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Hepatitis E virus (HEV) along the food chain: Investigations into spread, genetic diversity and molecular tracing

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Key words
Hepatitis E virus; pig; wild boar; zoonosis; herd screening; genetic diversity

Aim of the study
This research project aimed at assessing the occurrence and genetic diversity of Hepatitis E viruses (HEV) in Switzerland to gain knowledge on ways and sources of infection using molecular epidemiology.

Material and methods
The study was performed in two parts, i.e. two dissertations: Part 1 (thesis Isabelle Vonlanthen) comprising the testing and sequencing of samples from pigs, wild boar and meat products; part 2 focusing on the development of a sample collection method to screen pig herds for hepatitis E virus (HEV) (thesis Julia Lienhard).
Viral RNA was extracted by different methods depending on the type of sample material but mostly involving the Qiagen viral RNA mini kit or the Trizol method. Samples were tested for HEV RNA using an inhouse real-time RT-PCR according to Jothikumar et al. (2006) with minor adaptations and occasionally (mainly for processed meat products) the commercial CeeramTools HEV kit. For sequencing a 493nt long fragment of the ORF2 we used a nested RT-PCR protocol as recommended by the European HEVnet (the so-called “typing RT-PCR”). For next generation sequencing we followed our in-house developed protocol (Kubacki et al., 2021). Sequences were geno- and subtyped using the online typing tool by the European HEVnet https://www.rivm.nl/mpf/typingtool/hev/ as well as own phylogenetic analyses using published reference sequences. Complete sequences were submitted to the European HEV sequence repository (HEVnet database). For antibody testing of sera and meat juice the PrioCHECK HEV Ab porcine ELISA Kit was used.

Results and significance
Part 1: Prevalence and viral diversity in pigs, wild boars and meat products.
Of 192 pig livers provided by the three major pig slaughterhouses (Zürich, Basel, Courtepin) only one single liver was positive in the HEV real-time RT-PCR but 114 samples were antibody positive resulting in a virus prevalence of 0.5% and seroprevalence of 59.4% which is comparable to previous studies. In contrast, 7 of the 54 liver samples from two carcass collection points (mainly younger animals) were virus positive (13%) but only 14 (25.9%) were antibody positive. This finding confirms the fact that infection with HEV is a typical young-animal infection in pigs and usually over when they are slaughtered. In addition to the livers, also faeces and diaphragm of these animals were tested, and, in all cases, the highest viral load was observed in faeces followed by liver and diaphragm. Two diaphragm samples were even negative. Wild boar samples were provided by hunters from the cantons Schaffhausen (SH) and Ticino (TI). While 7 of the 75 livers from SH were virus-positive (9.3%) no viral RNA was found in any of the 46 samples from TI. To estimate the seroprevalence, 592 meat juice samples from the trichinella control were tested for antibodies against HEV. With 4.9% total virus prevalence and 12.3% seroprevalence the results were comparable to previous studies in Swiss wild boar. Interestingly, a marked regional difference in prevalence numbers was observed in wild boar but not in pigs. None of the 14 roe deer samples from SH was virus or antibody positive but the single sample from Germany (near the Swiss border) was antibody positive. Regarding meat products, we have received already positively tested samples from the BLV laboratory (provided by Dominik Moor, 21 samples) and the cantonal laboratory in TI (11 samples) for sequencing. These were mainly known high risk products such as Mortadella di fegato,

Leber-Salsiz, Saucisson, and liver patés. In addition, we have tested 32 samples of unknown HEV status. The 19 samples of fresh game meat and pork sausages from a major Swiss retailer were all negative as well as the majority of the 13 sausages and patés that we bought ourselves for testing. The only positive sample was a Figatellu sausage from France.
Of all positive samples, 29 were successfully sequenced in the ORF2 region using the 493nt long product from the typing PCR (14 animal samples and 15 meat products). Two liver patés of German origin contained HEV3c, the most prevalent HEV subtype in most European countries and one pig and one wild boar sample provided subtype 3o(p), which was also shown in Italy. However, most samples from pigs, wild boar and meat products contained HEV3s(p), which was previously proposed to be a Swiss-specific HEV3 subtype and is also the most prevalent in humans in Switzerland.

Part 2: Development of a HEV screening approach for pig herds.
We compared individual faecal samples, floor swabs, dust swabs and manure from 14 HEV positive farms out of the 21 sampled for a doctoral thesis at the pig clinic in 2017. In addition, manure samples from another 64 farms (26 farrowing, 25 fattening and 10 breeding farms) were tested. PCR results of the manure samples showed that HEV was mainly present in fattening farms (75%), followed by farrowing (19.2%) and breeding farms (10%) and that floor swabs were more representative for the actual status of infection and easier to access. Therefore, we asked the Swiss pig health service (Schweinegesundheitsdienst, SGD) for help to sample herds in different Swiss regions using sock swabs. Sock swabs from 85 pig herds (primarily fattening farms) were collected between October 2020 and May 2021 and 51 farms were found positive (60%) whereof 47 samples could be subtyped and resulted in 40 3s(p) and 7 3o(p) sequences.
In (very) short, the diversity of HEV in Swiss pigs, wild boars and meat products was found to be limited to HEV3s(p) and more rarely 3o(p) and sock swabs may present an easy way to screen pig herds for HEV.

Publications, posters and presentations
Oral presentations:

- 4. BfR-Symposium Lebensmittelassoziierte Viren, 7. November 2018, Berlin.
- 1st Essen Hepatitis E Symposium, 14.-16. Februar 2018, Essen.
- 22. Seminar Schweizerische Vereinigung für Schweinemedizin, 2019, Grindelwald.
- Inaugural thesis Isabelle Vonlanthen, accepted by the University of Zürich in Nov. 2020

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