on an every-day basis with dairy farmers in Cajamarca on both a clinical and an advisory/training basis. The FSVs work in agriculture stores where they sell, among other things, animal drugs over the counter (i.e., without a prescription). The typical clientele of both groups of participants consists of small, poor, rural farmers with one to five cattle that are housed and grazed on small pastures, manually milked and produce 5-10 kg of milk daily.

2.2 Data collection

The focus group moderator and interviewer, chosen for her knowledge of qualitative methods and veterinary medicine, used a written discussion guide and questionnaire developed based on a review of the literature and clinical veterinary expertise (available upon request from the corresponding author). FGDs and interviews were held in Spanish at Foncreagro offices and in feed-stores, respectively, and audio-recorded in July of 2012. Approval for this study was obtained from the Institutional Review Board of the University of Pennsylvania and from the Comité Ética of the Universidad Peruana Cayetano-Heredia.

2.3 Data management and analysis

Interviews were transcribed verbatim by a professional Peruvian transcriber, and transcripts were reviewed for accuracy. Transcripts were read *in toto* by a single investigator (LER) to identify key codes and to develop a coding dictionary using grounded theory [125]. Transcripts were then loaded into nVivo software (10.0), which allows text to be coded, sorted, retrieved and queried across sources (i.e., focus groups, interviews), individual participants and attributes (including gender, profession and years in the profession). The data were coded using the constant comparative method, which involves the revision of coding as themes emerge from the data and the checking of coding against that of other documents to establish similarities and differences across data sources and participants [126]. Briefly, information pertaining to a particular theme was labeled with a relevant code, and responses could be assigned as many codes as were applicable. For example, if a participant mentioned how economic hardships of a client prohibited them from buying the appropriate amount of an antibiotic, this quote was coded as "Inappropriate use of antibiotics", "Underdosing", and "Economic hardships". Codes were considered significant if they were mentioned more than five times or discussed by more than three participants. Codes could be compared, contrasted, re-defined or refined as new themes emerged from the data, or associated with a certain category of participant (e.g., veterinarians vs. feed-store vendor, men vs. women).

3. Results

3.1 Participants

Six veterinarians participated in each FGD (Table 1). The first group was composed of five men and one woman, and the second group was composed of four men and two women. Eight FSVs, all men, were interviewed. Veterinarians had been practicing for an average of 6.8 years (FGD1) and 5.5 years (FGD2), and FSVs had worked for an average of 6.2 years in their stores. Thematic saturation, or the replication of data collected from different participants that are similar and fit within the same category [127], was achieved with these two focus groups and eight interviews.

3.2 Definition of proper antibiotic use

Veterinarians identified four main criteria for proper antibiotic use (Table 3). First, antibiotics should be used in the correct clinical situation, i.e., for a bacterial infection, and not for any other type of illness where they would not be useful, such as a viral illness, a nutritional deficit, or an anatomical problem such as a prolapsed uterus. Conversely, other drugs – especially antiparasitics – should not be used in the place of an antibiotic when an antibiotic is called for. Second, the recommended dosage should be followed, both in terms of quantity (mg/kg) and duration of treatment. Third, microbiological tests should be used to identify the pathogen and its resistance profile. Fourth, drug withdrawal times should be respected to avoid the occurrence of antibiotic residues in milk.

When asked if antibiotics were currently used properly by their clients according to their own definitions, all veterinarians agreed that farmers generally failed to adhere to all four criteria.

For FSVs, the correct use of antibiotics consisted in specifically not confusing an antibiotic with an antiparasitic drug and using the proper dose (mg/kg). No mention was made of using tests or analyses, little mention was made of specific disease indications, and only one vendor mentioned the issue of antibiotic residues. Three of the eight vendors thought that antibiotics were more or less appropriately used among their clients, while five thought that antibiotic use was inappropriate and indiscriminate.

3.3 The role of prescribers in antibiotic use on dairy farms

Both veterinarians and FSVs directly and legally sell/dispense antibiotics to their clients. Veterinarians expressed that, because they are always able to examine the affected animal and make an informed clinical decision, their prescribing practices were generally appropriate; however, veterinarians did admit that certain constraints sometimes limited their ability to prescribe appropriately. FSVs, on the other hand, lamented that they were unable to observe the animal and had to depend on often vague descriptions of symptoms from owners; furthermore, all of the FSVs reported that at least half of their clients who purchased antibiotics requested a specific antibiotic instead of asking the vendor for guidance. FSVs therefore felt less able to ensure the proper use of antibiotics than veterinarians. However, both types of prescribers acknowledged that they sometimes felt obliged to prescribe antibiotics inappropriately. The most frequently cited factors associated with inappropriate prescribing are listed in Table 2 and illustrated in a conceptual framework in Figure 5. Relevant quotes detailing barriers to prescribing are shown in Table 3.

3.4 Determinants of antibiotic prescribing

3.4.1 Influence of the clinical situation

The clinical situation was the most important factor determining a prescriber's choice of antibiotic and decision to prescribe. Veterinarians asserted that a thorough examination of the animal and a detailed history were necessary before prescribing an antibiotic, and broad-spectrum antibiotics were generally the drugs of choice. Seven of the eight FSVs reported trying to obtain some sort of history of the sick animal before recommending an antibiotic and tended to consistently prescribe specific drugs for the most common illnesses (mastitis, metritis, diarrhea and pneumonia).

3.4.2 Availability of drugs/Prescriber's inventory

According to the veterinarians, oxytetracyclines are the most commonly prescribed antibiotics in Cajamarca, which has produced a relatively high prevalence of resistance to this drug. The veterinarians reported that the over-reliance on this antibiotic was in large part due to the widespread availability of this drug and the relative paucity of other drugs on the market in Cajamarca (Table 3). In addition, veterinarians reported that there tended to be a revolving selection of antibiotics available in feed-stores, with different names, packaging and active ingredients, which complicated the consistent use of antibiotics by both farmers, who often rely on visual memory of a drug, and veterinarians, who were not always able to subsequently locate a product they had used in the past. None of the FSVs believed that inventory in their own store was a problem that led to the inappropriate prescribing of antibiotics, and one of the vendors claimed that penicillin and sulfa antibiotics were sold as often as oxytetracyclines. However, one of the vendors mentioned that adulterated or counterfeit drugs were widely sold on the market and that many farmers were often sold inadequate products by other vendors.

3.4.3 Perception of clients' abilities

Veterinarians mentioned that the low education level of their clients sometimes influenced their prescribing practices. The majority of their clients in Cajamarca are illiterate farmers who understand little of drug indications, active ingredients or disease pathologies. As such, veterinarians often reported feeling unable to explain why a certain drug is needed for a certain period of time and at a certain dose; veterinarians maintained very low expectations that their instructions for use would actually be followed. Furthermore, farmers may not be able to tell a veterinarian which drugs they have used previously or how they have dealt with a clinical problem, which further limits a veterinarian's ability to prescribe an appropriate therapy. In contrast, half of the FSVs, while acknowledging that their clients abilities to understand drug indications and disease was limited, believed that the instructions they gave their clients when dispensing antibiotics were adequately followed.

3.4.4. Clients' needs and expectations

Major determinants of the type and quantity of antibiotic prescribed by both veterinarians and FSVs involved clients' expectations and needs, with economic needs taking on crucial importance. Farmers often have habits associated with antibiotic use, and financial constraints almost always dictate the type and amount of antibiotic administered. Firstly, farmers acquired customs from their family, friends and neighbors for treating their animals, and these customs often led farmers to purchase the same products, even if their efficacy was limited.

Secondly, financial constraints were a major limiting factor in the ability to effectively treat animals. The majority of farmers in the area are quite poor (earning less than $1.25 a day), and the sale of milk constitutes their sole source of income. Both veterinarians and vendors admitted to sometimes prescribing antibiotics they knew were not going to be effective because farmers could not afford anything else.

3.5 Attitudes towards other prescribers

Veterinarians felt pressure to compete with other prescribers (e.g., FSVs and drug promoters) in providing drugs and services to farmers. Their perception was one of being at a disadvantage compared to non-professional prescribers for whom economic motivation was a high priority and a lack of clinical knowledge often resulted in inappropriate prescribing practices. Veterinarians also expressed concern that farmers were more likely to buy their drugs from non-professional prescribers who were more likely to "accommodate" their preferences than a veterinarian, and that veterinary care was only sought in complicated clinical situations that could not easily be handled by a non-professional. Overall, veterinarians were mistrustful of non-professional prescribers.

When asked to describe their prescribing practices in general and in the scenario of a non-specific set of symptoms in particular, FSVs described trying to obtain a case history and prescribing an appropriate drug. However, if the case was complicated or if it was unclear what was wrong with the client's animal, all of the FSVs reported that they would defer to a veterinarian (when a veterinary practitioner was affiliated with their store) or encourage clients to seek veterinary help. Overall, FSVs appeared to view veterinarians as collaborators and valuable sources of clinical expertise for treating clients' animals rather than as competition.

4. Discussion

This study aimed to understand the perspectives and roles of antibiotic prescribers in antibiotic use and prescribing in Cajamarca, Peru, an agricultural setting dominated by small, poor, rural farms. The perspectives and opinions of veterinarians and FSVs on this topic were collected in focus group discussions and semi-structured interviews, respectively. To our knowledge, this is one of the first studies to seek the perspective of antibiotic prescribers on antibiotic use in livestock in a LMI country using qualitative methods.

Both types of antibiotic prescribers were mostly of the opinion that antibiotics were used inappropriately among their dairy farmer clients; however, veterinarians and FSVs had somewhat different definitions of what constituted proper antibiotic use, with vendors' definitions being more limited than that of veterinarians. Both types of prescribers admitted to sometimes inappropriately prescribing antibiotics for a variety of reasons, mostly having to do with the expectations and needs of farmers.

A number of other studies have described the use of antibiotics and the role of prescribers in human medicine in the developing world setting. In Sri Lanka, for example, Wolffers (1996) found that no prescriptions were needed to obtain antibiotics from pharmacies, no information on its proper use was provided with the sale of antibiotics and pharmacy personnel had no pharmaceutical qualifications and knew little about the drugs they were dispensing[37]. In Peru, Paredes et al. (1996) found that the practices of antibiotic prescriptions by physicians were more related to social expectations and perceptions of the physician's role than to medical/scientific reasoning[39]. Radyowijati et al. (2003) found that a lack of appropriate knowledge on the part of providers, lack of trust in delayed lab results, unstable or inadequate drug supply, economic incentives, fear of clinical failure/desire to stay on the safe side, desire to meet patient demand, folk beliefs, inadequate supervision and diagnostic uncertainty led to inappropriate antibiotic prescribing by physicians and pharmacists[41].

Given that veterinary medicine is less well regulated than human medicine in LMI countries, and given that livestock are often the principal – if not sole – source of income for a large number of farmers, it is not surprising that antibiotic use in dairy cattle was deemed inappropriate by antibiotic prescribers and that many of the same reasons for inappropriate prescribing in human medicine were found to be applicable to veterinary medicine.

Overall, prescribers felt that there was often little they could do to improve antibiotic use among their clients. Economic circumstances and entrenched attitudes made appropriate prescribing difficult if not impossible. Training/educating farmers on the appropriate use of antibiotics could be useful and was attempted by both veterinarians and vendors; however, such training was not always successful, especially since farmers sometimes received conflicting advice/information from a variety of prescribers. Furthermore, the appropriate use of antibiotics sometimes depended on other parties such as drug promoters who also prescribe antibiotics and milk companies that purchased the milk. Participants felt they could do little to influence these parties and expressed frustration with the lack of action on the part of these other groups in promoting the responsible use of antibiotics. Veterinarians expressed skepticism towards and mistrust of other non-professional prescribers (FSVs, drug promoters and technicians). FSVs, on the other hand, viewed veterinarians as important collaborators in improving animal health in Cajamarca. Improved relations and interactions between these groups of prescribers would likely be useful in dispelling potential misconceptions and could contribute towards the promotion of the improved use of antibiotics. In particular, the training of vendors by veterinarians in the appropriate use of antibiotics and the development of specific guidelines for appropriate prescribing and training of farmers could also be useful interventions.

also be useful interventions.

This was one of the first studies to investigate veterinarian and FSV perspectives on and rationale for antibiotic prescribing practices in LMI countries; however, several possible limitations of the study are worth mentioning. As in many qualitative studies, potential information bias and limited generalizability are important limitations. The participants may have been motivated by social desirability and therefore may not have been entirely forthright in their responses; however, because the focus group methodology is one of the best ways to promote candid discussion and peer interaction [124], information bias may have been mitigated.

The generalizability of the results of this study may be limited, as veterinarians and vendors were from a single geographic region. Furthermore, the veterinarians were employees of a non-profit organization that aims to improve farming practices on small dairy farms; participants may therefore have been especially attuned to concepts of best-farming practices and motivated by outcomes related to sustainable farming practices rather than strictly to animal health. The number of feed-store vendors interviewed, while appearing small, achieved thematic saturation, or the replication of data collected from different participants that are similar and fit within the same category [127]. Overall, a wide range of topics was discussed in the FGDs and interviews, and many overlapping themes were found in both groups; it is likely that aspects of the discussion are generalizable to the use of antibiotics in dairy cattle across Peru and throughout lower/middle income countries in Latin America.

These findings shed light on the perspectives of antibiotic prescribers in Cajamarca and the motivations and obstacles they encounter when prescribing antibiotics to dairy animals. This study also highlights the differences in prescribing between veterinarians and feed-store vendors; in particular, definitions of proper antibiotic use and opinions on whether antibiotics are used properly and whether instructions on how to use antibiotics were followed differed between veterinarians and vendors. While there was significant overlap in the description of obstacles to appropriate prescribing between the types of prescribers, some differences were noted, including the limited availability of certain drugs and competition from non-professional prescribers (in the case of the veterinarians) and the inability to examine the animal (in the case of the vendors).

As mentioned previously, qualitative data is useful for hypothesis generation and the understanding of behavioral processes. For example, it would be interesting to know how often veterinarians and vendors encounter particular obstacles to appropriate prescribing and to quantitatively determine whether inappropriate prescribing occurs more frequently with feed store vendors or with veterinarians. Finally, ascertaining the perspectives of farmers themselves on which factors are important in determining their antibiotic use would be of great interest.

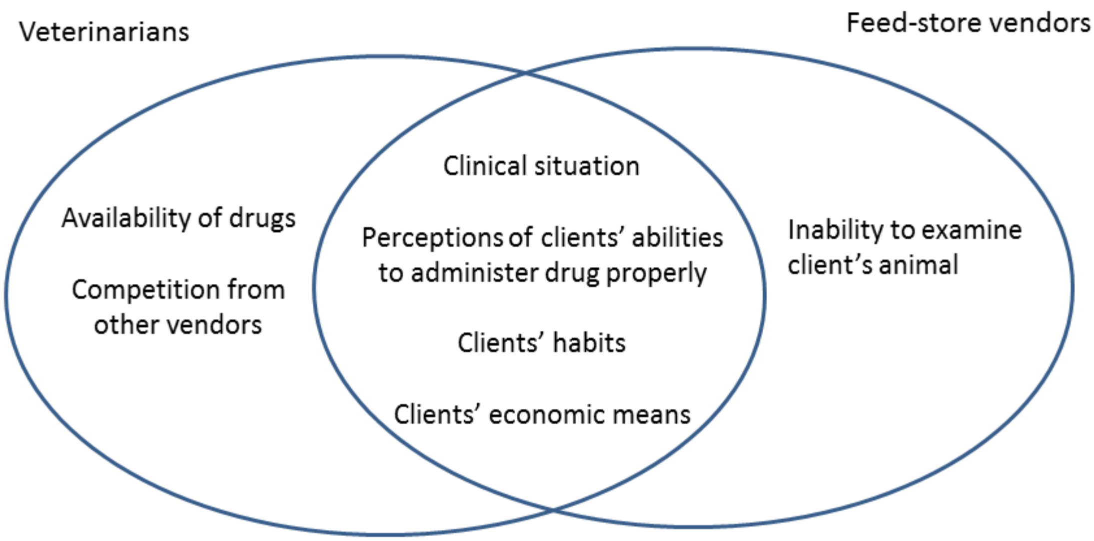

Figure 5 Conceptual framework describing the factors influencing the prescribing practices of animal health professionals

Table 10 Composition of Focus Group Discussions (veterinarians) and Interviews (feed-store vendors)

	Focus Group Discussion 1	Focus Group Discussion 2	Interviews
Gender	5 men, 1 woman	4 men, 2 women	8 men
Average time in the profession	6.8 years	5.5 years	6.3 years

Table 11 Frequency of discussion of essential points found to significantly influence antibiotic prescribing practices of veterinarians and feed-store vendors

	Number of times topic was discussed by veterinarians	Number of veterinarians referring to topic (number (percent))	Number of times topic was discussed by vendors	Number of vendors referring to topic (number (percent))
Importance of the clinical situation	6	4 (33)	5	3 (38)
Availability of drugs/Prescriber's inventory	13	6 (50)	0	0 (0)
Other prescribers	22	7 (58)	12	8 (100)
Perception of clients' abilities	12	7 (58)	14	7(88)
Clients' habits	12	10 (83)	5	2 (25)
Clients' economic means	17	10 (83)	7	7 (88)
Possibility (or lack thereof) of examining animal	4	2 (17)	6	5 (63)

Table 12 Quotes illustrating various topics discussed in focus group discussions and interviews

Topic	Relevant quote
Appropriate antibiotic use	
- Appropriate drug for clinical situation	"What we often see in the field is the misuse of antibiotics. For any disease, people reach for antibiotics without a proper diagnosis. We also see many cases where an antibiotic is confused with an antiparasitic drug - in some cases, people think they're dosing [against parasites] but they are in fact using an antibiotic." (Veterinarian, FGD 1)
- Proper dosing	"If, for example, we tell [a farmer] that they have to dose the animal with 1ml/kg, the farmer doesn't do so; some apply less, others apply more, saying "More is better", although not always. In the field, people often do not pay attention to indications [for the drug]". (Veterinarian, FGD 2)
- Use of diagnostic tests	"Commonly rural people or professionals in the field are not used to relying on or using a diagnostic laboratory test or microbiological diagnosis involving an antibiogram, because the owner can hardly afford that expense." (Veterinarian, FGD1)
- Avoidance of residues	"Most producers know that milk with residues should not be sent [to the milk company]. If the milk company doesn't say anything to them and we tell them they shouldn't send milk with residues, they send it anyway." (Veterinarian, FGD1)
Barriers to proper prescribing	
Perception of clients' abilities	"The problem here is that farmers have a very limited education. Most haven't even been to primary school. Even when they receive training [in proper drug usage], they get confused because each veterinarian from each laboratory has his own criteria, so the farmers easily get confused." (Feed-store vendor)
Clients' habits	"There are products that remain etched in the mind of the producer that they think can cure anything. […] Above all, they do things this way because their parents did things this way, so they continue doing so, and oftentimes it is difficult to get them to change." (Veterinarian, FGD 2)
Clients' economic means	"More than anything, I always say that the market of small producers has a lot to do with price. [Farmers] are always looking for the cheapest product. […] For example, penicillin is most expensive, oxytetracyclines are cheapest, and there are a large number of products of later generations which are much more expensive – impossible to use them in rural areas." (Feed-store vendor)
Drug Inventory	"It was very difficult to obtain penicillins on the market, which is why I say the market was not providing them. Oxytetracycline was used so often, and a study showed that Staph. Aureus was highly resistant to antibiotics. Animals responded well to penicillin, but it was hard to find that drug." (Veterinarian, FGD 1)

Chapter 5 Comparison of two methods for collecting antibiotic use data on small dairy farms

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ABSTRACT

Antibiotics are commonly used in animal agriculture; they can improve animal health and productivity, but their use may also represent a public health threat. Very little is known about antibiotic use on small farms in lower/middle income countries. To understand and improve antibiotic use on these farms, epidemiological data on antibiotic usage in livestock are necessary. However, acquiring such data can be difficult, as farmers are often illiterate (and therefore cannot participate in written surveys or keep treatment records), antibiotics can be obtained over-the-counter (in which case no prescriptions are generated) and monitoring and surveillance systems for drug use are often non-existent. The goal of this study was to compare two methods of acquiring epidemiologic data on antibiotic usage that are well-adapted to farms in lower-middle income countries: self-report and the collection of discarded drug packaging. A convenience sample of 20 farmers in Cajamarca, Peru, participated in the study. Farmers placed discarded antibiotic packaging in bins for six months. At the end of the six-month period, farmers were interviewed and asked to recall the antibiotic usage that occurred on their farm over the past month and past six months; these self-reported data were quantitatively and qualitatively compared to the bin contents collected in the last month and previous six months. We found that the agreement between the bins and self-report was relatively poor for both the quantity and types of antibiotics used. The bins appeared to perform better than self-report when bottles and mLs of antibiotics were measured, while self-report appeared to perform better for intra-mammary infusions. The bins also appeared to perform better when data pertaining to an extended time period (six months) were collected. The results of this study may provide guidance to investigators seeking to collect pharmacoepidemiologic data in similar environments.

Introduction

Antibiotics are commonly used in animal agriculture for growth promotion, the treatment of sick animals and the prophylactic treatment of healthy animals during periods of stress. These uses can improve animal health and productivity[10], but they may also promote antibiotic resistance among bacteria isolated from animals and humans, which can lead to infections with limited treatment options, greater mortality, and increased healthcare costs[115].

The misuse of antibiotics in human medicine in lower/middle income (LMI) countries has been extensively documented[41-43,45,128]. It is highly likely that antibiotics are also used inappropriately in animal agriculture in LMI countries. The US Centers for Disease Control and Prevention (CDC) reported that the widespread use of antibiotics in agriculture has resulted in increased resistance in infections in humans[33], and the propagation of resistant bacteria in animals and animal food products can increase the likelihood of the transmission of these bacteria to humans via food, the environment or direct contact with animals[129].

To understand the public health risk associated with antibiotic use in animal agriculture, epidemiological data on antibiotic use in livestock are necessary. Despite recommendations from the World Health Organization[34] to implement national surveillance programs for assessing antimicrobial usage in food animals, very little is known about the use of antibiotics in food animals in LMI countries. Furthermore, in LMI countries where sales records and on-farm treatment records are rarely kept, it can be difficult to accurately collect this type of data.

In general, data on antibiotic use in livestock have been collected at the national, regional, local and farm level from a variety of sources, including pharmaceutical companies, distributors, feed stores, pharmacies, over-the-counter retailers, veterinary clinics or farmers[65]. Examples of the different methods/sources used to collect information on drug use in both human medicine and veterinary medicine in previous studies are shown in Table 1.

Each source of data can be more or less accessible, especially in LMI countries where record-keeping and regulatory oversight may be limited. Data collected from the final user of the drug (or from the guardian or owner of the user – i.e., the farmer) are ideally suited for investigations on patterns of drug use[65]. However, the ascertainment of drug use data from consumers is subject to misclassification of drug exposure due to recall bias, reporting bias or social desirability bias[66]. Using data from prescriptions or sales records can also be unreliable, as such data do not take into account the adherence of consumers or the possibility of obtaining drugs from other sources (over-the-counter drugs, drugs sold on the black market, etc.)[66]. Enhancing the validity of pharmacoepidemiologic data obtained at the farm level is vital for using antibiotic use data to make inferences or design interventions aimed at improving use.

Few of the methods used in veterinary medicine can be applied in LMI countries where farmers are often illiterate, few (if any) treatment records or sales receipts are kept and national monitoring programs are nonexistent; as a result, the two methods most suited to small farms in LMI countries are in-person interviews with farmers and the collection of discarded drug packaging. The goal of this study was to compare the results obtained with these two methods on a sample of farms in a rural area of Cajamarca, a major dairy-producing region of Peru characterized by small peri-urban and rural farms (<15 cows/farm) with 30,000 registered milk producers[86] producing an estimated 307,187 kg of milk per day[87]. The farms encountered in Cajamarca are typical of small dairy farms in many other LMI countries, especially in Latin America.

1. Materials and Methods

2.1 Farms

The research team (a veterinary student from the United States and a Peruvian veterinary student) approached a convenience sample of owners of mid-sized farms in and around the city of Cajamarca to participate in the study. The purpose of the study was explained to the farmers, a consent form was read and farmers who agreed to participate provided verbal consent. Approval for this study was granted by the Institutional Review Boards of the University of Pennsylvania and the Universidad Peruana Cayetano-Heredia in Lima.

2.2 Demographic data collection and distribution of bins

Farmers who agreed to participate in the study were asked a series of questions to obtain demographic data and information on their farm and animals. Farmers were then provided with a 10-L plastic bin with a swinging lid labeled “Antibiotic packaging”. Farmers were instructed to place discarded packaging (bottles, boxes, empty infusion tubes) of any antibiotics (and antibiotics only) used on the farm (whether administered by the farmer or veterinarian) into the bin throughout the month.

2.3 Collection of bins and tallying of bin contents

At the end of each month, all of the farmers were visited and asked if they had used any antibiotic products in the previous month. Farmers who stated “Yes” and had items in the bin or who stated “No” and had no items in the bins were considered adherent to the study protocol. Farmers who reported “Yes” but had no items in the bins were considered non-adherent. The bin contents from each farm were emptied into a plastic bag labeled with the farmer’s unique ID number. The bin items were then tallied and the following information was recorded for each month: 1) number of bottles of antibiotic, 2) number of milliliters of antibiotic, 3) number of intra-mammary infusions, 4) names/active ingredients of all antibiotics, 5) names of any other type of (non-antibiotic) drug placed in the bin. Bin contents were collected six times during the six-month study period.

2.4 Final interview

At the end of the six-month period, the farmers or attendants-in-charge were interviewed by the research team to obtain self-reported drug use measurements. In particular, while being shown photos of antibiotics currently available on the market, farmers or attendants were asked the following questions:

1) How many bottles of antibiotics did you use in the past month?

2) How many milliliters of antibiotics did you use in the past month?

3) How many antibiotic intra-mammary infusions did you use in the past month?

4) What types of antibiotics did you use in the past month?

The same series of questions was then repeated, replacing “the past month” with “the past six months”. Half of the farmers/assistants were randomly assigned to answer the one-month series of questions first and the six-month series last; the second half of the sample was asked the questions in the opposite order.

2.5 Statistical analyses

Cohen’s kappas and McNemar odd ratios were calculated for the bin and self-report measures of categorical outcomes (presence/absence and reporting/non-reporting of specific drug types). Intraclass correlation coefficients for the bin and self-report measures of the continuous outcomes (bottles and mLs of antibiotics and infusions) were calculated.

Bland-Altman plots consisting of the mean number ((bin measure+self-report measure)/2) of continuous measures plotted against the difference between measures (bin measure - self-report measure) were constructed. The mean bias of the methods (bin measure - self-report measure) and the standard deviation of the bias were calculated, and the biases for the six-month and one-month period were compared with a Wilcoxon rank sum test. Ninety-five percent limits of agreement, calculated as the mean difference in drug measures ±1.96*SD, were calculated and labeled on the Bland-Altman plots. A horizontal line at y=0 was added to the plot to indicate the line of equality upon which all points would lie if both methods yielded the same results. Plots were then examined visually to identify any patterns in the data.

To investigate the potential for bias that is not constant across the range of values (proportional bias), linear regression models were fit for each measure of antibiotic (bottles, mLs, infusions) for each time period, with self-reported measures as the outcome variable and bin measures as the independent variable. The slope of the regression line was used to evaluate the extent of systematic bias between the two types of measurements. A Wald test was used to determine whether the slope of the regression line was significantly different from one. Scatter plots of the association between bin measures vs. the self-reported measures were constructed along with a line of equality (x=y) to illustrate the line upon which all points would lie in the absence of bias.

Ratios of six-month drug use to one-month drug use were calculated to examine the consistency of reported drug use by month. Ratios were compared for the two methods with the Wilcoxon rank sum test. P-values<0.05 were considered to indicate statistical significance. All analyses were conducted in Stata, v.11 (StataCorp. College Station, TX).

2. Results

3.1 Farm and farmer characteristics

Twenty-two farms were approached to participate in the study. One farmer declined to participate (participation rate of 95.5%) and one farmer, citing that the process was “too complicated”, dropped out of the study in the first month, resulting in a completion rate of 95.2%. Demographic data were collected on all farm owners at the beginning of the study (Table 13). The final interviews were conducted with six farm owners and 14 attendants-in-charge. Farms had an average of 33 lactating cows producing a total of 392 L of milk per day (12.4 L/cow/day). Farm owners had a mean age of 50.2 years and 60% of farm owners had completed university-level studies. Attendants-in-charge had a mean age of 40.6 years and, in general, were less educated than farm owners. Milk was sold to either large dairy processing companies (Nestlé or Gloria) or to local cheesemakers. On average, 3.2 men and 3.0 women worked on the farm in various capacities, including caring for the animals and the facilities, tending the fields or milking the animals (6.8 cows/worker).

3.2 Adherence to study protocol

Seventeen (85%) farmers were adherent to the study protocol (i.e., placed antibiotic packaging in the bins after having used antibiotics during the previous month) for the entire study period. One farmer was non-adherent for one month, one farmer was non-adherent for two months and one farmer was non-adherent for three months.

3.3 Bin contents

A total of 204 bottles, 34,665 mLs of antibiotics and 293 intra-mammary infusions ("chisguetes") were deposited in the bins during the six-month study. The active ingredients encountered (in order of decreasing frequency) were oxytetracycline, penicillin ± streptomycin, trimethoprim-sulfamethoxazole, gentamycin ± tylosin, enrofloxacin and cephalosporin. The intra-mammary infusions (with each infusion tube representing one dose) consisted mostly of cloxacillin and ampicillin; however, one farmer also used ceftiofur infusions. In addition, 16 of 20 farmers (80%) deposited non-antibiotic drugs in the bins, including vitamin complexes, anti-parasitic drugs, nutritional supplements (calcium, magnesium, phosphorus), diuretics, non-steroidal anti-inflammatory drugs, dexamethasone and oxytocin.

3.4 Measures of agreement for continuous variables

3.4.1 Intraclass correlation coefficients

The intra-class correlation coefficients (ICCs) evaluating the correlation between the number of bottles, mLs or intra-mammary infusions placed in the bin and the number of bottles, mLs or infusions reported by farmers were generally low (Table 14). The highest values were found for the infusions (0.585 and 0.521 for one month and six months, respectively), followed by the mLs (0.400 and 0.297 for one month and six months, respectively) and bottles (0.295 and 0.014 for one month and six months, respectively). In all cases, the confidence intervals of the ICCs for the one-month and six-month periods overlapped, suggesting that the correlations between measures were statistically similar for the two time points.

3.4.2 Bland-Altman analyses

The mean difference $\bar{d}$ (different from zero) between the number of bottles placed in bins and the number of bottles reported by the farmer represents the bias or lack of agreement between methods. The standard deviation of the difference $\bar{d}$ represents the variability of the differences and is used to calculate 95% limits of agreement between the methods. The 95% limits of agreement represent the range within which 95% of observations (i.e., differences between bin measures and self-report measures) fall. They do not represent confidence limits but function rather as a reference interval[130]. If the values of the differences within the range are considered "clinically acceptable", then the two methods could be used interchangeably. The mean differences in bottles, mLs and infusions, the deviations of these differences and the limits of agreement are reported in Table 14. Statistically significantly larger discrepancies were found for the six-month measures than for the one month measures for bottles and mLs (p=0.029 and p=0.066, respectively) but not for infusions (p=0.41). For both periods, the farmer reported having used more infusions than were placed in the bins ($\bar{d}$ <0), while more mLs were placed in the bin than were reported by farmers ($\bar{d}$ >0). No consistent trend was observed for bottles ($\bar{d}$ <0 for the one-month period and $\bar{d}$ >0 for the six-month period).

Bland-Altman plots, which plot the mean measure against the difference in measures, graphically display the biases ($\bar{d}$) and variability of the two methods relative to the 95% limits of agreement and the line of equality (y=0). Figures 6, 8 and 10 represent the Bland-Altman plots for bottles, mLs and infusions, respectively, for the one-month period; Figures 7, 9 and 11 represent the same respective plots for the six-month period.

According to the Bland-Altman plots, for all measures (bottles, mLs, infusions) both the one-month and the six-month periods, the absolute value of $\bar{d}$ grew as the mean number of measures increased. For bottles and mls, the mean difference tended to be positive more often than negative (i.e., the bins contained more measures than were reported by the farmers). Relatively few intra-mammary antibiotic infusions were used during the one-month period, and in all cases, the farmer reported using more infusions than the bin contained ($\bar{d} \leq 0$). For the six-month period, as the mean number of infusions increased, $\bar{d}$ values became more divergent and exclusively negative, that is, the farmers reported using more infusions than the bins contained.

3.4.3 Regression analyses

Given that the Bland-Altman plots displayed larger discrepancies between methods with higher values of the measures, we conducted regression analyses to examine the extent of any systematic bias between bin and self-reported measures. When self-reported measures are similar to the quantities from the bins, the regression line should be coincident with the line of equality (x=y), i.e., the slope of this regression line should be equal to one. The slopes of the regression lines comparing self-reported measures to bin measures are reported in Table 14. Figures 12, 14 and 16 show scatterplots of the bin measures vs. the self-reported measures for the number of bottles, mLs and infusions, respectively, used during the one-month period. Figures 13, 15 and 17 show the same respective scatterplots for the six-month period. Lines of equality (solid lines) and regression lines for the association between self-reported and bin values (dashed lines) are shown. For one-month bottles and one-month mLs, the slopes of the regression lines were not significantly different from unity (p=0.995 and p=0.536, respectively). For all other measures, the slopes were statistically significantly different from one, indicating poor agreement between the methods.

3.5 Consistency of antibiotic use

If antibiotic use is more or less consistent throughout the year, antibiotic use over the six-month period should be approximately six times as high as antibiotic use in the one-month period. We therefore compared the ratio of six-month antibiotic use to one-month antibiotic use for each method (Table 15). For bottles, the ratios were close to six (6.98 and 4.24 for bins and self-report, respectively) and not significantly different from each other (p=0.120). For mLs, the ratios were more divergent (19.3 and 5.62 for bins and self-report, respectively) and not quite significantly different from each other (p=0.089). For the intra-mammary infusions, the ratios were more similar (5.23 and 3.83 for bins and self-report, respectively) and not significantly different from each other (p=0.169). These results suggest that, with the exception of bin-mLs, antibiotic use on a monthly basis appeared relatively consistent.

3.6 Measures of agreement for drugs classes

Cohen's kappa provides a measure of inter-rater agreement for the two methods. Kappa is thought to provide a more robust measurement of agreement than simple percent agreement, as agreement that occurs by chance is accounted for. Kappa values for individual drug classes for the bins and self-report ranged from -0.29 to 0.52, indicating that agreement between the methods for individual drug classes was non-existent to fair (Table 16).

For injectable penicillin and oxytetracycline, one-month kappas were higher than six-month kappas. For the remaining types of antibiotic (intra-mammary infusions, trimethoprim-sulfamethoxadole (TMS) and all other types), six-month kappas were higher than one-month kappas. This suggests that the bins and farmers were more likely to agree on the most commonly-used drugs (penicillin and oxytetracycline) during the short-term (one-month) intervals; in contrast, farmers and bins were more likely to agree on the less-commonly used drugs for the longer six-month interval.

An analysis of the marginal homogeneity of the contingency tables displaying the presence/absence of a drug in the bin and the reporting/non-reporting of the drug by the farmer using McNemar’s test enabled us to calculate the odds ratios of a bin having a particular drug vs. the farmer reporting the drug (Table 17). An odds ratio greater than 1 means that the bin was more likely to contain a drug than the farmer was to report it. For the one-month period, the bins tended to contain fewer products than were reported by the farmer (odds ratios of 0.67, 0.67 and 0.2 for penicillin, oxytetracycline and TMS, respectively). In contrast, during the six-month period, the bins were more likely to contain a drug than a farmer was to report it (odds ratios of 1.5, 3.0 and 4.0 for penicillin, oxytetracycline and TMS, respectively).

3. Discussion

The goal of this study was to compare two methods of collecting antibiotic data on small and mid-sized farms in a peri-urban area of Peru. Because neither method has been established as a gold standard, we conducted an exploratory analysis examining the agreement between the methods for both drug amounts and drug categories. If one method tended to consistently report higher amounts of drugs than the other method (and the other method therefore appeared to be consistently under-reporting) or if one method reported the use of a particular drug type while the other did not, than that method could potentially be considered superior to the other and recommended for future studies on similar topics. Both methods were also evaluated for a short-term time frame (one month) and a longer-term time frame (six months), as it is possible that one method might perform better than the other when the collected data pertain to different time periods. In general, good adherence was achieved among farmers in depositing drug packaging in the bins and participating in the final interviews.

We found that the agreement between bins and self-report used to collect data on antibiotic use on farms was relatively poor; we also found that bins appeared to perform better than self-report for longer periods of time and that self-report appeared to perform better for more commonly used drugs on a short-term basis and for intra-mammary infusions.

The low intraclass correlations suggest that the continuous data (amounts of antibiotic) generated by each method were poorly correlated, while the low kappa values suggest that the agreement between methods for categorical data (drug types) was relatively low. Agreement tended to be worse for the six-month period than for the one-month period: all of the slopes of the regression lines generated by the regression of one method against the other were significantly different from 1 for the six-month period, whereas only the slope for infusions was significantly different from 1 for the one-month period. In addition, the mean biases of the Bland-Altman analyses tended to be larger during the six-month period: for bottles, mLs and infusions, the absolute differences were 10.6, 67 and and 4.7 times higher, respectively, during the six-month period than during the one-month period.

Better agreement (i.e., higher kappa values) was found for injectable penicillin and oxytetracyclines (the more commonly used drugs) during the one-month period, while the reverse was true for the less commonly used drugs (TMS, intra-mammary infusions of penicillin and all other types). Furthermore, for both time periods, the bin and self-reported measures tended to diverge (i.e., $|\bar{d}|$ increased) as greater volumes of products were used. In most cases, the limits of agreement were unacceptably large; for example, the difference between bin mLs and self-reported mLs could be as large as 3258 (six-month period), while the difference in number of infusions used could be as high as 112. Because these limits of agreement are far beyond what would be considered “clinically acceptable”, these two methods should not be used interchangeably.

A strength of the bins is that they were unable to “over-report” antibiotic usage; therefore, if a bin contained more products than a farmer reported, then the farmer was under-reporting (and therefore less accurate). However, if a farmer reported having used more products than were contained in the bin, we have no way of knowing if the farmer was over-reporting or if the bins were “under-reporting” (i.e., a farmer forgot to put packaging in the bin or only used part of what was in a bottle). Positive differences between bin and self-report measures ($\bar{d}$ >0) suggest that farmers tended to under-report drug use on their farm. For bottles and mLs, the differences tended to be positive (or only very slightly negative for one-month bottles) and the odds ratios for all antibiotics except intra-mammary infusions tended to be greater than 1 during the six-month period. As a result, for both bottles and mLs of antibiotics, the bins appeared to represent a more accurate way of measuring consumption than self-report, especially over longer periods of time (e.g., during the six-month period).

For infusions, the differences in measures were always negative and the odds ratio for one-month and six-month infusion use was always less than one; these results suggest that farmers either over-reported their use of infusions or did not place all of the used infusion tubes in the bins (or both). Given that 16/20 farmers placed no infusions in the bins during the one-month period but only 9/20 farmers reported having used no infusions during this time, it appears more likely that farmers simply did not place their used infusion tubes in the bin. Therefore, it appears that self-report was actually more accurate than bins for intra-mammary infusions.

Several studies have compared the collection of drug packaging to the use of treatment diaries on farms. Carson et al. compared these two methods of obtaining antibiotic use data on 24 beef farms in Ontario for a period of 12 months[102]. The authors found that the relative ranking of types of antibiotic used depended on the chosen metric and that the kg of active ingredients/1000 animals differed significantly by method for tetracycline, penicillin, florfenicol, tilmicosin, and spectinomycin. Overall, these authors recommended the use of bins over treatment diaries, as producers had problems recording routine treatments and treatments given during disease outbreaks; furthermore, the bin method was thought to be more accurate, as "producers were enthusiastic about having their "garbage" disposed of for them, protection of confidentiality created little reason for dishonesty, and throwing out empty containers did not take much effort."

Dunlop et al. assessed antimicrobial usage on 34 swine farms in Canada using on-farm treatment records and the collection of discarded drug packaging[84]. These authors also found that the bins represented convenient and fairly accurate ways of recording antimicrobial drug use and that the volumes of inventoried antimicrobials were highly correlated with the volume of antimicrobials listed in treatment records (r=0.90, p=0.0001); however, they also found the treatment records underestimated drug use by 35%.

In agreement with these studies, we found that the bins tended to perform better than self-report when data were collected over a longer period of time. However, both methods clearly have their limitations. Self-report can be affected by recall, reporting and social desirability bias. Bins may not contain all of the antibiotics that were used on the farm if farmers forget to place discarded packaging in them, if only a portion of the drug contained in a bottle is used (and the farmer or veterinarian obviously does not want to place a bottle with product remaining in the bin) or if the farmer does not inform all of the workers on the farm of the required protocol. In this study, the differences between bin and self-reported measures were not statistically significantly different for farm owners vs. attendants-in-charge, which suggests that the farm owners informed their workers of the protocol and that the workers were just as adherent to the protocol as the farm owners. However, we noted that 16/20 (80%) of farmers placed other types of drugs in the bin in addition to antibiotics; it is possible that farmers who saw a bin that was already "full" of other drugs may have been less likely to add antibiotic packaging.

This study had a number of limitations that warrant discussion. First, because a convenience sample of farmers was chosen, selection bias may exist; therefore, caution must be exercised before attempting to generalize these results beyond this sample of farms. Recall bias may have also affected our findings. Farmers were told that the study objective was to compare the two methods of collecting data on antibiotic use; they might therefore have thought about and remembered their antibiotic usage more carefully than if they had not been made aware of the study objective. The recall of farmers might have therefore been artificially high.

Second, the chosen metrics (bottles, mLs and infusions of antibiotics) may not be the most informative metrics to use when attempting to collect data on antibiotic usage, especially if there is no clear indication of the units of antibiotic contained in a bottle or in an ml. Other studies examining antimicrobial drug use on farms have used animal defined daily doses (the average daily on-label dosage multiplied by the approximate weight of an adult dairy cow)[94,95], antimicrobial drug usage rates (the number of animal defined doses used on a farm per 1,000 cows per day)[104], kg of active ingredients/1000-animal days[102] and individual-animal treatments[84]. In our study, the drug dosages indicated on the discarded packaging (which could have been used to calculate doses for a standard-sized animal) are rarely followed by farmers; in addition, given the range of sizes of animals on these farms, it would have been impossible to calculate the number of doses used from the information in the bins. The chosen metrics were the most readily available, and because farmers on small farms tend to think more in terms of bottles and mLs of antibiotics than mgs of active ingredients or defined daily doses, these metrics could actually be useful in settings such as those encountered in this study.

Third, it appeared that many farmers did not put “atypical” non-therapeutic antibiotics such as dry cow intra-mammary infusions in the bins despite the fact that they were told to place all antibiotic products in the bin. This may have been because farmers or attendants were not aware that these products contain antibiotics. Similar situations could occur in the case of medicated feeds given to calves (though none of the farms in this study administered such feeds to their animals). Therefore, great care would need to be taken to ensure that farmers understand which products contain antibiotics and to ensure that all such products would be put in the bin and/or mentioned in an interview.

Fourth, most of the farms placed non-antibiotic drugs in the bin despite the fact that bins were labeled “Antibiotics” and that the research team had instructed them to place only antibiotic products in the bin. We were unable to evaluate whether farmers understood the difference between “drugs” and “antibiotics” or whether farm owners may not have properly instructed their workers. Farmers in Cajamarca were often unable to define an antibiotic[116]; as a result, it is possible that a lack of awareness that intra-mammary infusions contain antibiotics may explain this finding. Finally, the relatively small sample size resulted in a number of non-significant associations in this study.

The strengths of this study included the relatively long follow-up period (six months), the good adherence of the participants to the study protocol, the high participation rate, the ability to compare one-month and six-month results and the use of photos of antibiotics available on the market during the final interviews to enhance recall by providing stimulatory visual cues.

The results of this study may provide guidance for investigators undertaking similar studies in similar environments. On a short-term basis (one month), the use of self-report can be recommended over the use of bins, which require considerable logistic support. However, for drug use data collected over a more extended period of time, bins, which appear to provide more accurate results than self-report, should be considered. In either case, great care should be taken to thoroughly explain the study protocol to farmers and farm workers and to ensure that study participants are aware of all products (including intra-mammary infusions and medicated feed) that may contain antibiotics or the active ingredient of interest. A system of inventorying drugs (especially bottles) present on the farm at the beginning and end of the study could also be implemented to account for discrepancies in bin and self-report data.

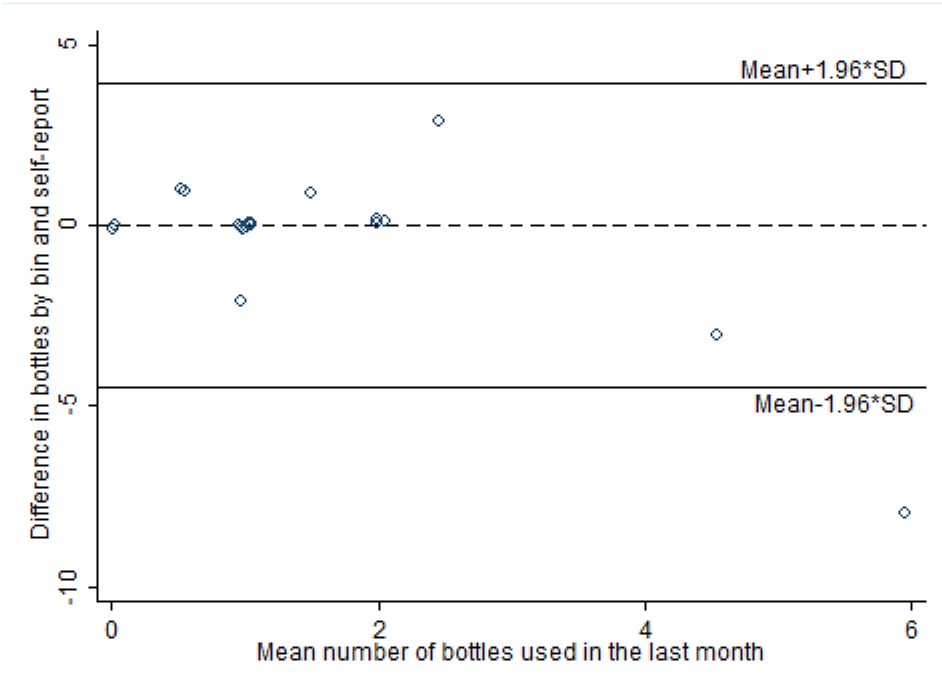

Figure 6 Bland-Altman plot of bottles of antibiotics collected in bins and reported by farmers on a sample of 20 farms in Cajamarca, Peru during a one-month period

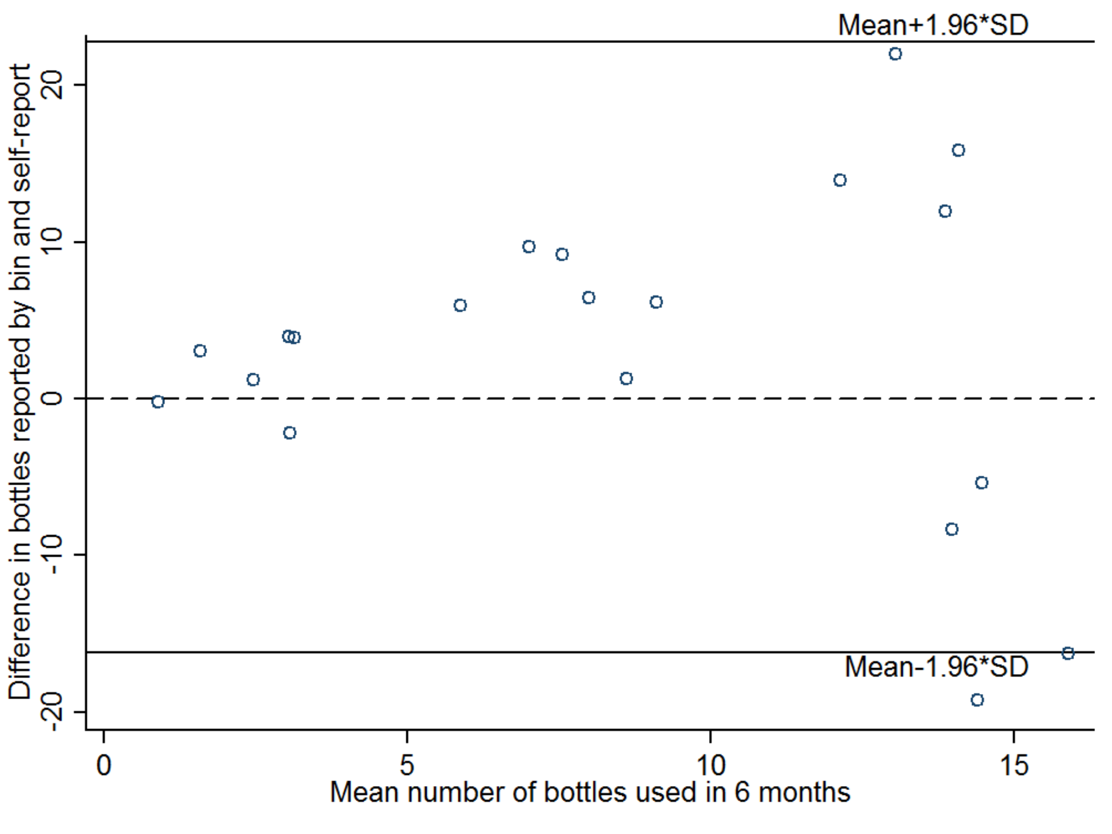

Figure 7 Bland-Altman plot comparing bottles of antibiotics collected in bins and reported by farmers on a sample of 20 farms in Cajamarca, Peru during a six-month period

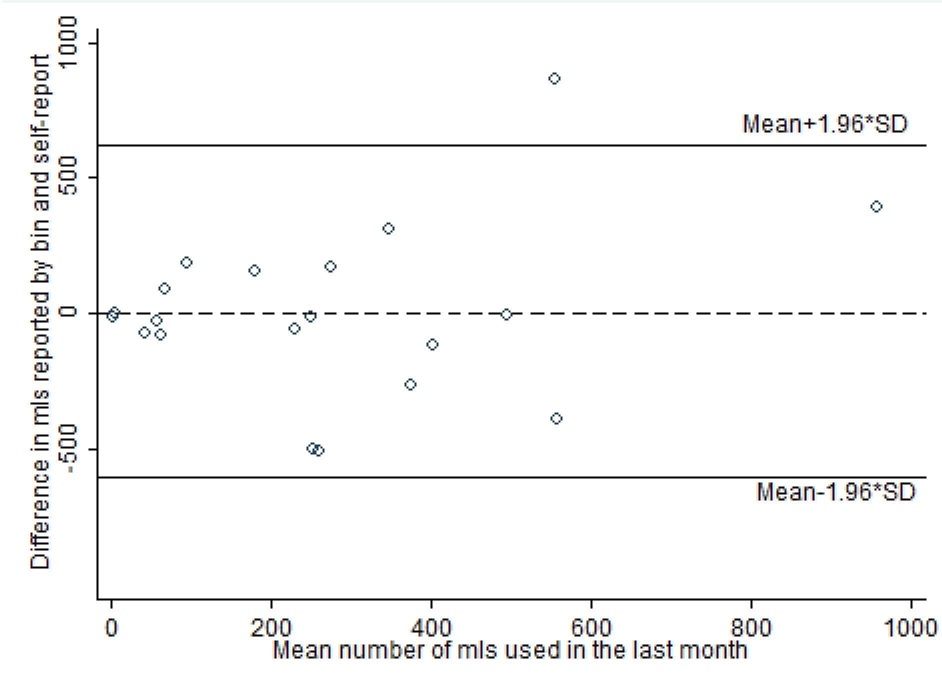

Figure 8 Bland-Altman plot comparing milliliters of antibiotics collected in bins and reported by farmers on a sample of 20 farms in Cajamarca, Peru during a one-month period

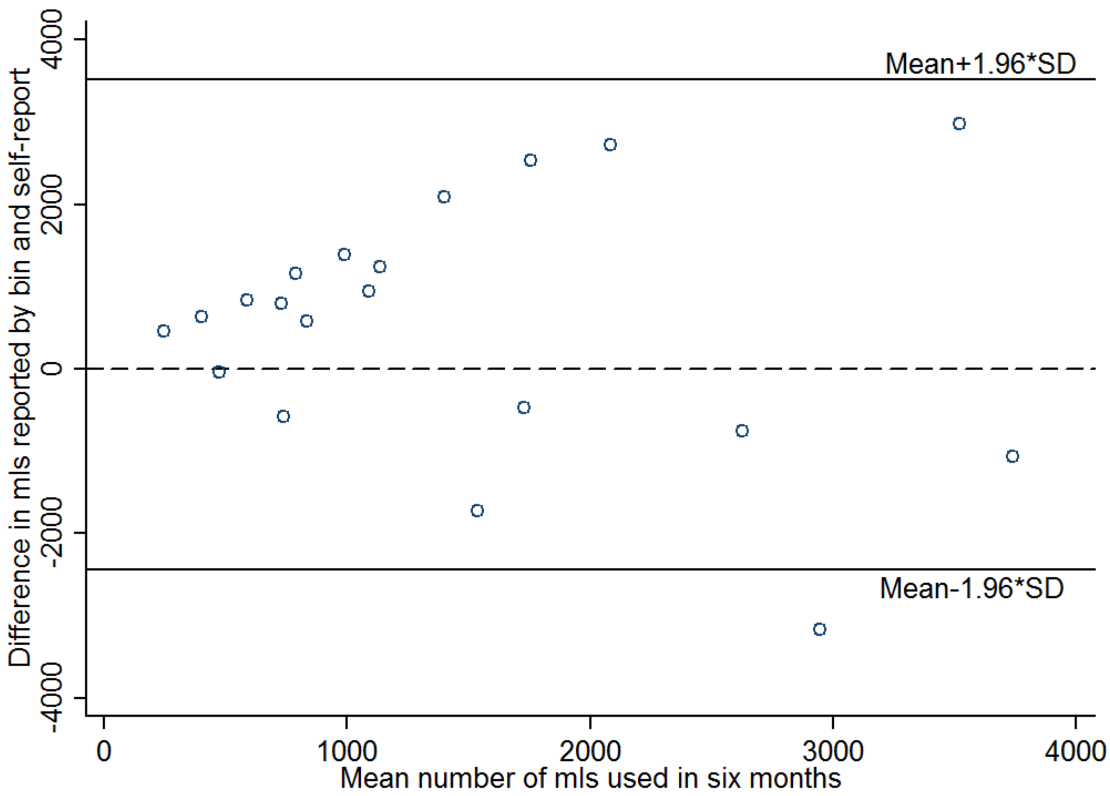

Figure 9 Bland-Altman plot comparing milliliters of antibiotics collected in bins and reported by farmers on a sample of 20 farms in Cajamarca, Peru during a six-month period

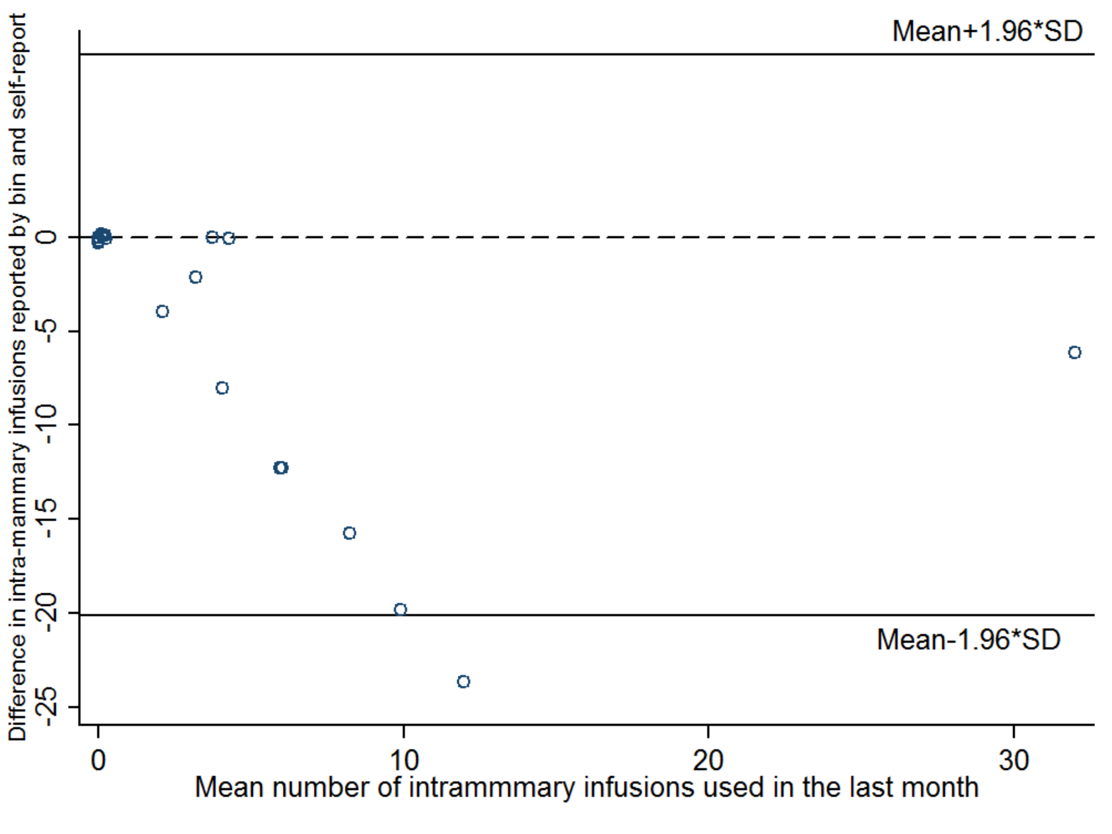

Figure 10 Bland-Altman plot comparing intra-mammary infusions of antibiotics collected in bins and reported by farmers on a sample of 20 farms in Cajamarca, Peru during a one-month period

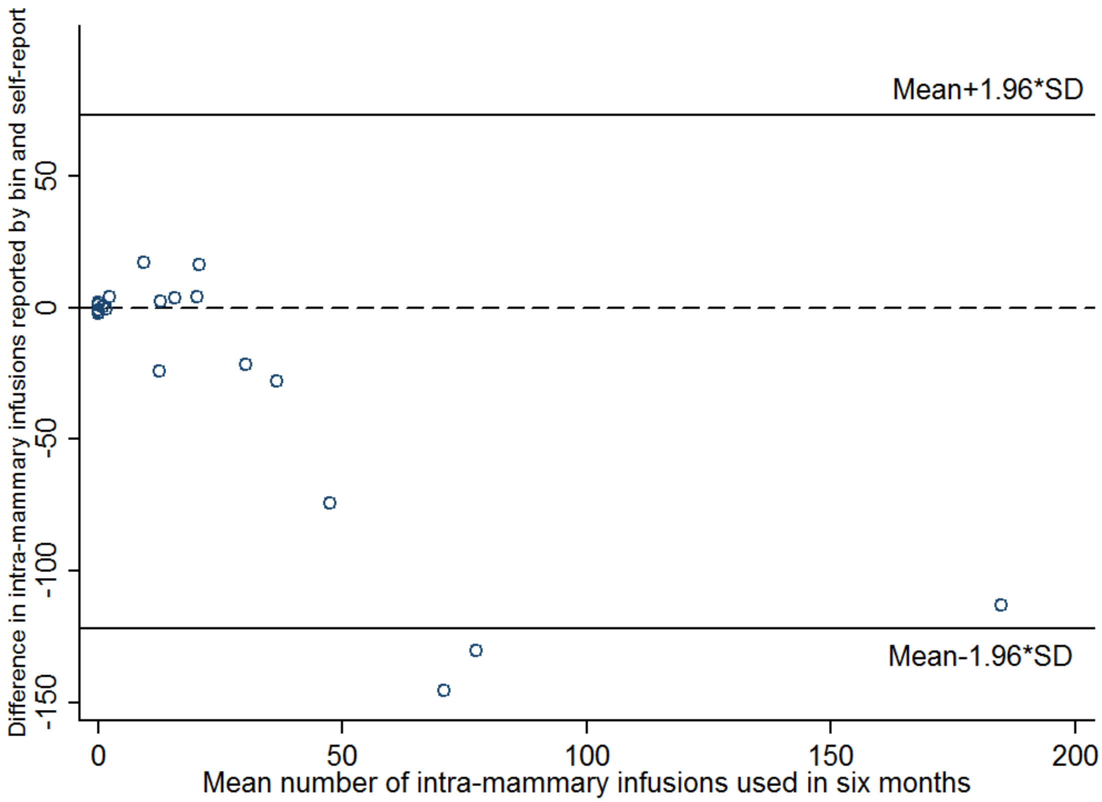

Figure 11 Bland-Altman plot comparing intra-mammary infusions of antibiotics collected in bins and reported by farmers on a sample of 20 farms in Cajamarca, Peru during a six-month period

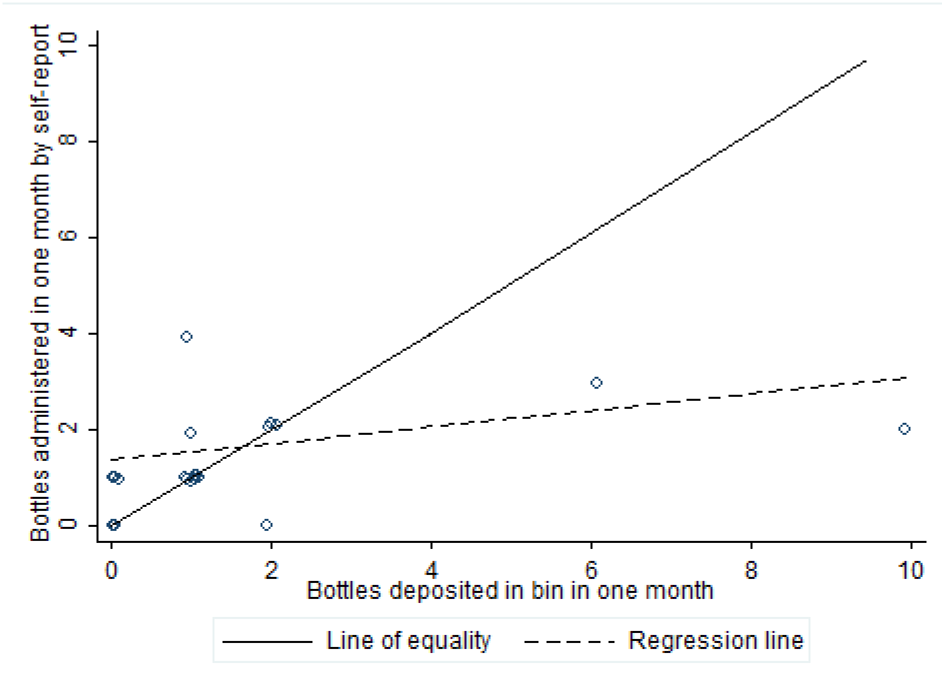

Figure 12 Bottles of antibiotics collected in bins and reported by farmers on a sample of 20 farms in Cajamarca, Peru during a one-month period

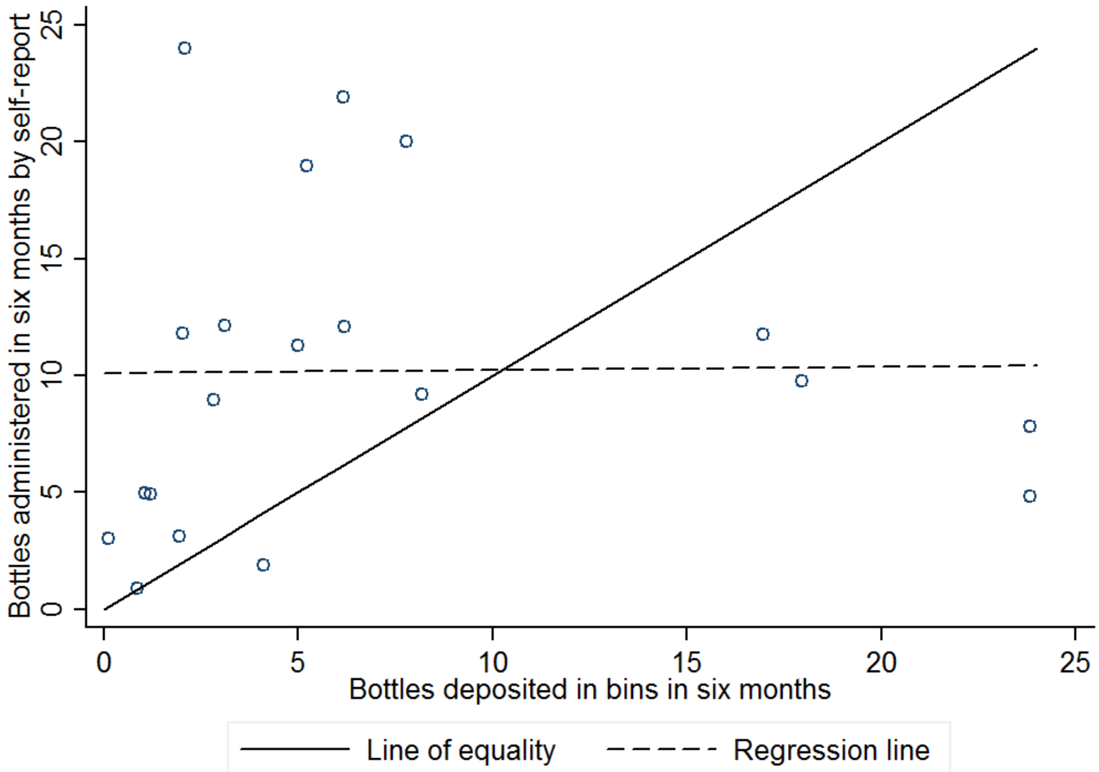

Figure 13 Bottles of antibiotics collected in bins and reported by farmers on a sample of 20 farms in Cajamarca, Peru during a six-month period

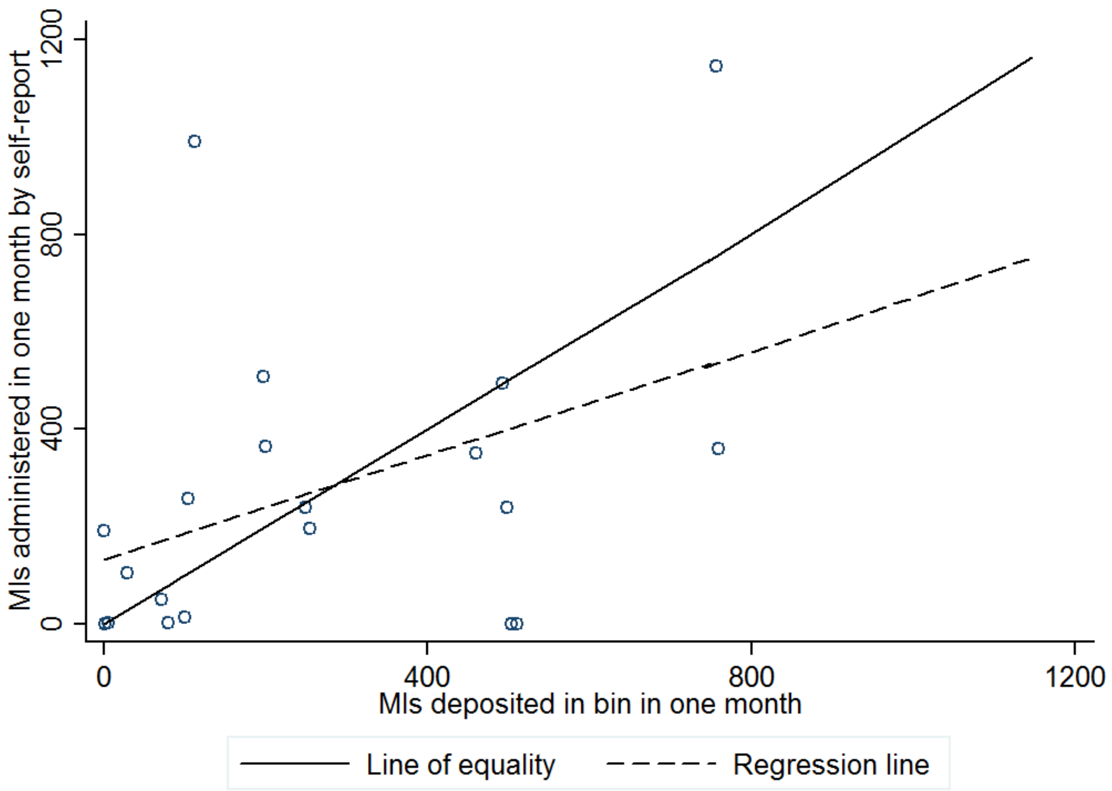

Figure 14 Milliliters of antibiotics collected in bins and reported by farmers on a sample of 20 farms in Cajamarca, Peru during a one-month period

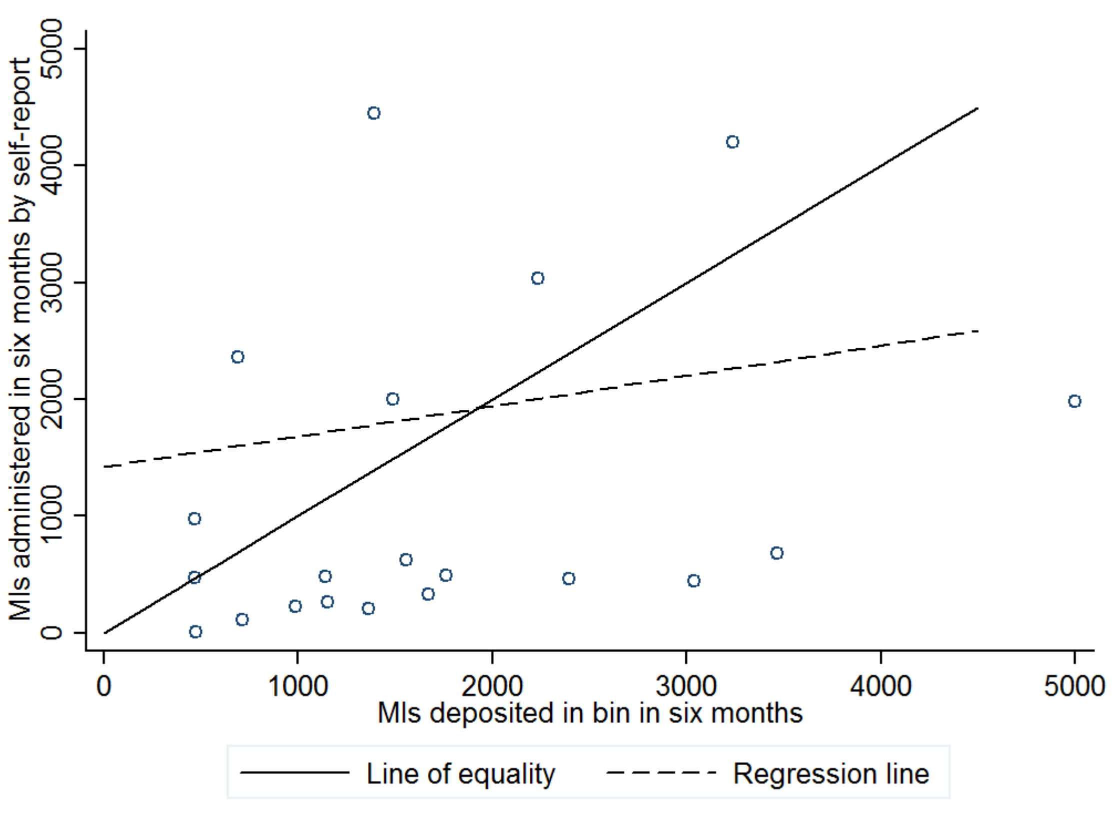

Figure 15 Milliliters of antibiotics collected in bins and reported by farmers on a sample of 20 farms in Cajamarca, Peru during a six-month period

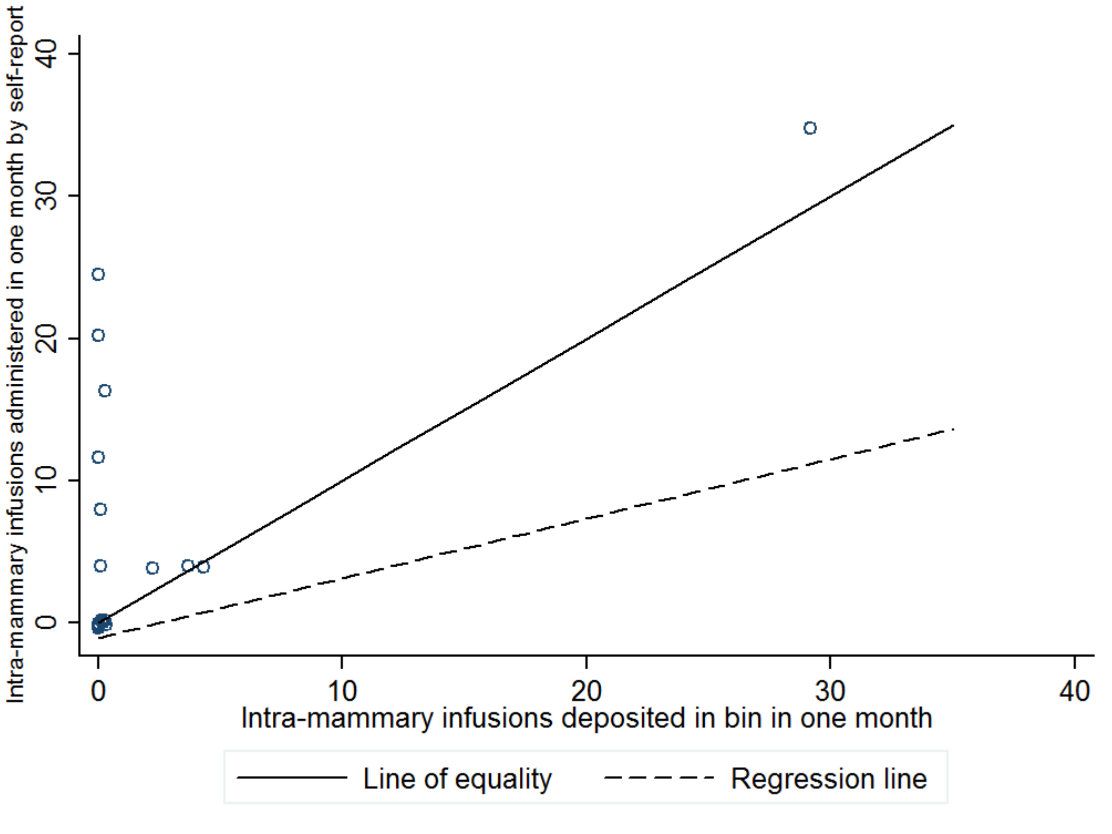

Figure 16 Intra-mammary infusions collected in bins and reported by farmers on a sample of 20 farms in Cajamarca, Peru during a one-month period

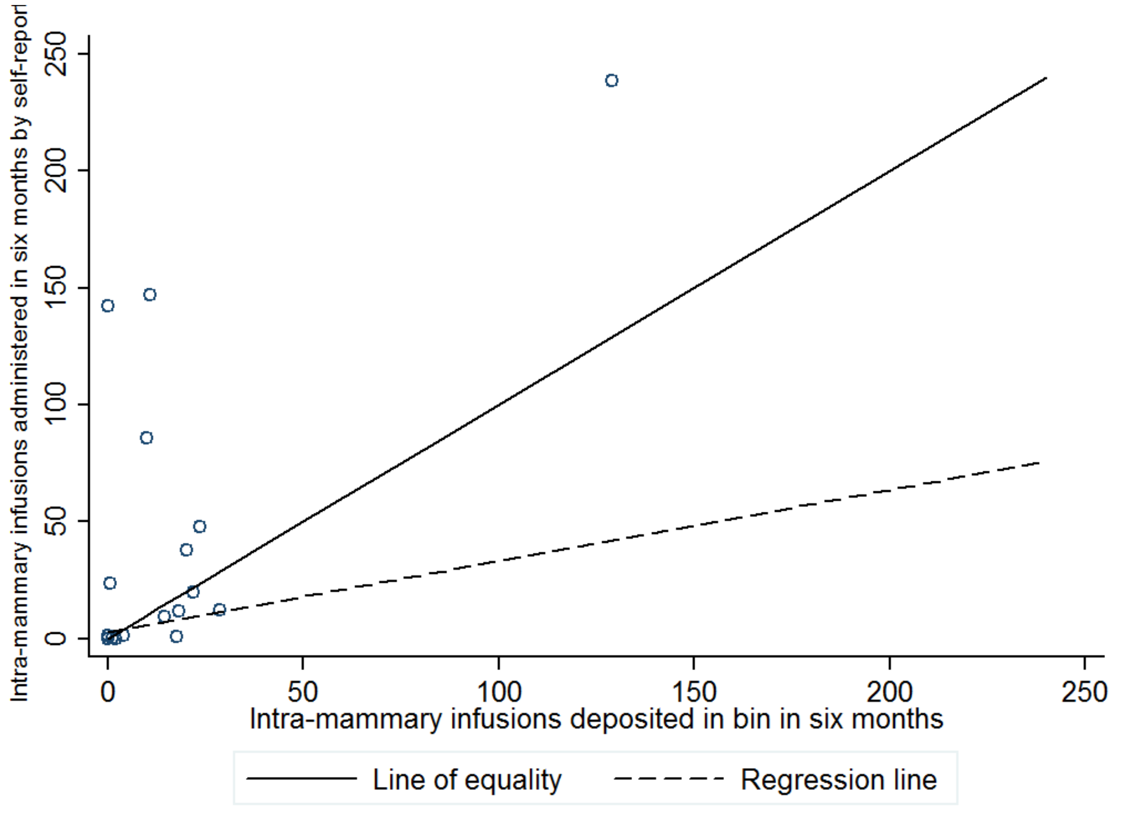

Figure 17 Intra-mammary infusions collected in bins and reported by farmers on a sample of 20 farms in Cajamarca, Peru during a six-month period

Table 13 Characteristics of farms and farmers (n=20) enrolled in a study comparing methods of acquiring antibiotic usage data in Cajamarca, Peru

Farm or farmer characteristic	No. of respondents
Mean (SD, min, max) number of lactating cows	33.0 (20.4; 6; 90)
Mean (SD, min, max) number of total cows	40.6 (25.1; 11; 120)
Mean (SD, min, max) number of calves	21.8 (13.6; 5; 50)
Mean (SD, min, max) daily milk production (L)	392 (231; 75; 1000)
Mean (SD, min, max) age of farm owner (n=20)	50.2 (11.7; 28; 67)
Mean (SD, min, max) age of attendant-in-charge (n=14)	40.6 (13.6, 16, 69)
Mean (SD, min, max) number of men working on farm	3.2 (2.0; 1; 8)
Mean (SD, min, max) number of women working on farm	3 (1.7; 1; 7)
Education level of farm owner (n(%))	
Primary school completed	4/20 (20)
Secondary school completed	4/20 (20)
University completed	12/20 (60)
Education level of attendant-in-charge	
Some primary school	6/14 (42.9)
Some secondary school	5/14 (35.7)
Some university	3/14 (21.4)
Destination of milk (n(%))	
Nestle	9/20 (45)
Gloria	5/20 (25)
Cheesemakers	7/20 (35)

Table 14 Intraclass correlation coefficients, slope of the regression line comparing self-reported measures and bin measures, and bias, variability of the bias and limits of agreement for bin measures and self-reported measures in a sample of 20 farmers asked to collect discarded drug packaging and recall antibiotic usage in Cajamarca, Peru

		ICC	95% CI of the ICC	Slope of regression line (bin measure vs. SR measure)	P-value indicating a significant difference from 1 for the slope	Mean bias[a]	SD	LL and UL[b] of agreement
Last month	Bottles	0.30	0.00-0.63	0.99	0.99	-0.3	2.15	-4.51; 3.91
	Volume (mLs)	0.40	0.11-0.69	0.54	0.11	8	313	-605; 621
	IMM infusions	0.59	0.370-0.80	0.42	<0.001	-5.2	7.61	-20.1; 9.72
Six months	Bottles	0.01	0.00-0.46	0.01	<0.001	3.2	10.0	-16.4; 22.8
	Volume (mLs)	0.30	0.00-0.63	0.26	0.001	536	1520	-2443; 3515
	IMM infusions	0.51	0.27-0.76	0.30	0.001	-24.4	49.8	-122; 73.2

[a]The mean bias represents the difference between bin measures and self-report measures
[b]The LL and UL represent the mean bias-1.96*SD and the mean bias+1.96*SD, respectively.
IMM=Intra-mammary, CI=confidence interval, ICC=Intraclass correlation coefficient, SD=standard deviation, LL=lower limit, UL=upper limit

Table 15 Ratio of six-month to one-month measures of antibiotic usage data collected on a sample of 20 farms in Cajamarca, Peru

	Ratio[a]	SD	P-value for Wilcoxon rank sum test comparing bin and self-reported measures
Bin bottles	6.98	5.27	0.120
Self-reported bottles	4.24	4.12	
Bin volume (mLs)	19.3	50.1	0.089
Self-reported volume (mLs)	5.62	7.51	
Bin intra-mammary infusions	5.23	1.21	0.169
Self-reported intra-mammary infusions	3.83	1.90	

[a] If antibiotic use is consistent over the six-month period, the expected ratio is 6

Table 16 Agreement between antibiotic use data collected in bins and self-reported by farmers (n=20) in Cajamarca, Peru, for individual drugs classes

		Prevalence in bin	Prevalence by self-report	Percent Agreement	Kappa	Kappa P-value
One month	Penicillin IM	0.40	0.45	0.75	0.49	0.0138
	IMM Penicillin	0.20	0.25	0.55	-0.29	0.902
	Oxytetracycline	0.40	0.45	0.75	0.49	0.0138
	TMS	0.15	0.35	0.70	0.24	0.106
	Other types of antibiotic	0.20	0.20	0.70	0.063	0.390
Six months	Penicillin	0.75	0.70	0.75	0.38	0.045
	IMM Penicillin	0.55	0.30	0.75	0.52	0.004
	Oxytetracycline	0.85	0.65	0.60	-0.013	0.526
	TMS	0.50	0.35	0.75	0.50	0.0095
	Other types of antibiotic	0.75	0.43	0.70	0.43	0.0098

IM=intramuscular, IMM=intramammary, TMS=trimethoprim-sulfamethoxazole

Table 17 Marginal agreement between bin and self-reported by farmers (n=20) for individual drug types in Cajamarca, Peru

		Odds ratio [a]	P-value
One month	Penicillin	0.67	0.65
	Intra-mammary penicillin	0.8	0.734
	Oxytetracycline	0.67	0.65
	TMS	0.2	0.10
	Other types of antibiotic	1	1
Six months	Penicillin	1.5	0.74
	Intra-mammary penicillin	0.92	0.025
	Oxytetracycline	3.0	0.16
	TMS	4.0	0.18
	Other types of antibiotic	Undefined	-

[a] The odds ratio compares the probability of a bin containing the drug to the probability that the farmer reported using the drug

Chapter 6 Summary and Future Directions

Antibiotic use in dairy production can contribute to the improved health and productivity of animals and therefore to the improved livelihood of the farmer. However, the inappropriate use of antibiotics can threaten the health of the animals, of the farmer and of the consumers of dairy products and compromise the livelihood of the farmer. The judicious use of antibiotics is therefore critical for farmers and consumers of dairy products. In Cajamarca, Peru, a large proportion of the rural population is engaged in dairy farming; the farms in this area are similar to small dairy farms in other parts of Latin America and in other lower/middle income countries (LMICs). Understanding the use of antibiotics and the farming practices on these farms can provide us with an indication of how these drugs are used on typical small dairy farms in LMICs and provide valuable information to stakeholders and policy makers in the region that can be used to improve drug use and farming practices on these farms.

We conducted a cross-sectional study to understand patterns of antibiotic use, farming practices and the extent of farmers' knowledge of antibiotics on rural farms in Cajamarca. We found that antibiotics were used relatively infrequently (0.477 episodes of treated disease per cow-year) on rural farms in Cajamarca and that few active ingredients were used (mostly oxytetracycline and penicillin). Farmers obtained their drugs from a veterinarian, from feed-stores (i.e., over the counter) or both, and cited the recommendation of the prescriber as the most important factor affecting their choice of drugs. A majority of farmers (80.2%) reported following the dosage recommended by the prescriber, but 29% of farmers also reported having observed complications when administering antibiotics to their animals, which suggests that the drug may not have been administered properly.

Farmers’ knowledge of antibiotics was adequate. While few were able to define an antibiotic or withdrawal times, most knew that there were risks associated with the administration of antibiotics for the animals and for the consumers of the dairy products. An increased incidence of treated disease on farms was negatively correlated with farmer income and with farm size and positively correlated with antibiotic knowledge. These associations might seem counter-intuitive; however, farm size and farmer income are positively correlated with a farmer's education level, and better-educated farmers appeared to have better management practices and therefore more healthy animals. Antibiotic knowledge, in turn, was correlated with a farmer's education level, with the practices of buying antibiotics from the feed-store and using antibiotics for prophylactic purposes and having observed complications in cows after having administered antibiotics.

All of these findings suggest that the use of antibiotics and animal health can be improved by increasing farmers' knowledge of and exposure to antibiotic use. The training of farmers on the judicious use of these drugs, on animal disease prevention and on adequate farm management can contribute to improved drug use and improved farming practices which, in turn, can improve animal health and productivity and farmer income. The implications of these findings on the public health aspect of antibiotic use are unclear. In particular, the degree of resistance to the most commonly used antibiotics in the field is unknown and would need to be studied. If the degree of resistance is low, than the continued use of a small number of active ingredients would not have a detrimental effect on public health. If the degree of resistance is high, then efforts would need to be undertaken to expand the registry of drugs available to farmers and more stringent efforts to ensure their appropriate use would be needed.

We also conducted a series of studies examining the issue of antibiotic residues in milk. The occurrence of antibiotic residues represents a health threat to consumers and a source of economic loss to the farmer and/or the dairy processing company. We found that on a given day, the point prevalence of contamination of milk with antibiotic residues for a given milk route was low (0-4.2%). However, we found that 91.6% of farmers who were treating their animals with antibiotics at the time sold milk with residues. Furthermore, 44% of randomly surveyed farmers admitted selling milk with residues, and those who reported withholding milk only did so for a mean of 2.2 days, which is less than the withdrawal times associated with oxytetracycline and penicillin (seven and five days, respectively). These findings suggest that a majority of farmers sell milk with antibiotic residues at one point or another. The factors associated with reporting to sell milk with residues were the purchaser of milk (the farmers who sold milk to cheese-making companies or Milk Company 2 were more likely to report selling contaminated milk than the farmers who sold milk to Milk Company 1) and the antibiotic knowledge score. This suggests that the issue of antibiotic residues could be mitigated in part by improving farmers' knowledge of antibiotics and drug withdrawal times; however, the issue is most likely to be resolved by the imposition of measures to incentivize the withholding of contaminated milk by producers (e.g., penalties for contaminated milk or the use of economic incentives) by the milk companies and, if necessary, governmental regulation of milk quality standards.

The issue of antibiotic residues in milk ultimately represents a significant challenge for farmers, dairy processing companies and policymakers. Farmers cannot afford to lose the income from the sale of milk that would need to be withheld to avoid antibiotic residues. At the same time, dairy processing companies are often unwilling to impose penalties for contaminated milk, as they would risk losing their providers to purchasers of milk with less stringent requirements and would therefore not be able to collect the necessary volume of milk to make routes to the rural areas of Cajamarca economically viable. One potential solution for this problem might involve the formation of milk cooperatives among the farmers in Cajamarca. Indeed, small farms face disproportionately high transaction costs, especially when costs are invariant with the quantities of milk sold[131]. The collectivization of smallholder dairy farms can reduce the per-unit transaction costs of selling milk[131] and provide farmers with greater bargaining power when negotiating the cost of milk[132]. Cooperative milk settings may provide farmers with more of an economic buffer, which could improve adherence to milk quality standards. The collectivization of milk supplies can also benefit the purchasers of the milk, as they are guaranteed a consistent supply of milk, and, in general, higher quality milk[133].

The judicious use of antibiotics – which includes the avoidance of residues – can be strongly influenced by the prescriber of the drug. To investigate the role of the prescriber in promoting the judicious use of antibiotics, we conducted a qualitative study consisting of focus group discussions and semi-structured interviews with veterinarians and feed-store vendors, respectively, in Cajamarca. Both groups of prescribers identified a number of barriers to the appropriate prescribing of antibiotics in Cajamarca, including farmers' habits, farmers' economic situations and farmers' ability to understand disease and drug use. Veterinarians also cited competition from other vendors (e.g., feed-store vendors) as a problem that perpetuated the inappropriate use of antibiotics in Cajamarca. In contrast, feed-store vendors perceived veterinarians as being valuable partners in promoting the health of dairy animals in Cajamarca and reported often referring their clients to veterinarians. Both veterinarians and vendors reported attempting to educate their clients on the appropriate use of antibiotics; however, all of the veterinarians and five of the eight vendors believed that antibiotics were inappropriately used by their clients. These findings confirm that promoting the judicious use of antibiotics on dairy farms in Cajamarca is difficult and that providers often feel powerless when attempting to do so. The development of more consistent and thorough educational interventions that can be used by these providers to educate their clients on the importance of appropriate antibiotic use and the promotion of enhanced communication between providers (veterinarians and vendors) might enable providers to overcome some of the barriers to appropriate prescribing.

Many of the findings of this study were obtained by interviewing farmers. The reported incidence of disease and antibiotic use on farms relied on farmers' ability to recall past drug use. However, self-report is not always reliable[66], and the quality of data obtained by self-report may be compromised by reporting, recall and social desirability biases. We therefore attempted to compare antibiotic drug use data obtained by self-report and by the collection of discarded drug packaging (the two methods most suited for small farms in LMICs) in our methods study. We found that neither method could be considered a gold standard and that both methods are flawed to a certain extent. Self-report can be used when the use data pertains to a relatively short period of time (e.g., one month) and is preferred when considering atypical antibiotics that do not come in a bottle or powder form (e.g., intra-mammary infusion tubes). In contrast, the collection of discarded drug packaging is preferable when the drug use data pertains to a prolonged period of time (e.g., six months); furthermore, this method has the advantage of not being able to over-report. However, the logistics required to implement this method render its application challenging.

The implications of these findings on the validity of the findings obtained in the other studies that relied on self-report are not overly discouraging. One of the important findings of the methods study is that the disagreement between self-report and the collection of discarded drug packaging increased as the mean number of antibiotics used on the farm increased. Because small farms were the primary focus of this research and because small farms tend to use few antibiotic products, the discrepancy between self-reported data and data obtained via the collection of discarded packaging might not be excessive. The validity of the antibiotic use data collected on the small farms is therefore likely acceptable. Furthermore, because the farmers were asked about antibiotic use data and the incidence of disease on their farms in a number of ways (including a method that involved the use of visual cues, i.e., pictures of antibiotics available on the market), it is likely that issues of recall bias and reporting bias were mitigated. These findings suggest that the methodology used in our study was appropriate and could be used by other investigators seeking to answer similar questions in similar settings.

This research was important and interesting for a number of reasons. First, we investigated a topic about which very little is currently known (antibiotic use on small farms in LMICs). Second, we addressed a broad scope of topics, including patterns of antibiotic use, the extent of contamination of milk with antibiotic residues, the perspective of veterinarians and feed-store vendors on the appropriateness of antibiotic use on dairy farms in Cajamarca and the methodology used to collect pharmacoepidemiologic data on small dairy farms in LMICs. Third, because all of our studies were characterized by high participation rates, we can conclude that our results may be generalizable to other farmers in this region (and potentially to farmers in other LMICs) and that the methodological approaches we used were acceptable to farmers. The data collected in these studies may be useful for milk processing companies, non-profit organizations, government institutions and other researchers and may contribute to improved animal health on small dairy farms in Cajamarca and better milk quality for the consumers of dairy products. Our data could also inform a number of logical follow-up studies, including an investigation into the degree of antibiotic resistance in the field and the implementation of educational intervention programs to improve farmers' knowledge of antibiotics and adherence to milk quality standards.

Appendix

Survey instrument used to interview farmers on antibiotic use patterns

*Note that italicized text in parentheses indicate measures of the test-retest reliability of a question assessed by re-interviewing a subset of 13 farmers two weeks after the first survey was administered. ICCs indicate intraclass correlation coefficients (for questions with continuous answers) and κ indicates kappa values for questions with categorical answers.

A. Farm   Farm ID:

1. Number of people working on the farm : Men   Women   *(ICC=0.800, p=0.03)*
2. Do you belong to an association of producers? Yes ☐ No ☐
3. How many animals do you have? *(ICC=0.82, p=0.038)*

Lactating cows	
Dry cows	
Calves/heifers	
Bulls/Steers	
Totál	

4. Do you sell or buy your cattle at the public auctions? Yes ☐ No ☐ *(κ=0.55, p=0.013)*
5. If so, how many did you buy/sell in the past year?
   Bought :   Sold :

6. How much milk does your farm produce per day? *(ICC=0.45, p=0.52)*
7. To whom do you sell your milk? *(κ=1.00, p=0.01)*

	Price/L
Gloria	
Nestle	
Cheesemakers	
Open market	

8. Who performs the following services on your farm ? :

	Not done	Veterinarian	Technician	Friend/Neighbor	Yourself
Artificial Insemination	☐	☐	☐	☐	☐
Vaccinations	☐	☐	☐	☐	☐
Antiparasitic dosing	☐	☐	☐	☐	☐
Disease treatment	☐	☐	☐	☐	☐

9. Would you like the vet to visit your farm more often ? Yes ☐ No ☐
10. If so, why do you not have the vet come more often ?

Price of services too high☐
Distance/difficult access☐
Difficult to contact the vet☐
Vet's treatments don't work☐
Unavailability of the vet ☐

B. ANTIBIOTICS/TREATMENTS:

11. Last year, did you administer antibiotics to your animals ? Yes ☐ No☐

12. If so, which ones?

13. Do you know what an antibiotic is ? Yes ☐ No☐ *(κ=1.00, p=0.002)*

If so, how would you define an antibiotic?

14. Where do you buy your antibiotics ? *(κ=1.00, p=0.001)*
Vet ☐
Feed store ☐
Promoters/drug reps ☐

15. Last year, how many of the following occurred ? *(ICC=0.80, p=0.028)*

	# episodes without Treatment	# episodes with Treatment	Name of drugs used
Mastitis			
Illness after parturition			
Respiratory illness			
Diarrhea			
Skin infections			
Bloating			

16. If you treat your animals yourself, where do you get information on how to do so?

Veterinarian☐ Feed store☐ Friends/neighbors☐ Training ☐ Other ☐

17. Do you give antibiotics to prevent disease? Yes ☐ No☐

18. If so, for what ?
After parturition☐
To dry the cow☐
When other animals on the farm are sick☐

20. When a cow is being treated with antibiotics, is she :

Visibly marked?☐ Milked last? ☐

21. When a Treatment doesn't work, what do you do ?

Increase the dose ☐
Change drugs ☐
Sell the cow ☐

22. When you buy antibiotics, what factors do you take into account?:

		Order of importance
Price	☐	
Brand	☐	
Packaging	☐	
Quality	☐	
Easy to obtain	☐	
Recommendation of the vet/vendor	☐	
Previous experience	☐	

23. Do you always give the dose/# of treatments recommended by the prescriber? Yes ☐ No☐
If not, why?
Not enough money ☐
The cow appears healed☐
The Treatment doesn't work☐
Because the milk production decreases☐
Because of side effects☐

24. When an animal is being treated with antibiotics, what do you do with her milk ?
Feed to calves ☐
Continue selling it ☐
Consumed by family ☐
Feed to dogs ☐
Thrown away ☐

(If any answer other than the second) For how long ?

25. Do you know what drug withdrawal/withholding times are? Yes ☐ No ☐ *(κ=1.00, p=0.002)*

If so, can you define them?

26. Do you believe that using antibiotics can result in any of the following?

Danger to the animal
Allergic reaction Yes ☐ No ☐ Don't know ☐ *(κ=0.75,*

	p=0.0001)
Using the same antibiotic frequently can diminish the effectiveness of the drug	Yes ☐ No ☐ Don't know ☐ *(κ=1.00, p=0.002)*
Danger to the consumer Pose a risk to consumers who drink milk from cows treated with Antibiotics	Yes ☐ No ☐ Don't know ☐ *(κ=1.00, p=0.002)*

27. Have you ever observed any complications/side effects after administering antibiotics ?

Yes ☐ No ☐ If so, which ones ? *(κ=1.00, p=0.002)*

C. MANAGEMENT

28.Do you clean the cow's udder before milking?
Never☐ Sometimes☐ Always☐

29. If so, with what ?
Water ☐ Water+soap☐ Disinfectant ☐

30.Do you wash your hands:

Before milking	Never☐	Sometimes☐	Always☐
Between each cow	Never☐	Sometimes☐	Always☐

31. Do you seal the cow's teats with iodine after milking?
Never☐ Sometimes☐ Always☐

32. Have you or your vet ever done tests to look for sub-clinical mastitis (CMT)?

Yes ☐ No ☐

33. Do you feed your cows concentrates? *(κ=0.52, p=0.017)*
Never ☐
Sometimes☐
Every day☐

D. FARMER

34. Age
35. What percent of your total income comes from sale of mlk? 0-50% ☐ 50-100% ☐
36. Monthly income *(ICC=0.97, p=0.004)*
37. Schooling attained:

Illiterate	☐	Secondary complete	☐
Primary incomplete	☐	University	☐

Primary complete	☐
Secondary incomplete	☐

38. Altitude of farm:
39. Location of the farm :

CMT results	☐ Negative ☐ 1 ☐ 2 ☐ 3	☐ Negative ☐ 1 ☐ 2 ☐ 3

Discussion guide for the focus-group discussions with veterinarians and for the interviews with the feed-store vendors

Demographic information:

How long have you been in practice or worked in your store?

What is your education level?
Did you have experience in farming / agriculture before becoming a veterinarian / to work in your store?
Tell us a little about your clients.
(Prompt: species of animal, size of farms, where the farmers live, educational level of farmer, farmer income)

Antibiotic use:

How would you define appropriate antibiotic use?

How are antibiotics typically used among your clients?

(Prompt: which ones? Are dosages followed? Are they used for prophylaxis or exclusively for therapeutic purposes?

Do you think your typical customer is knowledgeable about animal diseases?
(Prompt: Are they aware of the major diseases affecting animals? do they know how to treat or avoid these diseases?)

Do you think your typical customer is knowledgeable about drugs?
(Prompt: do they understand that drugs generally have specific indications? do they know how to administer them properly? Are they aware of the concept of residues?)

When a farmer purchases an antibiotic, does he/she tend to request a specific drug or describe symptoms and ask for an appropriate treatment?

How often do you prescribe antibiotics? How do you choose which antibiotic to prescribe?

Do you provide information to the farmer on how to use the drug correctly? Do you believe your instructions are followed?

Have you encountered problems with antibiotic resistance or treatment failure in the field?

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