TECHNICAL GUIDE

A Project of the research network CORE organic

Manure for Vegetables

Farm practice recommendations for minimizing human pathogenic bacteria contamination in vegetable production

In brief

To obtain sufficient yields in vegetable production, fertilization with nitrogen, phosphorus and potassium is necessary. Manure is a valuable source of these nutrients. This guide is an overview of the latest scientific information concerning potential food safety risks related to the application of manure in vegetable production, and gives recommendations for minimizing these risks. While the focus is on manure handling, other potential contamination sources are also briefly addressed.

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Introduction

To obtain a sufficient yield of vegetable crops, fertilization with nitrogen, phosphorus and potassium is necessary; and manure is a valuable source of these nutrients. The use of animal waste and by-products as a sustainable resource for crop fertilization and soil improvement, preferably recycling on-site, is a core component in organic agriculture [1,2]. According to IFOAM's principle of ecology, "inputs should be reduced by recycling and efficient management of materials and energy"[3]. Sources of mineral phosphorus are limited and it will become a rarely available nutrient in the near future. An alternative, renewable source that will become increasingly vital in the future is animal waste such as manure applied as crop fertilizers and soil amendments to optimize soil structure and microbial ecological balance.

Within the EU FP7 CORE Organic ERA-Net, the applied research project PathOrganic surveyed occurence of human pathogenic bacteria in manure and vegetable production systems, as well as transmission pathways from manure to soil and organic plant crops. Critical control points along the food chain (from sowing to farm gate) were defined and a simple risk model was developed.

The objective of this technical guide is to summarize the relevant information from reports, scientific papers and results of PathOrganic, and provide a current perspective on the occurence of human pathogens in manure and their possible transfer to organic vegetables. Although hygienic aspects during harvest and packing, rinsing produce with clean tap water and constant cooling chain after harvest are important, these points are not subjects of this technical guide (see e.g., "Global GAP Fruit and Vegetable" or Codex Alimentarius CAC/RCP 53-2003 "Code of hygienic practice for fresh fruits and vegetables").

Good application practice of manure

Human pathogens are primarily found in feces of wild and domestic animals. However, most bacteria (such as E. coli) live and propagate in the gastrointestinal tract of animals and humans without causing any health problems. The use of manure as fertilizer in agriculture implies a risk of introducing pathogens into vegetables and other crops either directly through the applied manure or indirectly via contamination of irrigation water, run-off from or flooding of neighboring fields[4].

Despite recent press attention, only a few of the foodborne human outbreaks associated with contaminated fresh produce can be linked directly to application of manure in vegetable systems[5]. Regardless, there is zero-tolerance and any incident can decimate markets. In order to ensure the safe use of manure as a resource in organic agriculture, research to understand pathogen life-cycles and developing risk-mitigation strategies is critical.

Current practices for using manure in vegetable crop production differ considerably among countries. For instance, in Germany and Austria fewer than 30 % of all vegetable growers use manure and large farms generally utilize other types of fertilizer. Many organic vegetable producers use green waste compost and commercial organic fertilizers (such as feather meal, which are heat treated to avoid pathogen transfer) to fertilize crops.

Using composted manure with shallow incorporation and before crop establishment is best practice to minimize pathogen survival and optimize nutrient efficiency. *(Picture: Martin Koller, FiBL).*

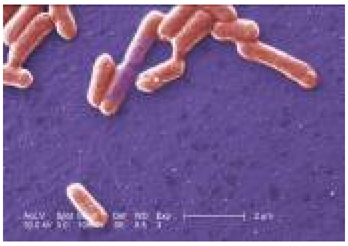
Short overview of outbreaks

According to the European Food Safety Authority (EFSA), very few outbreaks of foodborne diseases have been linked to vegetables[6]. In the European "Rapid Alert System of Food and Feed", only 30 out of 4'040 alerts of pathogenic microorganisms were associated with contaminated vegetable produce[7]. However, with vegetable produce, associated outbreaks were reported to increase recently, for example, in the USA[20a]. Organic produce has not been shown to differ in terms of risk from conventionally-grown produce1 (e.g., EFSA 2007), and often publicized links are ultimately traced to contaminated meat or other source[8]. However, increasingly popular ready-to-eat, bagged produce like baby leaf spinach or chopped salad may present an elevated risk via direct contamination (e.g., with manure or soil; contact with infected handlers/packagers) or indirect contamination (e.g., exposure to infested irrigation water or manure). Moreover, produce leaving the farm is required to be pathogen free because among other problems, pathogens grow exponentially when improperly stored at markets or by consumers.

In the USA, outbreaks traced to vegetables, feces-contaminated irrigation water[9a] or run-off of manure from neighboring fields has been implicated as suspected sources. However, the reactions to the recent outbreak in Europe with *E. coli* O104:H4[9b] readily considered links to the use of manure and slurry in organic farming. Therefore, examining the potential risk of manure use regarding human pathogens on vegetables is critical to maintain consumer confidence and market viability of organic produce.

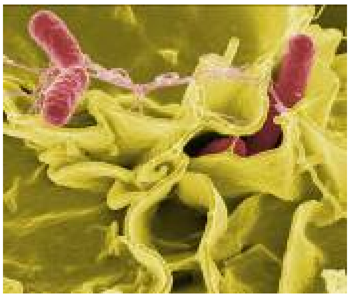
Pathogens Investigated in the PathOrganic Project

The five bacterial pathogens described below are among those that have most often been linked to outbreaks and were investigated in the PathOrganic project.



Escherichia coli is a rod-shaped, Gram-negative bacterium, which is a natural and generally unharmful inhabitant of the lower intestine of humans and animals. It is therefore used as an indicator for fecal contamination, e.g., in water. Some strains (e.g., enterohaemorrhagic *E. coli*, EHEC) produce toxins, which can cause serious and even life-threatening complications such as hemolytic-uremic-syndrome (HUS). Raw milk, undercooked meat and vegetables contaminated with feces are common sources of infection. Within PathOrganic, the EHEC strain O157:H7 was investigated.

Picture: Centers for Disease Control and Prevention / National Escherichia, Shigella, Vibrio Reference Unit, Janice Carr (United States Department of Health and Human Services).



Salmonella *serovars* are rod-shaped, Gram-negative, non-spore forming bacteria and are the bacterial foodborne pathogens most commonly linked to outbreaks. Infection leads to diarrhoea that can be life-threatening to labile persons or small children. This bacterium can survive outside the body of its host for weeks and was found in dry faecal matter for over 2 years. Poultry and eggs are often contaminated with *Salmonella*, although lately infections have been reported due the consumption of vegetables, such as rocket salad.

Picture: Rocky Mountain Laboratories, NIAID, National Institutes of Health (United States Department of Health and Human Services.)

Staphylococcus aureus is a ball-shaped, Gram-positive bacterium and it often appears in clusters. It is ubiquitous in nature and certain strains can cause a wide-range of diseases, from minor skin infections to sepsis. The bacterium can produce enterotoxins, which are heat stable and not destroyed by cooking, causing diarrhea and vomiting. Antibiotic-resistant strains of this pathogen are a serious problem in hospitals.

Picture: Centers for Disease Control and Prevention / Matthew J. Arduino, DRPH*, Janice Carr (United States Department of Health and Human Services).*

Listeria monocytogenes is a rod-shaped, Gram-positive, motile bacterium that is ubiquitously distributed in nature. It can be found in soil, water, plants and animals and is a classical foodborne pathogen. Infections occur mainly due to the consumption of contaminated food like raw milk, milk products, ice cream, untreated vegetables or raw meat. *L. monocytogenes* can cause serious infections in newborns, pregnant and immunocompromised persons. These individuals are at increased risk for infection. The symptoms range from diarrhea to live threatening meningitis and encephalitis in labile people.

Picture: Centers for Disease Control and Prevention Dr. Balasubr Swaminathan; Peggy Hayes *(United States Department of Health and Human Services).*

***Campylobacter* spp**. are spiral-shaped, Gram-negative bacteria that are sensitive to oxygen and dry conditions. They can be found in many animals, for example poultry, pigs, and cattle. Members of the genus *Campylobacter*, especially *C. jejuni* and *C. coli*, are poorly suited for growth in food, but the number of bacteria required for food poisoning on the other hand is low.

Picture: Centers for Disease Control and Prevention / Dr. Patricia Fields, Dr. Collette Fitzgerald , Janice Carr (United States Department of Health and Human Services).

Factors for Minimizing Risks in Animal Husbandry

Animal species, feeding regime and housing systems exert a high impact on the pathogen load in animals and their faeces.

E. coli (EHEC strains) is frequently present in ruminants[10a]. Especially high numbers are shed when concentrate feed rich in carbohydrates, dominates cattle diets[10b, 11]. Calves also shed high quantities of EHEC *E. coli*. *Listeria*, *Campylobacter* and less frequently *Salmonella* are also found in cattle manure[12].

Picture: Claudia Schneider, FiBL

Pigs are typically less frequent carriers of EHEC strains than cattle, but are often associated with *Salmonella* and *Campylobacter* [12]. In Scandinavian countries, a strict hygienic program leads to decreasing prevalence of *Salmonella* in pigs. However the manure survey in the PathOrganic project showed a low occurrence of *Salmonella* and *Campylobacter*, but in the majority of pig slurries *E. coli* O157:H7 was present.

Picture: Barbara Früh, FiBL

In poultry meat and eggs, infection with *Salmonella* and *Campylobacter* plays an important role. Free range systems lead to a higher prevalence of pathogens[13, 14]. In a manure survey of the PathOrganic project, there was no chicken manure sample contaminated with *Campylobacter* or *Salmonella* (sample size: n=7), probably due to dry conditions and a prolonged storage time. However, *Campylobacter* was present in slurries, when chicken manure was recently mixed with cattle slurry (n=2).

Picture: Klaus Böhler, FiBL

Sheep and goats are also potential EHEC carriers. However, with exceptions of some dairy sheep and goats, they are fed predominantly with grass (pasture) and hay and with only little or no concentrate feed, which offers poor growth conditions for EHEC.

Picture: Felix Heckendorn, FiBL

To our knowledge, the risk of horse manure being contaminated with bacterial pathogens is relatively low compared to cattle and pigs.

Picture: Barbara Früh, FiBL

Factors for Minimizing Risks in Manure Processing and Storage

Adequate manure storage and processing may have a significant impact on mitigating the pathogen load.

Stacked manure
During storage the population of pathogens decreases[15], but if new manure is added continuously (standard procedure), recontamination of the heap occurs. Storage time of 4 months or more (without adding new material) is suggested to ensure a significant decline of pathogens.

Picture: Res Schmutz, FiBL

Deep litter manure
Like stacked manure, deep litter manure shows pronounced anaerobic conditions. To allow a decrease of pathogens without recontamination, the manure has to be taken out of the stable and stored on heaps for 3-4 months[16].

Picture: Claudia Schneider, FiBL

Slurry
Slurry also shows anaerobic conditions, but in contrast to solid manures, a high content of easily available carbon sources is present, providing good conditions for pathogens. In the manure survey of the PathOrganic project, slurry was more frequently contaminated with *Salmonella*, *Listeria monocytogenes* and *E. coli*, compared with solid manure. Slurry was also the only manure type that contained *Campylobacter* according to the survey. In contrast, *Staphylococcus* was equally present in manure and slurry. In slurry stored for 3-4 months, the pathogen load decreased to a similar level as has been found in manure. Only when no fresh slurry is added during storage, a decrease in pathogen population takes place.

Picture: Martin Koller, FiBL

Manure composting (aerobic)
Under aerobic conditions, pathogens decrease faster than under anaerobic conditions (see below). If heaps are frequently turned and the temperature is allowed to rise up to 55 °C for 2 weeks, pathogen levels decrease below the detection limit[17, 29]. For *Salmonella* even lower temperatures are sufficient if the C:N ratio is lower than 20:1[18]. A disadvantage of such high temperature conditions and frequent turning is, however, an increase in the loss of nitrogen via NH_3 evaporation.

Picture: Martin Koller, FiBL

Fermented manure and slurry (anaerobic)
Pathogens are hardly decreased in mesophilic biogas fermentation plants (35-39 °C) with continual adding and removal of slurry (typical "agricultural plant"). Elimination could be achieved with pre-treatment at 70 °C for 1 hour or a semi-batch process of at least > 53 °C and 20 hours[19] (However, usually only for non-agricultural co-substrate is pre-treated in such ways).

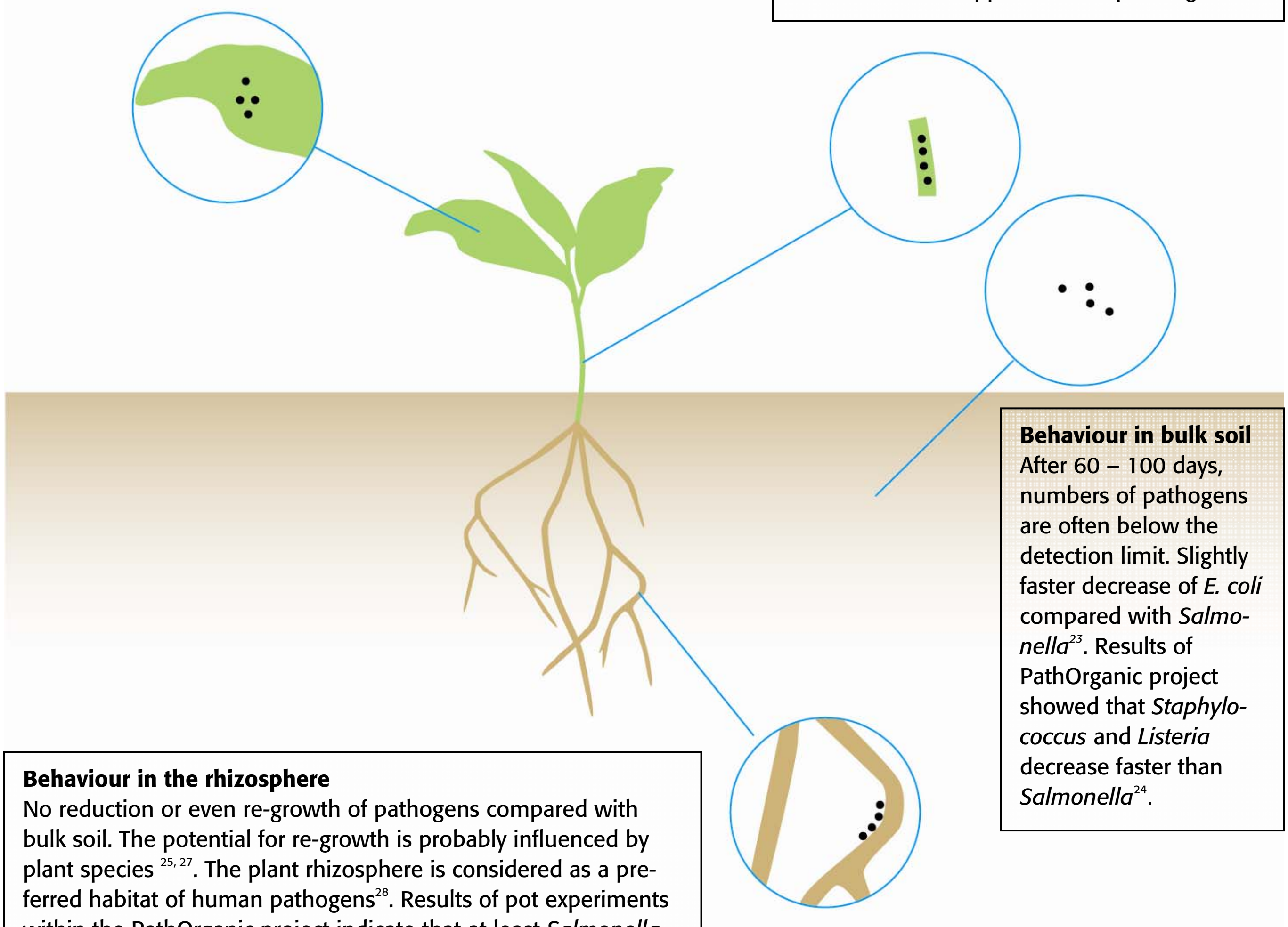
Picture: Jacques Fuchs, FiBL

Pathogens in Soil and on Plants

Human pathogenic bacteria can survive on the plants and in the soil. They may enter the plant through direct contact or splash contamination with the fertilizer, and may migrate within plant tissues. To which extent this migration is taking place is still a matter of debate[20a].

Direct or splash contamination
Pathogens on plant surface will decrease due to exposure to solar light (UV), but survival for almost one month has been reported (e.g., *E. coli*).

Bacteria in vascular bundle [20b, 21, 22] Internalization into roots and transportation in the plant is in some cases possible. Therefore, contamination of produce is possible, even when manure is applied before planting.



Behaviour in bulk soil
After 60 – 100 days, numbers of pathogens are often below the detection limit. Slightly faster decrease of *E. coli* compared with *Salmonella*[23]. Results of PathOrganic project showed that *Staphylococcus* and *Listeria* decrease faster than *Salmonella*[24].

Behaviour in the rhizosphere
No reduction or even re-growth of pathogens compared with bulk soil. The potential for re-growth is probably influenced by plant species [25, 27]. The plant rhizosphere is considered as a preferred habitat of human pathogens[28]. Results of pot experiments within the PathOrganic project indicate that at least *Salmonella* numbers can increase considerably there.

Graphic: Martin Koller and Claudia Kirchgraber (FiBL)

Existing Regulations and Recommendations

Current regulations for applying manure/slurry in vegetable production, in relation to human pathogens.

General instructions

Codex alimentarius:
"Organically Produced Foods" (GL-32-1999):
Annex A 50b) "Manure management practices that does not significantly contribute to contamination of water by nitrates and pathogenic bacteria"
"Code of hygienic practice for fresh fruits and vegetables" CAC/RCP 53-2003

Cross Compliance
Food that is not sure for consumption could not be laced to market.

LFGB §5 (Lebensmittel- und Futtermittelgesetzbuch)
It is prohibited to produce or treat food in a way that others might be endangered by consumption.

SR 817.0 LGV (Lebensmittel- und Gebrauchsgegenständeverordnung)
Human health should not be endangered by e.g. fertilization.

Concerning fertilizers

EC No 1069/2009
Laying down health rules concerning animal by-products not intended for human consumption…Organic fertilizers are only placed to market if treated after a method described in Annex V / Chapter III.

DüMV (Düngermittel Verordnung)
Fertilizers for vegetable production (§5) are only permitted to be placed on the market if they are free of *Salmonella* (in 50 g sample; §4)
Private organic regulations
The use of conventional slurry and chicken manure is prohibited (Bioland/Naturland/Gäa/Demeter/Biokreis)

NOP (National organic program) USA
NOP instruction 5006 on processed manure: Processed manure maybe used without further restriction, if a minimum temperature of 66 °C for 1 hour or maximum of 76 °C and dried to maximum moisture of 12 %. Processed manure products should not contain more than $1x10^3$ MPN (most probably numbers) fecal *E. coli* form per gram and three MPN *Salmonella* per 4 gram.

Applying manure

SJVFS 2006:66; 22 §
If applying manure between December 1st and February 28th, the fertilizer needs to be incorporated within 12 hours.

GLOBAL Gap
Time between manure application and harvest should be maximized, untreated organic fertilizer until 60 days before harvest (CB Annex CB.1: Microbial Hazard), organic fertilizers incorporated, prior to planting (FV 3.2.1).

Private organic regulations
Slurry and fresh manure is only permitted if they will be applied before planting/sawing vegetable crops (Bio Austria). Applying feces on growing plants for human consumption is prohibited (Gäa Germany).

Guides to Good Agricultural Practice

- Handbuch Gemüse 2008 (Switzerland): Slurry has to be incorporated before sewing/planting. In vegetable with a long cropping period, slurry is permitted, but leaf contact must be avoided, last application 6 weeks before harvest.
- Richtlinie für die kontrollierte Integrierte Produktion von Obst und Gemüse in der Bundesrepublik Deutschland (Germany): Application of organic fertilizer on germinated or planted vegetables is generally not allowed.
- Leitfaden für die Düngung von Acker- und Grünland (Germany): Slurry has to be incorporated after application.

NOP (National organic program) USA
NOP § 205.203: Manure has to be composted for a minimum of 15 days at between 55 -77 °C and turned five times. Uncomposted manure must be applied 120 days before harvest at the latest if crops are in contact with soil and 90 days when not in contact.

Safe Sources of Organic Fertilizers

Beside optimized crop rotation and green manure, green waste composts and organic fertilizers were used by the majority of vegetable growers to fertilize their crop.

Green waste compost

If compost based on green waste such as cuttings of hedges and lawn cuts is processed according to good practices (e.g., DE: Bundesgütegemeinschaft Kompost (RAL-GZ 251); CH VKS-ASIC: 2-3 weeks > 55 °C, several times turning and phase of ripening afterwards) the end product can be considered as hygienic (according to current research). However, re-contamination must be avoided.

Picture: Martin Koller, FiBL

Organic fertilizers

Animal based products, such as feather meal, horn chips are heat-treated, according to EC 1069/2009. Re-contamination has to be avoided.

Picture: Martin Koller, FiBL

Other Sources of Contamination

Applying manure is not the only potential source of bacterial pathogens. In fact, some of the best investigated outbreaks were tracked back to, for example, contaminated irrigation water.

Overhead irrigation with manure contaminated water

- Irrigation water from stagnant sources such as ponds or drainage ditches may be contaminated, particularly if they are reached by runoff from pastures, slurry lagoons or manure heaps.
- Importance: medium to high
- Control measures: Using safe sources or analyze irrigation water regularly (measure point of Global GAP). Use of drip irrigation is preferable.

Picture: Martin Lichtenhahn, FiBL

Unprotected rain water reservoirs exposed to feces from wild birds

- Importance: medium to low
- Control measures: If ponds are regularly visited by birds, use protection systems such as nets.

Picture: Martin Koller, FiBL

Wild animals in vegetable fields

- Wild animals can be carriers of human pathogens.
- If fields are frequently visited by wild animals, protection measures shall be installed to protect the harvest.
- Importance: low
- Control measures: Protecting fields with appropriate fences (see also Global GAP).

Picture: Claudia Daniel, FiBL

Runoff of manure from fertilized fields or from pastures

- Runoff could be considerably high, if direct slurry was applied prior to heavy rainfall on above laying fields.
- Importance: medium to high
- Control measures: Install buffer strips, such as hedges or grass strips.

Picture: Stefan Heller, FiBL

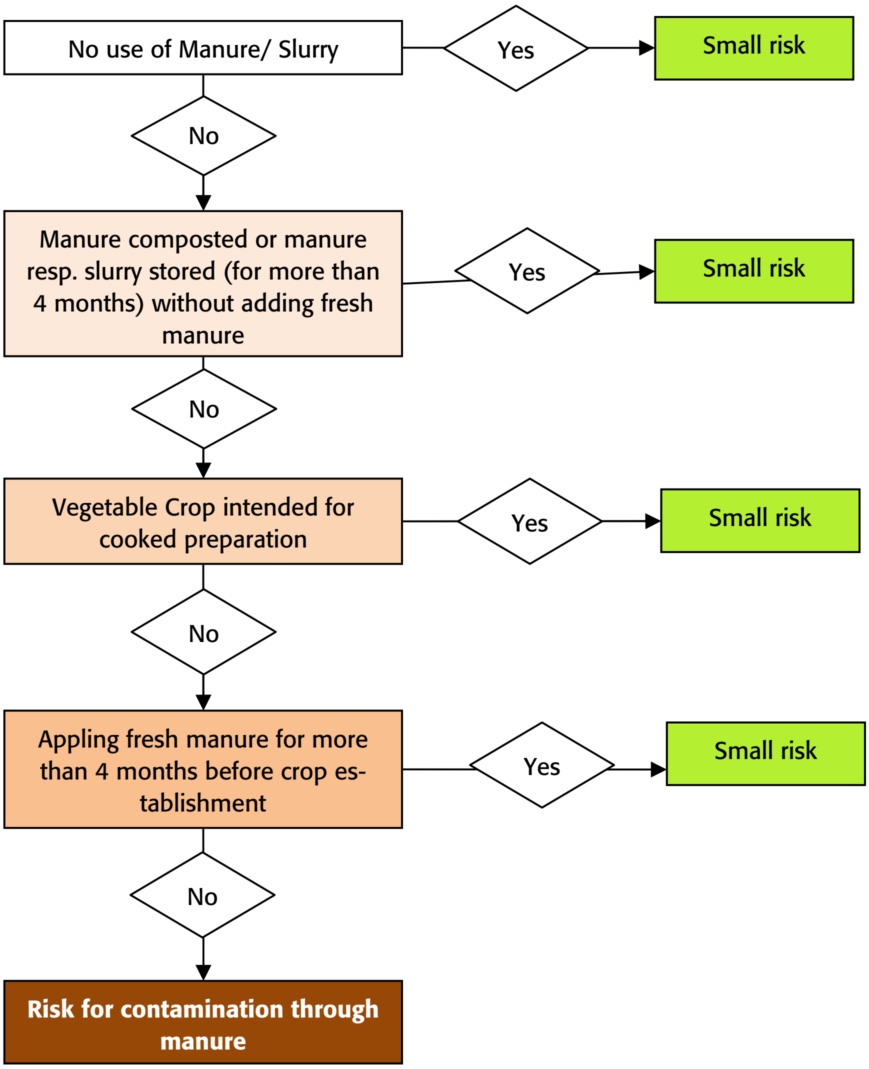
Recommendations for Using Manure for the Cultivation of Vegetable Crops

- Feeding roughage and not concentrates to cattle minimizes the propagation of pathogenic E. coli (including EHEC strains).
- Manure should be composted whenever possible.
- If not (aerobically) composted, storage of manure and slurry for 4 months without adding new material is recommended.
- Shallow incorporation, instead of deep ploughing or surface application. Surface application would allow a faster decrease of pathogens, but leads to higher ammonia evaporation.
- Solid manure is preferable to slurry.
- After planting and sowing, manure and slurry should not be applied and is even prohibited under certain organic regulations and Global GAP.
- In vegetable crops with a short cropping period (< 100 days), which are intended for raw consumption and for raw convenience food (e.g., chopped salad), fresh manure/slurry should be applied at the latest 4 months before crop establishment.

The rational for these recommendations is based on literature findings and research outcomes of PathOrganic, but these recommendations need further validation in the field.

Simplified Risk Assessment for Farmers

The following diagram serves to help identify potential risks regarding the use of manure. However, it has to be considered that other contamination sources with human pathogenic bacteria may occur as well.



PathOrganic Partners

Country	Institute		Project participants
Austria Project Coordination		AIT Austrian Institute of Technology GmbH	Angela Sessitsch Evelyn Hackl Claudia Fenzl
		University of Natural Resources and Life Sciences, Vienna / BOKU	Jürgen Friedel Thomas Rinnofner Agnes Schweinzer
Switzerland		Research Institute of Organic Farming/ FIBL	Paul Mäder Martin Koller Gabriela Wyss Bettina Landau
		Agroscope Changins-Wädenswil / ACW	Brion Duffy Kerstin Brankatschk
		Agroscope Reckenholz-Tänikon / ART	Franco Widmer
Germany		Helmholtz Zentrum München German Research Center for Environmental Health (GmbH)	Anton Hartmann Michael Schmid Andreas Hofmann
Denmark		Technical University of Denmark	Dorte Baggesen Annette Nygaard Jensen Maarten Nauta
		University of Copenhagen	Anders Dalsgaard
Sweden		Swedish University of Agricultural Sciences / SLU	Veronica Arthurson Lotta Jäderlund Janet Jansson
Netherlands		Wageningen UR (University & Research centre)	Ariena H. C. van Bruggen Carolien Zijlstra Hennie Halm

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Publisher Forschungsinstitut für biologischen Landbau (FiBL), Ackerstrasse, Postfach, 5070 Frick
Tel.: +41 (0)62 865 72 72,
Fax: +41 (0)62 865 72 73
info.suisse@fibl.org, www.fibl.org

Author Martin Koller (FiBL) and the PathOrganic consortium

Review Jacqueline Forster, Claudia Frieden (FiBL)

Editor Res Schmutz (FiBL)

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Links to further information

Report of the project will be available at www.orgprints.org

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