Mac Vet Rev 2013; 36 (2): 97–100

Available online at
www.macvetrev.mk

Original Scientific Article

UDC: 636.4.09:[616.36-002-02:578.891(497.4)

DETECTION OF HEPATITIS E VIRUS IN FAECES AND LIVER OF PIGS COLLECTED AT TWO SLOVENIAN SLAUGHTERHOUSES

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Received 30 June 2013; Received in revised form 22 July 2013; Accepted 22 August 2013

ABSTRACT

In recent years there have been numerous reports from different parts of the world describing hepatitis E virus (HEV) as a zoonotic agent, but the clinical cases in humans are still reported only sporadically. Domestic pigs represent the main reservoir of the HEV. Until recently it was believed that the HEV was transmitted only by faecal-oral route, but it has been proved that eating raw or undercooked pork meat and offal can cause acute HEV infection in human. This has triggered the alarm and many developed countries have already done a few studies to assess the percentage of infected pigs.

In this study the situation regarding the risk factor of HEV among pigs that enter the food chain in Slovenia was evaluated. At two different slaughterhouses 87 faeces and liver samples were collected from pigs within two age groups. 32 faeces and liver samples were collected from 3 months old pigs and 55 faeces and liver samples from 6 months old pigs. Animals were brought to the slaughterhouse from different farms located at the north eastern part of Slovenia, where the majority of the pig population is located. Collected samples were analysed with real-time RT-PCR method. Nucleic acids of HEV was found in 6 faeces samples from the younger age group (3 months of age), which represents 19% of examined samples. All liver samples from 3 months old pigs were negative. All samples of faeces and liver from 6 month old pigs were negative. The results were comparable with those from other European countries, where 7-30% of swine faeces samples were found HEV positive.

Key words: hepatitis E virus, pigs, slaughterhouse, undercooked pork meat, zoonosis

INTRODUCTION

Hepatitis E virus is a non-enveloped RNA virus with a polyadenylated, single stranded RNA genome. The genome has three open reading frames (1, 2). It has been classified in the genus *Hepevirus* in the family *Hepeviridae*. Based on the genome sequence analysis the virus is grouped into 4 genotypes, named genotype 1, 2, 3 and 4, that differ by their geographical distribution and host range. Genotype 1 and 2 cause infection only in humans, while genotype 3 and 4 infect also pigs and other mammals (3).

Antibodies for hepatitis E virus have been found in many animal species like pigs, cattle, sheep, goats, horses, cats, dogs, mice and rats. Most data is known about pigs, since they have been mainly investigated (4).

At first, HEV was only causing concerns in developing countries, their biggest problem regarding hepatitis E virus spread was poor sanitation. But lately infections have become a problem also in industrialized countries. It has been proved that eating raw or undercooked swine meat or offal can cause infection in humans (4).

Clinical symptoms in people infected with HEV are typical for hepatitis; abdominal pain, nausea, vomiting, followed by icterus and dark urine (5, 6). In most cases the disease disappears by itself in a few weeks, but if the patient has a history of

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Competing Interests: The authors have declared that no competing interests exist.

hepatic problems it can cause acute liver failure. It affects young to middle age adults and can be fatal in pregnant women, where the mortality is as high as 20% (6).

On the other hand, the symptoms in infected animals are non-existent, except for some hepatocyte necrosis and sometimes slightly enlarged mesenteric lymph nodes (7).

Although most of death cases related to HEV occur in developing countries, the interest in the disease in recent years has been growing in the developed countries. Many of the European countries have been collecting data about the infection status in pigs. From what has been published, we can estimate that approximately 20-40% of domestic pigs up to 6 months old are infected (8).

The situation in Slovenia has been quite poorly inspected so far, we have some data about the condition on a few Slovenian farms, but none from the slaughterhouses (9, 10). Since pigs that enter the slaughterhouse are the ones that end up on the consumers table, we were curious about the infection percentage of those pigs.

MATERIALS AND METHODS

From November 2012 to March 2013, samples of pig faeces and liver were collected at two different slaughterhouses in Slovenia. Pigs originated from 17 different farms, all of them located in the north eastern part of Slovenia (municipalities Ptuj, Gornja Radgona, Destrnik, Ljutomer, Murska Sobota, Tišina, Cerkvenjak, Benedikt, Ključarovci, Lokavec-Sv.Ana, Trate). Pigs that were sampled can be divided into two groups: younger age group – 3 months old pigs and older age group – 6 months old pigs. In total, 87 samples of faeces and 87 samples of liver were collected: 32 from the younger age group and 55 from the older age group. Faeces were sampled directly on the line from the colon of individual pigs. At the same time, a part of liver from the same pig was taken and stored in a separate sterile plastic centrifuge tube. Tubes where then put in a coolbox until being stored in a deep freezer at minus 70°C.

In the laboratory, 1g of samples were homogenized in 5 ml RPMI 1640 (Gibco, Invitrogen) with a homogenizer (IUL masticator). After homogenization, the suspensions of samples were centrifuged for 15 min at 2500 × *g*. The supernatant was recovered and used for the extraction of total RNA. Total viral RNA was extracted from samples using QIAamp® Viral RNA Mini Kit (Qiagen, Germany) according to manufacturer's instructions. For HEV RNA detection in samples rapid and sensitive real-time RT-PCR assay for the detection of four genotypes of HEV was used (11).

RESULTS

The results of real-time RT-PCR method in 3 months old pigs showed positive results (with cycle threshold value between 33,08 and 41,76) in six faeces samples, while all 32 pigs were negative in liver (Table 1).The results of 55 samples from the older age group (6 months old) were all negative in faeces and liver using real-time RT-PCR for HEV RNA detection (Table 2).

Table 1. Real-time RT-PCR results of fecal and liver samples, 3 months old pigs

	No. of samples	No. of positive samples	Percentage of positive samples (%)
Liver	32	0	0
Faeces	32	6	19

Table 2. Real-time RT-PCR results of fecal and liver samples, 6 months old pigs

	No. of samples	No. of positive samples	Percentage of positive samples (%)
Liver	55	0	0
Faeces	55	0	0

DISCUSSION

The majority of pigs that are brought to the slaughterhouses in Slovenia are around 6 months old. Since these are the animals that end up on the consumer's plate, we decided first to collect samples from this age group and have a look at the situation. All 55 samples of faeces and liver were negative for the presence of HEV RNA. The results did not come as a huge surprise to us: they were not much different from what was found in other European countries, although we didn't expect all the results to be negative. Because the number of samples in this study was rather small these results may not necessarily represent the real situation and further study on larger number of samples should be done.

While searching through the articles and gathering information about the infection of pigs in other countries, we came to the conclusion, that viral RNA is much more frequently found in younger animals. According to the literature the peak of viremia in pigs is from the first to the third month of age (12). As mentioned earlier, pigs slaughtered in Slovenia for consumption are mostly older, but there is also small percent of pigs that are slaughtered younger, at the age of 3 months.

From the 32 samples of faeces 19% (6 samples) were detected as positive in this study. These results are comparable with previous observations on samples collected between 2010 and 2011, when 25,2 % of faeces samples were detected as HEV positive in real-time RT-PCR in pigs between 2 and 4 month old (9).

All 32 liver samples from this study were negative for the presence of HEV RNA. The problem when collecting liver samples is that the HEV infection in liver is focal, so the chances of sampling the right part of the liver are much smaller (13). This could explain why the samples from animals that were tested positive in faeces were at the same time detected as negative for the presence of viral RNA in liver.

Finding viral RNA in faeces of slaughtered animals is alarming in many ways.

The most important is the possibility of cross-contamination of the meat during the evisceration. If such contaminated meat is not properly cooked, it is possible that the virus remains active and it can cause infection in human (12).

There is also a high risk of infection for the workers that are regularly exposed to infected animals. It has been proven that people which are in contact with pigs every day, like workers in a slaughterhouse, have higher prevalence of hepatitis E virus-specific antibodies (14).

Finally, there is also a risk of environmental pollution in case of not properly cleaned effluent.

REFERENCES

1. Vivek, C., Shikha, T., Manjula, K., Shahid, J. (2008). Molecular biology and pathogenesis of hepatitis. E virus. J Biosci., 33, 451-464.
2. Vasickova, P., Psikal, I., Kralik, P., Widen, F., Hubalek, Z., Pavlik, I. (2007). Hepatitis E virus: a review. Vet Med (Praha), 52, 365-384.
3. Song, Y. J. (2010). Studies of hepatitis E virus genotypes. Indian J Med Res., 132, 487-488.
4. Meng, X. J. (2010). Recent advances in hepatitis E virus. J Viral Hepat., 17, 153-161.
5. Chandra, V., Taneja, S., Kalia, M., Jameel, S. (2008). Molecular biology and pathogenesis of hepatitis E virus. J Biosci., 33, 451-464.
6. Teshale, E. H., Hu, D. J., Holmberg, S. D. (2010). The two faces of hepatitis E virus. Clin Infect Dis., 51, 328-34.
7. Lee, Y. H., Ha, Y., Ahn, K. K., Chae, C. (2009). Localisation of hepatitis E virus in experimentally infected pigs. Vet J., 179, 417-421.
8. Berto, A., Backer, J. A., Mesquita, J. R., Nascimento, M. S. J., Banks, M., Martelli, F., Ostanello, F., Angeloni, G., Di Bartolo, I., Ruggeri, F.M., Vasickova, P., Diez-Valcarce, M., Hernandez, M., Rodriguez-Lazaro, D., van der Poel, W.H.M. (2012). Prevalence and transmission of hepatitis E virus in domestic swine populations in different European countries. BMC Res Notes, 5, 190.
9. Toplak, I., Rihtarič, D., Jamnikar Ciglenečki, U., Hostnik, P., Grom, J. (2012). High diversity of hepatitis E virus detected in pig fecal samples in Slovenia. 9th International Congress of Veterinary Virology, Madrid, 2012. ESVV 2012. One world, one health, one virology. Madrid: European Society for Veterinary Virology, 163-164.

10. Naglič, T. (2010). Virus hepatitisa E pri domačih prašičih in v površinskih vodah v Sloveniji. Diploma thesis. University of Ljubljana, Biotechnical faculty.

11. Jothikumar, N., Cromeans, T.L., Robertson, B.H., Meng X.J., Hill, V.R. (2006). A broadly reactive one step real-time RT-PCR assay for rapid and sensitive detection of hepatitis E virus. J Virol Methods, 131, 65-71.

12. Pavio, N., Meng, X.-J., Renou, C. (2010). Zoonotic hepatitis: animal reservoirs and emerging risks. Vet Res., 41, 46.

13. Di Bartolo, I., Ponterio, E., Castellini, L., Ostanello, F., Ruggeri Franco, M. (2011). Viral and antibody HEV prevalence in swine at slaughterhouse in Italy. Vet Microbiol., 149, 330-338.

14. Krumbholz, A., Mohn, U., Lange, J., Motz, M., Wenzel, J., Jilg, W., Walther, M., Zell, R. (2012). Prevalence of hepatitis E virus-specific antibodies in humans with occupational exposure to pigs. Med Microbiol Immunol., 201, 239-244.

Please cite this article as: Raspor Lainšček Petra, Toplak Ivan, Kirbiš Andrej. Detection of hepatitis e virus in faeces and liver of pigs collected at two slovenian slaughterhouses. Mac Vet Rev 2013; 36 (2): 97-100.