

# Isolation *Escherichia coli* O157:H7 from dairy raw milk samples\*)

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## Summary

Microbiological screening tests for the presence of *E. coli* O157:H7 were carried out on 196 samples of raw cow's milk from different dairies in the East-Slovak region in the period of May-July 2006. Of the total number of examined samples, *E. coli* strains were isolated from two milk samples (1.02%) from dairies of the Trebišov district. In spite their relatively low incidence, the health consequences of the presence of this pathogen are so important that regular screening of raw milk seems to be necessary to determine the source and way of contamination as well as to make proposal for effective eliminative and precautionary measures.

**Keywords:** raw cow milk, *Escherichia coli* O157:H7, immunomagnetic separation (IMS), verotoxins

In the recent years a number of serious diseases associated with foods contaminated with *Escherichia coli* O157:H7 bacteria have been recorded in humans. Research on this type of bacteria has shown that a minimum of 10 to 100 of them is needed to cause a serious infection (5, 17, 27, 29, 41). Children infected with this pathogen show complications associated with a serious haemorrhagic-uraemia syndrome, which has lethal consequences in many cases (2). Most of the epidemics described to date have been directly related to consumption of foods of bovine origin, such as raw cow's milk and meat products which were subjected to insufficient thermal treatment (3, 32, 37, 38).

A range of studies conducted in previous years has shown that 0.0-2.2% of animal milk and meat (cattle herd) have been positive for *Escherichia coli* O157:H7 (9, 30, 39). The *Escherichia coli* O157:H7 serotype can be found in the intestinal tract of farm animals free of any health problems. The seasonal variations recorded in the occurrence of this pathogen have been associated with the animal feeding methods and the ability of *Escherichia coli* O157:H7 to survive in the outer environment. Because of the higher incidence (up to 60%) of *Escherichia coli* O157:H7 positive animals observed in summer and autumn (20, 28, 36). *Escherichia coli* O157:H7 were isolated from the faeces of healthy animals, particularly cattle. They are not pathogenic to calves older than 3 weeks; however, they can produce diarrhoea and typical lesions in intestinal mucosa during first few post parturient days (10).

The animals that are asymptomatic carriers of *Escherichia coli* O157:H7 become a source of contamination of both their environment and meat in slaughterhouses, which comes into contact with their intestinal content (7). It has been shown that these bacteria can survive for relatively long periods outside the animals. The study of McGee (26) suggests that horizontal transmission of *Escherichia coli* O157:H7 may occur during cattle housing and that animal hide may be an important source of transmission.

Apart from ground beef (13) and bovine meat products (37), the sources of *Escherichia coli* O157:H7 infections also include raw milk, milk products, (particularly yoghurt and cheeses). Furthermore, non-pasteurised fruit juices, dried fermented salami, game meat, poultry meat and poultry by-products, drinking water and water in swimming pools have been implicated as sources of outbreaks (1, 11, 12, 15, 16, 18, 33, 43, 47).

The pathogenicity of *Escherichia coli* O157:H7 is related to the production of one or two toxins: verotoxin 1 (VT 1) and verotoxin 2 (VT 2) (6, 21, 22). Despite the progress in modern food production technologies and growing efforts to ensure the harmlessness of foods (14, 31, 40, 48) the incidence of alimentary diseases is rising. Consumers are demanding products subjected to minimum treatment and this increases their risk to human health.

Contamination of carcasses with agents of alimentary diseases including *Escherichia coli* presents a serious hygiene problem. The reasons for the presence of these bacteria in animal carcasses vary. A healthy animal can have them on its skin, hair, in the digestive tract and in

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lymph nodes. The body surface can become easily contaminated during skinning and excrement removal and contaminated meat can, in turn, contaminate technological equipment used for its processing. If this equipment is not treated properly it may become a source of bacterial contamination of healthy raw materials, which are subsequently processed (4, 34).

For these reasons an effort has been made to develop a simple, rapid and sensitive method for detecting *E. coli* O157:H7 presence in raw materials and foods of animal origin (1-4, 8, 18, 19, 23, 35, 42, 44, 46).

A recently developed method of immunomagnetic separation (IMS) uses an immunomagnetic antibody-coated carrier of *Escherichia coli* O157:H7. IMS combines high sensitivity with a considerably shorter detection time as compared with previously used techniques. The IMS is simple and rapid and can prove the presence or absence of *Escherichia coli* O157:H7 in 24-48 hours (29).

There are now an increasing number of alimentary infections in which *Escherichia coli* O157:H7 plays an important role. The aim of the study was to research the detection of this pathogen in one of the basic raw materials of animal origin-raw cow milk.

### Material and methods

Samples of raw cow's milk for detecting *Escherichia coli* O157:H7 were taken from different dairies from 8 districts of the East-Slovak region.

Microbiological screening of *Escherichia coli* O157:H7 was carried out in the following way: samples of raw cow's milk (25 ml) were pre-cultivated in 250 ml of Tryptose phosphate broth (Oxoid) at 37°C for 18-20 hours. The pre cultivated samples were subjected to immunomagnetic separation using Dynabeads anti-*Escherichia coli* O157-magnetic particles coated with anti O157:H7 antibodies (DYNAL, Norway) and were cultivated in SNAC-CT (Sorbitol-MacConcey agar with cefixime and tellurit (Oxoid) at 37°C for 18-20 hours. The obtained sorbitol-negative colonies were used to prove the presence of *Escherichia coli* biochemically by means of the Enter-Screen test (Lachema, Brno). The sorbitol-negative *Escherichia coli* colonies were examined serologically for the presence of O157 and H7 antigens by means of a rapid latex agglutination test (WELLCOLEX) *Escherichia coli* O157:H7, (Murex). The *Escherichia coli* O157:H7-positive isolates were tested for production of verotoxins (V1 and V2) employing the VTEC-RPLA test (Oxoid).

### Results and discussion

The results obtained by microbiological screening were summarised in tab. 1 and tab. 2. A total of 196 samples of raw cow's milk obtained from various dairies in the East Slovakia region were examined for the presence of enterohaemorrhagic *Escherichia coli* (EHEC), serotype O157:H7, in the period of May-July 2006 (tab. 1). 4-8 sorbitol-negative colonies from each of the examined samples were obtained and characterized bio-chemically and serologically. *Escherichia coli* O157:H7 strains were found in samples from two dairies (1.02%) in the Trebišov district (tab. 2). Their specific feature was that they were characterized as sorbitol-positive during biochemical examination. This is similar to findings of (15, 25) who described sorbitol-positive *Escherichia coli* O157:H7.

The results of *Escherichia coli* O157:H7 strain prevalence in dairy cow herds in the East Slovakia region, obtained over the three month study period, suggested a very low occurrence (1.2%) of EHEC serotype O157:H7 in the samples of raw cow's milk obtained from dairies. Similar results from dairies (0-2.2%) were presented by Dargatz (9). The examination of isolated strains of *Escherichia coli* for the production of verotoxins by means of VTEC-RPLA test showed only poor production of small amounts of verotoxins in both VT 1 and VT 2.

Cattle have been identified as the main reservoir of *Escherichia coli* O157:H7. Prevalence of this EHEC O157:H7 ranging from 0.9% to 8.2% was described in a cattle herd in Great Britain (24). *Escherichia coli* O157:H7 enters the food chain as a source of infection either via faecal contamination of meat in slaughterhouses or by contamination of milk in the primary production stage – also confirmed by the results.

Due to the increasing incidence of alimentary diseases which develop with the active participation of the *Escherichia coli* O157:H7 pathogens for monitoring its presence were used in tests carried out on raw cow's milk – one of the basic foods of animal origin important for human nutrition. This monitoring was carried out in East Slovakia, where a total of 196 samples of raw cow's milk were taken from dairies located in 8 districts. *Escherichia coli* O157:H7 were detected by the immunomagnetic separation method, which has been reported to be more sensitive than conventional methods in isolating and identifying *Escherichia coli* O157:H7 strains (1, 45). Of the total number of 196 samples examined, *Escherichia coli* strains were isolated only from two dairies in Trebišov district. Although this is a relatively low incidence (1.02%), with regard to human health protection against this pathogen, it is important to note that the *Escherichia coli* O157:H7 isolated from two milk samples produced small amounts of verotoxins, which can play a key role in the pathogenesis of haemorrhagic colitis and haemolytic uraemia syndrome.

The results suggest that it is necessary to monitor pathogenic strain prevalence continuously in raw milk – a basic raw material, as early as in the stage of primary milk

**Tab. 1. The origin of examined raw cow's milk samples in East-Slovak regions**

District	No. of milk samples examined
Prešov	29
Trebišov	45
Bardejov	49
Kežmarok	34
Svidník	2
Rožňava	2
Humenné	21
Sabinov	14
Total	196

**Tab. 2. Incidence of *Escherichia coli* O157:H7 in raw milk samples of dairies**

Kind of samples	Total number of samples 196
Positive for <i>E. coli</i> O157:H7	2 (1.02%)
Positive for verotoxin 1 (VT1+)	2 (1.02%)
Positive for verotoxin 2 (VT2+)	2 (1.02%)

production, to determine the source and means of contamination. Furthermore, effective measures for its elimination should be proposed and applied including subsequent checking of their efficacy.

### Conclusion

Out of total number of examined milk samples strains *E. coli* O157:H7 were isolated only in two dairy cows in Trebišov district. Although complex evaluation revealed only low incidence (1.02%) in respect of health safety of the pathogen it is necessary to continually and in territorial range to observe its occurrence also in basic raw material raw milk as early as in the stage of primary milk production, to determine source and way of contamination and not only propose but also realize effective measures for its eradication with subsequent checking of their efficacy.

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