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Sorption, Leaching, and Surface Runoff of Beef Cattle Veterinary Pharmaceuticals under Simulated Irrigated Pasture Conditions

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Abstract

The use of veterinary pharmaceuticals in beef cattle has led to concerns associated with the development of antibiotic resistance in bacteria and endocrine disruption in aquatic organisms. Despite the potential negative consequences, data on the transport and mitigation of pharmaceuticals in grazed watersheds with irrigated pasture are scarce. The objective of this study was to assess the transport of common beef cattle pharmaceuticals (i.e., oxytetracycline, chlortetracycline, ivermectin) via surface runoff and leachate from manure amended to grass-vegetated soil boxes under irrigated pasture conditions. The transport of pharmaceuticals from animal manure in surface runoff and soil leachate was relatively low and appears to be limited by desorption and transport of pharmaceuticals entrained in the manure. In surface runoff, less than 4.2% of applied pharmaceuticals in manure (initial concentration: 0.2 mg kg⁻¹ of manure) were detected after three weeks of irrigation. Concentrations of pharmaceuticals in surface runoff and leachate never exceeded 0.5 µg L⁻¹. The major portion of pharmaceuticals (up to 99%) was retained in the manure or in the soil directly beneath the manure application site. Based on the minimal transport of oxytetracycline, chlortetracycline, and ivermectin, the risk of significant transport for these targeted beef cattle pharmaceuticals to surface water and groundwater from manure on irrigated pasture appears to be relatively low.

INTRODUCTION

The use of veterinary pharmaceuticals in beef cattle on grazed watersheds has led to concern associated with the development of antibiotic resistance in bacteria and endocrine disruption in fish (Ankley, et al., 2003, Bywater and Casewell, 2000). The development of antibiotic resistance, particularly in pathogenic bacteria, can pose a serious health risk to humans and animals (Gootz, 2010). Pharmaceuticals are often administered to livestock, for both therapeutic and prophylactic purposes, and is commonly considered a good herd management practice. However, due to the poor adsorption of pharmaceuticals within gut of the animal, up to 90% of administered pharmaceuticals can be excreted with animal wastes

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ASSOCIATED CONTENT

Supplemental Information

(Sarmah, et al., 2006). Upon irrigation or rainfall events, these antibiotics can potentially be leached from the manure pat and introduced into soil and water systems.

Transport of veterinary pharmaceuticals from livestock wastes into the ecosystem has been widely studied for highly intensive systems such as concentrated animal feeding operations (CAFOs). Significant concentrations of veterinary pharmaceuticals have been detected in soil and water in close proximity to CAFOs and in agricultural fields where livestock manure was applied (Sarmah, et al., 2006). For example, concentrations of tetracyclines detected in soils have been found as high as $170 \mu\text{g kg}^{-1}$ and concentrations in surface waters up to $1 \mu\text{g L}^{-1}$ (Sarmah, et al., 2006). At the same time, there is little data on the transport and mitigation of common beef cattle pharmaceuticals in irrigated pasture systems which provide critical forage to ranching enterprises in grazed watersheds throughout the western United States (Fernandez, et al., 2011). In these extensively managed (relative to CAFOs) systems, cattle density (less than 1 cow per hectare per year) and consequently the load of manure per unit area is significantly lower than in CAFOs. Also, in irrigated pastures, manure is excreted by cattle directly on grass covered soil, while in CAFOs manure is excreted to bare soil, stored in lagoons, and commonly applied to agricultural fields as a slurry. In addition, due to the reduced animal density, the amount of pharmaceuticals used per animal head is often lower on irrigated pasture systems. Finally, management of beef cattle on pasture is quite different from CAFOs and therefore differences exist in the pharmaceuticals used and administration method (e.g., oral in CAFOs vs. intramuscular or subcutaneous for pasture cattle). There is typically a more diverse use of pharmaceuticals in CAFOs, some of which are relatively water soluble (e.g., sulfonamides) and a greater risk for transport in the soil. The pharmaceuticals used for cattle on pasture are often formulated to be long acting and therefore different classes or compounds are often preferred. These and other differences in animal management in CAFOs and irrigated pasture systems limit the extrapolation of CAFOs based research findings to irrigated pasture systems.

Several approaches and various systems have been used to evaluate the transport of beef cattle pharmaceuticals and mitigate their exposure to non-target organisms (Fernandez, et al., 2011, Kay, et al., 2004, Kay, et al., 2005a, Kay, et al., 2005b, Kim, et al., 2010b, Rabolle and Spliid, 2000). Batch sorption experiments have been employed to evaluate the sorption of pharmaceuticals (oxytetracycline, sulphachloropyridazine, metronidazole, olaquinox, and tylosin) on soils and to calculate corresponding sorption coefficients, which aid in the mitigation of these pharmaceuticals. Transport of pharmaceuticals through soil has also been studied using packed soil columns (Kay, et al., 2005a, Rabolle and Spliid, 2000). While such an approach is helpful for defining antibiotic transport in strictly controlled conditions, it can be difficult to translate results from soil columns to natural settings due to small soil volume, absence of vegetative cover and uni-dimension of water flow. Also, the heterogeneity of soil and the channeling of water through soil macrospores under the field conditions have been shown to have a large influence on the potential for sorption and degradation (Kay, et al., 2004, Kay, et al., 2005b). A few studies on pharmaceutical leaching from soil systems have been completed under field or near-field conditions and scale (Fernandez, et al., 2011, Kim, et al., 2010b). Fernandez et al. (2011), examined Holstein cross cattle manure (1 kg) containing ivermectin applied to 5 m^2 leaching trays. Their results showed the highest

leaching rate of ivermectin only in the first rainfall events, with concentrations in runoff water ranging from 5 to 188 ng L⁻¹. The highest concentrations were detected in bare soil trays and the presence of grass reduced the concentrations of ivermectin in soil up to 10 times. Kim et al. (2010b) examined the transport of several commonly used antibiotics, including tetracycline, applied (209 mg) to 6 m² field plots subjected to rainfall of varying intensity. It was found that transport was affected not only by rainfall intensity, but primarily by the chemical properties of the antibiotics. Concentrations of chlortetracycline in runoff water ranged from 10 to 90 ng L⁻¹, with the majority of applied chlortetracycline remaining in the upper soil layer.

Due to the difficulties in conducting controlled *in situ* field studies and the specific limitations of laboratory column and batch experiment studies, we took an intermediate approach and designed grass vegetated, packed soil boxes based on designs previously used for phosphorus leaching studies as well as livestock microbial transport studies (Atwill, et al., 2002, Kleinman, et al., 2004, Tate, et al., 2004). We examined the transport of three commonly administered beef cattle pharmaceuticals (oxytetracycline, chlortetracycline, and ivermectin) under simulated irrigated pasture conditions (Table 1). Ivermectin, chlortetracycline and oxytetracycline are over-the-counter drugs which are used widely in beef cattle on grazed watersheds. Ivermectin is a macrocyclic lactone widely used in cattle to control parasitic worms (e.g., roundworms, threadworms) because it has a broad spectrum of activity, low toxicity and long duration of activity (Omura, 2008). Chlortetracycline and oxytetracycline are broad-spectrum antibiotics used to treat a wide variety of diseases (e.g. pneumonia, foot-rot, anaplasmosis, bacterial scours).

The primary objective of this study was to assess the transport potential of oxytetracycline, chlortetracycline, and ivermectin into surface runoff and leachate water from manure-amended grassed packed soil boxes reflective of irrigated pasture systems. The conditions for the study represent beef cattle (i.e., pharmaceuticals) and pasture management (i.e., irrigation) practices which are common in Northern California and reflect its Xeric moisture regime. Our specific objectives were to: 1) estimate the total amount of the pharmaceuticals transported with surface runoff and leachate upon simulated flood irrigation; 2) compare sorption affinity of the pharmaceuticals with two agricultural soils of similar particle size and contrasting parent material, and 3) assess the mitigation of the pharmaceuticals by manure and soil.

MATERIALS AND METHODS

Chemicals

Chlortetracycline, oxytetracycline, and ivermectin were purchased from Sigma-Aldrich (St. Louis, MO, USA), and 4-epitetracycline (IS, internal standard), 4-epianhydrochlortetracycline (recovery standard) were purchased from Acros Organics (Fair Lawn, NJ, USA). Optima grade methanol was used; all other solvents were of HPLC or LC/MS grade. Solvents and all other chemicals (at least of an analytical grade) were purchased from Sigma-Aldrich or Thermo Fisher Scientific (Pittsburgh, PA, USA).

Experimental Setup

Packed Soil Box Experimental Setup—Six wooden boxes (0.3 m wide, 1.22 m long, 0.2 m deep, 7% slope) were constructed and lined with stainless steel (Fig. 1). Molded glass beads (3.0 mm; Ceroglass, Columbia, TN, USA) were placed at the bottom of the box to improve drainage and a stainless steel wire mesh (0.5 mm) was placed on top of the beads. The boxes were then packed with two representative Northern California agricultural soils of contrasting mineralogy, Yolo silt loam (3 soil boxes) and Argonaut gravelly loam soil (3 soil boxes), to a bulk density of 1.2 g/cm³ (Table 2). This was achieved by placing a known mass of soil in each box in a series of layers and packing to the desired bulk density with a tamper. The process was repeated until the box was filled creating a uniform bulk density. Soil bulk density was confirmed, after experiments were run, via soil core sampling. Each box was equipped with a stainless steel gutter to collect surface water runoff and a drain at the bottom for leachate water collection. Each box was seeded with a mixed pasture grass blend and grass was grown for three months to establish the root system and provide complete vegetative cover of the soil surface. Transparent polymeric film was installed on top of the packed soil boxes (1 m above the grass level to allow for air circulation) to protect boxes from precipitation (none occurred over the course of the experiments). Irrigation water was applied using a 1.27 cm diameter, perforated irrigation pipe (20 cm long) connected to a water hose. The irrigation pipe was set at the top of the box and water flow was controlled using an inline flow meter that was set to the desired rate.

Prior to the application of manure, the soil was saturated with water and grass was trimmed to 5 cm. Cow manure (1 kg, 80% moisture) spiked with a mixture of pharmaceuticals (200 µg of each) was applied 10 cm from the front of each grassed soil box as a strip across the width of the entire box. The soil boxes were then irrigated at a 1 L min⁻¹ flow rate for 30 min. Thus, at each irrigation event, the total volume of water applied was equal to 82 L m². The 30 min irrigation was repeated weekly for three consecutive weeks. When conducting runoff box experiments one box for each soil type failed to produce runoff and thus both were excluded from the study. All data presented represent triplicate analyses from duplicate soil boxes.

Batch Sorption Experiment Setup—The batch sorption experiment of oxytetracycline and chlortetracycline on soils was performed as described by Jones et al (2005), and the batch sorption experiment of ivermectin on soils was performed using a modified method based on Krogh et al (2008). Briefly, 5 g of soil was equilibrated with 40 mL of aqueous antibiotic solution at an initial antibiotic concentration in the range of 0.005 to 1.25 mmol L⁻¹. Soils were equilibrated for 48 h at room temperature in the dark. After the equilibrium was reached, soil suspensions were centrifuged and supernatant was analyzed for the three pharmaceuticals by HPLC MS/MS. Concentrations of pharmaceuticals in water and soil were calculated and sorption isotherms were constructed. Linear, Langmuir, Freundlich, and Scatchard models were used for linearization and estimation of the sorption coefficients (Sithole and Guy, 1987, Sparks, 2003).

Sample Collection from Packed Soil Boxes

Water Samples—Water samples were collected by volume for each of the three irrigation events. Four samples (4 L each) of surface runoff were collected using 4 L glass amber bottles. After 16 L of surface runoff water was collected, irrigation was stopped. When runoff water ceased flowing, the total leachate volume (approximately 6 L) also collected. The contents of each bottle were mixed thoroughly, and an aliquot of 1 L was subsampled in a clean 1 L amber glass bottle. Water samples were stored at 4 °C and analyzed within 48 hours. All water samples were filtered through 1.6 µm and 0.7 µm glass microfiber glass filters (Whatman, Buckinghamshire, UK) to remove colloidal particles. Water filtrates were acidified to pH 4.0 with 1 M HCl. Ethylenediaminetetraacetic acid (EDTA; 0.25 g/L) was added to each sample to prevent complexation of tetracyclines with divalent cations. Pharmaceuticals were extracted from water samples and analyzed as described below.

Soil Samples—Soil samples were analyzed using HPLC MS/MS following pressurized liquid extraction (PLE) before the experiment to validate that no target pharmaceuticals were originally present in the soil (described below). Upon completion of the packed soil box experiments, soil samples were taken from each box. Soil samples were collected using 1 inch diameter soil probe. Within each box, three types of soil samples were collected: a) three soil samples taken directly under the manure deposit at three depths (0–5, 5–10, and 10–15 cm); b) soil sample taken 5 cm down slope from the manure deposit; and c) soil sample taken 15 cm up slope from the manure deposit. A total of five samples per box were obtained with each sample being a composite of five subsamples. The field-moist soil samples were sieved (2-mm sieve), mixed thoroughly, and then kept at 4 °C until analysis. Soil samples were analyzed within 48 h after processing. Soil pH values were determined by the soil saturated paste method (U.S. Salinity Laboratory Staff, 1954). Total organic carbon (OC) content of soil was determined using a Costech ECS 4010 nitrogen/protein analyzer (Costech Analytical Technologies Inc., Valencia, CA, USA) by dry combustion. Soil moisture content was determined gravimetrically after drying at 105 °C for 48 h.

Manure Samples—Samples of manure were obtained from the Dairy Teaching and Research Facility at the University of California (Davis, CA, USA). Manure samples were collected from hay fed cows which were not treated with oxytetracycline, tetracycline, or ivermectin for several months prior to collection. Manure samples were analyzed before the experiment to validate that no pharmaceuticals were originally present in manure. The moisture content of manure samples was adjusted to 80–85% by homogenizing about 600 g of manure with 300 mL of ultrapure water. Aliquots (200 µL) of 1 mg mL⁻¹ stock solution of oxytetracycline, tetracycline, and ivermectin were spiked into manure samples in a Nalgene bottle. Bottles were covered with aluminum foil and shaken at 300 rpm on an orbital shaker. Spiked manure was applied to the soil boxes within 1 hour. Upon the completion of the experiment, manure was collected from each box. Manure samples were broken up into smaller pieces, sieved (2 mm sieve), mixed thoroughly, and stored at 4 °C until analysis. Dry manure samples were analyzed within 48 h after processing.

Chemical Analysis

Pressurized Liquid Extraction—Antibiotics were extracted from soils and manure using a Dionex ASE 150 (Sunnyvale, CA, USA) PLE system. Five grams of the soil or manure sample was mixed with diatomaceous earth (Hydromatrix, Agilent Technologies, Inc., Palo Alto, CA, USA) and loaded into 22 mL stainless steel cell between two layers of Hydromatrix to occupy the dead volume in the cell. The stainless steel cell was lined with EDTA washed cellulose filter (1.9 μm pore size) to prevent material loss. Extraction was carried out at $1 \cdot 10^7$ Pa and 40 °C. Static extraction time was 20 min, with a flush volume of 60%, and purge volume of 60%. Two extraction cycles were performed using methanol:water (3:1, v/v) containing 0.25 mmol L⁻¹ EDTA and 50 mmol L⁻¹ sodium chloride at pH 8.0. Extracts were diluted with enough water to reduce the organic solvent concentration to less than 5% by volume in solution. The solution pH was subsequently adjusted to 4.0 with 1 mol L⁻¹ HCl. Antibiotics were concentrated by solid-phase extraction as described below and analyzed via HPLC MS/MS.

Solid-Phase Extraction—Antibiotics were extracted from water and diluted PLE extracts using solid-phase extraction (SPE). A Discovery® DSC-SAX cartridge was set up in tandem with an Oasis HLB phase cartridge. Oasis HLB cartridges (30 μm , 3 mL, 60 mg sorbent) were purchased from Waters Inc. (Milford, MA, USA). DSC-SAX cartridges (56 μm , 3 mL, 500 mg sorbent) were purchased from Sigma-Aldrich (St. Louis, MO, USA). The cartridges were activated with 2 $\times$ 3 mL of methanol and then conditioned with 3 mL of DI water and 3 mL of 0.04 mole L⁻¹ citric acid in water (pH 4.0). The water extracts were loaded on the SPE cartridges at 5 mm Hg negative pressure. After loading, the SPE cartridges were washed with 2 $\times$ 3 mL of 0.04 mole L⁻¹ citric acid (pH 4.0). The SAX cartridges were then removed, and the HLB cartridges were allowed to dry for 15 min at 5 mm Hg negative pressure. Pharmaceuticals were eluted from the HLB cartridge with 4 mL of 100% methanol by gravity. The methanol extracts were evaporated to dryness under a gentle steam of nitrogen in a water bath at 45 °C. The dry extracts were reconstituted in 300 μL of 50% aqueous methanol with 0.1% formic acid and analyzed with HPLC MS/MS.

HPLC MS/MS Analysis—HPLC-MS/MS analysis was performed using an Agilent series 1200 HPLC with diode-array and Agilent 6320 ion trap mass spectrometer detectors (Agilent Technologies, Palo Alto, CA). The MS parameters for a parent ion of each compound were optimized by direct infusion of 1 $\mu\text{g mL}^{-1}$ pharmaceuticals mixture in 50% aqueous methanol containing 0.1% formic acid at 30 $\mu\text{L min}^{-1}$. Optimized MS parameters are specified in (Supplemental Materials). MS data were collected in positive ESI MS/MS mode. The nebulizer temperature was 350 °C, nebulizer pressure was $3.4 \cdot 10^5$ Pa, and the drying gas flow rate was 10.0 L min⁻¹. Chromatographic separation was carried out on a reverse-phase Agilent Zorbax Eclipse XDB-C18 (250 $\times$ 4.6 mm, 5 micron) analytical column, which was protected by a guard column with the same stationary phase (12.5 $\times$ 4.6 mm, 5 micron) (Agilent Technologies, Palo Alto, CA). The column temperature was set at 40 °C, and the autosampler temperature was set at 4 °C. The mobile phase consisted of 0.1% formic acid in water (solvent A), and 0.1% formic acid in methanol (solvent B). The gradient elution program started with linear increase from 30% to 90% B in 18 min, followed by isocratic elution for 6 min at 90% B. The flow was diverted from the MS for the first 7 min

of the analysis to prevent contamination and ion suppression with salts and other polar species. Using this gradient elution program, baseline separation of the target analytes were archived with following elution times: 4-epitetracycline (8.3 min), oxytetracycline (10.2 min), chlortetracycline (12.5 min), ivermectin (17.1 min), and 4-epianhydrochlortetracycline (18.2 min). An injection volume of 5 μL at a flow rate of 0.5 mL min^{-1} was used. The diode-array detector was used for monitoring co-extracted humic materials and was set up to collect spectra from 190 to 400 nm with 2 nm step.

Method Quality Assurance and Quality Control (QA/QC) Procedures—All analytical protocols were evaluated prior sample analysis based on accuracy, precision, specificity, limits of detection and quantification, linearity, concentration range, reproducibility, and robustness (see Supplemental Material). Contamination-free apparatus, materials, and reagents for use were confirmed. Each sample analysis was performed at least in triplicate. Quality controls were carried through the sample preparation procedure and analyzed at the beginning and end of every sample batch. To document the precision and bias of a method in a given sample matrix, recovery samples were analyzed with each batch of samples, and spiked with an analyte standard at three or more representative concentrations prior to sample preparation. An internal standard was added to all samples prior to analysis to correct for analytical changes (retention time and response of HPLC MS/MS system) from run-to-run. Internal standard calibrations were conducted both before and after the analysis of every sample batch to ensure that interferences did not affect retention time and response.

RESULTS AND DISCUSSION

Packed soil boxes study

In experiments using packed soil boxes under simulated irrigated pasture conditions, the total amounts of applied pharmaceuticals detected in surface runoff and leachate were relatively low (Fig. 2). The cumulative amount of chlortetracycline detected in surface runoff water after three irrigation events was less than $7.4 \pm 1.1 \mu\text{g}$ (3.7% of the initially applied amount). Corresponding amounts of oxytetracycline and ivermectin in surface runoff water were even lower and accounted for 5.1 ± 0.6 and $1.4 \pm 0.1 \mu\text{g}$ (2.5 and 0.7% of initially applied amounts), respectively. The concentrations of pharmaceuticals in surface runoff (0.01 to 0.2 $\mu\text{g L}^{-1}$) and leachate (0.01 to 0.6 $\mu\text{g L}^{-1}$) water were of similar magnitude (Fig. 2). However, due to the differences in volumes (16 L of surface runoff water and 5.7 L of leachate water), the total masses of pharmaceuticals in leachate were 1.2 – 18.5 times lower than in surface runoff (Fig. 2). No additional biologically active metabolites of applied pharmaceuticals were present in the detectable amounts in the collected water. The presence of small amounts of leached pharmaceuticals is consistent with the previously reported soil column experiments, where oxytetracycline and tetracycline were detected in soil but not in leachate (Kay, et al., 2005a, Kim, et al., 2010a). In other studies, significant concentrations of tetracyclines were found in soil in areas of animal husbandries, but no detectable amounts of the antibiotics were detected in groundwater (Barnes, et al., 2008, Hirsch, et al., 1999, Lindsey, et al., 2001, Zhu, et al., 2001).

Desorption of pharmaceuticals from manure—Assuming the simplified model, desorption of pharmaceuticals from manure is the initial step in the overall transport process and one potential rate limiting step. For example, drawing an analogy to the well studied systems of photo- and bioremediation, the total mass of pharmaceuticals transferred from manure to water and soil comprise a series of processes with three major rate-limiting steps: desorption of pharmaceuticals from manure, leaching from manure into water and soil, and sorption of pharmaceuticals on soil can be described in terms of fluxes (Bosma, et al., 1997, Rijnaarts, et al., 1990). Desorption of pharmaceuticals from manure (desorption flux, $q_{desorption}$) is inversely proportional to the affinity of pharmaceuticals to manure organic carbon. Transport of pharmaceuticals (transport flux, $q_{transport}$) depends on the diffusion coefficient within the transport medium (manure, water, and soil). Sorption of pharmaceuticals on soil (sorption flux, $q_{sorption}$) depends on the sorption potential of the soils to studied pharmaceuticals. The reaction step with the largest kinetic resistance ($\frac{1}{k}$) limits the overall process rate and defines the time scale of the entire (Bosma, et al., 1997, Rijnaarts, et al., 1990).

$$\frac{1}{k} = \frac{1}{k_{desorption}} + \frac{1}{k_{transport}} + \frac{1}{k_{sorption}}$$

Animal manure typically has very high organic carbon (organic carbon content of the manure used was 33% by weight) and soluble salts content, both of which provide high capacity for organic contaminants sorption. For example, published data has shown that chlortetracycline can be sorbed by manure very strongly (Wang, et al., 2008). While maximum sorption of chlortetracycline on manure is achieved within an hour, the desorption process is very slow with less than 20% of chlortetracycline desorbed by water (Wang, et al., 2008). Similarly, a high sorption affinity of oxytetracycline to manure has been observed and attributed to interactions with divalent ions within the manure matrix (Loke, et al., 2002). In the current study, the mechanisms for tetracycline sorption on manure is believed to occur via ion exchange and/or partitioning in organic carbon. Sorption of highly lipophilic ivermectin is likely occurring through partitioning into lipophilic organic carbon moieties, with desorption into water being unfavorable.

Transport of pharmaceuticals desorbed from the manure is limited by the rate of permeation from sorption sites within the solid matrix and by diffusion through a liquid layer around the solid-phase particles (Rijnaarts, et al., 1990). The transport is primarily driven by a concentration gradient toward the surface of the manure. In the present study, 0.2 mg of pharmaceuticals was spiked into 1 kg of wet manure (ca. 0.2 kg on dry weight basis). With the maximum sorption potential of manure for the pharmaceuticals close to 500 L kg⁻¹, only a minute amount of potential sorption sites would be saturated (Wang, et al., 2008). In the case when manure is not saturated with pharmaceuticals (i.e., not all possible sorption sites are occupied), gradient driven transport is expected to be relatively slow. Extraction of low doses of the pharmaceutical studied from manure is challenging and yields low recoveries due to the high organic content of manure and drying of manure pats over time. For this

reason obtaining a mass balance for pharmaceuticals in the runoff box experiments is not possible.

Transfer of pharmaceuticals into water—With seven day intervals between irrigation events, diffusion of pharmaceuticals from the manure into runoff water was also affected by the changes in the manure moisture content over the time period. At the beginning of irrigation, manure contained ca. 80% water by weight. During a hot summer, the moisture content of manure left in the field can decline to 10% in just one week. In the runoff box experiment, moisture content in manure declined from 80 to 13% in one week, and then remained relatively constant for the experiment duration. In fact, by observation, manure pats were dry by the second irrigation event. A thick crust had formed around the manure pat by the third irrigation event. This hardening of the manure appears to further limit the transport of the pharmaceuticals into the irrigation water. During subsequent irrigation events, only the lower portion of the manure became moist while the remainder of the pat remains rather dry. In this study, the majority of oxytetracycline and ivermectin in runoff water was detected during the first irrigation event (Fig. 3). In contrast, the concentration of chlortetracycline in runoff water continued to increase during the two subsequent irrigation events. Similar to our results, in a study of ivermectin leaching under rainfall, ivermectin was detected in water only at the first rainfall event, while aging of manure pat likely prevented further releases of ivermectin (Fernandez, et al., 2011). In another field rainfall study, a steady decline of chlortetracycline leaching after the first 15 minutes of rainfall was observed (Kim, et al., 2010b).

Release of pharmaceuticals from the manure into irrigation water is another factor in the overall transport phenomena. For example, while the same amounts of pharmaceuticals were initially introduced to the soil boxes, the amount of chlortetracycline released into the water was twice the amounts of oxytetracycline and more than four times the amount of ivermectin (Figs. 2 and 3). This trend is consistent with the K_d sorption values (Table 3) and solubility of the pharmaceuticals in water with tetracyclines being moderately soluble and ivermectin only slightly soluble in water (Table 1) (Mougin, et al., 2003, Varanda, et al., 2006).

Sorption of pharmaceuticals to soil in packed boxes—To assess the amount of pharmaceuticals transported from the manure pat to the soil, soils and manure were analyzed for residual concentrations of pharmaceuticals at the end of the experiment. While the majority of the spiked pharmaceuticals remained in the manure, measurable levels of all three pharmaceuticals were detected in the soil (Fig. 4). The distribution of the pharmaceuticals within the soil followed a gradient with the highest concentrations detected in the upper 5 cm of soil directly under the manure pat and declined with increased depth. Residual concentrations of pharmaceuticals were also present in surface soil samples up-flow and down-flow of the manure pat, indicating horizontal diffusion of pharmaceuticals during or post irrigation. These results are in agreement with a rainfall simulation study, in which chlortetracycline was detected in up to 30 cm of soil with relatively uniform distribution over the soil depth (Kim, et al., 2010b).

Although the two soils studied were of the same textural class, the pharmaceutical concentrations were slightly higher in the Argonaut soil than the Yolo soil (Figure 4). This difference can be attributed to the higher organic C content of the Argonaut soil (Table 2) and compound hydrophobicity (e.g., water solubility; Table 1) which favors sorption to organic matter phases. In addition, the higher Fe-oxide content (weathered metavolcanic parent material) of the Argonaut soil provides positively charged sites for sorption of deprotonated pharmaceutical functional groups.

Concentrations of chlortetracycline directly beneath the manure (0–5 cm) in the Yolo soil were approximately seven times higher than concentrations of oxytetracycline and 13 times higher than concentrations of ivermectin (Fig. 4). In the Argonaut soil, corresponding concentrations of chlortetracycline were approximately three and five times higher than concentrations of oxytetracycline and ivermectin, respectively. This trend is consistent with the octanol-water partitioning coefficient (K_{ow}) values of the pharmaceuticals (chlortetracycline: -0.62 , oxytetracycline: -1.12 , ivermectin: 3.2) (Campbell, 1989, Loke, et al., 2002). Due to its higher water solubility, oxytetracycline is more likely to enter the water phase rather than partition into hydrophobic domains of soil organic matter. Ivermectin, on the other hand with its low solubility in water ($< 4 \text{ mg L}^{-1}$) is more likely to partition in the hydrophobic fractions of soil. In fact, the sorption strength of the pharmaceuticals to soil declined in the same way: ivermectin $>$ oxytetracycline $>$ chlortetracycline (Table 3). This trend was observed for both soil types and coincides with the increase of hydrophobicity of the pharmaceuticals.

Potential transport of pharmaceuticals on suspended solids—Although the concentrations of pharmaceuticals in filtered runoff water were quite low, there is potential for additional colloid facilitated transport. Extraction of pharmaceuticals from the small amounts of filtrate material collected was not possible with reasonable accuracy, and therefore estimations of the maximum potential pharmaceutical transport on suspended solids are provided. In the current study, the amount of particulate matter eluted with the runoff water ranged from 4.1 to 6.5 mg per each liter of runoff water. This is equivalent to 65.6 to 104 mg of particulate matter for 16 L runoff water collected at each irrigation event. The concentration of oxytetracycline, chlortetracycline, and ivermectin in soils was determined to be 2.2 to 25.4, 9.2 to 76.0, and 2.7 to 15.5 ng g^{-1} soil, respectively. Thus, the maximum mass of pharmaceuticals transported with particulate matter during each irrigation event would be 0.2 to 2.2, 0.8 to 6.9, and 0.2 to 1.4 ng of oxytetracycline, chlortetracycline, and ivermectin, respectively. The total mass of the corresponding pharmaceutical transported in the aqueous phase is significantly large (see Figure 2), with approximately 1000 to 5000 times higher mass loads as compared to the particulate phase estimates. Therefore transport of these pharmaceuticals on suspended solids is expected to be quite low for the conditions of our study. We also suggest that the water soluble fraction of antibiotics may be more important due to the higher potential to be transported to surface water sources. In addition, particulate matter bound pharmaceuticals are expected to have lower bioavailability due to the fact that they are bound to the solid matrix and are not likely to be desorbed in aqueous environment.

Batch soil sorption study

Batch sorption experiments were conducted to evaluate the extent of pharmaceutical sorption to these soils (Fig. 5). Although concentrations of pharmaceuticals were measured in soils from the packed soil box study, the high affinity of the pharmaceuticals for the manure resulted in different soil loadings of the pharmaceuticals. Therefore, batch experiments were needed to evaluate sorption affinity for the three pharmaceuticals with each soil. The data reveal the greatest sorption of ivermectin to soil and somewhat similar sorption of chlortetracycline and oxytetracycline. For chlortetracycline and oxytetracycline, sorption isotherms were nonlinear ($1/n < 1$) with a bi-Langmuir sorption character (i.e., Scatchard Model) which is in agreement with the previously reported sorption data (Jones, et al., 2005). The nonlinear character of sorption and the biphasic nature of Scatchard plots for tetracyclines suggested the presence of two types of sorption sites (Sithole and Guy, 1987). Scatchard plots also indicate greater sorption affinity to the Argonaut soil for both chlortetracycline and oxytetracycline as observed via the apparent binding constant (K_{ap} ; Table 3). Based on these and previously published data, it is hypothesized that these two different sorption sites are represented by the hydrophobic soil organic matter and the mineral component of the soil. It therefore follows that possible sorption mechanisms are partitioning into soil organic matter and binding via ion exchange, respectively (Pils and Laird, 2007, Sithole and Guy, 1987). It should be noted that confirmation of these mechanisms would require additional spectroscopic analysis which are beyond the scope of the current study.

Sorption isotherms and model parameters for ivermectin, on both Yolo and Argonaut soils, differ from those of tetracyclines (Table 3). Based on analysis of Scatchard plots, no biphasic sorption character for ivermectin was observed. The batch sorption isotherms of ivermectin can be characterized by the Freundlich model with $1/n$ values ranging from 0.93–0.99 (Table 3). In the case of linear sorption isotherms ($1/n = 1$), a partitioning mechanism is suggested (Sparks, 2003). Ivermectin is a highly hydrophobic compound and believed to bind to soil through partitioning into organic carbon domains (Mougin, et al., 2003). In the case of partitioning into soil organic carbon, the sorption affinity (i.e., K_F when $1/n = 1$) can be related to the fraction of organic carbon (f_{oc}) in order to normalize sorption based on carbon content via the organic carbon sorption coefficient (K_{oc}) (Sparks, 2003). The slightly greater organic carbon content of Argonaut soil (Table 2) may contribute to the K_{oc} for ivermectin which is about two-fold higher in Argonaut soil (Argonaut: 4380 L g^{-1} ; Yolo: 2220 L g^{-1}).

Concluding Remarks

The data from this study reveal that transport of three common beef cattle pharmaceuticals (chlortetracycline, oxytetracycline, and ivermectin) from manure to surface runoff water and soil leachate was low and limited by desorption and transport of pharmaceuticals entrained in the manure. The majority of target pharmaceuticals were retained in the manure or in the soil directly below manure. Water-soluble concentrations of the pharmaceuticals in runoff and leachate water did not exceed $0.5 \mu\text{g L}^{-1}$, which is below the minimal inhibitory concentration needed for development of tetracycline bacteria resistance and ivermectin LC/EC₅₀ values for aquatic organisms (Davies, et al., 1997, Halley, et al., 1989, Halling-Sorensen, et al., 2002, Wikkler, et al., 2006). It is also of note that water samples were

collected and analyzed immediately, while under the field condition runoff water is exposed to light and these photosensitive compounds can rapidly degrade to biologically inactive compounds. The half-life of tetracyclines ranges between 16 and 41 days (Loftin, et al., 2008), whereas ivermectin photodegradation is much faster with a half-life of about 8 days (Boonstra, et al., 2011). Thus, the runoff water concentrations of the studied pharmaceuticals in the flood irrigated rangelands are expected to be lower than what is reported here. It is therefore proposed that the risk of significant transport of chlortetracycline, oxytetracycline, and ivermectin to surface- and ground-water from cattle manure on irrigated pasture is relatively low.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviation List

HPLC	high pressure liquid chromatography
LC/MS	Liquid Chromatography Mass Spectrometry
MS	mass spectrometry
PLE	pressurized liquid extraction

REFERENCES

- Ankley GT, Jensen KM, Makynen EA, Kahl MD, Korte JJ, Hornung MW, et al. Effects of the androgenic growth promoter 17-beta-trenbolone on fecundity and reproductive endocrinology of the fathead minnow. *Environ. Toxicol. Chem.* 2003; 22:1350–1360. [PubMed: 12785594]
- Atwill ER, Hou LL, Karle BA, Harter T, Tate KW, Dahlgren RA. Transport of *Cryptosporidium parvum* oocysts through vegetated buffer strips and estimated filtration efficiency. *Appl. Environ. Microbiol.* 2002; 68:5517–5527. [PubMed: 12406745]
- Barnes KK, Kolpin DW, Furlong ET, Zaugg SD, Meyer MT, Barber LB. A national reconnaissance of pharmaceuticals and other organic wastewater contaminants in the United States - I) Groundwater. *Sci. Total. Environ.* 2008; 402:192–200. [PubMed: 18556047]
- Boonstra H, Reichman EP, van den Brink PJ. Effects of the veterinary pharmaceutical ivermectin in indoor aquatic microcosms. *Arch. Environ. Contam. Toxicol.* 2011; 60:77–89. [PubMed: 20422169]
- Bosma TNP, Middeldorp PJM, Schraa G, Zehnder AJB. Mass transfer limitation of biotransformation: Quantifying bioavailability. *Environ. Sci. Technol.* 1997; 31:248–252.
- Bywater RJ, Casewell MW. An assessment of the impact of antibiotic resistance in different bacterial species and of the contribution of animal sources to resistance in human infections (vol 46, pg 643, 2000). *J. Antimicrob. Chemother.* 2000; 46:643–645. [PubMed: 11020270]
- Campbell, WC. Ivermectin and abamectin. Springer-Verlag; 1989.
- Davies IM, McHenry JG, Rae GH. Environmental risk from dissolved ivermectin to marine organisms. *Aquaculture.* 1997; 158:263–275.

- Fernandez C, Angel Porcel M, Alonso A, San Andres M, Vicente Tarazona J. Semifield assessment of the runoff potential and environmental risk of the parasiticide drug ivermectin under Mediterranean conditions. *Environ. Sci. Pollut. Res.* 2011; 18:1194–1201.
- Gootz TD. The global problem of antibiotic resistance. *Crit. Rev. Immun.* 2010; 30:79–93.
- Halley BA, Jacob TA, Lu AYH. The environmental impact of the use of ivermectin: environmental effects and fate. *Chemosphere.* 1989; 18:1543–1563.
- Halling-Sorensen B, Sengelov G, Tjornelund J. Toxicity of tetracyclines and tetracycline degradation products to environmentally relevant bacteria, including selected tetracycline-resistant bacteria. *Arch. Environ. Contam. Toxicol.* 2002; 42:263–271. [PubMed: 11910453]
- Hirsch R, Ternes T, Haberer K, Kratz KL. Occurrence of antibiotics in the aquatic environment. *Sci. Total. Environ.* 1999; 225:109–118. [PubMed: 10028708]
- Jones AD, Bruland GL, Agrawal SG, Vasudevan D. Factors influencing the sorption of oxytetracycline to soils. *Environ. Toxicol. Chem.* 2005; 24:761–770. [PubMed: 15839547]
- Kay P, Blackwell PA, Boxall ABA. Fate of veterinary antibiotics in a macroporous tile drained clay soil. *Environ. Toxicol. Chem.* 2004; 23:1136–1144. [PubMed: 15180364]
- Kay P, Blackwell PA, Boxall ABA. Column studies to investigate the fate of veterinary antibiotics in clay soils following slurry application to agricultural land. *Chemosphere.* 2005a; 60:497–507. [PubMed: 15950042]
- Kay P, Blackwell PA, Boxall ABA. A lysimeter experiment to investigate the leaching of veterinary antibiotics through a clay soil and comparison with field data. *Environ. Pollut.* 2005b; 134:333–341. [PubMed: 15589660]
- Kim S-C, Yang JE, Ok Y-S, Carlson K. Dissolved and Colloidal Fraction Transport of Antibiotics in Soil under Biotic and Abiotic Conditions. *Water Qual. Res. J. Can.* 2010a; 45:275–285.
- Kim SC, Davis JG, Truman CC, Ascough JC, Carlson K. Simulated rainfall study for transport of veterinary antibiotics - mass balance analysis. *J. Hazard. Mater.* 2010b; 175:836–843. [PubMed: 19932562]
- Kleinman PJA, Sharpley AN, Veith TL, Maguire RO, Vadas PA. Evaluation of phosphorus transport in surface runoff from packed soil boxes. *J. Environ. Qual.* 2004; 33:1413–1423. [PubMed: 15254124]
- Krogh KA, Soeborg T, Brodin B, Halling-Sorensen B. Sorption and Mobility of Ivermectin in Different Soils. *J. Environ. Qual.* 2008; 37:2202–2211. [PubMed: 18948473]
- Lindsey ME, Meyer M, Thurman EM. Analysis of trace levels of sulfonamide and tetracycline antimicrobials, in groundwater and surface water using solid-phase extraction and liquid chromatography/mass spectrometry. *Anal. Chem.* 2001; 73:4640–4646. [PubMed: 11605842]
- Loftin KA, Adams CD, Meyer MT, Surampalli R. Effects of ionic strength, temperature, and pH on degradation of selected antibiotics. *J. Environ. Qual.* 2008; 37:378–386. [PubMed: 18268300]
- Loke ML, Tjornelund J, Halling-Sorensen B. Determination of the distribution coefficient (log K_d) of oxytetracycline, tylosin A, olaquinox and metronidazole in manure. *Chemosphere.* 2002; 48:351–361. [PubMed: 12146624]
- Mougin C, Kollmann A, Dubroca J, Ducrot PH, Alvinerie M, Galtier P. Fate of the veterinary medicine ivermectin in soil. *Environ. Chem. Lett.* 2003; 1:131–134.
- Omura S. Ivermectin: 25 years and still going strong. *Int. J. Antimicrob. Ag.* 2008; 31:91–98.
- Pils JRV, Laird DA. Sorption of tetracycline and chlortetracycline on K- and Ca-saturated soil clays, humic substances, and clay-humic complexes. *Environ. Sci. Technol.* 2007; 41:1928–1933. [PubMed: 17410786]
- Rabolle M, Spliid NH. Sorption and mobility of metronidazole, olaquinox, oxytetracycline and tylosin in soil. *Chemosphere.* 2000; 40:715–722. [PubMed: 10705549]
- Rijnaarts HHM, Bachmann A, Jumelet JC, Zehnder AJB. Effect of desorption and intraparticle mass transfer on the aerobic biomineralization of a-hexachlorocyclohexane in a contaminated calcareous soil. *Environ. Sci. Technol.* 1990; 24:1349–1354.
- Sarmah AK, Meyer MT, Boxall ABA. A global perspective on the use, sales, exposure pathways, occurrence, fate and effects of veterinary antibiotics (VAs) in the environment. *Chemosphere.* 2006; 65:725–759. [PubMed: 16677683]

- Sithole BB, Guy RD. Models for tetracycline in Aquatic Environments. II Interaction with Humic Substances. *Water Air Soil Poll.* 1987; 32:315–321.
- Sparks, DL. *Environmental soil chemistry*. San Diego, CA: Academic Press; 2003.
- Staff, USSL. pH reading of saturated soil paste. Washington, D.C: U.S. Government Printing Office; 1954.
- Tate KW, Pereira MDC, Atwill ER. Efficacy of vegetated buffer strips for retaining *Cryptosporidium parvum*. *J. Environ. Qual.* 2004; 33:2243–2251. [PubMed: 15537947]
- Varanda F, Pratas de Melo MJ, Caco AI, Dohrn R, Makrydaki FA, Voutsas E, et al. Solubility of antibiotics in different solvents. 1. Hydrochloride forms of tetracycline, moxifloxacin, and ciprofloxacin. *Ind. Eng. Chem.* 2006; 45:6368–6374.
- Wang R, Wei R-c, Liu T-z, Wang T. Sorption characteristics of veterinary antibiotics chlortetracycline on manure. *Huanjing Kexue.* 2008; 29:1363–1368. [PubMed: 18624208]
- Wikkler, MA.; Cockerill, FR.; Craig, WA.; Dudley, MN.; Eliopoulos, GM.; Hecht, DW., et al. Approved standard M7-A7. Clinical Laboratory Standards Institute; 2006. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically.
- Zhu J, Snow DD, Cassada DA, Monson SJ, Spalding RF. Analysis of oxytetracycline, tetracycline, and chlortetracycline in water using solid-phase extraction and liquid chromatography-tandem mass spectrometry. *J. Chromatogr. A.* 2001; 928:177–186.

Figure 1.

Scheme of the packed soil box. Wooden boxes (0.3 m wide and 1.22 m long, 7% slope) were constructed and lined with stainless steel. Boxes were packed with soil on top of a layer of glass beads. Each box was equipped with a stainless steel gutter for a runoff water collection and a drain at the bottom for leachate water collection. Soil was planted with a mixed pasture grass blend and grass was grown for 3 months to establish the root system.

Figure 2.

Total mass of ivermectin, oxytetracycline, and chlortetracycline in leachate and runoff water after three irrigation events. During each irrigation event, 16 L of runoff water was collected. Error bars represent the standard deviation of triplicate samples.

Figure 3. Cumulative masses of ivermectin, oxytetracycline, and chlortetracycline in runoff water. Data between vertical lines represent three irrigation events. Two trials were conducted for each type of soil.

Figure 4.

Concentrations of ivermectin, oxytetracycline, and chlortetracycline were consistently higher in soils under manure patches and lower in the bulk soil after a series of three irrigation events. During each irrigation event, 16 L of runoff water was collected.

Figure 5. Sorption isotherms of oxytetracycline, chlortetracycline, and ivermectin on Yolo (pH 6.7) and Argonaut (pH 5.3) soils.

Table 1

Chemical structure and selected properties of the studied pharmaceuticals (Boonstra et al., 2011; Clinical and Institute, 2006; Kotze et al., 2004; Loftin et al., 2008; Sanderson and Thomsen, 2009).

	Oxytetracycline	Chlortetracycline	Ivermectin
CAS Number	79-57-2	57-62-5	70288-86-7
Chemical Structure
Molecular Formula	C ₂₂ H ₂₄ N ₂ O ₉	C ₂₂ H ₂₃ ClN ₂ O ₈	C ₄₈ H ₇₄ O ₁₄
pKa	4.5, 10.8	4.5, 11.0	NA
Solubility in water, g L ⁻¹	25 (pH 1), 0.17 (pH 6), 999 (pH 9)	4.8 (pH 1), 0.048 (pH 6), 200 (pH 10)	0.004
K _{OW}	-2.1	-0.6	3.2
Half-Life, d	41	16	8
LD ₅₀	19 mg L ⁻¹	128 mg L ⁻¹	0.05 – 0.5 µM
MIC, µg L ⁻¹	16 µg mL ⁻¹	16 µg mL ⁻¹	NA

Table 2

Selected properties of studied soils.

Soil Property	Argonaut Soil	Yolo Soil
Taxonomy	Mollic Haploxeralf	Mollic Xerofluvent
Parent Material	Basic Metavolcanic Residuum	Sedimentary Alluvium
Clay, %	19	15
Silt, %	61	60
Sand, %	20	25
Organic Nitrogen, %	0.11	0.07
Organic Carbon, %	1.4	1.0
pH	5.3	6.7

Table 3
Selected sorption parameters of oxytetracycline, chlortetracycline and ivermectin on Yolo and Argonaut soils.

Compound	Soil	Single Point K_d [†] $L\ kg^{-1}$	Langmuir Model			Freundlich Model			Scatchard Model		
			K_s , $mmol\ l^{-1}$	q_{max} , $mmol\ kg^{-1}$	r	K_f , $L\ kg^{-1}$	$1/n$	r	K_{app} , $mol\ l^{-1}$	n_{app} , $mmol\ kg^{-1}$	r
Oxytetracycline	Argonaut	2580	50730	64	0.9610	68	0.50	0.9957	408	9	0.8754
	Yolo	1040	35180	53	0.9647	70	0.57	0.9650	88	16	0.9046
Chlortetracycline	Argonaut	280	36480	25	0.9648	81	0.52	0.9889	190	15	0.9174
	Yolo	440	57670	75	0.9587	82	0.53	0.9881	58	29	0.9342
Ivermectin	Argonaut	63020	NA	NA	NA	55549	0.93	0.9571	NA	NA	NA
	Yolo	21520	NA	NA	NA	30181	0.99	0.9311	NA	NA	NA

[†] K_d values calculated at an initial antibiotic concentration of 0.01 mmol L⁻¹.
 K_d - sorption coefficient; K - ; q_{max} - ; K_{app} and n_{app} - apparent binding constant and number of sites for the interaction, respectively;