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PathOrganic* – Risks and Recommendations Regarding Human Pathogens in Organic Vegetable Production Chains

Introduction

Outbreaks of human diseases associated with the consumption of vegetables have increasingly been reported. Evidently, human pathogens such as *Salmonella sp.* or *Listeria sp.* are able to invade and survive in living plant tissue. The use of organic manures for fertilization is a potential pathway of pathogen transfer from livestock to plant produce in organic agriculture. In a three years project, PathOrganic (<http://pathorganic.coreportal.org/>) evaluates food safety risks and provides guidelines for controlling bacterial contamination of organically produced vegetables.

Methods

Besides other activities, large-scale surveys of manures and vegetables were performed on 157 farms located in Austria (40), Denmark (31), Germany (6), Switzerland (40) and Sweden (40). Different types of animal manure were sampled and analyzed for the prevalence of *E. coli*, *Salmonella sp.*, *Staphylococcus aureus*, *Listeria sp.* and *Campylobacter sp.* by using cultivation dependent (ISO) as well as PCR based methods. In addition, *E. coli* counts and chemical analyses were done. Microbial DNA preparations from the samples were distributed among the participating labs, each specialized in specific PCR based analyses. Because of congruency with cultivation-dependent analyses and higher detection sensitivity, only molecular methods were used for analyzing vegetable plants subsequently grown on the fertilized fields.

Results

Human pathogens in organic manures

E. coli was detected in almost all manure samples, as expected. Some of the *E. coli*-positive cases, however, gave indications for the presence of enterohaemorrhagic *E. coli* (EHEC) based on PCR-detection of *rfbE* and *stx1*, *stx2* and *eae* virulence genes. A considerable amount of samples proved positive also for *Campylobacter sp.* and *Staphylococcus aureus*. *Salmonella sp.* was detected from time to time in manures from Austria and Switzerland but not from the Nordic countries (Fig. 1).

Human pathogens in spinach and lettuce plants

Vegetables from farms where manures have shown evidence for a risk of being contaminated with human pathogens were selected to be analyzed for the same pathogens as had been studied in the manures. A coherent scheme for both sampling and analysis was developed which concentrated on screening spinach and lettuce plants but included also carrots and cabbage (Fig. 2). Analysis of vegetable samples is still ongoing. Preliminary results suggest transfer of *Salmonella sp.* from manures to crops with potential residence of the pathogen in the soil. In the following, colonization behavior and competitive performance of human pathogens in vegetables will be studied in detail in greenhouse and field experiments.

Conclusions

1. Large-scale surveys of manure samples and raw-eaten vegetables such as spinach, lettuce, carrots and cabbage have been carried out in five countries (A, CH, D, DK, S).
2. Molecular techniques suitable for the detection of *E. coli*, *Salmonella sp.*, *Staphylococcus aureus*, *Listeria sp.* and *Campylobacter sp.* have been specifically adapted.
3. Most of the manure samples contained *E. coli*, and many samples proved positive for *Listeria sp.* and *Staphylococcus aureus*.
4. The significance of the prevalence of human pathogens for potential food safety risks will be analyzed in subsequent studies.

Part 1a

SAMPLE (A)	E. coli				E. coli EHEC*				Salmonella				Staphylococcus aureus				Listeria				Campylobacter			
	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-
A01MM																								
A02MK																								
A03MH																								
A04MK																								
A05MP																								
A06MK																								
A07MM																								
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A36SK																								
A37SK																								
A38MK																								
A39SM																								
A40MK																								

Part 1b

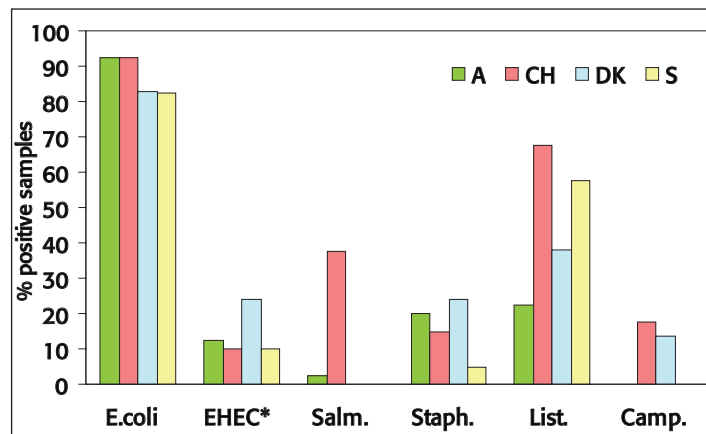


Figure 1. Part 1a Overview of PCR-based screenings of manures from organic vegetable farms in Austria (A), Switzerland (CH), Sweden (S) and Denmark (DK) for the prevalence of *E. coli*, *E. coli* EHEC, *Salmonella sp.*, *Staphylococcus aureus*, *Listeria sp.* and *Campylobacter sp.* Analysis of samples from Germany is still ongoing. Black versus white fields indicate positive versus negative PCR results.

* based on PCR results for *rfbE* and *stx1*, *stx2* and *eae* virulence genes.

Part 1b shows the percentage of samples from the individual countries that proved positive for the respective pathogens.



Figure 2. Sampling of lettuce plants for subsequent screening for the prevalence of human pathogens.

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