Effect of storage temperature and type of slurry on survivability of *Salmonella*

Wpływ temperatury składowania oraz typu gnojowicy na przeżywalność pałeczek z rodzaju *Salmonella*

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Abstract

The aim of this study was determination of the inactivation rate of *Salmonella* Senftenberg W_{775} and *Salmonella* Typhimurium in cattle and swine slurry stored under laboratory conditions at 4 and 20˚C. *Salmonella* bacilli underwent constant, gradual elimination from the slurry during its storage. This process occurred more efficiently in samples stored at 20˚C than at 4˚C. At both studied temperatures elimination of bacteria was faster in swine slurry than in cattle. Shorter survivability in research material, in both temperature variants and kinds of slurry, showed *Salmonella* Typhimurium.

Keywords: *Salmonella* Senftenberg W_{775}, *Salmonella* Typhimurium, slurry, survivability, storage temperature

Streszczenie

Celem badań było wyznaczenie tempa inaktywacji pałeczek *Salmonella* Senftenberg W_{775} and *Salmonella* Typhimurium w bydlęcej i świńskiej gnojowicy składowanej w warunkach laboratoryjnych w temperaturze 4 i 20°C. Pałeczki *Salmonella* podlegały stałej stopniowej eliminacji ze składowanej gnojowicy. Proces ten zachodził szybciej w próbkach składowanych w 20 niż 4˚C oraz w gnojowicy świńskiej niż bydlęcej. Krótszą przeżywalność w badanym materiale, w obu wariantach temperatury i typach gnojowicy, wykazały pałeczki *Salmonella* Typhimurium.

Słowa kluczowe: gnojowica, przeżywalność, *Salmonella* Senftenberg W_{775}, *Salmonella* Typhimurium, temperatura składowania

Streszczenie szczegółowe

Gnojowica jest cennym nawozem naturalnym, jednakże jej rolnicze zagospodarowanie może stwarzać zagrożenie sanitarno-higieniczne wynikające z obecności w tym nawozie znacznej liczby różnych mikroorganizmów, a także jaj pasożytów. Sprawia to, że czas i temperatura jej składowania są niezwykle istotne, jako główne czynniki wpływające na proces higienizacji.

Celem badań było wyznaczenie tempa inaktywacji pałeczek *Salmonella* Senftenberg W_{775} i *Salmonella* Typhimurium w bydlęcej i świńskiej gnojowicy składowanej w warunkach laboratoryjnych w temperaturze 4 i 20°C.

Materiał do badań stanowiła świeża gnojowica bydlęca i świńska, w której nie stwierdzono obecności pałeczek z rodzaju *Salmonella*. Pierwszym etapem było przygotowanie zawiesin bakterii *Salmonella* Senftenberg W_{775} i *Salmonella* Typhimurium w bulionie odżywczym, które po 24 godzinach inkubacji w 37°C zostały dodane do reaktorów z gnojowicą. Liczba pałeczek z rodzaju *Salmonella* była oznaczana przez 42 dni z 7-dniowym interwałem w oparciu o metodę NPL. Namnażanie wstępne bakterii *Salmonella* przeprowadzono z wykorzystaniem 1% zbuforowanej wody peptonowej (24 godz. w 37°C). Do namnażania selektywnego użyto bulionu Rappaporta (24 godz. w 43°C), a jako podłoża stałe zastosowano agar BPLS i XLD (24 godz. w 37°C). Identyfikację końcową pałeczek z rodzaju *Salmonella* przeprowadzono z wykorzystaniem poliwalentnej surowicy HM.

Badania wykazały stopniowy spadek liczebności populacji pałeczek *Salmonella* w trakcie składowania gnojowicy (tab. 1). Teoretyczny czas przeżycia bakterii *Salmonella* Senftenberg W_{775} w płynnych odchodach bydlęcych magazynowanych w 4°C był równy 114,24 dnia, a dla *Salmonella* Typhimurium 101,10 dnia, podczas gdy w 20°C wyniósł odpowiednio 43,06 i 41,06 dnia (tab. 2). Natomiast w gnojowicy świńskiej składowanej w 4°C teoretyczna przeżywalność pałeczek *Salmonella* Senftenberg W_{775} była równa 81,14 dnia, a dla *Salmonella* Typhimurium 75,05 dnia (tab. 2). Z kolei w 20°C teoretyczny czas przeżycia wynosił odpowiednio 32,50 i 30,27 dnia (tab. 2).

Powyższe rezultaty wykazały również, że przeżywalność pałeczek z rodzaju *Salmonella*, oprócz temperatury i typu gnojowicy, zależy także od serotypu bakterii.

Introduction

Due to the spread of the non-litter system of animal housing, slurry has achieved the greatest importance of all natural fertilizers. Despite the significant importance of slurry in soil fertilization, one should be aware that using slurry creates a potential microbiological risk. Slurry is a reservoir of a vast number of various microorganisms (Guan and Holley, 2003, Paluszak, 1998). Bacteria of the *Enterobacteriaceae* family (e.g. *E. coli*, bacilli of the *Salmonella* genus) and faecal streptococci are most frequently isolated (Paluszak, 1998). Pathogenic bacteria are also present, including the following genera: *Brucella, Mycobacterium, Leptospira, Chlamydia, Rickettsia, Campylobacter* and many others. Apart from bacteria, slurry microflora consists of viruses and fungi (Paluszak, 1998, Strauch, 1991). Also a remarkable amount of eggs and oocytes of parasites is found in this fertilizer (Bornay-Linares et al., 2006, Strauch, 1991).

Due to such variety of microorganisms present in slurry and their long survival time, the proper time and temperature of its storage necessary for sanitation process is of particular importance (Guan and Holley, 2003). *Salmonella* can survive in this fertilizer from 13 days to even 1 year (Munch et al., 1987). The fact that *Salmonella* bacilli make primary etiological factors of food poisonings (Adak et al., 2002), for which mortality can reach up to 30.6% (Kiessling et al., 2002), can bear evidence of the importance of the problem.

The aim of the study was to estimate the inactivation rate of the bacteria *Salmonella* Senftenberg W_{775} and *Salmonella* Typhimurium in cattle and swine slurry stored under laboratory conditions at 4°C and 20°C.

Material and methods

Fresh cattle and swine slurry was used in the experiment. No bacilli of the *Salmonella* genus were found in the input material.

The suspensions of *Salmonella* Senftenberg W_{775} and *Salmonella* Typhimurium in nutrient broth were prepared. After 24 hours of incubation at 37°C the 25 ml of suspensions per each 1 000 ml of cattle or swine slurry were added to the reactors. *Salmonella* Senftenberg W_{775} and *Salmonella* Typhimurium were introduced into different reactors to make easier its identification after sampling. The cattle and swine slurry contaminated with suspensions of the indicator bacteria were stored at 4°C and 20°C.

The number of *Salmonella* was determined at both temperatures for 42 days with 7 days interval. Enumeration of *Salmonella* was based on the MPN method. For the pre-multiplication of *Salmonella* 1% buffered peptone water was applied (24h at 37°C). Selective multiplication was carried out with the liquid Rappaport medium (24h at 43°C). Agar BPLS and XLD were solid growth media used in this research (24h at 37°C). The final identification of *Salmonella* bacilli was carried out on the basis of the serological test by means of the polyvalent serum HM.

The obtained results were verified and analysed statistically in the program SAS 9.1. The theoretical survival time and elimination rate of tested bacteria were determined on the basis of regression line equation. Differences between this parameters were checked with Tukey test.

Results

The study showed a gradual elimination of *Salmonella* from slurry stored both at 4 and 20°C. At the beginning of the experiment, the concentration of both *Salmonella* Senftenberg W_{775} and *Salmonella* Typhimurium, was 7.59×10^8 MPN/ml (tab. 1). The number of *Salmonella* Senftenberg W_{775} fell to 2.75×10^5 MPN/ml, and *Salmonella* Typhimurium to 5.75×10^4 MPN/ml during the 42 days of cattle slurry storage at 4°C (tab. 1). However in the same kind of slurry stored at 20°C the elimination rate was significantly higher (tab. 1). The number of *Salmonella* Senftenberg W_{775} at the last sampling time was equal to 0.25×10^1 MPN/ml, whereas *Salmonella* Typhimurium were not isolated from the slurry after 42 days of storage at 20°C (tab. 1).

The reduction in population of studied *Salmonella* serotypes was higher in swine slurry than in cattle one at both considered storage temperatures (tab. 1). The number of *Salmonella* Senftenberg W_{775} decreased to 1.17×10^4 MPN/ml, and *Salmonella* Typhimurium to 7.59×10^3 MPN/ml within 42 days of swine slurry storage at 4°C (tab. 1). On the 28th day of the experiment, the number of these bacteria was equal to 0.85×10^1 and 0.25×10^1 MPN/ml, respectively (tab. 1). The complete inactivation of tested *Salmonella* bacilli was observed after only 35 days of swine slurry storage at 20°C.

Table 1. Changes in the number of tested *Salmonella* bacilli [MPN/ml] during storage of cattle and swine slurry at 4 and 20 ° C
Tabela 1. Zmiany liczby badanych pałeczek *Salmonella* [NPL/ml] w trakcie składowania gnojowicy bydlęcej i świńskiej w 4 i 20°C

Sampling terms [days] Termin poboru próbek [dni]	Storage at 4°C / Składowanie w 4°C			
	Cattle slurry / Gnojowica bydlęca		Swine slurry / Gnojowica świńska	
	S. Senftenberg W_{775}	*S.* Typhimurium	*S.* Senftenberg W_{775}	*S.* Typhimurium
0	7.59×10^8	7.59×10^8	7.59×10^8	7.59×10^8
7	2.75×10^8	1.38×10^8	2.51×10^8	1.02×10^8
14	1.62×10^8	2.19×10^7	1.12×10^8	4.17×10^7
21	6.46×10^7	4.68×10^7	3.55×10^7	3.89×10^6
28	1.41×10^7	6.03×10^6	3.09×10^6	5.37×10^5
35	1.95×10^6	8.91×10^5	1.23×10^5	$5. 01 \times 10^4$
42	2.75×10^5	5.75×10^4	1.17×10^4	7.59×10^3
	Storage at 20°C / Składowanie w 20°C			
0	7.59×10^8	7.59×10^8	7.59×10^8	7.59×10^8
7	9.33×10^7	6.46×10^7	2.75×10^7	6.92×10^6
14	3.02×10^6	2.51×10^6	2.40×10^5	8.71×10^2
21	6.46×10^4	1.48×10^4	1.23×10^2	2.34×10^1
28	1.20×10^3	4.68×10^2	0.85×10^1	0.25×10^1
35	2.63×10^1	1.74×10^1	n.d. / n.w.	n.d. / n.w.
42	0.25×10^1	n.d. / n.w.*		

* n.d. / n.w. – not detected / nie wykryto

Theoretical survival times of *Salmonella* Senftenberg W_{775} and *Salmonella* Typhimurium in cattle slurry stored at 4°C were respectively equal to 114.24 and 101.10 days, with the elimination rate 0.08 and 0.09 log cycles/day (tab. 2). On the other hand, in cattle slurry stored at 20°C, the theoretical survival time of *Salmonella* Senftenberg W_{775} was 43.06 days and *Salmonella* Typhimurium was 41.06 days, with the elimination rate equal to 0.21 and 0.22 log cycles/day (tab. 2).

In the case of swine slurry, the theoretical survival times of *Salmonella* Senftenberg W_{775} and *Salmonella* Typhimurium, during it storage at 4°C, were at the level of 81.14 and 75.05 days, while at 20°C were equal to 32.50 and 30.27 days (tab. 2). The elimination rate of tested bacteria from swine slurry was 0.12 log cycles/day at 4°C and 0.27 log cycles/day at 20°C (tab. 2).

Table 2. Regression equations, the theoretical survival times and the elimination rates determined for tested *Salmonella* bacilli
Tabela 2. Równania regresji, teoretyczne czasy przeżycia oraz tempa eliminacji ustalone dla badanych pałeczek *Salmonella*

Bacteria Bakterie	Slurry type/Temp. of storage Typ gnojowicy/Temp. składowania	Regression line equation Równania prostych regresji	Elimination rate [log MPN/day] Tempo eliminacji [log NPL/dzień]	R^2	Theoretical survival time [days] Teoretyczny czas przeżycia [dni]
Salmonella Senftenberg W_{775}	Cattle / 4°C	y=-0.08x+9.1392	$0.08^{A,a,c}$	0.95	$114.24^{A,a,c}$
	Cattle / 20°C	y=-0.214x+9.2144	$0.21^{B,a,b}$	0.99	$43.06^{B,a,b}$
	Swine / 4°C	y=-0.1153x+9.3551	$0.12^{A,b,d}$	0.94	$81.14^{A,b,d}$
	Swine / 20°C	y=-0.2744x+8.9192	$0.27^{B,c,d,e}$	0.97	$32.50^{B,c,d,e}$
Salmonella Typhimurium	Cattle / 4°C	y=-0.0883x+8.9273	$0.09^{A,a,c,e}$	0.93	$101.10^{A,a,c,e}$
	Cattle / 20°C	y=-0.222x+9.1155	$0.22^{B,a,b}$	0.99	$41.06^{B,a,b}$
	Swine / 4°C	y=-0.12x+9.0064	$0.12^{A,b,d}$	0.99	$75.05^{A,b,d}$
	Swine / 20°C	y=-0.2666x+8.07	$0.27^{B,c,d}$	0.91	$30.27^{B,c,d}$

A,B,… - highly statistically significant differences ($p \leq 0,01$)
a,b,… - statistically significant differences ($p \leq 0,05$)

The differences found between slurry storage at 4°C and 20°C in the theoretical survival times and the elimination rate determined for both tested serotypes of *Salmonella* were highly statistically significant, however statistically significant differences in values of these parameters were shown between cattle and swine slurry (tab. 2). The differences in theoretical survivability and elimination rate observed between *Salmonella* Senftenberg W_{775} and *Salmonella* Typhimurium in the same kind of manure and similar temperatures were not statistically significant (tab. 2).

Discussion

The study showed that the main factor determining the survival of *Salmonella* in stored slurry was the temperature of storage. The rise of storage temperature resulted in reduction of tested bacteria survivability. This tendency confirmed the results obtained by Olszewska et al. (2011) who showed that the bacteria *Salmonella* Typhimurium survived almost 8 weeks in swine slurry stored at 20°C and more than 14 weeks at 4°C. Also Budzińska (2005) showed that in cattle manure stored at 4°C, *Salmonella* Senftenberg W_{775} survived for 42 days, and at 20°C for 36 days. These survival times were shorter than those obtained in own research.

The present experiment also demonstrated the impact of slurry type on the tested bacteria survival. *Salmonella* bacilli survived longer in cattle slurry than in swine one. Also Strauch (1991) isolated *Salmonella* Typhimurium from swine slurry for 39 days, and from cattle for 177 days. Burton and Turner (2003) found that the survival of *Salmonella* in the cattle slurry may be equal to 200-300 days, while in the swine just 90-120 days.

In own research, the impact of serotype of *Salmonella* bacilli on its survivability was observed. In each conducted experiments *Salmonella* Senftenberg W_{775} survived slightly longer than *Salmonella* Typhimurium. Also Jones (1976) observed the effect of serotype on survivability of bacteria and showed that *Salmonella* Dublin and *Salmonella* Typhimurium were isolated respectively for 90-113 and 113-140 from cattle slurry.

Conclusions

1. *Salmonella* bacilli underwent constant, gradual elimination from the slurry during its storage.
2. The survivability of tested bacteria during slurry storage was dependent on many factors.
3. The elimination rate depended on both the temperature and type of slurry. The process occurred much faster in samples stored at 20˚C than at 4˚C and in swine than in cattle slurry.
4. Shorter survival in research material, in both temperature variants and types of slurry, showed *Salmonella* Typhimurium.

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