

Survival of Salmonellas in Urine and Dry Faeces from Cattle – An Experimental Study

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Plym-Forshell L. and I. Ekesbo: Survival of Salmonellas in urine and dry faeces from cattle. – An experimental study. Acta vet. scand. 1996, 37, 127-131. – In order to contribute to the understanding of Salmonella transmission via animal excretes the survival of Salmonellas in cattle urine and in dry cow faeces was studied. It was shown that in urine, separated in the gutter without active mixing with faeces, Salmonella did not survive more than 5 days. In dry cow faeces on different stall surfaces *Salmonella Dublin* were found to survive for almost 6 years on the 4 tested surface materials.

cow; dehydration; bacterial cells; disinfection; environmental contamination.

Introduction

Knowledge of the tenacity of Salmonella bacteria in different environments is of great importance in order to understand the epidemiology of Salmonella infections. Knowledge is also vital for effective disinfection and sanitation procedures. The role of animal production in the epidemiology of Salmonella infections has met an increasing interest during the last decades. The aim of this study was to increase the knowledge of the survival of salmonellas in cattle urine and in dry cow faeces.

Materials and methods

Salmonella Senftenberg and *Salmonella Typhimurium* were used as test bacterias in the urine experiments and *Salmonella Dublin* in the faeces experiments. The strains were streaked on Purple Lactose Agar (Difco, Detroit, USA) and incubated at 37° C for 24 h. Two colonies of each strain were then transferred to 20 ml of Nutrient Broth (Oxoid, CM 67, Basinstoke,

G.B.) and again incubated at 37° C for 24 h. The concentrations of the Salmonella strains were estimated by making total viable counts on TGE-Agar (Oxoid, CM 127) incubated at 30°C for 72 h during which time the broths were kept at 4°C.

Urine experiments

Urine was collected from a urine pit in a dairy farm. Details of the farm have been previously described (Ekesbo 1979). The urine had been separated in the gutter and no dung-yard runoff nor silage silo drainage were mixed with the urine. The urine was seeded with the 2 Salmonella strains, both in concentrations of 10³ and 10⁴ cells/ml of urine. Experimental temperatures were 6° and 21°C.

10 ml samples were taken every 24 h. The pH of the urine was measured at the same time as sampling. The 10 ml samples were added to 90 ml of buffered peptone water and incubated at 37°C for 18-24 h. Five ml of this preenriched broth was added to 45 ml of selenite broth (Ox-

oid CM 395; Oxoid L 121) and incubated at 37°C. After 24 and 48 h 10 µl was streaked on the surface of Brilliant Green Agar (Oxoid CM 329) which was incubated at 37°C for 24 h. Colonies resembling Salmonella were tested biochemically and serologically and for sensitivity against 0-1 bacteriophage.

Faeces experiments

Fresh cattle manure taken in the gutter in a dairy herd was inoculated with *S. Dublin* to give a concentration of approximately 10⁷ cells/gram of manure. One kg of this infected manure was spread out on 0.5 m² respectively of 4 different stall surface materials. The materials studied were concrete, rubber and 2 types of non-woven polyester. The manure was removed after 15 min with a scraper. The following 26 days 1 kg of non-infected fresh cow manure was spread each day on the test surfaces and removed after 15 min. In this way an attempt was made to get an experimental design which was reasonably well reflecting a natural situation in which a carrier-cow could contaminate the indoor environment.

After day 26 no more manure was streaked out and successively the test surfaces started to dry out. Samples were taken weekly by removing approximately 10 grams of the dry manure with sterile scalpels. The samples were treated as described above with the exception that the selenite broth was incubated at 43°C. This temperature was chosen because we had seen a better detrimental effect on non-Salmonella at this temperature than at 37°C in faecal samples.

After 15 weeks sampling was made monthly and after 42 months every third month.

After 30 months the enrichment procedure was changed in such a way that 0.1 ml of the pre-enriched sample was transferred to 9.9 ml of modified Rappaport-Vassiliadis broth (Merck 7700 Darmstadt, Germany). This broth was incubated at 41.5°C for 24 h (Peterz *et al.* 1989).

The experiment was performed in an old barn no longer in use and the temperature and relative humidity in the barn correlated closely with outdoor conditions.

The experiment was stopped after getting 3 Salmonella-negative samples successively.

Results and discussion

Urine experiments

Survival of the tested Salmonella bacteria was found to be short (Table 1). The concentrations of the Salmonella strains used in the experiments were based on studies by Munch *et al.* (1987) which showed that the concentrations of Salmonellas in the slurry tanks in naturally infected herds were at the most 10⁴ cells/ml of slurry. It seemed fair to assume that in urine separated in the gutter the concentrations of Salmonellas would not be higher than in the slurry. The experimental situation therefore most likely represented what would in a natural situation be extreme concentrations.

We expected to find survival of *S. Typhimurium* at 6°C to be at least as good as at the higher temperature, and we cannot find a plausible explanation for this result. The other results of the experiment were in accordance with earlier studies of survival of Salmonellas in fresh urine (Scheffer *et al.* 1955, Meeser 1960, Blum 1968). Cow urine contains both bacteriostatic and bacteriocidal substances (Scheffer *et al.* 1955). One example being NH₃ which is rapidly formed by transformation of urea in the urine (Hansen 1941). Nevertheless Scheffer *et al.* (1955, 1956) stated that the bacteriocidal effect of urine is independent of nitrogen content.

In urine mixed with household sewage Best *et al.* (1971) found survival times of 5 different Salmonella strains to vary between 5 and 16 weeks. The temperature in the experiments varied between 4.5° and 13.5°C and the pH varied between 8.4 and 8.9. These results indicate that

Table 1. Survival of *S. Typhimurium* and *S. Senftenberg* at 6°C and 21°C in cow urine inoculated with 10³ and 10⁴ cells/ml.

Day	Urine pH	<i>S. Typhimurium</i>				<i>S. Senftenberg</i>			
		10 ³ cells/ml		10 ⁴ cells/ml		10 ³ cells/ml		10 ⁴ cells/ml	
		6°	21°	6°	21°	6°	21°	6°	21°
1	7.8	-	-	-	+	+	+	+	+
2	7.8	-	-	-	+	+	+	-	+
3	8.0	-	-	-	+	-	+	+	+
4	7.9	-	-	-	+	+	+	-	-
5	8.1	-	-	-	-	-	+	-	-
6	7.7	-	-	-	-	-	-	-	-
7	8.1	-	-	-	-	-	-	-	-
8	7.8	-	-	-	-	-	-	-	-

+ = viable *Salmonella* found; - = viable *Salmonella* not found.

the antibacterial effect of urine decrease when the urine is diluted. This is further supported by studies of the antibacterial properties of urine in other species, e.g. man, cat and rabbit (*Asscher et al.* 1966, *Mullholland et al.* 1969, *Lees et al.* 1979).

Quick and effective separation of the urine in the gutter and storage without further dilution or mixing with other farm effluents seems to be beneficial from a hygienic point of view.

Dry faeces experiments

Viable *S. Dublin* were found in the dry faeces on all tested materials for 68 months but not in the subsequent 3 samples taken. Already half a century ago studies by *Henning* (1939) and *Lerche* (1939) showed that the survival of *S. Dublin* and *S. Typhimurium* in dry faeces was considerable.

This ability of the *Salmonella* cell to survive for many years in dry environments has also been demonstrated in floor dust (*Robertson* 1972) and in hatcher chick fluff (*Miura et al.* 1964).

In dehydrated foods survival of *Salmonellas* is also reported to be long. For example *S. Eastbourne* survived for 13 years in chocolate and *S. Infantis* for 10 years in pasta (*D'Aoust* 1993).

Obviously the dehydrated *Salmonella* cell exhibits great resistance against some external factors. It is, for example, well known that *salmonellas* are not very heat resistant but nevertheless *Kirby & Davies* (1990) found a dramatic increase in heat resistance in dehydrated *Salmonella* cells.

The epidemiology of *S. Dublin* infections in cattle differs from other serovars in some ways. The most important difference is quite clearly that some infected animals become permanent carriers for many years if not for life (*Bulgin* 1983, *Wray et al.* 1989). Our own experience from Sweden indicates that *S. Dublin* infections generally persist longer in a herd than other serovars. This is also reported from England (*Wray et al.* 1989). Beside this, reinfections with *S. Dublin* are not rare in Swedish cattle herds. The remarkable capacity for survival under dry conditions can not be neglected when explaining these reinfections.

Recently we isolated *S. Typhimurium* from an empty slurry pit on a dairy farm. The pit had not been used for the last 4 years. Before the pit was taken out of use, infection with *S. Typhimurium* was verified in the herd.

The importance of careful and consistent clean-

ing and disinfection after Salmonella outbreaks thus must be stressed. This is also emphasised by McLaren & Wray (1991), who found that on some premises the organism was found to persist in the environment despite cleaning and disinfection. On some farms inadequate cleaning aggravated the contamination.

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Sammanfattning

Överlevnad av Salmonellabakterier i intorkad koträck och -urin. – En experimentell studie.

S. Typhimurium och *S. Senftenberg* tillsattes urin, passivt avskiljd i gödselrännan, i mängder av 10^3 resp. 10^4 celler/ml urin. Försöket utfördes vid +6° resp. +21°C. Längst överlevnad observerades för *S.*

Senftenberg, som överlevde i urinen 5 dygn vid 21°C. Färsk koträck tillsatt med *S. Dublin* applicerades på båsfall och tilläts så småningom torka in. Fyra olika båsfalltyper användes, betong, gummimatta och 2 typer av polyestermatta. *S. Dublin* överlevde 68 månader i den intorkade träcken på samtliga underlag. Resultaten diskuteras utifrån hygieniska synpunkter.

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