Milk and Milk Products

Pathogenic *E. coli*, an emerging foodborne pathogen

Shiga toxin producing *E. coli* (STEC) are foodborne pathogens that may cause serious illness in humans. Among the food related zoonoses, they are the fourth most occurring group in Belgium, but as far as human symptoms are concerned, they are one of the most dreaded organisms. Bovine animals, which are asymptomatic carriers, are the most important reservoir. Infection of humans is mostly caused by the consumption of infected foodstuffs derived from bovine animals, such as milk and milk products.

A wide range of serogroups is capable of provoking these human infections, the most important of them being O26, O103, O111, O145 and O157. The STEC bacteria owes its infective capacity to a combination of virulence properties, the most important of which are: the production of type I and II shiga toxins, which are responsible for kidney failure, the proteins encoded on the LEE locus, responsible for the modification and the intimate adherence to the gastro-intestinal cells, and enterohemolysin, which plays a role in the destruction of blood. The combination of these manifestations results in a complex pathology known as the hemolytic uremic syndrome (HUS). In Belgium, 50 human STEC infections per year are reported on average, 50 percent of which are caused by serogroup O157 and some 20 patients develop HUS (EFSA, 2009).

Generally, foodstuffs derived from bovine animals are at the origin of human infections, as well as raw vegetables and drinking water. Fecal contamination of carcasses, irrigation water or during the milking process may allow the entry of STEC bacteria into the food chain. Within the context of the monitoring program of foodstuffs on the Belgian market in 2009, no *E. coli* O157 was found in samples of cheese, butter and cream, except for one raw goat milk cheese sample that was found positive (Anonymous, 2010). On the other hand, a study of De Reu et al. (2004) and the non published results of De Reu et al. (2009), mention the occurrence of *E. coli* O157 in 0.7 % and 0.8 % of the Belgian raw farm milk samples, respectively. For foodstuffs, the prevalence rates are generally between 0 and 2%, as is shown in the reports of the European Union 2005-2007 (EFSA, 2009). But results may vary strongly, depending on the method used. If molecular detection methods are used that are based upon the detection of genetic material of the pathogen, very high prevalence rates of 5 to 30 % are found in raw milk and cheese (Jordan et al., 2010; Fach et al., 2001; Pradel et al., 2000; Vernozy et al., 2005). However, the detection of STEC DNA in a sample does not automatically reveal the presence of a viable, virulent organism. Nor has it been proved that the detected virulence genes are located within one single organism. This also goes for the isolates, which often do not contain a combination of *stx* and LEE genes and are in that case judged as being of relatively low virulence. These findings might explain the lower occurrence of human STEC infections. That one should be careful when consuming (raw milk) dairy products was shown in October 2007 when five children suffered serious renal damage after eating farm produced ice cream that was infected with STEC. In that particular case serogroups O26 and O145 were isolated, and not serogroup O157.

The non-sorbitol-fermenting (NSF) O157 strains have been studied most frequently. In fact, an internationally standardized isolation method for food and feed (ISO 16654) is available for that group and is considered as the gold standard. The method is based upon phenotypic characteristics of the organism, such as increased resistance and enzymatic properties. The NSF O157 strain is isolated in approximately 4 days using conventional culture. First, the sample is selectively enriched by adding antibiotics (novobiocin) for 6 hours at a relatively high temperature (41.5°C). Then, immunomagnetic separation (IMS) is used on the enriched sample. During that process, *E. coli* cells
belonging to serogroup O157 are captured by magnetic beads by means of antibodies (see figure 1). Then the beads are put on two selective agar media, among which CT-SMAC, which contains both selective (bile salts, crystal violet, cefixime and tellurite) and elective (sorbitol and BCI glucuronide) components in order to isolate and to recognize \textit{E. coli} O157. Confirmation of suspected colonies is obtained by means of the indole and agglutination tests. If no \textit{E. coli} O157 isolate is obtained, the isolation procedure should be repeated on the 24 hour enriched sample. As an alternative for these labour-intensive methods, the FASFC (Belgian Food Agency) has approved the use of certain other methods, i.e. Rapid'E. coli O157:H7, VIDAS ECO, VIDAS UP \textit{E. coli} O157 including H7, IQ-Check \textit{E. coli} O157:H7, GeneDisc \textit{E. coli} O157:H7, HQS \textit{E. coli} O157:H7, BAX \textit{E. coli} O157:H7 MP, BAX Real-Time PCR Assay \textit{E. coli} O157:H7 and MICROSEQ \textit{E. coli} O157:H7. The conventional isolation method Rapid'E. coli O157:H7 gives faster results and requires a smaller amount of agar medium. The VIDAS systems are based upon immunological detection of the pathogens, the IQ Check, GeneDisc, HQS, MICROSEQ and BAX systems are based upon DNA detection of the pathogens by means of PCR and only require an isolation procedure in the event of positive results. In short, the conventional ISO culture method is laborious and validated alternatives are available. But each method has some specific advantages and disadvantages.

\textbf{Figure 1:} Dynal sample mixer to provoke a binding between STEC bacteria from the enriched food sample and immunomagnetic beads that are coated with specific antibodies.
At present, there is no international standard method available for the detection and isolation of STEC strains that do not belong to serogroup O157. This is due to the lack of uniform phenotypic properties. Researchers use different enrichment media to which they add selective components as well as IMS for the most important serogroups (O26, O103, O111 and O145). PCR based methods have been described for the detection of the main virulent serogroup genes in samples. Different agar media have been described, including rhamnose-MacConkey Agar, enterohemolysin agar or the medium developed by the ILVO (Institute for Agricultural and Fisheries Research) and the Ghent University, described by Possé et al (2008) for STEC O26, O103, O111 and O145 (see figure 2). Furthermore a considerable number of alternative methods are used, such as colony hybridization or serological isolation techniques. We may, again, point out that each method has its advantages and disadvantages, but no standardized method has yet been developed for the other STEC serogroups, as opposed to E. coli O157.

Figure 2: Isolation medium for STEC O26, O103, O111, O145 and SF O157, as described by Possé et al. (2008).
References:


Karen Verstraete en Koen De Reu (ILVO T&V, Melle)
Karen.Verstraete@ilvo.vlaanderen.be en Koen.Dereu@ilvo.vlaanderen.be

Developments in the field of standards and legislation


Standards:

New:

- ISO 26462|IDF 214:2010 - Milk - Determination of lactose content – Enzymatic method using difference in pH
- ISO 29981|IDF 220:2010 - Milk products - Enumeration of presumptive bifidobacteria - Colony count technique at 37 degrees C
- ISO 2962|IDF 033:2010 - Cheese and processed cheese products - Determination of total phosphorus content - Molecular absorption spectrometric method

Withdrawn in 2010:

- IDF 027:1964 - Determination of the ash content of processed cheese products.
- IDF 098A:1985 - Milk - Determination of protein content Amido Black dye-binding method (routine method)
- IDF 112A:1989 - Butter - Determination of water dispersion value
Other interesting IDF publications:

• Bulletin of the IDF No. 447/2010 - New Applications of Mid Infra-Red Spectrometry for the Analysis of Milk and Milk Products
• Bulletin of the IDF No. 446/2010 - The World Dairy Situation 2010
• Bulletin of the IDF No. 445/2010 - A common carbon footprint approach for dairy - The IDF guide to standard lifecycle assessment methodology for the dairy sector

Koen De Reu (ILVO-T&V)  Koen.Dereu@ilvo.vlaanderen.be
Jessy Claeys (ILVO-T&V)  Jessy.Claeys@ilvo.vlaanderen.be