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Research Note

Detection of Hepatitis E virus (HEV) RNA in Raw Cured Sausages and Raw Cured Sausages Containing Pig Liver at Retail Stores in Switzerland

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Abstract

Hepatitis E virus (HEV) is the causative agent of an acute and self-limiting hepatitis and is increasingly detected in food products containing pork. In this study, 102 raw sausages containing pig liver (mortadella di fegato) and 18 raw pork sausages (salami type sausage) collected at retail level in a region of southern Switzerland, were screened for the presence of HEV by quantitative real-time RT-PCR. HEV was detected in 12 of 102 (11.8%) mortadella di fegato products, but not in any of the salami sausages. Viral loads in the mortadella di fegato sausages ranged from logHEV 2.3 to 5.7 genome copies per gram (gc/g) food product. This study identifies mortadella di fegato type sausages made with raw pig liver as a potential source of HEV infection in humans.

Hepatitis E virus (HEV) is the causative agent of an acute and self-limiting hepatitis and is commonly transmitted via the fecal-oral route *(5, 11)*. Belonging to the *Hepeviridae* family *(4)*, HEV is a non-enveloped positive-stranded RNA virus *(5)*, which is classified into four major human pathogenic genotypes with different host ranges and geographical distribution. HEV genotypes 1 and 2 are found exclusively in humans while genotypes 3 and 4 have been detected also in animals, and pigs and wild boars are considered the main reservoirs *(8, 9, 11)*. In Europe, seroprevalence rates (anti-HEV IgG) indicating previous HEV infection range from 58.8% to 71.3% in fattening pigs *(1, 17)* and from 12.5% to 41.3% in wild boars *(1, 13)*. The foodborne transmission of HEV has been described in Japan and in France reporting the presence of genetically related strains in both the food and the patient after the ingestion of contaminated game meat, wild boar and pig meat, or pig liver sausages *(2, 3, 15)*. Recently, the first confirmed foodborne HEV infection in Switzerland linked to the consumption of a raw sausage containing pig liver was described *(7)*. This sausage type called "Mortadella di fegato" is made with fresh pig liver, air-dried (cured) for two months, and is very popular in the canton of Ticino, located in southern Switzerland.

The aim of this study was to screen raw sausages containing pig liver and raw pork sausages collected at retail level in the Ticino region, to assess the occurrence and the viral load of HEV in these types of products.

Materials and Methods

Sample collection. In total, 120 raw sausages (102 mortadella di fegato type sausages and 18 salami type pork sausages) were collected between January 2016 and June 2017. The samples were either officially taken by food safety authority officers at production or retail level throughout the canton Ticino, or sent to the laboratory by the producers themselves,

within the framework of their self-check program according to Swiss food legislation. The samples originated from 73 producers.

HEV detection in the food samples. The food products were processed as described by Szabo *et al. (14)*. Briefly, 5 g of mortadella di fegato or salami which had been manually defatted, were placed in a 80 ml sterile filter bag (Interscience by Axon Lab, Baden-Dättwil, Switzerland) and 10 µl of Mengovirus solution [$1.61x10^5$copies/µl] as a process control (Mengovirus Extraction Control kit, bioMérieux, Geneva, Switzerland) were added and incubated at room temperature for 5 min. Thereafter, the sample was homogenized with 7 ml of TRI Reagent® (Lucerna-Chem AG, Luzern, Switzerland) using a blender (MiniMix®, Axon Lab, Baden-Dättwil, Switzerland) for 2 min. The rinse fluid was removed via the filter compartment of the bag and centrifuged at 10'000 x g for 20 min at 4°C to pellet residual food particles. A total of 1.4 ml chloroform (0.2 ml/ml TRI Reagent®) was added to the clarified supernatant and mixed for 15 sec. After incubation for 15 min at room temperature samples were centrifuged at 10'000 x g for 15 min at 4°C. The nucleic acids of a total of 1 ml of the aqueous phase containing the viral RNA were extracted using the NucliSENS® easyMAG system (bioMérieux, Geneva, Switzerland) according to the manufacturer's instructions and eluted in 60 µl of elution buffer. The extracts were either freeze-stored at -80°C, or immediately used for the viral RNA amplification. The quantitative real-time RT-PCR's for HEV and Mengovirus were performed with commercial kits (ceeramTools®, Mengo Extraction Control and hepatitis@ceeramTools®, bioMérieux, Geneva, Switzerland) according to the manufacturer instructions, on a Rotor Gene Q system (Qiagen, Basel, Switzerland). For the extraction method used in this study, the limit of detection (LOD) has been determined previously at $2.9x10^3$ HEV genome equivalents (GE) per 5 g raw sausage *(14)*. Quantitative real-time RT-PCR detected as few as 5 HEV genome copies per reaction. Positive food samples were retested using the same procedure for confirmation.

Results and Discussion

A quantitative risk assessment following the *Codex Alimentarius* principles was recently performed in Switzerland in order to predict the exposure of consumers to HEV through food consumption *(10)*. Pork products containing pork liver, in particular those sold raw, were identified as posing the highest risk for the consumer. Sausages of the "mortadella di fegato" type are a specialty in the canton Ticino. These sausages are made of ground pork, pork fat, and pork liver, and flavored with spices. The meat mixture is stuffed in natural casings and cured for about two months, and usually consumed raw (mortadella di fegato cruda). If cured for a shorter time, the mortadella di fegato might also be cooked (mortadella cotta). The sausages typically weigh between 300g and 2kg, with a liver content between 2% and 15%.

Here, we screened only raw sausages containing pig liver (mortadella di fegato cruda) and raw pork sausages without liver (salami) for the presence of HEV; cooked mortadella di fegato was not investigated. In total, 12 (10%) of the 120 products tested positive for HEV RNA (Table 1). The positive samples had been collected throughout the study period and their origins were not restricted to any specific producer. For all samples, Mengovirus recovery rates were >1%. None of the 18 salami type products tested positive for HEV. From a total of seven HEV positive samples, viral genome copies per gram (gc/g) were determined (Table 2). The logHEV in the samples ranged from 2.3 to 5.7 gc/g.

The prevalence (11.8%) of HEV in sausages containing pork liver in this study is higher than the estimated HEV prevalence of 4.6% for products containing pork meat and pork liver in Switzerland *(10)*. Furthermore, 4 (57.1% of the HEV-positive products) exceeded the highest estimated logHEV contamination level of 3.5 gc/g *(10)*. This suggests that mortadella di fegato products from this particular region in Switzerland expose the consumer to a higher than average risk of infection. However, our data remains lower than the prevalence of HEV in pork products in other European countries *(3, 12)*. Taking into account the large variabilty

of virus recovery from food, as demonstrated in this study by the Mengovirus recovery rates of 3.3%-71.4% (Table 2), our reported HEV prevalence as well as the HEV load may be underestimated.

The presence of HEV RNA alone does not confirm viral infectivity *(6)*. However, one may assume that products with high number of HEV gc/g are more likely to contain infectious HEV particles. Although information on infective doses of HEV for humans is not available, experimental studies with non-human primates indicate that the severity of infection with HEV is proportional to the infectious dose *(6, 16)*. Although there are currently very few studies that allow definitive conclusions about the role of food in human HEV infection, reports are emerging linking human infection to eating raw or undercooked pig liver *(7)*. Our results indicate that consumers at risk such as the immunocompromised, persons with underlying liver conditions and pregnant women should avoid eating products containing raw mortadella di fegato type sausages. Sequencing of the positive samples is in progress in order to clarify the genotypes of HEV detected in mortadella di fegato, and in one case described previously *(7)*, results show that HEV genotype 3 is involved (GenBank accession numbers MF346773 and MF346773).

Conclusively, our findings demonstrate that mortadella di fegato type sausages containing pork liver can harbor HEV, placing consumers at risk of infection. This study shows that it is important to identify and monitor high risk food products and develop risk management strategies, such as increasing awareness among consumers and health providers.

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Table 1: HEV-positive samples in screened pork sausage products

Product	No. samples tested	No. HEV RNA positive
Mortadella di fegato type sausages (with raw liver)	102	12 (11.8%)
Salami type sausages (without liver)	18	0 (0%)

Table 2: Determination of viral load in seven HEV-positive samples of mortadella di fegato

Sample ID	Product	Recovery of Mengovirus (%)	HEV log viral load (gc /g)
702	Mortadella di fegato	12.8%	5.7
703	Mortadella di fegato	11.0%	4.6
1813	Mortadella di fegato	27.7%	4.4
1815	Mortadella di fegato	3.3%	2.6
1818	Mortadella di fegato	14.6%	2.7
7123	Mortadella di fegato	71.4%	2.3
1811	Mortadella di fegato	28.6%	4.6