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Human noroviruses: detection in food and new transmission routes

Human noroviruses (HuNoVs) are yearly responsible for a large number of acute human gastroenteritis cases globally in all age groups. Typically, the virus transmits via the fecal-oral route from person to person, causing strong symptoms such as nausea, vomiting, and diarrhea, which usually disappear in a few days. However, HuNoVs cause also numerous food-related illnesses in developed countries, including Finland, inducing gastroenteritis outbreaks through contaminated water and foodstuffs. According to the reports of the European Commission, both in Europe and in Finland the most common foods causing HuNoV outbreaks are shellfish, berries (especially frozen raspberries), vegetables, and mixed foods, which most likely became contaminated by a sick food handler.

Noroviruses belong to the *Caliciviridae* family and are classified into seven genogroups. HuNoVs belong to genogroups I (GI), II (GII), and IV (GIV). Other genogroups contain only animal noroviruses. Noroviruses are generally regarded as host-species-specific, but the possibility of zoonotic transmission and infections has been discussed for over a decade for several genotypes.

The purpose of this study was to develop a simple and rapid method for detection of HuNoVs in food. The potential zoonotic nature of HuNoVs, particularly whether animals can serve as transmitters for these viruses, was also investigated.

In the past two decades, numerous methods for detecting HuNoVs in food have been developed. However, many of these are time-consuming and the sensitivity of the methods has been highly variable. In this work, four published extraction methods for detection of HuNoV in food (lettuce, ham, and frozen berries) were compared. The method based on alkaline elution and polyethylene glycol (PEG) precipitation was found to be the most reliable detection method for all three food matrices tested. The recovery efficiency of the method with frozen raspberries was on average 28%. Two rapid methods for detection of HuNoV in frozen raspberries were also presented. The rapid method based on direct RNA extraction yielded the same recovery levels (32%) as the PEG precipitation method. The method proved to be sensitive because it detected HuNoV also with a virus level of 10^2 genome copies in a 25 g sample. Moreover, the method detected HuNoV in naturally contaminated berry samples that were linked to outbreaks of disease.

A treatment with either a chloroform-butanol mixture or dilution of the food samples for the RT-PCR reaction was efficient in reducing the effect of PCR inhibitors. The same effect was achieved with PEG as a supplement in the food samples.

Thirty-nine frozen berry samples purchased from local stores in 2010, 2014, and 2017 were screened. All berries tested negative for HuNoVs GI and GII.

The possibility of zoonotic transmission of HuNoVs was investigated by analyzing fecal samples of birds, rats, mice, and pet dogs for HuNoVs. HuNoV genome was detected in the feces of 31 birds, two rats, and four dogs. The genotypes found in six bird samples and all dog samples were the same as those commonly found in human samples at the time of sampling.

HuNoVs can be detected in food samples also in small numbers using the rapid method presented in this study. The use of PEG as a supplement was found to reduce inhibition of the RT-PCR reaction in the two

rapid methods, and therefore, the commonly used chloroform-butanol treatment, which easily loses viruses during processing, could be omitted. The results of animal samples strongly indicate that wild birds, pet dogs, and possibly also rats may be involved in the transmission of HuNoVs to food, water, and surfaces.