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Enteric viruses in water samples from Brazilian dairy farms

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ABSTRACT

The contamination of water by adenoviruses (AdV), rotaviruses (genogroup A, GARV), and enteroviruses (EV), including bovine enterovirus (BEV), was assessed for the first time in Brazilian dairy farms. The area of study, at the municipality of Taquara, is highly representative of the dairy farms in Southern Brazil: the farms are small, high animal densities per grazing area are used, the management of manure is poor and the sanitation for the human excreta is also deficient. Conventional polymerase chain reaction (PCR) was practiced to detect AdV, GARV, EV, and BEV in water samples taken from 27 collection sites in 10 dairy farms; two collections were made, after wet and dry weather conditions. For the first collection of water samples, during wet conditions posed by 18 consecutive days of rain prior to the sampling, 6 (22.2%) samples gave positive results for AdV and only 1 sample was positive for GARV. On the other hand, during the second collection, made under dry conditions, 4 (16%) of the samples were positive for AdV, whereas 10 (40%) samples gave positive results for GARV. EV was found on lower rates (9.6%) of the samples and BEV was not detected. Fecal contamination of water bodies is widespread on these farms, which could be confirmed either by detection of fecal coliforms and enteric viruses found.

1. Introduction

The major concerns about the impact of dairy farming on water quality include the pollution of surface water and groundwater with nutrients, the misuse of water for cleaning of equipment and milking parlors and the contamination of watercourses by pathogens hazardous to human or animal health (Pullar et al., 2011; Schwarte et al., 2011; Weatherley et al., 2011).

The microbiological quality of water is mainly assessed by detection of fecal coliforms (de Medeiros and de Souza, 2009; Wu et al., 2011) a group of organisms that indicates the presence of fecal contamination, such as the bacterial groups thermotolerant coliforms or *Escherichia coli*. Hence, they only infer that there is a possibility of the presence of pathogens. The presence of *E. coli* in water samples indicates contamination of water bodies by feces and possibly by other parasitic and/or viral pathogens (Amaral et al., 2003; Wu et al., 2011). The absence of fecal coliforms does not necessarily exclude the presence of enteric viruses, since these microorganisms are more resistant to environmental degradation and disinfectants (Gerba et al., 1996; Griffin et al., 2003; Ley et al., 2002; Wu et al., 2011). Enteric viruses, such as adenoviruses (AdV), rotavirus (especially those belonging to genogroup A, GARV), and enteroviruses (EV) infects not only humans, but also domestic and wild animals are susceptible to the infection (Ahmed et al., 2010; Derbyshire and Brown, 1978; Griffin et al., 2003; Hunt et al., 2010; Jiménez-Clavero et al., 2005). These viruses are transmitted by the fecal–oral route and excreted in large amounts in the feces of both ill and asymptomatic animals or human beings, and may persist for longer periods in the environment than bacteria, due to their non-enveloped highly resistant capsid (Ansari et al., 1991; Derbyshire and Brown, 1978; Olszewska et al., 2008). Although asymptomatic individuals are expected to excrete lower viral loads in their feces compared to symptomatic patients, it has been reported high viral loads within asymptomatic carriers and they may consist of an important source of transmission (Ayukekbong et al., 2011; Cheon et al., 2010; Yezli and Otter, 2011; Yoshida et al., 2009; Zhang

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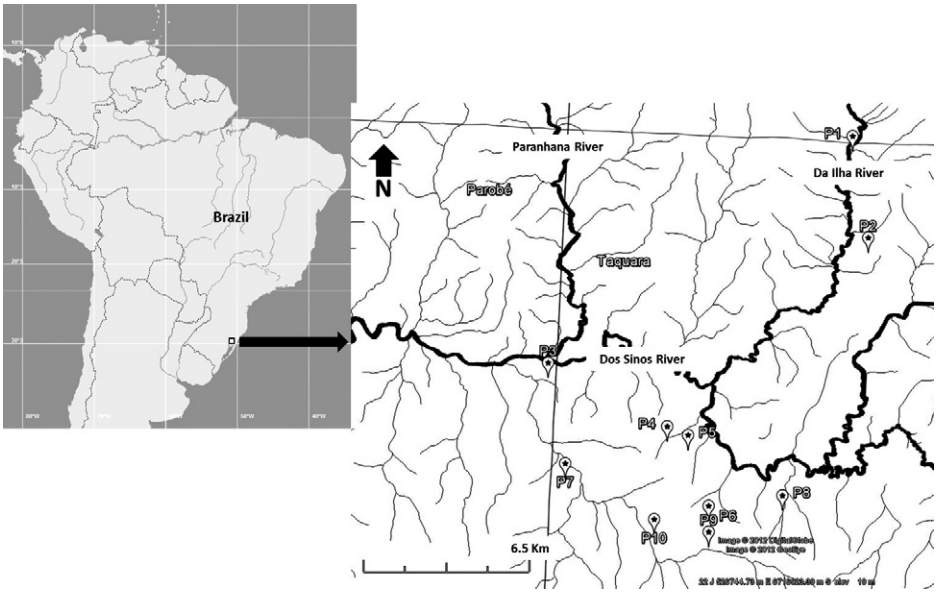

Fig. 1. Schematic representation of the location of farms chosen for the collection of water, showing the principal rivers and streams crossing the region.

et al., 2011). Bovine enterovirus (BEV) was proposed as a marker for fecal contamination of water by cattle feces from studies conducted in localities in the United States of America and Spain (Jiménez-Clavero et al., 2005; Ley et al., 2002); however, it was not tested on the Southern hemisphere, with different animal populations and diverging climate conditions. Other studies showed that AdV should be found as contaminants of water in rural areas, and may also be associated with the presence of fecal contamination (Ahmed et al., 2010; Verheyen et al., 2009; Wolf et al., 2010; Wu et al., 2011).

Most of the enteric viruses are host-specific, thus allowing tracking the primary source of fecal contamination on a given environment (Ahmed et al., 2010; Jiang et al., 2007). Many studies have shown that it is possible to determine and differentiate the participation of animal husbandry and human settlement as the sources of fecal contamination of water bodies (Ahmed et al., 2010; Jiménez-Clavero et al., 2005). Another possible advantage of the use of viral indicators is to better characterize the risk of consumption and contact with contaminated water by both human beings and animals, since many of these microorganisms are etiological agents for diarrhea and other diseases (Griffin et al., 2003).

Dairy farming is an important economic activity in the Southern region of Brazil (Heredia et al., 2010). There are growing concerns about the environmental impact which may be overcome, since the activity is mainly characterized by the production of milk on small-farms, using high animal densities (de Azevedo and Politi, 2008; Faye and Lancelot, 2006; Heredia et al., 2010). There are consequently problems with the intensive use of water and inappropriate disposal of large volumes of excreta generated by the animals, often used as a source of nitrogen and phosphorus for fodder cultivation, without prior treatment to eliminate hazardous microorganisms (Amaral et al., 2003; de Medeiros and de Souza, 2009; Faye and Lancelot, 2006). Although some studies were made to assess the fecal contamination of water on dairy farms in Brazil, they were based only on the detection of fecal coliforms, and contamination of surface and groundwater by enteric viruses were not performed so far on farms located in this region (Amaral et al., 2003; de Medeiros and de Souza, 2009). There are no reports on the dissemination of enteric viruses, such as AdV, GARV, BEV, and EV on the environment surrounding these farms and the possible impacts on environmental quality.

It is well known that both surface watercourses and groundwater on farms may contain enteric viruses and the dissemination of these pathogens may be highly influenced by the rain (through the superficial runoff of water passing over animal feces), animal densities and the use of excreta to enrich the nutrient content of the soil. The study area, the municipality of Taquara, has an annual overall amount of precipitation of 1700 mm per m^2, which is within the annual average of the north of Rio Grande do Sul (1500–1800 mm), where rainfall is evenly distributed throughout the year. The management of animal waste in the municipality of Taquara is often inappropriate and the animal densities are high, within small farms. Buffer vegetation on the margins of rivers, ponds, and lakes are poorly conserved, thus permitting the transport of nutrients and microorganisms into these water bodies (Spilki and Tundisi, 2010). This study assessed the possible impact of rainfall and the resulting wash off on the occurrence of different viral pathogens from animals and human beings on water bodies in this particular subtropical watershed. In addition, common problem of these farms is that the sanitation practices applied to human excreta is also poor or does not exist, thus the differentiation of EV from cattle and human beings was also analyzed to track the sources of fecal contamination in water samples.

2. Materials and methods

2.1. Sampling sites and samples

The Paranhana watershed comprises the municipality of Taquara, Rio Grande do Sul, and is highly representative for conditions in which dairy husbandry is performed in Southern Brazil. Three main rivers are present next to the sampling sites: Rio da Ilha, Rio Paranhana, and Rio dos Sinos (Fig. 1). Ten farms from 5 to 20 ha of total area, all using intensive farming, were chosen for collection and the geographic coordinates are given on Table 1. The farms were nominated P1–P10. Three farms had manure storage facilities, which may be considered ineffective due to problems of design and limited capacity to manage the load of excreta generated by the cattle. On the other 7 farms the excreta was not treated and its disposal on the ground was made *in natura*. The main activities were breeding of dairy cattle and other animals are found sporadically (chickens, rabbits, pigs, dogs, and cats) in a small number on these farms. Two collections were made; the first was on December 2009