# Supplementary material S1: Pathogen level calculations

### Cattle faeces

**Calculations of average amount of excreted pathogen from adult cattle (VTEC, salmonella) and calves (*Cryptosporidium parvum*)**

|  |  |  |  |
| --- | --- | --- | --- |
| Pathogen | Excretion from infected animal (bacteria/oocysts per gram faeces) | Proportion infected animals in each category  | Average pathogen excretion in faeces from an infected animal (n/g) |
|  | Low | Medium | High | Low | Medium | High |  |
| Salmonella | 10  | 100  | 10 000  | 0.1 | 0.8 | 0.1 | 1 081 |
| VTEC\* | 10  | 100  | 10 000 000  | 0.1 | 0.8 | 0.1 | 1 000 081 |
| *C. parvum* | 100  | 500 000  | 200 000 000  | 0.1 | 0.8 | 0.1 | 20 400 010 |
|  | 100  | 500 000  | 200 000 000  | 0 | 0.8 | 0.2 | 40 400 000 |

\*VTEC=verotoxin-producing *Escherichia coli*

**Prevalence of infected herds (coresponding to regions with highest herd prevalence):** salmonella 17%, VTEC 25% and C. parvum 100%

**Calculations of average amount of pathogen load in faeces from infected cattle herds (VTEC, salmonella) or an average cattle herd (C. parvum)**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Pathogen | Level of pathogen load | Within-herd prevalence a | Proportion of manure originating from calvesc  | Average amount in manure (n/g) from an infected herd (VTEC, Salmonella) or from all herds (*C. parvum*)  |
| Salmonella | Medium | 0.17 | - | 184  |
|  | High | 0.40 | - | 432  |
| VTEC\* | Medium | 0.17 | - | 170 014  |
|  | High | 0.30 | - | 300 024  |
| *C. parvum*b | Medium | 0.10 | 0.0022 |  4 400 |
|  | High | 0.10 | 0.0022 |  8 713 |

1. For salmonella and VTEC, the figure indicates prevalence in infected herds. For C. parvum, the prevalence corresponds to the average proportion of infected calves, in infected and non-infected herds
2. C. parvum is assumed to be present mainly in calves
3. For manure for fertilisation, 65% dairy and 35% beef was assumed, while for manure on pasture a 50/50 distribution from dairy and beef was assumed.

\*VTEC=verotoxin-producing *Escherichia coli*

### Infectious doses

**Calculation of concentrations required for infection of adult cattle and calves, respectively**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Pathogen | Animal category | Infectious dose (n bacteria/oocysts) | Reference | Daily water consumption (litre) | Pathogen concentration causing infection (n/litre)a |
| salmonella | Adult cattle | 10 000 | 1 | 50 | 200 |
|  | Calf | 5 000 | 1 | 10 | 200 |
| VTEC\* | Adult cattle | 10 000 000 | 3 | 50 | 200 000 |
|  | Calf | 300 | 2 | 10 | 30 |
| *C. parvum* | Calf | 5.8 | 4 | 10 | 0.58 |

1. Aceto H, Miller SA and Smith G 2011. Onset of diarrhea and pyrexia and time to detection of Salmonella enterica subsp enterica in feces in experimental studies of cattle, horses, goats, and sheep after infection per os. Journal of the American Veterinary Medical Association 238, 1333-1339.
2. Besser,TE, Richards BL, Rice DH, Hancock DD 2002. Escherichia coli O157[ratio]H7 infection of calves: infectious dose and direct contact transmission. Epidemiology and Infection 127, 555-560.
3. Cray WC Jr and Moon HW 1995. Experimental infection of calves and adult cattle with Escherichia coli O157:H7. Applied Environmental Microbiology 61, 1586-1590.
4. Zambriski JA. Nydam DV, Wilcox ZJ, Bowman DD, Mohammed HO and Liotta JL 2013. Cryptosporidium parvum: determination of ID(5)(0) and the dose-response relationship in experimentally challenged dairy calves. Veterinary Parasitology 197, 104-112.

\*VTEC=verotoxin-producing *Escherichia coli*

### Fertilisation scenario

**Basis for calculations of pathogen load after fertilisation of arable land**

|  |  |  |
| --- | --- | --- |
| Input |  | Source |
| Maximum yearly manure load allowed | 1.5 kg/m2 | Swedish legislation |
| Proportion of pathogens remaining on surface after ploughing  | 5% | Expert opinion |
| Proportion of pathogens remaining on surface if not ploughed | 100% | Expert opinion |
| Pathogen reduction during storage | 0 | Worst-case assumption |
| Proportion of manure originating from cattle herds | 90% | Expert opinion |
| Proportion of yearly manure production applied at time of simulation  | 50% | Worst-case assumption |

**Calculated pathogen loads from fertilisation of arable land, followed by heavy rainfall**

|  |  |  |
| --- | --- | --- |
| Pathogen | Level of pathogen load | Amount of pathogen per m2 |
| Salmonella | Medium | 21 653  |
|  | High | 50 948  |
| VTEC\* | Medium | 29 458 684  |
|  | High | 51 985 912  |
| *C. parvum* | Medium | 3 049 346  |
|  | High | 6 038 898  |

\*VTEC=verotoxin-producing *Escherichia coli*

### Grazing scenario

**Basis for calculations of manure load from grazing animals on pasture**

|  |  |  |
| --- | --- | --- |
| Input | Value | Source |
| Time period  | 2 weeks | Expert opinion |
| Number of adult cattle per hectare of pastureland | 7 | Swedish legislation |
| Number of beef calves per hectare pastureland that is grazed by beef cattle. | 0.5 | Swedish legislation |
| Amount of manure produced per beef cow (tonnes/year) | 5.33 | Swedish legislation |
| Amount of manure produced per dairy cow (tonnes/year) | 11.83 | Swedish legislation |
| Amount of manure produced per calf during 2 weeks on pasture (kg)  | 12.5 | Swedish legislation  |
| Proportion of pastureland grazed by beef cattle  | 0.5 | Expert opinion |

**Calculated pathogen load on pastureland from 2 weeks’ grazing**

|  |  |  |
| --- | --- | --- |
| Pathogen | Level of pathogen load | Number of microorganisms per m2 |
| Salmonella | Medium | 7 295  |
|  | High | 17 165  |
| VTEC\* | Medium | 9 925 073  |
|  | High | 17 514 835  |
| *C. parvum* | Medium | 637 500 |
|  | High | 1 262 500  |

\*VTEC=verotoxin-producing *Escherichia coli*

### Human wastewater

**Basis for calculation of pathogen concentrations in untreated wastewater, under ”normal” circumstances and from a fictitcious human ”outbreak”**

|  |  |  |  |
| --- | --- | --- | --- |
| Input | Salmonella | VTEC\* | Source |
| Incidence (cases per 100 000 inhabitants during a 6 month-period) | 17.96 | 2.56 | 3 |
| Multiplier correcting for under-reporting | 6.11 | 7.69 |  2 |
| Daily incidence corrected for under-reporting (cases per 25 000 inhabitants) | 0.15 | 0.03 | Calculations |
| Duration of excretion (days)  | 37 | 8 | 1 |
| ”Normal”: daily number of people excreting to wastewater  | 5.55 | 0.21 | Calculations |
| ”Outbreak”: daily number of people excreting to wastewater | 20 | 20 | Assumption |

1. Schönning C, Westrell T, Stenström TA, Arnbjerg-Nielsen K, Hasling AB, Høibye L and Carlsen A 2007. Microbial risk assessment of local handling and use of human faeces. Journal of Water and Health 5, 117-128.
2. Sundström K 2010. Samhällskostnader för salmonellos, campylobacterios och EHEC. Bilaga 9 i Betänkande Folkhälsa – Djurhälsa: Ny ansvarsfördelning mellan stat och näring. SOU 2010:106 <http://www.regeringen.se/49bbab/contentassets/85bc16894e354a5ba40187238673aa51/folkhalsa---djurhalsa-ny-ansvarsfordelning-mellan-stat-och-naring-del-c-bilaga-9-sou-2010106> Retrieved on 20 April 2018
3. Swedish Public Health Agency ([http://www.folkhalsomyndigheten.se/)](http://www.folkhalsomyndigheten.se/%29) Retrieved on 20 April 2018

\*VTEC=verotoxin-producing *Escherichia coli*

**Calculations of pathogen concentrations in untreated wastewater, under ”normal” circumstances and from a fictitcious human ”outbreak”.**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Input | Low | Central | High | Source |
| Daily amount of faeces per person (g) | 80 | 106 | 120 | 4 |
| Salmonella concentration in faeces from an infected person (bacteria/g) | 10^4 | 10^6 | 10^8 | 6 |
| Number of salmonella in wastewater during 24 h (”normal”)  | 10^6.65 | 10^8.76 | 10^10.82 | Calculations |
| Number of salmonella in wastewater during 24 h (“outbreak”) | 10^7.20 | 10^9.32 | 10^11.38 | Calculations |
| VTEC (verotoxin-producing *Escherichia coli)*concentration in faeces from an infected person (bacteria/g) | 10^2 | 10^2.52 | 10^3 | 6 |
| Number of VTEC in wastewater during 24 h (”normal”) | 10^3.24 | 10^3.88 | 10^4.41 | Calculations |
| Number of VTEC in wastewater during 24 h (“outbreak”) | 10^5.20 | 10^5.84 | 10^6.38 | Calculations |
| Release from wastewater treatment plant serving 25000 people (m3/h) |  | 570 |  | Expert opinion |
| Concentration of *Cryptosporidium* spp. in untreated wastewater (oocysts/litre) | 10^1.30 |  | 10^4.43 | 5, 7 |
| Proportion *Cryptosporidium parvum* of cryptosporidia in untreated wastewater  |  | 0.6 |  | Expert opinion |
| Number of *Cryptosporidium parvum* oocysts in wastewater during 24 h | 10^8.22 |  | 10^11.35 | Calculations |

1. Cummings KJ, Warnick LD, Elton M, Grohn YT, McDonough PL, Siler JD 2010. The effect of clinical outbreaks of salmonellosis on the prevalence of fecal Salmonella shedding among dairy cattle in New York. Foodborne Pathogens and Disease 7, 815-823.
2. Ottoson J, Hansen A, Westrell T, Johansen K, Norder H and Stenström TA 2006. Removal of noro- and enteroviruses, Giardia cysts, Cryptosporidium oocysts, and fecal indicators at four secondary wastewater treatment plants in Sweden. Water Environment Research 78, 828-834.
3. Schönning C, Westrell T, Stenström TA, Arnbjerg-Nielsen K, Hasling AB, Høibye L and Carlsen A 2007. Microbial risk assessment of local handling and use of human faeces. Journal of Water and Health 5, 117-128.
4. SMI (2011) Cryptosporidium i Östersund, Smittskyddsinstitutets arbete med det dricksvattenburna utbrottet i Östersund 2010–2011. Report in Swedish, available at: <https://www.folkhalsomyndigheten.se/contentassets/6ba0208adacc460b8aa203fadea39292/cryptosporidium-i-ostersund.pdf> Retrieved on 20 April 2018

# Supplementary material S2: Water quality modelling

The description below is an extract from our earlier publication: Sokolova E, Lindström G, Pers C, Strömqvist J, Sternberg Lewerin S, Wahlström H and Sörén K 2018. Water quality modelling: microbial risks associated with manure on pasture and arable land. Journal of Water and Health 16, 549-561. Refer to this publication for more information on model parameterisation and sensitivity analysis.

## Hydrological modelling

The HYPE model is a hydrological model for simulation of water and substances in soils, rivers and lakes. An early version of the model is described by Lindström et al. (2010), but model development is ongoing and the reader is referred to web sources (http://hypecode.smhi.se/) for the latest model version, open source code and documentation. Part of the model development has been the inclusion of routines (described below) for simulating the fate of pathogens in the environment.

The modelled domain is divided into sub-basins, each with a unique distribution of hydrological response units (HRUs). These HRUs are typically a combination of specific land uses and soil types. The soil profile of each HRU is divided into a maximum of three soil layers. Runoff of water and substances from all soil layers is calculated and summed for each HRU for each time step and then routed through the network of rivers and lakes in the model. Many parameters in the model are linked to either soil type (e.g., field capacity) or land use (e.g., evapotranspiration parameters), while others are general. The time step for most HYPE applications is 1 day, but there is a possibility to run the model with a shorter time step if forcing data (precipitation and temperature) with a higher temporal resolution are available.

HYPE considers several processes (Figure S1) affecting the fate of pathogens in soils and surface waters. These are: release from source, decay (the net effect of die-off and growth), adsorption, sedimentation and resuspension. The simulations for pathogens are very much affected by the hydrological simulations, especially by the partitioning between various flow paths and the turnover times in different model compartments.

Land-based sources of pathogens in the model are either application of livestock manure to the land or excrement from, e.g., grazing animals. Pathogens may also enter surface water directly through point sources. Pathogens in manure or excreta are applied to specific HRUs at a specific date or repeatedly on the same date each year. The total application can be spread in time over a user-defined number of days. Applied pathogens are added to a pool of pathogens on the soil surface. The user may specify ploughing dates, when incorporation of all or part of the pathogens into the top soil occurs. Usually the top soil layer in arable land in HYPE applications consists of the soil down to the ploughing depth.



Figure S1 Schematic representation of pathogen modelling in the HYPE model.

The symbol # is used to denote the number of pathogens.

Release of pathogens from the source occurs during days with rainfall or snowmelt (Equation (1)). The number of released pathogens (Frel, #) during a time step (Δt, d) is a function of the number of pathogens in the source (Nsource, #), rainfall and/or snowmelt during a time step (Vrel, mm) and a release parameter (prel, mm-1).

$F\_{rel}=N\_{source}∙\left(1-e^{-V\_{rel}∙p\_{rel}}\right)$ (1)

Pathogens released from the source either infiltrate into the top soil or enter the stream network directly through surface runoff, depending on the partitioning between the simulated flow pathways during the time step. Pathogens infiltrating into the soil may, to various degrees, sorb to soil particles. The number of pathogens adsorbing or desorbing is driven by the difference between the concentration in soil water and an equilibrium concentration (Equation (2)). The equilibrium concentration is calculated for each soil layer using a linear adsorption isotherm (Equation (3)). The equilibrium concentration is assumed to be reached during the time step. Pachepsky et al. (2006) suggest, in a review of process formulations for modelling manure-borne pathogens, that instantaneous adsorption may be more practical than kinetic adsorption for coarser scale applications, such as those intended for the HYPE model.

$F\_{sorb}=V\_{soil}∙(C\_{soil}-C\_{eq})$ (2)

where Fsorb is the number of pathogens adsorbed or desorbed in each time step (# km-2), Vsoil is the water content of the soil layer (mm), Csoil (µ# L-1) is the concentration in the soil water (before the process) and Ceq (µ# L-1) is the equilibrium concentration.

$C\_{eq}=\frac{N\_{tot}}{V\_{soil}+p\_{ads}∙ρ\_{bulk}∙d}$ (3)

where Ntot (# km-2) is the sum of pathogens in the soil layer either adsorbed to soil particles or in the soil water, pads is the partitioning coefficient (a model parameter, (# kg soil-1)/(# L-1)), ρbulk is the bulk density (kg soil m-3) and d is the thickness of the soil layer (m).

Pathogens may sediment in lakes and are then considered to be removed entirely from the system (Equation (4)). The amount of settled pathogens during a time step (Fsed,lake, #) depends on pathogen concentration in the lake (Clake, # m-3), the surface area of the lake (Alake, m2) and the settling velocity, a model parameter (psed, m d-1).

$F\_{sed,lake}=A\_{lake}∙C\_{lake}∙p\_{sed}∙∆t$ (4)

Sedimentation is also simulated in rivers, but here the pathogens sediment to a storage pool from where they may be resuspended to the water column at a later stage. The implemented process formulation is the same that was developed for particulate phosphorus by Rosberg (2003). The amount of sedimentation and resuspension is dependent on the flow in the rivers in relation to the bank full flow (calculated as the second highest simulated flow during a one-year period) (Equations (5)a and (5b)). At high flows, resuspension dominates, and at the bank full flow (or above), all previously settled pathogens are resuspended. With flow at half the bank full flow, the two processes are balanced, and no sedimentation or resuspension occurs during the time step. At lower flows, sedimentation dominates, and at zero flow, all pathogens in the water settle.

$F\_{sed,river}=a\_{sres}∙C\_{river}∙V\_{river}, a\_{sres}>0$ (5a)

$F\_{resusp,river}=-a\_{sres}∙P\_{sed}, a\_{sres}<0$ (5b)

where Fsed,river and Fresusp,river are net sedimentation and resuspension during a time step (#), Criver is the concentration of pathogens in suspension in the river (# m-3), Vriver is the river volume (m3), Psed (#) is the pathogens settled to the river bottom and asres is the fraction engaged during a time step (Equation (6)):

$a\_{sres}=MAX\left(-1,MIN\left(1,\left(\frac{Q\_{bank}-Q}{Q\_{bank}}\right)^{p\_{sres}}-\left(\frac{Q}{Q\_{bank}}\right)^{p\_{sres}}\right)\right)$ (6)

where Q (m3 s-1) is the stream flow at the current day, Qbank (m3 s-1) is the bank full flow and psres is a dimensionless model parameter.

Decay of pathogens is simulated in all model compartments, i.e., in the source, soil (both adsorbed and in the soil solute), lakes, rivers and river sediments. Decay is simulated as an exponential decay as suggested by Chick (1908) (Equation (7)):

$F\_{decay}=N\_{0}∙\left(1-e^{-μ∙∆t}\right)$ (7)

where Fdecay (#) is the number of deceased pathogens during a time step (Δt, d), N0 (#) is the number of pathogens in the pool before the process is calculated, and µ is the decay constant (d-1). µ is calculated (Equation (8)) from the model parameter phalflife (d).

$μ=\frac{ln⁡(2)}{p\_{halflife}}$ (8)

Pathogens from point sources may be added to the surface water system. They can be either constant during the simulation period or limited to a shorter period.

The HYPE models for the study areas (Lake Vombsjön, the rivers Skeboån and Svartån) were extracted from the national set-up of HYPE for Sweden, called S-HYPE. The original version of S-HYPE was described by Strömqvist et al. (2012), but the model is being developed continuously.

The parameter values for decay were estimated as the median of the values found in the literature for pathogen decay in water. The partitioning coefficient for sorption/adsorption varies greatly in the literature. The value for the partitioning coefficient was chosen based on a relatively low clay content (Pachepsky et al. 2006) in order not to underestimate the risks; the value was doubled for Cryptosporidium since oocysts to a high degree adsorb to soil (Petersen et al. 2012). The parameter for release from source was estimated from Shelton et al.’s (2003) data on manure-borne coliform bacteria; this is probably an overestimation for Cryptosporidium oocysts because of their higher sorption. The bacteria salmonella and VTEC were found to have similar decay rates and were assumed to have similar behaviour, thus the same parameter values were applied. The following values were used: the decay parameter phalflife (days) was assumed 3 for bacteria and 25 for Cryptosporidium; the partitioning coefficient pads (# kg soil-1)/(# L-1) was assumed 5 000 for bacteria and 10 000 for Cryptosporidium; and the release parameter prel (mm-1) was assumed 0.005 for all pathogens. Sedimentation and resuspension of pathogens were not included in the hydrological simulations, i.e., the parameter values were set to zero.

The S-HYPE model had previously been calibrated using the discharge observations from all of Sweden for the period 1999–2008. The performance was validated for Björkaån, using the observations from the only station within this study area (Eggelstad located in Björkaån) for the period 1981–2015. The Nash–Sutcliffe efficiency (NSE), which can range from −∞ to 1, with 1 representing a perfect match, was 0.858 and 0.836 for calibration and validation using daily discharge, respectively.

## Hydrodynamic modelling

To simulate the water circulation in Lake Vombsjön, a three-dimensional time-dependent hydrodynamic model MIKE 3 FM (MIKE Powered by DHI, https://www.mikepoweredbydhi.com/products/mike-3) was used. The MIKE 3 FM model is based on the numerical solution of three-dimensional incompressible Reynolds averaged Navier–Stokes equations using Boussinesq and hydrostatic assumptions. The model consists of continuity, momentum, temperature, salinity and density equations, and is closed using a turbulent closure scheme.

The modelling domain was approximated with prisms (triangles in the horizontal plane) using a flexible mesh approach. The mesh consisted of 817 nodes and 1 515 elements. The length of the triangles’ sides varied from approximately 75 to 190 m, and was adjusted to describe the coastline and bathymetry. Vertically, the lake was approximated by layers. The thickness of the two uppermost layers can vary depending on the water level in the lake; in an undisturbed state, the thickness of these layers is 0.5 m each. The other layers have a fixed thickness of 1 m.

The model was set up to simulate the period January–December 2012. The following input data were used: inflow from the tributaries Björkaån, Torpsbäcken and Borstbäcken (output from the HYPE model), outflow from the lake, water extraction for drinking water production, precipitation on the lake surface, wind speed and direction, air temperature, cloudiness (clearness coefficient), relative humidity. The initial test conditions in the lake were defined by the constant surface elevation and the flow velocity was set to zero. The model was set up to account for the hydrometeorological conditions (wind and precipitation on the lake surface), and to simulate the heat exchange between the atmosphere and the lake. The water density was formulated as a function of temperature. The bed resistance was described by a constant roughness height of 0.05 m. The horizontal eddy viscosity was simulated using the Smagorinsky formulation. The vertical eddy viscosity was simulated using the k-epsilon formulation. The model was run with default parameterisation.

In order to simulate the pathogen fate and transport in Lake Vombsjön, the microbial water quality model MIKE ECO Lab (MIKE Powered by DHI, https://www.mikepoweredbydhi.com/products/mike-eco-lab) was coupled to the hydrodynamic model of the lake. MIKE ECO Lab used flow fields from the hydrodynamic model to calculate the pathogen concentrations in the lake. The pathogen decay in the lake was described in the same way as in the HYPE model. Sedimentation of C. parvum oocysts in the lake was taken into account. It was conservatively assumed that the oocysts released into the lake were not attached to particles, thus the sedimentation velocity was specified as 0.03 m d-1, which is the value suggested for free oocysts (Medema et al. 1998). It was assumed that resuspension does not occur.

The performance of the model was validated using the daily observations of the water level in the lake for the studied period May–August 2012; the NSE was 0.895.

## References

Lindström G, Pers C, Rosberg J, Strömqvist J and Arheimer B 2010. Development and testing of the HYPE (Hydrological Predictions for the Environment) water quality model for different spatial scales. Hydrological Research 41, 295–319.

Medema GJ, Schets FM, Teuni, PFM and Havelaar AH 1998. Sedimentation of free and attached Cryptosporidium oocysts and Giardia cysts in water. Applied Environmental Microbiology 64, 4460–4466.

Pachepsky YA, Sadeghi AM, Bradford SA, Shelton DR, Guber AK and Dao T 2006 Transport and fate of manureborne pathogens: modeling perspective. Agricultural Water Management 86, 81–92.

Petersen HH, Enemark HL, Olsen A, Mostofa Amin MG and Dalsgaard A 2012. Transport of Cryptosporidium parvum oocysts in soil columns following applications of raw and separated liquid slurries. Applied Environmental Microbiology 78, 5994–6000.

Rosberg J 2003. Modelling Phosphorus Transport and Retention in River Networks. Master thesis, Uppsala University, Uppsala, Sweden.

Shelton DR, Pachepsky YA, Sadeghi AM, Stout WL, Karns JS and Gburek WJ 2003. Release rates of manureborne coliform bacteria from data on leaching through stony soil. Vadose Zone Journal 2, 34–39.

Strömqvist J, Arheimer B, Dahné J, Donnelly C and Lindström G 2012. Water and nutrient predictions in ungauged basins: set-up and evaluation of a model at the national scale. Hydrological Science Journal 57, 229–247.