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Identification and quantification of 12 pharmaceuticals in water collected from milking parlors: Food safety implications

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ABSTRACT

The introduction of drug residues into the food chain and their presence in drinking water has been recently investigated. The aim of this work was to monitor the presence of 19 active drugs in water samples collected from milking parlors of dairy farms located in Galicia (northwest Spain), one of the main Spanish milking areas. Overall, 65% of the samples tested positive for at least one of the compounds analyzed. A total of 12 drugs were measured, with concentrations ranging between 17 and 3,941 ng/L. Considering that a mixture of compounds may contribute to the overall effect of each compound and might increase or reduce its toxicity, it should be noted that 29% of the samples tested contained more than one pharmaceutical. To date, the effects of the continuous consumption of these mixtures of drugs in water or milk are unknown; however, antimicrobials may affect the human gut microbiota or have toxic effects in sensitive individuals.
Key words: residue, drinking water, dairy farms, antimicrobial

INTRODUCTION

Pharmaceuticals are important and essential elements in life because they are used in agriculture, human, and veterinary medicine. According to the European Federation of Animal Health (FEDESA, 1998), the annual consumption of antibiotics in the European Union (**EU**) is about 13,000 t, with about 65% used in human medicine, 29% in veterinary medicine, and 6% used as food additives. The use of these substances in animal production is a common practice, not only for therapeutic purposes but also for prophylactic treatments (Iglesias et al., 2014). Once consumed, pharmaceuticals are metabolized and part of the initial dose is excreted unchanged, with the higher percent excreted in the form of metabolites; both the parent compound and metabolites are excreted via feces and urine in both human and animals (Jjemba, 2006). These substances can therefore reach the aquatic environment through wastewater treatment plants because the plants are not able to remove them completely (Kosma et al., 2014). Another way of introducing these active compounds is through the use of contaminated manure in agricultural soils as fertilizer, contaminating rivers, lakes, and drinking water sources (Watkinson et al., 2009; Rodil et al., 2012). Additionally, during the manufacture and production of drugs, the environment can be contaminated with pharmaceuticals due to breaks, leaks, and uncontrolled washes (Rodil et al., 2012). Depending on the physicochemical properties of drugs and the characteristics of the water and soil, these active substances reach the environment, particularly the water system, through water runoff and leaking, contaminating aquifers, rivers, lakes, seas (Ayscough et al., 2000) and even human drinking water (Watkinson et al., 2009; Iglesias et al., 2014).

Farms across the European Union produced approximately 147,904 million tonnes of cow milk in 2014 (Eurostat, 2014); in particular, Spain produced 6,679 million tonnes. Galicia, in northwest Spain, is an area with high milk production at 2.65 million tonnes (36.6% of the total produced in Spain; MAGRAMA, 2014) and has 14,415 livestock and 450,271 head of cattle (IGE, 2014). Water is very important on dairy farms, not only because it is used to feed animals but also for cleaning the facilities. Inadequate quality of the water used on dairy farms can lead to food safety concerns, because water contaminated with pharmaceuticals can come in contact with milk during equipment cleaning (Llena, 2011). In general, milk farms use water from public distribution, but the use of water from water wells is also common practice in Spain, particularly in Galicia. European legislation states that "water for cleaning food facilities shall be potable water" (Regulation

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853/2004; EU, 2004), because this water is in contact with food. In Spain, health criteria for water quality for human consumption are regulated by the Spanish standard Real Decreto 1120/2012 (BOE, 2012) and EU Directive 2015/1787 (EU, 2015).

If even small amounts of water carrying pharmaceuticals remain in the milk tank after cleaning, the new fresh milk would also be contaminated with these pharmaceuticals. The amount of water that remains in the tank is variable, depending on the size of the tank and the quality of the water system tubing; leaks due to broken valves can result in more water remaining compared with the residual left after cleaning. Milk could also contain residues of pharmaceuticals as a result of overdosing cattle treatment, incorrect separation of milk from medicated and unmediated animals, dry cow therapy anomalies, and, to a lesser extent, inadequate withdrawal period (MAGRAMA, 2005). Thus, methods for analysis of pharmaceuticals in milk exist to control and prevent food safety problems as much as possible. Maximum residue limits of pharmaceuticals allowed in foods of animal origin within the EU are provided in Regulation 37/2010 (EU, 2004). However, pharmaceuticals are not usually controlled in the water used for cleaning facilities, although legislation states that cleaning water used on farms should be of the same quality as human drinking water.

Even if the concentration of pharmaceuticals in the final milk is low and below the level set for antimicrobials in Regulation 37/2010 (EU, 2004), the unknown consumption of pharmaceuticals in this way may lead to interactions with knowingly consumed drugs or by increasing or reducing the effects of the prescribed drugs (Pomati et al., 2008). In the particular case of antimicrobials, continuous consumption of low levels of these active compounds can lead to the development of bacteria with resistance genes in consumers, which may result in synergistic or antagonistic effects and the need to increase or decrease the dose of the treatment (De Liguoro et al., 2009). Additionally, these substances can have harmful effects on the environment; for example, the synergistic activity of sulfamethoxazole and trimethoprim results in greater inhibition of the growth of the alga *Selenastrum capricornutum* compared with the respective individual activities (Santos et al., 2010). Therefore, low levels of active compounds could have negative effects on sensitive consumers, such as infants, the elderly, and allergic individuals who are frequent consumers of milk.

In accordance with the above data, research into the presence of pharmaceuticals in water used to clean milking facilities is needed. The aim of this study was to collect water from dairy farms located in the province of Lugo, one of the main milking areas in Spain, and to investigate the presence of 19 pharmaceuticals by using solid-phase extraction and HPLC-tandem mass spectrometry (MS/MS).

MATERIALS AND METHODS

Study Area

The weather in the area is classified as continental oceanic with an average temperature of 13.6°C. The relief is very heterogeneous, constantly altering elevations and depressions, where the surface is broken by rivers or streams. The average rainfall is 678 mm/yr (Meteogalicia, 2015). Table 1 shows average climatological data (temperature, relative humidity, rain, light hours, and hydric balance) of the province of Lugo over a 17-wk period from February to June 2015. The region investigated was divided into areas (Figure 1): South (1 sample), Coast (6 samples), Mountain (7 samples), Flatland (15 samples), and Center (23 samples).

Chemicals, Reagents, and Stock Solutions

Methanol and acetonitrile (HPLC grade, ≥99.9%) were purchased from Scharlau Chemie (Barcelona, Spain), formic acid (purity, >99% for analysis) from Acros Organics (Geel, Belgium), and hydrochloric acid solution (0.1 *N* HCl) from Merck (Darmstadt, Germany). Nitrogen gas (purity, >99.98%) was generated by an in-house nitrogen generator from Peak Scientific Instruments Ltd. (Chicago, IL), and purified water was prepared in-house with a Milli-Q water system

Table 1. Climatological data for Lugo (Spain) during the months of water sample collection

Sampling month	Average temperature (°C)	Average relative humidity (%)	Rain (L/m^2)	Sunlight hours (h)	Hydric balance (L/m)
February	5.55	83.74	4.78	10.12	3.67
March	7.77	68.34	1.29	10.40	−0.70
April	11.84	75.82	3.55	12.98	−0.81
May	14.33	72.80	1.17	14.01	−3.32
June	17.36	73.53	0.56	14.71	−4.65
Average	11.37	74.84	1.61	12.44	−1.16