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Estimated exposure to hepatitis E virus through consumption of swine liver and liver sausages

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ABSTRACT

A quantitative risk assessment was undertaken following the *Codex Alimentarius* principles in order to predict the exposure of consumers to hepatitis E virus (HEV) through food consumption. Taking into account the tropism of HEV, fresh liver and liver sausages were regarded as having a higher risk of contamination. The model entailed a hypothetical food pathway and was based on worst case scenario where the intake of contaminated food derived from a 100% HEV-infected pig population was estimated. As no data on the prevalence of infectious HEV was available, the HEV-RNA prevalence in food matrices and the seroprevalence of HEV-specific antibodies in swine were assessed and adjusted for diagnostic misclassification and sampling uncertainty. Considering a HEV prevalence of 100% in pigs and excluding further cross-contamination events, a food portion consisting of 130 gr of liver or of 32.5 gr of sausage (containing 30% of liver) yielded an estimated exposure of 8047 and 210 RNA copies (median values), respectively. These findings take into account the effect of thermal treatment on the HEV-RNA concentration of food. Due to the lack of information concerning the correlation between HEV-RNA concentration and the amount of infectious virus as well as the dose-response relationship of HEV, the calculated RNA copies do not allow direct conclusions to be drawn on the risk of infection following ingestion of these food types. The true prevalence was estimated for Switzerland and Germany, leading to an overall prevalence of HEV-RNA in food of 6.2% (90% Highest Density Intervals (HDIs): 2.5%–11.2%). In comparison with fresh liver, liver sausages showed a higher prevalence, most likely due to the presence of more than one liver within the same sausage. The true prevalence of anti-HEV IgG ranged between 59.4% (HDIs 56.5%–62.4%) and 62.6% (HDIs 58.8%–64.3%) and between 7.6% (HDIs 3.3%–13.2%) and 30.5% (HDIs 23.2%–38.2%) in pigs and wild boars, respectively. The high prevalence of antibodies support the evidence that these animals can act as reservoirs for HEV and can contribute epidemiologically to the maintenance of the virus in the surroundings. This study is a preliminary investigation and highlights the major existing gaps needed to be filled in order to enable a refined HEV risk assessment that can drive future decisions for the implementation of food safety and of control measures.

1. Introduction

Hepatitis E virus (HEV) is the causative agent of an acute and self-limiting hepatitis and is commonly transmitted via the fecal-oral route (Bonney et al., 2012; Emerson & Purcell, 2003; Pavio & Mansuy, 2010). Belonging to the *Hepeviridae* family (Emerson et al., 2004), HEV is a non-enveloped positive-stranded RNA virus (Emerson & Purcell, 2003), which is classified into four major

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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 (Meng, 2010, 2011; Pavio & Mansuy, 2010).

Swine HEV infection is usually subclinical; pigs show no overt disease signs or pathological lesions. Pigs are normally infected at the age of 4–8 weeks resulting in a transient viremia and short fecal shedding (Pavio, Meng, & Renou, 2010). In Europe, seroprevalence rates (anti-HEV IgG) indicating previous HEV infection range between 58.8% and 71.3% in fattening pigs (Burri et al., 2014; Wacheck et al., 2012a) and between 12.5% and 41.3% in wild boars (Burri et al., 2014; Schielke et al., 2015).

In humans, clinical symptoms of hepatitis E are indistinguishable from other forms of acute hepatitis (Purcell & Emerson, 2001). The case fatality rate among patients is generally below 1–5% (Pavio et al., 2010), with the exception of pregnancy where rates up to 25% have been reported (Kumar, Beniwal, Kar, Sharma, & Murthy, 2004).

Although large outbreaks of hepatitis E seem to be confined to low-income countries as a consequence of poor water hygiene conditions, sporadic cases are reported globally, including in Europe. Infections in Europe are due to HEV-genotype 3, the most prevalent virus in humans and animal reservoirs. In Switzerland, hepatitis E is not notifiable; therefore the exact number of cases is unknown. Nevertheless, autochthonous cases were diagnosed in 2004 (Sudre, Delaporte, & Mezger, 2005, pp. 326–327) and in 2013 (Hiroz et al., 2013). Cases have been also reported after exposure to game meat (Joller & Gaudenz, 2015). Recently, in Germany, the number of notified hepatitis E cases has risen steeply. In 2014, 670 cases were reported with an increase of 46% compared to 2013 (Robert Koch Institute, 2015). Antibodies against HEV have been found in both the general population (Dremsek et al., 2012; Schnegg et al., 2013) and - with increased prevalence - in individuals with occupational exposure to swine and wild boars (Krumbholz et al., 2014; Schielke et al., 2015; Wilhelm et al., 2011). 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 (Colson et al., 2010, 2012; Tei, Kitajima, Takahashi, & Mishiro, 2003).

In order to estimate the exposure of an individual to HEV through the consumption of food, a quantitative exposure assessment was attempted following the *Codex Alimentarius* Commission principles. The model entailed a hypothetical food pathway and was based on a worst-case scenario where the intake of contaminated liver and liver sausages derived from a 100% HEV-infected pig population was assumed. This study also aims to identify current knowledge gaps.

2. Materials and methods

The overall scenario pathway for this quantitative exposure assessment to HEV via the consumption of food is represented with a three-module structure (Fig. 1). Two extra modules, that are not part of the food pathway, are also described in order to provide a broader view of the available knowledge of HEV. Input data for the development of the present study were obtained from the scientific literature.

2.1. Literature search

A review of the literature was performed to identify studies describing the HEV-RNA concentration in fresh liver and liver sausages (module 1). Only studies that quantified the RNA amount by real time PCR (RT-qPCR) per each of the positive samples were included. A search was also carried out to identify studies reporting the effect of the thermal treatment on RNA concentration (module 2). Studies in which the logarithmic reduction of a given viral load was measured were taken into account. Supplementary research on the prevalence of HEV-RNA in food was done (module a). The module aimed at the estimation of the prevalence (referred to as "true prevalence" from now on) and was attempted through adjustment for diagnostic misclassification and quantification of sampling uncertainties. Prevalence studies from Switzerland and Germany were eligible. The current knowledge on the seroprevalence of antibodies against HEV in pigs and wild boars was also reviewed (module b) with the aim of estimating the true prevalence of anti-HEV IgG in swine. Selection criteria were as follows: prevalence data obtained from commercial ELISA assays intended for serum and meat juice samples; studies carried out in the abovementioned countries within the last ten years. In addition to studies selected from the literature review, unpublished serological data from a one-year Swiss project were included (Table 4).

2.2. Initial concentration of HEV-RNA in food (module 1)

Following the previous selection criteria, a total of three and two studies were included in order to evaluate the initial concentration of HEV-RNA in fresh pig liver and liver sausages, respectively. Table 1 shows the number of positive samples and the corresponding titers. Concentrations were first converted into the same unit of measure (number of genome copies/gr) and then transformed into $\log_{10}$ copies/gr. In the study from Leblanc, Poitras, Gagné, Ward, and Houde (2010), the viral RNA titer per each positive liver sample was expressed as a range of values falling in the same log level. Thus, to gain a final value (in $\log_{10}$ copies/gr), a uniform distribution was applied with minimum and maximum values as observed in the studies. Thereafter, samples whose titers fell in the same $\log_{10}$ level were grouped and their relative and cumulative frequency calculated. The initial concentration of HEV-RNA was estimated using a cumulative distribution as described by Vose (1996). The initial concentration in liver sausages took into account the proportion of liver (on average 30%, modeled by Pert distribution) in the final product. Table 5 shows an overview of the distributions that were used in the present assessment. The software package @RISK™ version 5.5 (Palisade, Newfield, NY) for Excel™ (Microsoft Corp., CA) was used with ten thousand iterations and one simulation for all distributions. Bayesian analysis was used to reduce the uncertainty around the predicted exposure estimate.

2.3. Effect of thermal treatment (module 2)

To investigate the effect of thermal treatment on HEV-RNA concentration in fresh pig liver and liver sausages, a total of two and one studies were used, respectively (Table 2). Load reductions were grouped based on three core-temperature ranges: up to 60 °C (resembling rare-medium cooking), up to 69 °C (medium-medium well), and above 70 °C (well done). For each temperature range, a time range between 1 and 15 min was considered (Table 2). A Pert distribution was used to model the mean HEV-RNA log reduction (Log_{10}copies/gr) after thermal treatment (Table 5).

2.4. Final concentration of HEV-RNA in food (module 3)

The final concentration was defined as the number of viral RNA measured in Log_{10} copies/gr that remained within the food at the time of consumption quantified as the mean HEV-RNA load reduction after thermal treatment subtracted from the initial HEV-RNA concentrations.