Science of the Total Environment 427–428 (2012) 314–318

Contents lists available at SciVerse ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Microbiological quality indicators in waters of dairy farms: Detection of pathogens by PCR in real time

Diana Catalina Rodríguez *, Nancy Pino, Gustavo Peñuela

University of Antioquia, Laboratory Diagnostics and pollution control (GDCON), University Research Headquarters (SIU), Medellín, Colombia

ARTICLE INFO

Article history:
Received 22 November 2011
Received in revised form 19 March 2012
Accepted 20 March 2012
Available online 28 April 2012

Keywords:
Pathogens
Polymerase Chain Reaction (PCR)
Supply water
Wastewater
Dairy farms

ABSTRACT

When contaminated water is used to wash the udders of dairy cattle and milking utensils, raw milk may become contaminated with pathogens. Washing with high quality water is essential to reduce the microbial contamination of milk. Furthermore, the wastewater generated in dairy herds also contains high populations of pathogens, antibiotics and nutrients that more often are thrown into the water bodies without any treatment. In this work, both supply water and wastewater from 20 dairy farms from Antioquia, Colombia was monitored for 10 months to determine the presence of pathogenic microorganisms. Both *Cryptosporidium* and *Fasciola* were determined by the Polymerase Chain Reaction (PCR) technique in real time. The results showed that the supply water used for drinking and activities involving the herd, has high populations of *Fasciola hepatica* and *Cryptosporidium parvum*, with percentages of about 53.7% and 64.75% respectively. Additionally high populations of *Pseudomonas aeruginosa*, *Shigella*, *Salmonella*, total coliforms and Escherichia coli were found in both types of water, with values around 9.4×10^7, 2.1×10^7, 1.8×10^7, 1.9×10^{10} and 1.5×10^{10} UFC/100 ml respectively for the wastewater and 3.1×10^4, 1.9×10^4, 7.3×10^3, 1.2×10^5 and 6.2×10^3 UFC/100 ml for the supply water.

1. Introduction

Parasitic infections are a major cause of illness in animals and loss of productivity for farming around the world. These infections contribute to a decrease in productivity since they mainly affect younger cattle (Bradford and Schijven, 2002; Choperena et al., 2005). The economical losses caused as a result of these parasites are not only associated with severe clinical problems that can cause infection, but also to subclinical infections that prevent weight gain, cause growth retardation and decrease fertility (Sahoo et al., 2002; Dinander et al., 2003).

Cattle are natural hosts of many parasites. Among the parasitic infections that have a high prevalence in dairy farms around the world are cryptosporidiosis (Fayer et al., 2007; Atwill et al. 2003), that primarily affects growing animals, and fasciolosis, that is linked to grazing animals and affects any age. Cryptosporidiosis and fascioliasis are considered animal diseases and their control is indispensible for reducing human infections (Keeley and Faulkner, 2008).

Cryptosporidium spp. is one of the major etiologic agents of neonatal diarrhea syndrome in domestic ruminants (Feng et al., 2007), causing severe dehydration and growth retardation (Dixon et al., 2011). Even in the absence of other pathogens, infection by *Cryptosporidium* spp. produces high rates of morbidity and mortality that can reach up to 100% (Castro-Hermida et al., 2006). Its life cycle takes place in the intestinal mucosa, and after asexual or sexual multiplication, it produces infective oocysts which are very resistant. Elimination in the feces then allows the parasite to be dispersed in the environment. The infectious dose is very low (10–100 parasitic forms) and calves are infected by the ingestion of food and water contaminated with *Cryptosporidium* oocysts. In cattle, four species of *Cryptosporidium* were found: *C. parvum* (the most prevalent species), *C. muris*, *C. andersoni*, and *C. felix* (Fayer et al., 2000).

Fasciola hepatica, the causative agent of fascioliasis, is located in the liver of parasitized animals. It has an indirect life cycle in which fresh water snails intervene as intermediate hosts. Fascioliasis is a parasitic disease that affects many species of domestic and wild animals, and occasionally humans (Leclipteux et al., 1998). The existence of many wild animal species in which the parasite can remain favor the spread and perpetuation of the infection in the farming environment, hampering the fight against the disease (Marcos et al., 2007). The symptoms and severity of this infection depend on the intensity of the infection and parasitic phases that produce lesions in the animal. The Fascioliasis infection is closely linked to grazing in wetlands and marshes which contain drainage ditches, irrigation canals and springs as moisture is essential for the evolution and survival of the free-living stages.

The microbiological quality of water is measured by the presence of indicator organisms whose concentration or density is usually related to the health risk posed by this water. *Pseudomonas aeruginosa*, *Shigella* sp, *Salmonella* sp, *Escherichia coli* and total

* Corresponding author. Tel.: +57 4 2196570; fax: +57 4 2196571.
E-mail address: catalinarodriguez@udea.edu.co (D.C. Rodríguez).

0048-9697/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
doi:10.1016/j.scitotenv.2012.03.052

coliforms have been accepted as contamination indicator bacteria in treated, untreated and waste water. They have been critical factors in the incidence of several parasitic diseases especially in regions of poverty. One of the most important factors needed to achieve parasite control is the quality of water, since this is the medium by which many animals are infected (Marcos et al., 2007).

The aim of this study was to determine the presence of *C. parvum* and *F. hepatica* using the real-time PCR technique and *P. aeruginosa*, *Shigella*, *Salmonella*, total coliforms and *E. coli* by a method based on plate counting in dairy farm supply and wastewaters in Antioquia, Colombia, because this is important to the health of people and because of the absence of information about it. Wastewater and supply water were analyzed from 20 dairy farms located in eight municipalities of the Department of Antioquia, Colombia.

2. Materials and methods

2.1. Study area

The study was conducted in 20 dairy farms located in the north and east of Antioquia, Colombia (Fig. 1). The northern region is located on average at 2700 m above sea level and temperatures range between 13 °C and 19 °C. 38% of the land in the region is engaged in agriculture, 50% is used as pasture for cattle and 29.3% is cloud forest with a degradation rate of 0.6%. The eastern region comprises an area of approximately 2000 km^2 and consists of a plateau with elevations between 2000 and 2200 m above sea level. The average temperature (15 °C) does not undergo any change throughout the year and annual rainfall varies between 1700 and 2000 mm per year. 60% of the population of this region is engaged in activities related to agriculture and livestock. Both regions have abundant water supplies that provide water for consumption and energy.

2.2. Sampling

In the 20 dairy herds studied water was sampled for 10 months, at different points in the supply chain and wastewater chain, for a total of 270 samples for supply water and 200 for wastewater. Some farms had two sources of supply water, so they were monitored both. Sampling was conducted during the months of September to June in sterile containers. The samples were transported to the laboratory and refrigerated at 4 °C. Determinations of *F. hepatica* and *C. parvum* were conducted only during the months of April and June for a total of 54 samples analyzed for supply water and 40 for wastewater.

2.3. Detection of F. hepatica and C. parvum

2.3.1. Parasites

C. parvum oocysts were obtained from Waterborne Inc. (New Orleans, Louisiana), and stored in a phosphate buffer with antibiotics at 4 °C until used. *F. hepatica* parasites were obtained from the malacology laboratory collection program for the study and control of tropical diseases (PECET) at the University of Antioquia (Colombia).

2.3.2. Concentration of samples

1 L of supply water and wastewater was used for concentration according to Standard Methods APHA–AWWA–WEF, 2005 version. Water samples were subjected to flocculation (Vesey et al., 1993) by adding 100 ml of 1 M calcium chloride and 100 ml of 1 M sodium bicarbonate. After thorough agitation, the pH was adjusted to 10 with 2 M NaOH. If the sample had a pH greater than 10, it was not adjusted. After adjusting the pH, the samples were allowed to settle for a period of 24 h and the supernatant was removed by means of recovery in centrifuge tubes with a capacity of 50 ml. Additionally, bottles were washed with 200 ml of 10% sulfamic acid, 50 ml of PBS at pH 7.4 and 50 ml of 0.01% Tween 80. This dislodged any particles on the sides of the bottles, and the products of this washing were also put in the centrifuge tubes. The sediment was centrifuged at 3000 rpm for 10 min and the largest amount of sediment possible was recovered in one tube per sample, by washing with PBS. The concentrated samples were stored at −20 °C.

2.3.3. Real-time PCR

Quantification of parasites was carried out using a 1.5 LightCycler PCR system (Roche, Bogotá, Colombia). Genomic DNA was extracted from samples using the EZNA DNA extraction kit (Omega Bio-Tek,

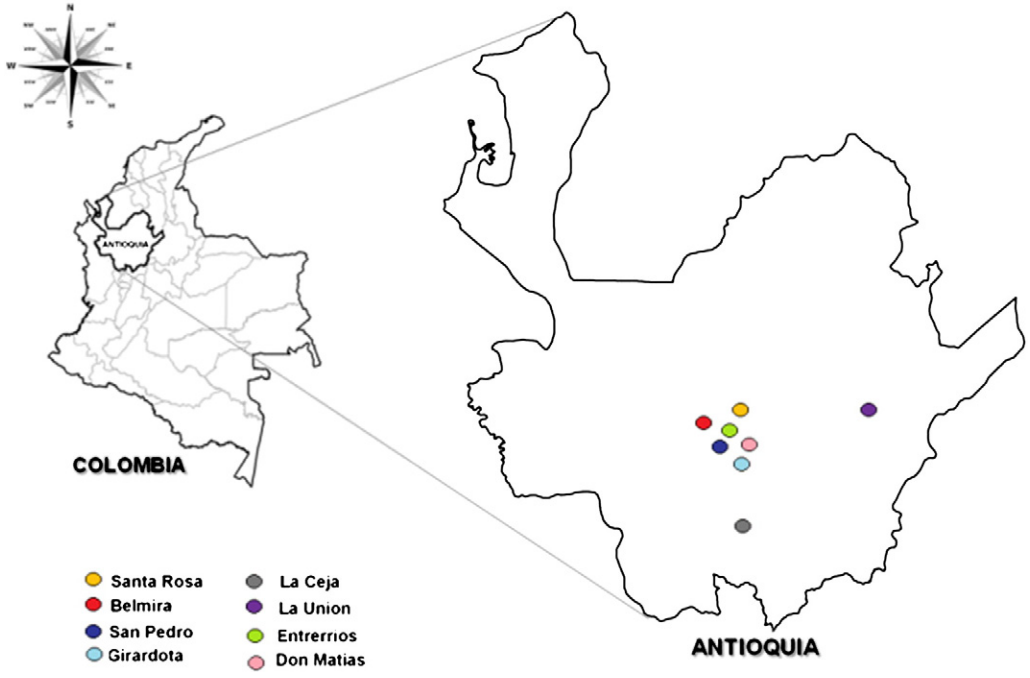

Fig. 1. Location of the municipalities in this study.