

## FAECAL MICROORGANISMS IN RUNOFF FROM CATTLE AND HORSE FARMS – QUANTIFICATION AND MITIGATION

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### SUMMARY

Nutrient runoff from agricultural sources to watercourses has been decreased in different ways during last decades. Indeed, until recent years, pathogen transmission via agricultural runoff waters has been under less concern. According our studies levels of faecal microbes can be extremely high (e.g.  $10^3$ – $10^6$  colony forming units of faecal coliforms in 100 mL) in runoff waters from exercising areas used by cattle or horses while the limit of good bathing water is <500 CFU/100 mL. The transfer of pathogens can be reduced e.g. by removing manure from lots, establishing of buffer zones and purification of waters.

**Keywords:** faecal indicator bacteria, faecal coliforms, horse paddocks, livestock, exercise yards, runoff, transport, water hygiene, buffer zones

### INTRODUCTION

Along with the recent growth in the size of cattle farms, their concentration in certain areas and increasing number of horses close to urban areas, hygiene problems with manure management and agricultural runoff are growing in Finland. Runoff waters from fields with slurry applied on the surface, yards used as exercising areas for cattle, and equine paddocks may contain high numbers of faecal microbes which can act as vectors of disease transmission from agricultural areas. This paper consists of levels of faecal coliforms, enterococci, presumptive *E. coli*, presumptive coliforms and sulfite-reducing clostridia which are used as indicators of potential microbial pollution in water, and how to mitigate pathogens from runoff water in cattle and horse farms. Results are compared with previous studies done at the MTT Agrifood Research Finland.

### MATERIALS AND METHODS

Study sites were 1) four exercise yards (covered with asphalt, woodchips or sand) and two open feedlots used by cattle, 2) a clay field with slurry applied on the surface and direct sowing, and 3) four horse paddocks. Because there was high variation in stocking rates (SR) and time animals spend in the yards (d), we calculated loading intensity (LI) according to the following formulas:

(1)  $LI = SRd$ , where

- LI=loading intensity (livestock unit day/hectare year; LSUday/ha yr),
- (1a) SR=stocking rate=LSU/A, where  
LSU=livestock unit (1 dairy cow or 1 horse = 1 LSU, 1 heifer or 1 bull less than 2-yr-old = 0.6 LSU), A=area of a lot (ha)
- (1b)  $d=tn/24$ , where  
 $t$ =exercising time/day (h), and  $n$ =number of exercising days/year.

Water samples were collected from sumps or drainage water wells adjacent to the exercise yards. Plant-soil-samples (10-cm-deep layers) were taken from (1) a clay field with a slurry application of  $20 \text{ t ha}^{-1}$ , (2) buffer zones (BZ) between the clay field and a ditch, and (3) four horse paddocks. The plant-soil-samples were used in a rain simulation study ( $20 \text{ mm h}^{-1}$ ) for 2 hours and surface water samples were collected during the rainfall simulation. The water samples were filtered for faecal coliforms, enterococci and both presumptive *E. coli* and coliforms through Millipore  $0.45 \mu\text{m}$  and for sulfite-reducing clostridia through Millipore  $0.22 \mu\text{m}$  filters. Faecal coliforms (SFS 4088), enterococci (SFS-EN ISO 7899-2) and *E. coli* were then cultivated on mFC agar (Difco), KF Streptococcus agar (Difco) and Harlequin *E.coli*/coliform medium (LabM), respectively. On the Harlequin medium, blue-purple colonies were counted as presumptive *E. coli*, and all blue-purple and magenta colonies were counted as presumptive coliforms. Sulfite-reducing clostridia were determined by SFS-EN 26461-2 and incubated in an Oxoid anaerobic jar. Bacteria counts were expressed as geometric means of colony forming units (CFU) per 100 mL of water.

## RESULTS

### *Exercise yards and outdoor feedlots*

Levels of faecal coliforms, enterococci and sulfite-reducing clostridia were the highest in surface runoff from asphalt yards (Table 1). Although some faecal micro-organisms were retained by woodchips on yard surface or by sand yards, the levels were still high in runoff. Soil filters used for cleaning runoff waters from asphalt yards were quite efficient at first but their purification capacity dropped if the suspended material was not removed from the water before purification (Uusi-Kämpä & Heinonen-Tanski 2000). After one year from the introduction of a 0.8-m-deep sand filter, faecal coliform levels from the asphalt yard runoff ( $7.0 \times 10^6$  CFU/100 mL) were decreased to outgoing runoff ( $3.6 \times 10^5$  cfu/100 mL). For enterococci and sulfite-reducing clostridia 10–100-fold lower values were obtained after the filtration of yard runoff by the 0.8-m-deep sand layer. Soil or sand floor of yards decreased bacteria levels compared with asphalt yards (Table 1). Also at a forested feedlot (2200 LSUday/ha yr), levels of faecal coliforms and sulfite reducing clostridia in a ditch water were small (220 and 44 CFU/100 mL, respectively; Uusi-Kämpä et al. 2006).

**Table 1.** Stocking rate, loading intensity, and geometric means of faecal indicator levels in runoff water from exercise yards and forested feedlots. (Number of samples is presented in parentheses)

Exercise yard /Feedlot	Stocking rate (LSU/ha)	Loading intensity (LSU day / ha yr)	Faecal coliforms (CFU/100 ml)	Enterococci (CFU/100 ml)	Sulfite-reducing clostridia (CFU/100 ml)
Asphalt yard (4) <sup>(a)</sup>	370–670	17,000–30,000	$4.8 \times 10^6$	$3.8 \times 10^6$	$4.6 \times 10^3$
Yard covered with woodchips (14) <sup>(b)</sup>	620–1100	28,000–50,000	$5.0 \times 10^4$	$7.8 \times 10^3$	150
Sand yard (3) <sup>(c)</sup>	330	45,000	$7.2 \times 10^4$	$2.3 \times 10^4$	120
Asphalt yard (1) <sup>(d)</sup>	770	30,000	$7.0 \times 10^6$	$3.1 \times 10^6$	$1.1 \times 10^4$
Asphalt lot covered with woodchips (2) <sup>(e)</sup>	140	30,000	$5.9 \times 10^4$	$1.9 \times 10^4$	$2.8 \times 10^3$
Forested feedlot (3) <sup>(f)</sup>	77	17,000	$1.1 \times 10^4$	$3.8 \times 10^3$	250

(a), (b) Established in 2000, studied between 2001 and 2006, ca 3 h exercising in the yard daily for 12 months/yr (Uusi-Kämpmä et al. 2006)

(c) Established in 2004, studied between 2005 and 2006, 24 h exercising in the yard daily for 4.5 months/yr

(d) Study in June 2000, ca 2 h exercising in the yard daily for 7.5 months/yr (Uusi-Kämpmä & Heinonen-Tanski 2000)

(e) Study in June 2000, rearing daily (24 h) for 7.5 months/yr (Uusi-Kämpmä & Heinonen-Tanski 2000)

(f) Established in 1995, rearing daily (24 h) for 7.5 months/yr, studied percolation water at 0.3 m, near feeding fences in 2000 (Uusi-Kämpmä & Heinonen-Tanski 2000)

### *Field runoff and surface runoff under rainfall simulation*

Levels of faecal coliforms were high in the surface runoff from a pasture in summer 2004 and 2005 decreasing quite soon in the cool autumn weather. For the faecal coliforms, 2–20-fold lower values were generally obtained in the surface runoff from pasture with a 10-m-wide cut grassBZ than from the pasture without a BZ (No-BZ). In the USA, Frantz et al. (2003) presented that a BZ system was able to reduce pathogen indicator levels from dairy wastewater by 100 to 10,000-fold. On an uncut vegetated BZ, the decrease of faecal coliform levels was less than on the grassBZ and sometimes the levels also might increase compared with the no-BZ. A reason for the increase of faecal coliforms might be voles living on the vegetated BZ. Sometimes they were found in the collected runoff water which was sub-sampled.

At a rainfall simulation study, the levels of enterococci, *E. coli*, coliforms and sulfite-reducing clostridia were high in surface runoff from 10-cm-deep plant-soil-systems two weeks after cattle slurry application (20 t/ha) (Table 2). The levels of hygiene indicators in surface runoff from BZs (not amended with slurry) were generally 100–1000-fold smaller compared to the levels of slurry-applied field runoff. In our study, levels of sulfite-reducing clostridia decreased below the detection limit in 6 weeks from the slurry application. The other indicator levels also decreased little by little during 6 weeks and especially after freezing the plant-soil-samples. In an earlier study, where slurry was applied on the surface of grass field with a 10-m-wide unmanaged BZ, the levels of total coliforms, enterococci and sulfite-reducing clostridia were in surface runoff water similar ( $1.9 \times 10^4$ ,  $4.8 \times 10^3$  and  $1.5 \times 10^3$  CFU/100 ml, respectively) as presented here (Heinonen-Tanski & Uusi-Kämpmä 2001).

**Table 2.** Geometric means for numbers of hygiene indicators in runoff water from plant-soil-samples under a rainfall simulation two weeks after a slurry application and direct sowing in September 2006. Slurry was applied on the field and on the No-BZ

Treatment	Enterococci (CFU/100 mL)	<i>E. coli</i> (CFU/100 mL)	Coliforms (CFU/100 mL)	Sulfite-reducing clostridia (CFU/100 mL)
Field (n=6)	$2.9 \times 10^6$	$5.2 \times 10^4$	$1.2 \times 10^6$	710
No-BZ (n=2)	$8.6 \times 10^5$	$2.5 \times 10^4$	$2.1 \times 10^5$	230
Cut grassBZ (n=2)	$3.8 \times 10^3$	91	$5.8 \times 10^3$	13
Uncut vegetated BZ (n=2)	$1.4 \times 10^5$	50	$3.3 \times 10^4$	<1

### *Equine paddocks*

Under a rainfall simulation, the levels of *E. coli* and coliforms were very high in surface runoff from soil samples taken from horse paddocks (Table 3). The samples were collected after a warm and dry summer in August 2006. The highest levels were measured from the paddocks 1 and 2 where the manure was not removed from the surface. Numbers of *E. coli* were also smaller in runoff from paddocks covered with woodchips or established on a clay soil. Addition of 10 t Ca(OH)<sub>2</sub> ha<sup>-1</sup> to the surface of the soil before rainfall simulation decreased coliform levels in the runoff (Table 3). In drainage water from the paddock 3, the levels of *E. coli*, coliforms and faecal coliforms were also rather high ( $1.3 \times 10^4$ ,  $5.6 \times 10^4$  and  $2.3 \times 10^4$  and CFU/100 mL, respectively) in the end of October 2006. Levels of sulfite-reducing clostridia were generally under the detection limit in the horse paddock runoff. According to Airaksinen et al. (2007) the levels of faecal coliforms, enterococci and sulfite-reducing clostridia were 5–4700, 18–2200 and <100 CFU/100 mL in surface runoff from two horse paddocks (37.5 animals/ha) in eastern Finland.

**Table 3.** Loading and geometric means for numbers of hygiene indicators in runoff water from paddock soil samples under a rainfall simulation in August 2006. (n=number of samples)

Place / Treatment	Loading (LSU day/ha yr)	<i>E. coli</i> (CFU/100 mL)	Coliforms (CFU/100 mL)
Paddock 1 (rubble cover and manure; n=2)	4400	$3.2 \times 10^7$	$7.8 \times 10^7$
Paddock 1 (rubble cover; n=2)	4400	$1.8 \times 10^5$	$9.2 \times 10^5$
Paddock 2 (sand cover with manure; n=2)	7000	$1.4 \times 10^5$	$1.4 \times 10^6$
Paddock 2 (sand cover; n=2)	7000	$1.1 \times 10^5$	$1.1 \times 10^6$
Paddock 2 + 10 t Ca(OH) <sub>2</sub> ha <sup>-1</sup> (n=1)	7000	<5	<5
Paddock 3 (covered with woodchips; n=2)	6000	$9.5 \times 10^4$	$2.6 \times 10^6$
Paddock 4 (clay soil; n=2)	8200	$2.0 \times 10^4$	$4.0 \times 10^5$

Paddock 1: 710 m<sup>2</sup>, 1–2 horses for 4 periods between 1.5 and 4.5 hours daily since 2005

Paddock 2: 520 m<sup>2</sup>, 1–3 horses for 4 periods between 1.5 and 4.5 hours daily since 2003

Paddock 3: 1900 m<sup>2</sup>, 7 horses for 11 hours daily since 2005

Paddock 4: 800 m<sup>2</sup>, 4 horses for 11 hours daily since 2001

A sedimentation pond with an addition of ferric sulphate was used for phosphorus retention from runoff waters from equine areas at Ypäjä, Southwest Finland (Närvänen et al. 2006). This system also seemed to decrease numbers of hygienic indicators in outgoing water compared with incoming runoff (Table 4).

## CONCLUSIONS

Most runoff waters contaminated by faeces would not fulfil the requirements for bathing waters, where faecal coliforms should be less than 500 CFU/100 mL and enterococci less than 200 CFU/100 mL. The feeding and drinking areas were the most contaminated areas both on feedlots (Uusi-Kämpmä & Heinonen-Tanski 2000) and horse paddocks (Airaksinen et al. 2007). Exercise yards and horse paddocks should be established so that the risks of pathogen transmission to waters can be controlled. Cleaning of paddocks, establishing buffer zones between source areas and waterways, and e.g. addition of calcium hydroxide to critical source areas decrease faecal microorganisms in runoff water. Filters blocked with suspended materials were not able to reduce levels of neither faecal microorganisms nor other pollutants from the yard runoff. More research is, however, needed to solve hygiene problems in agricultural source areas.

**Table 4.** Numbers of hygiene indicators in grab samples of incoming runoff water from equine areas and outgoing water purified by ferric sulphate in a sedimentation pond

Date/incoming/outgoing	Faecal coliforms (CFU/100 mL)	Enterococci (CFU/100 mL)	<i>E. coli</i> (CFU/100 mL)	Coliforms (CFU/100 mL)
15 Nov 2005 / incoming	$5.0 \times 10^4$	$9.4 \times 10^3$	n.a.	n.a.
15 Nov 2005 / outgoing	$3.2 \times 10^4$	$8.8 \times 10^3$	n.a.	n.a.
25 Oct 2006 / incoming	$7.2 \times 10^4$	n.a.	$2.7 \times 10^3$	$4.4 \times 10^4$
25 Oct 2006 / outgoing	$1.3 \times 10^3$	n.a.	<500	<500
7 Dec 2006 / incoming	n.a.	400	600 <sup>(a)</sup>	n.a.
7 Dec 2006 / outgoing	n.a.	0	0 <sup>(a)</sup>	n.a.
14 March 2007 /incoming	n.s.	n.s.	n.s.	n.s.
14 March 2007 /outgoing	n.a.	69	0	130

n.a.=not analysed, n.s.=not sampled

<sup>(a)</sup>=used the standard of SFS 3016 and confirmed by indole test

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