Centre for Environment Fisheries & Aquaculture Science

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Review of food-virus combinations of concern or potential concern for food safety in the United Kingdom

Report of the Foodborne viruses National Reference Laboratory

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Contents

Glossary

EU	European Union
FSA	Food Standards Agency
HAV	Hepatitis A virus
HEV	Hepatitis E virus
ISO	International Organisation for Standardisation
NRL	National Reference Laboratory
RT-qPCR	Reverse transcription – quantitative polymerase chain reaction
ssRNA	Single Stranded ribonucleic acid
dsRNA	Double Stranded ribonucleic acid
TBEV	Tick-borne encephalitis virus

1. Summary

At the request of the FSA, the National Reference Laboratory (NRL) for Foodborne Viruses conducted a review to identify and summarise the current emerging food-virus combinations of concern or potential concern for food safety in the United Kingdom.

Scanning of appropriate outbreak literature and other investigations identified 5 separate food-virus combinations (other than those covered by ISO 15216) of significant concern or potential concern for foodborne illness transmission in the UK, and Europe more broadly. These include non-ISO 15216 viruses in bivalve shellfish, fruit and vegetables; Norovirus in composite foods; Hepatitis A virus (HAV) in dates; Hepatitis E virus (HEV) in meat and meat products and Tick-borne encephalitis virus (TBEV) in milk and dairy products.

In each case published methodologies exist, often based closely on the existing standard methods detailed in ISO 15216. However, standardisation of these methods is either non-existent or at a very early stage.

2. Introduction

This review, requested from the National Reference Laboratory (NRL) for Foodborne Viruses by the Food Standards Agency (FSA), aims to identify and summarise the current emerging food-virus combinations of concern or potential concern for food safety in the United Kingdom. Food-virus combinations covered by ISO 15216, the international standard for viruses in foods (Anonymous, 2017), i.e., norovirus or hepatitis A virus (HAV) in bivalve shellfish, fruit and vegetables, or bottled water are not considered in this review. However, we have considered norovirus and HAV in other food matrices, and other viruses in bivalve shellfish, fruit and vegetables or bottled water.

Food-virus combinations of potential concern have been identified from three sources:

- Existing knowledge of NRL staff derived from literature scanning, attendance at conferences or other meetings, and personal communication with other experts;
- European Union summary reports on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2016 and 2017, and European Union (EY) One Health Zoonoses Reports for 2018, 2019 and 2020; these are available at EU One Health Zoonoses Reports (europa.eu);
- Titles and/or abstracts of papers submitted to Food and Environmental Virology (see Food and Environmental Virology | Volumes and issues (springer.com)) and Euro surveillance (Eurosurveillance | Eurosurveillance) as important journals within the foodborne viruses field from 2016 to date.

This process identified the following food-virus combinations as being of particularly frequent appearance in surveillance and scientific reports:

1. Non-ISO 15216 viruses in bivalve shellfish, fruit and vegetables;
2. Norovirus in composite foods;
3. Hepatitis A virus (HAV) in dates;
4. Hepatitis E virus (HEV) in meat and meat products;
5. Tick-borne encephalitis (TBEV) virus in milk and dairy products.

Several other food-virus combinations were reported at very low frequency, with no particular increase in recent years.

Each of the five food-virus combinations listed above was subsequently subject to a more comprehensive literature review, identifying reports of detection, reports of human illness outbreaks and sporadic cases, and available methods. These are summarised in this report.

3. Non-ISO 15216 viruses in bivalve shellfish, fruit and vegetables

An extensive range of human pathogenic viruses have been identified in bivalve molluscan shellfish samples from Europe and elsewhere in the world (summarised in Table 1).

Table 1:Human pathogenic viruses detected in bivalve shellfish

Virus	Family	Genome	Link to infection in consumers?
Astrovirus	*Astroviridae*	ssRNA	YES
Aichivirus	*Picornaviridae*	ssRNA	YES
Enterovirus	*Picornaviridae*	ssRNA	YES
Hepatitis A virus*	*Picornaviridae*	ssRNA	YES
Hepatitis E virus	*Hepeviridae*	ssRNA	YES
Norovirus*	*Caliciviridae*	ssRNA	YES
Sapovirus	*Caliciviridae*	ssRNA	YES
SARS-CoV-2	*Coronaviridae*	ssRNA	NO
Rotavirus	*Reoviridae*	dsRNA	YES
Bocavirus	*Parvoviridae*	ssDNA	NO

Virus	Family	Genome	Link to infection in consumers?
Adenovirus	*Adenoviridae*	dsDNA	NO

*Viruses included in ISO 15216

In addition to norovirus and HAV (included in ISO 15216) a number of these viruses (most frequently non-enveloped ssRNA viruses) have been linked epidemiologically to illness in bivalve consumers, or simultaneously detected in implicated shellfish and the stools of ill consumers, including astrovirus (Iritani et al, 2014; Le Guyader et al, 2008; Nakagawa-Okamoto et al, 2009), aichivirus (Iritani et al, 2014; Le Guyader et al, 2008; Nakagawa-Okamoto et al, 2009; Yamashita et al, 1991, Yamashita et al, 2000), enterovirus (Iritani et al, 2014; Le Guyader et al, 2008), hepatitis E virus (Koizumi et al, 2004; Said et al, 2009), sapovirus (Iizuka et al, 2010; Iritani et al, 2014; Nakagawa-Okamoto et al, 2009; Wang et al, 2015) and rotavirus (Iritani et al, 2014; Le Guyader et al, 2008).

In many of these reports, epidemiological evidence was relatively weak, or the viruses were present as co-infections with other viruses including norovirus, and it is therefore unclear whether infections of the non-ISO 15216 viruses resulted in illness symptoms, however the potential for these viruses to cause illness in shellfish consumers remains.

While detection of adenovirus (Formiga-Cruz et al, 2002; Fusco et al, 2019; Rodriguez-Manzano et al, 2014), bocavirus (Kumthip et al, 2021; La Rosa et al, 2018; Onosi et al, 2020) and more recently SARS-CoV-2 (Mancusi et al, 2022; Polo et al, 2021) in bivalve shellfish has been reported, to date no evidence of human infections with these viruses resulting from consumption of contaminated shellfish has been reported.

In fruit and vegetables, reports of detection of non-ISO 15216 viruses are less frequent than for bivalve shellfish, however some reports of detection of human pathogenic viruses including astrovirus (Prez et al, 2018), enterovirus (Prez et al, 2018; Purpari et al, 2019; Sergevnin et al, 2016), hepatitis E virus (Kokkinos et al, 2012; Loisy-Hamon and Leturnier, 2015; Maunula et al, 2013; Purpari et al, 2019; Terio et al, 2017), rotavirus (Parada-Fabián et al, 2016; Prez et al, 2018; Quiroz-Santiago et al, 2014; van Zyl et al, 2006) and adenovirus (Cheong et al, 2009; Gholipour et al, 2022; Kokkinos et al, 2012; Maunula et al, 2013) are available in the literature. To date, to our knowledge there have been no reports of illness or infection in consumers due to consumption of fruit and vegetables contaminated with these viruses.

Standardised methods for detection or quantification of these viruses in bivalve shellfish, or fruit and vegetables, do not currently exist. TaqMan reverse transcription – quantitative polymerase chain reaction (RT-qPCR) primer and probe combinations are available for aichivirus (Kitajima et al, 2013), astrovirus (Le Cann et al, 2004), enterovirus (Donia et al, 2005), hepatitis E virus (Garson et al, 2012; Jothikumar et al, 2006), sapovirus (Varela et

al, 2016) and SARS-CoV-2 (Centers for Disease Control and Prevention, 2020). In these cases it should in theory be possible to integrate such target virus-specific primers and probes into the ISO 15216 methods. For rotavirus, modifications to the RT-qPCR approach to allow denaturation of the dsRNA genome prior to reverse transcription are necessary (Freeman et al, 2008). For adenovirus (Formiga-Cruz et al, 2005) and bocavirus (La Rosa et al, 2016) the most commonly used molecular methods are conventional (gel-based) polymerase chain reaction (PCR) based approaches. However, these methods should be compatible with nucleic acid extracts generated using the ISO 15216 virus and nucleic acid extraction protocols.

4. Norovirus in composite foods

Composite or multicomponent foods are those that comprise a wide range of ingredients, normally including ingredients of both animal and non-animal origin, and often including processed ingredients.

Such foods are frequently linked to outbreaks of norovirus; these can originate from contamination of individual ingredients e.g., fruit and vegetables at the production stage, but often result from contamination of ingredients or finished meals by infected food handlers (de Wit et al, 2007; Franck et al, 2015, Zomer et al, 2010).

Because of the wide range of ingredients and processing methods used for such foods, the methods used for their analysis must be fairly generic and robust to the presence of difficult-to-extract components, RT-PCR inhibitors etc. A number of publications detail methods that have been successfully trialled with a wide range of different foods, including composite foods (Hennechart-Collette et al, 2021; Saito et al, 2015; Summa et al, 2010). The method described in Hennechart-Collette et al (2021) is based closely on the ISO 15216 method for soft fruit with some adaptations. In all cases the described methods for virus extraction can be used in concert with the RNA extraction and RT-qPCR methods described in ISO 15216.

While such methods can be applied to a wide range of foods with a reasonable degree of confidence in successful detection of any contaminating viruses, the extremely diverse nature of composite foods means that it should be anticipated that certain norovirus vehicles in food handler outbreaks will be very difficult to test using existing methods.

5. Hepatitis A virus in dates

Since 2018 a small number of outbreaks of HAV related to the consumption of dates have been reported in the UK and other European countries.

Between December 2017 and February 2018, 27 people in Denmark and 1 in Norway contracted HAV without having travelled to an endemic area. A case control study and traceback investigations identified dates from Iran as the probable source of infection (No 11 - 2018 (ssi.dk)). A positive result for HAV was obtained for 1 out of 10 boxes of dates tested, with sequencing providing a 100% match with the strain detected in the patients (Rajiuddin et al, 2020b).

A separate sequence-linked outbreak of HAV affected a total of 57 people from 6 European countries (39 from Germany with smaller numbers from France, the UK, the Netherlands and Sweden) between April and August 2018 (Germany bears brunt of Hepatitis A outbreak linked to dates | Food Safety News). Case control studies and interviews with cases identified consumption of contaminated dates from Morocco as the likely source of infection. The majority of ill people had travelled to Morocco during the incubation period, however 9 people from Germany and 2 from France had acquired the infection domestically.

Dates from Iran were again implicated in an outbreak of domestically acquired HAV affecting 9 people in Sweden from February to April 2019 (Dates from Iran linked to Hepatitis A outbreak for second time in 2 years | Food Safety News).

In this case although epidemiological evidence was strongly suggestive of dates as the source of infection, no positive RT-qPCR results were obtained in date samples.

Finally, an outbreak of HAV in the United Kingdom between January and April 2021 affected 31 people who had not reported travel to an endemic area. Epidemiological investigations identified consumption of dates from Jordan as the likely source of infection, and positive RT-qPCR results for HAV were obtained from two date samples (Garcia Vilaplana et al, 2021).

Although dates may be informally considered a soft fruit, the definition of soft fruit in ISO 15216: -

“Small edible stoneless fruit EXAMPLE Strawberries, raspberries or currants”

does not apply to dates, and the method for virus extraction in ISO 15216 was not designed or validated for date testing. Due to their high sugar content, dates provide some challenges for testing that ordinary soft fruits do not. Nevertheless, the ISO 15216 method for soft fruits was applied with some modifications to date samples involved in the UK

outbreak where positive results were obtained in a small number of samples (Garcia Vilaplana et al, personal communication).

Investigation of the Danish outbreak samples used an optimised direct lysis method previously developed for application to fruit and vegetables (Rajiuddin et al, 2020a). This method, which uses essentially identical RNA extraction and RT-qPCR methodology to ISO 15216, is considerably simpler and quicker than the full ISO method including the validated virus extraction protocol for soft fruit. The authors of the Danish outbreak sample testing report (Rajiuddin et al, 2020b) considered that the direct lysis method was more suitable for date samples. This finding was in agreement with a previous paper (Boxman et al, 2012) where the authors used a direct lysis method to carry out a survey of date and fig samples following a case of HAV in a person who frequently consumed dates. HAV RNA was detected in 1 out of 169 survey samples.

6. Hepatitis E virus in meat and meat products

Hepatitis E virus, being both an enteric virus that can be transmitted by the faecal oral pathway, and a zoonotic agent associated with various domestic and wild-hunted animals, is a potential risk for a wide variety of foodstuffs and different transmission pathways (summarised in Treagus et al, 2021). However, at present the most significant risk of foodborne HEV transmission is associated with consumption of meat, offal (particularly liver) and products containing meat or offal (sausages etc.).

Meat and products derived from domestic pigs and wild boar are most frequently linked to outbreaks and sporadic cases of HEV in Europe (Garbuglia et al, 2015; Guillois et al, 2016; Renou et al, 2014; Rivero-Juarez et al, 2017), however other species have been implicated in transmission through meat consumption elsewhere e.g., sika deer in Japan (Tei et al, 2003).

A high diversity of methods for extraction and detection of HEV from meat and meat products have been published – these are summarised by Cook et al (2022). Amongst the more notable publications, Szabo et al (2015) developed and optimised a method for detection of HEV in raw sausage and liver sausage that was applied to a retail survey in Germany. This method was subsequently subject to a multi-laboratory validation in 9 independent laboratories (Althof et al, 2019) making it one of the best characterised methods available in the literature currently. Elsewhere, Hennechart-Collette et al (2019) carried out a parallel evaluation of six different methods for detection of HEV in sausage, figatellu (Corsican pork liver sausage frequently implicated in HEV transmission) and pig liver, while Boxman et al (2019) reported a large survey of meat and meat products (including livers, liverwurst, liver pate, pork chops, fresh sausages and wild boar meat) in

the Netherlands using a relatively simple virus extraction method utilising mechanical homogenisation of the initial sample.

The significance of HEV as a food-borne pathogen in Europe (EFSA BIOHAZ Panel et al, 2017) and elsewhere, combined with the diversity of methods available for its detection, has resulted in initial steps towards method standardisation. An International Organization for Standardization working group, ISO/TC34/SC9/WG31, aiming to develop a standard method for detection of HEV in foods has recently been convened, with the first meeting of the group in January 2022, however, it should be anticipated that a standard method will not be available for some time.

7. Tick-borne encephalitis virus in milk and dairy products

Tick-borne encephalitis virus (TBEV) is an enveloped single-stranded RNA virus of the genus *Flavivirus*. Infections in humans can cause serious neurological symptoms (meningitis, meningoencephalitis, myelitis, paralysis, radiculitis) with long-term sequelae relatively common. The mortality rate of the European subtype of TBEV is estimated at 0.5-2%. This infection is of increasing concern in Europe, where there has been a large increase in cases over recent decades (Factsheet about tick-borne encephalitis (TBE) (europa.eu)).

In the large majority of cases, transmission of the virus to humans is via the bite of an infected tick of the genus *Ixodes*, however infected domestic animals such as goats, sheep and cows can excrete TBEV particles in their milk, and an increasing number of food-borne infections resulting from consumption of raw, unpasteurized dairy products has been noted across Europe. Traditionally TBEV has been considered endemic only in Central and Eastern countries within Europe and the majority of foodborne outbreaks and sporadic foodborne cases have been recorded in these countries also, including; Austria (Holzmann et al, 2009), Croatia (ilic et al, 2020; Markovinović et al, 2016), Estonia (Kerbo et al, 2005), the Czech Republic (Kríz et al, 2009), Hungary (Balogh et al, 2010; Caini et al, 2012), Poland (Król et al, 2019), Slovakia (Gresíková et al, 1975; Dorko et al, 2018) and Slovenia (Hudopisk et al, 2013). However, in recent years, some outbreaks have been reported from areas in Western Europe that were previously considered non-endemic, including France (Gonzalez et al, 2022) and Germany (Brockmann et al, 2018; Chitimia-Dobler et al, 2021). Infection with TBEV has also been identified in dairy herds in Norway (Paulsen et al, 2019) and Sweden (Blomqvist et al, 2021).

To date, to our knowledge no foodborne transmission or detection of TBEV in dairy animals has been reported in the United Kingdom, and the risk of tick-borne transmission

is considered very low for the general population and low for high risk groups (Qualitative assessment of the risk of TBE (publishing.service.gov.uk). However, TBEV has been detected in *Ixodes* ticks associated with deer herds in a small number of areas of England (Holding et al, 2019; Holding et al, 2020) and a single tick-borne case of TBEV infection in southern England has been reported (Kreusch et al, 2019). Given the general increase in both tick-borne and foodborne transmission of TBEV across Europe, there is a possibility of foodborne transmission in the UK due to consumption of either imported or domestically produced unpasteurised dairy products in the future.

Foodborne outbreak investigations have only rarely included testing of implicated dairy products, normally relying on epidemiological data to confirm linkage of illnesses to the implicated foods. Development and single-laboratory validation of a method for detection of TBEV in raw milk, based closely on ISO 15216, has been reported (Hennechart-Collette et al, 2022).

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