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SURVIVAL OF *ESCHERICHIA COLI* SEROTYPE O157:H7 IN CULTIVABLE AND MEADOW SOILS

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Survival of one strain of enterohaemorrhagic *E. coli* serotype O157:H7, isolated from milk in Poland, in cultivable and meadow soils at 5°C and 20°C was tested. It was found that the death rate of pathogenic bacteria depends on soil fertility and incubation temperature. At 5°C, in such soils as light loam and loamy silt, with humus content of 2.16% to 3.43%, bacteria died after 140-158 days of incubation, and in such soils as loamy sand with humus content of *ca*. 1.50% they died after 70–85 days of incubation. At 20°C, these periods for experimental options mentioned above were 70-90 days and 49–63 days, respectively.

INTRODUCTION

The first reports on food poisoning caused by *Escherichia coli* serotype O157:H7 date back to 1982 when in Oregon and Michigan 47 people fell ill after eating undercooked hamburgers originating from the same fast--food restaurant chain [Buchanan & Doyle, 1997].

Since that time, outbreaks of the illness have been noted after ingestion of contaminated water, raw milk, yoghurt, cheese, meat (mainly beef), fermented sausages, mayonnaise, sandwiches, potatoes, unpasteurized fruit juices, apple cider, vegetables (leafy lettuce, radish), alfalfa, soybean sprouts [Abdul-Raouf *et al.*, 1993; Buchanan & Doyle, 1997; Castro-Rosas & Escartin, 2000; Hara-Kudo *et al.*, 1997; Solomon *et al.*, 2002]. The risk of introducing these pathogenic bacteria with food into the human organism results from heat and acid tolerance of this serotype, its high survival rate in cooling and freezing conditions and its low sensitivity to low water activity and to some preservatives used in the food processing industry [Abdul-Raouf *et al.*, 1993; Benjamin & Data, 1995; Buchanan & Doyle, 1997; Kwiatek, 2000; Leyer *et al.*, 1995]. This organism causes a spectrum of disease symptoms of varying severity from haemorrhagic colitis manifested by bloody diarrhea to haemolytic uremic syndrome/thrombotic thrombocytopenic purpura leading in some cases to death [Buchanan & Doyle, 1997; Łękowska-Kochaniak *et al.*, 2002].

The reservoir of the *E. coli* O157:H7 bacteria is the intestinal tract of domestic animals (mainly cattle, but also sheep, goats, pigs, horses, poultry) and wild animals (deer and roe deer). These animals are only carriers of the enterohaemorrhagic serotype, which does not cause any disease symptoms to them [Buchanan & Doyle, 1997]. The prevalence of carrier status of *E. coli* O157:H7 is 3.2% in milk cows, and 1.6% in beef cattle [Zhao *et al.*, 1995]. According to Faith *et al.* [1996], this value, in general, for cattle is at the level of about 8.3%. Kudva *et al.* [1998] quoted after other authors values ranging from 0.3% to 6.1%. The studies by Uradziński [2001], conducted in the northern regions of Poland, indicated that 4 (0.73%) of 551 cattle were positive for serotype *E. coli* O157. This result is similar to the respective data from Norway (0.3%) and Sweden (1.1%) but different than the data from Italy (3.6%); England (4.0%); France (4.7%); Belgium (6.3%); Czech Republic (6.2%); Germany (10.8%), and Netherlands (10.6%).

The transmission of *E. coli* O157:H7 bacteria to the natural environment and the subsequent food contamination takes place, for example, through the faeces of infected subjects and fertilizers used for fertilization of cultivable soils. The studies by Kudva *et al.* [1998], on the survival of *E. coli* O157:H7 in fertilizers demonstrated that these bacteria were still present in sheep manure kept for 21 months under natural conditions, without aeration, and their number decreased from the level of 10^8 to $<10^2$ per g. The bacterial death rate increased on aeration of the manure; a similar level of contamination was obtained after a 4-month incubation of sheep manure and a 2-month incubation of cow manure.

In recent years, intensive studies have been conducted on the infiltration and survival of pathogenic bacteria in plant tissues and, subsequently, in processed plant foods (mainly in unpasteurized juice) [Natvig *et al.*, 2002; Solomon *et al.*, 2002; U.S. Food and Drug Administration, 1999]. It is claimed that these microorganisms penetrate into plants probably through the root system and stomatal apparatus through any mechanical damage to the plant (wounds, punctures, cuts, splits, hail damages, *etc*.) and damage connected with the excessively rapid growth of fruit or tuberous vegetables. It is known that the development of putrefaction bacteria or fungi in places of damage to fruit

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and vegetables causes intensive proliferation of pathogenic bacteria. The danger of the presence of pathogenic bacteria in the plant material results from the fact that chemicals used in disinfection act effectively against bacteria found on the surface of fruit and vegetables, and their effectiveness against microorganisms present in the deeper plant layers is negligible [Solomon *et al.*, 2002].

Considering the fact that pathogenic bacteria living in soil might contaminate plant material, the objective of this work was to determine the survival of *E. coli* serotype O157:H7 in this environment under temperature conditions typical of autumn-winter and spring-summer seasons.

MATERIAL AND METHODS

Target bacteria. In model experiments on the survival of the enterohaemorrhagic serotype in soils, the *Escherichia coli* strain O157:H7 isolated from milk by the employees of the Veterinary Institute in Puławy was used. Its pathogenicity (the presence of the genes: *stx1*, *stx 2*, *eae* and hly_{933}) was confirmed by the studies of Łękowska-Kochaniak *et al.* [2002].

Inoculum preparation for model experiments. Suspension of *E. coli* O157:H7 serotype necessary for soil inoculation was prepared after a two-stage culture process. In the first stage, 1 loop of the biological material was introduced into 10 mL of bioMerieux Trypcase Soy Broth and incubated for 6 h at 37°C. The entire content of the test tube was then transferred to 250 mL of the above-mentioned broth and incubated at the same temperature for 24 h. The prepared material contained about 10^8–10^9 bacterial cells per 1 mL (the count of bacteria was estimated on Trypcase Soy Agar).

Model experiments. Soil samples were taken from various sites in the Province of Mazowieckie. Soils were characterised with respect to their physicochemical properties and to the level of natural bacterial microflora. Aliquots of 20 g samples of the specific soil were put into sterile closed plastic 125-mL vessels. Each sample was contaminated with 1.5 mL of bacterial suspension. The vessels were incubated at 5°C and 20°C. The whole single soil sample was analysed within the indicated time. The frequency of analyses of the enterohaemorrhagic serotype survival depended on the death rate of the strain in the specific soil at the specific temperature. Before microbiological analyses were performed, 80 mL of normal saline (0.85%) was introduced into the vessel with a 20 g soil sample and after thoroughly mixing the level of the enterohaemorrhagic serotype in 1 mL of soil suspension at "0" and subsequent hours of incubation was determined. When the absence of the pathogenic serotype in the soil sample in question was established, humidity, pH and the level of natural microflora were determined in the tested sample, similarly as at the "0" hour of culture.

Microbiological analyses of soils. The count of *E. coli* O157:H7 bacteria was determined on bioMerieux Coli O157:H7 ID Agar with additional selective substances (0.5 g of sodium tellurite and 0.01 g of cefixim/L of the medium). Petri dishes were maintained at 37°C for 24 h. The total count of bacteria was determined on Oxoid Plate Count Agar. Petri dishes were maintained at 20°C for 72–96 h. Coliforms count and the count of *Escherichia coli* were determined on Oxoid *E. coli* /Coliform Agar. Petri dishes were maintained at 37°C for 24 h.

Confirmation procedure for determination of the presence of *E. coli* O157:H7 serotype in soils. Long-term incubation of soils affected the morphology of *E. coli* O157:H7 colonies. In doubtful cases, material from suspected colonies was transferred to Petri dishes with bioMerieux Endo Agar. After 24-h incubation at 37°C, the presence of metallic colonies was checked. These colonies were subject to testing with the use of a Merck Singlepath *E. coli* O157 test.

RESULTS AND DISCUSSION

The survival of *E. coli* O157:H7 serotype was evaluated in seven soil samples: three of them were intended for the cultivation of vegetables, one for cultivation of rye, and three were meadow soils. Four soils were classified to the granulometric group – loamy sand; two – to the light loam group, and one – to the loamy silt group (Table 1). The soils differed in pH values, humidity, sorption capacity and humus content (Table 1). On the basis of microbiological analyses of the soil samples, it was found that the total count of bacteria changed from 1.7×10^6 to 2.0×10^7 CFU/g of soil and the coliform count changed from 1.8×10^4 to 3.6×10^6 CFU/g of soil (Table 2). There were no *E. coli* bacteria in 0.1 g samples of soil.

Survival of the enterohaemorrhagic serotype was better at 5°C than at 20°C and better in soils with high humus content (Figure 1, Table 1). For example, at low temperature, in soils No. 1 and 2 – horticultural, and in soil No. 5 – meadow, with humus content ranging from 2.16% to 3.43%, the absence of pathogenic bacteria was noted after 140- to

TABLE 1. Physicochemical characteristics of soils used in the experiments on the survival *E. coli* O157:H7 serotype in this environment.

No. of soil	pH	Humidity (%)	Humus (%)	Sorption capacity (T)	Silt (%)	Clay (%)	Granulometric group
1 /1	7.1	15.93	2.16	12.77	30	35	Light loam
2/1	5.8	16.30	3.43	8.05	19	26	Light loam
3 /1	6.7	14.04	1.91	7.31	25	20	Loamy sand
4/2	6.8	10.02	1.90	6.83	22	14	Loamy sand
5 /3	6.2	24.26	2.74	7.51	67	23	Loamy silt
6 /3	7.7	9.31	1.52	18.44	19	14	Loamy sand
7 /3	6.3	7.23	1.57	6.61	12	16	Loamy sand

1/ horticultural soils; 2/ rye soil; 3/ meadow soils

TABLE 2. Microbiological characteristics of soils used in the experiments on the survival *E. coli* O157:H7 serotype in this environment.

No. of soil	Type of soil	Total count of bacteria	Coliform count (CFU/g of soil)	*E. coli* count (CFU/g of soil)
1	Horticultural, light loam	1.5×10^7	2.4×10^5	<10
2	Horticultural, light loam	2.0×10^7	3.6×10^6	<10
3	Horticultural, loamy sand	1.7×10^7	2.6×10^6	<10
4	Rye, loamy sand	1.6×10^7	2.6×10^5	<10
5	Meadow, loamy silt	1.9×10^6	6.5×10^4	<10
6	Meadow, loamy sand	1.7×10^6	1.8×10^4	<10
7	Meadow, loamy sand	3.2×10^6	2.6×10^5	<10

158-days of incubation. In poor meadow soils (No. 6 and 7) with humus content of *ca*. 1.5%, the death of bacteria occurred after 70-85 days. Similar regularities, although less clearly visible, were observed at 20°C, and the time of incubation during which total death of bacterial cells was observed ranged from 48 to 90 days. The tested serotype surviving in the soil at 5°C for about 5 months retained the virulence factors (data not presented).

It is difficult to compare the results of the present study with the results of other scientists, because the respective studies are currently ongoing. In an article written by Xiuping Jiang *et al.* [2002], *E. coli* O157:H7 bacteria were reported to have survived in soil fertilized with natural manure from 65 to 240 days and, according to the authors, their survival was better at 21°C than at 5°C. Internet data mentioned below are incomplete; the conditions of experiments on the survival of *E. coli* O157 serotype are not given and the data are not sufficient. However, Gagliardi *et al.* [2000], reported that this period is 30 days for unplanted soil and 60 days for soils planted with rye, alfalfa, hairy vetch or crimson clover. In the Final Report [2002] the survival of the serotype in question was indicated as ranging from 70 to 100 days. According to ScienceNet [2001], these bacteria can survive in the soil even up to 6 months. According to literature data [Anonym., 2001], the survival time of intestinal bacteria in soil depends on the properties of the latter: its humidity, moisture absorption capacity, temperature, pH, content of organic substances, and antagonistic action of soil microflora. Bacteria survive much longer in soils with high humidity. The high moisture--absorbing potential of the soil also contributes to this phenomenon (unfavourable conditions exist in sandy soils). The time of bacterial survival is prolonged at low temperatures (they survive better in winter than in summer) and at high content of organic substances in the soil. The bacteria die more rapidly in soils with pH 3–5 than in soils with alkaline pH. The presence of natural microflora accelerates the death rate of intestinal bacteria. The above data were confirmed to a large extent in the present studies.

The incubation of various soil samples at both temperatures caused an increase in their humidity and this could improve the survival of enterohaemorragic serotype (Figure 2). The pH values of soils at the end of the experiments did not significantly deviate from the initial values oscillating in most cases within the range of neutral or slightly acid pH (Figure 3), *i.e.* within the range optimal for survival of the majority of pathogenic bacteria. It seems that due to the low sensibility of the tested serotype to low environmental pH [Benjamin & Data, 1995; Leyer *et al.*, 1995], this parameter did not play a significant role in the survival of *E. coli*

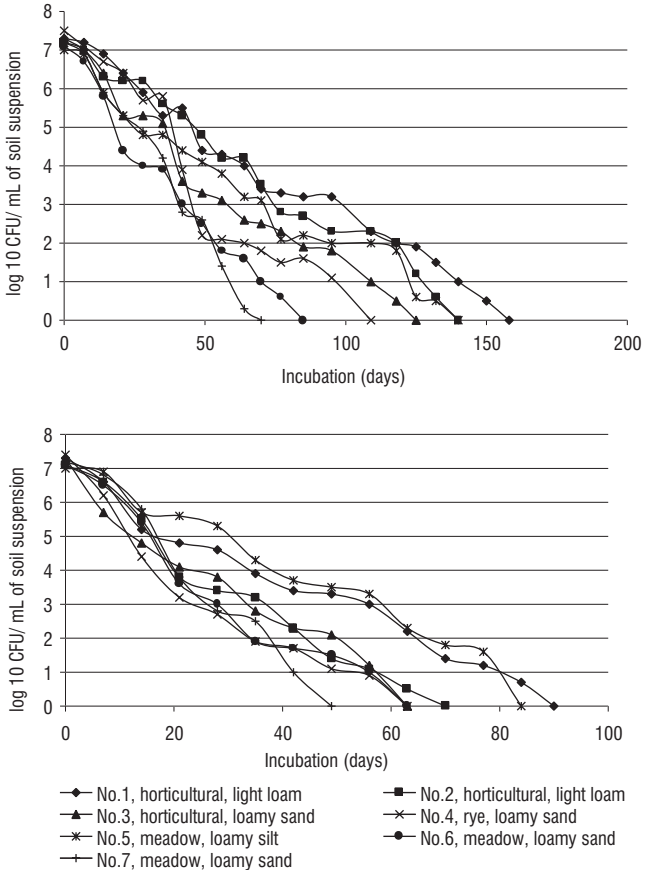

FIGURE 1. Survival of *E. coli* O157:H7 serotype in various types of soil at the temperature of 5°C (top curves) and at 20°C (bottom curves). Each point is the mean of values from duplicate experiments.

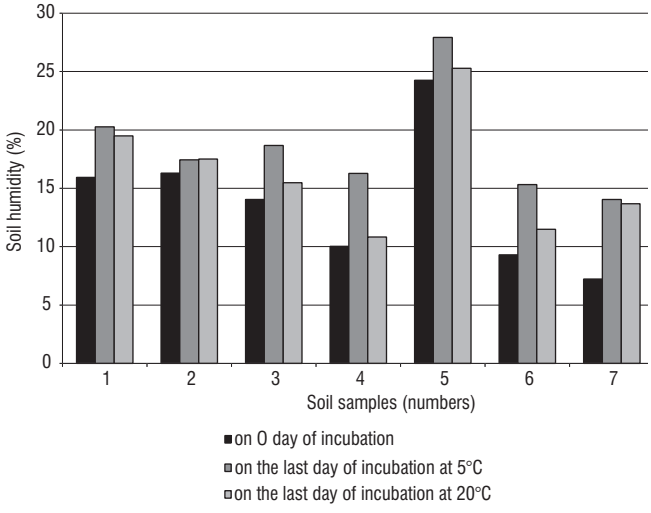

FIGURE 2. Changes in humidity of soil after incubation of samples at 5°C (No. 1 – 158 days; 2 and 5 – 140 days; 3 – 125 days; 4 – 109 days; 6 – 85 days; 7 – 70 days) and at 20°C (No. 1 – 90 days; 5 – 84 days; 2 – 70 days; 3, 4 and 6 – 63 days; 7 – 49 days).

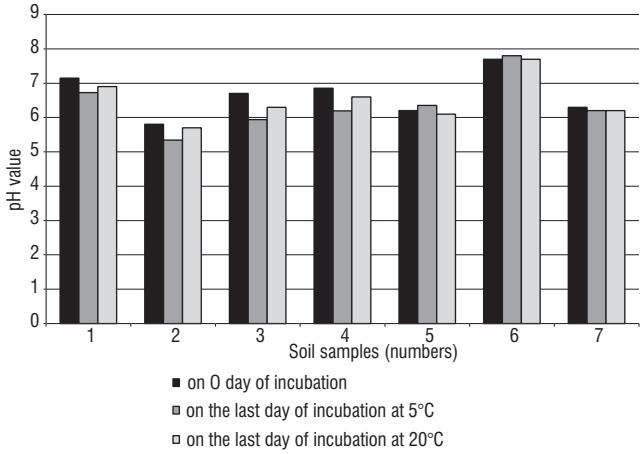

FIGURE 3. Changes in pH soil after incubation of samples at 5°C (No. 1 – 158 days; 2 and 5 – 140 days; 3 – 125 days; 4 – 109 days; 6 – 85 days; 7 – 70 days) and at 20°C (No. 1 – 90 days; 5 – 84 days; 2 – 70 days; 3, 4 and 6 – 63 days; 7 – 49 days).

O157:H7 in soil No. 2 with the initial pH of 5.8 which was further lowered during incubation.

No regularities were observed with respect to the changes in natural microflora count in the tested soil samples at the beginning and final periods of incubation (Figure 4). Changes towards an increase or a decrease in the specific bacterial population, irrespective of the incubation temperature used, rarely exceeded one logarithmic unit. These differences were greater in the test defined as "total count" than in the test of "Coliform count". It seems that the above may be explained by the cyclically repeated phenomena of decay and concurrent creation of new bacterial cells in the soil environment.

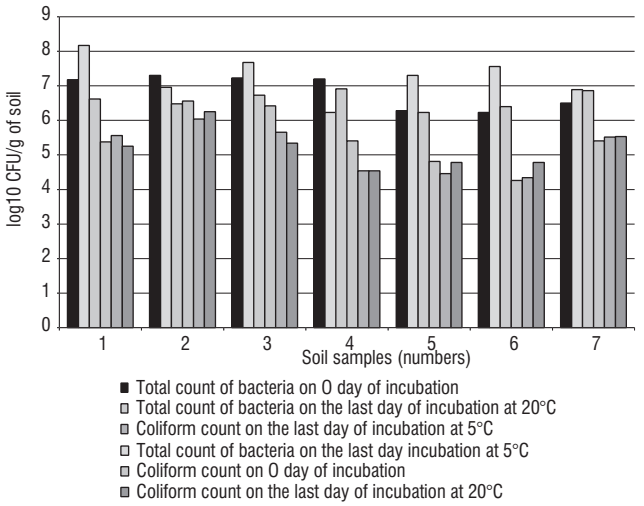

FIGURE 4. Changes in bacterial population soil after incubation of samples at 5°C (No. 1 – 158 days; 2 and 5 – 140 days; 3 – 125 days; 4 – 109 days; 6 – 85 days; 7 – 70 days) and at 20°C (No. 1 – 90 days; 5 – 84 days; 2 – 70 days; 3, 4 and 6 – 63 days; 7 – 49 days).

For food safety, it is important to keep track of all stages of food production chain "from farm to fork". Appraisal of survival of very pathogenic bacteria – *E. coli* O157:H7 – in soils is one of the elements of microbiological risk analysis. According to Natvig *et al.* [2002] as well as Xuiping Jiang *et al.* [2002], the period between the introduction of non-composted fertilizers into the soil and the harvesting of vegetables whose edible parts enter into contact with the fertilizer should not be shorter than 3–4 months. In our country, the period between introduction of fertilizers to the soil and the harvest of plants (*e.g.* early vegetables) is over 5–6 months. On the basis of the information mentioned above and the longest time of survival of the enterohaemorrhagic serotype in the examined soils (approximately 5 months), it can be concluded that the likelihood of the presence of pathogenic bacteria in plant material is very low. On the other hand, a very significant risk exists resulting from the relatively long survival of pathogenic bacteria in meadow soils.

CONCLUSIONS

Studies on the survival of enterohaemorrhagic *E. coli* O157:H7 serotype in soils should be continued, because only one strain and a limited number of soil samples were used in experiments. With regard to the fact that the target serotype has also been detected in milk, dairy products, meat and meat products as well as in water, experiments on the survival of enterohaemorrhagic serotype in sewage from dairy and meat industry and in appropriate activated sludges as well as in waters and in water sediments should be also performed.

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PRZEŻYWALNOŚĆ *ESCHERICHIA COLI* SEROTYPU O157:H7 W GLEBACH UPRAWNYCH I ŁĄKOWYCH

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Badano przeżywalność jednego szczepu, enterokrwotocznego serotypu *E. coli* O157:H7 wyizolowanego z mleka w Polsce, w glebach uprawnych i łąkowych, w temperaturze 5°C i 20°C. Stwierdzono, że szybkość zamierania chorobotwórczych bakterii zależała od żyzności gleby i temperatury inkubacji. W temperaturze 5°C, w glebach typu glina lekka oraz pył gliniasty, o zawartości próchnicy od 2,16% do 3,43%, bakterie zamierały całkowicie po 140–158 dniach inkubacji, natomiast w glebach typu piasek gliniasty o zawartości próchnicy ok. 1,50% – po 70–85 dniach inkubacji (rys. 1, tab. 1). W temperaturze 20°C, okresy te dla ww. wariantów doświadczenia, wynosiły odpowiednio 70–90 dni i 49–63 dni.