UNIVERSITY OF COPENHAGEN

Anne PETILLON – PRONK

Promotion 171

Internship report

# Semi-Quantitative Assessment of Risk Pathways for Spread of *Salmonella* Dublin through Biogas Plants linked to Dairy Cattle Farms in Denmark.

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Mission activity - 2nd year of agronomic engineering school (M1) - RENNES

September 2021 – February 2022

## ACKNOWLEDGEMENTS

This internship project is the product of my studies in the Department of Veterinary and Animal Science at the University of Copenhagen. It is a part of a main *Salmonella* project lead by SEGES.

I would like to thanks all the experts I met throughout this study for their time, their advices and discussions useful to extend my knowlegde on the topic. Thanks to all the residents of the Department for your help, your kindness and your warm welcome into your house. I also would like to thank all the biogas plants staff and farmers for having us in their company, to make us visiting their places and for patiently answering our questions. A big thank you for being so well adviced by all the members of the team work from SEGES. Thanks again for your patience and experience sharing. Finally, I fell extremly grateful for being so well supervised by my internship supervisor throughout this internship. Thanks to all the actors.

To **Liza ROSENBAUM NIELSEN**,

Professor in Veterinary and Preventive Medecine in the Department of Veterinary and Animal Science at the University of Copenhagen – my internship supervisor.

To **Lars PEDERSEN**,

Veterinarian - PhD student in biosecurity at SEGES.

To **Malene BUDDE**,

Veterinarian – Reseacher in biosecurity at SEGES.

To **Anders DALSGAARD**,

Professor in Food Safety and Zoonoses at the University of Copenhagen.

To **Anette BOKLUND**,

Veterinarian - Senior adviser in Animal Welfare and Disease Control at the University of Copenhagen.

To **Bruno SANDER NIELSEN**,

Chief Consultant of the Association Biogas Denmark.

To **Lis ALBAN**,

Veterinarian - Chief Scientist in Food Safety, veterinary matter and Risk Analysis at the Danish Agriculture & Food Council.

To **Lisbeth HARM NIELSEN**,

Veterinarian – Chief Consultant in Food Safety, veterinary matter and Risk Analysis at the Danish Agriculture & Food Council.

To **Pierre-Guy MARNET**,

Physiology of lactation, milk quality, milking and precision farming – my internship tutor in AgroCampusOuest.

To **Jeanne SERESSIA**,

Student at AgroCampusOuest – Intern with who I worked collaborativelly on the project. Her study was focused on risk assessment regarding the spread of *Salmonella* Dublin into dairy cattle farms.

## TABLE OF CONTENTS

## LIST OF FIGURES

## LIST OF TABLES

## LIST OF ACRONYMS

| | |
|---|---|
| *S*. Dublin | *Salmonella* Dublin |
| BGPs | Biogas Plants |
| FVST | the Danish Veterinary and Food Administration |
| UCPH | University of Copenhaguen |
| IVH | Department of Veterinary and Animal Sciences |

## LIST OF DEFINITIONS

**Dry matter** = Residues as sand and ground in biogas plants

**Manure** = Solid part of faecal wastes in husbandries. Manure is also called solid manure, matress, deep litter, bedding

**Livestock manure** = Solid manure and slurry

**Slurry** = Liquid part of feacal waste (mostly the dungs), also called liquid manure

**Systemic disease** = When a pathogen spread via the blood stream and enters different tissues in the body (usually with fever).

**Figure 1** : Picture of the University of Copenhagen – Frederiksberg Campus (Source: ku.dk)

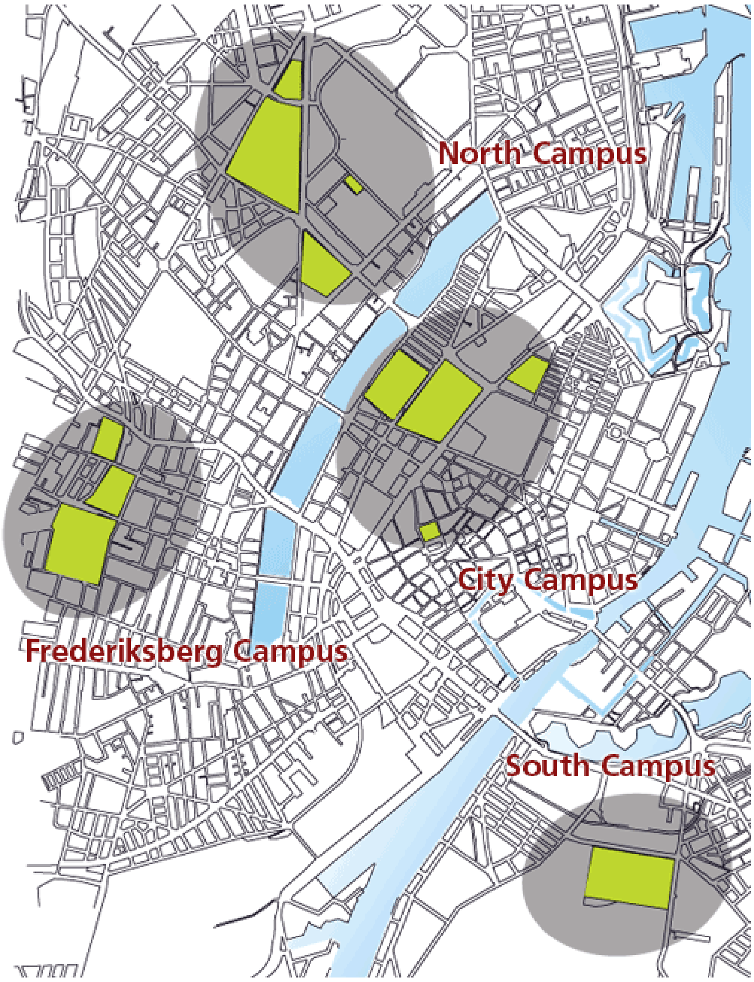



**Figure 2** : The University of Copenhagen divided in main 4 campuses

(Source: sciences.ku.dk)

# I. PREAMBLE

## 1. The internship institution and the study I took part

### 1.1. The University of Copenhagen and my host department

Founded in 1479 by the Danish king Christian I, the University of Copenhagen (KU) is the oldest University in Denmark and one of the oldest in Nothern Europe (**Figure 1**). The area of University of Copenhagen is around 940 464 m², which divided in 6 faculties in 4 different campuses within the city of Copenhagen (**Figure 2**). In 2007, the University of Copenhagen merged with the Royal Veterinary and Agricultural University and the Danish University of Pharmaceutical Sciences.

Nowadays, the University has more than 37 500 students and 9 500 employees – of whom around 5 000 are researchers – and generates revenues of 9 billion Danish Crown (DKK). The University has an internatonal research and study environment with 200 research centers and around 13 000 research works published, so it is highly ranked on the leading ranking lists of the world’s best universities.

The Department of Veterinary and Animal Sciences (IVH) covers a wide spectrum of animal and human disease biology. IVH is one of the biggest departments in whole the University, with around 360 employees, even bigger than the Human Health department. It creates new knowledge in among other things food safety, antibiotic resistance, animal models and welfare, immune system and lifestyle diseases. All the work is carried out in close collaboration with other departments at the University of Copenhagen.

### 1.2. The SEGES project, aim and financement

The project on which my internship is based is a part of a 3 year-project led by SEGES and the University of Copenhagen which are working accordingly to a collaboration agreement. SEGES is a part of the Danish Agriculture & Food Council. It is a knowledge and innovation organisation that provides solutions for agriculture and food clusters in Denmark and internationally. This structure works widely with research, development, and practical applications in the areas of animal health, welfare and reproduction.

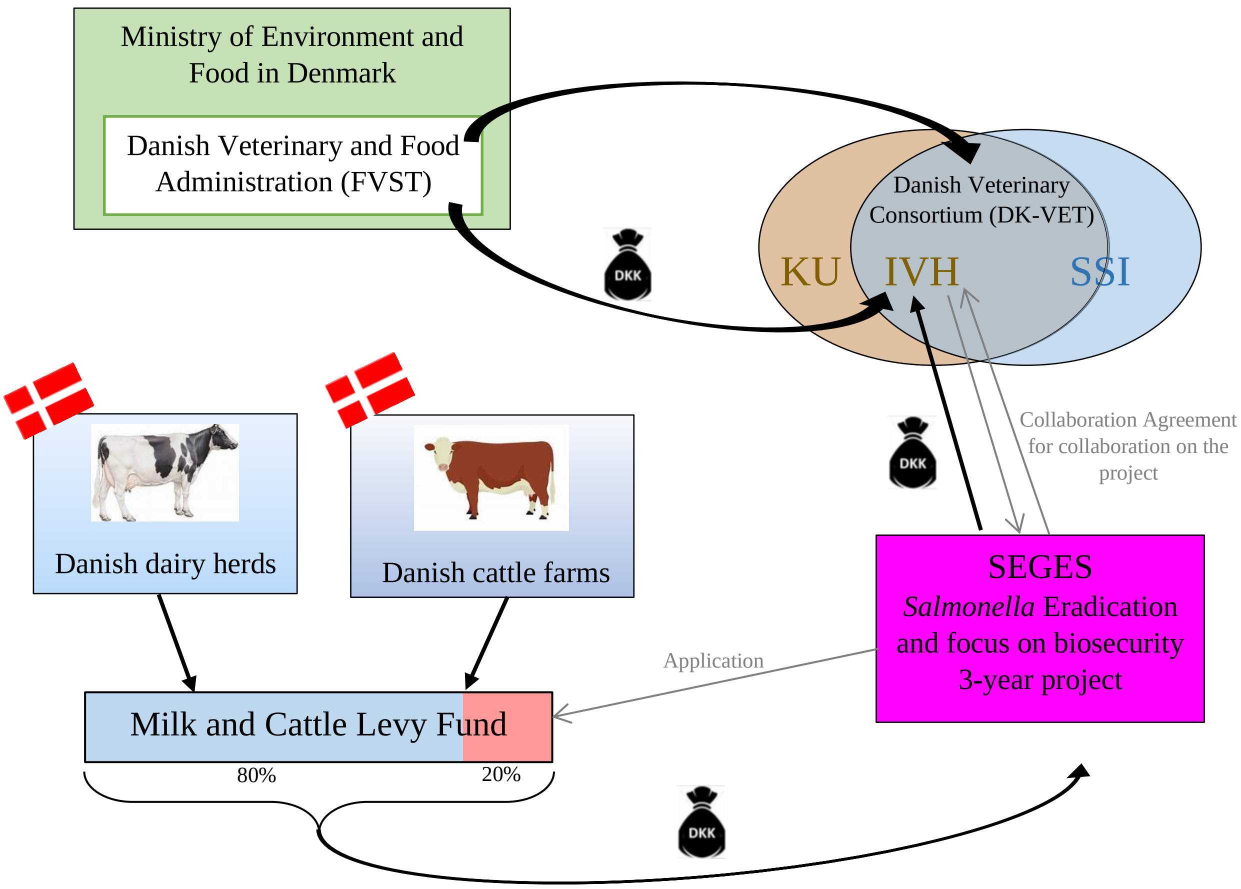



**Figure 3** : Diagram of the place of the *Salmonella* Eradication and focus on biosecurity 3-year project into the Danish organisation (

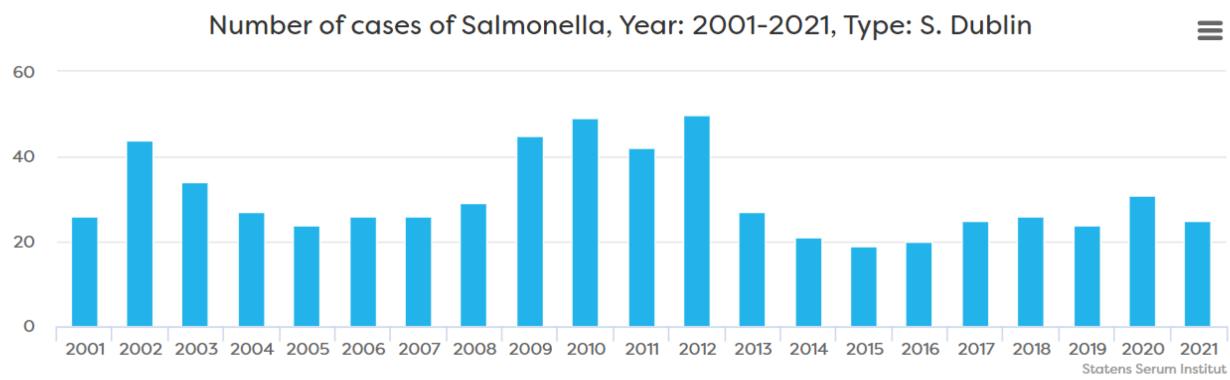



**Figure 4**: Registered human *Salmonella* Dublin cases in Denmark (Source: Statens Serum Institute).

The 3 year-project is not a part of the *Salmonella* Eradication programme per se, but it consists of exchange, communication with farmers and BGP, collect information about their processing, make them aware of the efforts to be made to improve biosecurity and supporting them in their current and future efforts to improve their systems to prevent the spread of the *Salmonella* Dublin (*S.* Dublin).

The Danish Veterinary and Food Administration finances the Danish Veterinary consortium's research projects, with around 90 million Danish crowns (12 M€) per year (**Figure 3**). The consortium allocates the amount of money regarding importance and needs of projects. In another way, farmers must pay a levy which is a low percentage of the meat or milk sold. This levy is called the Milk and Cattle Levy which reversed to the Milk and Cattle Levy Fund. These two boards decide and distribute the money to different research programmes, such as SEGES for example. For this, SEGES applies for money and receive 80% from Milk Levy Fund and 20% from the Cattle Levy Fund.

SEGES and the University of Copenhagen have signed a collaboration agreement that stipulates that they can provide information and collaborate together, but they cannot influence each other. This agreement allows the University to be involved in the project and it receives around 100 000 Danish crowns (13 K€) from SEGES in 2021, for helping with risk assessment of biogas plants (BGPs) in relation to spread of *S.* Dublin.

## 2. The background of *Salmonella* Dublin infection in Denmark

### 2.1. The *Salmonella* infection in humans

In Denmark the incidence of human salmonellosis increased on the 1990s. Human cases are characterised by systemic and invasive disease difficult to treat. (Nielsen, 2013), so the bacterium is a public health concern (Kudirkiene E et al., 2019). On the 1990's, more than 90% of serotypes have been named and characterised to have the potential, if they occur in feedstuffs, for infecting humans via animals or foods of animal origin, as consumption of contaminated beef. (Houe et al., 2014) (HC. Wegener et al., 2003). There are fewer than 60 annual human cases of salmonellosis recorded as caused by *S*. Dublin (**Figure 4**) and these are mostly attributed to consumption of contaminated imported as well as 90% by domestic beef in DK (Hald. T et al., 2006).

**Number of dairy farms contaminated by *S.* Dublin in % (Level 2)**

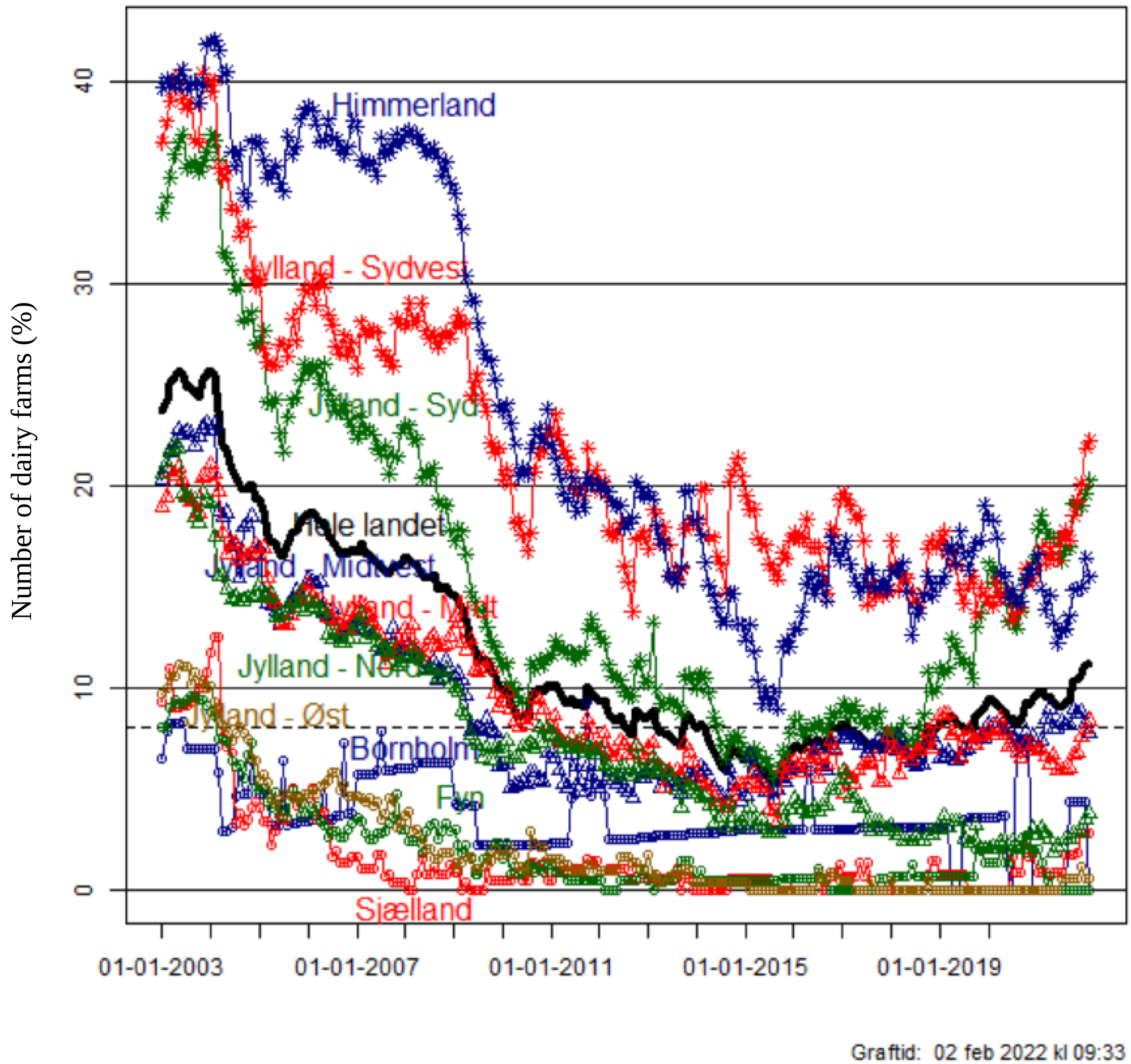



**Figure 5** : Graph of percentage of infected dairy farms (Level 2 farms) through time

(Source : kvaegvet.dk)

| |
|---|
| The coloured curves represent the average number of contaminated dairy farms in different regions of Denmark<br><br>The thick black curve represents the national average. |

### 2.2. The context of *Salmonella* Dublin issue in dairy farms

*Salmonella* Dublin is gram-negative bacterium that belongs to Enterobacteriaceae family. *S.* Dublin is the most common bacterium and a bovine adapted serotype which means that it is host-adapted to cattle (Nielsen, 2013). In cattle farms, *S.* Dublin is associated with increased morbidity, mortality and production losses including reduced milk yield in many infected cattle herds (Nielsen et al., 2013). Calves under 3 months old are more sensitive and more severe cases and important faecal shedding can be observed (Nielsen, 2013).

This increasing of bacteria issue led to the initiation of a targeted national control programme as was the case for poultry. (Bisgaard M, 1992).

### 2.3. The Cattle *Salmonella* Surveillance and Control programme

The infection effects animal health and welfare, production economics and food safety demonstrated the need to control *S.* Dublin in Denmark. The motivation for choosing a mandatory programme inclusive of all cattle herds was because of the high risk of transmission between cattle farms (Houe et al, 2014).The primary reasons for the mandatory programme provided were that, first, *S.* Dublin is an important zoonosis associated with morbidity in infected herds and which is difficult to get rid of. Secondly, the other primary sectors (poultry and pig) have already succesful *Salmonella* control programmes in place, and there was a national policy to reduce the incidence of salmonellosis.

Hence, a national surveillance programme for *S.* Dublin in Danish cattle has been launched in October 2002 by initiative of both the Danish Veterinary and Food Administration and the Danish Cattle Federation. At this time, 1/4 of Danish dairy herds were estimated infected (**Figure 5**). This rate distinctly decreased over the years to reach 6% of infected dairy herds in Denmark, around 2015. In the surveillance programme all Danish cattle herds are classified into two levels (Nielsen LR, 2003). The short-term purpose of the programme was to screen *S.* Dublin in both dairy herds and beef cattle herds to classify the herds according to estimated level of infection to control new infections in Danish cattle herds. Tested-positive dairy herds are classified “Level 1 farms” and tested-negative dairy herds are classified “Level 2 farms”. Furthermore, dairy herds with contact to L2-farms is considered L2 too. Finally, the long-term purpose was to reduce the prevalence of *S.* Dublin in Danish cattle and reduce the risk of human infection upon consumption of Danish cattle meat and dairy products.

Unfortunately, since about 2016, there has been an upsurge in dairy herds contaminated with *S.* Dublin. The ladder increased from 6% to more than 10% in 2021.

# I. GENERAL INTRODUCTION

## 1. *Salmonella* Dublin

### 1.1. *Salmonella* Dublin : spread and survival

*S.* Dublin survives easily in slurry and manure, depending on temperature, pH, microflora, slurry treatment and storage conditions (Jones, 1976; Jones et al., 1977). It has the potential to multiply outside the host, and it can survive for months in organic matter such as stored slurry, cattle manure, and soil and for years in dried-in faecal matter depending on climatic conditions (Nielsen, 2013). The bacterium grows particularly easily in water, which becomes a mean of diffusion within farms. Thus, in wet weather or during washing activities, *S.* Dublin becomes a fast-spreading infection, and the environment is an important reservoir of infection especially when water is contaminated with manure or slurry (Houe et al., 2014).

*S.* Dublin may also persist in the cattle herds, as animals of all ages may be infected from *S*. Dublin contaminated environment and some animals may become carriers excreting the organism in faeces for years, eventually for life (Wray C et al., 1989). These individuals become a source for spreading this disease between farms but also maintaining the disease once on the farm. It is very difficult to truly identify carriers of *S.* Dublin, but there is some evidence to suggest an antibody test to identify an animal as a potential carrier.

In order to avoid any outbreak of *S.* Dublin within their farms, farmers must clean and disinfected the rearing barns, use separate coveralls and boots in each barn, and provide new beddings once a week (T. D Nielsen et al., 2012).

### 1.2. *Salmonella* Dublin: uptake and dissemination in the host

*S.* Dublin can be ingested directly from contaminated feed, water, milk or the immediate, environment (Houe et al., 2014), but the main route is the faecal-oral transmission. Faeces contain the highest concentration in bacteria, and they are the most important vehicle of transmission. A carrier can shed millions of bacteria per day in faeces. However, shedding bacteria (from faeces) can be intermittent.

Usually, oral uptake of more than $10^6$ colony forming units (CFU) leads to clinical signs and/or shedding of bacteria in calves younger than 6 months old. However, an infection with *S.* Dublin may or may not result in the appearance of clinical signs. It depends on the number of bacteria ingested, the natural resistance of the individual, the infectious character and virulence of the strain (Nielsen, 2013).

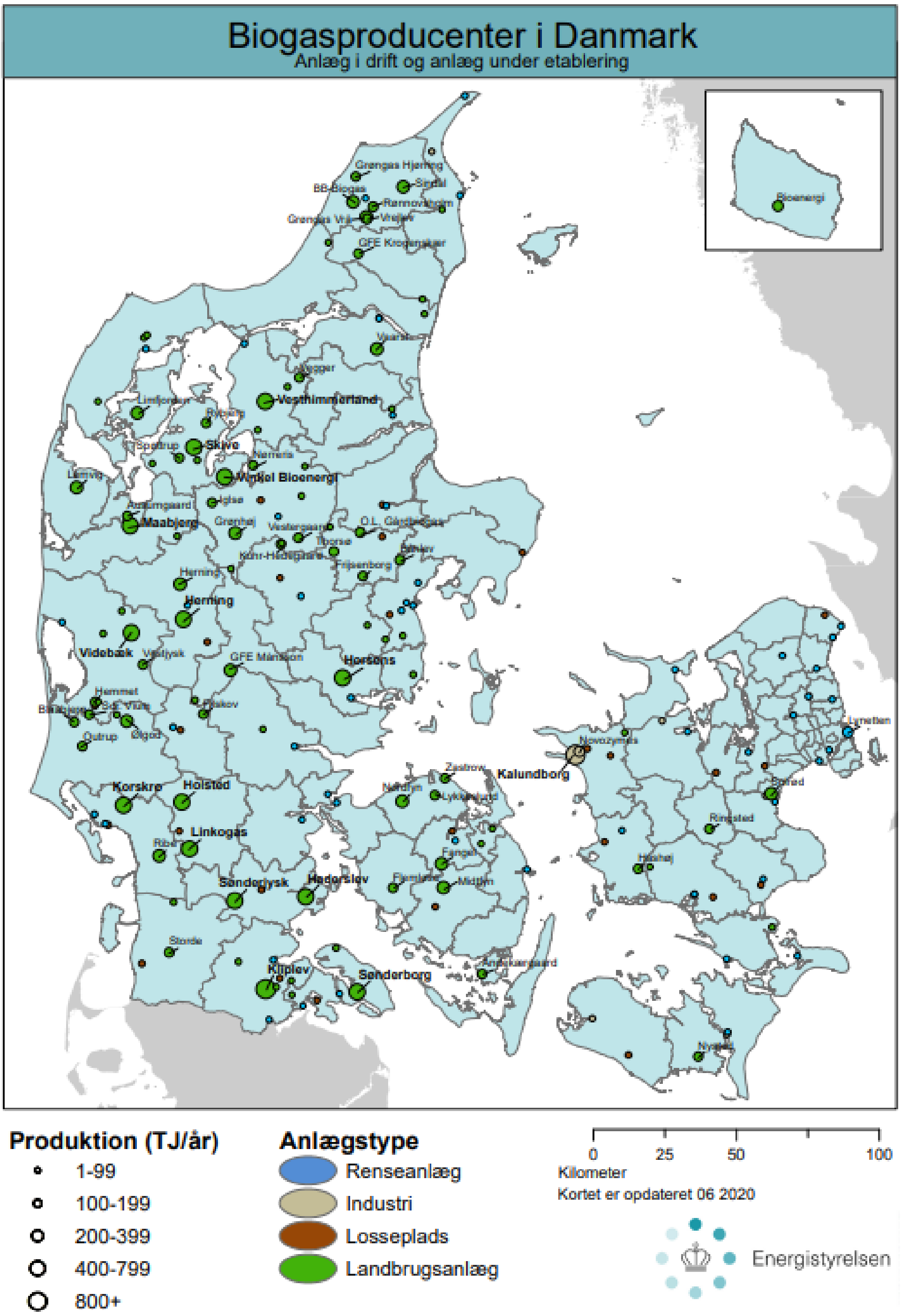



**Figure 6**: Map of distribution of biogas plants in Denmark in 2020 (Source: Danish Energy Agency)

- Wastewater treatment plants (51 sites)
- Industrial plants (4 sites)
- Landfills (27 sites)
- Agricultural plants (83 sites)

Upon digestion, the bacteria reach the gastrointestinal lumen, colonise and invade the gut epithelial cells as soon as 6 hours after uptake, and may be shed in faeces within 12-24 hours after exposure. The host is considered as infectious when it begins to excrete the bacterium (Houe et al., 2014). Sometimes, an asymptomatic animal can maintain infection on a farm by sporadically excreting the bacteria (Nielsen, 2013).

## 2. Biogas plants in Denmark

### 2.1. Background

The 1973 energy crisis and high-energy prices stimulated farmers, research centres and technology companies to investigate energy generation from manure (Raven et al., 2004).

Over the years, the biogas yields increased gradually and the economic feasibility improved throughout the 1990s (Raven et al., 2004). Also, livestock farmers needed to have a system to handle the livestock manure. A first BGP was established as a cooperative in 1993 by 16 farmers.

Nowadays, the digestion of manure and organic waste is a well-established technological practice in Denmark. This is an achievement compared to other European countries (Raven et al., 2004). Denmark is one of the leading biogas producers leader worldwide (**Figure 6**).

### 2.2. Description of biogas plant and the importance of agriculture

A BGP is a technical facility in which the biogas is made from a wide range of organic material such as manure and slurry from livestock, which represent the highest part of biomass used and other kinds of residues from industries, households and other wastes from agriculture (Raven et al., 2004). Biogas is the name of the mix of $CO_2$ and the inflammable gas $CH_4$, which is produced by bacterial conversion of biomass under anaerobic conditions (Raven et al., 2004).

The BGP picks the livestock slurry up on a farm, bring it to the BGP and it does not matter which farm is receives the treated biomass which is called digestate. About solid manure (mix of dungs, litter and straw), a majority of BGPs use the manure container from an external company to bring them the deep litter.

Livestock waste has always been considered as an important resource in Denmark. The country has one of the highest livestock densities in the world.

**Shares of bio-resources that are degassed – Biogas Denmark**

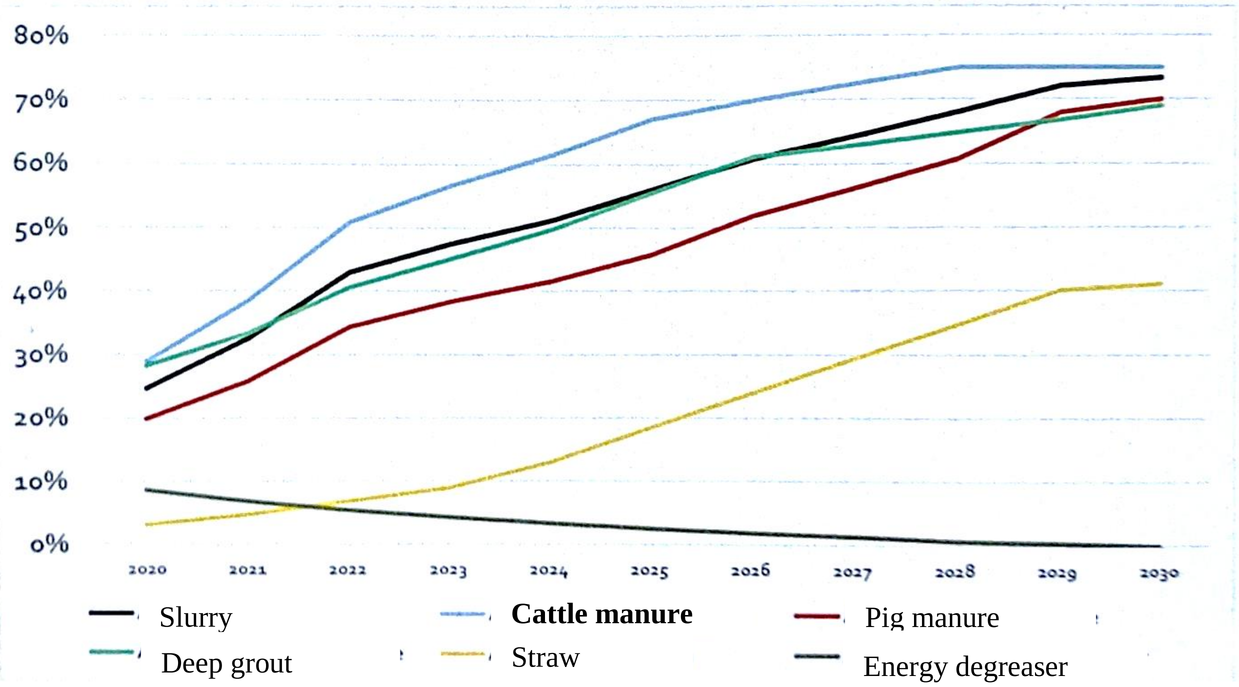



**Figure 7** : Anticipated shares of faecal matter resources (from husbandries) that will be degassed (Source: Biogas Denmark)

**Distribution of bioresources by weight – Biogas Denmark**

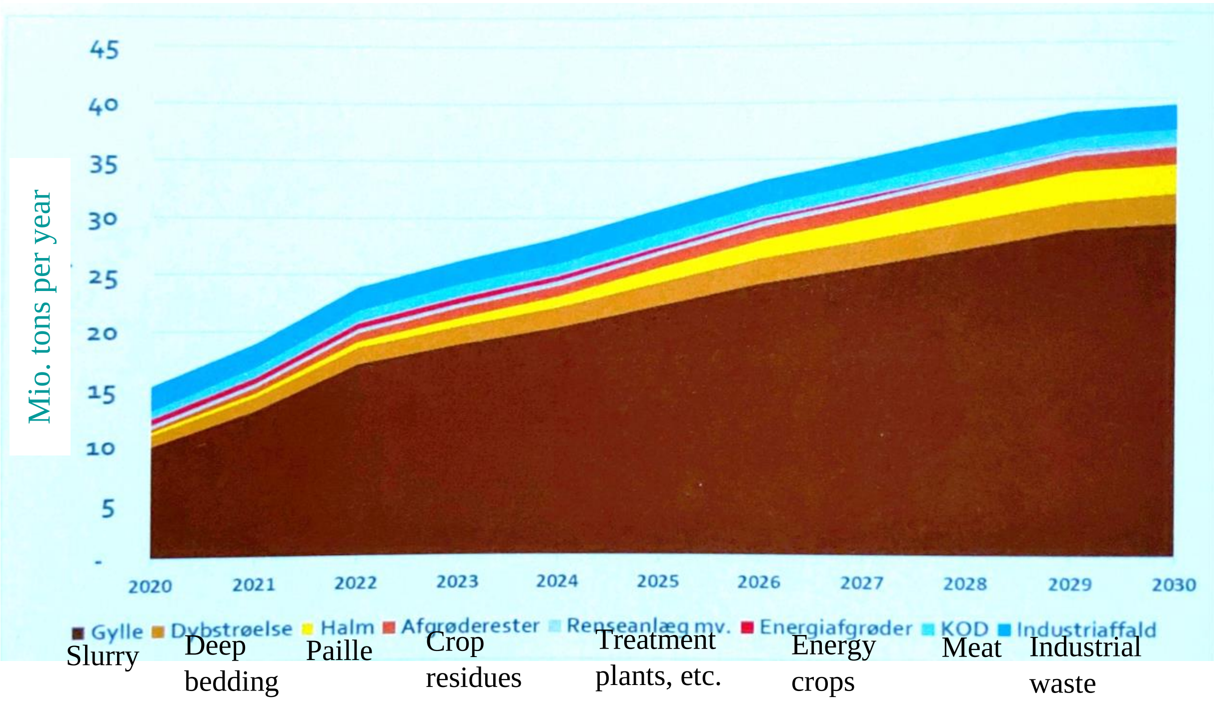



**Figure 8** : Projected distribution of bio-resources by weight received per year in millions of tons from now to 2030 (Source: Biogas Denmark)

Agriculture is heavily involved in the Danish economy, with large volumes of animal production, producing 35 million tonnes of livestock waste per year, particularly in cattle farming (**Figure 7**). Therefore, Denmark should invest in the development of innovative skills and technologies to enable the management of livestock manure and slurry while respecting the environment (Støckler, M. et al, 2020)

### 2.3. Importance and benefits of biogas plants use

BGPs bring benefits to the society by contributing to limit agricultural pollution and produce renewable energy. Also, digestate is less smelly than raw manure and has a higher fertilizer value, as it seeps into soils faster and the nutrients become more accessible by plants and the nitrogen rate is often higher in fields where biogas has been spread.

Methane ($CH_4$) can be used to replace the main source of fossil fuels energy system. The carbon dioxide ($CO_2$) is removed from the biogas and released in the air. This may seem a polluting process, but the $CO_2$ part is only 40% of the total biogas and the methane is a 25 times worse greenhouse gas than $C0_2$ whether it would also be released.

### 2.4. Biogas production forecasts regarding agriculture

Recommendations are that biogas production should cover 100 % of the planned production in gas consumption. In general, agriculture provides 75% of biogas production, the majority of which is livestock manure (Biogas Denmark). **Figure 8** reveals a significant increase in livestock manure use of up to 30 million tons or 75% of degassed biomass in 2030.

## 3. My internship study project, problem statement and process

One concern is the upsurge of *S.* Dublin which is affecting Danish cattle farms, it generates sick cattle and reduces production especially in dairy farms. At the same time, the demand and production of biogas is developing, so the national number of BGPs is also increasing. This leads to increased use of BGPs by dairy farms which supply manure and slurry and this increased use lead to more traffic between farms and BGPs which could increase the risk of transmission of *S.* Dublin.

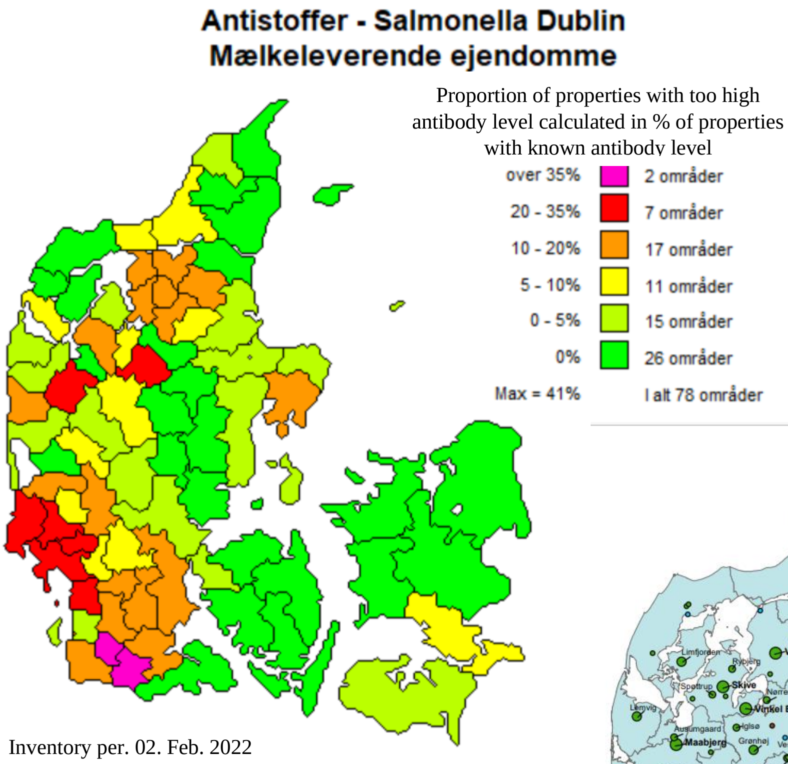
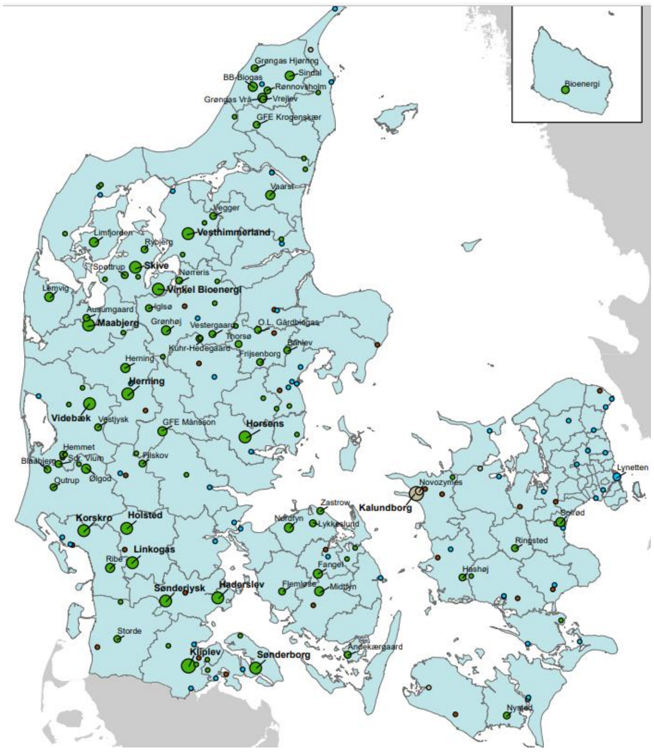



**Figure 9** : Map of distribution of level 2 dairy herds in Denmark in 2022 (Source: SEGES)

Omrader = areas

**Figure 6**



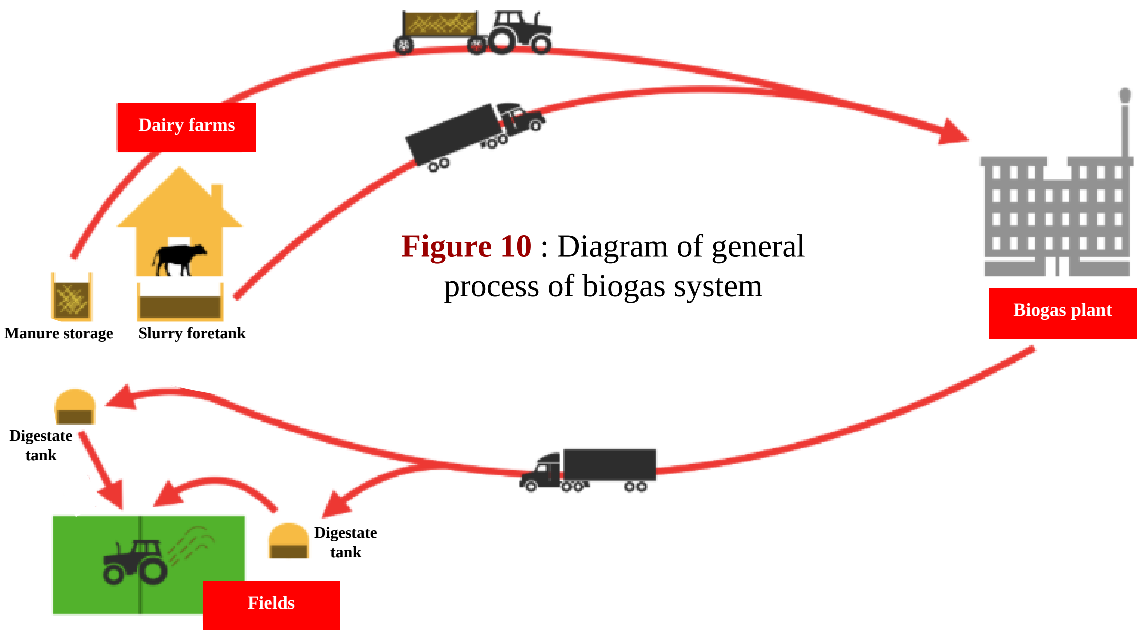



**Figure 10** : Diagram of general process of biogas system

According to observation, the localisation and amount of dairy farms contaminated with *S.* Dublin (**Figure 9**) seems tally with both the localisation and the size of BGPs using wastes from agriculture. That means that those BGPs use mostly faecal wastes as slurry and manure and they are represented with green points (**Figure 6**).

The comparition of these two maps allows to assume that the use of BGP by dairy farms could pose a risk in the increasing spread of *S.* Dublin in Denmark. The question is how to identify, estimate and become aware of the potential risk pathways that may lead to spread *S.* Dublin between dairy cattle farms through BGPs ?

A BGP and dairy farms interact with each other through fresh manure and/or slurry transport services from farm to BGP and delivery services for treated digestate, free of pathogens and better fertilization quality (**Figure 10**). Basically, trucks go to farms to collect fresh matter and bring it to BGPs where the matter will be degassed and treated by heating system. Then, the same truck go back to a farm (the same or another), to deliver digestate. The delivery could take place into the farm on slurry tanks or close to fields where farmers need to spread the fertilizer, then the cylce repeats.

The objective of the ongoing project I took part, was to define a structured approach/framework to assess the risk pathways for *S.* Dublin spread, within and out of BGPs, that exchange livestock manure with dairy farms. I focused my studies on developping a tool for a semi-quantitative assessment of risk pathways at BGPs for spread of *S.* Dublin through BGPs linked to dairy farms in Denmark, that can be used by researchers, advisors and assessing organisations to support communication and decision-making. I worked collaboratively with an other intern who worked on the developement a tool for risk pathways assessment for the introduction and establishment of *S.* Dublin into dairy cattle farms.

There is an important lack of knowledge on this topic and there has never been risk assessment achieved related to BGPs about *Salmonella*. To carry out the project, a lot of litterature review and concrete observation visits to BGPs have been required as well as discussions with different experts.

First, the process of the risk assessment framework is presented. Then, the resulting model, risk assessment and interpretation tools are described, illustrated and discussed. Finally, a conclusion and perspectives relate to the work carried out in this study and it included other projects.

| | Prob (Intro to delivery area via truckloads per week) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Prob(SD per truckload): | **0,00001%** | **0,0001%** | **0,001%** | **0,01%** | **0,1%** | **1,0%** | **2%** | **5%** |
| Frequency truckloads per week to Biogas Plant | | | | | | | | |
| **50** | 0,0005% | 0,0050% | 0,0500% | 0,4988% | 4,88% | 39,50% | 63,58% | 92,31% |
| **55** | 0,0005% | 0,0055% | 0,0550% | 0,5485% | 5,35% | 42,46% | 67,08% | 94,05% |
| **60** | 0,0006% | 0,0060% | 0,0600% | 0,5982% | 5,83% | 45,28% | 70,24% | 95,39% |
| **65** | 0,0006% | 0,0065% | 0,0650% | 0,6479% | 6,30% | 47,97% | 73,10% | 96,44% |
| **70** | 0,0007% | 0,0070% | 0,0700% | 0,6976% | 6,76% | 50,52% | 75,69% | 97,24% |
| **75** | 0,0007% | 0,0075% | 0,0750% | 0,7472% | 7,23% | 52,94% | 78,02% | 97,87% |
| **80** | 0,0008% | 0,0080% | 0,0800% | 0,7968% | 7,69% | 55,25% | 80,14% | 98,35% |

**Table 1** : Illustration of calculated probabilities of *S.* Dublin introduction in a BGP area through slurry trucks per week.

# III. MATERIAL AND METHODS

## 1. The sources of information

This project requires a significant amount of knowledge, whether for the characteristics and process of the spread of *S.* Dublin, for the management of BGPs with regard to biosecurity, but also to grasp how the risk assessment works in a brand-new field. Indeed, a whole bunch of literature addresses how to proceed with risk assessment in the processed food industry with tools that are adapted to it. However, the conditions were not sufficiently similar. Hence, part of the knowledge acquisition process was to have meetings with experts, specialists and professors in different fields as *S.* Dublin surveillance, pathogenesis, transmission, BGP organisation and development in Denmark, risk assessment methodology and biosecurity.

During those meetings, it was discussed what might be the most suitable framework of risk assessment in BGP, with relevant parameters, adapted to the situation. Several methods have been discussed including the qualitative assessment, because it is a logical first step to understand the system and identify risk pathways. It consists of estimating the occurrence of risks and then, linking it to consequences of various degrees of severity. Then, the interest turned to the semi-qualitative risk assessment i.e, a mix between qualitative and quantitative risk assessment. This is the current working method, which is described to later.

## 2. Model of probability of introduction of *Salmonella* Dublin to biogas plants areas

A calculation model was built, according to an epidemiology formula, using different probabilities of *S.* Dublin presence in the truckload depending on how many trucks move back and forth on farms over a week (**Table 1**). The probability of presence of *S.* Dublin in a truck is also linked to the proportion of contaminated farms (L2 farms) delivered by the BGP, however, a herd may be freshly infected by *S.* Dublin without being considered as a L2 farm. The objective is to foresee and estimate the potential risk of the introduction of *S.* Dublin into the BGP area from where it may contaminate other trucks or transportation in an out of BGP. Truck going into BGP can also drop faecal matter it has on the outside of the truck if the truck is not properly cleaned. For this second model, the same formula than previously, and new percentage parameter was added to account for the probability of *S.* Dublin-contaminated materials being dropped on the BGP area.


**Figure 11**: Map of Denmark and the area covered throughout visits to dairy farms and biogas plants in Jutland (Source: netmaps.net)

| | Date of the visit | Ownership | m3 biogas produced/year (in millions) | Process type | Number of farm suppliers | Cleaning method of trucks | Plant supplied by L2 dairy farms |
|---|---|---|---|---|---|---|---|
| **1** | 30/09/2021 | Cooperative | 36 | Thermophilic | 75 | Rinced | No |
| **2** | 10/11/2021 | Private | 19 | Thermophilic | 55 | Washed | No |
| **3** | 10/11/2021 | Cooperative | 22 | Thermophilic | 60 | Rinced | Yes |
| **4** | 16/11/2021 | Cooperative | 14 | Thermophilic | 35 | Rinced | Yes |
| **5** | 17/11/2021 | Energy company | 22 | Mesophilic | 140 | Washed | Yes |
| **6** | 23/11/2021 | Cooperative | 8 | Thermophilic | 45 | Rinced | No |

**Table 2:** Basic information collected throughout visits to biogas plants.

3. To identify potential risk pathways in the field

The majority cattle farms and BGPs are located the peninsula in Jutland (**Figure 11**), that means, far away from Copenhagen, where the university is. So, thanks to the project funding, a vehicle was loaned, as well as accommodation in Jutland, during the planned visits over 2 to 4 days.

3.1. Visits to BGPs

One of the meetings was with Technical Director of Biogas Denmark, who agreed to help in the approach. His contacts allowed a SEGES researcher, - whose work requires visiting some BGPs - to get in touch with different BGP managers and/or employees and arrange visit dates. Six BGPs were visited, and different types were selected to cover different characteristics as shown in the **Table 2.** During visits, information was collected about the BGP ownership, production capacity, treatment procedure, truck activity, hygiene management and contaminated suppliers. The second and main objective was to understand the organisation of BGP and identify risk pathways, to correctly understand the potential risks for the spread of the bacteria.

3.2. Visits to dairy farms

Nine dairy farms in Jutland were visited, which are all suppliers to a BGP and some of them were chosen because of their relation to visited BGP. The objective was to observe and analyse potential contamination pathways. A training in biosecurity assessment and estimation of the risk of introduction and establishment of the bacterium on different areas of the farm was necessary and carried out by a PhD student from SEGES. For some information, interviews with farmers were required.

4. Risk assessment

The risk assessment is the component of the analysis which estimates the risks associated with the hazard. In this study, the hazard is employed in the clearly definite meaning of exposure to a specific biological agent capable of causing an adverse health effect: *S.* Dublin. The risk assessment has never been conducted on BGP about *S.* Dublin spreading issues.

The semi-quantitative assessment of risk pathways table has been created on Excel. For this table, a stepwise procedure has been conducted, indeed, the pathways and risks descriptions took different versions before the one estimated to match best.

Step 1: Self-suggestions were made regarding simple based knowledge about generated digestate in BGP.

Step 2: BGP employees' suggestions about what can lead to a potential risk into the plant were collected.

Step 3: Concrete observations and analyses were done during visits at BGPs that operate differently. Moreover, different opinions were discussed with SEGES veterinarians and researchers with biosecurity expertise on cattle farm.

Step 4: A scoring system of each risk mentioned in this table was added. The objective was to describe the situation at BGPs as good as possible, giving several criteria for each risk. The current scoring ladder is extended from 0 (lower risk) to a maximum of 10 (highest risk) according to (Nielsen and Nielsen, 2012). This scoring system is not evidence-based but I attempted to make it logical so the absolute numbers cannot be used for statistical purposes.

Step 4: The risk assessment scheme was discussed for improvement with SEGES veterinarians and researchers.

Step 6: The effectiveness and the relevance of the risk assessment scheme were tested throughout the last visits of BGPs. Some adaptations and improvements were achieved on the scheme, which lead to the last version of the risk assessment prototype.

Step 7: Below the table, a more accurate description of each single risk is added to make the use of this tool easier by the operator. So, the use with both the scoring system and background information is created to focus attention of biosecurity decision makers in the plant on the control of the spread of the bacteria (Nielsen and Nielsen, 2012).

## 5. Interpretation of the result

To interpret the results of the scoring risk assessment table, a graph was created in Excel. The objective of this tool was to illustrate the results for the BGP being assessed. The best scenario is the smaller the red zone as possible, which indicates lower risk of the BGP exposed to the propagation of *S.* Dublin. With this tool, it is possible to visualise and become aware of main sources of potential risk, making it possible to then, try to change these.

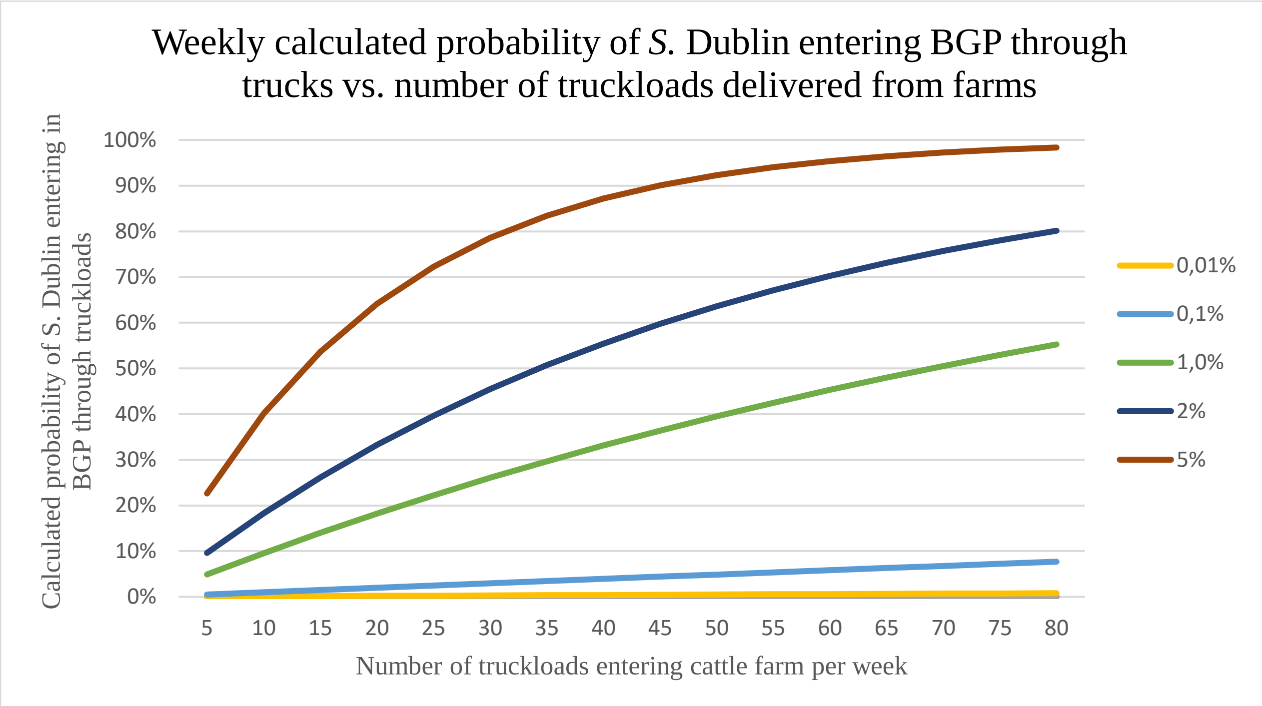



**Figure 12** : Graph of the calculated probability of the introduction of *Salmonella* Dublin into biogas plant.

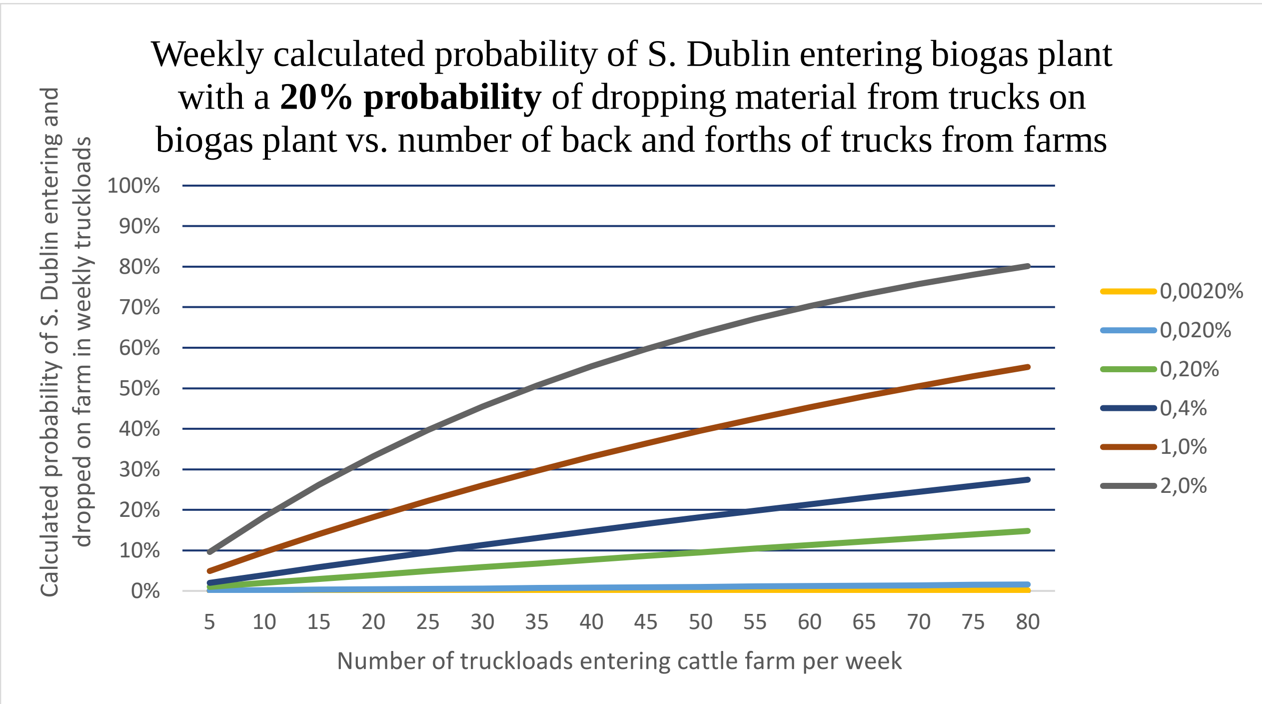



**Figure 13** : Graph of the calculated probability of the introduction of *Salmonella* Dublin with the probability of dropping material from truck into biogas plant.

# IV. RESULTS

## 1. Calculated probability of introduction of *Salmonella* Dublin within biogas plants

A calculation model has been built to estimate and quantifiate the risk of the introduction of *S*. Dublin in a BGP, depending on the number of back and forth of trucks from farms per week (**Figure 12).** For instance, only a 5% probability that the bacterium is present into the truckload could reach 90% after the 45$^{th}$ truck coming back, at the end of the week. Furthermore, a larger plant which trucks would double the traffic, again for presence of 5% of *S*. Dublin on truck, would reach nearly 100% of the introduction of the bacterium by the end of the week. This calculation model illustrates that *S.* Dublin can be introduced quickly in few truck back and forth into BGPs.

In addition, as trucks come back dirty on the outside (bottom and wheels) from farms, they can easily drop some potentially contaminated faecal matter into the BGP, and then, spread *S*. Dublin in there. Therefore, the previous probability were corrected with the probability for a truck to drop matter at the BGP (**Figure 13**). This new probability were estimated at 20% randomly (it can be easily changed). For instance, a 2% probability that the bacterium is present into the truckload and that the truck drop faecal matter, could reach 70% after the 60$^{th}$ truck coming back, at the end of the week.

## 2. How to use the risk assessment table ?

The **Table 3** (on following pages), shows the semi-quantitative risk pathways assessment tool for spreading *S.* Dublin in BGPs. This tool should be used by researchers, advisors and assessing organisations to support communication and decision-making.

To use the risk assessment table, the operator must choose the criteria the most representative of the current situation for each risk during a BGP assessment. However, if the situation being assessed would be better defined by an intermediate score in the table, then the operator is free to grant an intermediate score.

The maximum score reachable is 10, however for some risk pathways, the highest score does not reach 10, if the operator assessed the situation being assessed to be riskier than the riskiest criteria announced, he is free to rate lower.

| The scoring system will aid in identifying routines or areas in the biogas plant that facilitate the spread of *Salmonella* bacteria that spread via faeces. |
|---|

| Date | / / | Biogas Plant ID | XXXXX | Operator's name or ID : | XXXXX |
|---|---|---|---|---|---|
| Treatment of biomass | | Number of cattle farms delivered | | Number of slurry trucks | |
| Temperature of treatment | | Number of dairy farms delivered | | Number of truck for manure (deep litter) | |

| Risk pathways categories | Risk pathways sub-categories | Risk pathways | Estimated score | Score | Guiding criteria for each score |
|---|---|---|---|---|---|
| Contaminated farms (Level 2 farms) | 1- When the truckload is contaminated | 1.1- Management of L2 farm suppliers | | 0 | L1 frams only |
| | | | | 1 | One truck is all the time reserved to visit only L2 farms |
| | | | | 1,5 | Each day, one truck is reserved to visit only L2 farms |
| | | | | 2 | There is no specific organisation to visit L1 or L2 farms, but trucks from L2 farms are systematically washed coming back to the plant |
| | | | | 5 | L2 farms are visited by trucks at the end of the day |
| | | | | 10 | There is no difference of management between L2 farms and L1 farms |
| | | | | | I don't know |
| | | 1.2- There is a procedure afterward found out *Salmonella* Dublin by testing | | 0 | A hygiene enhancement procedure is immediately established |
| | | | | 4 | There is a procedure, but it is rarely implemented |
| | | | | 7 | Nothing |
| | | | | | |
| Hygiene of different types of trucks transporting manure and slurry | 2- Slurry trucks' and other trucks hygiene | 2.1- Frequency of **cleaning the outside** of trucks | | 0 | Washed between each back & forth (same farm) |
| | | | | 1 | Washed between different farms |
| | | | | 6 | Washed once a day or more |
| | | | | 8 | Washed less than once a day |
| | | | | 10 | Never washed |
| | | 2.2- **Cleaning method** and tools for the **outside** of trucks | | 0 | Trucks are washed using at least one of this possibilities : soap, brush, desinfectant |
| | | | | 1 | Trucks are rinced using hot high pressure water AND wash using soap, brush, detergent **at the end of the day** |
| | | | | 3 | Trucks are rinced using hot high pressure water (70°C ) |
| | | | | 10 | Nothing |
| | | 2.3- Frequency of **cleaning the inside** of trucks | | 0 | Washed between each back & forth (same farm) |
| | | | | 1 | Washed between different farms |
| | | | | 4 | Washed once a day or more |
| | | | | 6 | Washed less than once a day |
| | | | | 8 | Never washed |

**Table 3 (*Part 1*)**: Table of risk pathways in biogas plants using scoring system *(to be continued)…*

**Figure 14**: Muddy unconcrete path used by slurry trucks in a dairy farm (Left: slurry tank / Right: Cow barn) (Source: Personnal picture)

3. Description and accuracies for each risk (**Table 3**)

3.1. Contaminated farms (Level 2 farms) (**Table 3 (*Part 1*)**)

**1.1-)** Level 2 (L2) farms are herds registered to have been infected by *S.* Dublin. Even if level 1 farms could be infected, there is a higher probability for L2 herds to generate contaminated livestock manure. In this case, the more the BGP is supplied by L2 farms, the greater the risk of spreading the bacteria within the BGP. However, if a BGP is supplied by L2 farms, it is still possible to adapt truck visits schedule in order to limit the propagation of *Salmonella* into non-contaminated farms (level 1 farms).

**1.2-)** According to the legislation, each BGP must test the presence of *S.* Dublin in faecal matter once a year. The aim of this testing is to enhance hygiene procedures for manure and slurry handling when the bacterium is found out into the truckload. If no hygiene enhancement is decided afterward a contaminated sample, the bacterium could disperse very quickly within the BGP, and then, it could lead to infected dairy herds.

3.2. Hygiene of different types of trucks transporting manure and slurry

**2.1)** When slurry trucks deliver digestate to farms, they sometimes pass through farms, taking mud paths or dirty concrete paths, potentially contaminated by *S.* Dublin. So, the outside of the truck - mostly the wheels and the back – must be washed between several trips to one farm or between trips to 2 farms, otherwise, it could lead to a risk of spreading the bacteria into the BGP and from a farm to others and through other trucks which are also delivering to other farms.

**2.2)** If slurry trucks are not rinsed with hot high pressure water, the pathogen could survive and there is a risk to contaminate other farms that the truck will visit later. The cleaning is more efficient using soap, brush and detergent. A standardise washing procedure should be introduced in each BGP using livestock manure.

**2.3)** When a truck unloads slurry at the BGP, the truck tank should be drained as well as possible in order to empty the truckload. Despite this, potentially contaminated fresh slurry residues remain in the truck tank. Right after, trucks load digestate to bring it back to the farm. If the inside of the truck is not rinced regularly, *S.* Dublin from fresh slurry, could spread into the digestate. However, the digestate strongly dilutes the bacterium, this risk pathway is not estimated as a serious risk, because of the very light concentration of the pathogen. The treated slurry could become risky for the farm, specially if the container is into the farm, near cattle or cross the feed path.

| | | | | | |
|---|---|---|---|---|---|
| Hygiene of different types of trucks transporting manure and slurry | | 2.4- The cleanliness of leaving trucks (from the plant) is assessed | | 0 | Yes, the cleanliness assessment is done **everytime** |
| | | | | 3 | Yes, the cleanliness assessment is done **at least occasionally** |
| | | | | 6 | Never |
| | | 2.5- Cleaning of other trucks (for slaughterhouse wastes for instance) | | 0 | Washed everytime |
| | | | | 1 | The company that own trucks wash them regularly |
| | | | | 2 | Washed only if a risk is announced |
| | | | | 6 | Never washed |
| | | 2.6- Assessment of cleanliness of tools used to wash trucks | | 2 | No residues of slurry, mud or dust on cleaning tools AND the floor is immaculate |
| | | | | 6 | Mostly no residues on cleaning tools BUT some residues left on tools and on the floor |
| | | | | 9 | Residues of slurry, mud or dust are easily visible on tools AND the floor is dirty after washing |
| | | | | | |
| | 3- Hygiene and management of manure containers **used by an external company** | 3.1- Manure containers management procedure during delivery | | 0 | Never touch the ground |
| | | | | 3 | Come into contact with **clean ground** |
| | | | | 4 | Come into contact with **unclean ground** AND it is **at least rinced before** going back to the farms |
| | | | | 10 | Come into contact with **unclean ground** and it is **not rinced before** going back to the farm |
| | | 3.2- Where is stored the manure container before going back to farms | | 0 | The manure container is not stored into the biogas plant, it leaves directly afterward delivering |
| | | | | 3 | At least the back of the container is rinced and stored in a clean (safe) place |
| | | | | 6 | The manure container is directly stored in a clean (safe) place |
| | | | | 9 | The manure container is left near the manure storage area |
| | | 3.3- Cleaning of external companies' containers | | 0 | Wheels and back of the container are at least rinsed with high pressure water **before AND after** delivering solid manure |
| | | | | 3 | Wheels and back of the container are at least rinsed with high pressure water **before OR after** delivering solid manure |
| | | | | 5 | The external company handle the washing of the containers |
| | | | | 10 | The container is **never or very occasionelly** washed |
| | | | | | I don't know |

**Table 3 (*Part 2*):** Table of risk pathways in biogas plants using scoring system *(to be continued)…*

**Figure 15:** Tractor lifting a manure container to empty it in biogas plant (Source: Personal picture)

**2.4)** Here, the point is to know whether BGP staff check the cleanliness of trucks right after being washed and just before it leaves the BGP. Doing this, BGP staff can interpret the effectiveness of their washing procedure, and even change it if trucks are assessed "not clean" before departure. However, even a truck which appears clean at a glance does not mean that it is free of *Salmonella*, so this risk pathway is not one of the main concerning. (That is why the worst criteria does not reach 10) (**Table 3 (***Part 2***)**)

**2.5)** BGPs are supplied by livestock manure, slurry, agricultural wastes, factory residues, slaughterhouse and household wastes. All of that matter is brough to the BGP by different types of trucks and tractors– owned by different structures. So, all trucks that cross paths into the plant could disseminate the pathogen, specially to trucks doing back and forth to farms without be washed between, and then, infect dairy herds. It would be more secure to clean those machines at the BGP, or to have a certification from external companies on the washing of their machines to reduce the dissemination of *S.* Dublin.

**2.6)** The assessing operator requires to observe the cleanliness of the tools used to wash trucks. If tools are not kept clean, their use could become inefficient in the washing of trucks, by contaminating the washing area and trucks. Finally, truck could contaminated dairy farms by transporting feacal matter.

**3.1)** Most of the time, manure (solid faecal matter) is transported to the BGP through containers. The most convenient way to empty a containers is to lift it above the ground. Nevertheless, it is possible to deposit the container on the ground, and this process could contaminate the underside of containers which are going back to farms to be fulled again. The ground is often dirty and wet, which is optimal for *S.* Dublin spreading and this could infect dairy herds.

**3.2)** The manure container is transiting between different farms and BGPs to deliver manure. So, if the manure containers is not cleaned regularly and/or stored in a potentially contaminated place at BGPs, it could lead to a risk of spread *S.* Dublin through many dairy farms.

**3.3)** This risk pathways refers to the frequency of cleaning containers and tractor owned by an external company. Thoses containers are rented by famers to bring their manure to the BGP. Indeed, a same container can go into different BGPs and in various farms throughout the same day and without being washed. This process is highly risky regarding the dissemination of *S.* Dublin, that is why, BGPs should demand the container driver to often wash his machinery while he does back and forth. (The best scenario would be to wash the container to a BGP between each back and forth with a farm).

| | | | | | |
|---|---|---|---|---|---|
| Transport between dairy farms and biogas plant | 4-Traffic data between farms and biogas plant | 4.1- Average number of back and forths for **1 slurry truck** per week | | **0** | Less than 10 back and forths by truck |
| | | | | **3** | 10 to 20 back and forths by truck |
| | | | | **5** | More than 20 back and forths by truck |
| | | 4.2- Average number of **farms visited for 1 slurry truck** per week | | **0** | Less than 10 back and forths by truck |
| | | | | **3** | 10 to 20 back and forths by truck |
| | | | | **5** | More than 20 back and forths by truck |
| | | 4.3- Average **total** number of **visited farms** per week | | **0** | 1 to 10 |
| | | | | **3** | 11 to 30 |
| | | | | **4** | 31 to 50 |
| | | | | **5** | More than 50 |
| | | 4.4- Trucks deliver digestate | | **0** | To one farm at time |
| | | | | **10** | To more than one farm with the same truckload |
| | | 4.5- Average distance of farms from the BGP | | **0** | 0 to 15 km |
| | | | | **3** | 16 to 40 km |
| | | | | **5** | |
| | | 4.6- Means of transporting livestock manure | | **0** | Slurry only |
| | | | | **2** | Manure brought by trucks provided for this purpose |
| | | | | **2** | Slurry and manure brought by trucks provided for this purpose |
| | | | | **5** | Manure brought by containers from external company |
| | | | | **10** | Manure brought by farmers (with their own tractor) |
| | | 4.7- Means implemented to prevent the spread of dirt into the BGP | | **0** | Washing or rinsing structure at the entrance of the plant |
| | | | | **1,5** | Rinsing structure for trucks as a truck shower tunnel |
| | | | | **2** | The delivery area is the washing place for trucks. The delivery area is placed in outskirt of the plant. |
| | | | | **8** | No means is implemented |

**Table 3 (*Part 3*)**: Table of risk pathways in biogas plants using scoring system *(to be continued)…*

### 3.3. Transport between dairy farms and biogas plants (**Table 3 (*Part 3*)**)

**4.1 & 4.3)** At some farms, slurry trucks need to multiply consecutive back and forth to empty the slurry container completely. Otherwise, trucks take a single trip per farm and come back to the BGP between each farm. Doing like this, a slurry truck could do a lot of back and forth in in one week, however, this pathways is size-dependent of the plants, so it may be hard to do anything about but it is important to but be aware of the risk and to handle the best as possible. Obviously, more a BGP generates traffic, more it increases the risk of spreading *Salmonella* into the plant.

**4.2)** Like the previous risk, this pathway depends on the size of the BGP, but the higher the average number of visited farms, the more back and forth there is into the BGP, which increases the risk of spread *S*. Dublin among farm suppliers. However truck schedules could be managed in order to visit fewer farms than possible per day without reducing BGP activity. For instance, by visiting several times consecutively, and therefore less often, the same farm.

**4.3)** See **4.1)**

**4.4)** When a truck delivers at least 2 neighbouring farms with the same truckload without going back to the BGP, the truck is not at least rinsed, so this procedure could dangerously disseminate the pathogen to farms, though the outside of the truck, specially dirty wheels and it could infect dairy herds. This risk pathway is one of the most concerning, therefore slurry trucks must be washed between at the BGP before going in different farms.

**4.5)** The further the average distance travelled by trucks to deliver, the greater the risk of spreading the *S*. Dublin to a wide perimeter. However, this risk pathways is BGP size dependant and it is not possible to rebuke a plant because of its size, so the worst criteria is lightly scored.

**4.6)** The more the transport of livestock manure is controlled and managed by machineries solely used for this purpose, the less likely it is to disseminate *S*. Dublin.

**4.7)** When a truck enter in the BGP, it crosses paths of other vehicles (containers, cars, other trucks) and especially the weighing scale. By the way, all other machinery transporting wastes need to pass on it. The crossing area becomes a risk pathway for spreading *S.* Dublin within the plant and in dairy farms through trucks. So, implement a washing or rinsing area for trucks at the entrance (at the level of the weighing scale), of the BGP, could reduce this risk.

| | | | Score | |
|---|---|---|---|---|
| Human activities in biogas plants | 5- Farmers' delivery process regarding solid manure | 5.1- Farmers get off the tractor during the delivery | 0 | No farmer into the BGP |
| | | | 7 | Farmers never get off the tractors |
| | | | 9 | Farmers get off the tractors only if somthing wrong |
| | | | 10 | Everytime |
| | | 5.2- Farmers wash their machinery (tractor + manure container) | 0 | Yes, the delivering farmers wash **everytime** their machinery after coming into the BGP |
| | | | 6 | Yes, some farmers wash occasionelly their machinery |
| | | | 10 | No farmer wash their machinery at the BGP, **but the BGP structure allow them** to do it |
| | | | 10 | No, the BGP structure don't allow them to do it |
| | | | | I don't know |
| | | 5.3- Cleaning of farmer machinery (tractor + manure container) | 0 | Wheels and back of machinery are at least rinsed with high pressure water **before AND after** delivering solid manure |
| | | | 3 | Wheels and back of machinery are at least rinsed with high pressure water **before OR after** delivering solid manure |
| | | | 5 | Farmers certify to wash their machinery at the farm |
| | | | 10 | Never washed |
| | | | | I don't know |
| | 6- Drivers coming in farm | 6.1- General procedure for drivers getting off the trucks in farm | 0 | Never |
| | | | 2 | The driver wears equipment (wellies, gloves) + access to a cleaning area |
| | | | 8 | The driver does not wear equipment without acces to cleaning area |
| | 7- Visitors (from a farm or to a farm) | 7.1- Cleaning the outside of visitor vehicles | 0 | Everytime |
| | | | 2 | Only if visitors came from a farm |
| | | | 6 | Never |
| | | 7.2- Presence of a parking for visitors | 0 | The parking right after the entrance, isolated from trucks and tractors paths and waste area |
| | | | 2 | The parking after the entrance, not really isolated from trucks and tractors paths and waste area |
| | | | 6 | Visitors' vehicles can park everywhere into the BGP, most of the time visitors' vehicles are very close to crossing area |
| | | 7.3- BGPs provide equipment for visitors | 0 | Gloves, suit, wellies, especially when they come from a farm |
| | | | 3 | Partiel equipment |
| | | | 6 | Never |
| Management of slurry and manure delivery places | 8- Delivery areas | 8.1- The place of loading and delivery of slurry areas | 0 | Not in the same area |
| | | | 4 | In the same area |
| | | | 7 | Very close to each other |
| | | 8.2- How is cleanliness maintained around slurry delivery area ? | 0 | The area is at least rinced **everytime a truck comes deliver** |
| | | | 4 | The area is at least rinced **at the end of the day** |
| | | | 7 | The area is at least rinced **less than once a day** |
| | | | 8 | The area is never washed |
| | | 8.3- Assessment of the cleanliness of the slurry delivery area | 0 | The area is clean, **with no residue** of slurry on the ground |
| | | | 3 | The area is quite clean, **with little residues** of slurry on the ground |
| | | | 6 | The ground is dirty, large residues and dry traces |
| | | 8.4- How is cleanliness maintained around pick up and delivery areas (manure) ? | 0 | These areas are at least rinced **everytime** a truck/machinery dirty them |
| | | | 4 | These areas are at least rinced **once a day**, afterward trucks/machineries dirty them |
| | | | 7 | These areas are at least rinced **less than once a day** |
| | | | 8 | These areas are never washed |
| | | 8.5- Assessment of the cleanliness of the manure delivery area | 0 | The area is clean, **with little residues** of manure on the ground |
| | | | 3 | The area is quite clean, **with somes residues** on the ground **BUT** people are currently working |
| | | | 6 | The ground is dirty with large residues and **no one is currently working** |

**Table 3 (*Part 4*)**: Table of risk pathways in biogas plants using scoring system.

**Figure 16** : Slurry truck delivering slurry in the unloading point through mechanical arm

(White arrow: digestate pumping point (to full the truck tank))

(Source : Personal picture)

### 3.4. Human activities in biogas plants (**Table 3 (*Part 4*)**)

**5.1)** Some farmers deliver livestock solid manure themselves with their own machinery at the BGP. This way of proceeding could greatly increase the risk of spread the bacterium and even more so if farmers get off their machinery during the delivery stage.

**5.2)** Farmers who deliver livestock solid manure with their own container can easily disseminate the bacterium from the farm to the BGP and vice versa whether container and tractor are not cleaned between back and forth. In some BGP, it is possible for farmers to wash their machineries, and therefore, washing the truck could be a safer procedure regarding regarding the spread of *S.* Dublin into farms through the use of BGPs.

**5.3)** With regard to **5.2),** in BGPs, if the cleaning area can be used by farmers, the point is to determinate the frenquency of tractors and containers cleaning.

**6.1)** When trucks come in farms, drivers can get off the truck to ensure the proper functionning of the loading or unload of the digestate. At this moment, if drivers do not wear any personal equipement as suit, gloves or wellies and whether he has no access to a cleaning/rinsing area, there is a risk to spread the bacteria within the BGP when he comes back to the BGP. Without any hygiene procedure, he could contaminate other farms and also employees of the BGP.

**7.1)** & **7.2)** When visitors came into the BGP, they easily can carry the bacteria onto the outside of their vehicles, specially when they come from a farm or other potentially contaminated place. Therefore, a rinsing facility and a suitable parking for visitor at the entrance, could lead to reduce the risk of disseminate *S.* Dublin into BGPs.

**7.3)** If visitors are required to enter in areas of transit or storage of organic matter, they should wear some personal equipments, as suit, gloves or at least wellies lended by the BGP to avoid transporting the pathogen outside the BGP (potentially in farms).

### 3.5. Management of slurry and manure delivery places

**8.1)** If loading and delivery areas of slurry are too close to each other, the potentially contaminated fresh slurry could easily disseminate *S.* Dublin in the treated slurry when trucks make the transition.

**Illustrated results of the risk assessment in a BGP and identification of risky zones for each risk into the "Traffic data between farms and BGP" category**

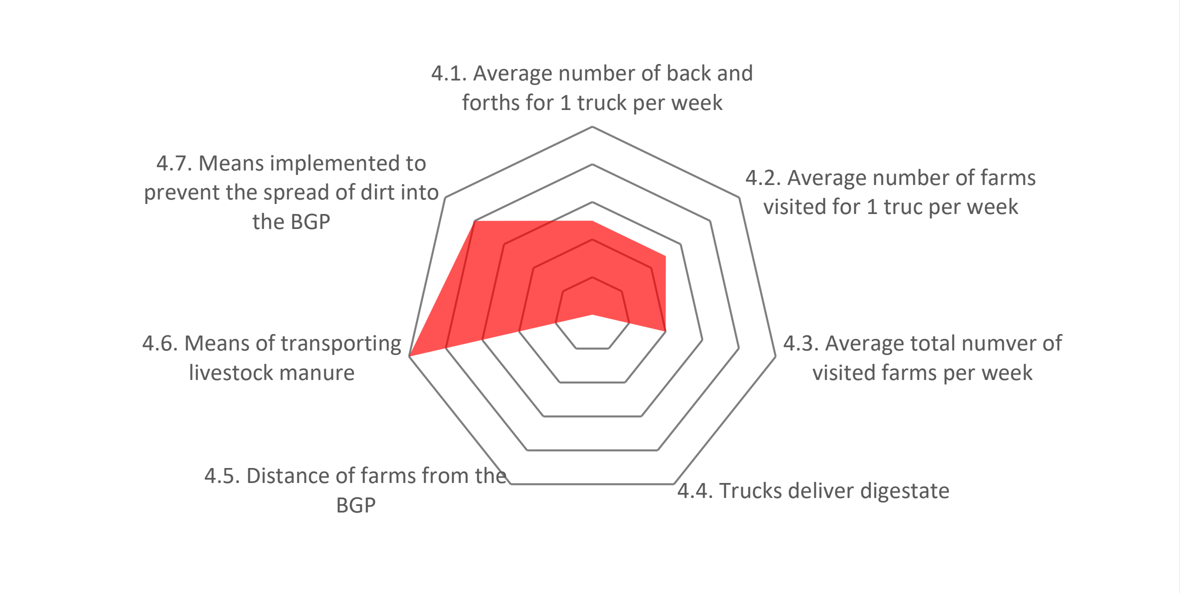



**Figure 17**: Illustration graph of the results of the risk assessment in a BGP and identification of risky zones for each risk into the "Traffic data between farms and biogas plant" category.

**Illustrated results of the risk assessment in a BGP and identification of risky zones for each risk for the total risk score of each category.**

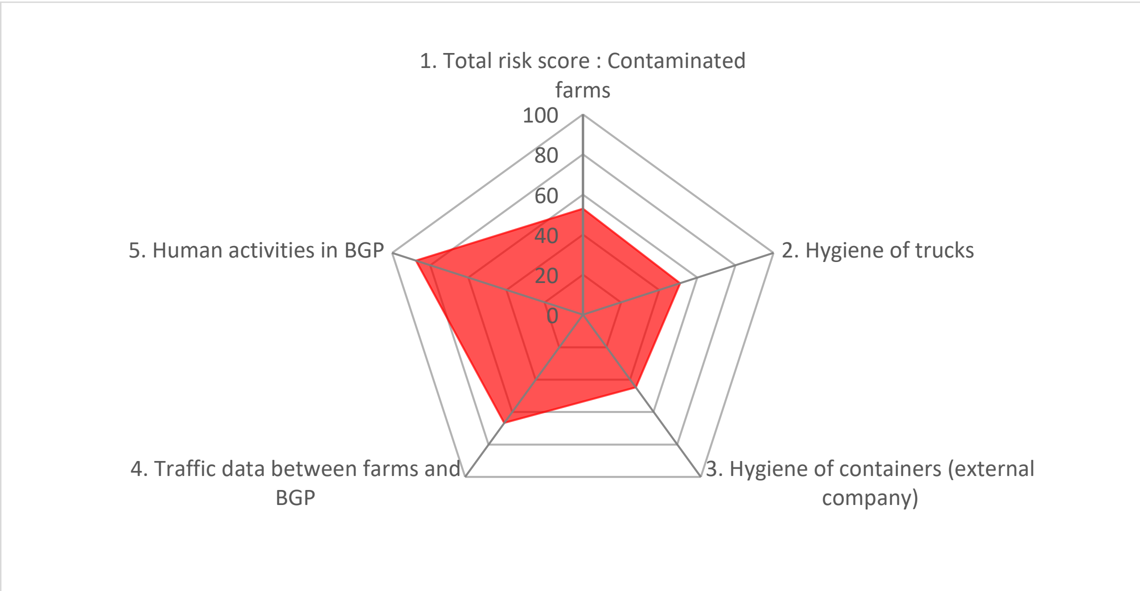



**Figure 18** : Illustration graph of the results of the risk assessment in a BGP and identification of risky zones relative to the total risk score of each category

**8.2)** The slurry is stored in a container related to an unloading point at the BGP. Each time a truck unload fresh slurry, some matter drops from the mecanical arm of the truck. In this way, if the area around the unloading slurry point is not rinsed and/or disinfected, it could lead to a risk to spread the bacteria through others trucks which are coming to deliver slurry.

**8.3)** This point refers the previous one (**8.2**) and it requires the operator to observe and assess the state of the cleanliness of the slurry delivery area by himself.

**8.4)** Most of the time, the manure is stored as a pile (or in a pit) in the BGP and parts of manure are added in the mixer step by step for the treatment process. All the back and forth carried out for this, as well as the passage of trucks to delivery the manure, inevitably make the area around the manure pile dirty. Thereby, more this area is dirty, the greater is the risk to disseminate the bacteria through workers, trucks or other vehicles. Finally, the contamination of vehicles could lead to infected dairy herds when containers (with dirty wheels) come back to farms.

**8.5)** This point refers the previous one (**8.4**) and it requires the operator to observe and assess the state of the cleanliness of the slurry delivery area by himself.

## 4. Interpretation of the results

The **Figure 17 and 18** allowed BGP operators to easily visualise areas for improvement. The red area represents the current situation of a BGP afterward assessment. The larger the red area, the greater the associated risk for the BGP being assessed. These figures are examples and the risk 2.6. seems to be concerning of thas BGP (**Figure 17**). Then, when scored sumarised, the global result of the assessment can be visualised. In **Figure 18**, “4. Traffic data between farm and BGP” refers of the mean of the scores presents in **Figure 17**.

## V. DISCUSSION

Risks into biogas plants

This observation study highlights the most concerning risk pathways of spreading *S.* Dublin in dairy cattle farms through BGPs. These are mainly related to unceasing traffic between farms and BGPs caused by slurry trucks and especially tractors and manure containers owned by farmers or external companies. Also, hygiene management regarding transport vehicles is not established by any standardised procedures, in particular with regard to the method and frenquency of truck cleaning. Therefore, these risk pathways should become the main concerns regarding the diffusion of the pathogen.

This study only highlights the responsibility of BGPs in reducing the spread of *S.* Dublin, however, farm management is also involved in this issue, such as manure management and layout of paths used by trucks/tractors within farms. A similar study has been carried out by Jeanne, student at AgroCampusOuest, focussing on dairy cattle farms. She worked on a tool based on semi-quantitative assessment of the risk of introduction and establishment of *S.* Dublin in dairy farms using BGPs. Our observation studies are therefore complementary, and they should both be read both to understand the entire system.

The risk assessment tool

The risk assessment table has been built based on concrete observations, so it is not evidence-based. Determinate seriousness of each risk pathways to rank them and to score all criteria for each risk was the most difficult part of the study. Remember that this tool has never been carried out before, so expert advices, analyse of situations and discussion with BGP staff were essential to reduce uncertainties of this tool as much as possible. Moreover, too many risk pathways requires questioning BGP staff to assess, and not observing BGP areas.

Limitations

To test the assessment tool, BGPs were visited only one time. This is defentively not enough to well assess a BGP. It is required to visit one several times to have a result closer to reality. Because few BGPs were visited throughout this study, certainly the risk identified are not really representative of reality. Furthermore, a large majority of dairy herds are mainly located in Jutland, so, all the visited BGPs are also located in this area of Denmark. From UCPH, our destinations were 200 to 300 km away. Even if we had the opportunity to group the visits on 2 to 3 consecutive days, transport time has taken an important place for trips.

Some ideas to emphasise

Troughout visits to BGPs, we found out a lack of procedure on different sectors. First, there is no cleaning procedure for slurry trucks and other machinery. Each single BGP manage the cleaning of trucks independently, without any standardisation. It exists a legislation which refers the hygiene on trucks but, the eight BGPs visited seems to ignore it. Secondly, few BGPs use from time to time the same truckload of digestate to deliver in two neighbouring farms, without coming back to BGPs between them two to clean the truck (Risk 4.4. from the Table 3). This process is forbidden but, as previously, none seems informed. More commonly, slurry trucks come back to BGP between different farms to deliver fresh slurry but the truck is not cleaned (or rinsed) everytime. These two situations seem as risky as each other for the spread of *S.* Dublin in dairy herds through BGPs.

However, it is required to test the presence of *S.* Dublin in the digestate freshly treated to control the effectiveness of the treatment which heat the biomass at 70°C or 90°C and these temperatures are enough to kill the bacteria. Therefore, digestate is the least risky pathways of the BGP, that is why, it would be interesting to test fresh slurry and deep litter when trucks and containers collect them from farms. Furthermore, most BGPs seem ignore which farms they deliver are contaminated farms (L2 farms), so they do not take any precaution, such as visiting L2 farms at the end of the day and wash the slurry truck using soap, brush and detergent when it returns to the plant. Very few of them gave us the number of L2 farms they deliver, which mean that they probably do not take precautions. Nevertheless, herds can be infected by *S.* Dublin, and not being registered as L2 farm if the infection is too recent.

Globally, this study highlights the importance of fostering communication. First, BGPs, farmers and drivers from external companies could sometimes meet together in order to discuss the means to reduce de dissemination of the pathogen and structure preventive actions. Through meetings, they could share their opinion, advices and ideas regarding biosecurity in farms and BGPs. For instance, the BGPs washing area is sometimes available for farmers and drivers from external company to clean their manure containers, but they often ignore it. In this situation, a reminder could encorage farmer and other drivers to clean their equipments in BGP more frequently. Likewise, as the legislation of hygiene management seems unknown by BGPs, it would be interesting for BGPs managers and staff to exchange with people behing the legislation, in order to have standadised procedures for optimise hygiene processes. Obviously, communication is not enough to remove any responsability for the spread of *S.* Dublin by BGPs, other more effective and costly means should be considered.

## VI. CONCLUSION

The objective of this observational study was to investigate if the use of BGPs could pose a risk in the increasing spread of *S.* Dublin in Danish dairy herds Denmark**.** It consisted to communicate with farmers and BGPs staff, collect information about their processing and make them aware of the efforts to be made to improve biosecurity in order to support them in their current and future efforts to restrain the spread of *S.* Dublin.

The modelling of the introduction of the bacterium into the BGPs showed that the probability of presence of *S.* Dublin could increased very quicky. Then, this study was mainly focused on the creation of a semi-quatititative risk assessment tool, regarding spreading of *S.* Dublin into BGPs. The final version table is still a prototype and contains 30 risks pathways designed to be used by researchers, advisors, assessing organisations to support communication and decision-making.

Therefore, the spread of *S.* Dublin into BGPs is facilitated due to truck traffic between farms and BGPs and to the hygiene management, especially trucks and containrers cleaning. If BGPs ignore risk indications, this could lead to an increase in the spread of *S.* Dublin on farms, and particularly dairy farms where the main concern is. In this way, BGPs could pose a risk into dairy farms.

Finally, this type of work has never been conducted before, so we had to deal with a lack of knowledge about *Salmonella* issues related to BGPs. Thereby, this work could be helpful for future, by being, for instance, analysed jointly with a risk assessment study based dairy farms using BGPs, also regarding the spread of *S.* Dublin.

## VII. PERSPECTIVES

The point of this study for current and future concerns

This study is a subpart of a 3-year project, whose the purpose is to communicate with dairy farmers and BGP managers about the *Salmonella* issues and to make them aware of potential improvements to be made from a biosecurity perspective. In this way, it could be include in the *Salmonella* Surveillance and Eradication programme to increase the importance of the projet and reduce the lack of knowledge in this sector.

Moreover, this study could help to sensitise people involved in the biogas production (BGP managers, staff, drivers, farmers) about the risk pathways to disseminate pathogens in dairy cattle farms through BGPs, which are not trifling. Also, this study could lead to foster communication on how to reduce these risk pathways. For example, it could lead to establish an efficient washing routine for dirty trucks and containers at the entrance of plants, and a cleaning training for all drivers. Furthermore, BGPs are currently growing and they are in a sustainable development approach by promoting the preservation of the environment by reducing greenhouse gas emissions. Thereby, they should not be considered responsible for the spread of *S.* Dublin in dairy cattle farms, however, BGPs have several risk pathways regarding biosecurity and that is why it is important to reduce these risk pathways as mush as possible.

The point of the carried out tools for current and future concerns

The tool based on a semi-quantitative assessment of risks, carried out throughout this study, is an initiation to understand an overall system that include a variety of risks pathways about the spread of *S.* Dublin. It is a very first prototype and it should be tested by several operators in BGPs, such as researchers, advisors and assessing organisations to enhance the accuracy of the tool and generate further concrete results and less uncertainties. Also, the risk assessment tool could be improved using quantitative factors. For instance, taking samples of livestock manure in differents BGP areas to determine the presence of *S.* Dublin could reveals the riskier practices and places. However, it is difficult to have *S.* Dublin in samples, even from a contaminated environment, thereby, several samples are required to prove the contamination.

The risk assessment table and the interpretation graph could be used to benchmark biogas plants. The aim would be the identification the better structures and managements of BGPs regarding the reduction of spreading of *S.* Dublin in order to guide and standardise future BGPs to be build.

## VIII. EPILOGUE

The penultimate version of the risk assessment tool was tested in two different BGPs, in Jutland, to assess the relevance and the effectiveness of the tool to determinate riskier areas regarding the propagation of *S.* Dublin. These assessements of risk pathways were achieved by a veterinarian researcher who mentionned points to improve.

The feedback of tests reveals that the risk assessment table is an easy-to-use tool and the scoring could be done without any hesitation. So, the assessment is quite fluid, even for an inexperienced operator who has never read the table before. This table contains 30 different risk pathways regrouped in 5 categories and 8 risk pathways sub-categories. So, the table is quite fast to fill up.

Nevertheless, the risk assessment table has been supplemented with some other risk pathways, mainly regarding the management of manure containers owned by external companies into BGPs. Moreover, some risk pathways have been changed to be more accurate and clear, but some risks remain too simple. For instance, the table proposes only one cleaning method with only one frequency of cleaning the outside of trucks, whereas, the last BGP visited has two different cleaning method at two different frequency for the outside of trucks.

In my opinion, fill an assessment form in front of the BGPs'staff requires to ask a set of questions to the person, without really discussing and it could be considered to a brake on natural communication. However, this risk assessment tool can be used as a communication support for the advisor or researcher and it can be fill afterward the meeting. For sure, the assessing operator need to know the content of the risk assessment table he uses.

Finally, the development of this tool based on semi-quantitative assessment of risk pathways of the spread of *S.* Dublin within BGPs is a first step within this brand new domain of study, but it needs to be reworked and improved in future studies on the topic.

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## ABSTRACTS


### Semi-quantitative assessment of risk pathways at biogas plants for spreading of *Salmonella* Dublin to dairy farms in Denmark

*S.* Dublin concerns Danish farmers by its rapid spread which cause in particular, drops, in milk yields. Currently, Denmark is experiencing an increasing imprevalence in dairy farms. Simultaneously, the national number of new BGPs has increased a lot. This development fit with farmers' needs to handle their growing manure and slurry production. So, the use of BGPs by farms could be part of the upsurge explanation. The objective of this study is to develop a a tool on a semi-quantitative assessment of risk pathways for spreading *S.* Dublin for biogas plants and a tool for interpreting the outcome of the assessment. Both are at the prototype stage and this study is an initiation. To do this, the operation of some BGPs was observed, analysed, discussed with different actors and project members, and the tool tested. Efforts of communication have been required. Thus, some major risks indentified concern the transport of livestock solid manure and the hygiene management on truck within BGPs.



Keywords: *Salmonella* Dublin, Upsurge, Risk assessment, Semi-quantitative, Biogas plant.



### Evaluation semi-quantitative des risques de la propagation de *Salmonella* Dublin dans les usines de méthanisation, fournies par les fermes laitières au Danemark.

Pays connu pour sa production laitièrer intensive, le Danemark connait une recrudescence de *S.* Dublin au sein de ces cheptels de bovin lait. Ce sérotype bactérien se propage très rapidemment et est notamment à l'origine de fortes baisses de rendemment laitiers. Simultanément, le nombre d'usines de biogaz à fortement augmenté ces dernières années. Cette émergence coïncide avec les besoins des éleveurs de gérer leur production croissante de fumier et lisier. Alors, l'utilisation des usines de méthanisation par les fermes pourraient en partie expliquer cette recrudescence. L'objectif de cette étude est d'élaborer un outil d'évaluation semi-quantitative des voies de risques présents dans les usines de méthanisation ainsi qu'un outil d'interprétation des résultats de l'évaluation. Tous sont des prototypes et cette étude est une initiation. Pour ce faire, le fonctionnement de quelques usines de biogaz a été observé, analysé, discuté avec différents acteurs et membres du projet, et l'outil a pu être testé. Ainsi, certains risques majeurs identifiés concernent le transport des matières faecales et la gestion de l'hygiène au sein des usines de méthanisation.



Keywords : *Salmonella* Dublin, Recrudescence, Evaluation des risques, semi-quantitative, Usine de méthanisation.