Line Fredslund

PATHOS

Leaching of pathogens from manure to drainage water – assayed using classic and DNA/mRNA based methods

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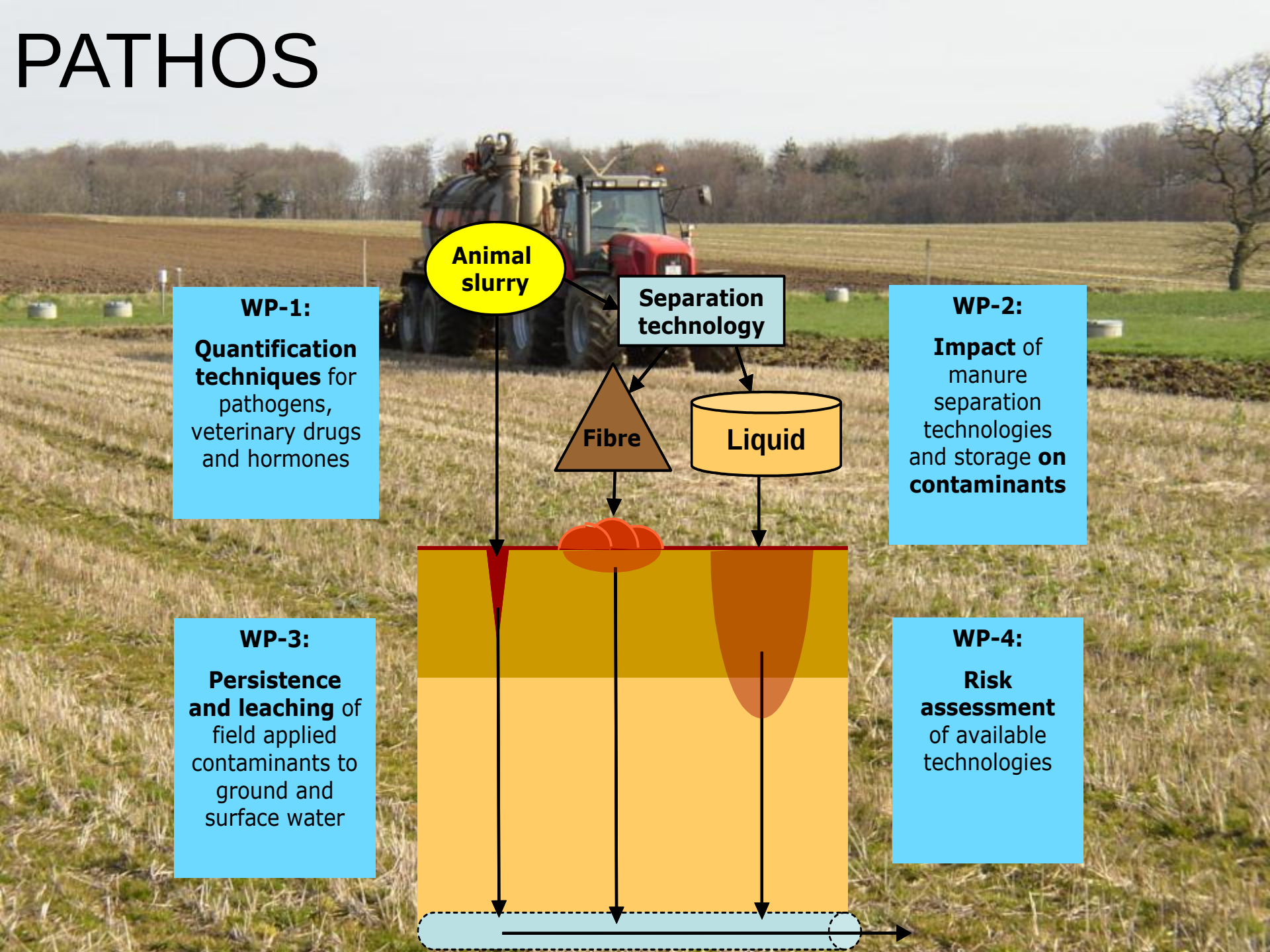

WP-1:

Quantification techniques for pathogens, veterinary drugs and hormones

WP-2:

Impact of manure separation technologies and storage **on contaminants**

WP-3:

Persistence and leaching of field applied contaminants to ground and surface water

WP-4:

Risk assessment of available technologies

Background National investigation

GEUS pesticide survey on clayey areas found pathogen indicator bacteria in ¼ private water supply

Bacterial parameter	Treshold value	Drillings / installations		% drills / installations ≥ treshold	Max. value
		Number of examined	Number above tresholds		
Kimtal ved 37 ˚C PCA	20	618	200	**32**	>2.000
Kimtal ved 21 ˚C Kings B	200	416	151	**36**	>2.000
Coliforme bacteria 37 ˚C	Non measurable	619	164	**26**	>160
Thermotol. coliforme bacteria 44 ˚C	Non measurable	619	94	**15**	>160
All bacterial parameters	-	619	297	**48**	>2.000

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Background International investigations –
Pathogens survival and leaching after manure application

- In the city of Walkerton (Ontario, Canada) manure contaminated drinking water supply with *E.coli* O157 and *Campylobacter jejuni*. 2300 persons received medical treatment, 7 died, (Goss et al., 1998)
- Between 1000 and 10.000 *E.coli* O157 per ml drainage water could be found after disposal of manure on an Irish field (Vinten et al., 2002)
- Pathogenes like *E.coli, Salmonella* sp., *Campylobacter* sp. and *Listeria* sp. dies out in 2-8 days in old-fashioned manure heaps, but they survive in high numbers after 6 month in an anaerobic manure tank (Nicholson et al., 2005).
- But what is the influence of the method we use to asses the survival ? mRNA, DNA or CFU ?

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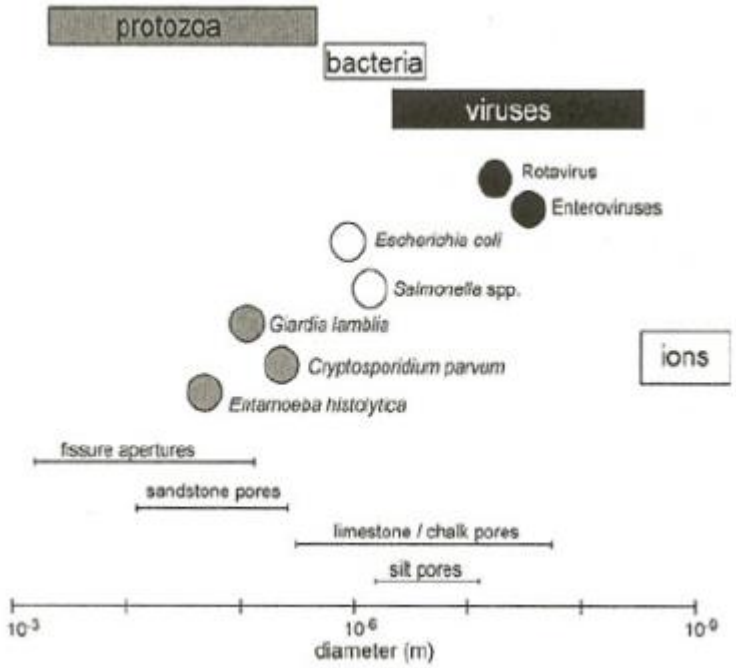
Zoonotic pathogens in PATHOS

- Bacteriophages
- Bacteria
  - *Salmonella*
  - *E. coli*
  - *Campylobacter*
  - Tetracycline resistant bacteria
- Protozoa
  - *Cryptoporidium*
  - *Giardia*



(Taylor et al (2004): FEMS microbiology vol. 49)

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Survival of slurry pathogens in soil

- The physiological state of the microorganism
- Biological interactions (competition from indigenous microflora)
- Physical and chemical nature of soil water:
  - **pH, soil water content, SOM, availability of nutrients, adsorption properties**
- Atmospheric conditions
  - **sunlight, precipitation, temperature**
- Application method
  - **technique, frequency of application, organisms concentration in manure***

Microorganism	Soil type	manure type and inoculation	Survival (day)	T_{90}
S. *Typhimurium*	Unknown	Cattle manure (10^7)	231	32.8
S. *Typhimurium*	Loamy sand	Cattle manure (10^5)	119	23.6
S.*Typhimurium*	Sandy loam	pig manure (10^6)	56	9.2
S.*Typhimurium*	Loam	Cattle manure (10^7)	56	7.9
Salmonella sp.	Clay	pig manure (10^5)	180	35.8
S. *Newport*	Silt loam	Cattle manure (10^7)	332	47.3

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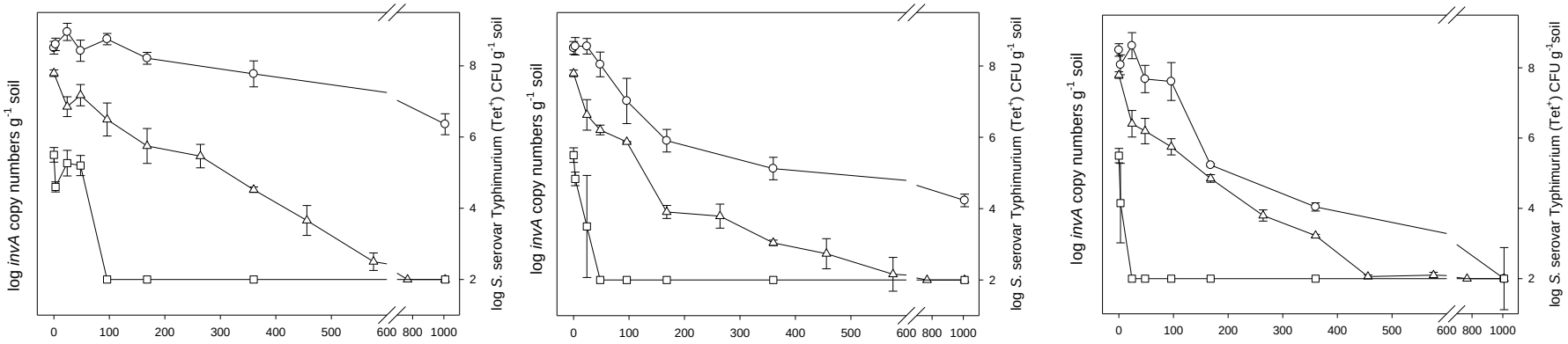
Lab experiments – Survival of *Salmonella* ser. Typhimurium in soil mixed with slurry

5ºC **15ºC** **25ºC**



Evolution of *S*. serovar Typhimurium (Tet+) measured by quantitative PCR and plate counting in manure-amended soil samples at three different temperatures.

( ) **log *invA* DNA** copies g^{-1} soil;
( ) **log *invA* mRNA** copies g^{-1} soil;
(Δ) **log CFU** g^{-1}.

Detection limit = 10^2. Error bars represent standard error of the mean. Please note the broken *x* axes

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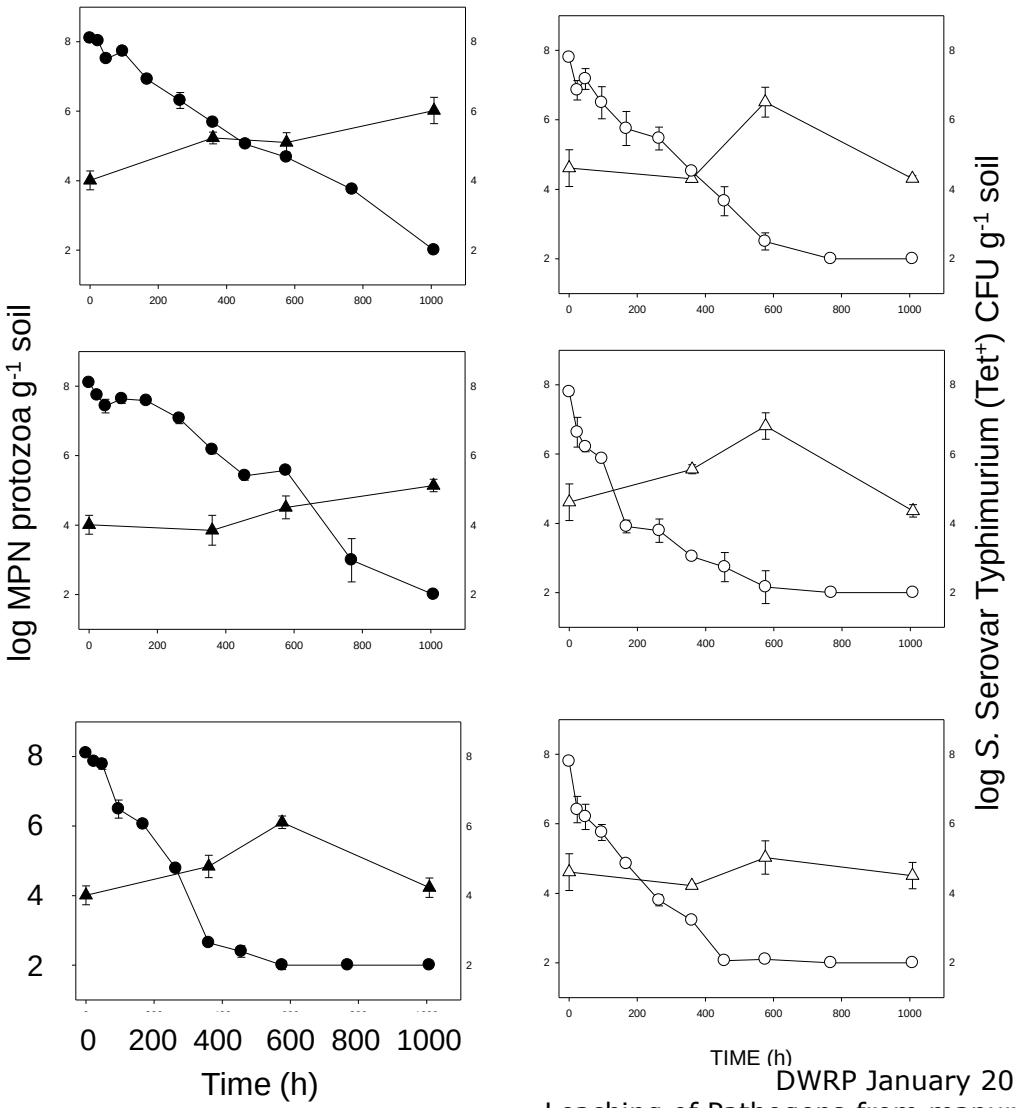
Protozoan predation may explain *Salmonella* disappearance in this system



Evolution of *S.*serovar Typhimurium CFU (,) and protozoan levels (Δ,) in soil samples (closed symbols) and manure-amended soil samples (open symbols) incubated at three different temperatures.

Protozoa counting were made at 360, 576 and 1008 hours after the beginning of the assay.

Protozoan levels at time 0 were determined before the addition of *Salmonella*. Error bars represent standard error of the mean.

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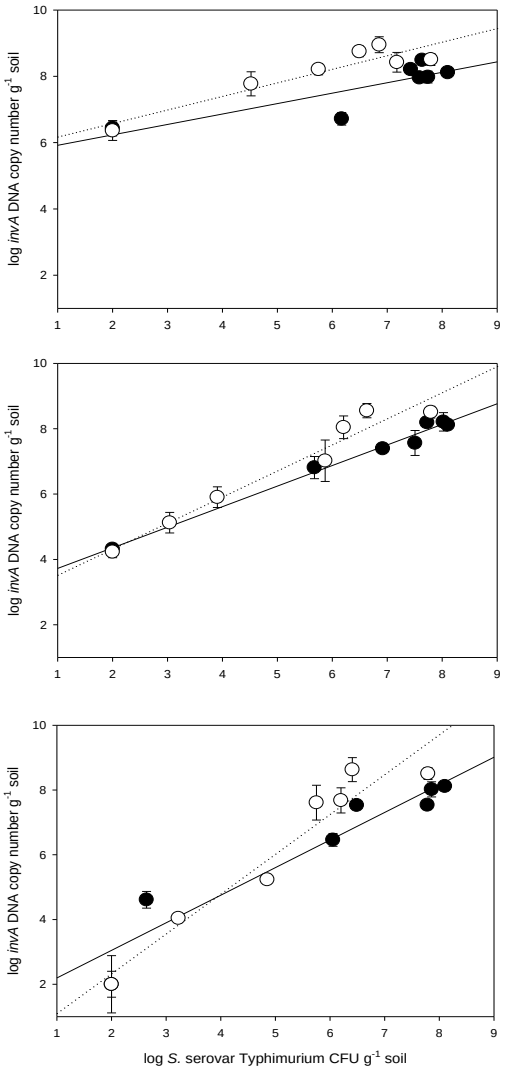
CFU counts and DNA quantification give the best fit



Linear regression of the levels of *Salmonella typhimurium* quantified by plate counting and qPCR.

The logarithm *invA* DNA copy number is plotted versus the logarithm of *S. typhimurium* per gram of soil at three different temperatures: 5º C, 15 ºC and 25 ºC;

( ) soil; ( ) manure-amended soil. Error bars represent standard error of the mean.

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Leaching through monolits

- 4 large monolits (soil columns) in Foulum in spring 2006.
- Monolits 60 cm in diameter and 100 cm high.
- Two filled with fracture moraine soil from Sealands Odde and two with sandy soil from Jyndevad in Southern Jutland.
- By introducing a tetracycline resistant *Salmonella enterica* DSMZ554 to manure leaching could be quantified
- DSMZ554 was cultured in a liquid medium and a density of 8x107 cells ml-1 manure was added to the monolites

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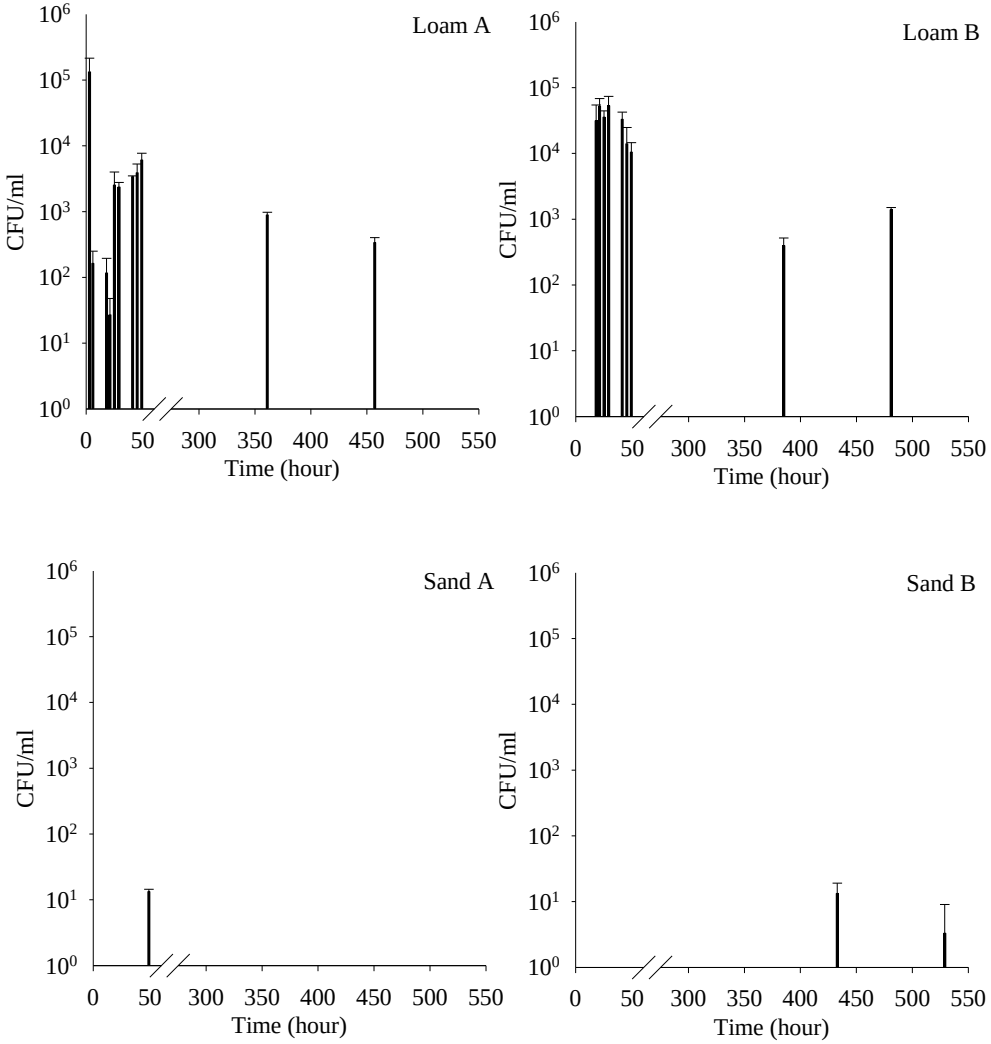
Leaching of *Salmonella enterica* is fastest in structured clay soil



- 8x10^{7}CFU of *S. enterica* DSM554 per ml manure (< 1% of total CFU)
- Fast leaching from clay-monolits up to 5x10^5 CFU per ml water!
- Leaching through sand monolits was slow (detection level reached after long time)

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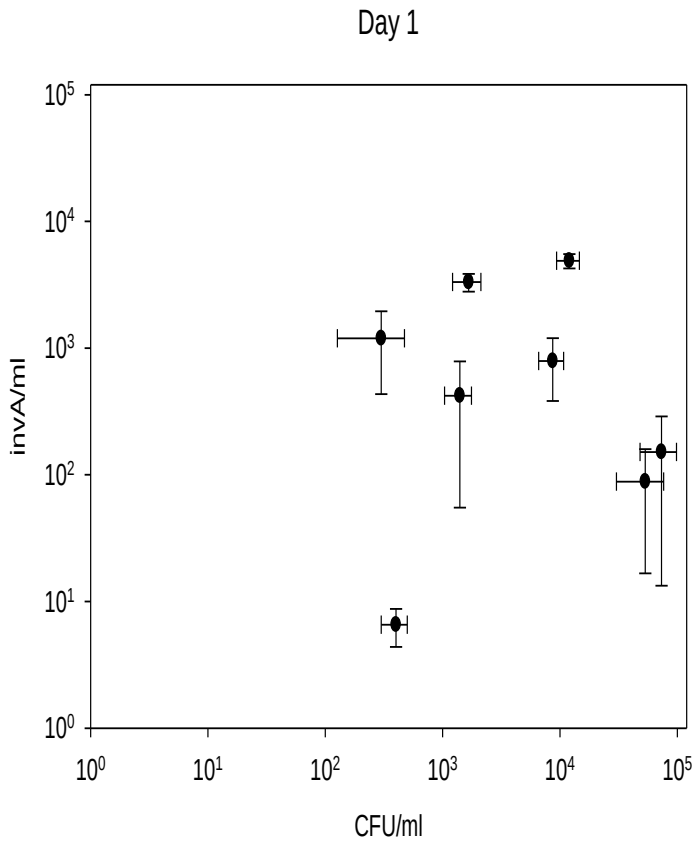
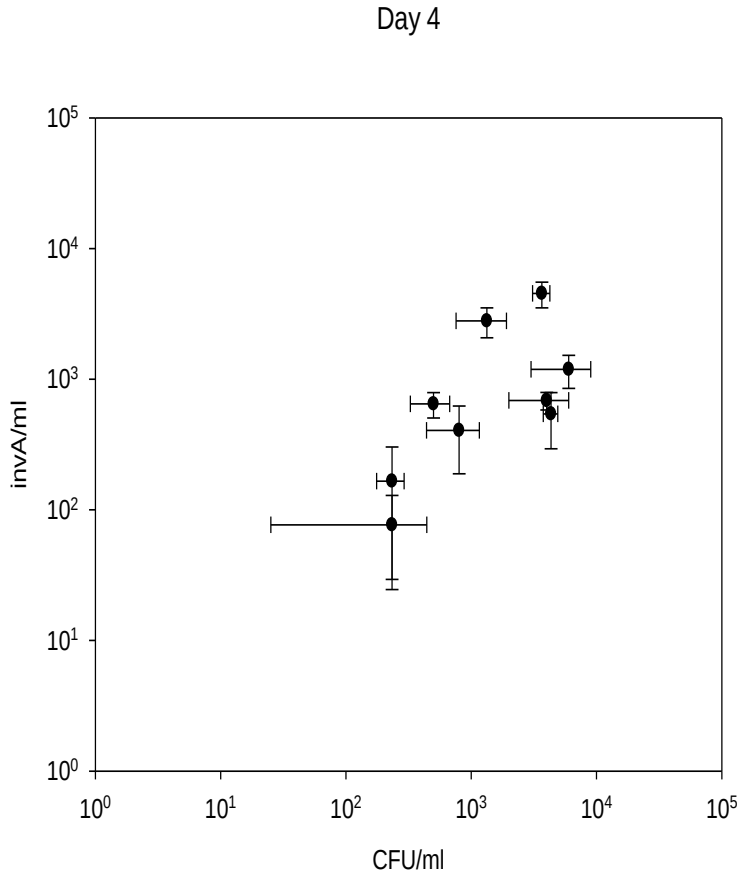
Comparing PCR on *inv*A-DNA and selective plating in quantifying *Salmonella* sp. in water leaching from column experiments.





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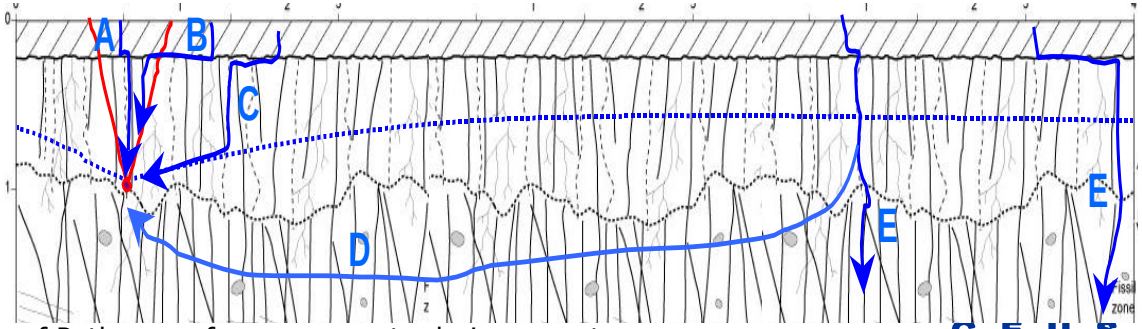
Upscaling: The VAP Field experiment

Questions

- Will pathogens leach to drains when applied to agricultural fields with slurry?
- By which pathways do pathogens enter the drainage system?



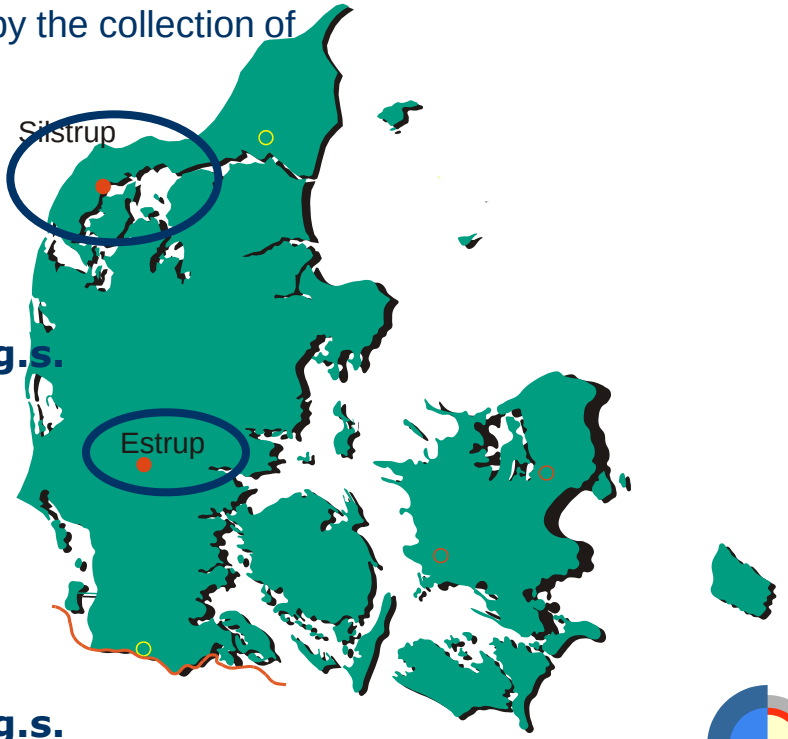
Field experiments - VAP

Two loamy field sites

- Manure from was injected primo April and September 2009
- Leaching has been monitored during a 9 month period
- Drainage water was sampled proportional to the flow and collected weekly
- Selected storm events was intensively monitored by the collection of subsamples for every 2 mm drainage runoff

- Silstrup
- **1.7 ha**
- **18-26% clay**
- **866 mm/year**
- **Gwt: 1-3 m b.g.s.**
- **Tile drained**

- Estrup
- **1.3 ha**
- **10-20% clay**
- **862 mm/year**
- **Gwt: 1-3 m b.g.s.**
- **Tile drained**



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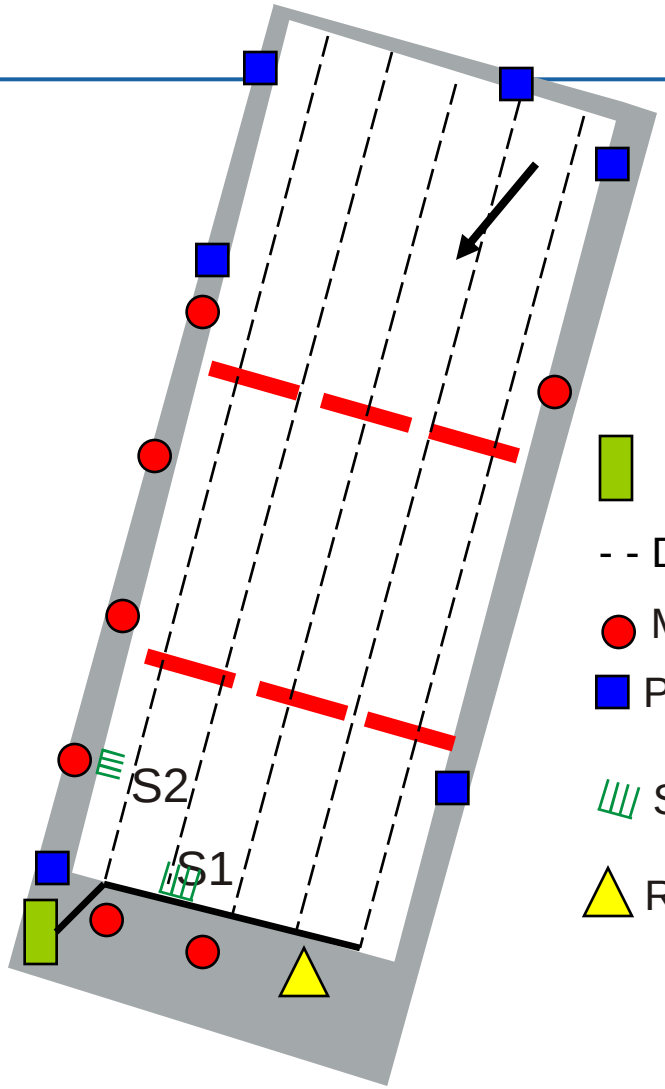
Monitoring design - loamy site -



Shed

- - Drainage system

Monitoring wells

Piezometer

Suction cups, TDR and pt-100

Rain Gauge

0 75 m

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Manure characteristics

Site	Dry matter	total N	Ammonium N	Phos-phate P	Potassium K	Cubber Cu	Magnesium Mg	E. coli	Tetracycl. resistant bacteria
	%	kg/ton	kg/ton	kg/ton	kg/ton	g/ton	g/ton	CFU/mL	CFU/mL
Estrup	0,79	2,86	2,34	0,16	1,17	1,4	98,5	20.000	35.000
Silstrup	6,34	4,65	2,95	1,33	1,77	32,3	1090	100.000	300.000

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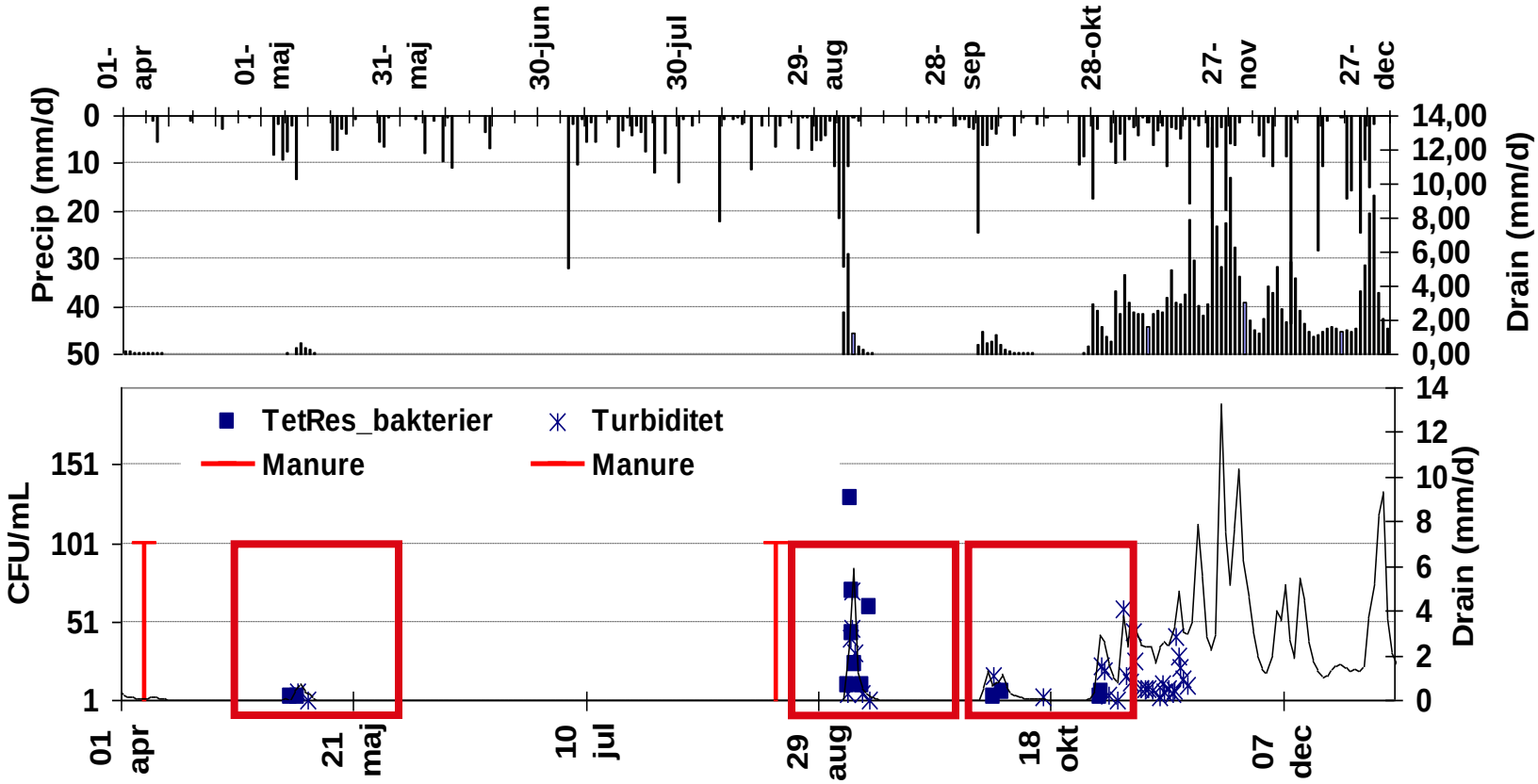
VAP Leaching of tetracycline resistant bacteria from 1/4 - 31/12 2009



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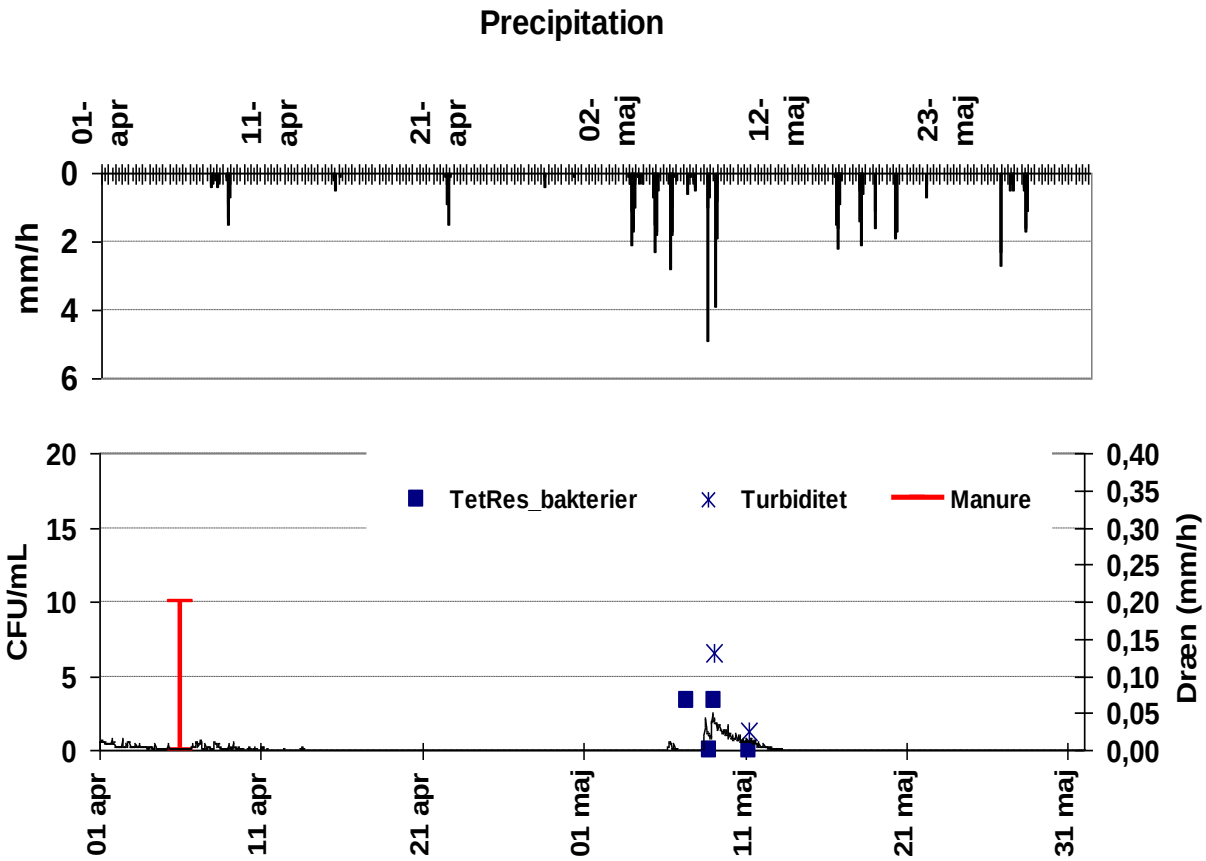
1. Event


Precipitation
01-apr
11-apr
21-apr
02-maj
12-maj
23-maj
mm/h
0
2
4
6
CFU/mL
20
15
10
5
0
TetRes_bakterier
Turbiditet
Manure
Dræn (mm/h)
0,40
0,35
0,30
0,25
0,20
0,15
0,10
0,05
0,00
01 apr
11 apr
21 apr
01 maj
11 maj
21 maj
31 maj

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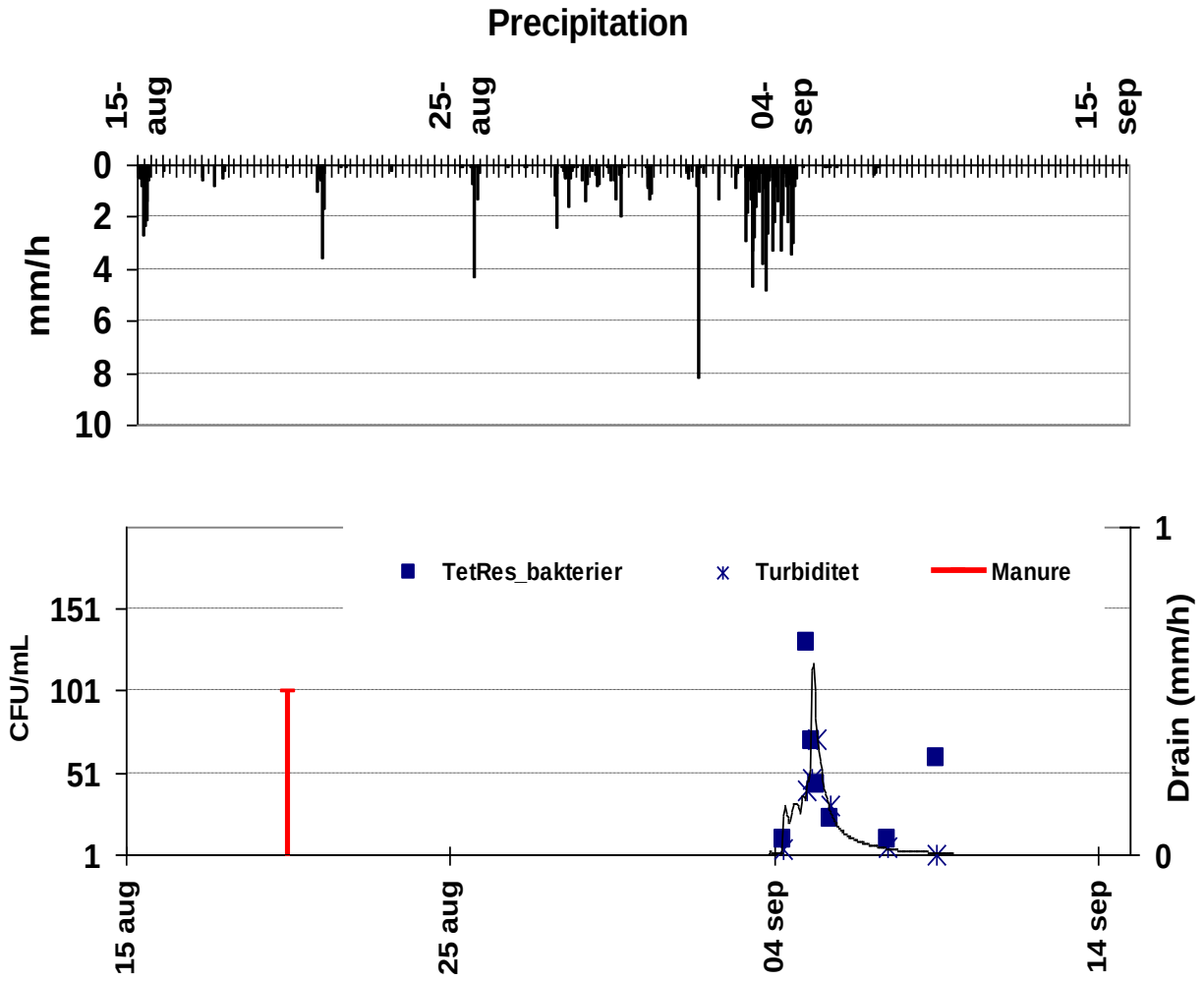
2. Event


Precipitation
15-aug
25-aug
04-sep
15-sep
mm/h
0
2
4
6
8
10
TetRes_bakterier
Turbiditet
Manure
CFU/mL
151
101
51
1
Drain (mm/h)
1
0
15 aug
25 aug
04 sep
14 sep

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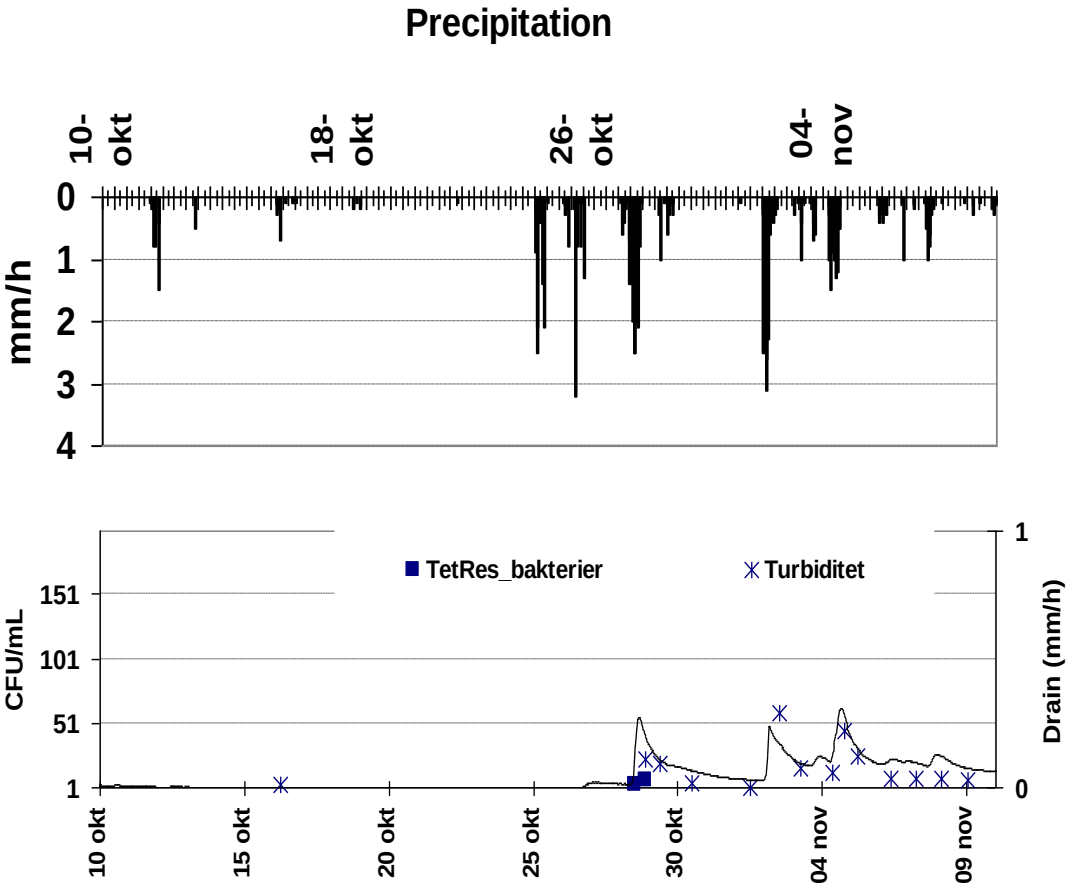
3. and 4. Event



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Conclusion

- Quantitative RT-PCR of mRNA in soil is indeed a possibility to assess pathogens in the environment!
- Leaching of *Salmonella* sp. are faster through fractured clay soil than sand
- Tetracycline resistant bacteria are leached from natural manure applied to the field
- By detecting pathogens using mRNA based methods we can provide data on risk of infectivity, provided that the relation between mRNA and infectivity is established
- Further development and validation of DNA/mRNA based detection systems is needed

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