TITLE: ASSESSMENT OF VIRAL CONTAMINATION OF BRAZILIAN ARTISANAL RAW MILK CHEESE

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ABSTRACT:

Enteric viruses such as noroviruses has been the main cause for acute gastroenteritis outbreaks due to the consumption of fresh and ready-to-eat foods such as sandwiches, deli meat and dairy products. This study evaluated virologic naturally contamination from 100 Brazilian artisanal raw milk cheese samples obtained from different agroindustries from Minas Gerais and Piauí states, from October 2017 to April 2018. The cheese samples were processed by using swab and eluting in PBS buffer as described in ISO 15216-1:2017. Viral RNA was extracted from 140 µL of the concentrated samples, using QIAamp viral RNA mini kit® (Qiagen), according to the manufacturer’s instructions. Synthesis of complementary DNA was performed using random primers (Invitrogen®, USA) for RNA virus detection. Murine norovirus 1 was spiked in all samples as internal process control (IPC). Norovirus genogroup I and II, human adenovirus and murine norovirus 1 were investigated by qPCR using TaqMan® system (ABI PRISM 7500, Applied Biosystems) using a set of specific primers and probes. For all determination a specific standard curve to each virus was synthetized by a 10-fold serial plasmid dilution (10⁷–10⁰ genomic copies (gc)/µL). Forty cycles were used in the reaction and samples with a cycle threshold <40 showing a characteristic sigmoid curve were regarded as positive. From a total of 100 samples, viruses were detected in 43%, being 17 adenoviruses and 29 norovirus GI strains. Viral concentrations ranged from 6.17x10⁴ to 1.44 10⁷ gc/L (µL) and IPC success rate of recovery was 100% with recovering efficiency of 10%. Previous studies investigating noroviruses in cheese have been conducted highlighting the importance to monitor virus in those matrices, including human adenovirus screening, as it is the candidate viral marker of human fecal contamination. The difficulty in establishing the origin of viral food contamination is well recognized, being often attributed to food handlers at the end of the production chain. In this study we investigated viral contamination as a first step to assess the risk that this contamination may pose to the consumer of these products, as well emphasize the need for good manufacturing practices, quality control systems in the dairy industry and markets.

Keywords: raw milk cheese, norovirus, adenovirus.

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