

Hepatitis E

Hepatitis E Virus (HEV) is an important but extremely under-studied pathogen. It is a small, non-enveloped RNA virus (single-stranded positive-sense RNA) that is transmitted primarily via the faecal-oral route. HEV infection has a long incubation period in humans (up to 10 weeks) and symptoms that include jaundice, anorexia, hepatomegaly (enlarged liver), and mortality in pregnant women.

In recent years there has been a rise in indigenous (non travel-related) HEV infections in the UK. Indigenous infection can be transmitted in three ways: consumption of contaminated food or water, via person-to-person, or by direct contact with infected animals. Evidence indicates that HEV is widespread in the domestic pig population and that the virus has been detected in pork products. A study by DEFRA in 2012 reported that 10% of pork sausages tested at the point of sale from UK retailers were found to contain detectable HEV.

It is believed that one of the routes of HEV transmission in pig herds is through infected pigs shedding HEV in their faeces, which can then contaminate communal feed and water supplies. The handling of infectious meat post-slaughter in butcheries and using meat from multiple animals in processed pork products have also been a suggested route of contamination. There is currently no available vaccination for the virus, although studies have begun to investigate possible vaccines. These could be used as an additional control against HEV infection and contamination of pork products.

One of the main controls used to eliminate any pathogen from raw foods product, is cooking. Some viruses appear to be relatively heat stable and difficult to inactivate in this way. The effect of heat and cooking on HEV in foods has been investigated during domestic cooking and manufacturing heat processes. Studies have found that heating pork liver pate to an internal temperature of 71 °C for 20 minutes is necessary to completely inactivate HEV, however further studies are required to confirm that these times and temperatures are effective for all foods. Products made with pork liver, such as liver sausages have several components, including up to 30% fat. It is possible the composition of the food product may affect HEV resistance to thermal treatment and so more research is needed.

Limited information is available on effective cleaning and decontamination procedures for HEV in the food factory environment. A safety sheet published online by the Public Health Agency of Canada (2011) states that HEV is sensitive to 1% sodium hypochlorite, 5% formalin in water, and glutaraldehyde. It is also thought that HEV is also susceptible to iodinated disinfectants (1% iodine). The inactivation of HEV through chlorine in water has been investigated by Girones et al., (2014). This study suggests the use of chlorine disinfection as an effective strategy to control HEV waterborne transmission. Further studies are required in this area, however, as the effectiveness of these chemicals on decontamination in the food manufacturing industry and their ability help reduce the prevalence of HEV infection in pigs is uncertain.

Ergot mycotoxins

The European Food Safety Authority has proposed that maximum levels will be set for ergot alkaloids in unprocessed grain in 2017. Ergot is the name given to the sclerotia of the fungus *Claviceps purpurea* which is widespread and infects many cereals, including wheat, rye, barley and oats and the related *Claviceps fusiformis*, which infects millet. Ergots are hard, dark tuber-like bodies which are visible to the naked eye and produce mycotoxins. The sclerotia are harvested together with the cereals and can lead to contamination of cereal-based food and feed products with ergot alkaloids.

Ergots produce mycotoxins composed of up to 40 related alkaloids, collectively called ergolines. The toxicity of ergot alkaloids has been recently reviewed by EFSA. They have moderate acute oral toxicity and can also cause issues via long-term dietary exposure. Ergot alkaloids are neurotoxic and act on several neurotransmitter receptors. Ergots are larger, darker and less dense than grain. The industry has almost a zero tolerance for presence of ergot sclerotia in grains. Any sclerotia visible on inspection will lead to the entire consignment of grain being rejected. Although grains can be sorted by colour or density to remove sclerotia which results in a considerable reduction in the levels of ergot alkaloids in the grain, the toxins can still remain on the grain.

Ergot can infect a range of commercially important cereals in Europe and North America. Livestock which consume grain contaminated with ergot can also be affected. While human epidemics are now very rare, incidents due to contaminated animal feed do still occur because the sclerotia can concentrate in the by-products of cereal milling.

In June 2012, following a request from the European Commission, EFSA's Panel on Contaminants in the Food Chain (CONTAM Panel) delivered a scientific opinion on the risks to human and animal health related to the presence of ergot alkaloids in food and feed. EFSA have derived a group acute reference dose (ARfD) of $1\mu\text{g}/\text{kg}$ body weight and a group tolerable daily intake (TDI) of $0.6\mu\text{g}/\text{kg}$ per body weight per day. EFSA has estimated the chronic dietary exposure in the adult population in the EU to range between 0.007 and $0.08\mu\text{g}/\text{kg}$ body weight per day for average consumers and 0.014 and $0.19\mu\text{g}/\text{kg}$ body weight per day for high consumers.

Campden BRI has developed a sensitive mass spectrometry-based method to detect the six major ergot alkaloids as defined by EFSA (ergometrine, ergotamine, ergosine, ergocristine, ergocryptine and ergocornine) and their corresponding epimers at levels as low as $1\mu\text{g}/\text{kg}$. The validated method has been accredited by UKAS to ISO 17025. Campden BRI is one of the few companies currently providing this testing service in the UK.

Julian South, Head of Chemistry and Biochemistry at Campden BRI comments: "We have been carrying out ergot testing for several years. EFSA has proposed that in 2017 maximum levels will be set for alkaloids in unprocessed grain. Our method will help manufacturers working with cereals and grains to maintain the quality and safety of their products."

Novel DNA methods

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New developments in DNA methods means we can expect more user-friendly, smaller, quicker and cheaper detection devices to be the norm for authenticity and adulteration testing within the next five years. Currently, DNA approaches are used to determine animal species, breeds and genotype, as well as plant species and varieties. They involve DNA amplification by PCR, detection by gel-electrophoresis or by real-time PCR, with the latter technique offering the ability to quantify the amount of target DNA in a sample. Standard Sanger DNA sequencing is also used for species identification, but has limitations when applied to complex food ingredients containing a range of species. In the last few years, alternative DNA amplification and next generation sequencing approaches have been developed which offer potential benefits, not only for use by food analysts but also for on-site testing by food companies.

Isothermal amplification

Non PCR-based target amplification techniques, such as LAMP (Loop Mediated Isothermal Amplification) and RPA (Recombinase Polymerase Amplification) have recently been used in a number of applications. They use a different enzyme system which makes them fast, very sensitive and they do not require purified DNA so they can work on relatively crude preparations from samples and swabs. The total time from sampling to result generation can be less than 30 minutes.

Though they are still in their infancy, these types of assay offer the prospects for industry based screening of raw materials, if combined with simple detection systems. Also, as their endpoint is based on fluorescence detection, isothermal amplification assays can be used on standard real-time PCR instrumentation, which allows high throughput. Dedicated kits and instruments have been developed by a few companies.

Next Generation Sequencing (NGS)

There has been a rise in interest in non-targeted detection of species, using next generation DNA sequencing approaches. This is particularly relevant for highly processed meat, fish or herb products, as this approach enables screening for any species DNA that may be present. Standard NGS Instrumentation routinely found in genomics research laboratories are now being used by food analysts. However a new approach, based on nanopore sequencing, lends itself for use in even the most basic laboratories. It uses a technique in which single strands of DNA move rapidly through a synthetic nanopore on a chip and generates sequence data in real-time. The sequencing is performed on a sensor instrument that is smaller than a mobile phone, connected to a laptop which uses cloud-based software to enable DNA sequence determination and species identification. Use of simple DNA extraction and preparation techniques could enable delivery of a result within a few hours.

At Campden BRI, we will continue to assess the use of these technologies via our ongoing research project looking at next generation techniques for microbiological and chemical food safety.

Toxigenic E. coli

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The E. coli species consists of a diverse range of strains. While E. coli do originate in the animal gut, they are widely distributed within nature and will be found in the soil and other environmental sources so their presence in a food is not necessarily indicative of faecal contamination. The presence of low levels of general E. coli in many fresh foods does occur and is not usually harmful; indeed their presence at low levels in some foods is recognised and allowed within European legislation. We know that there are particular strains of E. coli that are highly pathogenic. The first strains of this type were isolated from hospitalised patients in the early 1990s and were identified as E. coli O157:H7. This serogroup of E. coli was originally linked to minced beef products, particularly improperly cooked burgers. A number of large outbreaks occurred and many of those affected had to be hospitalised and a number died.

This led to much research on the organism and a number of recommendations of how to cook minced beef products which, unlike whole muscle cuts of beef, have large numbers of bacteria distributed throughout the whole product, and need a more thorough cook to kill these deeply embedded bacteria. As research continued, outbreaks of O157:H7 were recognised from non-meat products including unprocessed apple juice (in the USA referred to as apple cider), raw or improperly pasteurised milk (or pasteurised milk that had become cross contaminated with raw milk due to poor dairy hygiene), fresh produce (leafy greens, spinach etc) and sprouted seeds (bean sprouts). The link in all of these was that at some point the product had become contaminated from the environment, and was then consumed without thorough cooking. Worse was to come, as similar severe outbreaks of illness were recognised that were caused by E. coli that did not belong to the O157 serogroup. The common factor was that all of the E. coli concerned contained the genetic information to produce one of two toxins and were named accordingly Verocytotoxin producing E. coli (VTEC) or Shigatoxin producing E. coli (STEC). In both the USA and Europe, legislation was produced that required testing and the absence of these organisms in certain types of food. Serogroups O26, O111, O121, O103, O145, O45 and O104 were all considered potent food hazards. The range of foods concerned has widened over time, and we have a situation at present where a very large amount of unprocessed flour is being recalled in the USA due to its association with an outbreak of E. coli O121.

Testing for this group of E. coli requires specialist skills and facilities. In Europe toxigenic E. coli are classed as Hazard Group 3 pathogens and can only be handled under enhanced containment, which few laboratories have. Testing also requires knowledge in the use and interpretation of polymerase chain reaction methods. We have to understand that they cause a potent illness and have a low infective dose. We must take care to cook foods properly and prevent any cross contamination between cooked and raw products. Fresh produce has to be produced with a view to preventing contamination with this group, by controlling and managing growth, irrigation, harvesting and transport conditions.

Six steps to success

Richard Leathers and Louise Harris,
Quality Management Systems Specialists, Campden BRI

Audits are crucial to maintaining food safety standards and play a fundamental role in certifying that proper food safety practices are being adhered to. Auditing continues to be a headache for many clients, but following some simple steps can make the process easier.

1. Fail to prepare – prepare to fail

Preparation is key to a successful audit. First ensure you know what is going to be covered in the scope and criteria of the audit. Make sure you understand the clauses of standards where issues are being raised and what the auditors are expecting to see as conformance for a particular clause. This will put you in a stronger position. Be prepared to challenge your auditor as much as they challenge you. Check that hazard and risk assessments have been conducted and that all the relevant food safety and quality procedures are documented. It is vital that you establish and conduct internal 'mock' audits. The internal audits should follow an annual schedule which takes risk and previous audit findings into account. You also need to demonstrate that your customer requirements are documented, understood and reviewed.

2. Prerequisites

The prerequisite programmes in your food business operations are important. Without prerequisites, the rest of your food safety management system is not supported. Cleaning and control of chemicals, as you will see later, come out in the top five non-conformances against many audits carried out annually.

3. A team effort

A competent team is required to manage an audit. The team should be trained and able to challenge company procedures and systems to ensure you comply with the standards you are aiming for. Unannounced audits are becoming more common, so it is advisable to be 'audit ready' 365 days of the year. Strong management reviews, effective HACCP reviews and internal 'mock' audits can help with this. Senior management commitment is becoming more important and you will need to provide evidence that members of the senior management team are actively participating in audit meetings and driving food safety within your businesses.

4. Keep up to date

Make sure you are aware of any changes in standards and legislation, for example ISO 22000 Food Safety Management System is under review and the new version is expected soon.

5. Learn from others' mistakes

Auditing has been carried out against BRC Version 7 since 1st July 2015. The top five most frequent non-conformities found up until the end of 2015 were supplier approval, raw material risk assessment, supplier risk assessments, vulnerability assessments, and cleaning and control of chemicals.

6. Continuous improvement

The standard should become ingrained in your organisational culture to ensure continuous improvement is driven internally. Arrange your next audit prior to certificate expiration.

Installing new technology

Emma De-Alwis, Hygiene Specialist, Campden BRI

In order to produce high quality and safe food products, suitable hygienic design, maintenance and use of food production equipment is required. The design of equipment should be based on a good balance of operational requirements (personnel and process safety) and hygienic requirements (food safety). There are many key hygiene questions to take into consideration when installing new equipment, for example:

- **What are the risks or hazards?**

The risks to the consumer may be biological, physical, chemical or allergenic. The severity depends on the product, the shelf-life, consumer and many other aspects. The equipment must not bring about unacceptable change in the food composition, deteriorate organoleptic characteristics or present danger to human health.

- **Where is the equipment located?**

Equipment must be installed in a way that allows adequate cleaning and maintenance of the equipment and the surrounding area. EHEDG (European Hygienic Engineering Design Group) suggests that a distance away from the floor, wall, ceiling and other structures, is dependent on the equipment width. For items smaller than 90cm wide a 20cm clearance is advisable, whereas for items above 210cm a clearance above 60cm is desirable.

- **How is it designed, maintained and cleaned?**

Directive 2006/42/EC states that machinery intended for use with foodstuffs must be designed and constructed in such a way as to avoid any risk of infection, sickness or contagion. The materials used must be suitable for the application to which they are intended and must be cleanable before each use. Smooth surface finishes and limited angles, corners or crevices help prevent microbial or insect niches and areas for organic matter to gather, while also making the task of cleaning easier. Sufficient drainability is important, as stagnant water or cleaning chemicals within a piece of equipment present a risk to the product. It is also important that no ancillary substances (e.g. lubricants) can come into contact with food. When a food manufacturer purchases a piece of equipment they may not anticipate the damaging effect the harsh environments within its factory may have (through humidity, fluctuating temperature and cleaning chemicals). Planned preventative maintenance is important to establish when installing a new piece of equipment in order for that machine to last and continually produce safe food. It is vital before commissioning a piece of equipment, to consider the time it will take to clean and the suitability of the cleaning method. The more nooks, crannies and difficult to reach areas; the longer cleaning times: thus longer down time. Validation of cleaning methods is an important step when designing cleaning schedules.

- **Who can I ask for advice?**

Standards such as EN 1672-2:2009 and EN ISO 14159:2008 provide information in relation to hygienic design of machinery and support the objectives of the directive. Campden BRI provides wide-ranging advice and guidance on food and drink production, including hygiene. EHEDG also provides helpful guidance and practical advice in implementing legislation into design practices and manufacturing processes.