Recovery of salmonella serotypes from swine faecal samples using ISO 6579

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Introduction

Standard procedures to examine the contamination of faecal samples for salmonella are usually focussed towards the isolation of a single salmonella culture. Isolation procedures for salmonella consist of four distinct phases: 1) non selective pre-enrichment; 2) selective enrichment; 3) selective isolation and elective growth to produce suspect isolates; 4) confirmation of the isolates. In most cases only one suspect colony is picked out for confirmation. In previous experiments we observed that more than one salmonella strain could be isolated if more than one suspect colony from the same sample was investigated. Growth rates and sensitivity towards selective compounds during isolation procedures could influence the recovery of individual salmonella strains in a sample. The objective of this study was to determine whether the recovery rate of different salmonella strains/serotypes, present in one sample, was equal for all serotypes.

Materials and methods

Two experiments were carried out with salmonella strains of 4 different serotypes, all having different O-antigens: *S. typhimurium*, *S. panama*, *S. london* and *S. infantis*. The four strains were grown on Brain Heart Infusion broth (oxoid CM255) for 20 hours at 37°C.

In the first experiment 6 samples of sterile pig faeces of 25 gram each were inoculated with 10 colony forming units of 2 different serotypes of salmonella (all combinations of the 4 serotypes, see table 1). Isolation of salmonella, according to ISO 6579, was started immediately after inoculation of 6 samples. Duplicate samples were stored overnight at 25°C, whereafter isolation procedures were started. In the second experiment 6 samples of sterile pig faeces of 25 gram each were inoculated with four different salmonella serotypes in different ratios (see table 2). Isolation of salmonella was started immediately after inoculation. In both experiments, 10 suspect colonies were picked from the brilliant green agar plate and cultured overnight on plate count agar at 37°C. The confirmation step was not carried out, because salmonella was the only inoculated organism in the sterile faeces. Isolated salmonella strains were typed with monovalent specific "O" anti-sera (Pro-lab Diagnostics) to identify O-antigens and thus serotypes.

Results

Salmonella was isolated out of all samples in both experiments. The results of the first experiment showed that *S. infantis* and *S. london* were recovered more frequently than *S. typhimurium* and *S. panama* (see table 1). *S. infantis* and *S. london* were isolated in equal amounts in experiment 1, whereas *S. london* was isolated more frequently than *S. infantis* in experiment 2. The recovery rate of the different salmonella strains was not influenced by the incubation time of salmonella in the faeces. The results of the second experiment showed that *S. london* was the most frequently

Table 1: Experiment 1

Sample no.		Inoculum (in CFU per gram faecal sample)	Serotypes of 10 resulting colonies
A1	Isolation started immediately after inoculation	10 S. typhimurium + 10 S. infantis	10 x S. infantis
B1		10 S. typhimurium + 10 S. panama	6 x S. typhimurium + 4 x S. panama
C1		10 S. typhimurium + 10 S. london	10 x S. london
D1		10 S. infantis + 10 S. panama	10 x S. infantis
E1		10 S. infantis + 10 S. london	7 x S. infantis + 3 x S. london
F1		10 S. panama + 10 S. london	10 x S. london
A2	Isolation started after overnight storage at 25°C after inoculation	10 S. typhimurium + 10 S. infantis	10 x S. infantis
B2		10 S. typhimurium + 10 S. panama	10 x S. typhimurium
C2		10 S. typhimurium + 10 S. london	10 x S. london
D2		10 S. infantis + 10 S. panama	9 x S. infantis + 1 x S. panama
E2		10 S. infantis + 10 S. london	5 x S. infantis + 5 x S. london
F2		10 S. panama + 10 S. london	10 x S. london

Table 2: Experiment 2

Sample no.	Inoculum (in CFU per gram sample)				Serotypes of 10 resulting colonies			
	S. typhi-murium	S. infantis	S. panama	S. london	S. typhi-murium	S. infantis	S. panama	S. london
1	10	10	10	10	0	2	0	8
2	50	10	10	10	0	1	0	8
3	10	50	10	10	0	0	0	10
4	10	10	50	10	0	2	0	8
5	10	10	10	50	0	4	0	5
6	50	50	10	10	0	4	0	5

Discussion

From these preliminary results we can conclude that salmonella strains differ in recovery rate during standard isolation procedures. This implies that the presence of certain strains can be underestimated in samples that appear to be contaminated with more than one salmonella strain. It is even possible that, when 2 different salmonella serotypes are present in one sample, a particular salmonella strain will not be isolated, even if it was the major salmonella strain in the sample.

In these preliminary experiments we found a clear hierarchy in recovery rate of the 4 salmonella strains that were used (decreasing recovery rate) : *S. infantis/london – S. typhimurium – S. panama*. In the experiments no other micro-organisms were present in the faeces, so there was no competition between salmonella and other bacteria. If a competitive flora had been present in the faeces, the recovery rate of salmonella may have been different. Additional research should be conducted to verify these data.