This project has received funding from the European Union's Horizon 2020 research and innovation programme under Grant Agreement No 773830.

BIOPIGEE Information Material

Science is a complex enterprise. Therefore, we want to share what we did and inform about our project. In this information material, we briefly explain the BIOPIGEE project, refer to some of our studies and outputs and talk about a few of the most important biosecurity measures to control faecal-orally transmitted pathogens like *Salmonella* and hepatitis E Virus (HEV). At the end of this document you will find many helpful links if you want to know more about biosecurity

What is the BIOPIGEE project?

The BIOPIGEE project (Biosecurity practices for pig farming across Europe) aimed to generate knowledge on effective biosecurity measures for reducing the occurrence of *Salmonella* and HEV in pigs. The project included experts from various fields and institutions from 12 European countries. The project group used a variety of scientific methods to collect and produce knowledge on how to prevent transmission of the selected pathogens best in the context of pig production.

What is biosecurity?

The key concept of biosecurity is to prevent transmission. Biosecurity measures can be categorized into external and internal biosecurity measures. External biosecurity measures aim at preventing pathogen introduction through the farm perimeter and into the herd. Internal biosecurity measures aim at preventing a pathogen from spreading within the farm across animals, multiple pens or production stages. Biosecurity is believed to be the single best way of controlling *Salmonella* and HEV in pig farming.

Why Salmonella and HEV?

The two pathogens, *Salmonella* and HEV, share some similarities which is why they were investigated together in this project. Salmonellosis was the second most commonly reported foodborne gastrointestinal infection in humans in EU countries in 2021 with 60,050 confirmed human cases. With 21,000 acute clinical cases and an overall 10-fold increase of reported cases over the last 10 years, HEV is an important infection in humans in EU/EEA countries.

Salmonella	**HEV**
Salmonella is a bacterium. There are many subspecies of *Salmonella*, which are further divided into so-called serovars. Not all serovars are equally dangerous to human or animal health. While some of them are usually harmless to humans, some others can result in enteritis, which is commonly known as food-poisoning, and some few can lead to typhus, which is very dangerous and often deadly.	HEV is a virus. The hepatitis-e viruses are further divided into genotypes. Some genotypes only infect humans and are well known in countries with poor hygiene conditions. Others, the ones we are most interested in here in Europe, are the genotypes 3 and 4 which can infect both humans and animals. While infections with these genotypes are often asymptomatic in humans, people with a weakened immune system are at a higher risk of developing acute or chronic hepatitis or maybe even death.

Commonalities

- Zoonotic pathogens (can infect humans and animals)
- Present on many pig farms and in many countries worldwide
- Can stay infectious for a long time in the environment
- Mostly asymptomatic infections in pigs
- Can lead to asymptomatic, mild or severe disease in humans
- Infections mainly via contaminated, undercooked pork meat or liver, but also through direct or indirect contact with contaminated faeces

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Project Outputs

The goal of the project was to collect and summarize existing knowledge as well as generating new insights into the benefits of biosecurity measures. You can find links to the tools below, at the end of this document, on our BIOPIGEE webpage or search for them directly on Zenodo.org.

Checklists

We constructed two checklists based on the existing scientific knowledge about effective measures against *Salmonella* and HEV on pig farms. The checklists can be used to identify gaps and by filling these improve biosecurity against *Salmonella* and HEV on pig farms. The HEV checklist contains seven measures, the *Salmonella* checklist contains 12 measures. The checklists are for farmers, veterinarians, or agricultural consultants. If you are interested in improving biosecurity on your farm or want to know more about the science behind the measures, you can use the checklists and the literature backing them up. You can find them here: **Link**

Slaughterhouse Guidance Manual

Contaminations of pigs and pig carcasses may very well happen during transport or in the slaughterhouse. The slaughterhouse guidance manual can be used to improve biosecurity against *Salmonella* and HEV in slaughterhouses. The slaughterhouse guidance manual contains 35 recommendations. The guidance manual is for slaughterhouses, veterinarians, or consultants. As soon as it is published (2023), you can find it here: **Link**

Biosecurity Protocol

The biosecurity protocol is a questionnaire that can be used for further insights into farm biosecurity measures against *Salmonella*, HEV or other faecal-orally transmitted diseases like *E. coli*. It contains 56 questions on measures, that can be actively changed by the farmer and that are likely to influence the transmission of these pathogens. It can be used by researchers, veterinarians or consultants. As soon as it is published (2023), you can find it here: **Link**

Cost-Efficiency Support Tool

One of the important questions associated with implementing biosecurity measures in pig farming are the associated costs and benefits. We developed a cost-efficiency tool with which can be used to estimate when the implemented measures may pay-off. The support tool can be used to estimate costs and benefits of biosecurity measures against *Salmonella* and HEV on pig farms or slaughterhouses. The support tool contains 12 measures. The support tool in its current status is mainly for policy makers, advisor, or further research. As soon as it is published (2023), you can find it here: **Link**

Do you want to improve farm biosecurity?

During our farm visits we found many good examples of implemented biosecurity measures. In the following sheets we want to show some good examples of biosecurity and also point out, what is so important about these measures. If you are interested in the studies that found effective biosecurity measures, follow the **links** at the end of this document.

<u>The biosecurity measures address some aspects of the following topics:</u>

Hygiene locks
Cleaning and disinfection procedures
All-in/all-out
Water system cleaning
Rodent control
Carcass management

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Hygiene Locks: Floor Barrier/Shoes

In a study on French pig farms, using farm-specific boots was associated with lower risk of samples being positive for HEV. In a German study, cleaning the "dirty" side of the hygiene lock was associated with lower risk of *Salmonella*.

A hygiene lock is a measure to prevent pathogen introduction into the farm or individual barn. Hygiene locks can be at the entrance of the farm, the barn or between barn sections. For the hygiene lock to be effective, it should be the only entrance to the area it is leading to, and it should be used by everyone entering that area, consistently and without exceptions.

Picture: This image represents a basic hygiene lock that may be enough to prevent the introduction of some pathogens. There is a narrow corridor with a block dividing the floorspace between the door and the rest of the corridor at about shin-height. The black door has a sign reading "STREFA BRUDNA", which means dirty area.

Just follow the links to the measures:

Hygiene locks: Floor Barrier/Shoes
Hygiene locks: Compulsory Shower
Hygiene locks: Farm-specific Clothing
Hygiene locks: Boot Cleaning
Hygiene locks: UV Decontamination
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A hygiene lock marks the border between a "dirty" area and a "clean" area. To prevent pathogens from crossing into the clean area and depending on level of biosecurity and pathogens to control, actions taken may be some or all of the following:

- Changing of shoes or boots
- Changing of clothes
- Washing hands
- Showering

Staff's own shoes are kept on the dirty side, while farm specific footwear is kept on the clean side. Since *Salmonella* and HEV are both shed in faeces and are faecal-orally transmitted, it is very likely that people carry these pathogens unknowingly around at the bottom of their shoes.

Picture: Here the solid obstacle doubles as a bench to sit down. This hygiene lock is at the entrance of a bigger farm with more workers. The entrance on the left leads to a changing room, where workers remove any outside clothes.

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Hygiene Locks: Compulsory Shower

In a study on Spanish pig farms, shower-in entry to farm was associated with lower risk of the farms being positive for *Salmonella*.

On some farms, people must use the shower before entering the farm or an individual barn. The shower is necessary to remove any pathogens that might rest anywhere on the body, including the hair. Pathogens have been found in the hair of truck drivers, veterinarians and farmers. Taking a shower can be considered as a measure of high biosecurity.

Picture: A compulsory shower on a Dutch farm. Cleary visible is the exit door on the other side to enter the clean area.

Just follow the links to the measures:

Hygiene locks: Floor Barrier/Shoes
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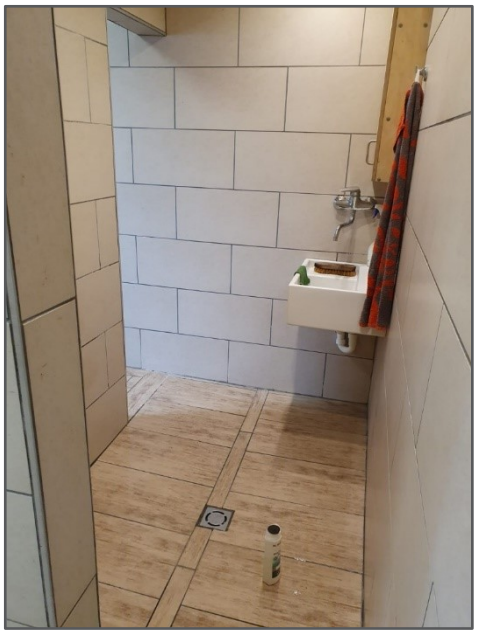
The procedure would be as follows: the person takes of their shoes and clothes and stores them on the "dirty" side of the changing rooms. Next, the staff walks into the shower and washes the full body (hair included) with water and soap. The shower is left ideally through a second exit into a separate "clean" area (not back into the dressing room), in which fresh, work-specific clothes and shoes are put on.



Picture: A compulsory shower on an Austrian farm. In the one-way system, workers step in from one side, shower and leave through the other side to a clean area. This makes introduction of pathogens very unlikely.

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Hygiene Locks: Farm-specific Clothing

Farm-specific clothes can be considered as external and internal biosecurity measures, depending on the context. To prevent pathogen introduction, any person entering the farm needs to be supplied with clean, farm-specific clothing or a disposable overall.

A high level of biosecurity would be the use of production stage-specific clothing to prevent pathogen spread within the farm e.g. by a person going from fattening to the nursery. To support compliance and prevent mistakes, color-coded clothing can be used, so that the chance of accidental trespassing is reduced.

Picture: This image shows how to properly store fresh and clean clothes used for the different production stages.

Just follow the links to the measures:

Hygiene locks: Floor Barrier/Shoes
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Hygiene Locks: Boot Cleaning

Swine production-specific boots were associated with lower risk of HEV in a study on French farms. In another study on English pig farms, disinfection of footwear was associated with lower risk of *Salmonella*.

Cleaning the working boots properly and regularly is essential for internal biosecurity. Inside the farm buildings containing pigs, the boots are always in contact with the ground and pig faeces, and are most likely contaminated. To properly clean the boots after working, brushes, soap and high pressure water is required to effectively clean the outside and sole of all visible residues. Slatted flooring is necessary for the dirty water to run-of.

Picture: This image shows an example of a boot cleaning station with a cold-water high pressure cleaner and a hygiene lock demarcation on the ground. The demarcation provides a physical barrier between the cleaning station and the outside area. An elevated stack for the cleaned boots enables any dirty water to run away whilst the boots dry.
On the other side of the hygiene barrier, a sink with soap is provided on the wall in the clean section of the room for handwashing after cleaning boots. This is an example of a high biosecurity measure.

Just follow the links to the measures:

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Picture: Boot cleaning station, with brushes, a shower head and slatted flooring.

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Hygiene Locks: UV Decontamination

In the study on the Reunion Island, disinfection of equipment was associated with lower risk of *Salmonella*.

An ultra-violet (UV) light decontamination box can help prevent pathogen introduction into the farm or barn by destroying pathogens on the surfaces of glasses, cellphones, keys, lunch boxes or portable equipment. As well as skin, hair, shoes and clothing, other items carried by persons can also be contaminated. Many pathogens can stay infectious on surfaces for a significant period. UV-light has been shown to be effective at reducing pathogen presence on surfaces, and appropriate radiation doses have been established for some swine pathogens including *Salmonella* and HEV. When used and maintened properly, UV chambers can be an effective component of biosecurity programmes. However, training on use and staff compliance are fundamental to ensure maximum efficacy.

A cycle in a UV chamber lasts about 15 minutes. Items need to be placed in the chamber and may stay there for multiple cycles before removal. When there is no metal grit, objects need to be turned around so that the UV-light can reach every part of the object. Objects should also not be stacked upon each other and be placed with enough place in-between.

Just follow the links to the measures:

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Picture: A UV-decontamination chamber for personal items or equipment. The items can be retrieved after the procedure from the inside of the building.

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Cleaning and Disinfection

In a German study, sows had a lower *Salmonella* risk, when the troughs and the feeding system was cleaned. Cleaning of drinkers was associated with lower *Salmonella* risk in the study on the Reunion Island. In our own study, wet cleaning was associated with a lower risk of HEV, as was the use of a hygienogram at the end of the cleaning procedure.

A full cleaning and disinfection procedure should always consist of:

- Dry cleaning - sweeping/removing organic matter (dust, bedding, faecal matter)
- Wet cleaning - with high pressure water and detergent (to loosen and remove organic matter)
- Drying time
- Disinfection - with an approved disinfectant for the specified contact time (disinfection must only occur after all debris has been removed)
- Downtime of **at least** three days

It is also good practice to check how effective the cleaning and disinfection procedure was by collecting samples to test for the presence of bacteria left in the area. Cleaning procedures need to be applied to all surfaces, as well as fixed and non-fixed equipment. Written biosecurity plans and procedures can also help increase adherence to the protocols by all staff.

Dry cleaning is necessary to remove **all of the** leftover bedding, faeces, dust and other organic matter in the area. This step becomes important later, as organic matter can deactivate disinfectants and stop them from working properly.

Using detergents when *wet cleaning* is also vitally important as it helps to remove leftover organic matter, and is able to breakdown biofilms, which is an oily film produced by bacteria which can help them to survive disinfectants. Ceilings, walls, floors, drinkers/feeders, etc. need to be soaked with cold water, before a detergent is applied under low pressure. Let it soak for at least 30 minutes or overnight. In a following step, hot high-pressure water is used from the ceiling to the walls and floors to wash and clean all surfaces and to not re-contaminate. Everything else must be cleaned by hands and don't forget the hard-to-reach spaces.

Just follow the links to the measures:

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Pictures top and bottome: These images show examples of what a barn should look like after emptying and cleaning. The walkways and corridors need to be cleaned as thoroughly as the pens and compartments.

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After the area and all objects are *completely dry*, disinfectant can be applied. When using disinfectant it's important that the area is dry to increase their effectiveness, and that it is mixed to the correct concentration before using. It is also important to use the right disinfectants for the pathogens present in the barn, since the disinfectant efficacy is often pathogen specific.

Picture: Sows with piglets in a clean pen. Here we see a sow with her piglets in a farrowing pen with fully slatted plastic flooring. The area is clean, with no dropped feed or faeces visible in the pen. This is best practice to help prevent the spread of pathogens.

Downtime is the period of at least three days where the area after cleaning and disinfection has a break and does not hold pigs. This is a practice which helps to reduce the amount of bacteria and viruses left on surfaces.
Finally, after the proper cleaning and disinfection procedure, it is crucial to not re-contaminate the room. This means, that the area is only re-entered by anyone, when the restocking with pigs occurs.

Picture: This image shows an example of how clean to keep pens once they contain pigs, specifically at the weaning stage. Here we see weaning pigs in a pen with metal grid separation, fully slatted plastic flooring and an enrichment toy (chain coming from the ceiling). The floor is clear and free from any feed or faeces. The pigs also appear in good health with no injuries. First infections of HEV occur commonly during the weaning phase, and it is therefore important to clean and disinfect the pens thoroughly as well as all objects that can be reached and touched by the weaning pigs to reduce spread within the herd and the farm.

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All-in/all-out

In the Reunion Island study, all-in/all-out was associated with lower risk of *Salmonella*. Not mingling pigs was found to be associated with lower risk of HEV in a French study.

An all-in/all-out (AIAO) system operates where all pigs enter the shed/building/paddock at the same time. An important aspect is that the **same pigs are kept together** in groups as they move through each phase of the pig production cycle.

After all pigs of the group have left the building, there is now an opportunity to clean and disinfect the building, which may not have been possible in a continuous flow system. This can help to reduce the spread of disease between batches of pigs. It is also easier to keep a closer eye on indicators of disease, known as 'key performance indicators' (KPI's), in a set group of pigs. These can give early indications of disease, for example how much feed and water the pigs are consuming and how fast they are growing.

Just follow the links to the measures:

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Mixing Pigs

By mixing pigs from different groups, there is a possibility the animals may have different levels of immunity and exposures to different pathogens. This introduces the risk for disease spread. It only requires a single pig shedding bacteria or virus in its faeces to infect the whole pen or building. Therefore, mixing pigs increases the risk of introducing pathogens into an area and spread within it.

Picture left top and left bottom: These images are another example of what barns and pens should look like after cleaning and disinfection. Here we see an empty pig compartment with cleaned and solid separations between pens, fully slatted plastic flooring, cleaned feeding troughs and cleaned enrichment objects (the yellow ball). No pigs can be seen as they have all been removed. In the second picture following the AIAO procedure, the barn has been restocked by pigs from the same batch. Of note, the solid walls between each pen also add an additional level of biosecurity by preventing snout-to-snout contact between the pigs in different pens.

AIAO systems can also reduce the stress of the pigs, because they are not being mixed with new pigs. Pigs are social creatures and in confined conditions, such as those found in farms, they can form a hierarchy as young as one week of age. Therefore, mixing different pigs together once a hierarchy has been formed can increase fighting and aggression. This can be stressful for the pigs, and stressed pigs are often more susceptible to diseases. Henceforth, stable groups can create a less stressful environment which can in turn assist with disease control.

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Water System Cleaning

The use of clean and/or filtered water was associated with lower risk of HEV in a study on French pig farms.

The drinking water system can be a source of infection, especially in the case of faecal-orally transmitted pathogens that survive well in water or wet environments, such as HEV and *Salmonella*. Therefore, it needs to be cleaned and disinfected with appropriate machinery and chemicals. The cleaning needs to cover the pipes and the troughs and other drinker types that are used by the pigs. Cleaning the water system should take place at least once per year. If non-public or non-main water sources are used, like private wells or boreholes, it is necessary to ensure that the water quality is sufficient for use in animal production.

Picture: This image shows an example of how to reduce the risk of contaminated water entering the pens. Here we see the equipment used for cleaning and disinfecting the drinking water system. The chemicals used to clean the system are stored appropriately and should be checked regularly for any signs of leakage or damage.

Just follow the links to the measures:

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Rodent Control

In a study on Spanish pig farms as well as in our own study on protective factors against *Salmonella*, rodent control was associated with lower risk of the farms being positive for *Salmonella*.

Rodents can help spread pathogens between buildings on a farm. Rodent control should cover the appropriate use of rodenticide and traps, and an assessment should be undertaken on rodent location to identify appropriately positioned traps/ baiting points. The position and type of controls should be changed to maximise effectiveness, and to ensure rodents do not get used to them and learn to avoid them. Additionally, control includes the removal of material between buildings, that provide cover and harbourage sites for rodents. Feed bins should not allow rodent access and dropped feed should be removed from buildings, so as not to attract rodents.

Just follow the links to the measures:

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Carcass Management

The disposal of dead pigs outside of the barn was associated with lower risk of *Salmonella* in the Reunion Island study.

Proper carcass management is very important for any farm. First and foremost, a dead animal needs to be disposed of as quickly as possible and the removal company notified to collect the carcass. There are many important aspects of the carcass container to serve its purpose fully and not directly or indirectly increase the risk of pathogen spread on farm. The carcass container should be big enough for the one or multiple carcasses to be stored. It should be robust and easy to clean once the carcasses have been removed. It should not leak to ensure that no pathogens the animal is contaminated with can escape the container. Once closed, no pets or other animals on the farm and especially not wild birds or rodents should be able to access the container or come into contact with the carcasses. Pathways up to and around the container should be made of concrete or otherwise sealed so that the environment cannot accidentally be contaminated. One possibility is to have it slightly below ground which lowers the temperature. Ideally, to slow down the decomposition process, the container should be located in a shady location, especially in summer.

Picture: This image shows how to store pig carcasses prior to removal from the premises. Here, the sealed metal carcass container is also underground. This helps to prevent any contaminated material from also contaminating the farm environment.

Just follow the links to the measures:

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The carcass container should be built in a way that is optimal for the removal company to grab and empty the container to prevent the container from damage and possible leakage in the environment. Finally, the carcass container should be situated away from the farm building and optimally so close to the farm perimeter that the removal company can drive close to the fence and empty the container without crossing the border onto the farm.

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Some Final Points and Where to Find More

Keep in mind that not every biosecurity measure is of equal importance to every farm. Each farm needs to tailor their biosecurity measures to their specific needs and threats. Biosecurity also always consists of a set of collective measures that reduce pathogen risk. All biosecurity measures should be optimal and adhered to at all times, as pathogens will always find the weakest spot of a farm's biosecurity and exploit it. Therefore, it is good practice to always look for continual improvement by adjusting the measures applied to the pathogens that are most relevant for your farm. A final note will be on education, training and compliance. Everyone working or visiting a farm needs to understand the measures applied and comply with the procedures. Without constant adherence and understanding of the procedures, these measures may not be as effective and may lull you into a false sense of security. If you are unsure about how to improve biosecurity at your farm, please contact your veterinarian or agricultural advisor.

In this information material, we point out how certain biosecurity measures can be best implemented. There are many aspects we could not cover and some biosecurity measures we couldn't mention. It is therefore important that when improving biosecurity on your farm, to keep updated on the latest suggestions and guidelines, which are often available online. If you are interested in learning more about the BIOPIGEE project and the research articles we used to collect this information, you will find links and sources at the end of the material.

Further Links and References

More information on the BIOPIGEE project you can find on the OHEJP website of BIOPIGEE: Link.

BIOPIGEE project outputs and results will all be published on Zenodo by the end of the project: Link

Link to the BIOPIGEE glossary where important terms of the field and for the research were defined by the project team: (Glossary)

European Union Health Zoonosis Report (by EFSA): Link

Public health risks associated with hepatitis E virus (HEV) as a food-borne pathogen (by EFSA): Link

References of the studies that found effective biosecurity measures can be found in the project deliverable or in the checklists: Link, Link

Pigs are social creatures: Link

On proper cleaning procedures and disinfectants: Link

Detergents effective against *Salmonella*: Link

UV radiation against Salmonella: Link

HEV stability on surfaces: Link

UV radiation against HEV: Link

UV, implementation and best practices regarding UVC in swine production: Link