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Chloramphenicol residue levels of marketed farm gate milk in Senegal

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ABSTRACT

The risk of consuming marketed milk containing Chloramphenicol (CAP) residues was investigated by testing 41 dairy milk samples collected at retail outlets of farms at Dakar (Senegal) from March to November 2013. Analysis for CAP residues was performed at the Veterinary Drugs Control Laboratory (LACOMEV) of the Inter-States School of Veterinary Sciences and Medicine (EISMV) of Dakar using reverse-phase HPLC with UV/Visible detection at 278 nm. The results showed that 78.04% of marketed milk samples contained CAP drug residues suggesting that this veterinary drug is used in dairy cows in Senegal even though it is not approved in food producing animals. Investigations on veterinary drugs importations have showed that 82.82% of powders for injectable solution were associated with CAP while this compound was included in 27.32% of other solid tetracycline formulations.

Although this pilot study is not representative of the general situation, the findings are alarming and reflect a misuse of antibiotics in dairy farms in Senegal.

1. Introduction

In Senegal, the government is committed to protect the health of animals through vaccination campaigns and the use of veterinary drugs. Among the various molecules that are commonly used antibiotics occupy an important place in the veterinary drugs market in Senegal. However, the distribution and use of these drugs is most often by unqualified people (Walbadet, 2007). Unfortunately, the improper and uncontrolled use of these products leads to the presence of their residues in animal foodstuffs especially in the milk (Maghuin, Janosi, Peteghem, Sanders, & Eehckout, 2002). Previous investigations on antibacterial substances residues in certain foods in the region of Dakar showed the presence of residues of these compounds (Abiola et al., 2005; Bada Alambedji, Cardinal, Biagui, & Akakpo, 2004). The dangers of these residues for consumers have been widely described (Derache, 1986; El Bahri, Ben Hassine, & Belguith, 2007).

These residues are the source of potential diseases ranging from simple allergies to cases of acute toxicity causing death. This includes residues of Chloramphenicol (CAP) (Milhaud, 1985). CAP is known for its multiple therapeutic benefits. And it is the compound which shows the best diffusion in most tissues and biological liquids (Mercer, 1980).

Moreover, the presence of CAP residues in food leads to hematological disorders characterized by irreversible aplastic anemia and depression of erythropoiesis (Milhaud, 1985).

Owing to the considerable risks that veil multiple therapeutic qualities of this molecule, it was banned for use in livestock in many countries such as those of the EU and those members of *Codex Alimentarius*, among which the state of Senegal may be mentioned.

However this prohibition is not fully respected in Senegal. Indeed, a study conducted by Abiola and al. in 2005 (Abiola et al., 2005) revealed the presence of CAP in the livers and gizzards of broiler chickens sold in Senegal.

In this context, this study aimed at investigating the presence of CAP in raw milk marketed in Senegal.

2. Material and methods

2.1. Study area and sampling period

This study was conducted in the region of Dakar specifically in the city of Dakar and its suburbs. The region of Dakar is located in the extreme west of the country on the peninsula of *Cap Vert*. It covers an area of 550 km^2 and is the coolest part of the country because of the almost constant presence of the trade winds (cool

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and wet wind). The region of Dakar is also characterized by its physical conditions (climate, soil, and hydrogeology) that are favorable to the development of many agricultural activities including dairy farming. Milk production is quite developed in this region, particularly in the suburban areas. It is marked by a type of intensive production system characterized by the presence of modern dairy farms breeding cows of high productivity (Diao, 2004).

Dairy farms namely from A to G, located in Keur Massar (A), Niacoulrab (B), Sangalkam (C), Niaga (D, E and F) and Keur Moussa (G) were visited during our investigations where pooled raw milk samples were collected.

The most important companies distributing veterinary drugs are also located in the city of Dakar. The surveys were conducted from March to August 2013.

The investigation phase consisted in inquiries that targeted both dairy farms and some veterinary drugs distribution companies in the region of Dakar through questionnaires, interviews and formal discussions. Then disease types and various imported quantity of antibiotics were investigated.

2.1.1. Sampling

The sampling was performed based on the methodology recommended by the *Codex Alimentarius* guidelines which prescribe random, statistically and risk based sampling through the selection of substances (Codex Alimentarius, 2009). Given the lack of data on the subject, it was considered a pessimistic and realistic assumption that the rate of positive samples would be 15% corresponding to the estimated prevalence. With a confidence interval of 99%, a minimum of 29 samples was needed (Codex Alimentarius, 2009). However, it was decided to collect 42 samples. For that, seven (7) dairy farms that hold outlets of raw milk and dairy products were identified. These dairy farms were constituted by four (4) intensive types and three (3) extensive types. Samples were taken at the outlet of each farm and they were constituted of raw milk pooled by farmers.

Sampling was performed by collecting one sample (500 mL of pooled raw milk) per outlet and per day during three consecutive days. This protocol was repeated one month later at the same outlets. Six pooled milk samples were collected per outlet, except at one outlet where only five samples were taken. In total, 41 pooled raw milk samples were collected. The samples were stored in a cooler with ice for transportation to the Veterinary Drugs Control Laboratory (LACOMEV) of the Inter-States School of Veterinary Sciences and Medicine (EISMV) of Dakar where they were stored at −20 °C.

2.2. Laboratory analysis

The LACOMEV is a specialized laboratory in analysis of veterinary drugs. Its primary mission is the quality control of veterinary drugs in sub-Saharan Africa. It also performs the determination of residues of chemical contaminants in food and feed.

LACOMEV was officially recognized as a reference laboratory by the World Organization for Animal Health (OIE) since May 2004 (EISMV, 2013). The collected samples were analyzed from June to November 2013.

2.3. Equipment

The material used in the laboratory consisted of glasses of different capacities, analytical balance type PRECISA 205 A SCS and equipment worthy of a control station of veterinary drugs residues. The High Performance Liquid Chromatography (HPLC) technique was used with WATERS chromatographic system Type 2695 equipped with a Photodiode Array Detector (PDA), a column oven, a thermostatically controlled vials chamber, a quaternary pump and an automatic injector. The unit is controlled by EMPOWER 3 software with a PC type THINKSTATION LENOVO Intel Xeon and printer HP Office jet 6100 category. The stationary phase was an ACE C18 column with the following characteristics: pore size 300 Å, length 15 cm, internal diameter 4.6 mm, particle size 5 μm.

2.4. Reagents

The reagents used were of HPLC grade: ethyl acetate (Daejung), methanol (Sigma–Aldrich), chloroform (Merck), hexane (Carlo Erba), acetonitrile (Carlo Erba), 5 M hydrochloric acid, 5 mM ammonium dehydrogenate phosphate. A reference substance of CAP purchased from Sigma and milk free of CAP residues as blank control were used.

2.5. Analysis

The estimated Limit of Detection (LOD) was 25 ng/mL (25 ppb). The LOD was calculated by using the intercept of the calibration curve of the standards around the detection limit. Then the standard deviation of the response (σ) and the slope (S) of the calibration curve were determined (EDQM., 2005). The LOD was calculated as follow: $LOD = (3.3 \times \sigma)/S$.

This method used in LACOMEV was validated as a qualitative screening method for the detection of CAP in milk (European Commission, 2002). The principle is based on four stages, namely: preparation of standard solutions, sample extraction by ethyl acetate, purification of the extracts with a mixture with chloroform/hexane, and qualitative determination by high performance liquid chromatography with a Photodiode Array Detector (PDA).

2.5.1. Standard preparation

A Stock Solution (SS) of CAP standard (500 μg/mL) and an Intermediate Solution (IS) of 5 μg/mL were prepared in methanol and HPLC grade water (HGW).

The stock solution was prepared with 50 mg of CAP diluted in 2 mL of methanol and 50 mL of HGW. After sonication and mixture, the solution was made up to 100 mL with HGW.

The intermediate solution (IS) was prepared by diluting 1 mL of stock solution in 100 mL HGW.

A calibration curve was prepared with 5 different concentrations of CAP standard (25, 50, 100, 500 and 1000 ng/mL) prepared by diluting the IS with HGW.

2.5.2. Sample preparation

To extract the CAP from raw milk, samples and blank control milk were first thawed, and then homogenized to thoroughly mix the cream with the milk using an ultrasonic bath. Then, individually, 170 μL of milk were transferred in a 1 mL eppendorf tube to which were added 2 μL of 5 M hydrochloric acid solution. After vortexing during 1 min, 500 μL of ethyl acetate were added to the mixture which was vortexed again during 1 min. Then the mixture was centrifuged at 4000 rpm/min for 5 min. The supernatant was transferred into hemolysis tubes and evaporated to dryness under a nitrogen stream at 70 °C. The oily residue remaining in the test tube was dissolved with 660 μL of a mixture of hexane and chloroform (1:1, v/v) and vortexed vigorously for 15 s. A volume of 340 μL of HGW was added and the mixture vortexed for 2 min. The resulting mixture was centrifuged at 7000 rpm/min for 10 min. The upper hexane layer was discarded, and finally about 200 μL of aqueous lower layer were aspirated into micro-vials. Then 100 μL of this solution were injected into the chromatographic system running in