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## **FAECAL BACTERIA AND COLIPHAGES IN RUN-OFF FROM DAIRY FARMS**

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### **Introduction**

Along with the recent growth in the size of dairy farms, problems with slurry management have increased in Finland. Leaks of slurry during storage, transport and spreading, and water flows from pastures and outdoor yards can act as vectors of disease transmission from agricultural areas. Microorganisms may enter surface water via overland flow pathways, by subsurface transfer routes in highly permeable soils or through artificial field drainage (4). Here, observations were made of hygiene indicators (faecal coliforms, enterococci, sulphite-reducing clostridia and coliphages) in waters in the surroundings of large dairy farms.

### **Materials and methods**

The study consisted of 19 recently enlarged dairy farms (47–168 milk cows/farm) located in Central Ostrobothnia and North Savo, Finland. A total of 92 water samples from open ditches, drain pipes and drain wells were collected adjacent to modern loose housing systems, farmyards, silage stores, slurry tanks, outdoor yards for cattle, fields and pastures, and drain outlets for household wastewater in 2002 and 2003. Control samples were taken from near-by ditches and lakes at more distant farms. Owing to the exceptional drought during the study, samples were taken only during the spring snow melt.

In another study at Jokioinen, surface run-off samples were collected from an experimental pasture (Lintupaju field) with either a 10-m wide grass buffer (GB) or a scrub buffer (SB) in 2003–2005. The GB was cut and the residue was removed annually but the SB growing grass and scrub plants was not harvested. Results from the pasture with 12–13 year-old GB and SB were compared with those from the pasture with a newly planted buffer grazed by cattle (GrB).

Water samples were filtered for faecal coliforms and enterococci through Millipore 0.45 µm and for sulphite-reducing clostridia through Millipore 0.22 µm filters. Faecal

coliforms were then cultivated on mFC agar (Difco™) and confirmed by oxidase test (8). Enterococci were cultivated on KF streptococcus agar (Difco), and colonies were confirmed with 3% H<sub>2</sub>O<sub>2</sub> (7). Sulphite-reducing clostridia were determined according to the European Norm on self-made media (1) and incubated in an Oxoid anaerobic jar. Water hygiene was further studied by counting somatic and RNA coliphages (*E. coli* ATCC 13706 and 15597 as hosts) according to the method of Grabow and Coubrough (2), as modified by Rajala-Mustonen and Heinonen-Tanski (6). All bacteria counts are expressed as geometric means of CFU per 100 ml and coliphage counts as PFU per 100 ml. For geometric means, half of the detection limits, 0.5 CFU/100 ml or PFU/100 ml, was used when the count was 0/100 ml.

### Results and discussion

The highest counts of all indicator microbes were measured in open ditches to which household wastewater had been piped from farm houses or in waters near outdoor yards (Table 1). Eight samples were also taken from surface run-off water from outdoor yards. There the counts of faecal coliforms, enterococci and sulphite-reducing clostridia were 6600, 150 000 and 64 CFU /100 ml, respectively, whereas the values of coliphages ATTC 13706 and 15597 were 66 and 13 PFU/100 ml, respectively. Very high faecal coliform values (700 000–120 000 000 CFU/100 ml) were also observed in run-off from an asphalt exercise yard for 100 milk cows in South Savo in 2001–2002 (5).

Fairly high enterococci counts were obtained from the surroundings of slurry tanks (22 000 CFU /100 ml), farmyards (6300 CFU /100 ml), silage stores (5100 CFU /100 ml) and loose housings (2500 CFU /100 ml) on the dairy farms. However, the counts of faecal coliforms and sulphite-reducing clostridia and coliphages were quite low in these samples. The lowest counts of faecal indicators were measured in waters from fields and pastures and in control samples. Note that, in this study, water was sampled only in spring during or after the snow melt. As the samples were often taken from open ditches near farm houses (surroundings of loose housings) they may contain waters from different sources.

In the grass field with buffers, the faecal coliform counts were low in surface run-off water before animals were grazed in the experimental area. During grazing, the counts increased, being at their highest in July and September in the rainy year of 2004 (Table 2). The 10-m wide GB (cut annually) reduced the faecal coliform counts by 21–96% compared with the pasture with a 10-m wide GrB. The 10-m wide SB (not harvested) frequently reduced the counts by 46–89%.

**Table 1. Geometric means for counts of faecal microorganisms in waters sampled from open ditches, drain wells and drain pipes on modern dairy farms in spring 2002 and 2003.**

<i>Source of impact</i>	N <sup>1)</sup>	Faecal	Enterococci <b>13706</b> CFU/100 ml	Sulphite- <b>coliforms</b> clostridia <sup>2)</sup>	ATTC <b>reducing</b> <b>15597</b>	ATTC
Household wastewater	9	680 000	510 000	230	100	5.8
Outdoor yards	5	970	72 000	150	83	12
Slurry tanks	8	39	22 000	1.0	11	5.7
Farmyards	7	300	6 300	10	0.6	0.5
Silage stores	11	54	5 100	12	1.3	0.9
Loose housings	20	68	2 500	4.7	3.1	1.6
Fields and pastures	17	9	1 200	2.3	1.2	0.5
Control areas	15	16	1 900	1.5	0.7	0.6

<sup>1)</sup> Number of samples

<sup>2)</sup> Total number of samples 85, but 92 in other analyses.

The faecal indicator microbes counts were quite low in most of the samples taken on modern dairy farms in spring. In summer and autumn, the counts may rise when manure is applied to fields, and cattle are grazed on pastures or given access to outdoor yards. The faecal indicator counts were highest in water samples taken from areas where there was dung or human faecal material, such as outdoor yards and household wastewater outlets. High values of faecal coliforms, enterococci, sulphite-reducing clostridia (880, 4800 and 1500 CFU/100 ml, respectively) and coliphages ATCC 13706 (2700 PFU/100 ml) were observed in surface run-off water sampled four days after surface broadcasting of cattle slurry to grass (3).

Table 2. Faecal coliform counts and (in parentheses percentage for reduction of coliforms) in surface run-off from a pasture with three different buffer zone types: grazed grass buffer (GrB), annually cut grass buffer (GB) and unmanaged scrub buffer (SB) growing natural grass and scrub plants.

Date	<b><i>Faecal coliforms (CFU/100 ml)</i></b>		
	GrB	GB	SB
26 Mar 2003	68	81	69
<b>19 Nov 2003</b>	<b>93</b>	<b>63 (32%)</b>	<b>28 (69%)</b>
5 Apr 2004	5	3	3
<b>1 Jul 2004</b>	<b>24 000</b>	<b>16 000 (33%)</b>	<b>27 000 (- 13%)</b>
27 Sep 2004	35 000	1 800 (95%)	4 000 (89%)
<b>29 Sep 2004</b>	<b>2 000</b>	<b>110 (94%)</b>	<b>710 (64%)</b>
12 Jan 2005	240	190 (21%)	130 (46%)
19 Jan 2005	1 200	49 (96%)	5 000 (- 317%)

## Conclusion

Although the indicator numbers were mainly quite low, there was a severe risk of transfer of pathogens to the environment, especially when household wastewater or surface run-off water from outdoor yards was poorly purified and allowed to flow into ditches and watercourses. More research is needed to establish the risk of pathogen transmission from livestock farms in different environments and seasons. Outdoor yards should be built so that they do not cause a risk of pathogen transmission to waters. In the future, household wastewater must be purified before it is released to the environment. The existence of buffer zones between fields and watercourses may reduce the numbers of faecal microbes in surface run-off water.

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