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Biosecurity considerations for the Baltic Sea Region

Focus on transmission of microorganisms from livestock to the aquatic environment

Baltic Compass, Work Package 5

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Preface

Surface water is an important resource for drinking water, irrigation water and recreation. It is therefore necessary to protect it from pollution deteriorating its quality. Whereas several environmental directives have been implemented at the European level, *e.g.* Nitrate Directive (91/676/EEC) and Water Framework Directive (2000/60/EC), animal and human health is poorly protected. The Bathing Water Directive (2006/7/EC) was implemented in order to reduce human exposure to potential hazards, but rather than being a tool to improve water quality it is used merely as a means to locate public beaches and their access. Agricultural practices (*e.g.* manure and sludge fertilisation, animal grazing) and wastewater outlets are the main sources of faecal pollution of surface water, along with risks for further transmission of human and animal gastro-intestinal diseases. Therefore, biosecurity aspects were included in the Baltic Compass project as an important component of sustainable agriculture. This report summarises the main messages on biosecurity risks within the Baltic Sea drainage area, and how these risks can be reduced by best management practices.

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Introduction

Biosecurity is a broad concept encompassing all efforts to minimize the prevalence and spread of infectious disease agents. According to the FAO it is: “A strategic and integrated approach that encompasses the policy and regulatory frameworks for analysing and managing relevant risks to human, animal, and plant life and health, and associated risks to the environment”(www.fao.org). Within Baltic Compass, the biosecurity work has focused mainly on transmission of pathogens from manure to water. This was to enable comparison with corresponding leakage of nutrients from manure to water. It was anticipated that adding health considerations to the eutrophication (nutrient over-enrichment) discussion could result in a stronger willingness to implement regulations regarding measures important for reducing the risk of nutrient leakage to surface waters.

Due to intensified animal production in the Baltic Sea Region (BSR), and to a changing climate, there is potentially an increasing risk of transmission of infectious disease agents from animal farms to the water environment, *e.g.* via land application of manure and via animals on pasture, with consequent risks for animal and human health. However, land application of manure serves several purposes. Not only is it a practical solution to waste handling, but it also contributes to recycling of valuable plant nutrients such as nitrogen, phosphorus, sulphur and potassium, as well as some essential micronutrients such as nickel, zinc and copper. Addition of manure can further improve soil properties such as structure and water-holding capacity (Williams *et al.*, 1995; Lavelle, 1988; Oades, 1984). However, recycling of manure may also serve as a transmission route for pathogens to the environment and into the food chain (Albihn & Vinnerås, 2007). Large outbreaks of infectious animal diseases may be rare, but structural changes in animal production may lead to higher risks and more devastating consequences of disease outbreaks. Thus, measures that prevent or minimise the risk of spread of infectious disease agents are increasingly important. The overall aim of the biosecurity task within Baltic Compass Work Package 5 was to establish a connection between nutrient and biosecurity/health considerations, in order to provide additional impetus for measures, investments and integration of policies.

The biosecurity work has been integrated with the work on nutrients, and has resulted in several joint publications. Identification of measures that combine mitigation of nutrient losses and biosecurity risks has been published in the report “Implementation and status of priority measures to reduce nitrogen and phosphorus leakage, Summary of country reports” (Salomon & Sundberg, 2012). Moreover, methods to identify biosecurity risk areas have been published in “Mapping erosion- and phosphorus vulnerable areas in the Baltic Sea Region – data availability, methods and biosecurity aspects” (Tattari *et al*., 2012). The aim of this report is to highlight the biosecurity issues separately and more thoroughly.

Background

Livestock manure and health risks

Livestock manure may contain pathogenic (disease causing) microorganisms, some of which are zoonotic (*i.e*. transmitted between animals and humans), such as *Campylobacter*, *Salmonella*, *Listeria*, *Escherichia coli* O157, *Cryptosporidium* and *Giardia* (*e.g.* Mawdsley *et al*., 1995; Pell, 1997), which can cause serious illness in humans. Cattle are recognized as reservoirs for *E. coli* O157:H7 (Dean-Nystrom *et al*., 1999). *Campylobacter* is part of the gut flora of a wide range of vertebrate hosts, including many domestic and wild animals. Poultry are considered to be an important source of human infections, but this can be controlled with strict measures (Hansson *et al*., 2007). Despite reduced prevalence in poultry, domestic cases in Sweden are increasing, and water is an important transmission route (Schönberg-Norio *et al*., 2004; Sopwith *et al*., 2008). As *Campylobacter* and Salmonella are found in many livestock species, many countries have instituted successful control programmes for food-producing animals (EFSA, 2012). Thus, the prevalence of faecal pathogens in livestock varies between regions. Moreover, biowaste is another source of pathogens that can end up in the aquatic environment. This is of importance because transboundary animal diseases cause large outbreaks, and eradication measures include proper disposal of large volumes of biowaste, including manure and animal carcasses (Regulation (EC) No 1069/2009 with amendments). Table 1 lists examples of zoonotic and epizootic diseases that can spread via biowaste.

Some of the enteric pathogens mentioned above are opportunistic, and may be considered part of the normal gut flora, while others are pathogenic, and can cause disease in many host species, including wild and domestic animals as well as people. The composition of the gut flora of domestic animals is affected by individual factors such as host immunity, but is mainly affected by herd-level factors such as feeding, use of antibiotics, health, and management factors, etc. Host animal species may affect the pathogen load in manure in various ways, and the spectrum of different pathogens differs between host species. The prevalence of pathogens usually varies between different animal species in different regions. The concentrations also vary, so that animal species that produce large volumes of manure can have a lower concentration in the manure and animals with small manure volumes may have higher concentrations. In general, however, it may be assumed that animal species that produce large volumes of manure also contribute a larger amount of pathogens to the environment per animal (assuming that they are infected). On the other hand, if some species are kept in large herds with less stringent biosecurity, these herds may be expected to produce a larger amount of pathogens than species that are kept in smaller herds. Further, young animals are considered to be more associated with zoonotic pathogens. This has been shown for *C. parvum* and *E. coli* O157 in Swedish cattle herds, with higher incidences in calves shedding higher numbers (Silverlås, 2012; Eriksson, 2010). This has also been shown for Hepatitis E virus genotype 3, with 23% of piglets infected shedding viruses in their faeces (Widén *et al*., 2011).

Table 1. Examples of zoonotic* and epizootic† agents that can be transmitted via manure and biowaste (modified from Albihn *et al*., 2012).

Agent	Disease	Most common host species
Bacteria		
Bacillus anthracis[*,†]	Anthrax	Multiple[‡]
Brucella spp.[*,†]	Brucellosis	Multiple[‡]
Campylobacter spp[*]	Campylobacteriosis	Multiple[‡]
Coxiella burnetii[*,†]	Q-fever	Ruminants
Escherichia coli[*]	Enterohaemorragic *E. coli*	Ruminants, swine
Listeria monocytogenes[*]	Listeriosis	Multiple[‡]
Mycobacterium bovis[*,†]	Bovine tuberculosis	Cattle
Mycobacterium avium subsp. *paratuberculosis*[†]	Paratuberculosis	Ruminants
Salmonella spp.[*]	Salmonellosis	Multiple[‡]
Yersinia enterocolitica[*]	Yersiniosis	Swine
Viruses		
African swine fever virus[†]	African swine fever	Swine
Aujeszky's disease virus[†]	Aujeszky's disease	Swine
Bovine herpesvirus type 1[†]	Infectious bovine rhinotracheitis	Cattle
Classical swine fever virus[†]	Classical swine fever	Swine
Foot-and-mouth disease virus[†]	Foot-and-mouth disease	Cattle, swine
Goat pox virus[†]	Goat pox	Goat, sheep
Hepatitis E virus[*]	Hepatitis	Swine
Avian influenza virus[*,†]	Avian influenza	Poultry
Newcastle disease virus[†]	Newcastle disease	Poultry
Porcine reproductive and respiratory syndrome virus[†]	Porcine reproductive and respiratory syndrome	Swine
Sheep pox virus[†]	Sheep pox	Sheep
Swine vesicular disease virus[†]	Swine vesicular disease	Swine
Parasites		
Cryptosporidium parvum[*]	Cryptosporidiosis	Cattle
Giardia spp.[§]	Giardiasis	Multiple[‡]
Toxoplama gondii[*]	Toxoplasmosis	Cat
Trichinella spp.[*]	Trichinellosis	Swine

[‡] Most warm-blooded animals can be infected
[§] Transmission between animals and humans is not clear

Exposure Assessment

When faecal microorganisms are released to the environment, they tend to decrease in numbers/viability due to natural die-off (ageing, low re-growth), unfavourable environmental conditions (UV-light, desiccation, fluctuating temperatures), dilution, and predation. By increasing the retention on the soil surface or within soils, more reduction can be achieved. Further removal will take place in sedimentation processes (attachment to mineral and organic particles) and by soil adsorption during infiltration. However, a key risk mitigation measure is the proper collection and storage of manure. Despite the measures that have been implemented (further discussed below), some pathogens will survive and be transmitted to water either as sub-surface run-off or via hydrological pathways, such as infiltration through soils, bypass flow in cracks or macropores, and artificial soil drainage. When pathogens end up in surface water, humans can be exposed through recreational activities such as swimming, consumption of leafy greens irrigated with contaminated water, and drinking tap water produced from contaminated water.

Humans can be exposed to manure-contaminated water during recreational activities such as bathing. Epidemiological studies have shown that the rates of some adverse health outcomes, such as gastrointestinal disease, are higher in swimmers compared with non-swimmers and are correlated to the

faecal contamination of the water (Prüss, 1998; Wade *et al*., 2006). A risk assessment by Soller *et al*. (2010) estimated that the risks associated with exposure to recreational waters contaminated by fresh cattle faeces may not be substantially different from waters impacted by human sources, mainly due to the prevalence of *E. coli* O157:H7 in cattle.

Faecally contaminated surface water used for irrigation ends up on the surface of edible crops. Some inactivation of pathogens on the crop surface will take place over time, as an effect of UV light, temperature and other environmental factors (Ottoson *et al*., 2011). Presently, there are no European guidelines either on irrigation water quality or on holding time between irrigation and harvest. Several *E. coli* O157 outbreaks from freeland cultivated leafy greens have been reported (CDC, 2006; Söderstrom *et al*., 2008). However, after a lettuce outbreak in Sweden in 2005, a government commission recommended that irrigation water should at least comply with bathing water quality, and that irrigation 48 h prior to harvest should be of drinking water quality (Anon, 2007).

Surface water is also extensively used as source water for drinking water production. In the water treatment plant, controlled removal and estimated inactivation takes place during disinfection processes. However, a specific challenge is the chlorine-resistant protozoan parasites *Cryptosporidium* and *Giardia*, unless filtration processes are optimized (Ottoson, 2012). Groundwater treatment plants rely on the removal of pathogens through soil passage in order to provide water with high quality, and disinfection is rarely employed in Sweden. However, increased surface water impact of groundwater can take place during high flow events, and pathogens like, *Campylobacte*r and EHEC can potentially cause problems in cattle-dense areas, and Hepatitis E virus in pig-dense areas (Ottoson, 2012). A waterborne outbreak from cattle manure contamination took place in Walkerton, Canada, where 2300 people fell ill to *Campylobacter* and EHEC, and with seven deaths (Auld *et al*., 2004).

Current changes affecting disease transmission

Structural changes in livestock production

The expected trend in livestock production is that farm size will generally increase while the number of holdings decreases. This is also the case in the BSR, where the number of cattle holdings in Estonia, Latvia, Lithuania, Poland, Finland and Denmark decreased by 2 – 57 % between 2007 and 2010 (Eurostat, 2012). At the same time, there have been few changes in the number of cattle (Table 1). In Sweden this is illustrated by an increase in average dairy herd size from 59 to 65 dairy cows between 2009 and 2011 (SJV, 2012).

Table 1. Cattle populations in the Baltic Sea Region.

	No. of cattle holdings			No. of cattle		
	2007	2010	change	2007	2010	change
Estonia	990	560	-43%	241	236	-2%
Latvia	880	860	-2%	399	380	-5%
Lithuania	4 870	2 100	-57%	788	748	-5%
Poland	38 600	20 430	-47%	5 406	5 562	+3%
Finland	1 900	1 320	-31%	903	909	+1%
Sweden	30	30	0%	1 517	1 475	-3%
Denmark	100	NA		1 545	1621	+5%

NA=not available

The increase in herd size presents new challenges for maintaining efficient biosecurity and reducing the spread of infectious diseases. Large livestock holdings generally have a better biosecurity than smaller holdings; however, once an infection is introduced it may spread rapidly within the herd and cause substantial animal- and subsequent economic losses. For example, outbreaks of transboundary animal diseases, such as classical swine fever, affected several large pig herds in the Baltic region in 2011 (OIE, 2012). During severe disease outbreaks in a herd, followed by increased mortality, proper disposal of carcasses is of utmost importance, to prevent spread to other farms or to wildlife. If carcasses are deposited in the environment or buried under non-suitable conditions, there is a risk of pathogen spread.

The intensification of cattle farming also contributes to an enormous production of manure within limited geographical areas, with potentially serious negative effects. Spreading manure on farmland is a common practice, but this may also contribute to the spread of infectious diseases, for example during water runoff to neighbouring livestock or to wildlife. In cases where manure is dumped in the environment due to lack of arable land to fertilize, or if commercial fertilizers are used instead, several negative consequences can be expected. In these areas there is a potential impact on biodiversity, for example through nutrient overload, and a potential problem with accumulation of pathogens.

Changes in wildlife populations

Structural changes in society affect the interface between domestic animals and wildlife. It is well known that habitat fragmentation reduces overall species diversity and alters species abundance, often with cascading effects on ecological processes and community structure. An important aspect of this is how habitat fragmentation alters the way in which hosts and pathogens interact, and how this affects the ability of the host to survive and prosper. Habitat fragmentation may also affect the interface between wildlife and domestic animals and people, increasing direct and indirect contacts between species.

Changes in the wildlife population, such as an increase in wild boars and increased ecological pig-keeping, will lead to more contact between species with subsequent risks for disease transmission. The introduction of new domestic animal species such as camelids and buffaloes, as well as new game farms with deer and wild boars, may lead to increased opportunities for transmission of new diseases between different host species.

Diseases of wildlife occur in many different forms in a wide range of animal species and populations. Diseases, when expressed in free-ranging animals, can have a significant effect on wildlife ecology. Whilst some diseases exist as symptomless, subclinical infections without obvious ecological impact, and of no consequence for domestic animals or humans, occasionally there are dramatic epizootic outbreaks characterised by high morbidity and mortality. In addition, wild animals, such as insects, invertebrates, fish, birds and mammals, can be reservoirs for highly contagious and severe diseases, as well as other important diseases that infect domestic animals or humans. Reports of illnesses or deaths involving many animals from a free-living population may represent the initial alert to the likely presence of a new disease agent.

Transmission of infectious diseases between livestock and wildlife is also a matter of increasing concern from human perspectives. The problem is accelerating due to new emerging diseases, increased global trade and changing strategies of production. Protection of natural borders, such as fields, forest, and rivers, are key elements to reducing the risks for transmission of pathogens between wildlife and domestic animals.

Climate change

Climate change will lead to a rise in temperature, changes in the amounts and pattern of precipitation, and a rise in sea level in the countries surrounding the Baltic Sea basin (Baltadapt, 2012). For example, during the 2000s the average temperature in Sweden has increased by 0.6° C, which is regarded as a fast and substantial increase (SMHI, 2012). It has also been reported that extreme rains (here defined as >90 mm precipitation / 24h) in Sweden have, most notably in the eastern part of Sweden, increased in number during the last 40 years, as can be seen in Figure 1 below.

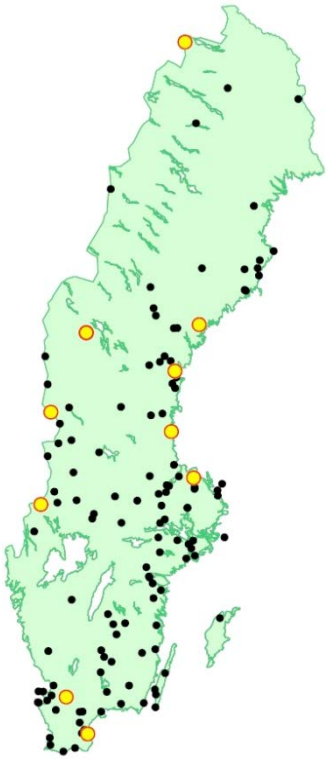

Figure 1 The map shows locations with >90 mm precipitation / 24 h on one occasion (black dots) and locations with >90 mm precipitation / 24 h on at least two occasions, during the period 1961-2011.

Source: SMHI (http://www.smhi.se/nyhetsarkiv/skyfall-har-blivit-vanligare-1.23063)

The changes that are reported, as well as predicted, are crucial to take into account in a societal planning perspective, but also when it comes to introduction and spread of infectious diseases in the environment. In a warmer climate, the conditions for introducing new pathogens are likely to increase, as new disease carrying vectors may become established. However, climate change may have impacts not only on the distribution of disease carrying vectors. Some diseases are associated with water, and may be exacerbated by flooding. Flooding may also provide favourable conditions for insects that can act as vectors for infectious diseases. Spore-forming pathogens (such as clostridia and *Bacillus anthracis*) are also affected by extreme weather conditions, as changes in soil structure, flooding, etc. may cause residual spores to reach the surface and infect grazing animals. Also, the survival and growth of pathogens, either in the environment or in a host, will be affected depending on the characteristics of the pathogen. More torrential rains will likely cause higher levels of surface runoff, thereby leading to increased spread of potential pathogens from the manure-fertilised fields to the water.

Monitoring biosecurity risks

Monitoring animal health

Disease surveillance must be fit for the purpose, and may be conducted as passive or active surveillance. Passive surveillance means that disease reporting forms the basis for the surveillance and no active sampling or investigations are performed. In general, passive surveillance is considered to have low sensitivity, but it may work well for diseases with a clear clinical picture. Active surveillance includes active sampling or clinical observations of the entire population or, more commonly, a representative sample of the population.

For wildlife, passive surveillance usually involves post-mortem surveillance of animal carcasses submitted for investigation. Active surveillance may involve capturing and sampling of wildlife as well as targeted hunting activities or intensified searching for carcasses to sample.

International disease reporting aims to facilitate safe trade without spreading animal diseases (www.oie.int). There has been a fear of a negative impact on trade if diseases are reported, but, nowadays, proper disease reporting is generally considered as a prerequisite for trade.

Disease surveillance is an important tool for disease prevention. In order to assess the risks and detrimental effects of animal diseases, the prevalence of disease in the population must be known. Without knowledge of the animal population, as well as the regional disease patterns, appropriate disease control cannot be delivered and risks of environmental spread cannot be addressed.

Monitoring microbial water quality

Due to legal, financial or practical constraints, on-farm monitoring of biosecurity risks is not always possible. Under such circumstances, monitoring microbial water quality in the vicinity of animal farms may give valuable information regarding potential biosecurity risks, and may provide a basis for advice on how to mitigate risks and for motivating such measures.

Monitoring data for microbial water quality in surface waters influenced by agricultural activities in the BSR is not performed on a regular basis, despite the risks from exposures via irrigation, drinking water and recreation. Bathing waters are monitored for microbiological water quality according to the Bathing Water Directive (2006/7/EC); however this is limited to the summer season and to beaches decided upon by the municipality (EU-flag). There are almost 200 different pathogenic microorganisms possibly transmitted by water. Analysis can be cumbersome and is generally expensive. Therefore, microbial water quality is determined by enumerating faecal indicator bacteria, such as *E. coli* and enterococci. Faecal indicators in surface water indicate that enteric pathogens also may be present, and therefore are a health risk. Enumeration of indicator bacteria will give information about faecal contamination as an indirect measure of the health risk (FAO, 1994). However, some pathogens will behave differently in the environment, *e.g.* viruses and parasites, having longer survival times. Therefore, pathogens may be found even though indicators are not found. On the other hand, all faecal indicators do not come from individuals shedding pathogens, which is why the presence of indicators and pathogens does not necessarily correlate. However, it has been shown that faecal indicator bacteria can be used to predict gastrointestinal disease amongst swimmers (Wade *et al*., 2006). An inclusion of the bathing water directive in the Water Framework Directive (2000/60/EC) of the EU (WFD), to state a minimum level for “good microbiological status”, would protect waters everywhere, not only where humans may be exposed when bathing at public beaches. Until this is a reality, we suggest that nations include a microbiological parameter (*e.g.* faecal indicator) in their

own implementation of the WFD for the monitoring, classification and management of water quality, and to increase the information to the public about water quality.

Controlling transmission routes

Transmission of diseases can be monitored and controlled at various points in the transmission cycle. Figure 2 illustrates transmission pathways, and includes possible points for control measures and monitoring.

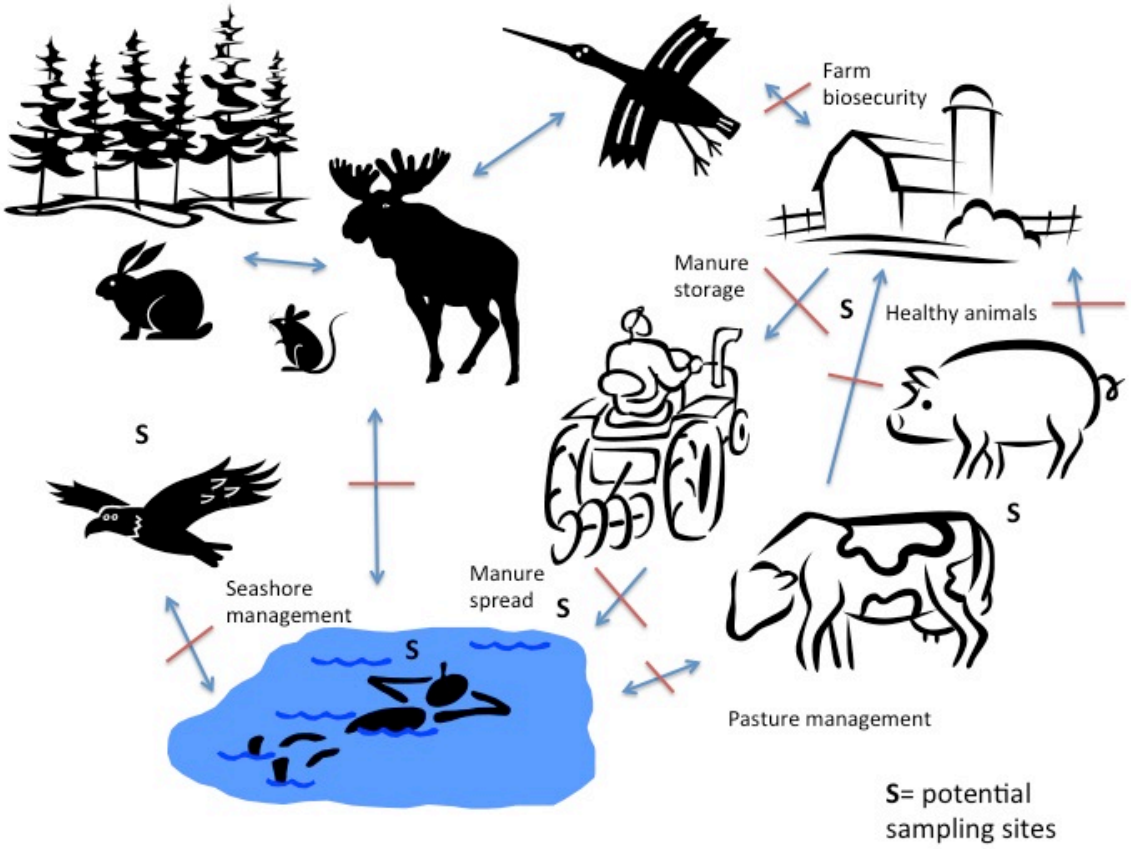

Figure 2. Transmission pathways (blue arrows), possible points for control (red), and monitoring (S) of diseases.

Connection between eutrophication and biosecurity

It was anticipated that if nutrient and biosecurity risk areas coincide, and if there are measures beneficial for reduction of nutrient and pathogen leakage to surface waters, the biosecurity/health aspects could further motivate measures and investments regarding farming practices in the BSR.

Identification of biosecurity risk areas

To be able to identify possible overlaps with defined agricultural nutrient loss risk areas and HELCOM point and diffuse source Hot Spots within the BSR, we have in a previous report proposed criteria and methods for identifying biosecurity risk areas (Tattari *et al.*, 2012). Biosecurity risk areas are in this context defined as areas where there is a risk of pathogens leaching to water and subsequent risks for human and animal health, *i.e.* areas where high pathogen load coincides with high nutrient transport risk to waters (Figure 3). The amount of pathogens in manure is mainly related to disease prevalence in the animal population, and the duration and conditions of storage before land application. Transport risk of microorganisms to surface

waters is mainly related to surface runoff, erosion and bypass flow (Tyrrel & Quinton, 2003; Oliver *et al*., 2005).

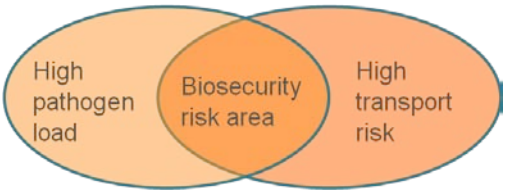

Figure 3. Potential biosecurity risk areas – areas where high pathogen load coincides with high transport risk areas.

Animal density has been suggested as one important factor for identification of biosecurity risk areas, indicating potential high pathogen load. High animal density is associated with higher disease prevalence and higher frequency of animal movements, and thus higher incidence of introducing infectious diseases. Infectious diseases are also spread more easily in dense populations. An increased animal density will likely be associated with excess manure at the farm level, which potentially could result in transport of manure to other regions, with a risk of disease transmission. The map in Figure 4 shows the livestock density index in the BSR, which is the ratio of livestock units per hectare of utilised agricultural area. A higher index means that a higher amount of manure is available per ha of utilised agricultural area, which indicates a higher pressure of livestock farming on the environment, and a potentially increased risk of nutrient and pathogen leaching. In addition to livestock density, farming practices, such as manure handling, also affect the risk of nutrient and pathogen leaching. Thus, the livestock index does not necessarily lead to environmental degradation, and should not be used as the only indicator of possible risk areas.

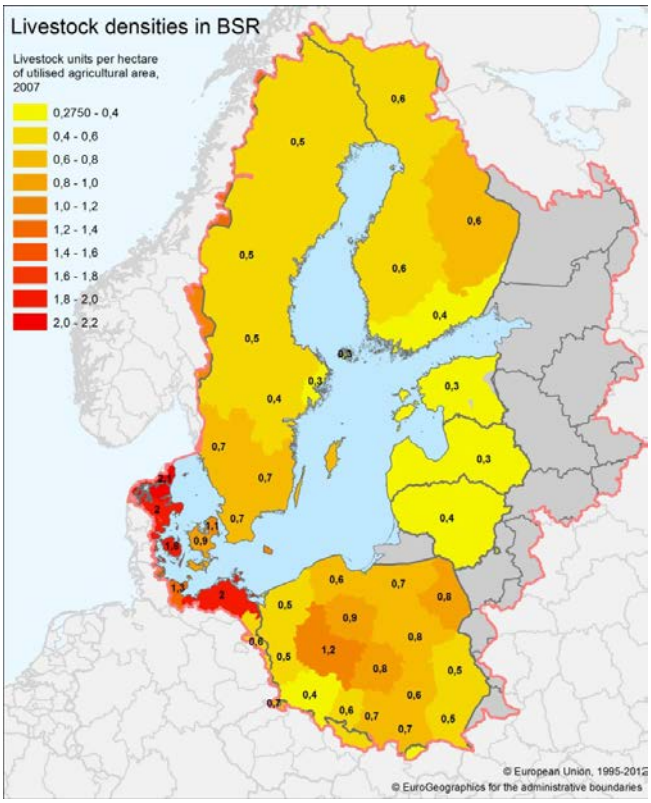

Figure 4. Livestock density index (livestock units per hectare of utilised agricultural area) in 2007 in the Baltic Sea Region.

Data about animal density is available for most countries in the BSR, although at varying levels of detail and spatial accuracy, whereas data about other biosecurity-relevant factors (*e.g*. manure handling practices and routines for animal husbandry) is more difficult to obtain. Therefore, identification of potential biosecurity risk areas within the BSR may initially be done by combining the available data about animal density with information about nutrient vulnerable areas, which indicate high transport risk. To confirm these potential high-risk areas, more accurate and detailed data on animal density, manure handling practices and disease prevalence is needed, as well as monitoring of biosecurity risks (animal health, microbial water quality).

Measures which combine mitigation of nutrient losses and health risks

Nitrogen (N) is more easily transported through soil than are pathogens, and hence a larger part will leak through drainage water and to groundwater. Phosphorus (P), on the other hand, binds harder to soil particles, and in that sense is more likely to be reduced in the same pattern as many pathogens. The difference is that, while pathogens are inactivated over time, P in the soil is in a stable ion form, so that erosion is relatively more important for P than for pathogen leakage. The common denominator for nutrients and pathogens is surface runoff, and reducing the risk of surface runoff will therefore have multiple benefits. A study of surface waters in Australia showed that the majority of pathogen impacts on reservoirs arose from rainfall-induced runoff in most catchments, and microbial load estimation showed that as much as 300 years' worth of dry weather pathogen contaminant loads could be exported during 1 day in a single small event (Roser & Ashbolt, 2007). Thus, the importance of reducing surface runoff is pertinent for avoiding nutrient and pathogen leakage from the field to water; and the following measures were rated the highest within Baltic Compass (Salomon & Sundberg, 2012):

- Avoiding manure spread during high-risk periods
- Avoiding manure spread in high-risk areas
- Storage of manure
- Buffer zones
- Incorporation of manure into soil.

Generally, surface runoff from fields is the most important transport route for viable pathogens, but transport through soils may be significant in areas with a risk of fast transport, *e.g.* via by-pass flow. Avoiding the application of manure on land with a risk of runoff to surface water (*e.g*. fields with a significant slope, adjacent to water, or draining to nearby water), and the spreading of manure during high-risk periods (*e.g*. heavy rainfall, flooded or snow covered fields, and during frost) would significantly reduce the risk of nutrient and pathogen leakage via surface runoff. Fields close to water with a substantial slope (>10%) should preferably not be fertilised at all.

Sufficient storage makes it possible to avoid application of manure during high–risk periods, and during storage a time-temperature inactivation will take place. According to Guan & Holley (2003) a storage time of 90 days at 25 °C is enough for acceptable inactivation of most zoonotic agents. A model based on semi-continuous daily filling over a year would lead to 98% reduction of *E. coli* despite emptying the tank the same day as the last filling (Elving, 2012). However, the inactivation of pathogens may differ a lot, and sanitization treatment of manure is suggested for a more controlled inactivation, for example when manure is handled according to the Regulation on Animal By-products (2009/1069/EC) or if several farms share a facility (Albihn *et al*., 2012). This could, for example, be achieved by composting (Elving *et al*., 2012), thermophilic digestion (Sahlström, 2003), urea (Ottoson *et al*., 2008) or lime disinfection (Nyberg *et al*., 2011), for a five log reduction of bacterial pathogens and three log reduction of heat-resistant viruses.

While composting and liming are effective in reducing pathogen levels, nitrogen losses to air may limit their suitability. Proper storage has been identified as being one of the most important measures to reduce nutrient leaching (Sundberg & Salomon, 2012). Improper storage is considered to be one of the main causes of hot-spot pollution as regards leaching of nutrients from intensive livestock production (Foged, 2010).

Vegetated buffer zones along watercourses are used to reduce the surface runoff of nutrients and pathogens from fields. Reduction of microorganisms across vegetated buffer zones depends on width, vegetation and slope (Dufour *et al*., 2012). Buffer zones of ≤ 6 m width have, in lab-scale and field studies, been shown to reduce pathogen run-off by up to 99% (range 56 – 99%, 1 – 6 m) (Larsen *et al*., 1994; Goel *et al*., 2004; Atwill *et al*., 2002).

The amount of nutrients available in runoff water can be reduced by incorporation of manure during land application (*e.g*. Hill *et al*., 2005). For pathogens, incorporation of manure will, on the one hand, increase the survival time in soil compared to when manure is left unincorporated on the soil surface (Hutchison *et al*., 2004) but, on the other hand, leaving manure on the soil surface will increase the risk of disease transmission via surface runoff to waters. Furthermore, the possibility of pathogen spread via wild animals, such as insects and birds, will increase if manure is left on the surface. Due to the risks associated with surface runoff, and the risk of disease spread to the wider environment, incorporation of manure has here been considered as a measure beneficial for biosecurity.

Other measures beneficial for nutrient and pathogen leakage are constructed wetlands and sedimentation ponds. Sedimentation ponds make the water move more slowly and, due to sedimentation and UV inactivation, the transport of pathogens from drainage water will decrease. Pathogen removal in constructed wetlands is effected by predation, die-off through UV disinfection and sedimentation. Up to 99.9% reduction over a wetland has been reported (Dufour *et al*., 2012). However, the risk of pathogen accumulation in wetlands and sedimentation ponds has not been evaluated. If this risk is substantial it may counteract the benefits of pathogen removal from water.

Sampling of microbial water quality in two case areas within the BSR

As part of the Baltic Compass project, water samples from the Aurajoki river basin in Finland and the island of Öland in Sweden were collected during 2011-2012. The main objective of this pilot study was to illustrate how to investigate microbial contamination of surface waters influenced by agricultural activities, and how to combine the nutrient and biosecurity aspects. Below is a summary of the pilot study. For a more detailed description, see Appendix 1.

The Aurajoki river basin was chosen as a case area because it belongs to the Archipelago Sea catchment area, which has been identified by HELCOM as a high risk area for nutrient loss to water, and it is known to have high yearly nutrients losses from fields to waterways. The Island of Öland was chosen based on its high cattle density, the extensive grazing activity (often close to water), and the relatively high prevalence during recent years of salmonella outbreaks among the cattle farms. In general, though, both the Swedish and Finnish salmonella situation is very favourable, with national prevalence in domestic animals close to zero. In both areas, land application of manure in spring and autumn are common practices. However, since both areas are within nitrate-vulnerable zones according to the Nitrate Directive, there are limitations concerning when and where manure can be applied, in order to avoid the risk of nutrient leaching into water. For instance, manure should not be spread in periods when lands are frozen or water saturated, or on fields with steep slopes adjacent to watercourses.

Surface water samples were collected from sites both upstream (reference sites) and downstream of animal farms during different seasons and flow conditions. Potential high-risk events were included, such as snow melting and manure spreading periods. Water samples were analysed for the presence of faecal indicator bacteria (*E. coli* and enterococci), pathogenic zoonotic bacteria (*Salmonella*, *Campylobacter* and VTEC), and antimicrobial resistance among indicator bacteria and salmonella, using standard methods.

The lowest concentrations of indicator bacteria were found in the reference areas and the highest in small watercourses in Öland, where grazing cattle had direct access to the sampled water stream. In Aurajoki, the highest bacterial counts were detected downstream of large farms in connection with high-risk contamination events, *i.e.* high-flow conditions during snow melting, and within manure application periods. The lowest levels were found in June at low-flow conditions. Zoonotic pathogens were not detected in water samples at the reference sites, but were at sites downstream of animal farms in both areas. The highest prevalence was found for *Campylobacter* (Öland 9 of 41 samples, 22%; Aurajoki 3 of 20 samples, 15%), which is in line with previous investigations in the Aurajoki area (Hörman *et al*., 2004). Salmonella was detected in one sample from Aurajoki, whereas VTEC was never found. Bacteria resistant to antimicrobials were found both at the reference sites and at locations downstream of animal farms. In 16% of the samples from Aurajoki and Öland, bacteria resistant to more than one antimicrobial were found, all of them downstream of animal farms. In comparison, investigation of wastewater from seven Swedish sewage treatment plants during 2011 revealed that bacteria resistant to more than one antimicrobial could be detected in about 40% of the effluent water samples (unpublished data).

The higher bacterial counts in surface waters downstream of animal farms, compared to upstream sites with minor human and domestic animal impact, indicate possible faecal contamination linked to agricultural activities. Besides animal farms, faecal contamination may also emanate from wastewater treatment plants, on-site sanitation, or run-off from wastewater sludge fertilization. The enumeration of faecal indicators does not distinguish between these sources and manure, and the further development and implementation of methods for tracking sources of faecal pollution are warranted. No municipal wastewater treatment plant is situated upstream of any of the sampling sites in either Aurajoki or in Öland. However, there are several smaller on-site sanitation facilities, including private sewers; and, at least in the Aurajoki area, spreading of sewage sludge to fields is a common practice. Another confounding factor that cannot be excluded is faecal contamination from wild animals.

In conclusion, the most contaminated waters were found in the Swedish area. This could be explained by the smaller water courses in Öland than in Aurajoki, and the sampling sites were situated closer to farms. However, the most important factor is probably grazing animals having direct access to the sampled water stream at some of the Swedish sites. In Aurajoki, the relatively high bacteria counts at some sites downstream of animal farms, at snow melting and at medium flow conditions within manure spreading periods, highlight the importance of the transport risk (high water flow, steep slope) for leakage of microorganisms from fields to surface water. The investigated sites in this pilot study, on some occasions, did not comply with bathing water standards showing potential runoff from agricultural practices or affected by wastewater/sludge. Further investigations of microbial water quality of surface waters influenced by agricultural activities in the BSR, using a larger sample size, would add important information for estimating the risk of disease transmission from animal farms to the water environment.

Conclusions

Sustainable agriculture is a prerequisite for future food production, the environment, and animal and human health. Managing risks for eutrophication and disease transmission is crucial for the future of the BSR.

Biosecurity is important to prevent spread of diseases that may otherwise have detrimental consequences for the human and animal population (domestic and wildlife). This is of particular importance in view of the structural changes in the agricultural sector and climate change, which will affect animal health and welfare as well as animal productivity and the environment. It is important that scientists and policymakers are prepared to meet these challenges.

Monitoring of biosecurity risks is important for prevention. To be prepared for new challenges, the monitoring system has to be flexible and continuous. To be sensitive and cost effective, monitoring should be conducted as close to the source as possible.

Biosecurity measures at the farm level may prevent the introduction of pathogens, and consequently reduce the risk of shedding pathogens into the environment. Successful control programmes reduce the pathogen load in manure, and thereby also the level of environmental contamination. The risk of spreading the infectious agents present in manure to the environment can be managed by various means. These include proper storage, sanitisation, proper spreading techniques, and other measures to reduce surface runoff, such as buffer zones along watercourses, and avoiding the spread of manure during high-risk periods and in high-risk areas.

We have identified apparent synergies between nutrient and biosecurity mitigation measures. These are important to consider ensuring cost-effective management of both types of risks. A broader view of such risks is beneficial for both aspects, and can provide a further incentive for measures and investments in the BSR.

Future needs

Some future needs for research and policy development have been identified. These are listed below.

To gather more detailed data at the farm level on animal density, disease prevalence, and manure handling practices in the BSR, in order to identify larger geographical risk areas from the perspective of animal and human health considerations.

To monitor water quality in the BSR to confirm potential risk areas. One way could be to include microbiological status (*e.g.* faecal indicators) in the implementation of the Water Framework Directive (2000/60/EC). Investigating microbial water quality of surface waters influenced by agricultural activities in the BSR would provide important information for estimating the risk of disease transmission from animal farms to the water environment.

To assess the role of different sources (wastewater, agriculture or other) for microbial contamination in the BSR. Methods to identify sources of pollution should be developed and implemented, *e.g*. microbial source tracking methods and models to predict pathogen kinetics and dispersion.

To investigate a selection of infectious diseases in different farming structures and in different regions. This baseline data can be used for developing monitoring and control programs, with the ultimate goal of promoting animal health and reducing the load of pathogens in the environment.

To study the dynamics of infectious diseases at the interface between wildlife and domestic animals, in order to monitor and control disease transmission within different agricultural practices.

To investigate potential transmission routes of zoonotic microorganisms and antibiotic resistance bacteria from sediments and water into food webs, by studies of organisms at different levels of biological organization, including protozoa, invertebrates, and different classes of vertebrates from fish to mammals.

To consider survival and transmission of zoonotic microorganisms and antibiotic resistance bacteria based on abiotic water parameters, such as pH, eutrophication, salinity and oxygen, and to link such parameters with the impact of emerging factors, *e.g*. climate change, new chemicals, pharmaceuticals, invasive species and thereby modifications in feed webs.

To elucidate knowledge gaps about whether and how antibiotic resistant genes are transmitted between animals through the environment and via feed webs.

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Appendix 1: Case study

Introduction

Monitoring data for microbial water quality in surface waters influenced by agricultural activities in the Baltic Sea Region (BSR) is not performed on a regular basis, despite the risks from exposures via irrigation and drinking water and recreation. Bathing waters are monitored for microbial water quality, but this is limited to the summer season and to beaches decided upon by the municipality. As part of the Baltic Compass project, water samples from the Aurajoki river basin in Finland and the island of Öland in Sweden were collected during 2011-2012. The water samples were analysed for the presence of bacterial indicators of faecal contamination, pathogenic bacteria known to cause water-borne disease, and antibiotic resistance among the bacteria. The main objective with this pilot study was to illustrate how to investigate microbial contamination of surface waters influenced by agricultural activities (parameters, methods), and how to combine nutrient and biosecurity aspects. Furthermore, although limited as regards number of samples and study areas, the pilot study contributes valuable monitoring data.

Description of study areas

The Aurajoki river basin was chosen as a case area because it belongs to the Archipelago Sea catchment area, which has been identified as a high risk area for nutrient loss to water by HELCOM, and it is known to have high yearly nutrient losses from fields to waterways. The Island of Öland was chosen based on its high cattle density, the extensive grazing activity (often close to water), and the relatively high prevalence of salmonella outbreaks among the cattle farms during recent years.

Animal production

In general, animal density is higher in Sweden than in Finland. This is illustrated in the map (Figure 1) showing livestock density, which is the ratio of the number of livestock units per hectare of utilised agricultural area. A higher livestock density means that a higher amount of manure is available per ha of utilised agricultural area, which indicates a higher pressure of livestock farming on the environment and a potentially increased risk of nutrient and pathogen leaching.

On the island of Öland, cattle are the predominant species for animal production. Most herds are dairy herds but there are also some beef herds. A particular feature of cattle production in this region is the use of common pasture on Alvaret, a nature reserve that provides a rich habitat for wildlife, and where grazing is part of the nature conservation. This use of common pasture is regarded as one of the reasons for the circulating infection with *Salmonella* Dublin in the area. Öland is a small island with comparably barren vegetation in pasturelands, leading to a more intense contact pattern between grazing animals than is expected on richer pasturelands.

Based on Finnish conditions, the Aurajoki river basin has a moderate animal density, with the highest density in the northern part. Based on livestock units, pigs represent approximately 41% of the animals in the area, with poultry approximately 31% and cattle approximately 14 %.

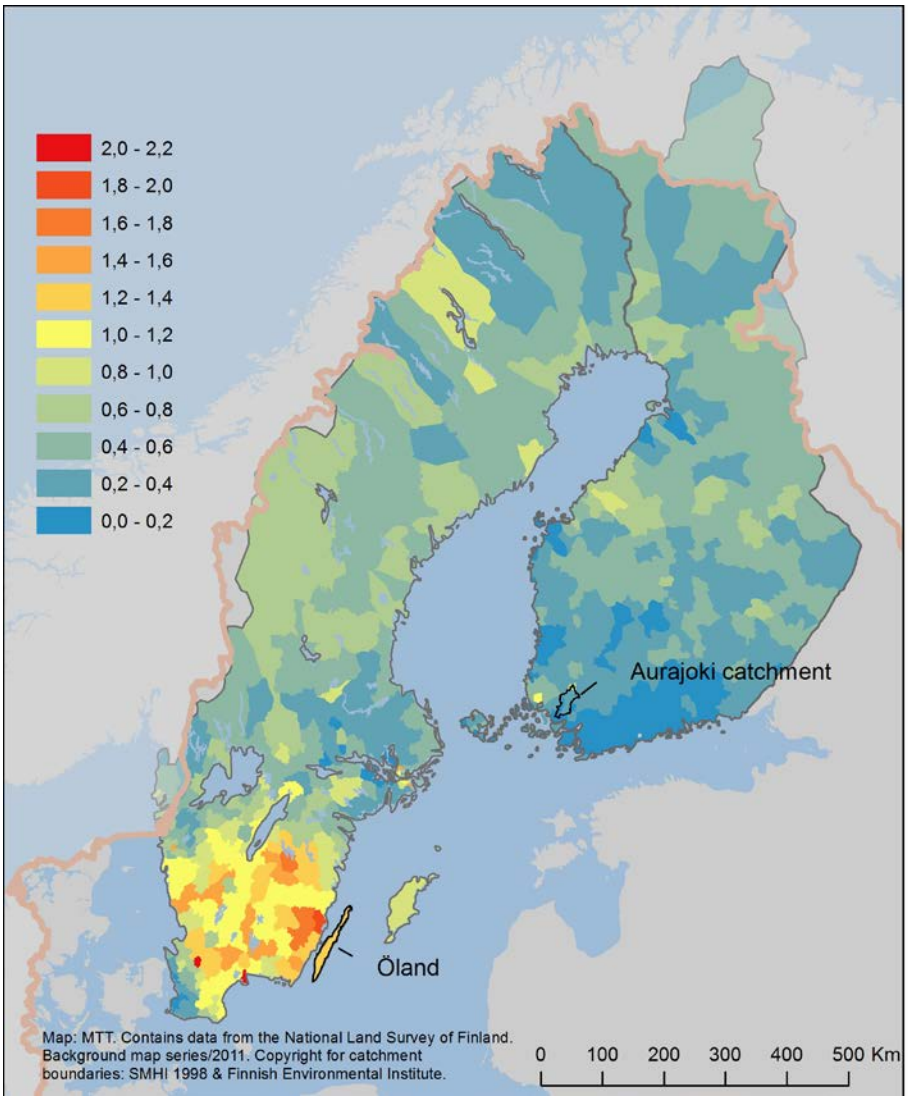

Figure 1. Livestock density index (livestock units per ha of utilised agricultural area) per municipality in 2010 in Sweden and in Finland.

Manure handling practices

Both Öland and Aurajoki have been designated as nitrate vulnerable zones (NVZ) according to EU's Nitrate Directive (91/676/EEC) (Figure 2), and thus manure handling practices are regulated to avoid risk of leakage of nutrients to water. Within NVZ, manure should, for instance, not be spread in periods where lands are frozen or water saturated, which indirectly means that sufficient manure storage is needed. On Öland, most of the pig and cattle farms have slurry storages, whereas most of the poultry farms have dry manure storages. Manure application to land in spring and autumn are common practices. In the Aurajoki catchment, most of the poultry farms have dry manure storages. In addition, most of the poultry farms spread the manure on fields, as usually only broiler farms (which are few, and none are located close to the sampling points) have contracts with industry. Most of the pig farms have slurry storages. For cattle, the situation is more evenly distributed between dry manure and slurry storages. Because of the Nitrate Directive, the storages both on Öland and in Aurajoki are well maintained, to enable spreading of manure only when allowed.

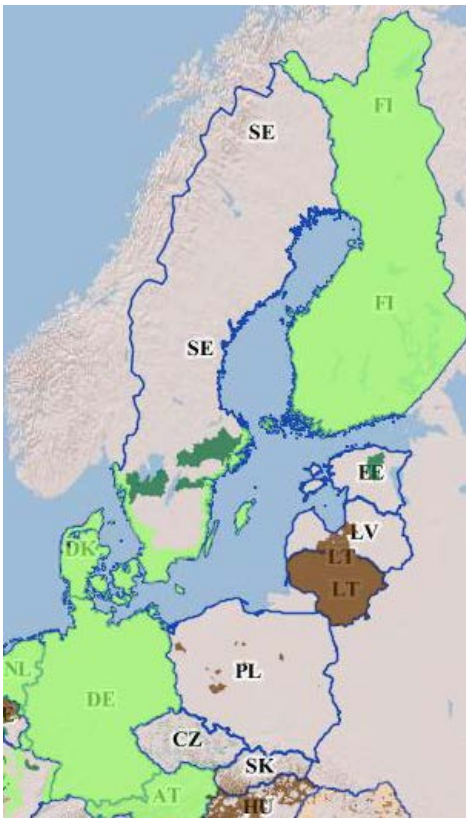

Figure 2. Nitrate vulnerable zones designated in Member states around the Baltic Sea.

Animal disease prevalence

The Swedish and Finnish salmonella situations are very favourable, with national prevalence in domestic animals close to zero. In both countries there are only around 4 -13 yearly salmonella outbreaks on cattle farms. In the case of salmonella positive farms, measures to eliminate the infection and trace the source are always implemented. In Sweden, the prevalence of salmonellosis in cattle is higher on Öland compared with other parts of the country, due to circulation of *Salmonella* Dublin.

Antimicrobial use in animals

In Sweden and Finland, prudent use of antimicrobials is an important feature of veterinary as well as human medicine. For example, in veterinary medicine there are policies and guidelines for all animal species (Swedish Veterinary Association, 2012), and antimicrobial resistance issues are high on the agenda. There is continuous surveillance of antimicrobial resistance in animal pathogens as well as indicator bacteria, and antimicrobial usage is also monitored (SVA, 2012). Antimicrobials for animals are only available with veterinary prescription, and antimicrobial growth promoters have been banned since 1986. In Finland, the situation is similar, although antimicrobial growth promoters were used until a decade later. There is now an EU-wide ban on antimicrobials for growth promotion. Results from the Finnish surveillance of antimicrobial resistance and monitoring of antimicrobial consumption can be found at the Finnish Food Safety Authority (Evira, 2012).

Climate, soil type, and land use

The total arable area on Öland in 2011 was 40,812 ha (ca 30% of the total area), and the main crop (in percentage of total arable land) was ley (54%), followed by spring barley (9%), winter wheat (9%), and maize (5%) (SJV, 2012). The dominant soil types in the sampling areas on Öland are clayey till, (5-15% clay), sandy soils, gravel, and limestone pavement (alvarmark) (SGU, 2012). Öland is one of the driest regions in

Sweden. The average precipitation in 2011 (mean value of data from two weather stations, one at the north and one at the south part of Öland) was 369 mm, and the number of days with precipitation was 135.

In the Aurajoki catchment, the total field area, calculated from the databases of the Information Centre of the Ministry of Agriculture and Forestry and The Agency for Rural Affairs, was 30,825 ha (35% of the total area) in year 2011. The main crop was spring wheat (24% of the total field area). The share of all spring cereals was 71% (grass 18%, autumn cereals 11%). The dominant soil type, according to the database of the Geological survey of Finland in the Aurajoki catchment, is clay (55%), which explains the high proportion of fields. According to the Finnish Meteorological Institute (FMI, 2012), the average yearly precipitation in the Aurajoki catchment is 717 mm.

Other possible sources of microbial contamination

No municipal wastewater treatment plant is situated upstream of any of the sampling sites either in Aurajoki or on Öland. However, there are several smaller on-site sanitation facilities, including private sewers, and, at least in the Aurajoki area, spreading of the end-product of the bio-gas process, where at least some part of the starting material is sewage sludge, to fields is a common practice. At least two companies have delivered the product to the Aurajoki fields, but unfortunately it was not possible to obtain the precise amounts to specific fields. Another confounding factor that cannot be excluded is faecal contamination from wild animals.

Water sample collection

The sampling strategy was to collect water both upstream (reference site) and downstream of animal farms, sampled during different seasons and water-flow conditions, and including sampling during high-risk events, *e.g*. at snow melting and within manure spreading periods.

In the Aurajoki river, water samples were collected from one reference site, which is a forested area with minor human and domestic animal impact, and from four locations downstream of animal farms (mainly pig, poultry and cattle). No farms were contacted in advance, since the sampling did not take place close to any particular farm. Rather, the samples were intended to provide an idea of the influence of many upstream farms. There were four sample times: in April 2011 water samples were collected at high flow during snow melting but before spreading of manure, in June 2011 at low flow approximately one month after manure spreading, in September 2011 at medium flow within the period of harvesting/spreading of manure, and in May 2012 water samples were collected at medium flow within the period of sowing/spreading of manure. Due to the fact that the Aurajoki catchment is quite big and thus the timing of field practices differs from north to south, it was difficult to sample all of the places during one day at the best possible time concerning, for example, spreading of manure.

On Öland, water samples were collected from different small waterways in the north-eastern and south-eastern parts, at a reference site upstream of animal farms with minor human and domestic animal impact, and at several sites downstream of cattle farms, both in grazing and non-grazing areas. There were three sample times: in June 2011 water samples were collected at low flow approximately one month after manure spreading, in August/September 2011 at medium flow within the period of harvesting/spreading of manure, and in October 2011 water samples were taken at medium flow within the period of sowing/spreading of manure.

In Aurajoki, four out of five (at low flow three out of five) samples were collected by Limnos water sampler (Figure 3). All water samples on Öland, as well as the sample at the Aurajoki reference site and one of the

other sites at low flow in Aurajoki, were collected manually (Figure 4). Every sample was taken without disturbing the bottom sediments.

Figure 3. Water sampling in the Aurajoki catchment 12.4.2011. Photo by Sirkka Tattari.

Figure 4. Taking a water sample during low flow at one of the sampling sites 13.6.2011. Photo by Elina Jaakkola.

At each sampling site, 1 L of water was collected in sterile bottles. Water samples were transported cold (2-8 °C) to the laboratory within 24 h, and analysed for detection of faecal indicator bacteria (*E. coli* and enterococci), pathogenic bacteria (*Salmonella*, *Campylobacter* and verotoxin producing *E coli*; VTEC), and antimicrobial resistance among indicator bacteria and salmonella.

Water analysis

Indicator bacteria

Water was serially diluted in peptone water and 100 ml was membrane filtered (0.45 µm pore size; filtertratt micro funnel, Pall Cooperation; VWR, Sweden). Filters were put onto Lactase TTC agar with sodium heptadecylsulfate (Fluka; Sweden) and Slanertz-Bartley (Slaba; Oxoid) agar for the enumeration of *E. coli* (SS-EN ISO9308-1) and enterococci (SSEN ISO7899-2), respectively. TTC plates were incubated at 44 °C for 24 h. Typical yellow or orange colonies capable of forming gas from lactose at 44 °C and indole-production were counted as indicators of *E. coli*. Slaba agar plates were incubated at 44 °C for 48 h, and typical blood-red or purple colonies capable of hydrolysing aesculin were counted as enterococci.

Zoonotic bacteria

Salmonella was analysed by NMKL 71, 5, 1999 or NMKL 187, 2007, as indicated in Tables 2 and 3. In the first method (NMKL 71, 5, 1999), 25 ml water was pre-enriched in 225 ml buffered peptone water (BPW) at 37 °C for 18 (± 2) h. Then 100 µL of the pre-enrichment was transferred to 10 mL rappaport vasiliadis soya broth (RVS) and incubated at 41.5 °C for 24 (± 3) h. From the RVS, 10 µL was streaked out on xylose lysine dexosycholate (XLD) agar and brilliant green (BG) agar, and incubated at 37 °C for 24 (± 3) h. In the second method (NMKL 187, 2007), 300 ml water was pre-enriched in 50 ml BPW at 37 °C for 18 (± 2) h. Then 100 µL of the pre-enrichment was placed with three drops separately and equally spaced on the surface of modified semisolid rappaport vasiliadis (MSRV) agar, and incubated at 41.5 °C for 24 (± 3) h. If the plates were negative after 24 h, they were incubated for another 24 h at 41.5 °C. From presumptive salmonella positive plates, approximately 1 µL of the opaque zone was streaked on XLD and BG and then incubated at 37 °C for 24 (± 3) h. For both method A and B, typical salmonella colonies on XLD agar plates (reddish colour with a black centre) were serologically confirmed.

Campylobacter were detected by ISO 17995:2005.

For detection of VTEC (*E. coli* O157), 100 ml water was membrane filtered (0.45 µm pore size; filtertratt micro funnel, Pall Cooperation; VWR, Sweden). Filters were put into 100 ml of tryptone soya broth (TSB), incubated at 41.5 °C for 24 h, and further analysed at the Swedish National Veterinary Institute (SVA) for detection of *E. coli* O157 (NMKL 164, 2, 2005).

Antimicrobial resistance

Antimicrobial susceptibility tests were performed following the standards for microdilution of the Clinical and Laboratory Standards Institute (CLSI, 2008). The microdilution panels used, VetMic, were produced at SVA. From positive water samples, the resistance profile (Table 1) of one *E. coli,* one enterococci and one Salmonella colony was determined with VetMic™ GN-mo (version 4), VetMic™ E-cocci (version 3), and VetMic™ Salmonella, respectively (SVA-production; Uppsala, Sweden). Minimum inhibitory concentrations (MIC), *i.e.* the lowest concentration of an antimicrobial that inhibits bacterial growth, were recorded. For interpretation of results, epidemiology cut-off values issued by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) (http://www.escmid.org) were used.

Table 1. Antimicrobial substances tested for resistance in *E. coli,* enterococci and salmonella, and cut-off values.

E. coli		*Enterococcus* spp.		*Salmonella enterica*	
Substance	Cut-off value [mg/L]	Substance	Cut-off value [mg/L]	Substance	Cut-off value [mg/L]
Ampicillin (Am)	> 8	Ampicillin (Am)	> 4	Ampicillin (Am)	> 8
Nalidixic acid (Nal)	> 16	Erythromycin (Em)	> 4	Nalidixic acid (Nal)	> 16
Gentamicin (Gm)	> 2	Gentamicin (Gm)	> 32	Gentamicin (Gm)	> 2
Streptomycin (Sm)	> 16	Kanamycin (Km)	> 1024	Streptomycin (Sm)	> 16
Tetracycline (Tc)	> 8	Tetracycline (Tc)	> 4	Tetracycline (Tc)	> 8
Florfenicol (Ff)	> 16	Chloramphenicol (Cm)	> 32	Florfenicol (Ff)	> 16
Colistin (Cs)	> 2	Vancomycin (Va)	> 4	Trimethoprim (Tm)	> 2
Trimethoprim (Tm)	> 2	Bacitracin (Ba)	> 32	Chloramphenicol (Cm)	> 16
Chloramphenicol (Cm)	> 16			Kanamycin (Km)	> 16
Kanamycin (Km)	> 8			Cefotaxime (Ctx)	> 0.5
Cefotaxime (Ctx)	> 0.25				

Results and discussion

In general, the lowest concentrations of indicator bacteria were found in the reference areas, *i.e.* upstream of animal farms in areas with minor human and domestic animal impact, and the highest concentrations were in waters where grazing cattle had direct access to the sampled water stream (Tables 2 and 3). Higher concentrations were found downstream of animal farms. On Öland, in small watercourses close to grazing cattle, up to 500,000 colony forming units (CFU) *E. coli*/100 mL and 13,000 CFU enterococci/100 mL were found. In Aurajoki, the highest bacterial counts (up to 1,600 CFU *E. coli*/100 mL and 2,900 CFU enterococci/100 mL) were detected downstream of large farms in connection with high-risk contamination events, *e.g.* after snow melting and within manure application periods. The lowest levels were found in June under low-flow conditions. The Aurajoki results highlight the importance of the transport risk (high water flow, slope) for leakage of microorganisms from fields to surface water.

Amongst the pathogens, *Campylobacter* was detected in 12 of 61 total water samples in Aurajoki and Öland; salmonella was found in one, whereas VTEC was never found. All pathogens were detected in samples downstream of animal farms. A higher percentage of *Campylobacter* positive samples was found on Öland (22%) compared to Aurajoki (15%). A similar prevalence of *Campylobacter* in the Aurajoki river basin, *i.e.* 17%, has previously been reported (Hörman *et al*., 2004). The presence of faecal indicator bacteria indicates potential pathogen contamination. Pathogens occur in much lower numbers in manure compared to indicator bacteria, and are therefore more difficult to detect within a water sample. Two different methods were used for analysis of salmonella in the present study. The method that was used in the latter part of the study (NMKL 187, 2007) is considered being the most sensitive, due to a larger water volume and a more selective pre-enrichment.

Resistant bacteria were found both at the reference sites and at locations downstream of animal farms (Tables 2 and 3). In 16% of the samples from Aurajoki and Öland, bacteria were found resistant to more than one antimicrobial drug, all of them downstream of animal farms (Tables 2 and 3). In addition to the antimicrobial substances listed in Table 1, the susceptibility testing also included sulfamethoxazole for *E. coli*, and virginiamycin, streptomycin, narasin and linezolid for enterococci. However, for different reasons discussed below, the results have been omitted for these substances. For *E. coli* and salmonella, a remarkably high prevalence of resistance to sulfamethoxazole was noted; almost all tested isolates were found resistant. This result may, however, be due to a methodological error. Susceptibility testing of bacteria to sulphonamides using microdilution methods is, in some cases, difficult and should be

interpreted with caution. Since no distinction between *Enterococcus faecalis* and *Enterococcus faecium* was made, and since the cut-off values differ between the two species regarding virginiamycin, streptomycin, and narasin, it was not possible to interpret the test outcomes, and the results for these substances are not presented. Some cases of possible linezolid resistance were noted among the Swedish water samples; however, further confirmation is needed. A high MIC for vancomycin was seen for enterococci in one water sample from Öland downstream of a cattle farm; however, since it is not known whether the resistance trait belongs to *E. faecalis* or *E. faecium*, it is difficult to determine the significance of this result.

Table 2. Number of indicator bacteria (*E. coli* and enterococci), presence of zoonotic bacteria, and antimicrobial resistance among indicator bacteria, in water samples collected in the Aurajoki catchment in 2011 and 2012.

Site description	Sampling time	*E. coli* (CFU/ 100mL)	enterococci (CFU/ 100mL)	Zoonotic bacteria[1,2]	Antimicrobial resistance		
					E. coli	enterococci	*Salmonella*
Downstream several large farms	April 2011	200	160				
	June 2011	10	96			Km/Ba	
	Sept 2011	500	2900		Am/Nal/Gm/ Ctx	Tc	
	May 2012	1300	25			Ba	
Downstream several large farms	April 2011	1400	420				
	June 2011	<1	<1		-	-	
	Sept 2011	100	61				
	May 2012	1600	55	*C. jejuni*		Em	
Upstream (reference)	April 2011	2	2				
	June 2011	70	10			Ba	
	Sept 2011	260	150			Tc	
	May 2012	30	<1			-	
Downstream several farms	April 2011	150	230			Tm/Em	
	June 2011	<1	<1		-	-	
	Sept 2011	10	13		Am/Nal/Gm/ Tc/Ctx	Tc	
	May 2012	400	43			Em/Ba	
Downstream some smaller farms	April 2011	30	310	*C. jejuni, C. coli*			
	June 2011	10	36			Tc	
	Sept 2011	360	133		Am/Nal/Gm/ Tc/Tm/Ctx/	Tc	
	May 2012	9	3	*C. jejuni, S. typhimurium*		-	

[1]Zoonotic bacteria analysed: *Salmonella*, *Campylobacter*, and verotoxin producing *E. coli* (VTEC)

[2]Water samples collected in April and June were analysed for *Salmonella* by NMKL 71, 5, 1999; samples collected in September were analysed by NMKL 71, 5, 1999 and NMKL 187, 2007; samples collected in May were analysed by NMKL 187, 2007.

Table 3 Number of indicator bacteria (*E. coli* and enterococci), presence of zoonotic bacteria, and antimicrobial resistance among indicator bacteria, in water samples collected on Öland in 2011 upstream or downstream of animal farms.

Site description	Sampling time	*E. coli* (CFU/100ml)	enterococci (CFU/100ml)	Zoonotic bacteria[1, 2] [positive/negative]	Antimicrobial resistance	
					E. coli	enterococci
Upstream	June 2011	<1	10		-	
	August 2011	12	9			
	October 2011	<1	<1		-	
Downstream	June 2011	<1	5	*C. jejuni*	-	Ba
	August 2011	560	350			
	October 2011	22	19			
Downstream	June 2011	<1	<1		-	
	August 2011	5	8			
	October 2011	10	<1			
Downstream[a]	June 2011	>500000	3000			
	August 2011	26000	13000			
	October 2011	65000	3700			
Downstream	June 2011	10	<1			-
	August 2011	840	1000			
	October 2011	54000	5800	*C. jejuni*		
Upstream	June 2011	<1	<1			
	August 2011	250	24			
	October 2011	-	-			
Downstream	June 2011	<1	26		-	
	August 2011	260	290	*C. jejuni*		
	October 2011	2500	1800	*C. jejuni*		
Downstream	June 2011	120	36			
	August 2011	150	80	*C. coli*		
	October 2011	180	66	*C. coli*		
Upstream[b]	June 2011	<1	3		-	Km/Tc/Cm
	August 2011	1200	150			
	October 2011	33	16			
Downstream[a]	June 2011	3300	880	*C. coli*		Cm
	August 2011	260	12			
	October 2011	30	9			Va
Downstream	June 2011	<1	15	*C. coli*	-	Gm/Km/Ba
	August 2011	130	14			
	October 2011	16	16			
Common grazing area[a]	June 2011	11000	19	*C. jejuni*		
	August 2011	200	70			
		110	70			
		51	35			
		10	<1			
		100	54			
	October 2011	50	9			
		23	3			
		100	5			

[1]Zoonotic bacteria analysed: *Salmonella*, *Campylobacter*, and verotoxin producing *E. coli* (VTEC)

[2]Water samples collected in June were analysed for *Salmonella* by NMKL 71, 5, 1999; samples collected in August and October were analysed by NMKL 71, 5, 1999 and NMKL 187, 2007.

[a]Cattle had direct contact with the watercourse

[b]No grazing animals at the time of sampling; grazing had occurred previously.

The Aurajoki case was an example of how the biosecurity aspects can be considered together with nutrient vulnerable areas. In Figure 5, classifications of water sampling sites in the Aurajoki river basin regarding microbial water quality (Bathing Water Directive, 2006/7/EC), into Good, Sufficient and Poor, is plotted on a map showing the modelled risk of nutrient leakage as calculated by the USLE-method (Räsänen, 2010).

(Figure 5). The model takes into account erosion and manure application risks. The erosion factor is based on soil, slope, land use factor and dominant field plants; and the manure application is estimated based on the number and type of animals in farms, and assuming that the manure that they produce is spread evenly in the surrounding fields within a zone that is based on the amount of the produced manure. The bathing water directive classifications should, however, preferably be based on evaluation of samples for the last four-year period, taking into account the variability in microbial pollution. In this investigation, the water quality evaluation was based on only four samples during one year.

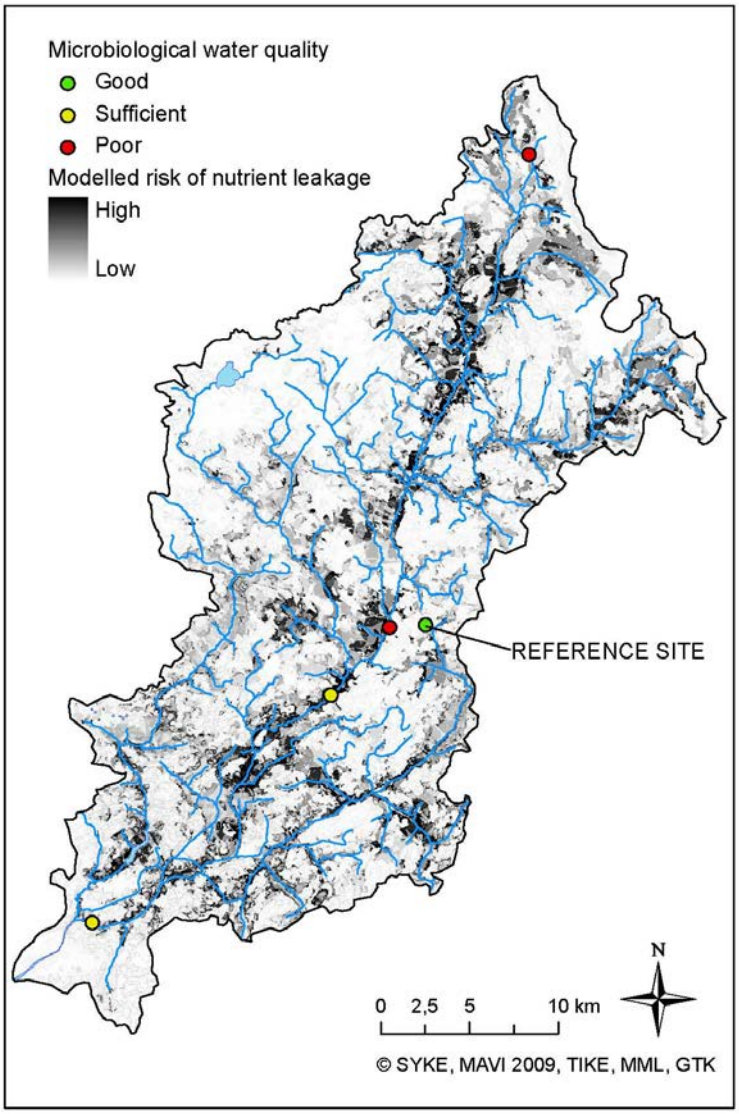

Figure 5. Modelled risk of nutrient leakage to surface waters in the Aurajoki river basin, and classification (Bathing Water Directive, 2006/7/EC) of the water sampling sites into Good (green), Sufficient (yellow) and Poor (red) regarding microbial water quality.

Conclusions

The most contaminated waters were found in the Swedish case area. This could be explained by the smaller water courses on Öland than in Aurajoki, and that the sampling sites were situated closer to farms. However, the most important factor is probably grazing animals having direct access to the sampled water

stream at some of the Swedish sites. In Aurajoki, the relatively high bacterial counts at some sites downstream of animal farms, during snow melting and under medium flow conditions within manure spreading periods, highlight the importance of the transport risk (high water flow, steep slope) for leakage of microorganisms from fields to surface water. The sites investigated in this pilot study at some occasions did not comply with bathing water standards, showing potential runoff from agricultural practices or being affected by wastewater/sludge. Further investigations of microbial water quality of surface waters influenced by agricultural activities in the BSR, using a larger sample size, would add important information for estimating the risk of disease transmission from animal farms to the water environment.

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