Hepatitis E virus: the analytical challenge

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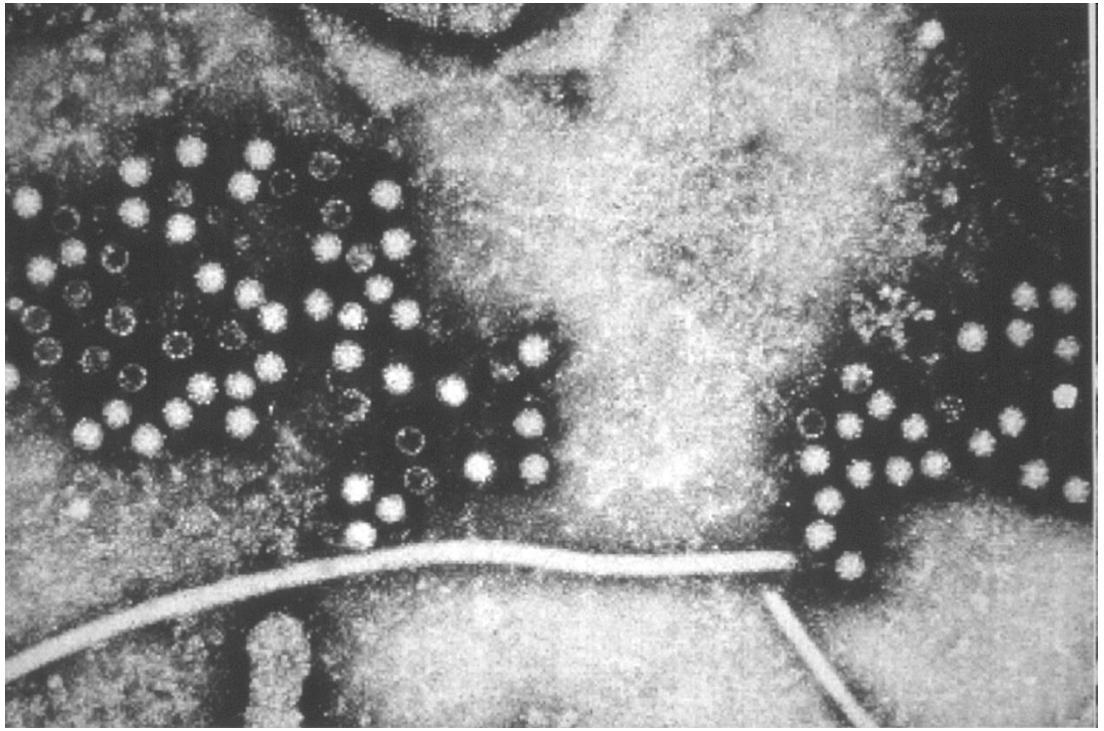
Hepatitis E virus

- Family *Hepeviridae*, genus *Orthohepevirus* (1 other genus: *Piscihepevirus*)



HEV structure

- 35 nm diameter
- Single-stranded RNA genome
- 7 Kb long
- 3 ORFs, encoding non-structural polyprotein, capsid protein and a phosphoprotein

HEV classification - genotypes

Eight genotypes (based on genetic diversity):

- gt I (humans)
- gt II (humans)
- gt III (humans, pigs, deer, rabbits)
- gt IV (humans, pigs)
- gtV (wild boar)
- gtVI (wild boar)
- gtVII (camels, humans)
- gtVIII (camels)

Further divisions as subtypes

Limited diversity at amino acid level – only one serotype

HEV - pathology

- Virus replicates in intestine
- Travels to liver
- Replicates in hepatocyte cells
- Damage to liver cells through immune mechanisms
- Released into bile and bloodstream

HEV – symptoms

- Jaundice
- Fever
- Malaise
- Nausea
- abdominal discomfort
- dark urine

HEV – symptoms

- Onset 4-5 weeks after ingestion
- Duration 1-4 weeks (6 months in some cases).
- No specific treatment. Ribavirin reported useful for chronic infection
- Mortality up to 4 % (25 % in pregnant women) (only known for gt1&2)

HEV – susceptibility

- HEV infection rarely detected in children
- Increasing severity as age increases
- Genotypes 1 and 2 highly endemic in developing regions of the world, with peak incidence among 15-35 year-olds
- More common in men
- In developed countries, males over 55 high risk group

Hepatitis E - epidemiology

- HEV gt I and II cause ~3.4 million cases of acute hepatitis and ~70,000 deaths globally

- In developed countries, HEV gtI and II infections are generally attributed to travel to endemic regions

Autochthonous hepatitis E

Autochthonous: indigenous, native, formed or originating in the place where found

Autochthonous HE is not linked to travel to endemic regions; the infection is acquired in the patient's home country. Several studies have reported autochthonous cases

Most autochthonous cases due to gt3 with a few gt4

Hepatitis E in Europe

- Estimated 3,000 – 9,000 cases per annum in Europe

- Number of reported cases in UK has been increasing

HEV in pigs

- Domestic pigs are a reservoir of gt3
- Seroprevalence in pig herds is high (up to 100 %)
- Weaner pigs become infected around 12 weeks
- Up to 90 % pigs infected by 18 weeks

HEV epidemiology - pigs

- Brazil – anti-HEV antibody in 80 % swine farms
- Japan – anti-HEV antibody in 93 % swine farms
- Netherlands – 55 % swine farms HEV in feces
- New Zealand – anti-HEV antibody in 90 % swine farms
- Poland - anti-HEV antibody in 50 % wild boars
- UK - anti-HEV antibody in 75 % pigs; 25 % fatteners excreting virus

HEV in pigs

- Virus shed 3 – 7 weeks
- Virus replicates in liver
- Viremia normally 1 – 2 weeks
- No symptoms of infection in pigs
- 6 months pigs go to slaughter, can still be infected

HEV in the food supply chain

- Domestic pigs naturally infected at early age
- Can still harbor virus in liver at slaughter
- Some pigs can still be viremic (virus circulating in blood) at slaughter
- No indication of infection – cannot be detected at slaughterhouse
- No official control policies

HEV in pork products – intrinsic contamination

Could as many as 10 pork sausages be carrying a deadly hepatitis E virus? | Mail Online - Mozilla Firefox

Mail Online health

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One in ten sausages may carry the hepatitis virus: Cases of rare deadly strain have rocketed 40% in a year

- Once considered very rare, cases have risen by nearly 40 per cent in a year
- 1 in 50 of those infected will die, rising to one in five pregnant women
- Sausages most dangerous pork product - they contain liver meat

By SOPHIE BORLAND

PUBLISHED: 17:52, 15 September 2013 | **UPDATED:** 22:35, 15 September 2013

1,095 shares 181 comments

As many as one in ten sausages could be infected with a potentially deadly virus that causes liver damage, scientists warn.

They are concerned that rising numbers of Britons are being struck down with hepatitis E after eating contaminated pork.

The infection was once considered very rare but cases have risen by nearly 40 per cent in a year and there were 657 in 2012.

The virus usually causes only relatively mild symptoms such as sickness, a temperature and muscle pain, which clear up by themselves within a month.

But it can be fatal for the elderly, cancer victims, pregnant women and others with existing liver problems.

Around one in 50 of those infected will die, rising to one in five pregnant women.

Experts say sausages have to be cooked at 70C (158F) for at least 20 minutes to kill the virus but they say that most Britons do not leave them in the oven for this long.

Tests have showed that it can survive at 60C (140F) after an hour.

A report published last week by the Department for Environment, Food, and Rural Affairs says 10 per cent of sausages sampled were found to contain the virus.

It states that there is 'increasing evidence' that hepatitis E is a food-borne infection.

Deadly porker: One in ten sausages could be carriers of hepatitis E

HEV infection has been linked to pork product consumption

- Czech republic – tripe sausages
- France –raw figatelli sausages, undercooked pig liver
- Germany – offal, boar meat
- Hungary – home-prepared pork sausage
- The Netherlands – dry raw pork meat sausages
- Spain – pork meat, wild boar meat
- UK – Consumption of pork pies, ham and sausages (from a major supermarket chain) significantly linked to infection

HEV recognised as zoonotic

- European Food Safety Authority (2011)
- UK DEFRA zoonosis report (2013)

HEV zoonosis

- UK blood donor study (Hewitt et al. (2014). The Lancet http://dx.doi.org/10.1016/S0140-6736(14)61034-5)
- 225,000 donations tested
- 79 donations– HEV gt3 genome detected
- Indication that at least 80,000 infections annually in England
- Most infections subclinical, but number of cases rising (124 in 2002, 249 in 2009, 1202 in 2019)

HEV infection linked to pork products

- European Food Safety Authority (EFSA) has concluded that consumption of raw or undercooked pork meat and liver is the most common cause of hepatitis E infection in the EU.

Research currently underway

- to fully identify where links exist between food consumption and hepatitis E
- to quantify the risks posed by HEV in foods
- To develop methods to detect the virus in foods
- To determine the prevalence of HEV in the food supply

HEV detected in pork products

- Canada – pate, liver
- France –foods containing raw pork liver
- Germany - liver, sausages
- UK – sausages
- USA - liver

Testing of retail pork-based products

- Can result in some negative media activity

- Impact on political and economic exportation policies - in 2014, UK / China food exports temporarily halted following a BBC website report*

*White, K. The Grocer. 2017. *https://www.thegrocer.co.uk/food-safety/pig-industry-fears-loss-of-china-access- after-hep-e-reports/553257.article*

Testing of meat and meat products for HEV

- A range of various methods developed in various laboratories
- Majority based on RT-PCR
- Most used the universal HEV assay of Jothikumar et al. (2006)

  J. Virol. Methods 2006, 131, 65–71.

- No standard method

General steps of a method for detection of viruses in food

2 basic parts – sample treatment, and detection assay

Removal of viruses from the foodstuff

Removal of food substances

Nucleic acid extraction

Assay

VITAL* method for HEV detection in pork meat

1 cm^3 pieces chopped,

4 ml lysis buffer added

homogenised by grinding

250 ml homogenate + 1 ml buffer + 2.5 g zirconia beads

mechanical disruption

centrifugation

800 ml supernatant processed by commercial NA extraction kit

300 ml extract

10 ml used for RT-PCR

* EU FP7 project

Meat is a challenging matrix

A variety of sample treatments have been used *

Matrix	Sample Size (g)	Sample Treatment (Prior to NA Extraction)	Reference
liver	5–20 g	Blending in PBS	[41]
liver	150 mg	Homogenisation by scalpel, bead disruption, proteinase K	[32]
liver	0.1 mg	Homogenisation by beating with zirconia beads, lysis reagent, chloroform, centrifugation, gel separation	[56]
dried and liquid blood products	200 mg	Mixing with glycine buffer + beef extract	[53]
figatellu	10 mg	Fat discarded, homogenisation in PBS, centrifugation	[26]
liver, kidney, heart	1 cm^3	As [32] then lysis reagent and chloroform extraction	[36]
liver, sausage, figatellu	3 g	Cell disruption in dH_2O	[47]
liver, meat	10 mg	Bead disruption	[22]
liver sausage	3 g	Stomaching in dH_2O, centrifugation	[44]
liver, pate, raw sausages		Homogenisation (ultrasonication?) in Glycine buffer pH9.5, filtration, centrifugation, PEG precipitation, lysis reagent	[55]
liver	1–10 g	As [32] then ultrafiltration	[39]
salami, boar liver	salami, 5 g; boar liver, 2 g	Stomaching in 7 mL lysis reagent centrifugation, chloroform extraction	[48]
liver	10–20 mg	Homogenisation by mortar and pestle	[27]
liver, chops	liver, 312 mg; chops, 262 mg	Mechanical disruption in lysis buffer, centrifugation	[23]

From: Cook et al. (2022). Microorganisms 10 428.

A standard method is required for HEV detection in meat

- Ensure reproducibility between laboratories
- Compare data from labs using different methods
- More industry confidence in data
- Facilitate regulations

ISO / TC34 / SC9 / WG31 “Hepatis E virus”

- Project began November 2021
- 26 members
- 12 countries
- Belgium (1 member), Canada (1), Finland (2), France (3), Germany (2), Italy (1), Kenya (2), Netherlands (4), Spain (2), Sweden (1), UK (4, including the Convenor), USA (3)
- 3 meetings already held

HEV standard under development

- Scope will focus on meat and meat products, including liver and liver products
- Will be based on RT-PCR.
- Will be focused on detection (not quantification).
- Will not include methods for assessing infectivity

HEV standard

- Draft title: Microbiology of the food chain -determination of hepatitis E virus in meat and meat products, and liver and liver products, using real-time RT-PCR

- Could mirror ISO 15216-2: 2019 “Microbiology of the food chain - Horizontal method for determination of hepatitis A virus and norovirus using real-time RT-PCR - Part 2: Method for detection”

HEV standard – 3 options

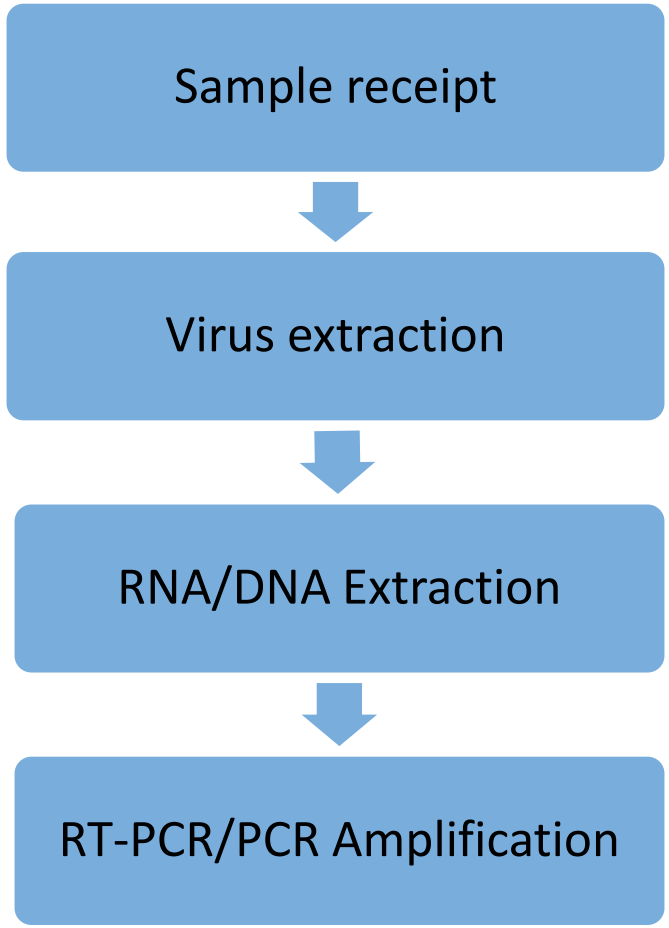
- Adopt already validated method as basis
- Adopt forthcoming method once validated
- Merge existing published and in-house methods

HEV standard - controls

- The chosen method may require additional controls

- The format / composition of key controls will be decided

Flow of controls in detection of foodborne viruses



Sample Process Control

Negative Process Control (if possible)

Negative extraction control

Negative PCR control
Positive PCR control
Internal Amplification Control OR
External Amplification Control

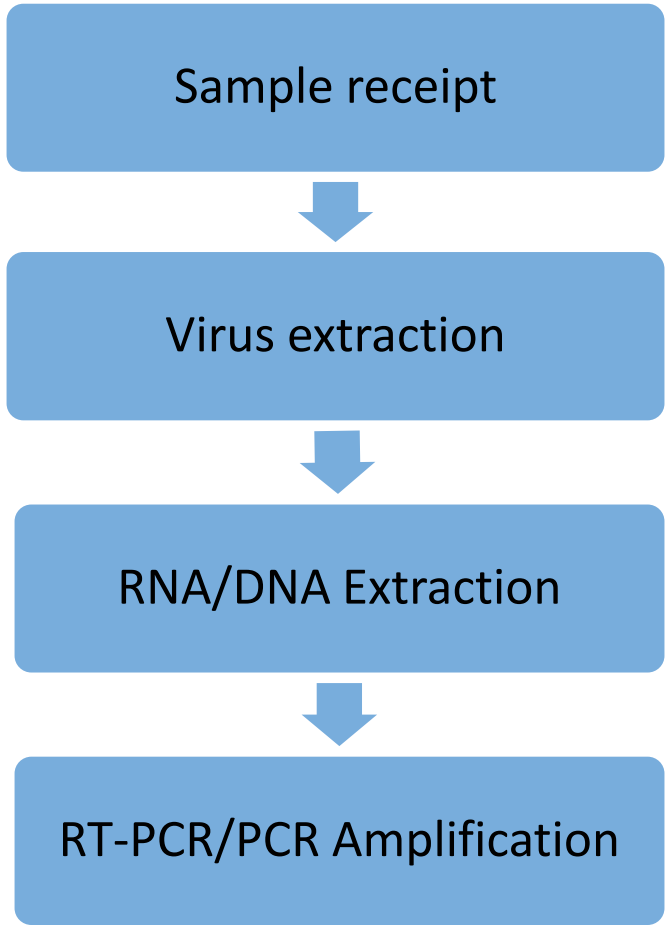
Sample process control virus (SPCV)

- Purpose: To verify that sample treatment has functioned correctly, and evaluate recovery efficiency of target
- Description: It is a non-target virus, added to every test sample at the start of analysis (upon receipt)
- Interpretation: The SPCV must be detected in every sample into which it has been added, and at an agreed recovery efficiency

Which is best SPCV for the HEV standard?

- Mengovirus
- Murine norovirus
- Feline calicivirus
- Porcine teschovirus
- Fish hepevirus

Flow of controls in detection of foodborne viruses



Sample Process Control

Negative Process Control (if possible)

Negative extraction control

Negative PCR control
Positive PCR control
Internal Amplification Control OR
External Amplification Control

Amplification control

- Purpose: To verify reactions which have functioned correctly, and identify those which have been inhibited
- Description: RNA containing target virus sequences
- 2 possible formats – external (EAC) or internal (IAC)

Which is best AC for the HEV standard?

- EAC - used in ISO15216, avoids competition with target, but doubles reactions and may cause false +ves

- IAC – 2 types:

  - ❖ homologous – uses same primers as target, requires optimization

  - ❖ heterologous – uses different primers to target, may not fully control for inhibition

WG31 future steps

- Decision on basis of standard by December 2022
- 1st working draft of standard to be completed by Spring 2024
- Interlaboratory validation, possibly 2025/26 via EURL
- Full standard in 2026/27?

A British standard?

- FSA project FS307033 “Optimising extraction and RT-qPCR-based detection of hepatitis E virus (HEV) from pork meat and products (HEVdetect)”
- Fully controlled RTPCR-based method developed
- Validation complete by March 2023

Thankyou for your attention