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Livestock-associated MRSA CC398 survival in manure

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Background. **The application of manure to agricultural production is common practice in many countries. In Denmark alone, 30 million tons of swine manure are spread on Danish fields as fertilizer on a yearly basis. This fertilization is of great importance for a sustainable agriculture, but it may also constitute a potential dissemination route of pathogenic microorganisms and resistance genes to the environment.**

Purpose. The present study was undertaken to examine the presence of LA-MRSA in manure along with the decimation time of LA-MRSA in swine manure. The aim of the study was to evaluate whether LA-MRSA is likely to survive the passage of manure from the pig house to the farm land.
Knowledge on the decimation time of LA-MRSA in swine manure at different temperatures, will facilitate analysis on how and where to impose control points to avoid that LA-MRSA reaches the environment via manure.

Method. The survival of LA-MRSA in natural positive manure samples was investigated at three temperatures, 5 ˚C, 15 ˚C and 37 ˚C. Additionally, decimation times of two LA-MRSA isolates were investigated in spiked samples at four temperatures 5 ˚C, 15 ˚C, 25 ˚C and 37 ˚C. Isolates of *spa* type t011 and t034 were inoculated in swine manure at a low (~10^3) and high (~10^7) spiking level, calculated as colony forming units (CFU) per ml. Unspiked control samples were included at every temperature. The bacterial load of every sample was determined at multiple time points until LA-MRSA was no longer detectable by direct plating.

Table 1. **Overview of T90 at different temp. and initial LA-MRSA loads.**

Sample ID.	Temp. (°C)	Initial load CFU/ml	T90 (Days)
t011	5	7.40E+02	25
t034	5	9.10E+02	25
t011	5	1.72E+07	16.5
t034	5	2.83E+07	30.5
Natural pos.	5	2.00E+01	24
Natural pos.	5	1.05E+02	32
t011	15	6.75E+02	13.5
t034	15	7.55E+02	11.5
t011	15	1.67E+07	6.5
t034	15	2.87E+07	6.5
Natural pos.	15	1.15E+02	15
t011	25	7.30E+02	4.5
t034	25	8.55E+02	6.5
t011	25	2.13E+07	2.5
t034	25	2.62E+07	2.5
t011	37	5.85E+02	1.6
t034	37	8.45E+02	1.6
t011	37	1.82E+07	1.1
t034	37	2.76E+07	1.1

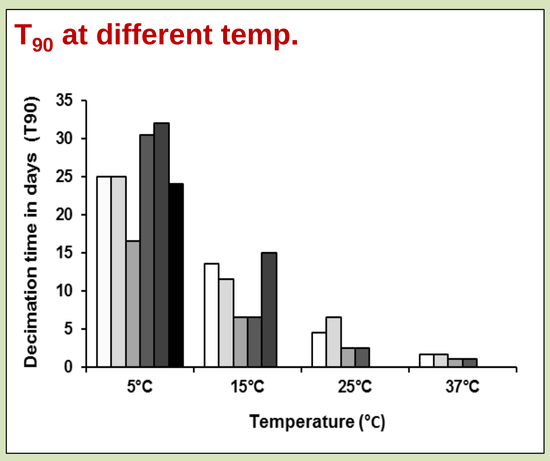

Figure 1. **Decimation times of various initial LA-MRSA loads at different temp.** White = t011 10^2 CFU/ml, light grey = t034 10^2 CFU/ml, grey = t011 10^7 CFU/ml, semi-dark grey = t034 10^7 CFU/ml, dark grey = natural sample 100 CFU/ml, black = natural sample = 20 CFU/ml.

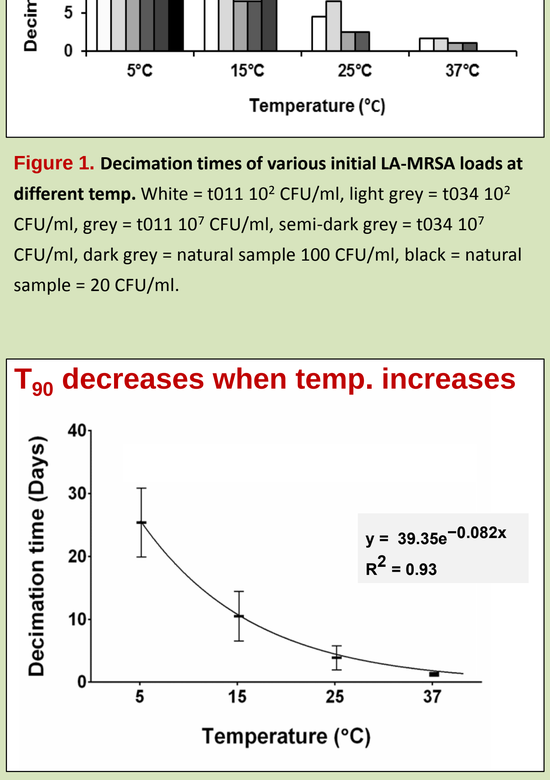

Figure 3. **Decimation times for samples with various initial LA-MRSA loads at different temperatures explained by exponential regression.**

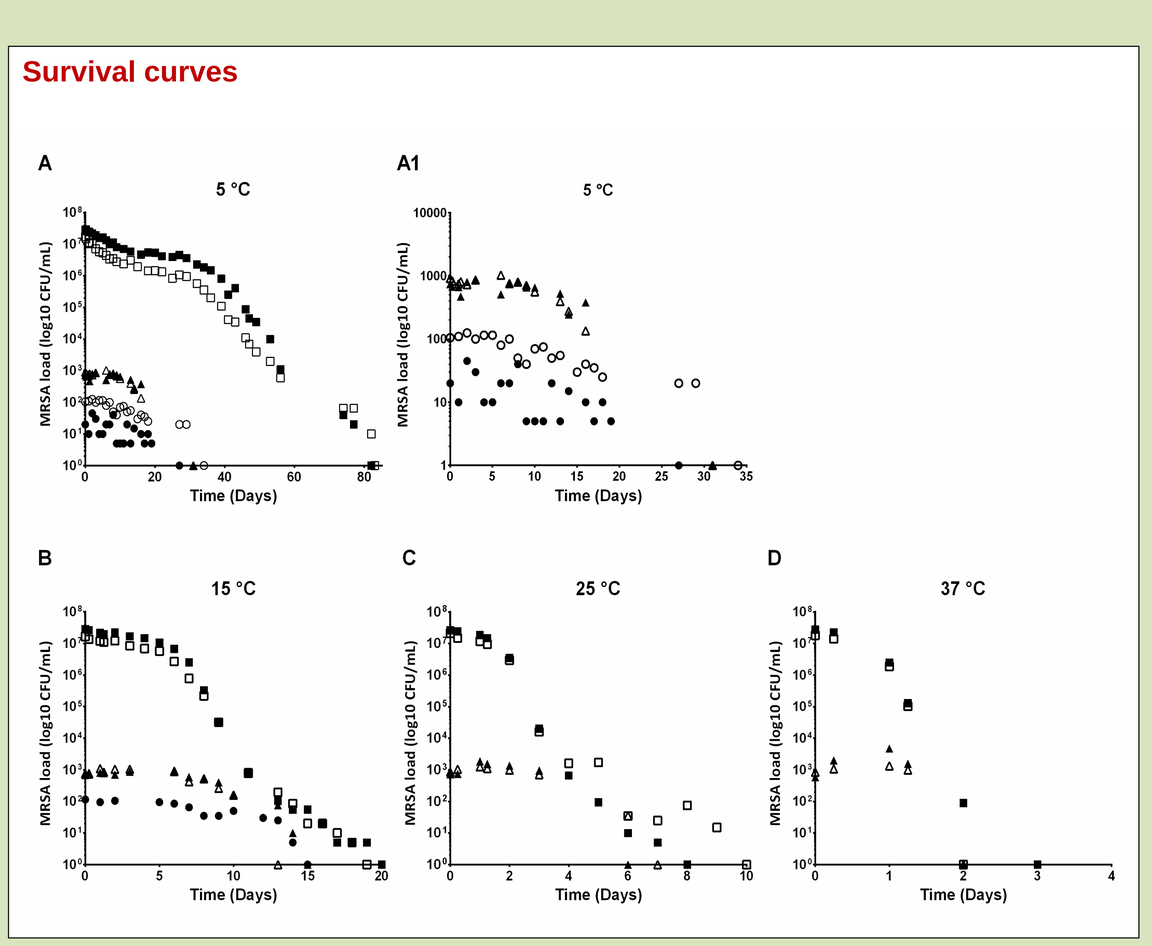

Figure 2. **Survival curves of LA-MRSA in natural positive samples and spiked samples at four different temperatures.**
All figures: Closed square = t034 10^7 CFU/ml, open square = t011 10^7 CFU/ml, closed triangle = t011 10^2 CFU/ml, open triangle = t034 10^2. (A) 5 ˚C, closed circle = naural pos. sample 20 CFU/ml, open circle = natural sample 100 CFU/ml. (A1) 5 ˚C, close-up of low levels from A. (B) 15 ˚C, circle closed = natural sample 100 CFU/ml. (C) 25 ˚C. (D) 37 ˚C.

Results. Our results show that LA-MRSA in non-spiked natural LA-MRSA positive swine manure was able to survive for at least 32 days at 5 ˚C and 16 days at 15 ˚C with a natural load of 5.00 – 1.90 $\times 10^2$ CFU/ml. At 37 ˚C LA-MRSA was undetectable already after 24h. In spiked swine manure the survival of *spa* type t011 and t034 was similar and was found to be temperature dependent (Table 1). The decimation time (T_{90}) was found to depend highly on the temperature and to a lesser extend on the initial LA-MRSA load as seen in Figure 1. The initial load of LA-MRSA however had an influence on the decay rates.
With a high spiking level the decay of LA-MRSA, until decimation time, at 5 ˚C could be explained by exponential regression, whereas the decay at 15 ˚C, 25 ˚C and 37 ˚C could be explained by linear regression. The survival in samples with low spiking, irrespective of temperature, was less predictable and the decay could not be described by either linear or exponential regression. At 5 ˚C LA-MRSA was stable until day 7, whereas an increase in LA-MRSA was observed at 15 ˚C, 25 ˚C and 37 ˚C, before a continuous decay of LA-MRSA could be detected (Figure 2).
The detected decimation times could, irrespective of initial load, be explained by exponential regression dependent on temperature with $R^2 = 0.93$ (Figure 3).

In conclusion: **The survival of LA-MRSA in manure was shown to be temperature dependent. Furthermore, the duration of LA-MRSA survival for at least 16 days at temperatures likely to prevail during normal manure storage (15˚C), may constitute a risk that the LA-MRSA can transit the manure storage tank and thereby reach the manured fields in a viable state during seasons of manure application.**

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