027

Hepatitis E - analyzing the occurrence in slaughter pigs for a risk assessment of raw meat products

Dzierzon J.[1], Oswaldi V.[1], Merle R.[2], Langkabel N.[1], Meemken D.[1]

[1]Freie Universität Berlin, Institute of Food Safety and Food Hygiene, Berlin, Germany, [2]Freie Universität Berlin, Institute for Veterinary Epidemiology and Biostatistics, Berlin, Germany

Introduction

The hepatitis E virus (HEV) of genotype 3 and 4 is known as a zoonotic agent. In this context, the pig was identified as the main animal reservoir. In Europe, the consumption of raw or undercooked pork products represent a potential risk for HEV infections in humans. In humans, HEV infections can cause acute hepatitis, which is usually self-limiting. Chronicity in immunocompromised patients and a high mortality rate of up to 28% in pregnant women have been reported (Meng 2011).

In Germany, according to § 7 of the German Infection Protection Act (IfsG, 2019), the direct or indirect detection of HEV in humans must be reported to official health services. In 2018, a total of 3,275 cases of hepatitis E was reported to the Robert Koch Institute (RKI 2019).

As pigs are a main reservoir of HEV several studies were performed identifying the antibody status of fattening pigs across EU member states. With a seroprevalence of up to 96% (Wutz et al. 2013), HEV shows a wide distribution among fattening pigs in Germany. Nevertheless, national studies examining the occurrence of HEV RNA in liver or muscle samples from pigs are rare.

The objective of this study was to estimate the risk of HEV entering the food chain via pork products based on serological tests and on the analysis of pork liver and muscle samples from the same animal used for the production of pork liver and pork meat products.

Materials and Methods

In 2018, a total of 250 fattening pigs from 25 farms (10 pigs per farm) were sampled in an abattoir in North-West Germany. One sample of ham muscle, one sample of liver tissue and one sample of the muscle of the diaphragm pillar were collected from each pig during the slaughter process. Each animal was tagged individually and samples were taken at different stages of the slaughter line. Livers were collected and stored in boxes during the slaughter process as usual until sampling. All samples were chilled and transported to the institute's laboratory. Muscle samples from the diaphragm pillar were stored at -30 °C and liver and ham muscle samples were stored at -80 °C until laboratory examination.

To determine the seroprevalence, meat juice from the diaphragm pillar samples was serologically tested for HEV antibodies using the Priocheck™ HEV Antibody porcine ELISA Kit (Thermo Fisher Scientific®, USA) according to the manufacturer's manual. The liver and muscle samples were analysed for the presence of HEV RNA by real-time RT-PCR according to Jothikumar et al. (2006) after RNA extraction with the RNeasy® Mini QIAcube Kit (QIAGEN®, Germany).

For each pig the antibody status will be gathered and herd status will be analysed, too. Afterwards, the presence of HEV antibodies for each animal will be compared with the presence of viral RNA in the liver and the muscle.

Results

In total, 62% (155/250) of the meat juice samples were positive for antibodies against HEV at a single animal basis. At herd level, 72% (18/25) of the herds were positive. Herds were considered to be positive, if at least one of the ten samples was positive.

For the herd seroprevalence four groups, according to the serological detection rate, were defined. The herds investigated were allocated to one of these groups using their antibody prevalence (Table 1).

Table 1: Allocation of herds according to the antibody status

serological detection rate	Proportion of herds (n/N)
0% (HEV seronegative)	28% (7/25)
10%-30% (low prevalence)	8% (2/25)
60%-90% (high to very high prevalence)	16% (4/25)
100% (all samples are HEV seropositive)	48% (12/25)

Table 2: Detection of HEV in liver and ham muscle from slaughter pigs

Number of analysed samples	Number of HEV positive tested samples	Viral Prevalence
liver: 126	18	14 %
muscle: 133	0	0 %

Analysed so far, HEV RNA was detected in 14 % (18/126) of the liver samples (Table 2), which came from HEV seropositive pigs. Whereas in liver samples from HEV seronegative pigs, HEV RNA could not be detected, until now.
So far, all investigated muscle samples were negative (0/133) for HEV RNA (Table 2).

Conclusion

The serological results show that HEV antibody prevalence is relatively high in fattening pigs included in this study (62 %). The sporadic presence of HEV in liver samples indicates that pig liver or pig liver products may represent a potential risk for HEV infection if consumed raw or undercooked or if the rules of kitchen hygiene are not observed.
In addition, HEV positive livers do not seem to be associated with HEV positive ham muscles.
Based on the results obtained so far, it appears possible to use serological tests to predict the presence of HEV RNA in the liver of fattening pigs.

References

IfsG (2001): Infection Protection Act in the version of January 1, 2019
Jothikumar, N., T. L. Cromeans, B. H. Robertson, X. J. Meng and V. R. Hill (2006): A broadly reactive one-step real-time RT-PCR assay for rapid and sensitive detection of hepatitis E virus. J Virol Methods, Vol. 131, 65-71
Meng, X. J. (2011): From barnyard to food table: the omnipresence of hepatitis E virus and risk for zoonotic infection and food safety. Virus Res, Vol. 161, 23-30
Robert Koch-Institut, Epidemiological Bulletin (1/2019) January 3, 2019
Wutz, K., Meyer V. K., Wacheck S., Krol P., Gareis M., Nolting C., Struck F., Soutschek E., Bocher O., Niessner R. and Seidel M. (2013): New route for fast detection of antibodies against zoonotic pathogens in sera of slaughtered pigs by means of flow-through chemiluminescence immunochips. Anal Chem, Vol. 85, 5279-5285