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Short communication

Influence of temperature on survival of *Escherichia coli* O157:H7 in stored cattle slurry with respect to environmental biosafety

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Abstract

The aim of the study was to determine the influence of temperature, i.e. 4 and 20°C on the *Escherichia coli* O157:H7 survival time in a stored cattle slurry in a laboratory model experiment. The results of this investigation indicated that the tested microorganisms underwent a gradual elimination in the cattle slurry, whereas their inactivation rate was clearly dependent on the temperature. A higher survival rate was found in *Escherichia coli* O157:H7 at 4°C where a theoretical survival time of these microorganisms, determined using a regression analysis, amounted to 83 days. Our study indicates that there is a necessity for the slurry to undergo hygienization processes and that a constant monitoring of liquid animal excreta in search for pathogenic microorganisms is required.

Key words: *Escherichia coli* O157:H7, temperature, survival, cattle slurry, environment

Introduction

Escherichia coli serotype O157:H7 is an important zoonotic pathogen causing haemorrhagic colitis (HC), a haemolytic-uraemic syndrome (HUS) and a thrombotic thrombocytopenic purpura (TTP) in humans. A major and natural reservoir of *Escherichia coli* O157:H7 is healthy cattle (Yoon and Hovde 2008, Whitworth et al. 2010). According to studies conducted both in Poland, the United Kingdom and the United States, *Escherichia coli* O157 isolation frequency from cattle is 0.73-35.8% (Uradziński 2001, Inat and Siriken 2010). A carrier state and excreting these bacteria by the cattle is transient and lasts 3-4 weeks on average (LeJeune et al. 2001). Due to this, an essential role in the transmission of *Escherichia coli* O157:H7 is played by the environment, especially by infected cattle slurry, in which these microbes may survive for a long time. The use of cattle slurry, not pre-treated in the appropriate hygienization processes, for fertilization purposes constitutes a potential health hazard for people and is the cause of infections in livestock (Fremaux et al. 2008). The aim of our experiment was to determine the influence of temperature on the *Escherichia coli* O157:H7 survival time in the stored cattle slurry.

Materials and Methods

A standardized non-toxigenic strain of *Escherichia coli* O157:H7 (NCTC 12900) was used in our study. In order to launch the experiment cattle slurry was poured into two glass containers with a volume of 1000 cm^3 each. Then samples of slurry were inoculated with *Escherichia coli* O157:H7 bacterial suspension. One hour after the start of the experiment, the level of bacteria in 1 cm^3 of slurry was estimated in all the samples. Following that, the containers were

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Table 1. Regression equations characteristic of inactivation rate of *Escherichia coli* O157:H7 in the cattle slurry stored at 4 and 20°C.

Microorganism	Temperature (°C)	Regression equation	R^2	Theoretical time of survival (days)
Escherichia coli O157:H7	4	y = -0.13x + 10.68	0.85	83
Escherichia coli O157:H7	20	y = -0.16x + 7.89	0.84	50

placed in chambers with varied temperature values, i.e. 4 and 20°C. On fixed days of the experiment the count of *Escherichia coli* O157:H7 was determined in separate slurry samples on the basis of the most probable number of microorganisms (MPN). The experiment was conducted in three replicates. The evaluation of *Escherichia coli* O157:H7 survival rate in cattle slurry was carried out according to the following procedure. The first step was an initial proliferation of the tested bacteria in mEC-broth with Novobiocin (Merck) (incubation at 37°C for 24 hours). At the next step, inoculations were carried out from the culture into a selective solid medium: Sorbitol-MacConkey agar (SMAC agar, Merck). Incubation of the inoculated media was carried out at 37°C for 24 hours. Final identification of the determined microbes was made using biochemical and serological tests. The results of the survival rate of *Escherichia coli* O157:H7 in cattle slurry were statistically analysed with the aid of STATISTICA 8.0 software.

Results and Discussion

Initial concentration of *Escherichia coli* O157:H7 in the research material was 109 MPN · cm^{-3} on the first day of the experiment. On the day 55 of the experiment mean number of these microorganisms at 4°C has got reduced by 7.95 log, i.e. from the level of 9.0×109 down to 9.5×101 MPN · cm^{-3}. Yet, at 20°C the kinetics of the inactivation regarding *Escherichia coli* O157:H7 proceeded considerably more rapidly and on the 28th day of the experiment their count ranged from 9.0×10^1 to 1.0×10^2 MPN · cm^{-3}, whereas at 4°C this parameter value was 1.5×10^8 – 5.5×10^8 MPN · cm^{-3} on the same day of the experiment. On a day 46 of the study a mean level of bacteria in the slurry dropped by 8.80 log (from 9.5×10^9 to 1.9×10^1 MPN · cm^{-3}) at 20°C. On the last day of the experiment no microbes were isolated from the studied material.

Values of the daily rate of inactivation and the survival time of *Escherichia coli* O157:H7 in the stored cattle slurry have been listed in Table 1. Based on the regression analysis, the elimination rate of the tested microorganisms in the research material at 4 and 20°C was 0.13 and 0.16 log per day, respectively. We have found that the lower temperature exerted a stabilizing effect on the population of the studied bacteria, which favoured the elongation of their survival time. Results similar to our own were achieved by Wang et al. (1996), who were isolating these microorganisms from bovine faeces at 22°C for 56 days, however at 5°C they were detecting them even until the 70th day of the experiment. In our own investigations a theoretical survival time of *Escherichia coli* O157:H7 in the cattle slurry, established on the basis of regression analysis for 4 and 20°C was 83 and 50 days, respectively.

In summary, in our study we have observed a relatively high survival rate of *Escherichia coli* O157:H7 in the stored cattle slurry under the influence of variable temperature. Our findings unequivocally indicate that the slurry should necessarily undergo hygienization processes. Furthermore, a constant monitoring of pathogenic microorganisms in liquid animal faeces is also required in order to minimize environmental contamination and, consequently, to eliminate epidemiologic and epizoonotic risks.

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