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Pathogens and Piggery Effluent - An Updated Review

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Executive Summary

Background

Intensive animal farming can impact both on aspects of human health, either at a farming level or as a consequence of managing wastes generated. The concentration of animal farming adjacent to urban areas, soil and water environments can be impacted by commonly adopted farming or waste management practices. This means that the pig industry needs a solid scientific basis to demonstrate safe and sustainable use, both at a farming and waste (effluent) management level. Food-safety i.e. the environmental movement of food-safety pathogens both within and external to the environment is increasingly becoming an emerging area of concern. The significance of this issue was recognised nearly 20 years ago, and in response, the pig industry funded two projects, that were undertaken at the Department of Agriculture and Fisheries (DAF) Animal Research Institute, Yeerongpilly , Queensland from 1998 - 2004. The two studies (a) "Pathogens and piggery effluent" (DAQ 60/1353) and (b) "Establishing guidelines for the safe application of piggery waste to pastures" (Project no. 1797) provided literature knowledge, research studies and explored approaches to address microbial risks. This work has been summarised and addressed in the context of both previous and recent literature, to provide a comprehensive summary on the environmental movement and management of food and water-borne pathogens as a consequence of piggery effluent re-use.

Pathogens of concern in piggery effluent

The preliminary study identified the pathogens of significance, through a review of literature following whereby pathogens were chosen as high priority were subsequently quantified in piggery effluent (via a survey). Key pathogens chosen as high priority were both *Salmonella* and *Campylobacter* (along with the indicator organism *Escherichia coli*.) The food-safety focus identified 20 years ago remains relevant where there is an increase in the use of animal wastes in food agriculture, which can lead to environmental transmission. In updating previous studies, whilst key pathogens were a focus of several studies in piggery housing, quantitative data on their levels were limited (and not as detailed as the Australian study undertaken). The Australian data remains comprehensive (and comparable) and thus can be used for purposes of developing guidelines or addressing risk. During the previous study, the same pathogen data was used to address risks via Quantitative Microbial Risk Assessment (QMRA). Updated literature on pathogens and piggery effluent/waste has been included to demonstrate the type of studies undertaken in this area to help manage various risks.

Pathogens, aerosols and human health

This is an important and sensitive area directly related to human health both on-farm and for communities adjacent to piggeries. Australian studies included testing within piggery housing to address concerns around effluent flushing and health of workers. Only *E. coli* was captured at low levels across studies undertaken on four farms. These *E. coli* levels used via (QMRA) demonstrated that the risks from pathogen inhalation during piggery effluent spray irrigation to residents at 500 m away from a spray irrigator (on piggery) was within the allowable United States Environmental Protection Agency risk (i.e. 1 infection per10,000 people per year). It should be noted that food-borne pathogens are pathogens of the gastro intestinal tract and once inhaled need to be swallowed (at the infective dose) to initiate infection in humans (and this is what is imperative). There were limited international studies that quantified food-borne pathogens within piggery housing. Several pathogen studies were a focus of in-shed hygiene and addressing risks downwind, to neighbours. The work carried out in Australian poultry sheds was compared to the original piggery pathogen testing undertaken. Based on the studies across both industries, the risk from the food-borne pathogens via the aerosol pathway inside piggery housing is not a major risk (based on the type of open piggery

housing studied). Updated literature on studies quantifying food-borne pathogens inside piggery housing and other studies focusing risks downwind to piggeries targeting risks to neighbours is summarised.

Pathogen survival in food crop, pasture, turf

The original studies were carried out when effluent from piggeries was commonly used to irrigate pasture. The interest at the time was the withholding period for effluent irrigated pasture, as consequence of pathogen survival on leaf (grass) surfaces. Both Gaussian plume and the Model for Effluent Disposal Using Land Irrigation (MEDLI) model were able to predict conditions that supported a 2-log reduction after 24 hours of effluent application to a leaf surface. Currently, the use of effluent is of relevance due to its use to irrigate food-crops. The review summarised studies linked to food-borne pathogen survival in water, soil, leaf surface and the potential for pathogens to internalise in leaf and root crop and be a food-safety risk as a consequence of using animal waste for food agriculture.

Pathogens survival in effluent treated soils

Previous work was summarised detailing studies carried out across four piggeries (during winter and summer) addressing pathogen die-off in effluent irrigated soil. The pathogen die-off time was longer in winter than summer. One of the key outcomes across the studies is that the commonly used indicator organism, *E. coli*, (for pathogen presence) was resident in soils in piggery environments with potential to re-grow. Thus, the organism's prior presence in soil around piggeries and the potential to re-grow makes the organism an unsuitable indicator to address compliance in effluent irrigated soils. Based on previous studies, the use of *Arcobacter* is suggested as a better marker of recent piggery effluent exposure (than the *E. coli*). Updated literature on survival of pathogens in animal manure amended soils and the associated concerns has been included.

Mobilisation of bacteria in effluent irrigated soils following potential heavy rainfall

Previous studies addressed pathogen run-off via a simulated condition related to effluent overfill (and effluent irrigation to contain overfill which is followed by heavy rain). The study included the comparison of the use of vegetative filter strips (VFS) to manage run-off. Both *E. coli* and *Arcobacter* were collected in run-off under a simulated "heavy rain event", where the filter strips used failed to contain both *E. coli* and *Arcobacter*. However, the use of appropriate VFS can contain pathogen movement. The guidance provided in the Australian guidelines (The National Environmental Guidelines for Indoor Piggeries 2018) for nutrient management and the summary of recent literature on pathogens provide guidance on VFS to help manage pathogen run-off into sensitive areas. However, there is a need to consider the possible interference of established background populations in conforming to guidelines that may use *E. coli* as an indicator organism for run-off water. Irrespective of all this (and as addressed in guideline section), on-farm risk management protocols can help to proactively identify and manage risks as an on-going risk management tool.

Antimicrobial resistance from soils exposed to piggery effluent

The previous study undertaken via the testing of common soil organism against commonly used antibiotics for pigs, demonstrated that there were no population shifts in bacteria isolated from soil from organic and conventional farming environments. This is detailed in a manuscript entitled "Impact of antibiotics on fluorescent *Pseudomonas* group and *Bacillus cereus* group isolated from soils exposed to waste from conventional and organic pig farming" that is ready to submit for publication.

Updating guidelines and Quantitative Microbial Risk Assessment

Quantitative Microbial Risk Assessment (QMRA) was reviewed highlighting the complexity involved and the applicability for its role in addressing risks for piggery effluent re-use. QMRA remains a potential approach but offers little practical advantages. The guidelines included in the previous study were not relevant and updated guidelines summarised, focus on a microbial risk management approach. This revised summary includes both national and international guidelines. The risk management approach demonstrated in some of the guidelines can be adopted for piggery effluent. A flow diagram that illustrates this approach along with a "tentative table" was created as a basis for discussion and input. The risk management approach identifies hazards as critical control points that can be monitored as part of an on-going process for risk management.

Other organisms of interest

The study of *Arcobacter* was undertaken and literature updated, because it is an emerging pathogen. *Arcobacter* is widely distributed in waters, and due to its status of listing in the UNESCO "Global Water Pathogen Project" and its presence in high numbers in Australian piggery effluent. A watching brief should be maintained on the pathogen status of this organism. *Clostridium difficil*e remains a pathogen of uncertain significance as there is no conclusive evidence based on literature on the organisms' zoonotic potential. A watching brief should be maintained on this organism. *Leptospira* is a pathogen of significance as an occupational risk and is based on direct contact with an infected pig. This is evidence that the effluent pathway is not relevant for this organism. For *Burkholderia pseudomallei* there is no published evidence of any relevance of the effluent pathway. The widespread presence of this organism in sub-tropical and tropical environment means it would be difficult to connect the organism to the piggery effluent pathway. For *Pfiesteria piscicida* the industry needs to be aware of the significance of this organism based on fish kills that have occurred in California due to piggery waste spills.

Overall summary

Almost 20 years ago, the food and water borne pathogens and their potential environmental pathways within a piggery were identified to addresses the various challenges that were likely to occur via the re-use of piggery effluent as it occurs within Australian piggeries. These concerns are current today. Thus, both past Australian studies and the updated studies (presented as a literature review) provide a basis for addressing and managing some of these risks, in a factual and scientific manner to arrive at practical solutions.

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1. Background to Research

In a country such as Australia with limited water resources, waste water (animal or human) is a valuable resource for agriculture or other purposes, provided safe re-use is addressed. Piggery effluent is typically subjected to minimal treatment (stored in one or more anaerobic uncovered ponds), before the effluent is re-used. The re-use options include use in the immediate environment or inside piggery housing. Animal wastes (such as piggery effluent) can be a potential source of pathogens which may impact human health. The movement of such pathogens through the environment (following re-use) can occur via direct (or indirect) pathways. There is thus a need to address potential risks that may arise from time to time, to support both safe and sustainable re-use practices. Such an understanding can contribute to more informed decision making processes by both the piggery industry and the local and state regulators. These decisions can then be made, with a realistic understanding, of the complex microbial interactions that are a part of biological environments. An absence of scientific understanding can lead to unrealistic (or non-science driven regulatory policies). This could impact on the potential for the pig industry to re-use effluent within the constraints of current pig farming practices adopted under Australian conditions. Irrespective of all these factors, there is a need to address safety concerns related to human health as a result of pathogen movement to the human food chain.

The significance of this issue was recognised nearly 20 years ago, and in response, the pig industry funded two projects, which were undertaken at the Department of Agriculture and Fisheries (DAF) Animal Research Institute, Yeerongpilly , Queensland from 1998 - 2004.

Listed below are the projects (and their objectives):

1.1 Pathogens and piggery effluent (DAQ 60/1353) – 1998 – 2001

Details of this project are listed in the relevant APL project (Blackall 2001)
Objectives:

- To perform an extensive and critical literature review on the presence of pathogens in piggery effluent.
- To quantify the pathogen concentrations in piggery effluent from typical piggeries
- To validate a quantitative risk assessment computer model that will allow predictions of the risk of infections under a variety of atmospheric conditions from spray irrigation of piggery effluent.
- To extend the quantitative risk assessment computer model by performing pathogen die-off studies allowing a prediction of the risk of infections arising from pastures irrigated with piggery effluent
- To provide an assessment of the risk associated with re-cycling piggery effluent
- Suggest cost-effective disinfection methods to reduce the risk of infection

1.2 Establishing guidelines for the safe application of piggery waste to pastures. (Project no. 1797) – 2001 – 2004

Details of this project are listed in the relevant APL project (Blackall 2004)
Objectives:

- To determine the die-off of pathogens (such as *Campylobacter* and *Salmonella*) as well as indicator organisms (*Escherichia coli*) in pasture soils over time after effluent irrigation.
- To assess the levels of antimicrobial resistance in selected non-pathogens (*Pseudomonas* and *Bacillus* species) from soils that have received piggery effluent as well as soils that have not received piggery effluent.
- To assess the level of pathogen (such as *Campylobacter* and *Salmonella*) as well as indicator organism (*Escherichia coli*) mobilisation in run-off water from land that has been treated with piggery effluent.
- To use the results generated in the above objectives to develop guidelines for the sustainable re-use of piggery effluent.

The above two studies provided literature knowledge, research studies and explored approaches to address microbial risks. This early research was carried out in collaboration with the Department of Natural Resources (DNR) (Resource Science Centre), Queensland. This team had expertise in risk management and modelling approaches (specifically for water). Prior to the APL work, both teams (from DAF and DNR) had completed a study on the "re-use of human effluent for the purpose of sugar cane irrigation". Thus, the prior outputs/knowledge adopted for human effluent provided valuable insight in addressing issues relevant to piggery effluent. This was a time when addressing risks attributed to animal wastes in the environment was emerging in Australia.

The current research summary is in response to a call from Australian Pork Limited in 2017 due to similar concerns expressed by regulators, as was the situation nearly 20 years ago. There is thus a need to re-visit and summarise the in-depth work carried out at the time and address any new emerging concerns.

2. Objectives of the Research Project

1. To assemble a comprehensive overview of previous pig industry research in the area of health risks associated with piggery effluent re-use

2. To review the literature to identify new information on the potential pathogens present in piggery effluent and the availability of more recent quantitative data on pathogens in piggery effluent.

3. To update where necessary and where possible the recommendations on guidelines on piggery effluent made in the early 2000s

4. To identify any risks that have emerged since the last active Australian based research in this area

5. To identify gaps in knowledge, if present, where additional research on pathogens and their levels are required to improve the management of health risks associated with piggery effluent re-use.

3. Introductory Technical Information

This research summary is built upon the previously funded APL research undertaken from 1998 – 2004, that comprehensively addressed risks attributed to piggery effluent re-use as adopted by the pig industry.

Following is a summary of the research undertaken.

- A literature review which enabled the identification of zoonotic pathogens of concern (linked to pigs)
- The review prioritised those pathogens of most concern to humans
- Key food-borne pathogens, both *Salmonella*, *Campylobacter*, *E. coli* (as an indicator) along with rotavirus were identified as high priority
- The levels of these high risk organisms were enumerated in ponded piggery effluent from a representative set of 13 farms across South East Queensland
- The pathogen survey provided prevalence and levels of the key pathogens, *Campylobacter* and *Salmonella,* rotavirus was not detected.
- The work that followed focused on these two key pathogens along with the indicator organism *E. coli* to address risk
- The aerosol pathway was identified as a concern both within pig housing (effluent flushing) and the external environment to a piggery (spray irrigation of pasture or crop) – both common industry practices
- Both in-shed pathogen studies and risk assessment modelling approaches were undertaken to quantify potential risks (to humans) attributed to the transfer of pathogens via aerosols
- Transmission via direct irrigation of both pasture and crop were identified as potential pathways
- This was addressed by undertaking pathogen survival studies on effluent irrigated foliage (under laboratory conditions) and risks were quantified by adopting risk modelling approaches
- Survival in soil was identified as a pathways of concern due to the possibility of movement of pathogens by large irrigated pasture and the need to understand withholding periods for human (recreational) or animal (grazing) activities
- A literature review was undertaken; *Arcobacter* was identified as an emerging pathogen at the time. There was very little evidence at the time suggesting linkage to pigs
- Studies carried out across assessing piggery effluent (and irrigated soil) suggested that *Arcobacter* was found to be well distributed in Australian piggery effluent (the levels were also quantified; this included the first report of a new species, *A. cibarius* linkage to pigs
- The soil survival studies addressed, pathogen die-off in piggery effluent irrigated pasture soils
- The die-off of both *Campylobacter* and *Arcobacter* (due to their appreciable levels in piggery effluent) was studied across four piggeries. *Salmonella* was not included due to the organism's infrequent presence and low levels in piggery effluent (survey outcome)
- Using the pathogen survival data from soil, time taken for log reduction (T_{90}) a common approach to addressing die-off was calculated and die-off periods across seasons established
- The risk of overland pathogen mobilisation that can occur during "a random heavy rain event" was simulated (using a rainwater machine on effluent irrigated land) to understand run-off
- Common antibiotics used by the industry were tested from organic and conventional piggery environments that had a long history of re-use for their ability to demonstrate population shifts (in two common soil organisms)

- Australian and international human effluent guidelines were summarised to assist and address / managing pig effluent re-use
- Quantitative Microbial Risk Assessment (QMRA) was suggested as a best approach to quantify risks based on varied application/uses as adopted by the Australian pig industry
- This approach requires numerical data of pathogens levels (*Salmonella*, *Campylobacter* and *E. coli*, as an indicator) and was generated across all the previously listed studies
- Both studies summarised outcomes of research undertaken in a manner to provide background, data (and knowledge) that addressed potential risks relevant to common practices adopted under Australian pig farming conditions.
- Some of the early work was presented at conferences and published via peer review. Results from this work remains to be published and is currently being completed for peer review. The abstracts of all published work are included in this review.

4. Research Methodology

This study is a desk top study summarising and updating research carried out 20 years ago on (a) "Pathogens and piggery effluent" (DAQ 60/1353) and (b) "Establishing guidelines for the safe application of piggery waste to pastures" (Project no. 1797).

The following approach was adopted:

4.1 Summary of previous studies

The summary of previous research is presented in the following manner:

- The listed studies (a) and (b) formed the basis for the up-dated research summary in the broad category "pathogens and piggery effluent"
- Majority of that previous work has been summarised in a manner suitable for this document
- Selected tables were revised and some data has now been presented as graphics, to provide comparative summarised data to support the research summary
- Where relevant, methodologies have been summarised, however all detailed experimental designs, farm and microbiological methodologies (adopted for the various trials undertaken), and statistical analysis are presented in the original project documents and relevant peer review publications (to date)
- Should further detail be required, the original reports -(DAQ 60/1353); (Project no. 1797) (and publications) can be referenced

4.2 Literature review to update previous work

The literature review was undertaken based on the following criteria

- All key study areas across the two projects were identified (e.g. effluent, aerosols, guidelines) as major areas to provide the framework for the updated review
- Updated introductory summaries are presented for each of the identified study areas to provide context
- This is followed by providing reviewed summaries for most sections in a table format (where relevant) or via a simple research summary
- Some areas, such as the guideline section were updated with new information as the previous information was no longer relevant
- Suggestions for adopting some of the approaches adopted across the various guidelines have been summarised as a flow diagram and a tentative table to provide some background for piggery effluent
- Selected emerging pathogens are addressed and others updated

4.3 Overall objective of the literature review

- Provide updated knowledge concerning the safe re-use of piggery effluent that addresses both human health via the movement of pathogens in the environment
- Assist the industry to address management options that are verified by research studies (to demonstrate safe and sustainable re-use of piggery effluent)

5. Results

5.1 Intensive farming and the movement of pathogens in the environment

Both intensive animal farming and food agriculture are rapidly growing sectors, and so are the environmental challenges. Such challenges can be complementary with intensive farming of animals and animal manures used in food agriculture. Other factors such as methods of re-use in irrigation practices and the storage of solid manure in compost can contribute to the movement (and adaptation) of zoonotic pathogens from intensive animal farming in the environment. The size (and density) of animal populations are an important factor influencing the spread of microbial loads in the environment with potentially increased risks of exposure to humans (and animals), (European Food Safety Authority 2011). Manures, composts, irrigation water, and runoff water can be pathways for the introduction of pathogenic bacteria to people, leading to either gastro-intestinal or respiratory illnesses. The key food/water-borne pathogens, (*Salmonella* and *Campylobacter*) are responsible for the highest numbers of gastro-intestinal diseases in Australia (number of cases of campylobacteriosis and salmonellosis for 2014 being 19,931 and 16,358 respectively) (National Notifiable Disease Survalilance System, annual report working group 2016).

Australian piggeries adopt an integrated approach to effluent re-use. Pigs can be a source of pathogens and transmission from a piggery can occur via indoor livestock units (leaching of contaminated water) and/or waste treated field (run-off), affecting water catchments (Hooda et al. 2000). Figure 1 illustrates potential direct (or indirect) environmental pathways for pathogen movement (within or surrounding a pig farm), which can impact human health. The fate of key food-borne pathogens associated with intensive pig and poultry farming environments is detailed in Chinivasagam (2014).

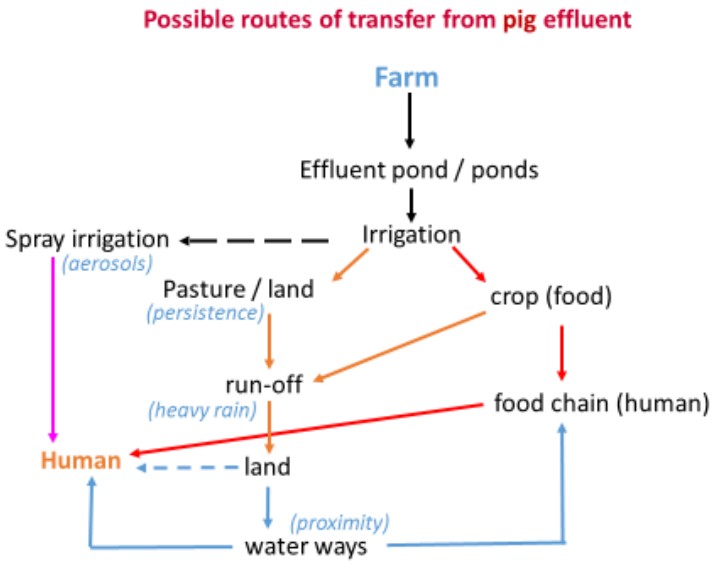

Figure 1 Pathogen movement in a piggery environment – possible pathways

5.1.1 Pathogens in piggery effluent – identifying the organisms of concern

The preliminary project (DAQ60/1353) conducted almost 20 years ago was initiated (by APL) following concerns related to *Salmonella* in piggery ponds in Australia (Henry et al. 1995; Henry et al. 1983). A limited survey of three piggery ponds resulted in the isolation of *Salmonella* and a range of

Salmonella serovars (from piggery ponds) (Henry et al. 1995). It was unclear, if this was broadly applicable to Australian piggeries (Blackall, 2001). At the time, ponded piggery effluent was re-used within Australian piggeries, for flushing purposes. Regulatory changes were being adopted in countries such as Denmark, prohibiting the re-use of effluent contaminated with *Salmonella* species within piggeries (Blackall 2001).

There was a perceived need to understand the comprehensive pathogen situation in piggery effluent to address managing risks. Thus, via an extensive literature review, the pathogens of importance were identified (Blackall 2001). In order to narrow down the list, the pathogens were classified as being a "high", "medium" or "low" risk priority (based on their potential presence in pigs/piggery effluent and the risk posed to humans), Table 1.

Table 1 Classification of pathogens based on risk, source (Blackall 2001)

High Priority	**Moderate Priority**	**Low Priority**
Salmonella spp. ✓	Porcine parvovirus*	*Listeria monocytogenes*✓
Erysipelothrix rhusiopathiae ✓	Rotavirus ✓?	*Leptospira* spp. ✓
Campylobacter spp. ✓	Swine Hepatitis E Virus ✓?	*Yersinia pseudotuberculosis* ✓
*Serpulina hyodysenteriae**	*Lawsonia intracellularis**	*Eimeria* spp.*
Escherichia coli ✓	*Serpulina pilosicoli* ✓??	*Giardia duodenalis* ✓??
	Cryptosporidium ✓??	
	*Isospora suis**	
	Balantidium coli ✓	

✓denotes those pathogens recognised as potential zoonoses.
✓**?**denotes that evidence for the zoonotic role of these agents is still unclear or the subject of current scientific debate
✓**??** Parasite agents where the evidence is unclear or uncertain about pathogenicity for humans
*denotes organisms that pathogens strictly of pigs and/or other livestock**.**

Amongst the pathogens identified as being of "high priority" were the zoonotic organisms, *Campylobacter jejuni/coli, Erysipelothrix rhusiopathiae, Salmonella, Escherichia coli (*as an indicator organism) and thermotolerant coliforms (indicators of both human and animal waste). One viral pathogen (rotavirus), was placed in the medium priority ranking. Both *Salmonella* (Argüello et al. 2018) and *Campylobacter* (Weijtens et al. 1999) are also organisms reported associated with pig production. Whilst not a direct comparison, an European Union (EU) summary report (European Food Safety Authority 2011) based on national monitoring programmes for key pathogens in pig meat (and products) from member countries reported, low prevalence of *Salmonella* (only 0.7% tested positive in 2009 and 0.8% in 2008) and *Campylobacter* (0.6% tested positive in 2009 and 0.5% in 2008). These results provided insight of these pathogens in post slaughter product at an international level, though a need to understand their status in effluent from an EU context is further required.

5.1.2 Survey of pathogen levels in ponds

In order to understand the presence and levels of pathogens identified (as high priority), a survey of representative piggery effluent (ponds) was conducted. A total of 13 piggeries were selected across South East Queensland. Table 2 presents the description of the piggeries and the location (pond type

or sump) tested. A total of 29 samples across sumps, primary, secondary and tertiary ponds were tested. A quantitative approach (not just presence / absence) was adopted to assess the levels of key pathogens. A summary of this segment of the work is detailed in (Chinivasagam et al. 2004). The levels of *E. coli, Campylobacter* and *Salmonella* are presented in Figures 2, 3 and 4. Whilst *E. coli* the indicator organism was dominant across effluent ponds (and sumps) tested, *Salmonella* emerged intermittently (at low levels), compared to *Campylobacter*. *E. coli*, the indicator organism is present at high levels across all piggeries, but is not a good indicator of the pathogen, *Salmonella*. Both rotavirus and *Erysipelothrix rhusiopathiae* were not detected in effluent, narrowing down to the food-borne pathogens such as *Salmonella* and *Campylobacter* which need to be a focus in piggery effluent.

Table 2 Description of production system and the types of samples collected

Piggery	Production System	Number of Sows	Number of standard pig unit (SPU)	Description of type of treatment unit tested
A	Farrow to Finish	210		No. 1 – Anaerobic pond inlet No. 2 – Anaerobic pond No. 3 - Effluent lagoon
B	Farrow to Finish	90		No. 4 – Surface water
C	Farrow to Finish	1200	13,365	No. 5 – Secondary pond No. 6 – Primary pond
D	Farrow to Finish	1000	9596	No. 7 – Final pond No. 8 – Primary pond No. 9 – Sump
E	Farrow to Finish	430		No. 10 – Pond effluent No. 11 – Sump
F	Breeder Facility	2700	4900	No. 12 – Final pond No. 13 – Primary pond
G	Grow Out Facility Only	NA	9212	No. 14 – Tertiary pond No. 15 – Inlet of primary pond
H	Farrow to Finish	-	13,487	No. 16 – Tertiary pond No. 17 – Primary pond
J	Farrow to Finish	-	15,477	No. 18 – Primary pond No. 19 – Recycle pond
K	Farrow to Finish	1200	14,992	No. 20 –Tertiary pond No. 21 – Primary pond
L	Farrow to Finish	1000	12,497	No. 25 - Final pond No. 26 – Primary pond No. 27 – Primary pond inflow
M	Weaner Facility	-	2900	No. 25 - Final pond No. 26 – Primary pond No. 27 – Primary pond inflow
N	Grow Out Facility Only	-	2699	No. 28 – Sump No. 29 - Final pond

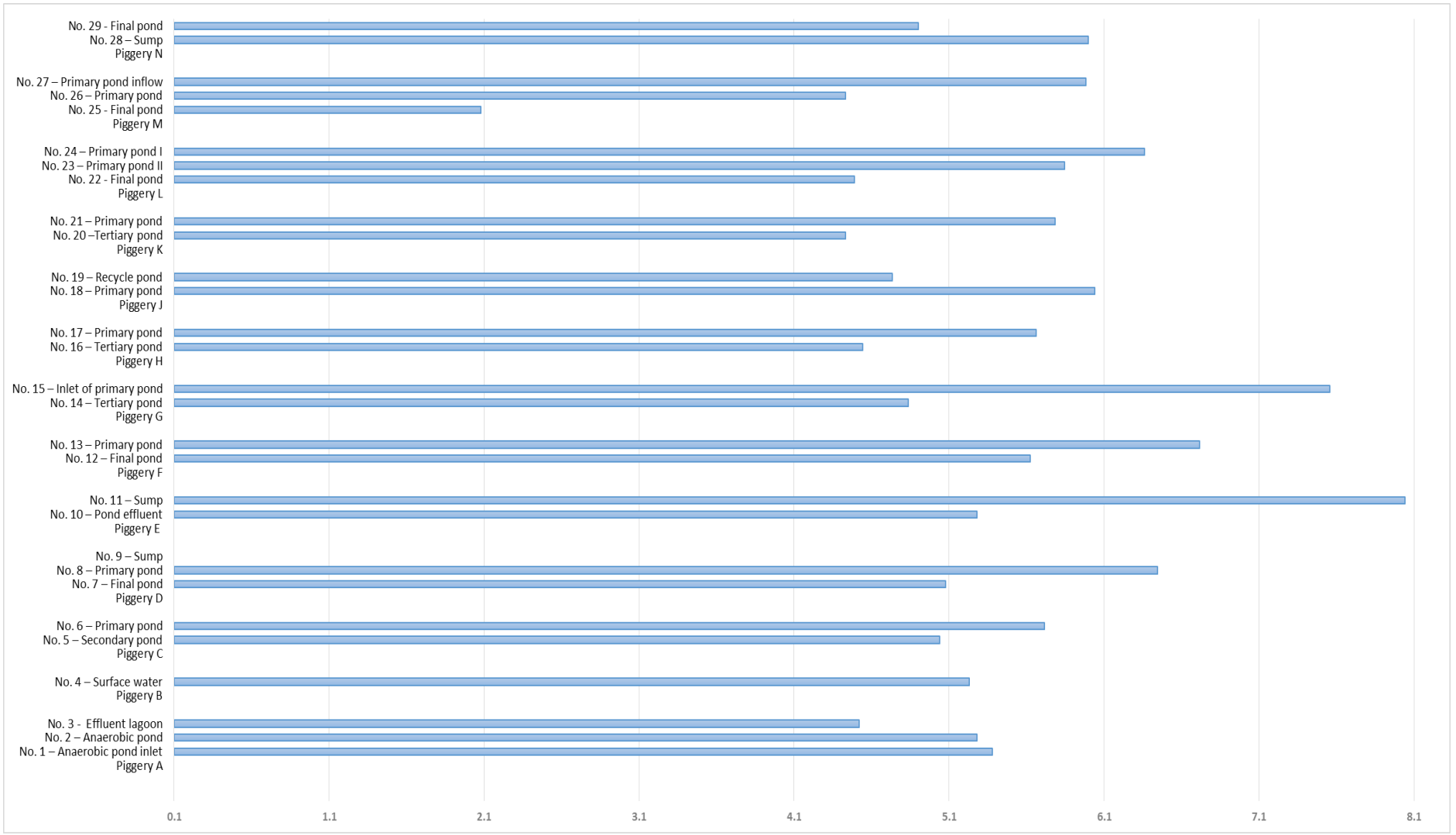

Figure 2 E. coli levels (CFU/ 100 ml) across 13 piggery effluent samples across piggeries in South East Queensland

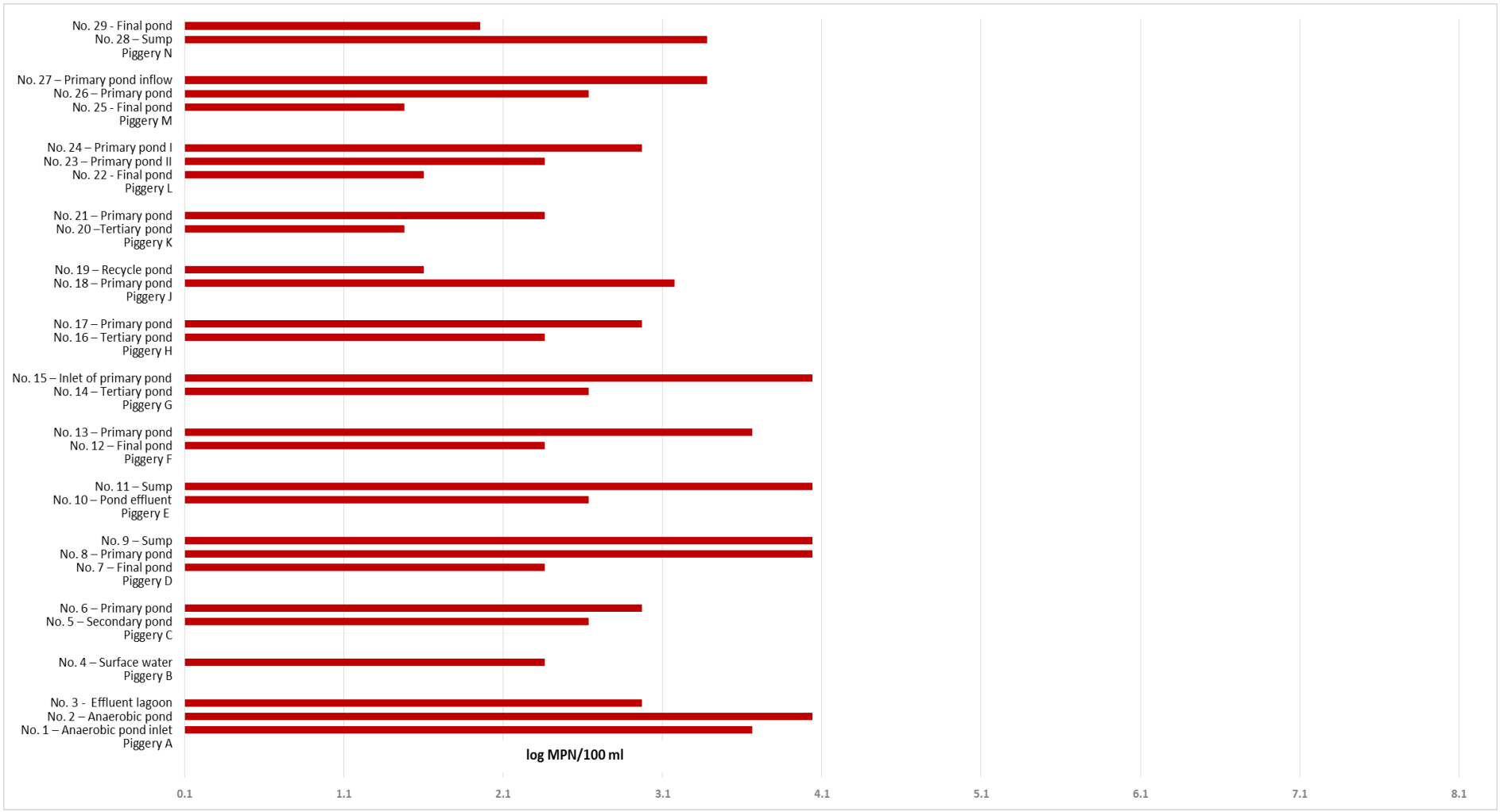

Figure 3 Campylobacter levels (MPN per 100 ml) across 13 piggery effluent samples across piggeries in South East Queensland

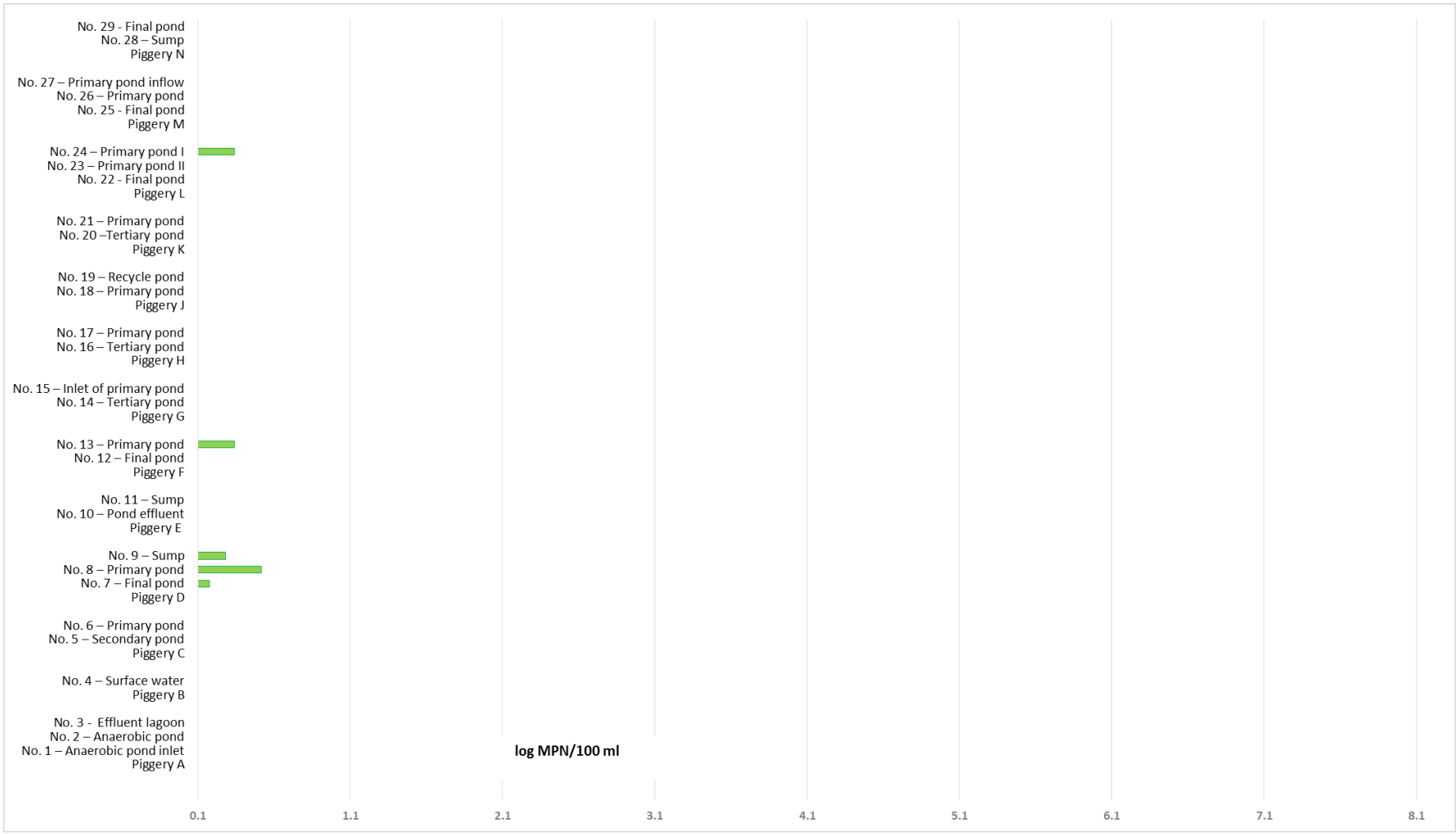

Figure 4 Salmonella levels across 13 piggery effluent samples across piggeries in South East Queensland

As means of updating this work, a literature search (1982 – 2017) was undertaken to compare the original study to any recently published work, where comparable enumeration of pathogen levels was undertaken. Table 3 lists the source, country and year relevant to the various studies compared, along with the available quantitative data, The Australian data is highlighted (for comparison). Based on Table 3, enumerating *Campylobacter* in piggery effluent was not common, (studies, 1982 – 2017) only one other study enumerated *Campylobacter*. Four studies enumerated *Salmonella* and reported low levels, comparable to the Australian study (Chinivasagam et al. 2004).

Due to the limited studies that quantified these organisms in piggery effluent, literature was also reviewed to assess other work undertaken, that addressed both *Salmonella* and *Campylobacter* in pig farm environments. Table 4 lists different types of studies that ranged from inactivation studies, movement of pathogens to waterways. This includes some early Australian work (highlighted). Thus, these two pathogens were of importance and were addressed in a range of studies that considered the importance of managing the movement of these pathogens.

Table 3 List of studies quantifying Salmonella and Campylobacter in piggery wastes (including effluent)

Source	**Country**	**Year**	***Salmonella* levels**	***Campylobacter* levels**	***E. coli* levels**	**Reference**
Effluent from swine manure	Brazil	2017	<3 - 7.4 X 10^{1} MPN/100 mL	not tested	7 X 10^{3} - 16.8 X 10^{4} MPN/100 mL	Bilotta et al. (2017)
Final effluent – collected following secondary settling	Brazil	2015	artificially introduced	not tested	10^{3} CFU mL^{-1}	Viancelli et al. (2015)
Separated liquid fraction of raw manure	Ireland	2015	detected in manure, not in effluent	not tested	log 2.1 CFU/mL	McCarthy et al. (2015)
Pig slurry Biogas effluent	Vietnam	2014	not enumerated not enumerated	not tested not tested	~ log 6.0 – 7.0 ~ log 4.0 – 5.0	Huong et al. (2014)
Final effluent (from a swine manure treatment system)	Brazil	2013	0.28 ± 0.13 log MPN mL^{-1}	not tested	2.31 ± 0.31 log CFU mL^{-1}	Viancelli et al. (2013)
Effluent from anaerobic swine manure lagoon	USA	2012	log 0.87 – 2.83 MPN100 mL^{-1}	log 3.59 – 4.92 MPN100 mL^{-1}	log 5.46 – 6.36 CFU 100 mL^{-1}	McLaughlin et al. (2012)
Final effluent (following integrated wetland treatment)	Ireland	2011	not enumerated	not tested	1.0 log CFU/ 100 mL	McCarthy et al. (2011)
Lagoon liquid used to flush manure from barns	USA	2005	log 2.61 CFU/mL	not tested	not tested	Vanotti et al. (2005)
Piggery A No. 2 – Effluent lagoon	Australia	2004	*not detected*	log 2.97 MPN/100mL	log 4.52 CFU/100mL	Chinivasagam et al. (2004)
Piggery B No. 4 – Surface water / pond	Australia	2004	*not detected*	log 2.36 MPN/100mL	log 5.23 CFU/100mL	Chinivasagam et al. (2004)
Piggery C No. 5 – Secondary pond	Australia	2004	log 0.05 MPN/100mL	log 2.63 MPN/100mL	log 5.04 CFU/100mL	Chinivasagam et al. (2004)
Piggery D No. 7 – Final pond	Australia	2004	log 0.18 MPN/100mL	log 2.36 MPN/100mL	log 5.08 CFU/100mL	Chinivasagam et al. (2004)
Piggery E No. 10 – Pond effluent	Australia	2004	*not detected*	log 2.63 MPN/100mL	log 5.28 CFU/100mL	Chinivasagam et al. (2004)
Continued……..						

Piggery F No. 12 – Final pond	Australia	2004	0.09 MPN/100mL	log 2.36 MPN/100mL	log 5.62 CFU/100mL	Chinivasagam et al. (2004)
Piggery G No. 14 – Tertiary pond	Australia	2004	*not detected*	log 2.63 MPN/100mL	log 4.84 CFU/100mL	Chinivasagam et al. (2004)
Piggery H No. 16 – Tertiary pond	Australia	2004	*not detected*	log 2.36 MPN/100mL	log 4.54 CFU/100mL	Chinivasagam et al. (2004)
Piggery J No. 19 – Recycle pond	Australia	2004	*not detected*	log 1.60 MPN/100mL	log 4.73 CFU/100mL	Chinivasagam et al. (2004)
Piggery K No. 20 –Tertiary pond	Australia	2004	*not detected*	log 1.48 MPN/100mL	log 4.43 CFU/100mL	Chinivasagam et al. (2004b)
Piggery L No. 22 - Final pond	Australia	2004	*not detected*	log 1.60 MPN/100mL	log 4.49 CFU/100mL	Chinivasagam et al. (2004)
Piggery M No. 25 - Final pond	Australia	2004	*not detected*	log 1.48 MPN/100mL	log 2.08 CFU/100mL	(Chinivasagam et al. 2004)
Piggery N No. 29 - Final pond	Australia	2004	*not detected*	log 1.95 MPN/100mL	log 4.90 CFU/100mL	Chinivasagam et al. (2004)
Effluent (various ponds)	Australia	1995	not enumerated	not tested	not tested	Henry et al. (1995)
Aerobically treated pig slurry	Australia	1882	not tested	not tested	not tested	Ginnivan and Chandler (1982)

The reported APL study *(Chinivasagam et al. 2004)* is highlighted in grey.

Table 4 List and type of studies where Salmonella and Campylobacter were studied in piggery environments

Reference	Year	Nature of studies where indicators or pathogens were studied
Fongaro et al. (2018)	2018	Inactivation studies/swine effluent sludge (Studied introduced *Salmonella*, human adenovirus and phage)
Argüello et al. (2018)	2018	Surveillance /Disease control (Studied *E. coli* - Swine diarrhoea, *Salmonella*)
(Fongaro et al. 2016)	2016	Settling/survival of pathogens in swine effluent lagoon (Studied introduced *Salmonella*, human adenovirus and phage)
Giacoman-Vallejos et al. (2015)	2015	Pathogen removal /experimental wetlands (Studied total coliforms, faecal coliforms, enterococci and *Salmonella*)
Brooks et al. (2014)	2014	Screening bacterial pathogens antibiotic resistance genes in three swine manure management systems. (analysed genes *Salmonella* spp., *Campylobacter* spp., antibiotic resistance)
Bilotta and Kunz (2013)	2013	Alkaline control and UV radiation/ swine effluent post-treatment. (Studied, Total coliforms and *E. coli* and introduced *Salmonella*)
McLaughlin et al. (2012)	2012	Dynamics of faecal indicators/zoonotic pathogens in anaerobic swine manure lagoon water. (Studied *E. coli*, enterococci, *Clostridium perfringens (*tested to understand pathogen stratification), *Campylobacter*, *Listeria*, *Salmonella*, and staphylococci)
Choi et al. (2011)	2011	Impact of pig slurry on water quality of stream. (Studied *E coli, Salmonella)*
Fablet et al. (2006)	2006	Validation of a microbiology method (MPN) for *Salmonella* for pig farm effluents (Studied *Salmonella*)
Hill and Sobsey (2001)	2001	Constructed wetland for removal of *Salmonella* (Studied *E. coli*, enterococci, *Clostridium perfringens* spores (as an indicator for the removal of protozoan and helminth parasites), somatic coliphages, F-specific coliphages, *Salmonella*.
Henry et al. (1983)	1983	Factors affecting *Salmonella* survival in anaerobically fermented pig waste – laboratory study
Chandler et al. (1981)	1981	Persistence of faecal coliforms and faecal streptococci on land used for disposal of piggery effluent
Chandler and Craven (1981)	1891	Persistence *Salmonella* (and faecal coliforms) in naturally contaminated pig effluent disposal site
Chandler and Craven (1980a)	1980	Persistence of *Erysipelothrix rhusiopathiae* (faecal indicators) in pig effluent disposal site
Chandler and Craven (1980b)	1980	Soil moisture and the survival of *E. coli* and *Salmonella* in soils – Experimental studies
Chandler and Craven (1978)	1978	Factors affecting survival of introduced *E. coli* and *Salmonella* in land used for effluent disposal

Australian studies are highlighted in grey.

5.1.3 Summary of pathogens and piggery effluent in Australian piggeries

The outcome on pathogens and piggery effluent has been also presented as a peer reviewed publication as presented below.

Abstract: Microbiological status of piggery effluent from 13 piggeries in the South East Queensland region of Australia (Chinivasagam et al. 2004)

Aims: To assist in the development of safe piggery effluent re-use guidelines by determining the level of selected pathogens and indicator organisms in the effluent ponds of 13 South-East Queensland piggeries.

Methods and Results: The numbers of thermotolerant coliforms, *Campylobacter jejuni/coli*, *Erysipelothrix rhusiopathiae*, *Escherichia coli*, *Salmonella* and rotavirus were determined in 29 samples derived from the 13 piggeries. The study demonstrated that the 13 final effluent ponds contained an average of 1.2 X 10^5 colony-forming units (CFU) 100 ml^{-1} of thermotolerant coliforms and 1.0^3 X 10^5 CFU 100 ml^{-1} of *E. coli*. The *Campylobacter* level varied from none detectable (two of 13 piggeries) to a maximum of 930 most probable number (MPN) 100 ml^{-1} (two of 13 piggeries). *Salmonella* was detected in the final ponds of only four of the 13 piggeries and then only at a low level (highest level being 51 MPN 100ml^{-1}. No rotavirus and no *Erysip. rhusiopathiae* were detected. The average log_{10} reductions across the ponding systems to the final irrigation pond were 1.77 for thermotolerant coliforms, 1.71 for *E. coli* and 1.04 for *Campylobacter*.

Conclusions: This study has provided a baseline knowledge on the levels of indicator organisms and selected pathogens in piggery effluent.

Significance and impact of the study: The knowledge gained in this study assisted in the development of guidelines to ensure the safe and sustainable re-use of piggery effluent.

5.1.4 Overall summary – pathogens and piggery effluent

The key pathogens *Salmonella* and *Campylobacter* have been quantified in piggery effluent, this can form the basis for addressing guidelines or adopting risk management approaches for piggery effluent re-use. Few other international studies have quantified all pathogens, though they have been used as target organism across various studies including inactivation studies and pathogen movement in the environment. *Clostridium perfringens* has been used either as surrogate for parasites or means of understanding pathogen stratification lagoon water. Coliphages have been used as a viral surrogate in piggery environments. During the previous APL survey the virus of concern was identified as rotavirus and was directly detected without the need for a surrogate, such as coliphage. This approach is adopted for human effluent where there are a range of potential viruses, which in some instances are difficult to detect (and thus a need for a surrogate such as a coliphage). This approach has been demonstrated (i.e. using MS2 coliphage, as a surrogate for assessing the contamination of fruit and soil irrigated with treated human effluent (Chinivasagam et al. 2008).

5.2 Aerosols and risk to human health

Intensive animal farming can be source not only of aerosolised bacterial pathogens but also endotoxins and dust, which are of concern to human health (Pillai and Ricke 2002). However, this summary only focuses on bacteria. Aerosols can be generated during farming activities. Some examples include animal housing and manure management during intensive farming (Millner 2009), land application of biosolids (downwind) (Tanner et al. 2008; Brooks et al. 2004), mechanically ventilated broiler sheds (Chinivasagam et al. 2009b), mechanically ventilated swine barns (Predicala et al. 2002), and spray irrigation of wastewater (Donnison et al. 2004). Animal farming generated aerosols are a mixture of organic material, biological active components and microorganisms (Seedorf et al. 1998). The size of

bioaerosol particles can vary. Respirable particles < 7 µm, are of greatest concern (Agranovski et al. 2004), due to their ability to be inhaled when present in aerosols. In addition another area of concern are bacteria (and fungi), with the composition and concentrations of these populations being influenced by source, dispersal mechanisms and environmental conditions (Pillai and Ricke 2002). Modelling studies addressing risk in general, take into account the route of transfer, inhalation, deposition or swallowing of bacterial (or viral pathogens) when using dose response models to calculate risks (to workers / others) (Dowd et al. 2000).

5.2.1 Detection and enumeration of bacteria in the air of piggery sheds

Ponded piggery effluent is used within piggeries to flush sheds (following treatment in effluent ponds). The study was undertaken to address industry concerns regarding risk (via inhalation) to piggery workers during flushing of sheds with piggery effluent. The organisms of concern for the shed aerosol studies were based on the outcomes of the piggery effluent survey. *Salmonella* was only intermittently present, and when present was at low levels (in effluent) which meant it was highly unlikely to detect the organism in aerosols. The organisms of choice were thus *Campylobacter*, *E. coli* and total bacteria (which can occur at high levels). *Campylobacter* was not isolated during the first piggery sampling), so it was decided to focus on *E. coli* and heterotrophic (total) bacteria. *Campylobacter* is a fragile organism (Klancnik et al. 2009) thus it was unlikely the organism can survive aerosolisation stress and subsequently be present in high numbers in air.

The capture of aerosols for the purpose of sampling requires that the organism undergo minimum stress (as they are already stressed in the air/aerosol environment). Thus, during work undertaken during the previous study, extensive microbiology validation methodology was initially undertaken. This included comparison of aerosol samplers using the impaction (agar plates) (six stage Anderson Sampler) or impingement (liquid) (AGI- 30 impinger) approach to capture aerosolised micro-organisms with minimum stress. The use of suitable additives to maintain survival (of captured organisms) was also explored. Finally, the shed aerosol trials were undertaken. The outcomes are presented in (Chinivasagam and Blackall 2005).

Table 5 lists studies (from 1976 – 2014) that had a focus of enumerating pathogens in aerosols within piggery sheds. Other than the Australian study, one other study (Yuan et al. 2010) enumerated the organisms within pig shed environments. Table 5 presents the data from four Australian and Chinese piggeries. The levels of *E. coli* in air within sheds is comparable across both studies. The limited number of these studies is because most other studies focused on aspects other than in-shed risks. The common focus is either normal shed hygiene or most importantly risks at distances from the farm. These studies were also reviewed for comparison with the in-shed studies (where pathogens levels were specifically enumerated).

Table 5 Available data for bacterial levels from aerosols generated from piggery sheds

Source	Country	Year	Sampler	*E. coli* levels	Other bacteria/fungi	Reference
INSHED						
Natural ventilation (semi enclosed) Farm A	China	2010	Anderson	35, (range 13 -76) $CFUm^{-3}$ (lower levels detected at, 50, 100, 200 m, not detected at 400 m downwind)		Yuan et al. (2010)
Mechanical ventilation (completely closed) Farm B	China	2010	Anderson	23, (range 19 - 58) $CFUm^{-3}$ (lower levels detected at, 50, 100, 200 m, not detected at 400 m downwind)		Yuan et al. (2010)
Mechanical ventilation (completely closed) Farm C	China	2010	Anderson	27 (range 10 - 67) $CFUm^{-3}$ (lower levels detected at, 50, 100, 200 m, not detected at 400 m downwind)		Yuan et al. (2010)
Mechanical ventilation (completely closed) Farm D	China	2010	Anderson	21 ((range 9 - 47) $CFUm^{-3}$ (lower levels detected at, 50, 100, 200 m, not detected at 400 m downwind)		Yuan et al. (2010)
Growers, naturally ventilated sheds (Piggery G) normal - pig activity	Australia	2005	Anderson AGI	3 $CFUm^{-3}$ (8:30 am); (same day) 10 $CFUm^{-3}$ (8:30 am)	4.3×10^5 $CFUm^{-3}$	Chinivasagam and Blackall (2005)
Growers, naturally ventilated sheds (Piggery G) - Flushing	Australia	2005	Anderson AGI	42 $CFUm^{-3}$ (9:00 am); (same day) 21 $CFUm^{-3}$ (9:00 am)	6.0×10^5 $CFUm^{-3}$	Chinivasagam and Blackall (2005)
Growers, naturally ventilated sheds (Piggery G) normal - pig activity	Australia	2005	Anderson	*Not detected* (8:45 am – 13:20 pm – hourly sampling); (same day)	3.5×10^4 $CFUm^{-3}$ 4.5×10^4 $CFUm^{-3}$ 1.0×10^5 $CFUm^{-3}$ 6.0×10^4 $CFUm^{-3}$ 6.0×10^4 $CFUm^{-3}$ 8.7×10^4 $CFUm^{-3}$	Chinivasagam and Blackall (2005)
Continued…						

Growers, naturally ventilated sheds (Piggery G) - Flushing	Australia	2005	Anderson	*Not detected* (8:45 am – 13:20 pm hourly sampling); (same day)		Chinivasagam and Blackall (2005)
Growers, naturally ventilated sheds (Piggery B) normal - pig activity	Australia	2005	Anderson	59 - 9 $CFUm^{-3}$ (10:35 am); (11:55 am) (same day)	7.8×10^5 $CFUm^{-3}$ 1.7×10^5 $CFUm^{-3}$	Chinivasagam and Blackall (2005)
Growers, naturally ventilated sheds (Piggery B) - flushing	Australia	2005	Anderson	24 $CFUm^{-3}$ (11:35 am) (same day)	2.6×10^5 $CFUm^{-3}$	Chinivasagam and Blackall (2005)
Growers, naturally ventilated sheds (Piggery W) normal - pig activity	Australia	2005	Anderson	32; 35 $CFUm^{-3}$ (11:00 am); (13:30 pm) (same day)	2.0×10^5 $CFUm^{-3}$ 2.7×10^5 $CFUm^{-3}$	Chinivasagam and Blackall (2005)
Growers, naturally ventilated sheds (Piggery W) - Flushing	Australia	2005	Anderson	47 $CFUm^{-3}$ (11:50 am) (same day)	3.2×10^5 $CFUm^{-3}$	Chinivasagam and Blackall (2005)

Australian studies are shaded in grey

5.2.2 Other studies on aerosols and piggeries

Table 6 lists additional studies from piggeries with a focus on managing shed hygiene or addressing risks to neighbours (at downwind distances to the shed). In summary, these studies were targeted at addressing human health (workers and neighbours) via in-shed management for a range of measures i.e. pollutants, antibiotic resistance, respirable particles and endotoxins (which are known to be generated by gram negative bacteria) and respiratory disease (to pigs). These studies lacked quantitative data relevant to the Australian study (which are also listed for comparison). Some studies were undertaken across various pig farming stages (e.g. farrowing, weaning etc.).

Table 6 Nature of studies that have looked at aerosols in piggery environments

Reference	Year	Focus of study and organisms of concern
Li et al. (2014)	2014	The focus was to assess hygiene in swine houses. Total aerobic and anaerobic bacteria, fungi and total gram negative bacteria enumerated to address environmental management.
Hong et al. (2012)	2012	The focus was worker and animal health attributed to airborne bacteria and associated antibiotic resistance genes in pig farrowing, gestation, weaning and finishing units. Microbial community profiles in building was assessed to address risks attributed to production phase.
Yuan et al. (2010)	2010	The focus was to address health of neighbours and pigs. Quantified *E. coli*, inside shed and at distances up to 400 m.
Letourneau et al. (2010)	2010	The focus was exposure to workers in swine confinement buildings. Viable *C. perfringens*, *Enterococcus*, *Y. enterocolitica* detected in aerosols from 16, 17, 11 and 6 of the 18 facilities. Either viable (or non-viable) culturable or non-culturable *Campylobacter*, *C. perfringens*, *Enterococcus, E. coli*, *Y. enterocolitica* detected in aerosols from 10, 6, 15, 18 and 2 barns, respectively. Also tested nasal flora for pathogens and resistant bacteria.
Duan et al. (2009)	2009	The focus was impact to neighbours. *E. coli* Isolated from indoor air and at distances of 10 and 50 m from five swine houses. Results showed that some *E. coli* strains isolated from downwind and indoor air originated in the swine faeces, rather than those isolated upwind (though levels in air was not quantified).
Ko et al. (2008)	2008	Aerosol testing (to assess risk from swine farms to workers and neighbours). Two farms using conventional lagoon spraying and 10 farms using alternative waste treatment were assessed. Isolated 5% faecal coliform, 1.2% *E. coli*, 22.2% *Clostridium perfringens* 12.3 coliphage and no *Salmonella*. Bacterial pollution was detected downwind from farms.
Banhazi et al. (2008)	2008	To address air pollutant concentrations in piggery buildings. Quantified total airborne bacteria, respirable endotoxins, ammonia, and respirable and inhalable particles were monitored inside 160 piggery buildings in Australia.
Chinivasagam and Blackall (2005)	2005	To address risk to workers in pig sheds during effluent flushing. Quantified total bacteria and *E. coli* in four piggeries in Australia.
Chapin et al. (2005)	2005	To address if air from piggeries are a source of multidrug-resistant bacterial pathogens transferring from swine to humans.

		Continued
Agranovski et al. (2004)	2004	To address air pollutant concentrations and risk to workers inside piggery buildings. Quantified total bacteria, fungi and total viable particles total respirable particles (< 7 µm) (including respirable fine particles, (< 2.5 µm)) in a grower shed with flushing in Australia.
Zucker and Muller (2002)	2002	Studied four pig houses, assessed airborne bacteria to assess relationship with endotoxins
Chang et al. (2001)	2001	Focused on aspects of hygiene /management health of workers. Enumerated viable and gram negative bacteria plus fungi in open air pig buildings from six farms i.e. breeding, growing, finishing, (open air) and nursery and farrowing (closed), found no difference.
Zucker et al. (2000)	2000	Concern, respiratory disease among farming community, multiple animal housed including pig fattening house tested. Enumerated total aerobic/anaerobic and total bacteria in air, *E. coli* was linked to animal houses that did not use litter.
Duchaine et al. (2000)	2000	Focus building hygiene and risk to workers. Inside swine confinement buildings (8) (winter and summer); enumerated total bacteria and fungi and endotoxins to assess hygiene.
Seedorf et al. (1998)	1998	Focus, respiratory disease, animal and humans. Enumerated total bacteria, fungi Enterobacteriaceae for sows' weaner and fattening pigs inside house to assess emission rates. Endotoxins tested.
Platz et al. (1995)	1995	Focus respiratory disease in pigs. Enumerated total bacteria in 13 fattening pig houses at distances (from house across seasons to understand contamination.
Lavoie et al. (1995)	1995	Focus, human health. Enumerated total bacteria (and assessed fungi) within a grower-finisher pig building using the deep-litter method to address risks from fungi.
Crook et al. (1991)	1991	Focus, respiratory health of pig farmers. Total airborne microorganisms were enumerated in 6 pig farms.
Elliot (1976)	1976	Focus swine respiratory health. Studied air-borne *Salmonella* and *Staphylococcus* from two swine finishing-growing units. No *Salmonella* and few coagulase positive *Staphylococcus* isolated; the risk from these organisms were deemed low.

Australian studies shaded in grey

5.2.3 Outcomes from studies of Australian poultry sheds – Salmonella and Campylobacter in air

Studies were conducted in mechanically ventilated poultry sheds, to understand the distribution (and presence) of both *Campylobacter* and *Salmonella* in aerosols (Chinivasagam et al. 2009b). Though the situation was different to piggeries during extensive sampling (from 2005 – 2007), across four farms, six broiler cycles (~50 days per cycle), weekly sampling i.e. 42 sampling dates, *Campylobacter* was captured only once at low levels and only inside the sheds and not outside (at 10m distances from the fan). Similarly, *Salmonella* was captured twice and at low levels inside the shed (and not outside). The levels of *Campylobacter* (Chinivasagam et al. 2016; Chinivasagam et al. 2009b) and *Salmonella* (Chinivasagam et al. 2012) enumerated in poultry litter can be higher or comparable to final or

secondary ponded effluent (Chinivasagam et al. 2004). Thus, the likelihood of isolating the organisms in shed piggery effluent flushing environments is quite low.

The piggery studies were all in-shed studies and were based on industry concerns to address risks to workers, whilst the poultry studies were driven by community concerns focused on risks outside and at distances to shed. The use of a "marker organism" to define distance travelled downwind from the fan-end of the poultry sheds was also demonstrated (Chinivasagam et al. 2010). The outcomes from the poultry studies showed that there was minimum risk to neighbours from both *Salmonella* and *Campylobacter* which were not captured at 10m from the fan end of the sheds.

5.2.4 Summary of work undertaken in Australian piggeries

Abstract: "Investigation and application of methods for enumerating heterotrophs and *Escherichia coli* in the air within piggery sheds." (Chinivasagam and Blackall 2005).
Aims: To investigate methods for the recovery of airborne bacteria within pig sheds and to then use the appropriate methods to determine the levels of heterotrophs and *Escherichia coli* in the air within sheds.
Methods and results: AGI-30 impingers and a six-stage Andersen multi-stage sampler (AMS) were used for the collection of aerosols. Betaine and catalase were added to impinger collection fluid and the agar plates used in the AMS. Suitable media for enumerating *E. coli* with the Andersen sampler were also evaluated. The addition of betaine and catalase gave no marked increase in the recovery of heterotrophs or *E. coli*. No marked differences were found in the media used for enumeration of *E. coli*. The levels of heterotrophs and *E. coli* in three piggeries, during normal pig activities, were 2.2 x 10^5 and 21 Colony Forming Units (CFU) m^{-3} respectively.
Conclusions: The failure of the additives to improve the recovery of either heterotrophs or *E. coli* suggests that these organisms are not stressed in the piggery environment. The levels of heterotrophs in the air inside the three Queensland piggeries investigated are consistent with those previously reported in other studies. Flushing with ponded effluent had no marked or consistent effect on the heterotroph or *E. coli* levels.
Significance and impact of the study: Our work suggests that levels of airborne heterotrophs and *E. coli* inside pig sheds have no strong link with effluent flushing. It would seem unlikely that any single management activity within a pig shed has a dominant influence on levels of airborne heterotrophs and *E. coli*.

5.2.5 Summary of work undertaken in Australian poultry sheds

Abstract: "Mechanically ventilated broiler sheds: a possible source of aerosolized *Salmonella*, *Campylobacter*, and *Escherichia coli*." (Chinivasagam et al. 2009b).
This study assessed the levels of two key pathogens, *Salmonella* and *Campylobacter*, along with the indicator organism *Escherichia coli* in aerosols within and outside poultry sheds. The study ranged over a three year period on four poultry farms and consisted of six trials across the boiler production cycle of around 55 days. Weekly testing of litter and aerosols was carried out through the cycle. A key point that emerged is that the levels of airborne bacteria are linked to the levels of these bacteria in litter. This hypothesis was demonstrated by *E. coli*. The typical levels of *E. coli* in litter were (~10^8 CFU g^{-1}) and, as a consequence, were in the range of 10^2 to 10^4 CFU m^{-3} in aerosols, both inside and outside the shed. The external levels were always lower than the internal levels. *Salmonella* was only present intermittently in litter and at lower levels (10^3 to 10^5 most probable number MPN g^{-1}) and consequently present intermittently and at low levels in air inside (range of 0.65 to 4.4 MPN m^{-3}) and

once outside (2.3 MPN m^{-3}). The *Salmonella* serovars isolated in litter were generally also isolated from aerosols and dust, with the *Salmonella* serovars Chester and Sofia being the dominant serovars across these interfaces. *Campylobacter* was detected late in the production cycle, in litter at levels of around 10^7 MPN g^{-1}. *Campylobacter* was detected only once inside the shed and then at low levels of 2.2 MPN m^{-3}. Thus, the public health risk from these organisms in poultry environments via the aerosol pathway is minimal.

Abstract: "The aerobiology of the environment around mechanically ventilated broiler sheds." (Chinivasagam et al. 2009a).
Aim: To investigate the aerobiology of the environment around mechanically ventilated broiler sheds with the aim of understanding dispersion in the surrounding environment.
Methods and Results: Aerosol samples were collected weekly on four different commercial broiler farms through the cycle of 55 days from 2005 to 2007. Samples were collected inside the shed and at varying distances from the sheds. Litter and dust from within the shed were also examined. Members of the genera *Staphylococcus* (and to a lesser extent Corynebacterium) dominated (10^6 CFUm^{-3}) in the outside air at 20m from the fan and were shown to decrease with distance. At distances of around 400 m, the levels of staphylococci/coryneforms returned to levels typical of those present before the placement of chickens. *Escherichia coli* levels were low (maximum 100 CFUm^{-3}) at 20m. Fungi were present at uniform levels across the broiler cycle.
Conclusions: Staphylococci are the dominant organisms present in the air around mechanically ventilated broiler sheds and have the potential to act as an airborne 'marker organism'.
Significant impact of the study: The outcomes of this study suggest that the impact of aerosols emitted from broiler sheds could be monitored and managed by examining the levels of staphylococci/coryneforms. (Whilst staphylococci/coryneforms can act as a "marker organism" for poultry due to their presence in features, the concept of using a "marker organism" to assess distance travelled, could be adopted using an organism such as *E. coli*)

5.2.6 Overall summary

A summary from literature around and after the period (until recent) has revealed there are limited studies that quantified either key food-borne pathogens or *E. coli* within pig shed environments. Most studies (as listed) however, did address shed hygiene (and risk to workers) from aspects such as endotoxins and particulate matter. Other studies looked at mechanically ventilated sheds and risks to neighbours downwind from the operation. The Australian pathogen data could be used for risk management purposes.

5.3 Quantifying risks to neighbours and piggery workers

Quantitative Microbial Risk Assessment (QMRA) was used as a means of providing a quantifiable risk for pathogen transmission via the aerosol pathway. The activities targeted were the application of piggery effluent, via spray irrigation to land and the transmission of pathogens during flushing of sheds with effluent. This has been demonstrated in the two examples (presented below) using actual levels of pathogens from the pathogen survey of effluent ponds and from aerosols (as in previously described studies undertaken).

5.3.1 Quantifying pathogen risks during the use of piggery effluent via spray irrigation to neighbours off-farm

This section demonstrates the use of Quantitative Microbial Risk Assessment (QMRA) for quantifying risks due to the use of piggery effluent for spray irrigation. The piggery that was used for this purpose

was located in Toowoomba, Queensland. This piggery was already using a specific travelling irrigator to irrigate turf for about eight hours, mainly at night; with the irrigator travelling a distance of 200 m. The turf was irrigated as needed i.e. twice in summer and once in winter depending on rainfall and effluent availability. Effluent was a valuable resource for this purpose.

The inputs for modelling the health risk assessment included the type of pathogen, their levels present in effluent, meteorological conditions (at time of irrigation), the irrigation system used, its location (in relation to targeted people), and their activities plus their likelihood of succumbing to infection.

The following pathogen levels (Table 7 from Queensland pathogens survey) were used to calculate risks. Table 7 presents the minimum and maximum counts of the three main organisms in Queensland piggery effluent as obtained during the effluent pond survey. The counts reported for piggery A is also included (as this piggery was a part of the survey).

Table 7 Minimum and maximum counts of the three main organisms found in Queensland piggeries (plus piggery A) (Source (Blackall 2001)

Final Effluent Pond reading	*Escherichia coli* cfu/100ml	*Campylobacter* spp. MPN*/100mL	*Salmonella* spp. MPN/100mL
Survey Minimum	120	40	0
Survey Maximum	530000	>11000	2.3
Piggery A	33000	930	0

* MPN – Most probable number.

In summary, the risks to personnel were calculated in the following manner:

(a) A Gaussian plume model was used (this model takes into account both the droplets that aerosolise as well as those that travel normally though a centre line. However note that this model does not account for spray drift).
(b) The Model for Effluent Disposal Using Land Irrigation (MEDLI model) was then used. This model is the tool recommended by the environmental regulator in Queensland to make or assess decisions about how to dispose of effluent by land irrigation. (https://www.des.qld.gov.au/science/government/science-division/medli/).
(c) The MEDLI model thus addresses spray drift (droplet deposition and evaporation and size).
(d) Final predictions were arrived at using MEDLI and an enhanced Gaussian plume model.
(e) Pathogen concentrations in the air at various distances from the irrigator were calculated and used to assess health risks.
(f) The modelling approach is able to predict survival, based on various inputs including bacterial die-off to assess risks under worst case scenarios.
(g) Bacterial die-off (with time), droplet dispersal and deposition were some of the parameters included to evaluate risks.

The risk of infection was calculated to decrease with increased separation distance and that the separation distance also depended on the pathogen levels in effluent (along with wind speed). Environmental conditions, (at the time of irrigation) such as atmospheric stability and relative humidity, also played a role in predicting pathogen risks.

The final analysis presented for Piggery A indicated no bacterial infections to residents housed at 500 m away from the irrigator. Based on the levels of *Campylobacter* (for piggery A,) a low risk i.e. "1 infection per 10,000 over a year" Figure 5 was calculated based on the input parameters. The minimum separation distances for farm workers was recommended as 300 m (for minimum health risks) under normal wind speed conditions, and the need to curtail irrigation during strong wind. Thus, this risk assessment showed that the irrigation practices used were not causing any unacceptable risk to either the near-by neighbours or the farm workers. Specific recommendations to ensure that minimal public health risk were provided.

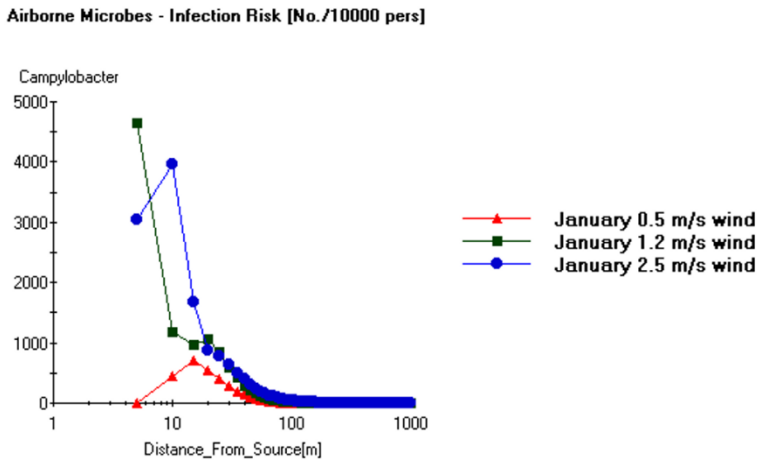

Figure 5 The infection of risk as a result of inhaling aerosols

Figure 5 presents the likely number of infections per 10,000 persons from irrigating effluent containing *Campylobacter* at 930 MPN /100mL for various separation distances from the irrigator and three wind speeds. (Source (Blackall 2001)

5.3.2 Quantifying pathogen risks during in-shed flushing to in-shed piggery worker

A simple risk assessment was done to demonstrate the calculation of risks to pig workers. The levels of *E. coli* (indicator organism) was used as a means of demonstrating risk to workers, based on the levels captured from aerosols within the piggery.

The risk of infection to pig works was calculated as follows: (Blackall 2001)

- An acceptable risk set was one extra infection per 10,000 people per year. This was proposed by USEPA for drinking water (EPA 1989). This level was used in the absence of any other accepted risk for aerosols.
- QMRA was used to understand risk
- The outcome suggested that the *E. coli* levels obtained from the aerosol study needed to be 15 times higher than the highest recorded value for an unacceptable level of risk to be achieved.
- In summary, the risk assessment performed showed that for every 10,000 workers who may spend 3.8 years of continuous time in a grower shed, one worker will get an infection with exposure to *E. coli* from aerosols in the shed.

- This is an acceptable risk – as the USEPA is 1/10,000 people per year
- Please note *E. coli* is not a pathogen but an indicator organism – but the levels were used to demonstrate risk, using QMRA.

5.3.3 Summary, modelling risks of infection from aerosols

Using pathogen data from both effluent and (in-shed studies) modelling was carried out to address risks. To address risk from droplet dispersion during spray irrigation both the MEDLI and a Gaussian Plume model were used. To address risk from droplets generated within a shed, the QMRA approach was used. Both approaches i.e. using effluent for in-shed flushing or spray irrigation (up to 300 m) met the recommended US EPA criteria of "1 infection per 10,000 over a year" (EPA 1989).

5.4 Survival of Escherichia coli on plant surfaces irrigated with piggery effluent

The identification of possible routes that lead to enteric pathogen contamination of food crop (or other) is vital to develop appropriate intervention strategies. The mode of contamination of crop in most instances can occur via the use of contaminated waters, effluent, manures or composts (splashing can occur during irrigation). Thus, minimising contact from contaminated irrigation waters (or manures) is an important food-safety intervention measure. Studies carried out under experimental field conditions have shown introduced *Salmonella* to persist for 161 (to a maximum of 231 days) in soils amended with contaminated composts on which lettuce and parsley were grown (Islam et al. 2004b). Subsequently, the organism persisted in lettuce and parsley for 63 and 231 days respectively (Islam et al. 2004b). Studies have also shown that pathogens such as *Salmonella* and *E. coli* 0157 could survive superficially, on the surfaces of protected leaf locations of the cabbage rhizosphere, (cultivated with artificially contaminated manure amended soil) (Ongeng et al. 2011).

There are concerns that pathogens such as *Salmonella* can survive on plant surface biofilms and, as a consequence, internalise within fruits (Heaton and Jones 2008). *Salmonella* is known to be able to attach or internalize into vegetables and fruits (Hanning et al. 2009). Tomatoes irrigated with contaminated water resulted in leaf contamination and contaminated fruit (up to 10^5 CFU per fruit) (Barak et al. 2011). Spraying leaf surface with contaminated water (with *E. coli* 0157) resulted in the organism surviving on the leaf surface for 27 days post spraying plus internalising within the leaf (for 14 days) (Erickson et al. 2010). *Salmonella* survived for 203 – 231 days in plots treated with contaminated composts and irrigation water, subsequently being detected in root vegetables (radishes and carrots) up to 84 and 203 days (post sowing) (Islam et al. 2004a).

Whilst the following study targeted the survival of *E. coli* on grass (pasture) or grass, it is also possible that effluent re-use on crops can have implications should pathogens of concern be present. The following study was carried out to understand the survival of the indicator organism *E. coli* on leaf surfaces

5.4.1 Survival of E. coli on leaf surfaces

Contamination of crop following pond effluent irrigation is a concern due to the potential presence of pathogens in piggery effluent. This study was undertaken to understand the die-off of pathogens on effluent irrigated leaf surfaces or more specifically to address withholding periods, following the irrigation process with ponded piggery effluent.

With regards to bacterial die-off, a 99.9% reduction (3-log reduction) is regarded as an acceptable reduction during treatment processes (Gilbert et al. 1976). This study was designed to provide an understanding of the impact of key environmental conditions on pathogen die-off.

The study details are summarised as follows.

a. The study was undertaken under controlled environmental conditions in a plant chamber (where the environmental conditions could be simulated) and a glass house, (which was dedicated to plant growth studies), the study conditions are listed in Table 8.
b. Potted Mondo grass was used for the study.
c. Initially piggery effluent was collected from final pond (experiments A and D) but in order to achieve a higher *E. coli* count and be able to calculate die-off, effluent was directly collected from the piggery drains for the later studies. (Table 9).
d. Sunlight was simulated using a light source that provided UVA and UVB components (as in sunlight). This light source consisted of two True-Lite lamps (Interlectric Corporation, Warren, PA USA) and was located 40 cm from the test plants. At this distance, the UVA emission was 9.06×10^{-2} W/m^2, and UVB emission was 1.18×10^{-2} W/m^2, as measured by the manufacturers. In the zero-UV experiment (control), a fluorescent lamp was used as the light source. For the glasshouse experiments, the UV dose received from the sun in hourly increments was obtained from the Australian Radiation Protection and Nuclear Safety Agency.
e. *E. coli* was introduced to grass by spraying with natural piggery effluent (under laboratory conditions). *E. coli* levels were assessed under varying UV, relative humidity and temperatures (which could be simulated in the cabinets).
f. The time achieved for 99% reduction (2-log) was estimated for the combinations studied.

Table 8 presents the conditions used for the nine controlled – survival experiments conducted, under varying "simulated" environmental conditions

Table 8 Environmental conditions for controlled survival experiments (after (Blackall 2001)

Experiment	Facility Used	Temperature	Relative Humidity	UV Source
A	Plant chamber	28^{0}C	75%	True-Lite
B	Plant chamber	28^{0}C	85%	True-Lite
C	Plant chamber	28^{0}C	78%	None
D	Plant chamber	28^{0}C	60%	True-Lite
E	Plant chamber	33^{0}C	56%	True-Lite
F	Plant chamber	34^{0}C	60%	True-Lite
G	Plant chamber*	34^{0}C	60%	True-Lite
H	Glasshouse	34^{0}C	65%	Natural sunlight
I	Glasshouse	29^{0}C	63%	Natural sunlight

* In this experiment, six hours of 34° C was used followed by an ambient temperature overnight period.

Figure 6 presents simple die-off data in the glass house under natural weather conditions. More specifically, under constant humidity (63-64%) rapid die-off is achieved on a 29°C (cloudy, rainy day), compared to 34° C (a sunny day), in this instance higher temperature not being the driver for die-off. This indicates the complex nature of the interactions contributing to die-off (i.e. type of organism, temperature and relative humidity). The modelling approach was adopted to get a clearer picture and is described below.

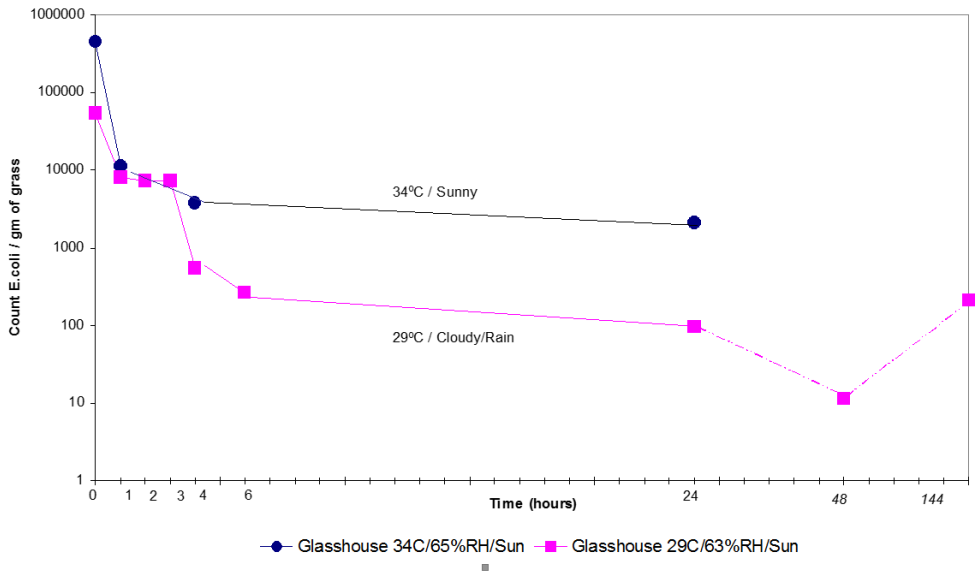

Figure 6 Change in E. coli levels in Mondo grass, during variable temperature and constant humidity in a glasshouse

Risk modelling was carried out to provide in order to be able to obtain simulated outcomes of *E. coli* die-off on leaf surfaces. The detailed die-off and other associated parameters are presented in Table 9. The environmental conditions used for the study were incorporated into a risk model to predict die-off, for given experimental conditions adopted during the study.

Table 9 Levels of E. coli in source (effluent) and leaf and time taken for 2 log (99%) reduction

	***E. coli* levels**								
	A 28°C 9.5mbars 42.5J/m^2 UV (UV lamp)	**B** 28°C 5.7mbars 42.5J/m^2 UV (UV lamp)	**C** 28°C 8.8mbars 0J/m^2 UV (UV lamp)	**D** 28°C 15.2mbars 42.5J/m^2 UV (UV lamp)	**E** 33°C 22.2mbars 42.5J/m^2 UV (UV lamp)	**F** 34°C 19.2mbars 42.5J/m^2 UV (UV lamp)	**G** 34°C 21.3mbars 42.5J/m^2 UV (UV lamp)	**H** 34°C 18.7mbars <910J/m^2 UV (Sunlight)	**I** 29°C 14.9mbars <770J/m^2 UV (Sunlight)
Effluent (CFU/100ml)	7.1X10^5 (final pond)	2.5X10^8 (Shed)	4.7X10^7 (Shed)	5.5X10^6 (final pond)	7.5X10^7 (Shed)	1.3X10^8 (Shed)	1.3X10^8 (Shed)	1.3X10^8 (Shed)	5.0X10^8 (Shed)
Leaf (MPN/g)	4,000	76,666	96,666	3,833	15,1666	460,833	460,833	460,833	53,333
Time					**% Die-off**				
1 Hour	90.000	76.087	72.414	86.955	65.934	90.597	90.597	97.541	84.687
2 Hour	96.000	90.001	77.328	97.052	92.528	91.320	91.320	-	86.562
3 Hour	98.200	76.087	96.035	95.956	92.803	97.541	97.541	-	86.562
4 Hour	96.125		99.400	97.469	92.638	87.342	87.342	99.185	98.969
6 Hour	-	-	99.307	96.348	98.352	94.883	94.883	-	99.499
24 Hour	-	98.522	99.586	97.991	99.275	99.054	99.054	99.543	99.819

The highlighted areas present 99 – 99.9 die-off

5.4.2 Model development to predict E. coli die-off

The data generated from the above experiments were used by our collaborating team to develop a model to predict survival of pathogens under a range of environmental conditions. Whilst all details are already described (Blackall 2001), in summary the model incorporated environmental effects (relative humidity, vapour pressure deficit and UV radiation dose) to create a survival equation.

Some details from the model are presented as follows

- The model looked at environmental effects and improve the model created for *E. coli* die off by adding in the effects of temperature, relative humidity and UV radiation dose to interpret survival
- Statistical analysis indicated mean survival obtained for each experiment was not statistically significant, showing no clear effects of temp, Vapour Pressure deficit (VPD) and UV
- For the purpose of modelling temp was averaged and was shown that *E. coli* die-off increases with temp
- No significant trends with VP and UV were seen
- This suggests that under conditions of around 30°C, a 2-log reduction in *E. coli* may be seen only after 24 hours.
- Under cooler conditions (20°C), this reduction may require 40 hours.
- Based on the model data, time was finally regarded as being the key factor for withholding period and the model indicated a 2-log was achieved after 24h (under experimental conditions)
- The model outcome also suggested that dryness may inhibit pathogen transfer and hence minimise risk

Some final comments suggested that low relative humidity and high temperature can promote pathogen death, with the possibility of survival increased by cool moist conditions. The ultimate risk of infection depends on any pathogen that remains on the leaf being ingested in doses high enough to cause an infection (and disease).

5.4.3 Summary, survival on leaf surfaces and modelling E. coli die-off

Leaf surface survival studies were undertaken both in controlled atmosphere (in a cabinet) and glasshouse conditions to understand the survival of *E. coli* in effluent irrigated leaves subjected to varying temperature and humidity conditions. The data was initially analysed graphically, indicating the complexity of interactions between bacterial survival and environmental conditions. Subsequent modelling studies using the same data demonstrated the weather parameters that played a contributory role to achieve a 2-log reduction of *E. coli* on leaf surfaces. These approaches could be used to predict / address / manage withholding periods on irrigated leaf / crop surfaces following piggery effluent application.

5.5 Arcobacter and piggery effluent, an updated summary

The interest in *Arcobacter* is because it is an emerging food-borne pathogen (Phillips 2001) with a pig association. *Arcobacter* is a relatively newly classified organism and was originally classified under *Campylobacter* (Vandamme et al. 1992). The main difference being, unlike *Campylobacter*, these organisms are able to grow under aerobic conditions. The organism is also reported to be prevalent in humans (Ramees et al. 2017), though the role of the organism in human disease, is still unclear (Collado and Figueras 2011). *Arcobacter* is recognised as zoonotic organism, linked with causing both

bacteremia and diarrhoea in humans (Collado and Figueras 2011). *Arcobacter butzleri* is the most commonly reported human pathogen (among the other *Arcobacter* species) (Mansfield and Forsythe 2000).

At present, 25 species of *Arcobacter* are recognised, and have been isolated from a wide range of environments (Ramees et al. 2017). *Arcobacter* has been isolated from broiler carcasses (Houf et al. 2002), raw poultry, meat, and meat products (Mor-Mur and Yuste 2010), animal faeces i.e. pigs, cattle, and sheep (Van Driessche et al. 2003). The organism has been shown to be present in foods such as poultry, beef, dairy products, seafood, pork, lamb, rabbit and vegetables (as well as food-processing environments) (Hsu and Lee 2015).

In the early years following classification, the organism was originally shown to colonise neonatal pigs (Wesley et al. 1996) and isolated from aborted pig foetuses (de Oliveira et al. 1997). *Arcobacter* can also be present in pork (Zanetti et al. 1996; (Van Driessche and Houf 2007). Finishing pigs can intermittently (i.e. time and numbers) excrete *Arcobacter* irrespective of age or farm hygiene/ management practices, whilst showing no signs of clinical disease (De Smet et al. 2011a). Three of the common species *A. butzleri, A. cryaerophilus* and *A. cibarius* have been isolated from both piggery effluent and effluent-irrigated soils from Australian piggery environments (Chinivasagam et al. 2007). Since then, three new species have been isolated from pigs i.e. *Arcobacter thereius* (kidney and liver of an aborted pig foetus) (Houf et al. 2009), *Arcobacter trophiarum* (faecal samples of pigs) (De Smet et al. 2011b) and *Arcobacter lanthieri* (pig manure tank) (Whiteduck-Leveillee et al. 2015) but no further details are currently available.

The interest in *Arcobacter* and piggery effluent is the potential for the water-borne transmission route of this organism. Water has been shown to be a route of transmission of the three major species (*A. butzleri, A. cryaerophilus,* and *A. skirrowii*) (Collado et al. 2008). This includes sewage (Collado et al. 2008; Talay et al. 2016; Merga et al. 2014), fresh water, sea water, (Collado et al. 2008), river and spring water (Talay et al. 2016). Arcobacters have also been isolated from drinking water treatment plants (Jacob et al. 1998), ground water (Rice et al. 1999) and seawater (including plankton) (Fera et al. 2004). They are able to attach to water distribution pipes (Assanta et al. 2002). The highest concentration of *Arcobacter* have shown to be present in waters contaminated with faeces (Collado et al. 2010). The organism's presence in waters has been partially linked to faecal contamination of animal origin (Lee et al. 2012). Thus, different water bodies are a common reservoir and can be a source of transmission. Hsu et al. (2015) commented that in an era of climate change and extreme precipitation events, monitoring of waters / treatment require the consideration of this emerging waterborne pathogen.

Piggery effluent is unique in terms of other animal wastes (and in some respects similar to human effluent) from a perspective of pathogen monitoring/management that address human health. The key difference being the source pathogens. A recent (2017) review by "global water pathogen project" (International Hydrological Programme (IHP) of the United Nations Educational, Scientific, Cultural Organization (UNESCO), and Michigan State University) has included *Arcobacter* as a pathogen of concern (Banting et al. 2017). The review, however does acknowledge the "gaps in knowledge" of the organism's prevalence, genetics of virulence and infectious dose to humans, and thus, a lack of understanding of risks linked to this organism's exposure to humans (Banting et al. 2017). The comprehensive review lists *Arcobacter* species across two matrices, "wastewater/ sludge" and "other water matrices".

Amongst the 34 listings reported there is only one single report originating from animal, or more specifically, "piggery effluent" (under "wastewater/ sludge"), an Australian study published in 2007 (Chinivasagam et al. 2007). The rest are categorised as sewage and allied sources. This is the only study (based on the review) which reported *Arcobacter* prevalence/levels in both piggery effluent and effluent irrigated soils. The other water matrices included are irrigation, estuarine, canal, tap, ground, surface, lake, beach, spring, river, and drinking water. The common species identified across all waters are *A. butzleri, A. cryaerophilus,* and *A. skirrowii.* Both *A. butzleri* and *A. cryaerophilus* have been also isolated from piggery effluent. (Chinivasagam et al. 2007) is the only published study on quantifying *Arcobacter* in both piggery effluent and effluent treated soils. In summary, based on recent literature and the recognition of this organism in other waters (of concern), there is a need to consider *Arcobacter*. Additionally, due to the high numbers in piggery effluent this organism could also be used as an indicator to assess treatment.

This work was presented in a peer reviewed publication as follows:

Abstract: Detection of *Arcobacter* spp. in piggery effluent and effluent-irrigated soils in southeast Queensland. (Chinivasagam et al. 2007)
Aims: To investigate the occurrence and levels of *Arcobacter* spp. in pig effluent ponds and effluent-treated soil.
Methods and Results: A Most Probable Number (MPN) method was developed to assess the levels of *Arcobacter* spp. in seven pig effluent ponds and six effluent-treated soils, immediately after effluent irrigation. *Arcobacter* spp. levels in the effluent ponds varied from 6.5×10^5 to 1.1×10^8 MPN 100 ml^{-1} and in freshly irrigated soils from 9.5×10^2 to 2.8×10^4 MPN g^{-1} in all piggery environments tested. Eighty-three *Arcobacter* isolates were subjected to an abbreviated phenotypic test scheme and examined using a multiplex polymerase chain reaction (PCR). The PCR identified 35% of these isolates as *Arcobacter butzleri*, 49% as *Arcobacter cryaerophilus* while 16% gave no band. All 13 nonreactive isolates were subjected to partial 16S rDNA sequencing and showed a high similarity (>99%) to *Arcobacter cibarius*.
Conclusions: *A. butzleri, A. cryaerophilus* and *A. cibarius* were isolated from both piggery effluent and effluent-irrigated soil, at levels suggestive of good survival in the effluent pond.
Significance and Impact of the Study: This is the first study to provide quantitative information on *Arcobacter* spp. levels in piggery effluent and to associate *A. cibarius* with pigs and piggery effluent environments.

5.5.1 Summary, Arcobacter and piggery effluent

There is a need to consider *Arcobacter* due to its status as an emerging pathogen which may be present in high numbers in piggery effluent. The UNESCO global water pathogen project has recognised *Arcobacter* to be an organism of concern across waters. The high levels present in piggery effluent means this organism (or *E. coli*) can be used to address treatment efficacy of piggery effluent. This organism was also used to address pathogen survival in-soil and run-off in previous APL studies.

5.6 Survival of pathogens in animal manure amended soils, the concerns

Pathogens can be present in manure amended soils, thus there is a need to consider both environmental contamination and animal recycling (of the organism), by setting withholding periods with consideration to pathogen levels (in manures) and application rates (Holley et al. 2006). More specifically, setting defined intervals between application prior to harvest of food crop (or providing

access to grazing animals) provides time for the significant decline of pathogen levels (Nicholson et al. 2000).

Factors such as soil temperature and addition of manure and predation by protozoan parasites can influence *Salmonella* survival in soil (Garcia et al. 2010). *Salmonella* from soil can transfer to fresh-water either due to run-off or preferential flow paths in soil (Jacobsen and Bech 2012). Contaminated compost or irrigation water (with *E. coli* O157:H7) can contaminate subterranean crops (carrot and onions) (Islam et al. 2005). Fresh produce can be contaminated from water splashes during rain events with potential for the organism to attach to the surface or internalise within the plant (Jacobsen and Bech 2012). Studies have shown that *Salmonella* can transfer (from a point source) to tomatoes during a 30 minute rain event via aerosols at levels that can be of concern to humans (Cevallos-Cevallos et al. 2012).

Waste type, rather than the rhizosphere (which can influence soil nutrients), was shown to impact on *E. coli* O157:H7 survival in soil amended with animal waste (Williams et al. 2007). Interestingly, *E. coli* survival in soils that received organic fertilizers (with high C/N ratio leading to low nutrient release) were lower than soils that received artificial fertilizers (Franz et al. 2008). Both *Salmonella* and *E. coli* demonstrated a death rate of 14 days per log cycle in Australian podzol grey loam top soil with the ability to re-grow in moistened soil (Chandler and Craven 1980b).

The survival and transfer of pathogens in soils receiving animal (organic) waste is complex. Reddy et al. (1981) in a comprehensive review on the behaviour of pathogens in soils treated with organic wastes defined bacterial decay rates using first order kinetic equations to predict die-off rates. The decline of *Salmonella* enterica, serovar Newport in manure amended soils was shown to follow a first order kinetic model, i.e. for a 1-log reduction, 14 – 32 days, a 2-log reduction 28 -64 days and for a 3-log reduction 42 – 96 days (You et al. 2006). Die-off of bacteria can thus be defined by decimal reduction time, (T_{90}), which is the time taken for the viable bacterial count to decrease by 1-log unit (log_{10}) i.e. a 90% reduction in population. Kearney et al. (1993) demonstrated an initial rapid decline of bacterial population in animal slurry, during which it was possible to calculate T_{90}, and a subsequent "equilibrium period" (with no reduction in bacterial numbers by 90%).

Faecal bacteria such as *E. coli* is the commonly adopted indicator to assess die off (Crane et al. 1980). It is uncertain if faecal bacteria can sufficiently predict the pathogen response, as not all faecal coliforms originate from faeces, with the possibility that non-environmental sources can complicate estimating the fate of pathogens from animal waste (Sobsey et al. 2006). However, *E. coli* (rather than faecal coliforms) have been shown to be a good indicator of drinking water, surviving 4 – 12 weeks depending on environmental conditions and prevalent microflora (Edberg et al. 2000). The survival of faecal bacteria depend both on conditions prior to manure application and after, such as the competitive interactions with native soil flora (Unc and Goss 2004).

A study addressing the survival of pathogens in piggery effluent irrigated soil was undertaken during the previous APL study. The aims of the study were:

- understand die-off of the selected organisms
- Assess the suitability of using *E. coli* (over a pathogen) as an indicator, applicable to assessing piggery effluent treated soils.

The organisms of choice to be monitored during the trials undertaken in four piggeries were *Campylobacter* and *Arcobacter* (along with *E. coli* as an indicator). *Salmonella* was not considered, due to the organism's intermittent presence in piggery effluent (and low levels, when present). Unlike *Salmonella* and *Campylobacter*, *Arcobacter* being present in high levels in effluent is a useful organism to monitor the impact of pathogens in effluent irrigated soils. The study compared the die-off of *Arcobacter*, *E. coli*, and *Campylobacter* in effluent irrigated pasture soils. The study was carried across two farms during winter and summer.

5.6.1 Farm trials to assess pathogen survival in effluent irrigated soils

Trials were carried out on four piggeries W, G, K and R through summer and winter (to compare seasonal survival). The farm conditions at the four piggeries are presented in Table 10.

The area chosen for the trial had not received piggery effluent in the recent past, although all sites were on piggeries and past irrigation events cannot be excluded. The effluent was sourced from the effluent ponds on-farm.

Table 10 Farm conditions at piggeries W, G, K and R

Farm	Year	Soil type	Ground cover	Ponds
Piggery W	2002	Clay	Couch grass, thick and even	1 pond, effluent removed before irrigation
Piggery G	2002	Volcanic	Rye grass, tall in summer, patchy in winter	1 pond, effluent removed before irrigation
Piggery K	2003	Heavy black	Couch grass	3 (effluent used 1st pond)
Piggery R	2003	Clay	Couch grass	3 (effluent used 1st pond

5.6.2 Design of field trials carried out on piggeries

The field trials were carried out using mini-plots and the study plots were separated by a similar size buffer (to prevent cross contamination). Figures 7 and 8 illustrate the experimental designs for the summer and winter trial.

Figure 7. Mini plot design for summer trials, piggery G, W, R, and K

Figure 8 Mini-plot – winter trial – piggery G and W (inclusive of wetting)

The experimental conditions for the summer trials are summarised as follows:

- Each plot was 1m X 1m and a 1m square metal template was pushed into the soil to contain the water within the plot.
- Piggery effluent from ponds on relevant farms were used to irrigate the "treated plots". The "control plots" were not irrigated.
- The rate of effluent application was set with the aim of achieving a 30 mm irrigation event – meaning 30 L of effluent was added per plot.
- On day 0 all plots were hand irrigated and soil samples were collected in a random manner.

The experimental conditions for the winter trials are summarised as follows:

- The experimental conditions were similar to the summer trials, with the exception of having a wetted plot for both treatment and control.
- Wetting plots was used as a means of maintaining moisture levels, following the irrigation.
- Thus, in addition to the "treated" and "control" as in the summer treatment two additional plots i.e. "control – wetted" and "treated – wetted" plots were assigned. The only difference being that the "wetted plots were irrigated initially with 30 L of water on day 0.
- Every Monday, Wednesday and Friday the "treated – wetted" and "control – wetted" plots were re-irrigated with a volume of water so that the moisture levels of these plots returned to the moisture levels as prevailed on day 0 (received 30L), thus maintaining constant moisture levels.

Soil sampling

- Pathogens: A 2 cm diameter stainless steel core sampler cored to a depth of 4 cm, removing two cores from each plot. The core samples from three replicate plots were combined to form a composite sample. Samples were cored in a structural manner so that a previous spot was not re-sampled.
- The levels of *Campylobacter*, *Arcobacter* and *E. coli* were enumerated on the set sampling days
- Soil moisture: A Theta Probe, (AT Delta-T Devices Ltd) was inserted to a depth of 5 cm in combination with a type HH2 hand held moisture reader, (an average of four readings were taken)
- Relative humidity was measured during sampling

Statistical Analysis

- Non-linear regression analysis was performed using the package Genstat

$T\log_{90}$ the time taken for the bacterial count to decline to 10% of the initial count in log numbers was calculated.

5.6.3 Detailed pathogen survival data

Appendix 1 contains the detail pathogen survival data presented in the following tables, which can be used for risk assessment or other purposes

- Table 18 Comparative bacterial levels over time in soil (from pasture) irrigated with pond effluent, in piggeries G and W in summer
- Table 19 Comparative bacterial levels over time in soil (from pasture) irrigated with pond effluent, in piggeries G and W in winter

- Table 20 Survival of *E. coli* over time in soil irrigated with pond effluent (with re-wetting) piggeries G in winter (Including moisture levels).
- Table 21 Survival of *E. coli* over time in soil irrigated with pond effluent (with re-wetting) piggery W in winter (Including moisture levels).
- Table 22 Comparative bacterial levels, soil moisture, temperature and RH in soil irrigated with pond effluent in piggery R in summer and winter
- Table 23 Comparative bacterial levels, soil moisture, temperature and RH in soil irrigated with pond effluent in piggery K in summer and winter

5.6.4 Summary of findings – piggeries G and W – summer and winter

Effluent

- Not much difference in the levels of *Arcobacter* (~log 6.0 CFU/100 mL), *Campylobacter* (~log 3.0 CFU/100 mL)and *E. coli* (~log 5.0 CFU/100 mL) in effluent in summer across both piggeries
- In comparing winter and summer, the levels of *Arcobacter* and *E. coli* and *Campylobacter* were around a log cycle higher in both piggeries in winter than summer.

Die-off in soil

- There was a rapid die-off of *Campylobacter*, thus no exponential modelling was possible
- Exponential decay of bacterial die-off was demonstrated by both *Arcobacter* and *E. coli* and regression modelling was undertaken for piggeries G and W. The number of days required to reduce the population to one-tenth of the original was calculated (T_{90}), Table 11. This time period was longer in summer than winter
- *E. coli*, (the indicator organism) was present in both piggeries, pre effluent treatment (maximum 1.63 MPN/100 mL), whilst the other two organisms were not detected either in summer or winter
- In summer, the high rainfall at piggery G may have supported non-die off of *E. coli* up to the last day (log 3.38 MPN/g). This was not seen in piggery W (treated plots) which did not have rainfall
- The higher than day 0 *E. coli* levels at piggery G (compared to the final day) and fluctuating *E. coli* levels at piggery G and W, in the irrigated plots in summer suggests that re-growth was occurring
- Winter wetting of plots had no impact on the survival of any organism, though natural summer rain seemed to contribute to *E. coli* re-growth. It is thus possible that higher soil temperatures in summer could have played a contributory role (rather than rainfall, the difference in soil temperatures between both seasons being 12 degrees).
- Longer survival of both *Arcobacter* and *E. coli* were observed in winter though there was a difference in both piggeries, for example *Arcobacter* survival was 28 days in piggery W compared to 42 days in piggery G.

Table 11 Summary of T_{90} estimates – Piggery G and Piggery W, winter and summer*

	E. coli (days)			*Arcobacter* (days)	
	Winter		Summer	Winter	Summer
	treated	Treated - wetted	treated	treated	treated
Piggery W	7.1	5.6	5.1	2.9	0.9
Piggery G	8.9	6.4	not fitted	3.7	1.3

* T_{90} is the number of days required to reduce the population to one tenth of the original

5.6.5 Summary of key findings, piggeries R and K

Effluent

- There were no major differences in the levels of *Arcobacter* and *E. coli* and *Campylobacter* in effluent across both seasons

Die-off in soil

- There was a rapid die-off of *Campylobacter* in both piggeries across summer and winter
- Resident *E. coli* (the indicator organism) was present in both sites, pre-treatment
- It was unusual to detect a low background level of *Arcobacter* at piggery R (prior effluent treatment). This could be attributed to the long history of grazing cattle at the site
- The resident population of *E. coli* (in summer) at piggery R (represented by the control *E. coli*) may have been the reason for the shorter time (day 23) to reach background, compared to Piggery K (33 days) (die-off was calculated based on the time to reach the background levels)
- In winter *E. coli* never reached background levels at piggery K even by day 85, possibly due to the on-going presence of the resident *E. coli* population (remaining at log 3.22 MPN/g). This was in contrast to piggery R (reached background by day 28)
- When comparing seasonality there was a variation in the times *Arcobacter* reached background, which seemed to be piggery dependent; in winter the duration was at piggery R, 7 days and at piggery K, the period was 20 in winter days In contrast, in summer it was 14 days at piggery R and 7 days at piggery K. As with *E. coli* there were no interfering resident populations
- No regression modelling for bacterial die-off was possible at piggeries K and R, because of the absence of exponential decay for all organisms, over the full sampling periods
- The reason being rain fall as soil moisture caused a rise in *E. coli* levels and not continuous decay
- A stable population of continuous presence of *E. coli* is evident in piggery K, in winter, in the presence of rain events with no die-off, (Figure 9)
- At piggery R, in winter, the increases seen in *E. coli* levels seemed responsive to rain (or other parameters not included in the study) Figure 9
- This overall phenomenon is not seen for *Arcobacter* in both piggeries K and R
- Overall, the survival of *E. coli* and *Arcobacter* in winter in both piggeries were longer than summer, the soil temperature variation across both seasons being 12◦C

5.6.6 Overall summary, soil survival studies

- Tables 18 - 23 in Appendix 1 contain detailed soil survival data
- There was a presence of a resident soil *E. coli* population in the soil at all sites
- The levels of this *E. coli* resident population varied across farms
- Based on *E. coli* levels the time to reach background was dictated by the varying *E. coli* levels present across farms and seasons
- *E. coli* populations can show re-growth in the environment
- The variations such as soil type, other possible site-specific factors may impact on *E. coli* survival and levels.
- In most, but not all trials, a rise in soil moisture – due to rainfall – was accompanied by a re-growth of *E. coli* in effluent treated soils. 1

- In controlled experiments looking at the effect of moisture on the re-growth of *E. coli* by artificial wetting, did not reproduce the phenomenon, where artificial wetting resulted in a reduced survival rate for *E. coli*.
- Neither *Campylobacter* spp. nor *Arcobacter* spp. are present at detectable levels in soil that has not recently received piggery effluent
- *Campylobacter* spp. levels are very low after effluent application and drop to undetectable levels very quickly
- *Arcobacter* spp. levels drop to an undetectable level at a rate slower than that of *Campylobacter* spp., but at a faster rate than *E. coli*
- Pathogen die-off rates are higher in summer than winter
- Pathogen die-off rates can vary from piggery to piggery
- The use of *Arcobacter* may be a better marker of recent piggery effluent exposure than the *E. coli*

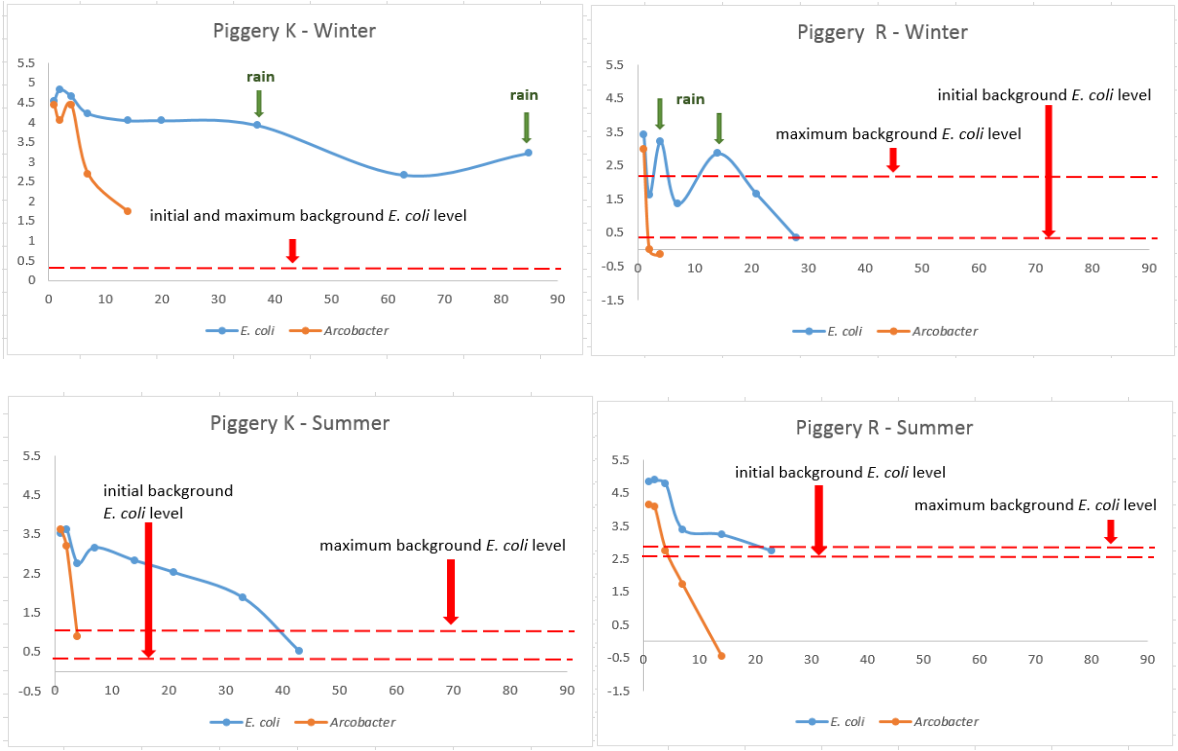

Figure 9 Survival of E. coli and Arcobacter, in piggeries K and R, with E. coli showing tailing effect

5.7 Mobilisation of bacteria in effluent irrigated soils following potential heavy rainfall

Heavy rainfall can contribute to mobilisation of pathogens in areas of recent re-use or due to a risk of overflow of storage facilities with indirect impacts to human health. Major food-borne outbreaks in fresh produce has been linked to waters contaminated with animal wastes. For example, in 2006 an outbreak involving *E. coli* O157 (linked to bagged baby spinach) spanning 26 states in the USA (with 205 cases of illness and 3 deaths) was traced to a single farm/processing unit. On investigation of the farm, the outbreak strain was also cultured from animal faeces (cattle and feral pigs), surface water, soil and pasture, a demonstration of risks due to proximity of animal waste to food agriculture (Jay et al. 2007). Whilst multiple sources other than water can contribute to environmental contamination of pathogens, this process can be regarded as a dynamic process involving multiple sources and varying

methods (Cooley et al. 2007). Thus, there is a need to address reasons that drive overland transport of pathogens, from lands receiving faecal waste, in order to address management (Tyrrel and Quinton 2003).

Soil contaminated with animal waste can be a source of pathogens, though mobilisation is a complex process; this depends on pathogen concentration, adhesion pattern to various soil types, soil particle size, soil water flux and finally rainfall intensity (Reddy et al. 1981). In summary, mobilised bacteria due to run-off from pasture (through filter-strips) as a result of rainfall can be trapped on the soil surface, infiltrated into soil or deposited as sediment, finally contributing to overland flow to waterways, (Olilo et al. 2016a). The effectiveness of vegetative buffer strips to reduce coliform levels following run-off from feed lots has been demonstrated (Young et al. 1980). In contrast, Entry et al. (2000) did not see a reduction in coliform bacteria following the movement through riparian filter strips (a combination of grass and forest) across seasons. The US EPA recommended level for run-off is 126 cfu/100 mL for an average of five samples collected over 30 days, or 235 cfu/100 mL for a single grab sample (Knox et al. 2007). The use of vegetative filter strips are beneficial as they can reduce suspended solids, total nitrogen and bacteria (*E. coli*) following overland water flow and can improve water quality (Olilo et al. 2016b).

The National Environmental Guidelines for Indoor Piggeries (2018) has detailed filter strip design based on the work of Redding (2003) to reduce nutrient entry. The recommendations in the guideline for the use of vegetation/filter strips to control nutrient flows has been developed for Australian conditions. The guidance provided for filter strips in this document to reduce nutrient entry can also contribute to pathogen movement. They are: (a) the location of the filter strip below the re-use area with consideration for path for run-off, (b) the use of non-clumping grasses that provide good ground cover that prevent run-off entering adjacent water courses or areas deemed sensitive (c) The construction of filter strips that prevent soil loss (due to the slope of the land) (d) The size of filter strips to contain soil erosion based on terrain being addressed and (e) The impact of the rain intensity on soil erosion. These factors can contribute to managing pathogen movement via soil or water. However, other considerations also need to be taken into account.

Table 12 presents examples of filter-strips used to contain pathogen movement and in some instances both pathogen and nutrients as a consequence of animal waste in the environment has mixed results. However, based on the studies of Beck et al. (2013), Cardoso et al. (2012) and Fox et al. (2011) (Table 12) there can be a difference between the use of filter strips for nutrients and bacteria. High *E. coli* levels in run-off could be a result of *E. coli* populations already establishing in vegetative / soil areas that continuously receive animal waste, the shaded vegetative areas enhancing survival overtime leading to the potential for the vegetative filter strips to support residual *E. coli* populations. Other factors include limited infiltration in of *E. coli* in already saturated soils. The presence of residual *E. coli* populations in soils around piggeries has been demonstrated by the previous APL studies in Australian piggeries (reported in the earlier section). Background *E. coli* populations were present in soils adjacent to piggeries with the potential to re-grow which also was influenced by rain. These could be some of the reasons for the mixed results for the use of filter strips where *E. coli* is chosen as an indicator.

The best option would be to adopt appropriate on-farm management strategies to contain overland flow and in instances where filter strips are in place or adopted be aware of the possible limitations with regards to *E. coli* – should the organism be an indicator of choice in future guidelines. However, as demonstrated in the following trial undertaken during the previous APL study (reported below),

the filter strips were studied to address a "contingency event" – such as an overflow of effluent from the pond due to a random heavy rain event. Such situations would not deem using the filter strips on an on-going basis (as shown in some of the studies in Table 12) e.g. run-off from areas using routine manure applications or feedlots. However consideration need to be applied in the routine use for pasture application. Ultimately it all comes down to topography which will dictate suitable vegetation and design of strips depending on proximity to watercourses that may be impacted and the potential guidelines being addressed for run-off water.

Table 12 The use of vegetative filter-strips (VFS) to manage pathogen movement, as a consequence of animal waste in the environment

Aim / other features of study	**Waste**	**Rain simulation**	**Nature of filter strip**	**Pathogens reduced/not reduced**	**Reference**
The effectiveness of a grass hedge in reducing microbial transport following manure application. Assessed microbes, total Phosphorus (P) and total Nitrogen (N)	Cattle manure applied to plots of silty clay loam	Simulation of rainfall 30 min at an intensity of 70 mm h^{-1}	1.4 m wide single switchgrass hedges planted along a contour	Significantly reduced levels of phages, E. coli, and enterococci in runoff.	Durso et al. (2019)
Assess VFS to remove suspended sediment, total N, P and *E. coli* to improve receiving water quality standard (due to overland flow)	cattle manure spread to simulate a grazing field 14 m away from filter strip	Predicted natural generated overland flow. Rainfall intensity recorded, 54 mm.	A combination of 2 strips, i.e.10-m Napier grass draining into 20-m Kikuyu grass and 10-m Kikuyu grass draining into 20-m Napier grass (VFS III)	1-log reduction of *E. coli* that reduced surface flow concentrations below the 200 (CFU 100 mL-1) recommended water quality standards,	Olilo et al. (2016b)
To Assess enteric microbial pathogens originating from agricultural practices	Cattle manure laden water	1 artificial flooding 2 Simulated (4d) and natural rainfall 2, 46, 94, 97, and 111 days after the application	Established 3 types of grass, 1 X 10 m	During 1 and 2, higher *E. coli* concentrations than in manure slurry in exiting water Reason: high *E. coli* levels already present Moist shaded vegetation conditions enhanced *E. coli* survival over time	Beck et al. (2013)
To assess the efficacy of VFS to reduce bacterial runoff from land-applied swine manure.	Swine manure with inoculated *Salmonella* and *E. coli* and Bromide tracer for water movement		Filter strips, 6.5 X 3.9 m (5% slope); loamy topsoil, clay loam or loam subsoil; grass vegetation	Limited infiltration of pathogens in wet soil and management of levels via retention in soil, due to hydrological conditions of soil VFS should account for the soil water saturation and soil water storage capacity	Cardoso et al. (2012)
To meet Total Maximum Daily Load (based on clean water act) – reduce – to	Feedlot waste	Run-off from working feedlot	Filter strips 14 to 15 m, 2% average slope fescue grass,	*E. coli* 0.8 to 1.0. log reduction achieved at 30m, thus reduced surface-flow levels of *E. coli* to below the 200	Douglas-Mankin and Okoren (2011)

reduce faecal bacteria, N, and P using VFSs from feedlot			Newtonia silt loam soil. 150 m long	CFU/100 mL (coliform-forming units, CFU) water-quality standard for Kansas	
To evaluate if VFS effectiveness of *E. coli* removal from runoff is related to inflow rate, infiltration capacity, and flow concentration.	Diluted swine manure assessing in flow rate, infiltration capacity, and flow concentration.	Under laboratory conditions	200 cm long, 100 cm wide, 7.5% slope – soil box	No sedimentation during transport Non attachment to soil particles due to manure origin of bacteria Need to address infiltration of bacteria Should prevent concentrated flow, that can limit total infiltration Filter strips can be source of residual *E. coli* from previous run-off	Fox et al. (2011)
To assess initial effectiveness of an integrated grass/tree strip system to filter runoff and drainage water from crop fields fertilized with liquid swine manure.	Spreading liquid swine manure to corn plots	A 5-year study under natural rainfall and normal agricultural activities associated with growing corn	30 X 5 m Corn with 5 X 5m grass only or grass + tree filter strip	Grass strip reduced *E. coli* by 48%; grass + tree filter reduced *E. coli* by 57%, overall 25% *E. coli* reduced by run-off and drainage. Vegetation reduced run-off and increased infiltration	Duchemin and Hogue (2009)
To examine the ability of small wetlands to filter *E. coli* in runoff from irrigated, grazed pastures.	Irrigated pastures with grazing cattle	Naturally irrigated tail- water run-off from pasture	Natural wetland to manage pasture run-off	*E. coli* levels reduced filtering run-off through natural wetland (directly adjacent to water course) to reduce run-off rates and letting pasture rest from grazing at least a week before next irrigation. 98% samples above U.S. EPA recommended level of 235 cfu/100 mL.	Knox et al. (2007)

5.7.1 Trials to address run-off using filter-strips (following the application of piggery effluent)

This study was carried out to evaluate the ability of vegetative filter strips to reduce the movement of bacteria from piggery effluent irrigated soils following an intense rain event. The experimental design was based on the following principles:

- Good irrigation principles mean that run off would not occur in an effluent application period, as the irrigation is controlled to prevent run-off.
- The aim was to test if the effluent treated area would mobilise bacteria, if a rainfall event, beyond the control of the farmer/irrigator occurred after irrigation.

Three trials were carried out on a single site in Toowoomba. The soil in the selected area was black cracking clay soil (black vertosol). Trial 1 was carried out to validate methodology. Trials 2 and 3 were carried out to address the objectives described below.

Objective for trial 2 – run-off due to heavy rain based on a simulation described below

The following scenario was simulated to address the objective of trial 2.

- Heavy rain occurs on a pig farm, filling the effluent ponds.
- Irrigation becomes necessary due to over-topping concerns.
- Weather reports are fine, so irrigation of a moderate amount of effluent is completed to reduce levels in the ponds.

Despite weather forecasts, a short, high intensity, rainfall event occurs the next day.

Study design - trial 2

- A bare paddock that had been tilled 2 months before and ready for crop production was used
- A mini plot design was adopted and a rain simulator was used to simulate rainfall (Figure 10)
- Four set of twin plots (1 X 2m) were prepared, one of each twin plot was marked "treated" and the other "control"
- The plots were initially irrigated with pathogen free rainwater to reach saturation and then allowed to drain for two days (prior to effluent application)
- A metal frame to contain water was dug into each plot receiving effluent/or rainwater so as to contain and let drain in, then subsequently removed
- A tray to collect drip was placed at the end of each of the plots
- The treated plot received 20 L of piggery effluent and the control 20 L of rain water, both irrigated by hand
- Rainfall simulation occurred the next day across the plots (to simulate the described condition)
- A 20 minute 90 mm/hr high intensity rain event was simulated on each of the twin plots
- Run off from each plot was collected at 3, 6, 9, 12, 15 and 18 minute, intervals
- The simulated rain was equal to "1 in 10 year" rainfall intensity event for a 20 minute storm at the trial location

Figure 10 Mini-plot and rain irrigator

Trial 3 objective – Assessing the use of grass filter strips to reduce pathogen run-off

Experimental design - Trial 3

- A bare paddock, with soil ready for crop production, a day before the trial was used for the study
- Eight plots (1 X 6 M length) were used; the lowest 1 M area, functioned as the filter strip, with or without grass.
- Figures 11 and 12 illustrate the design of the plots with and without grass filter strips
- The plots were irrigated to saturation point with pathogen free rain water and allowed to drain for 2 days
- The filter strip area was separated from the main plot via a metal divider to ensure that the effluent (or rain water) was contained within each plot (away from the filter strip)
- Two days after wet-up, the upper 5 m length of all plots was irrigated with 60 L of effluent.
- Once the effluent settled in the metal plates were removed
- Rain was simulated using a larger rain simulator
- Rain simulation was carried out over 2 days to reach saturation point, leading to run-off (
- The simulation was a rainfall intensity of 90 mm/hr was applied for a total of 35 minutes.
- Subsequent run-off was then captured from either bare soil or grass (i.e. testing effect of a grass verge as compared with a bare earth verge).

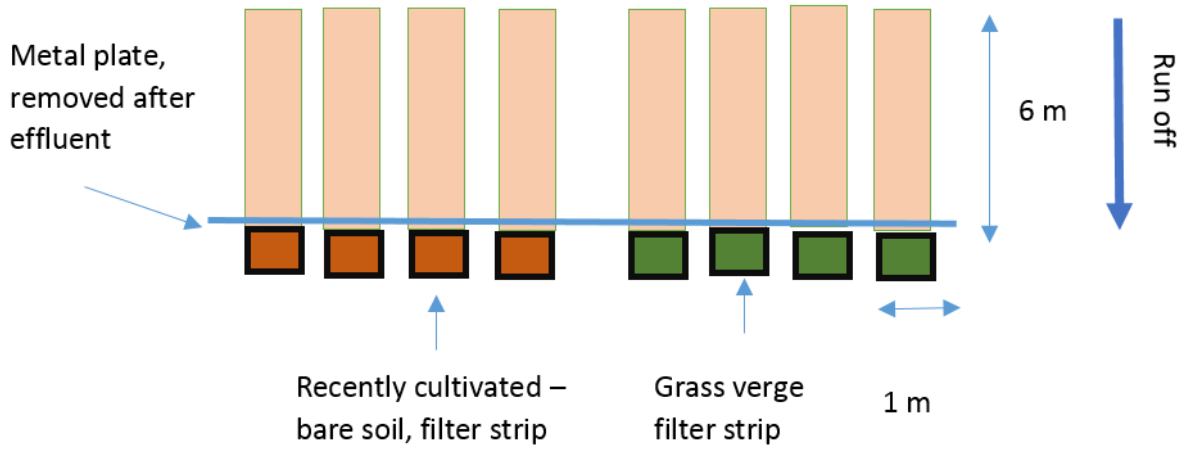

Figure 11 Design of plots with and without grass filter strips

Figure 12 Design of plots with and without grass filter strips

5.7.2 Summary of outcomes - Trial 2

The levels of *Arcobacter* and *E. coli* in effluent, effluent treated soil and the subsequent run-off is presented in Figure 13. There is a about a 2-log cycle difference between the levels in effluent (MPN/ 100 mL and soil (MPN/g). *E. coli* was detected in the run-off samples from the effluent treated plot 2 and based on the levels of *E. coli,* there had been cross contamination between effluent treated and rainwater treated plots. Similarly, the *Arcobacter* was not detected in control plots but was detected (at a lower level) in run-off, also suggestive of cross contamination between plots. Nevertheless, from an overall perspective there were higher levels of both *Arcobacter* and *E. coli* in run-off water from the treated plots compared to the controls, following run-off after (simulated) heavy rain. The levels of *Arcobacter* were higher than those graphically presented (under estimated at planning stage at lab).

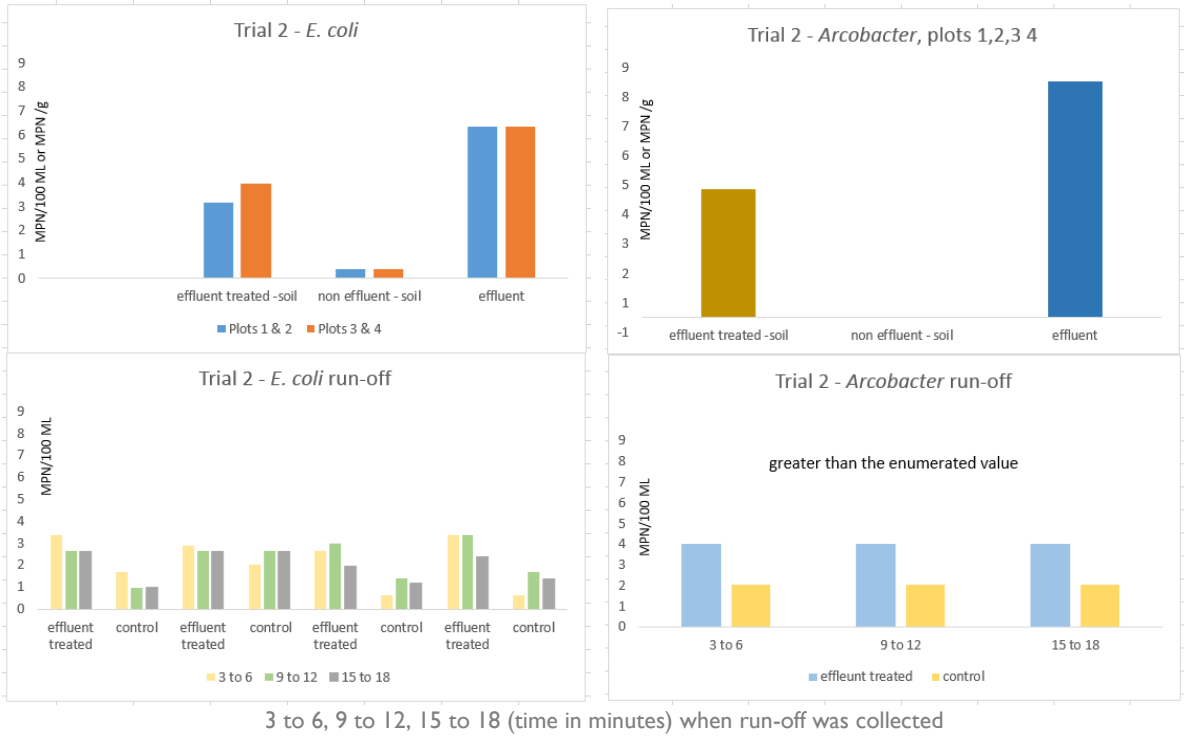

Figure 13 Trial 2 run-off - E. coli and Arcobacter

5.7.3 Summary of outcomes - Trial 3

The levels of *Arcobacter* and *E. coli* in effluent, effluent treated soil and subsequent run-off is presented in Figure 14. There is approximately 4-log cycle difference between the levels in effluent (MPN /100 mL) and soil (MPN/g) for *Arcobacter* and 3-log cycles for *E. coli*. There was no obvious difference between the levels of *E. coli* or *Arcobacter* spp. in the run-off water from the plots with a vegetative filter strip and those plots with no such vegetative strip. The volume of run-off water collected at the end of each of the plots is shown in Figure 15, as a function of time. The graph shows that there was no reduction in run-off water volume associated with the presence of the filter strips.

5.7.4 Summary, Trials 2 and 3

The objective of the overall study was to assess if rain water could mobilise bacterial of faecal origin from soils that had received piggery effluent. The second stage of the study was to assess if vegetative filter strips can either minimise or prevent the mobilisation of bacteria (*E. coli* and *Arcobacter*). The conditions of the experiment were to simulate a significant 1 in 10 year rainfall event of significant strength. This was achieved using a rainwater simulator over land (used for cropping).

Based on the outcomes of trial 2, there is clear evidence that a heavy rainfall event, within 24h of effluent application to soil, will result in bacterial contamination of run-off, which originated from effluent. Trial 3 demonstrated that the vegetative filter strip (when compared to non-use) was not able to contain bacteria that originated from effluent. There were some concerns that the filter strip used to contain the bacteria may not have been suitable. This is also demonstrated by the run-off volumes, which were not altered by the filter strip used in the trial. During both studies, the soil was irrigated to saturation, where pathogens could not be trapped by the infiltration of soil. In the second study, a filter strip was available to capture directly from overland run-off, which failed to reduce pathogen run-off. The study used a simple filter strip. This outcome suggest the importance of addressing design based on some of the conditions / constraints discussed earlier. The designs adopted for nutrient run-off also can contribute to managing pathogen run-off. Soil, type and saturation that support infiltration, vegetation (and slope) to trap and contain movement all play a role.

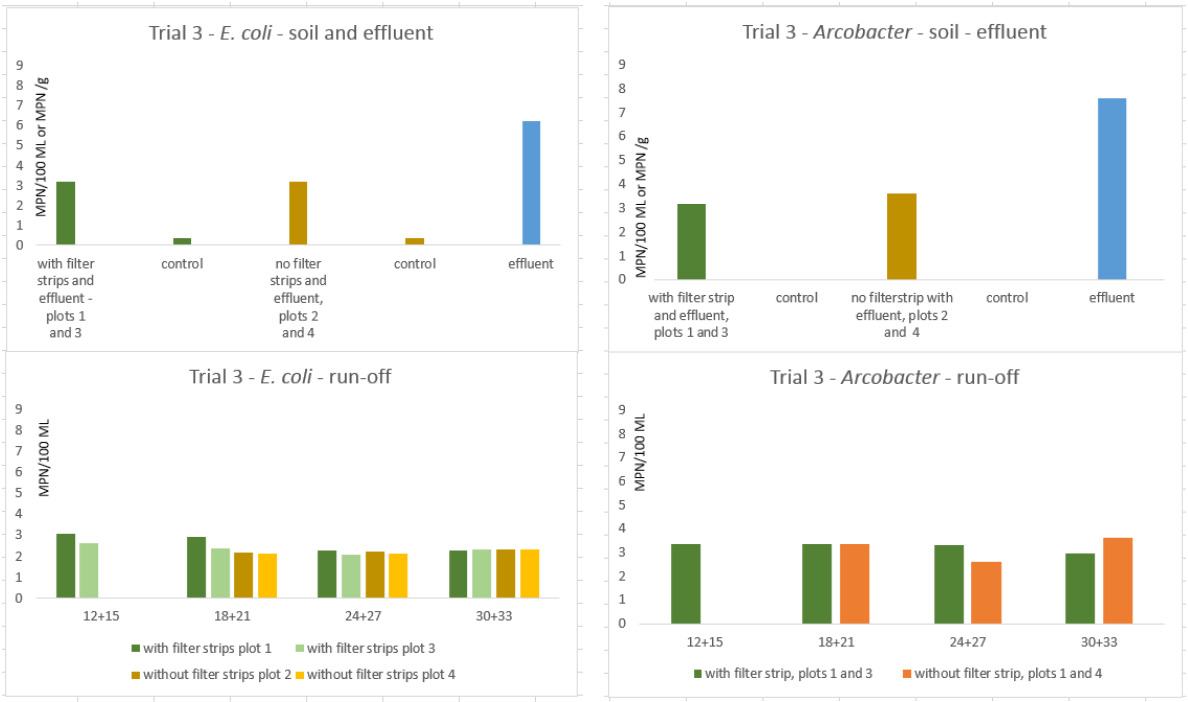

Figure 14 Trial 2 run-off - E. coli and Arcobacter

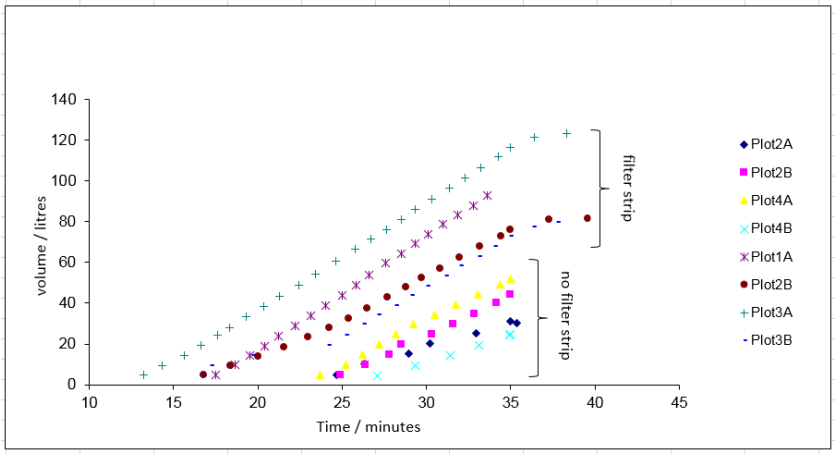

Figure 15 Change in volume of water with time on plots with a simulated rainfall event

5.7.5 Overall summary, the use of filter strips

The filter strips have a role of containing organism flow by infiltration and acting as a simple barrier. Additionally, soil type, vegetation and slope used to prepare the filter strips seem also to play a contributory role in managing pathogen levels during run-off. There is a need for the consideration the role vegetative filter strips may place in the establishment and possible maintenance of residual *E. coli* populations when used on a long term basis. The guidance provided for nutrient management using vegetative filter strips in the Piggery Environmental Guidelines for indoor piggeries can also support pathogen movement.

The present set of studies demonstrated pathogens such as *Arcobacter* and the indicator organism did mobilise in run-off from effluent treated soil (during soil saturation) and with the type of filter strip

used. The filter strips placement and location would be site and vegetation dependent taking into consideration the risks addressed. For example, a random heavy rain event or on-going usage for agricultural irrigation and the proximity to a watercourse.

5.8 Antimicrobial resistance among fluorescent Pseudomonas and the Bacillus cereus group isolated from soils exposed to piggery effluent

In food animal production antibiotics are commonly administered therapeutically on individuals or groups as well sub-therapeutically for growth promotion (Venglovsky et al. 2009). It is well accepted that the use of antibiotics in pig production can result in the presence of antibiotic resistant bacteria in the faeces of the treated pigs (Aarestrup et al. 1998a; b). Some classes of drugs used for animal health belong to the same classes as those used in human medicine (Guardabassi and Courvalin 2006), a further cause for such concern among some quarters with respect to the development of resistance to key antibiotics (Collignon 2009). It has been hypothesised that either the pathogens, or their resistance genes, may be transferred from the piggery to soil or the food chain via land application of effluent to crops (Chinivasagam and Blackall 2006). Sengelov et al. (2003) linked tetracycline resistance levels in soil to the immediate period when the pig manure was being added, with the resistance levels returning to background after five weeks, suggesting only transient impacts in soil. Higher antibiotic concentrations than that usually present in natural ecosystems can be found in soils treated with manure (Martinez, 2009) contributing to antibiotic resistance selection pressure. Such antibiotics can be bound to soil and still be active (Chander et al. 2005), although composting does degrade the antibiotics (Dolliver et al. 2008). Overall, residual antibiotics, resistant microorganisms and their associated resistance genes can be transferred via waste and affect the soil community with impact to the human food-chain (Thiele-Bruhn 2003).

The application of waste from pig ponds to land can be a source of resistant forms of gastrointestinal bacteria and a source of resistant genes as well as the actual antibiotics (Chee-Sanford et al. 2009), all of which can contribute to resistance among soil flora. Survival studies on Australian soils that received piggery effluent have shown a 90% die-off time (T_{90}) of 14 and 15 days for introduced *Salmonella* and *E. coli*, suggesting a low probability of long time survival bacteria of enteric origin in soil environments (Chandler and Craven 1980b). Chee-Sanford et al. (2001) have also shown the potential for indigenous soil microbiota to mobilise tet(M) genes in ground water associated with pig effluent lagoons. Thus, there is the potential for antibiotic resistant genes to move from the bacteria of animal origin to indigenous soil bacteria, creating an unwanted reservoir of antibiotic resistant genes (Jensen et al. 2001). The *Pseudomonas* and *Bacillus cereus* group are common across four ecosystems, water, soil, humans and animals (Nwosu 2001) and have been used to study antibiotic resistance in soil environments that had received piggery waste (Jensen et al. 2001).

A summary of this work undertaken during the previous APL study is presented in the following abstract – ready for submission.

Abstract: Impact of antibiotics on fluorescent *Pseudomonas* group and *Bacillus cereus* group isolated from soils exposed to waste from conventional and organic pig farming
H.N. Chinivasagam, P. Pepper, P.J. Blackall
This study evaluated the impact of antibiotics on fluorescent *Pseudomonas* spp and the *Bacillus cereus* group in soils exposed to piggery effluent (via re-use for irrigation purposes) or piggery waste (as a consequence of free-range pig production). The study looked at soils from six different sites that had

a history of exposure to piggery effluent (or waste) – i.e. four sites exposed to effluent from conventional piggeries that routinely used antibiotics (as required) and two piggeries that adopted organic production (no use of antibiotics). Isolates of fluorescent *Pseudomonas* spp. and the *B. cereus* group from each environment were examined by a disc diffusion methodology using nine antibiotics. The results were analysed from a population distribution aspect (and not a sensitive/resistant viewpoint) in order to understand bacterial population patterns for each antibiotic. Each population (farm/organism combination) was statistically analysed to determine whether the mean diameters were significantly above a selected interpretation point (the sensitive break point as defined by the disc manufacturer was used as the "goal post"). The key outcome of the study was that bacterial populations of both bacterial species sourced from the two different environments (i.e. exposed to both normal effluent and organic waste) did not show any distinct statistically significant population distribution pattern that could be associated with the use of antibiotics. The outcomes of this study will help address concerns arising as a result of re-using piggery effluent/waste in the environment.

5.9 To update where necessary and where possible, the recommendations on guidelines on piggery effluent made in the early 2000s

Both state, national and international guidelines were summarised during the previous APL study (Blackall 2001).
They are:

- National Health And Medical Research Council, 1995
- Victorian EPA, 1996
- NSW, recycled water committee, 1993
- Interim guidelines for declared waste water, QLD DNR 1996
- WHO guidelines for using treated waste water in agriculture – 1989 (Blumenthal et al, 2000)
- Recommended revised guidelines for using treated waste water in agriculture ((Blumenthal et al, 2000)

In analysing those guidelines, the study concluded that the relevance of the various guidelines (surveyed at the time) were not applicable (or suitable) for piggery effluent, due to the following:

- The range of pathogens discussed in guidelines that addressed human effluent were not relevant to piggery effluent.
- It was recommended that any guidelines for piggery effluent should not be directly adopted from human effluent.
- The recommendation was that piggery effluent guidelines be developed based on a Quantitative Microbial Risk Assessment (QMRA) approach

A chapter addressing suitability of adopting a complex process such as QMRA for piggery effluent is presented in the following section.

The recent National Environmental Guidelines for Indoor Piggeries (NEGIP) (2018) did note some of data produced by the APL project (Blackall 2001) such as the presence/absence and/or levels of some key pathogens and food safety pathogens. However, the National Environmental Guidelines for Indoor Piggeries (NEGIP) (2018) did not provide any specific guidelines or recommendations that focussed on the issue of pathogens in effluent re-use scenarios.

In order to update the recommendations given in the early 2000s, both state and national health related guidelines (as relevant to those reviewed during the previous study) that have since emerged are reviewed. In summarising, the key focus was placed on the microbiological criteria (and approaches adopted to manage bacterial risks). The guidelines summarised (below) are for sewage (and other recycled waters, i.e. storm, grey and roof waters). None of the guidelines specifically address animal waste water (following intensive farming). Most of the later guidelines have adopted the risk assessment / management approach to address human health, rather than a prescriptive approach (i.e. the use of indicator levels for application purpose). The reason the newly adopted approaches can contribute to piggery effluent re-use, includes the fact that some of the end applications that recycled waters target, are similar to the end uses of piggery effluent, e.g. food crop, pasture etc. These approaches could contribute to managing risks to both human health and the environment following piggery effluent re-use.

In undertaking this summary, the key focus is on the microbiological criteria (whilst the rest of the detail can be sourced from the relevant documents). The available guidelines are summarised as follows:

5.9.1 Australian guidelines for water recycling: managing health and environmental risks -2008 and 2009

Below are two more recent guidelines,

A. Australian guidelines for water recycling: managing health and environmental risks (phase 2) augmentation of drinking water supplies (Environment Protection and Heritage Council the National Health and Medical Research Council and the Natural Resource Management Ministerial Council. 2008). This comprehensive guideline extends aspect of phase 1 of this guideline and supports the sustainable recycling of sewage, grey and storm water, which are part of the national water quality management strategy.

B. Australian guidelines for water recycling: managing health and environmental risks (phase 2). Stormwater harvesting and re-use. (Natural Resource Management Ministerial Council, the Environment Protection and Heritage Council, and the National Health and Medical Research Council. 2009).

Following is a summary for the 2008 guideline **(A)** managing health and environmental risks, of sewage, grey and storm water

In summary, the guideline addressed below has moved away from complying with the stipulated levels of a bacterial indicator organism to a "risk management approach".
Some key points:

- The microbial risk is addressed by Disability Adjusted Life Years (DALYs) which considers the severity and impact of infection (and subsequent illness), (thus, differentiating between mild and severe illness) to humans; possibly because of achieving drinking water status.
- Risks of recycled water are differentiated between inputs (i.e. agricultural residential, industrial), water source (sewage or storm water).
- Microbial risks for recycled water are addressed across the distribution chain (e.g. infrastructure, treatment/disinfection, storage and distribution).
- The "hazard identification and risk management" approach is adopted, e.g. for stormwater catchments, entry of livestock or heavy rain).

- The microbiological hazards (specific pathogens of relevance and concern) are identified for human sewage. They are *Salmonella*, *Campylobacter*, pathogenic *E. coli* and various viruses and protozoa)
- The potential for variability of pathogens (and their concentrations) in sewage have been recognised (i.e. depending on the rates of human illness in the population, at various times)
- Such variability is also possible in storm water (e.g. influence of other sources, human and animal activity in catchments, seasonal and rainfall patterns).
- The organisms of choice being addressed via Microbial Risk Assessment in this guideline are *Campylobacter* (representing bacteria), rotavirus and adenovirus combination (representing enteric viruses) and *Cryptosporidium* (representing protozoa and helminths)
- The risk from ingestion of water as drinking water is then calculated (also taking into account DALY's) to assess the required log reduction (for sewage) to produce safe water for drinking. This was calculated for *Campylobacter* to be 8.1 log (i.e. representing bacteria).

Following is a summary for the 2009 guideline (**B)** (Storm water harvesting and re-use with a focus on roof harvesting)
In summary:

- This guidelines includes both roof water (non-residential) and storm water (collected run-off from drains, water ways or run-off)
- Re-use of stormwater for small to-medium scale application includes open-space irrigation (including playing fields, golf courses, bowling greens, parks and gardens)
- Re-use of stormwater for larger schemes, include home use, food crop irrigation (home grown), food crop irrigation (commercial).
- Both environmental and human health risks are recognised in using recycled stormwater
- Additional treatment for storm water is not required in order to minimise "environmental risks" (under stipulated conditions) but is required to address "human health risks" as presented in Table 13 (via log reductions) for bacteria.

Table 13 Stormwater treatment criteria for public, open-space irrigation (no access control) — managing health risks (Environment Protection and Heritage Council 2009)

Parameter	Stormwater treatment criteria
Disinfection	>1.5 $\log_{10}$ (96%) reduction of viruses and bacteria >0.8 $\log_{10}$ (82%) reduction of protozoan parasites *E. coli* <10 colony forming units (CFU)/100 mL (median)

- For stormwater disinfection options (amongst others cited) include the use of constructed wetlands, but the absence of data on the retention of the "reference pathogens"(in wetlands) makes this an option with high variability (though data could be sourced from literature)
- It is recognised that both "storm events" and "run-off" (from land) can contribute to variability in thermotolerant coliforms and *E. coli* levels in catchments storing stormwater
- The lack of statistical correlation between faecal indicators and human pathogens in storm water is recognised and thus the need to monitor a pathogen is required, (though this is a costly approach).
- The situation is different for roof water where the main faecal contributors are birds and small mammals. Again, due to the lack of statistical co-relation between pathogens and *E. coli*, direct

pathogen monitoring is preferred with, *Campylobacter* the organism of choice, the most commonly detected pathogens in roof water.

- Health risk assessment for storm water is conducted, via quantitative risk assessment, using the maximum observed MPN level in storm water i.e. 15 MPN/L "reference pathogen" (*Campylobacter*), and was calculated to be 0.038 infectious bacteria/person/year.
- The above risk assessment approach is preferred over the use of an indicator bacterial organism (*E. coli*), due to the poor correlation between the "reference pathogen" and the indicator bacterial organism.

5.9.2 Victorian Guideline for reclaimed water (EPA Victoria 2003)

- The guideline addresses pathogen risks of reclaimed water via exposure routes to human health, through food-safety (i.e. food crop and /or livestock)
- The potential for ground and surface waters to contribute to pathogen risks to the environment is recognised
- The treatment processes adopted should address appropriate reductions in pathogen levels, in order to protect human and livestock exposure from reclaimed waters.
- The land receiving reclaimed water cannot be used as an extension for the pathogen treatment process
- For food-safety risks, the exposure routes were specified as contact between food crop and pathogens and reclaimed water and soil
- The water quality objective for "tertiary pathogen reduction" is specified as < 10 *E. coli* /100 mL e.g. human food crops consumed raw (among some other uses)
- The water quality objective for "secondary pathogen reduction" is specified as < 100 *E. coli* /100 mL e.g. dairy cattle grazing (among some other uses)
- The water quality objective for "secondary pathogen reduction" is specified as < 1000 *E. coli* /100 mL e.g. human food crops cooked/processed, grazing/fodder for livestock (among some other uses)
- The water quality objective for "secondary pathogen reduction" is specified as < 10000 *E. coli* /100 mL e.g. non-food crops including instant turf, woodlots, flowers (among some other uses)
- The guideline further addresses in detail specific irrigation methods that target the acceptable agricultural uses, livestock access and food safety controls along with stipulations for the various classes of reclaimed water.

5.9.3 New South Wales Guidelines (NSW Department of Primary Industries - Office of Water 2015)

- This guideline targets a risk management approach and is a detailed document addressing the various steps
- Where minimal risks are envisaged for both public and environmental health, a desktop risk assessment can be undertaken which includes low exposure uses i.e. irrigation of produce that will be cooked, pasture for livestock, crops for fodder etc. and areas of restricted access.
- Log reduction targets for "bacteria" (not specified) have been provided based on "end-use".
- "Indicative log reduction values, based on treatment, is provided for "bacteria" (not specified). e.g. for "lagoon storage" = 1- 5 log reduction; "wetland surface flow" = 1 log reduction; wetland subsurface flow = 1-3 log reduction
- Non-treatment barriers such as subsurface irrigation of above ground crops, drip irrigations for raised crops (no ground contact) fruits (e.g. apple) a 4-log reduction value is specified for "bacteria (not specified) in contrast to a lesser risk (drip irrigation with crops with limited ground contact (e.g. tomatoes and capsicums).

- As a part of risk management, a hazard is identified (e.g. bacteria) and critical control points (CCP) are specified, (as means of managing risks). Amongst others listed, a CCP specified is "lagoon retention time – in days" which allows the specified hazard to be addressed.
- Finally, verification is part of a process for risk management – with monitoring for microbial indicator organisms. The organisms listed as typical are *E. coli* and *Clostridium perfringens* (the frequency of testing depending on the level of exposure).

5.9.4 Queensland water recycling guidelines (Environmental Protection Agency, Queensland 2005)

- A risk assessment approach is adopted (i.e. hazard identification, exposure and dose response assessment, identification of critical control points as is usually adopted for this process.
- The possible health impacts for bacterial pathogens is addressed via "Quantitative Microbial Risk Assessment", which recognises the "minimum infective dose" for pathogens
- The possible pathways that can impact on human health are addressed as being "direct" ingestion of contaminated water, droplets or airborne particles, foods, inhalation of contaminated water droplets (and aerosols) and finally licking and direct contact with skin
- Microbial indicators are used to "classify" water classes (for various uses) and provide "treatment efficacies" stipulated via the use of an indicator organism. (Table 14)
- *E. coli* is used as the bacterial organism of choice for recycled water but was not a preferred indicator once recycled water was in open storage, due to potential contribution faeces from animals and birds
- *Clostridium perfringens* is used as an indicator for parasites (due to spores of *Clostridium perfringens* being in similar size and resistance to cysts of *Giardia lamblia* and the oocysts of *Cryptosporidium parvum*.
- Following is the microbiological (*E. coli*) criteria for various classes of water and amongst the various other examples, those that may be of relevance as listed for the various classes of water

Table 14 Microbiological water quality specifications classed A - D recycled water

E. coli cfu /100ml			
Class A	Class B	Class C	Class D
>10	>100	>1000	>10,000
(1) Irrigation of public open spaces Golf courses (2) food crops consumed raw or minimally processed (3) Above ground food crops with *above ground* irrigation (4) Root crops	(1) pasture/fodder for dairy animals without withholding period (2) Wash-down of hard surfaces in agricultural industries	(1) Controlled access or subsurface irrigation (2) Above ground food crops with *below ground* irrigation (3) Pasture/fodder for dairy animals with withholding period of five days (4) Pasture/fodder for other grazing animals except pigs with withholding period of four hours	(1) silviculture, turf, cotton, wholesale nurseries with controlled access and other safeguards to protect the health of workers or neighbours

5.9.5 South Australian recycled water guidelines (Government of South Australia 2012)

- Incorporates sewage, stormwater, and greywater and roof water and is the only guideline to incorporate "animal waste water".
- Animal wastewater is defined as originating from animal industries (inclusive of abattoirs, sale yards, dairies and feedlots).
- The pathogens of concern are *Salmonella* (in addition to the parasites *Cryptosporidium* and *Giadia*)
- It is mentioned that whilst the overall guideline does not apply specifically to animals, the "generic approach", adoptable for all classes of recycled water was recommended.
- The most relevant are listed in Table 15 is the "indicative minimum treatment required for various uses of recycled water".

Table 15 Indicative minimum treatment required for various uses of recycled water

Indicative log removal (Bacteria…….)	Microbiological criteria†: *E. coli* (median org/100mL)	Typical Treatment Process Train	Scheme Class/type
landscape irrigation	<1000	Secondary treatment or primary treatment with lagoon detention	Class C
Non-food crops e.g. trees, turf, woodlots	<10000	Primary sedimentation plus lagooning, or Full secondary	Class D

5.9.6 EU guidelines (Alcalde-Sanz, and Gawlik 2017).

The above guidelines titled "Minimum quality requirements for water reuse in agricultural irrigation and aquifer recharge - Towards a legal instrument on water reuse at EU level" (Alcalde-Sanz and Gawlik 2017), has comprehensive details. Listed are some points that can be relevant to piggery effluent re-use:

- Minimum quality requirements for re-use should be developed based on a risk management frame work as recommended by the World Health Organisation (WHO), which means managing risks in a pro-active manner
- The Australian guidelines for water recycling and the Australian drinking water guidelines (NHMRC-NRMMC, 2011) are cited as an example
- In addressing health and environmental risks for water reuse in agricultural irrigation, the types of crop categories are identified
- The steps addressing health risks (hazard identification, dose response, exposure assessment and risk characterisation are recommended
- In addressing environmental risks, via hazard identification, environmental matrices such as soils, related ecosystems and crops recommended international guidelines (Food and Agriculture Organisation, WHO and United States Environmental Protection Agency)
- The exposure of the environmental end-points such as likelihood to exposure to a hazard needs to be identified. For example, limits of heavy metals in soils receiving sewage sludge
- The estimation of the consequences to a hazardous event such as determination of the consequence of an event, which can be addressed via a risk assessment matrix. For example, prevention of adverse effects to surface or ground water
- Finally, adopting preventative measures. For example, treatment options, reducing exposure by preventative or restrictive measures

- Details of water treatment, such as, irrigation and storage options are listed. For example microbial re-growth or natural decay in open or closed storage reservoirs
- In the section "Health and environmental risks considered for agricultural irrigation" the use of reference pathogens to address log reductions is suggested. Based on WHO guidelines - water reuse and drinking water, they are *Campylobacter* for bacteria, rotavirus for viruses and *Cryptosporidium* for protozoa. The log reductions for these based on worst case scenario (i.e. irrigation of lettuce with reclaimed water) are as follows *Campylobacter* 5 $\log_{10}$ reduction, rotavirus 6 $\log_{10}$ reduction and *Cryptosporidum* 5 $\log_{10}$ reduction.
- Further details could be sought from the above document

The details of the above have been discussed in the following document. "Summary form Request for scientific and technical assistance on proposed EU minimum quality requirements for water reuse in agricultural irrigation and aquifer recharge" (Allende et al. 2017) and "Proposed EU minimum quality requirements for water reuse in agricultural irrigation and aquifer recharge: SCHEER scientific advice." (Rizzo et al. 2018). Whilst the details could be sought from the three relevant documents some of the points of interest, amongst others from (Allende et al. 2017) are as follows:

- Better documentation of the rationale of the decision for the suggested microbiological requirements
- The use of an indicator is recommended when there is clarity to the choice of such indicator to predict the probability of a pathogen, as the use of indicators remains controversial and at times discredited
- However the use of indicators for treatment efficacy was acceptable, where performance of established disinfection treatments are validated
- From a microbiological perspective the use of both a verification monitoring using specific levels of *E. coli* (cfu/100 mL) as well as using additional preventative measures is recommended
- The need to consider human health from possible contaminated water being a source of pathogens to fresh fruit and vegetables (and pasture and fodder crops via the consumption of animal products) when used for agricultural irrigation
- The need to consider animal health (e.g. pasture or feed crops used to feed animals) and the need for minimum quality requirements to protect animal health

5.9.7 Possible risk management option for piggery effluent based on the summarised guidelines

Most of the guidelines summarised in this section have adopted a risk management approach to help address bacterial pathogens and their risks to human health. This includes hazard identification and risk management along the various pathways linked to effluent re-use scenarios. A flow diagram Figure 16, (adapted from Environment Protection and Heritage Council, the Natural Resource Management Ministerial Council and the Australian Health Ministers' Conference 2006) was created for piggery effluent. This flow diagram (Figure 16), sets out potential pathways that can impact on human health as a consequence of storing (or re-using) piggery effluent in and around a piggery. As stated in NSW guidelines (NSW Department of Primary Industries - Office of Water 2015) and as part of the risk management process, once a hazard is identified, e.g. bacterial levels in piggery effluent lagoon, a critical control point (CCP) can be specified to manage that risk (e.g. lagoon resident time). This can be followed by monitoring *E. coli* to address conformity to the required *E. coli* levels. The EU guidelines specify the need to protect animal health (pasture or feed crops). Thus, the flow diagram addresses

both human and animal health with consideration to environmental movement as a means of providing a framework for risk management.

Some of the outputs related to summarising key microbiological criteria for recycled water addressing human health are presented in Table 16. These include treatment, options for pathogen reduction, environmental risks, indicator organism and treatment based log reduction and end use criteria (or water class classification). Thus, Table 16 was prepared as a starting point. The dominant treatment approach for piggery effluent is recognised as on-site storage in lagoons (or ponds), taking into account that initially pig effluent is resident in the primary pond (for a specified period), then follows to the secondary, and in some cases a third pond. The log reductions are arbitrary as they were adapted from the various guidelines for recycled waters previously summarised. The potential for other treatment options (other than ponding) to become available has been recognised and the required end use criteria have also been included.

5.9.8 E. coli *as an indicator for treatment efficacy*

As was the situation for most recycled waters, for piggery effluent in Australia, *E. coli* can be the organism of choice to address treatment efficacy where performance of established disinfection treatments are validated (Allende et al. 2017). The organism is in sufficiently high numbers in piggery effluent and *Salmonella* being only intermittently present in low numbers and *Campylobacter* can die-off rapidly (Chinivasagam et al. 2004). For example, in piggery effluent irrigated pasture soil *Campylobacter* die-off was 0 -4 days in summer and 0 – 7 days in winter (compared to *E. coli* die-off, 14 - 23 days in summer and 28 – >85 days in winter (Chinivasagam et al. 2005). Levels of *E. coli* are commonly used across various guidelines to achieve a water class status for re-use categories, based on the levels of risk or defined levels for stipulated end-use applications (Table 16).

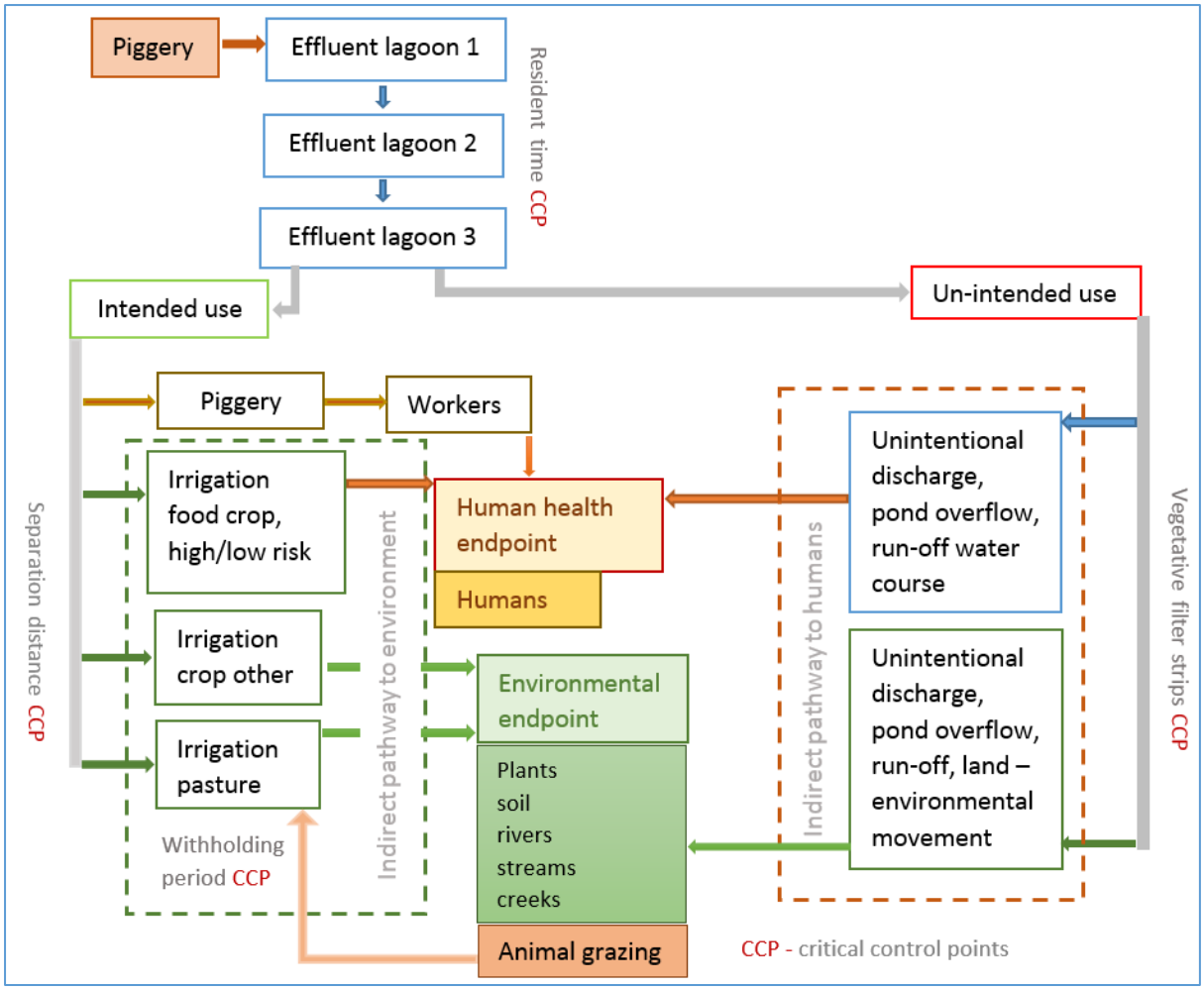

*Figure 16 Flow diagram to address potential risk management in piggery**

(*adapted from Environment Protection and Heritage Council, the Natural Resource Management Ministerial Council and the Australian Health Ministers' Conference 2006)

5.9.9 Choice of E. coli *as an indicator organism for* Salmonella *and* Campylobacter

There are also difficulties in using *E. coli* as an indicator for presence of persistence of pathogens in piggery effluent and in soils irrigated with piggery effluent. As previously described, Allende et al. (2017) have outlined that the suggestion of a recommended indicator be done when there is clarity to the choice of such indicator to predict the probability of a pathogen. *E. coli* is consistently present in high levels in piggery effluent (Chinivasagam et al. 2004). In contrast *Salmonella* is not commonly present and when present is at low levels in piggery effluent. Thus, *E. coli* is a poor indicator organism for *Salmonella*. *Campylobacter* is consistently present in effluent but dies off rapidly in re-use scenarios such as effluent irrigation (Chinivasagam 2005). In, contrast, *E. coli* shows prolonged survival during re-use scenarios such as effluent irrigation. Hence *E. coli* is not a good indicator for the presence of *Campylobacter* in piggery effluent re-use scenarios.

Indeed, for both effluent and effluent irrigated soils the use of *E. coli* (as an indicator of a pathogen) seems not to be a suitable option. Firstly *E. coli* can be present naturally in soils before the application of piggery effluent (Blackall 2004). Secondly, based on previous APL work (Blackall 2004), *E. coli* has been shown to re-grow in effluent irrigated soils with such soil *E. coli* levels increasing with rain.

5.9.10 Direct measurement of a pathogen

Some guidelines suggest the measurement of the true pathogen in re-use scenarios. As an example, the 2009 guidelines for storm water harvesting and re-use with a focus on roof harvesting, (Environment Protection and Heritage Council 2009) have outlined the need to monitor a pathogen, due to the lack of statistical correlation between faecal indicators and human pathogen, though they acknowledge it is costly. Thus, the same guideline uses *Campylobacter* as a reference pathogen to undertake quantitative risk assessment using available pathogen levels (Environment Protection and Heritage Council 2009).

Should the need arise, such a QMRA approach could be adopted for piggery effluent the relevant organism would be the emerging pathogen *Arcobacter*. It is present in high numbers 10^8 MPN/ 100 and can be quantified (Chinivasagam et al. 2007). However it would be costly to monitor on a routine basis and can be used as previously described for the purpose of quantitative risk assessment as described. *Arcobacter* is also recognised as an emerging human pathogen of concern in waters (as previously discussed).

Table 16 Merging of options adopted for recycled waters with piggery effluent to assist address microbiological (bacterial pathogen) risks

Pond Treatment	**Options for pathogen reduction**	**Environmental or human risks**	**Indicator organism**	**Treatment based log reduction**	**End use criteria (or Water class classification?)**	**End uses**
For assessing treatment efficacy *E. coli* can be used levels dependable, naturally present in pigs Quantitative Australian data available Table 1 (effluent), Table 3 (aerosols), Tables 18, 19, 20, 21, 22, 23 for die-off (pasture soils)						
Primary pond	Resident time (in pond)??	Storm event/ overflow, run-off, = pathogen levels??	NA	Need to flow through to secondary pond	NA	NA
Secondary pond	Resident time (in pond??)	Storm event/ overflow, run-off = pathogen levels?? Safe guard worker health	*E. coli*	Log reduction? To reach required *E. coli* level to address end use	(a) "secondary pathogen reduction" < 10,000 *E. coli* /100 mL (b) "secondary pathogen reduction" < 1000 *E. coli* /100 mL	(a) non-food crops, turf, trees (safeguards to protect health of neighbours and workers) (b) Landscape irrigation. Above ground human food crop with below ground irrigation cooked/processed, grazing /fodder for livestock. Pasture for dairy, withholding period five days.
Secondary pond (storage)	Resident time (in pond??)	Storm event/ overflow, run-off = pathogen levels?? Aerosols transmission pathway	*E. coli*	Log reduction? (	Flushing of pig sheds	e.g. Seasonal water requirements
Other improved treatment option	Description of validated treatment??	Based on treatment design / time / type??	*E. coli*	Log reduction?	(a) "secondary pathogen reduction" < 100 *E. coli* /100 mL (b) tertiary pathogen reduction" < 10 *E. coli* /100 mL	(a) Pasture/fodder for dairy animals with withholding period for five days (b) Human food crops consumed raw or minimally processed. Above ground food-crops with above ground irrigation. Root crops.

5.10 Quantitative Microbial Risk Assessment (QMRA)

QMRA is a process that evaluates risks to humans based on exposure to specific pathogens to defined scenarios. It characterises pathogens and their exposure, for example, to treated reclaimed water. The risks to an exposed human population is estimated using a dose response model which includes the probability of infection from the chosen pathogen. Finally, risks per year is estimated via the exposure frequencies that can occur over that time and then compared to a health risk target set by a regulator, Hass et al. (1999). The risk is often expressed as disability-adjusted life-years (DALYs), an approach that takes into account risk of infection and illness including the quality of life (which can be impacted by the severity of that illness).

However, there are a number of limitations and problems with the use of QMRA. Firstly, QMRA requires a high level of technical knowledge and resources and has been most widely used in high-income countries where government agencies have promoted and supported the use of the approach (WHO 2016). A further key limitation is that QMRA requires detailed information on the fate, transport and die-off of the pathogens of interest (WHO 2016). This lack of data is often overcome by using worst case scenarios (WHO 2016), a situation that is now recognised as resulting in an overestimation of risk (WHO 2016). Irrespective of these limitations, there have been significant updates in the software available to undertake QMRA. Thus, the absence of the use of QMRA for piggery effluent re-use scenarios is not based on a lack of appropriate support tools, but rather the complex inputs that may be needed to model risks for the various situations, that have to be targeted.

An Australian study (Ahmed et al. 2017) has demonstrated the use of QMRA to provide a range of management options for source separated urine to be used as liquid fertilizer. This required complex mathematical modelling inputs to arrive at the set objectives. In the Ahmed et al (2017) study, the actual inactivation data for *E. coli* and MS2 phage were used as indications for the inactivation of *Campylobacter* and rotavirus respectively. In the case of piggery effluent, a simple risk management approach (rather QMRA) would thus be appropriate (and has been described in the section for guidelines). As there is only a limited range of recognised pathogens associated with piggery effluent including the re-use options currently adopted, it would appear that one of the strengths of QMRA, an ability to deal with poorly characterised risks, is not relevant to the effluent re-use scenario.

Based on European Union (EU) (Regulation 2160/2003) that targets the reduction of zoonoses and zoonotic agents at varying stages of the food chain, QMRA has been adopted for the "pig food process chain". The European Food Safety Authority (EFSA) was commissioned to carryout QMRA for *Salmonella* in slaughter and breeder pigs with the aim of providing recommendations for *Salmonella* control along the food-process chain (Romero-Barrios, et al. 2013). This study included the modelling of the whole food-process chain i.e. microbial loads from "farm to consumption" (Romero-Barrios, et al. (2013). This exercise has been carried out for the various steps for a food-process chain, which is a well-established and documented process, driven by regulation to address pathogen management along the food chain. The re-use options for piggery effluent at present are varied and do not have the complexity as they would for a food-process chain, as is for pork meat.

While drinking water QMRA applications are now common (WHO 2016), government agency support for QMRA applications on piggery effluent is never likely to reach the level provided for this situation. Hence, the support and technical resources necessary for QMRA approaches to managing the re-use of piggery effluent seem unlikely to be available in the near future. It is important to note that the WHO suggests that there is no justification to support adopting exceedingly high level risk assessment

methods such as QMRA in situations where appropriate less technically demanding methods are relevant and applicable (WHO 2016).

A search of the literature has shown that there appears to have been no use of QMRA as a means of providing formal guidance on the re-use of piggery effluent. Further, while this review has identified some possible pathogens (e.g. *Arcobacter*) and some additional low level pathogens (*B. pseudomallei*), these pathogens have no available data on dose response (a key requirement for a QMRA approach). As well, even for those pathogens with well-known dose response characteristics (e.g. *Salmonella* and *Campylobacter*), there would be a need for considerable extra data such as the effect of different irrigation methods, seasonality and meteorological conditions on pathogen persistence and transport.

Overall, QMRA remains a potential approach but offers little practical advantage and has several problems (specifically a lack of data in a number of key areas that would require considerable additional research). A watching brief should be maintained on the potential use of QMRA but there is no current strong argument for the use of QMRA. A possible risk management approach as illustrated in the previous chapter could be a more practical approach to manage risks in piggery effluent.

5.11 Other pathogens of interest that have arisen since the APL study

Three pathogens are discussed here, they are *Clostridium difficile, Leptospira* and *Burkholderia pseudomallei*. Both *Leptospira* and *Burkholderia pseudomallei* have been included in this report due to recent public interest in these organisms. *Pfiesteria piscicida* was part of the original study and has been updated with Australian literature

5.11.1 Clostridium difficile*, pathogen of uncertain significance*

Clostridium difficile is a spore forming strict anaerobe, distributed in the environment with a wide host range and the ability to colonise the intestinal tract of animals and humans (Rodriguez et al. 2016). The organism can cause gastrointestinal diseases in humans and animals with a predominance of disease causing ribotype 078 being dominant among various animals (Martin-Burriel et al. 2017). There is no direct evidence of transmission from animals to humans though circumstantial evidence exists for zoonotic transmission (Hensgens et al. 2012). *C. difficile* infections were originally restricted to hospital environments, but recent molecular studies implicate both animals and food. This has changed the perception of the epidemiology of this organism, due mainly to the presence of toxigenic strains in the environment (Rodriguez-Palacios et al. 2013).

Pigs are known to be a reservoir of *C. difficile*. On comparing age groups in a vertically integrated piggery the highest incidence was among suckling piglets (50%), lactating sows (23%), effluent from farrowing barn (8.4%), nursery (6.5%), pork products (3.9%) and finally grower finishers and breeding boars (3.9%) each (Norman et al. 2009). In Ireland, a study has isolated PCR ribotype 078 toxic to humans from pigs with the organism prevalent in 77% pig litter and 31% sow samples (Stein et al. 2017). The organism was isolated from manure compost across 14 pig farms, including the toxigenic ribotype 078 (Usui et al. 2017). The organism has been isolated from pig slaughter houses, pig stools, colons, carcasses and scalding water were genetically closely related though there was no evidence of food-borne transmission .(Wu et al. 2017).

The overlap of *C. difficile* ribotypes between humans and animals is suggestive of transmission between these environments (which include soil, water, and sediment) (Rodriguez Diaz et al. 2018). They have been isolated from ready to eat raw vegetables (Eckert et al. 2013). The food-borne route is implicated

due to the fact that ribotypes isolated from diseased humans are also present in foods, though no food-borne outbreaks attributed to *C. difficile* have been reported, thus the role of the environmental reservoir in transmission remains unclear (Keessen et al. 2011). *C. difficile,* fits the criteria of a food-borne pathogen due to the organism's presence in foods such as meat, seafood and fresh produce, but no food-borne outbreaks have been linked to the organism (Warriner et al. 2017). In addition, there is no conclusive evidence that the various food matrixes can support spore germination (Warriner et al. 2017). *C. difficile* is regarded as an "un-specified food-borne agent", because though the organism is found in a range of food products there is no conclusive evidence that these foods are of risk to consumers (Candel-Pérez et al. 2019) The infectious dose of the organism to humans remains unknown (Moono et al. 2016).

C. difficile has been isolated from neonatal pigs in Australia (Knight et al. 2015). Australian studies have isolated common ribotypes in both human and pigs, thus suggesting potential food-borne transmission. This has led to highlighting potential risks attributed to piggery effluent re-use (seen as mode dissemination) with suggested pathways being the agricultural recycling of piggery effluent, including pig waste compost (i.e. land application for agriculture, pasture or in-shed re-use, vegetable growing, compost in community settings). This Australian study however, acknowledges that the human cases studied were not linked to livestock occupations (or those that lived closed to piggeries) with the mode of transmission between humans and pigs remaining unclear (Knight et al. 2017). However, there is no conclusive evidence based on literature with respect to the zoonotic potential of the organism and the specific role of pigs. More data is required to understand whether these agents are a risk or not.

5.11.2 Leptospira*, pathogen of significance as occupational risk*

Leptospirosis, is a zoonotic disease that affects both humans and animals caused by the pathogenic strains of *Leptospira* which can penetrate the skin via cuts and abrasions (Fernandes et al. 2016). Based on the previous APL literature review *Leptospira* was identified as a pathogen of "low risk" (Blackall 2001). Rodents are considered the primary reservoir (and permanent carrier), from which other animals including pigs can act as a carrier (Tilahun et al. 2013). In developed countries, animal production (including pigs) along with agriculture are considered as risks due to the organism's ability to transfer via the soil – water pathway, which can be a source of human contamination (Adler and Moctezuma 2010). Pathogenic strains are also known to survive in low nutrient environments (i.e. moist soil and fresh water) for extended periods (Evangelista and Coburn 2010). Pigs can get infected via contaminated soil (Alexander et al. 1964). Wet seasons can be a higher risk, as the organism can survive in stagnant water, ponds, slow moving streams, surface pools, waterways leading to human exposure via contaminated water and soil (Srivastava 2008; Smith and Self 1955). The organism has been isolated from piggery effluent (Tomescu et al. 1974) and can survive in pig effluent pond from 24 to 48 hours with a maximum 96 hours (Minzat and Tomescu 1975). In Australia, incidences of leptospirosis has been reported from South Johnstone river in North Queensland, following heavy rain and proximity to rivers and low lying areas, where urine of carrier animals can be a source of contamination (Smith and Self 1955). There is no evidence of movement via the food-chain.

5.11.3 Burkholderia pseudomallei*, pathogen of low significance*

Burkholderia pseudomallei (responsible for melioidosis) is regarded as a saprophytic environmental organism that can occur in wet soils and stagnant waters in regions where this organism is regarded as endemic (Galyov et al. 2010). Melioidosis is endemic to South East Asia and northern Australia and

linked to natural disasters such as floods, (which is likely to increase due climate change) via exposure to contaminated soil and water (Paterson et al. 2018; Millan et al. 2007). Melioidosis is thought to have arrived in northern Australia due to importation of animals from countries where it is endemic; though isolates have also been sourced from Queensland, Southwest and Western Australia (Samy et al. 2017). The disease in Northern Australia is linked with higher than average rainfall (Parameswaran et al. 2012) with possible transmission via aerosols during rain and strong wind (Wiersinga et al. 2012). Other pathways include transmission via soil-water and infection occurs via ingestion, inhalation or inoculation (Limmathurotsakul and Peacock 2011). *B. pseudomallei* is classified as an emerging swine zoonosis (Khan et al. 2013), though not a lot of pig related incidences are reported in overseas literature (or Australian literature).

5.11. 4 Pfiesteria piscicida *(update and brief summary from original review)*

Pfiesteria and "*Pfiesteria* like dinoflagellates" are known to occur over extensive geographic and environmental ranges and are responsible for fish kills as well as impacting human health. The generally held belief is that pollution from human sewage, urban run-off, animal wastes and farm run-off, can play a role. For example, a piggery waste spill (over 40,000,000 litres) reached a small receiving river and estuary in North Carolina, USA. After two days, the 29 km freshwater segment that the waste had traversed was anoxic with 4000 dead fish (Burkholder and Glasgow 1997). Exposure to waters contaminated with toxic forms of *Pfiesteria* can cause memory loss, confusion, acute skin burning, headaches respiratory irritation, skin rashes and gastro-intestinal problems in humans, i.e. nausea, vomiting, diarrhoea, and abdominal cramps (Bever et al. 1998; Golub et al. 1998). Whilst there are reports originating from the US, there were no reports in Australia at time of writing the last review in the early 2000s.

Since then, *Pfiesteria piscicida* has been isolated from Australian waters with specific concerns to the marine environment. The National System for the Prevention and Management of Introduced Marine Pest Incursions has identified seven harmful bloom, toxic dinoflagellate species one of which is *Pfiesteria piscicida* (Dias et al. 2015). Table 17 lists the reported isolations.

Table 17 Pfiesteria piscicida *detection in Australia*

Location detected	Comments from publication	Reference
Tasmania	Concerns, fish kills and human illness; organism detectable by PCR in water and sediment	Cited in (Dias et al. 2015)
Australia	Two *P. piscicida* strains isolated from ballast water suggesting possible introduction of *P. piscicida* into Australian estuaries via ballast water.	(Park et al. 2007a)
Tasmania	*P. piscicida* was detected only once, in May 2005	(Park et al. 2007b)

Management plans controlling pollution from all sources, including intensive animal production facilities, would reduce the excessive nutrient load to aquatic systems that is regarded as being primarily responsible for *Pfiesteria* outbreaks. There has not been any evidence of the presence of *P. piscicida* in piggery effluent ponds. The unplanned release of piggery effluent can, and has, resulted in nutrient overload situations that have triggered the proliferation of *P. piscicida* in North Carolina, USA (Burkholder et al. 1997). There remains a need for the industry to be aware of the potential of this organism (and similar relatives) to cause problems for the pig industry and to ensure effluent handling processes are used that prevent nutrient overloading of ecosystems.

5.12 Appendix to soil survival studies

Table 18 Comparative bacterial levels over time in soil (from pasture) irrigated with pond effluent in piggeries G and W in summer*

	Piggery G**						**Piggery W*****					
Day	*E. coli* test	*E coli* cont.	*Arcobacter* test	*Campylobacter* test	Moisture %	Temp °C	*E. coli* test	*E. coli* cont.	*Arcobacter* test	*Campylobacter* test	Moisture %	Temp °C
	Effluent (MPN / 100 mL)						**Effluent** (MPN / 100 mL).					
	4.95	-	6.63	2.97			4.95		5.97	2.36		
	Soil MPN/g of soil						**Soil** MPN/g of soil					
0#	1.63	1.63	nd	nd	19.8	24.0	0.98	0.98	nd	nd	16.2	30.5
0##			*-	*-	34.0	nd	nd	*-	*-	*-	35.2	*-
1	2.89	*-	3.73	0.52	29.4	22.0	2.38	*-	3.52	-0.40	33.6	25.1
2	2.66	*-	3.76	0.34	27.7	22.0	1.97	*-	3.18	nd	28.9	26.9
4	3.40	*-	2.66	nd	22.8	21.1	1.51	*-	1.08	nd	20.8	25.0
7	2.22	*-	0.76	nd	20.0	22.0	1.36	*-	nd	nd	12.6	26.6
14	3.38	*-	nd	nd	24.7	20.5	0.99	*-	nd	nd	12.0	26.0

* 30 L of pond effluent applied evenly over each of six 1 sq. M plots of pasture and soil samples taken from a depth of 4 cm. Soil moisture and temperature collected as described

**Piggery G grass height 20-50 cm. from 0-14 days. Rainfall of 2, 2, & 18 mm at days 1, 2, & 14.

***Piggery W grass height 4-25 cm from 0-14 days. Rainfall of 5 mm only on last day

before ## after irrigation of pasture on day 0 *- not done nd not detected

Table 19 Comparative bacterial levels over time in soil (from pasture) irrigated with pond effluent in piggeries G** and W*** in winter*

Day	Piggery G				Piggery W			
	E. coli control	*E coli* irrigated	*Arcobacter* irrigated	*Campylobacter* irrigated	*E. coli* control	*E. coli* irrigated	*Arcobacter* irrigated	*Campylobacter* irrigated
	Effluent (MPN / 100 mL)							
	*-	5.83	7.40	3.83	*-	6.38	6.66	4.04
	Soil (MPN/g of soil)							
0#	0.6	0.6	nd	nd	2.12	2.12	nd	nd
1	*-	3.28	4.40	1.00	*-	2.83	4.14	nd
2	*-	2.73	3.21	nd	*-	3.69	4.13	nd
4	1.11	2.83	3.15	nd	1.36	2.82	3.22	nd
7	1.30	2.54	3.30	*-	0.90	2.94	3.37	nd
14	1.40	1.99	1.83	*-	0.60	2.10	1.08	nd
21	1.11	1.83	1.20	*-	0.78	*-		*-
28	*-	0.78	0.85	*-	*-	1.15	nd	nd
35	*-	0.85	-0.40	*-	*-	*-	*-	*-
42	0.30	0.30	*-	*-	*-	*-	*-	*-

***** 30 L of pond effluent applied evenly over each of six 1 sq. M area of pasture and soil samples taken from a depth of 4 cm. *E. coli* control results are the levels of *E. coli* in the untreated plots. nd = not detected

******Piggery G grass height 4-22 cm from 0-42 days. Rainfall of 20 and 26 mm from days 14 and 19 respectively.

*******Piggery W grass height 2.5-6 cm from 0-42 days. No rainfall.

Before – results before irrigation on Day 0

*- not done

Table 20 Survival of E. coli over time in soil irrigated with piggery effluent (with re-wetting) piggeries G in winter (Including moisture levels).*

days	Irrigated *E. coli*	Irrigated wetted *E. coli*	Control *E. coli*	Control wetted *E. coli*	irrigated moisture	Irrigated wetted moisture	Control moisture	Control wetted moisture
				MPN/g of soil				
0[#]	0.6	0.6	0.6	0.6	7.5	8.2	6.7	6.0
0[##]	*-	*-	*-	*-	22.5	23.6	*-	14
1	3.28	3.26	*-	0.97	18.3	16.8	8.1	14.1
2	2.73	*-	*-	*-	18.4	14.8	8.2	15.5
4	2.83	2.94	1.11	0.63	14.0	20.5	6.8	17.3
7	2.54	2.66	1.30	0.97	11.8	17.8	6.9	16.6
9	*-	*-	*-	*-	12.2	20.1	6.0	15.5
12	*-	*-	*-	*-	10.2	20.5	5.1	15.8
14	1.99	2.14	1.40	0.52	25.7	32.4	21.2	32.5
16	*-	*-	*-	*-	30.8	32.5	26.5	30.4
19	*-	*-	*-	*-	33.3	32.1	28.7	34.7
21	1.83	1.10	1.11	0.52	24.0	25.2	23.7	25.4
28	0.78	*-	*-	*-	16.3	*-	17.0	*-
35	0.85	*-	*-	*-	10.2	*-	10.1	*-
42	0.30	*-	0.30	*-	10.1	*-	8.4	*-

***** 30 L of pond effluent applied evenly over each of six 1 sq. M area of pasture and soil samples taken from a depth of 4 cm.
nd = not detected

******Piggery G grass height changed from 20 to 50 cm over trial. Rainfall of 2, 2, 0, 0 and 18 mm recorded on Days 1, 2, 4, 7, & 14.
[#] Before and [##]after irrigation on Day 0 *- not done

Table 21 Survival of E. coli over time in soil irrigated with piggery effluent (with re-wetting) Piggery W in winter (Including moisture levels).*

days	Irrigated *E. coli*	Irrigated wetted *E. coli*	Control *E. coli*	Control wetted *E. coli*	Irrigated moisture (%)	Irrigated wetted moisture	Control moisture (%)	Control wetted moisture
				MPN/g of soil				
0#	2.12	2.12	2.12	2.12	22.2	24.0	*-	22.2
0##	*-	*-	*-	*-	36.0	39.8	24.0	37.3
1	2.83	2.97	*-	1.28	33.2	35.6	25.8	33.9
2	3.69	*-	*-	*-	32.2	34.5	25.0	30.4
4	2.82	2.69	1.36	0.78	26.6	32.0	22.9	29.2
7	2.94	2.52	0.90	1.68	21.5	28.7	16.9	26.7
9	*-	*-	*-	*-	18.4	30.2	15.0	28.6
11	*-	*-	*-	*-	17.3	33.8	15.3	31.4
14	2.10	1.83	0.60	1.11	13.5	30.5	12.2	27.8
28	1.15	*-	0.78	*-	8.9	*-	9.7	*-

***** 30 L of pond effluent applied evenly over each of six 1 sq. M area of pasture and soil samples taken from a depth of 4 cm.
******Piggery W grass height changed from 4 to 25 cm over trial. Rainfall of 5 mm recorded on Day 21.
Before and ## After irrigation on Day 0 *- not done

Table 22 Comparative bacterial levels, soil moisture, temperature and RH in soil irrigated with pond effluent in piggery R in summer and winter*

SUMMER										
day	***E. coli***		***Arcobacter***		***Campylobacter***		**Moisture**		**Temp**	**[$]RH**
	Effluent (log MPN /100 mL)									
	6.20		6.16		3.89					
	Soil (log MPN/g of soil)									
	irrigated	control	irrigated	control	irrigated	control	irrigated	control		
1	4.83	2.83	4.15	0.13	0.11	nd	19.59		25.3	52
2	4.89	2.76	4.08	nd	0.69	nd	37.92	20.32	21.4	58
4	4.78	2.37	2.76	nd	nd	nd	36.74	24.53	21.9	54
7	3.40	2.37	1.73	nd	nd	nd	32.96	21.73	22.8	76
14	3.24	0.52	-0.46	nd	nd	nd	34.88	24.66	nd	nd
21	*-	*-	*-	*-	*-	*-	*-	*-	*-	*-
23	2.74	1.48	*-	*-	*-	*-	30.64	19.8	20.3	
33	*-	*-	*-	*-	*-	*-	34.34	32.44	nd	68
43	*-	*-	*-	*-	*-	*-	31.26	20.54	nd	nd
WINTER										
day	Effluent (log MPN /100 mL)									
	5.76		5.81		3.32					
	Soil (log MPN/g of soil)									
1	3.42	0.48	2.98	nd	-0.05	nd	3.89	*-	*-	*-
2	1.63	2.34	0.52	nd	nd	nd	13.18	5.22	16.3	36
4	3.21	2.07	-0.15	nd	nd	nd	7.32	4.21	18.0	95
7	1.36	1.13	nd	nd	nd	nd	5.60	4.47	14.4	57
14	2.86	0.36	nd	nd	nd	nd	15.01	12.18	16.7	78
21	1.65	0.52	*-	*-	*-	*-	12.72	13.01	15.9	48
28	0.36	0.36	*-	*-	*-	*-	4.98	5.15	*-	*-
37	*-	*-	*-	*-	*-	*-	4.19	4.90	*-	*-

***** 30 L of pond effluent applied evenly over each of six 1 sq. M plots of pasture and soil samples to a depth of 4 cm. [‡]Piggery R rainfall on Days 1, 2 and 23

[$]RH = Relative Humidity *-= Not done nd = not detected

Table 23 Comparative bacterial levels, soil moisture, temperature and RH in soil irrigated with pond effluent in piggery K in summer and winter*

SUMMER										
day	***E. coli***		***Arcobacter***		***Campylobacter***		**Moisture**		**Temp**	**$RH**
	Effluent (log MPN /100 mL)									
	5.92		6.63		3.89					
	Soil (log MPN/g of soil)									
	irrigated	control	irrigated	control	irrigated	control	irrigated	control		
1	3.52	0.36	3.61	nd	nd	nd	36.4	24.2	*-	*-
2	3.63	0.52	3.20	nd	nd	nd	27.8	14.6	26	47
4	2.76	0.36	0.90	nd	nd	nd	24.2	17.1	25.6	65
7	3.16	0.76	nd	nd	nd	nd	20.8	14.5	26.0	82
14	2.83	0.97	*-	*-	*-	*-	18.6	13.8	25.9	92
21	2.52	0.52	*-	*-	*-	*-	21.5	24.0	*-	*-
23	*-	*-	*-	*-	*-	*-	23.9	26.5	*-	*-
33	1.89	0.36	*-	*-	*-	*-	14.5	16.7	21.5	49
43	0.52	nd	*-	*-	*-	*-	13.4	17.9	21.7	53
WINTER										
day	Effluent(MPN/ 100 mL)									
	7.04		6.63		4.38					
	Soil (log MPN/g of soil)									
1	4.54	0.36	4.44	nd	-0.05	nd	21.78		15.9	46
2	4.83	0.36	4.04	nd	-0.46	nd	43.69	22.60	15.0	58
4	4.66	0.36	4.45	nd	nd	nd	37.07	21.67	16.5	58
7	4.22	0.36	2.69	nd	nd	nd	34.22	19.31	15.7	57
14	4.04	0.36	1.75	nd	*-	*-	28.30	16.25	12.1	32
20	4.04	0.36	nd	nd	*-	*-	22.67	14.29	14.0	91
21	*-		*-	*-	*-	*-	15.57	11.87	*-	*-
28	*-		*-	*-	*-	*-	11.76	9.49	*-	*-
37	3.92	0.36	*-	*-	*-	*-	22.15	23.95	15.0	40
63	2.66	0.36	*-	*-	*-	*-	6.05	6.70	*-	32
85	3.22	0.36	*-	*-	*-	*-	35.1	35.4	*-	41

***** 30 L of pond effluent applied evenly over each of six 1 sq. M plots of pasture and soil samples to a depth of 4 cm. †Piggery K rainfall on Day 7 $RH = Relative Humidity
*- not done nd = not detected

6. Discussion

Intensive animal farming can impact on aspects of human health, either at a farming level or as a consequence of managing wastes generated. The concentration of animal farming adjacent to urban areas, soil and water environments can be impacted by commonly adopted farming and waste management practices. One of the major issues facing all intensive animal industries is sustainability. More specifically, organically sourced animal wastes are increasingly viewed as an area of risk. This means that the pig industry requires a sound scientific basis to demonstrate safe and sustainable use, both at a farming and waste (effluent) management level. In addition, the environmental movement of food-safety pathogens both within and external to the environment is increasingly becoming an emerging area of concern. At the same time there is a need for the industry to use effluent (and waste solids) in a manner that can derive financial benefits to the pig farmer/industry, which simultaneously addresses sound on-farm practices. This review addresses previous research and updates the relevant areas to provide a comprehensive summary to address the potential environmental movement of pathogens as a consequence of re-using piggery effluent.

6.1 Pathogens and piggery effluent

The preliminary study identified the pathogens of significance, by a review of literature following which those chosen as high priority were enumerated in piggery effluent (using a survey). Based on the priority pathogens selected, the focus was then directed to food and water-borne pathogens. The food-safety focus identified 20 years ago is still current. Where there is an increase in the use of animal wastes in food agriculture there is the potential for environmental transmission. A review of the available literature of studies that provided quantitative data on *Salmonella*, *Campylobacter* (and *E. coli* as an indicator) in piggery lagoons were limited. *Salmonella* is not widely distributed across Australian piggery pond effluent and when present is found at low levels. This suggests that *Salmonella* is a pathogen of lesser risk than *Campylobacter* in piggery effluent ponds. *Campylobacter* is a fragile organism (Klancnik et al. 2009) with potential for die-off, compared to *Salmonella,* which has better survival strategies in the environment (due the organisms' potential for resistance to stress response) (Spector and Kenyon 2012). The pathogen data extracted from other studies are comparable to the levels of *Salmonella*, *Campylobacter* and *E. coli* levels in Australian piggery effluent *(*Chinivasagam et al. 2004*)*. This suggests that this data can be used for purposes of developing guidelines or addressing risk management approaches, as it is more relevant Australian data.

Though not enumerated, the key food-safety pathogens were a focus of a range of studies addressing the survival or movement of these pathogens in piggery environments. Early Australian studies, (1979 – 1983) addressed *Salmonella* in effluent disposal to land and survival in effluent treated soils. Other pathogens of food/water borne relevance included were *Listeria* (commonly distributed in the environment), staphylococci, coliforms and faecal coliforms were assessed across constructed wetland studies and swine manure lagoons pathogen dynamics. *Salmonella* survival/inactivation was widely studied in swine effluent sludge, swine lagoons, wetlands, the validation of treatment efficacies (UV) and survival during the land disposal of effluent. *E. coli* as an indicator was also widely used across studies. *Clostridium perfringens* was used as an indicator for bacterial stratification studies or as a surrogate for protozoans. In summary, the food-borne organism focus is an important area and the summarised studies provide background to the types of issues addressed for piggery effluent/waste.

6.2 Pathogens, aerosols and human health

This is an important (and sensitive) area directly related to human health both on-farm and to communities adjacent to piggeries. The Australian data (Chinivasagam and Blackall 2005) was compared with other in-shed studies that enumerated similar organisms (*E. coli*, total bacteria, fungi and viable particles). There was just one other study that undertook in-shed testing similar to the Australian study and the *E. coli* levels were comparable. Only the Australian study addressed in-shed effluent flushing and the risk was found to be minimal due to the low *E. coli* levels captured in aerosols. No *Campylobacter* was captured. *Salmonella* was not tested due to the organism's low and infrequent presence in effluent and thus unlikely presence in aerosols. The risks (assessed via QMRA) for inhalation by pig workers was an acceptable risk when compared to the US EPA levels, (1infection per 10,000 people per year). Using the levels of *E. coli* from the effluent study, QMRA also demonstrated that the risks from pathogen inhalation during piggery effluent spray irrigation to residents at 500 m away from the irrigator was within the allowable US EPA risk (1 infection per10, 000 people per year).

It should be noted that food-borne pathogens are pathogens of the gastrointestinal tract and once inhaled need to be swallowed (at the infective dose) to initiate infection in humans (and this is what is imperative). Particles of >7µm are trapped in the upper respiratory tract regions, nose and throat and can thus gain access to the gastrointestinal tract (Hatch 1959). This process requires the ingestion of sufficient organisms to cause an infection. The amount required to cause an infection is measured as the number of cells at which 50% the normal human population will get infected (ID_{50}). For *Salmonella* the infectious dose is 10^5 - 10^6 organisms (Shuval et al. 1986) and for *Campylobacter* it is 500 organisms (Robinson 1981). Thus, the risks of direct illness, is based only on the fraction of the airborne pathogens that are ultimately swallowed. Present studies (Chinivasagam and Blackall 2005) conducted in piggeries demonstrate minimal risk. Even in chicken sheds based on the reported Australian studies (Chinivasagam et al. 2009b; Chinivasagam et al. 2009a), the risk was deemed low, though both *Salmonella* and *Campylobacter* are captured in litter at similar or higher levels than in piggery effluent. Unlike most piggeries, chicken sheds are mechanically ventilated operations that move large volumes of air from birds (feathers and dust). The chicken studies focused both in-shed and at distances external to the fan (downwind) due to most concerns being from neighbours.

Whilst few pig shed studies reviewed quantified pathogens, several studies compared shed contamination levels both within and at downwind distances to shed, some of which were mechanically operated. The focus ranged from shed hygiene to worker (and animal health), impact to neighbours, testing for multi-drug resistant organisms, endotoxins (produced by Gram negative organism) and respiratory disease among workers. Thus, there is a need to be aware of the some of the emerging issues such as endotoxins and dust all of which may be occupational risks.

6.3 Pathogen survival in food crop, pasture, turf

The original studies were carried out at a time when effluent from piggeries was commonly used to irrigate pasture. Some piggeries included grazing cattle and turf. The irrigation of food-crops did occur in some instances. The interest at the time was the withholding period for effluent irrigated pasture, as consequence of pathogen survival on leaf surfaces (grass). The present summary included a review of literature for food-crops. Contaminated soil via animal waste application or manure treated water to irrigate crops are considered a potential risk to food crops, especially sensitive crops (vegetables eaten raw). Both water (and soil) can be contaminated by organisms such as *Salmonella*, which have been shown to survive in soil. The review has briefly discussed studies that have demonstrated leaf

surface survival as well as potential for pathogens such as *Salmonella* to internalise in both leaf and root crop and be a food-safety risk in vegetables eaten raw.

The initial APL study undertaken in the early 2000s, assessed piggery effluent treated grass (under laboratory conditions and a glasshouse) using varying environmental conditions such as UV or sunlight, temperature and relative humidity. These factors in combination, can impact pathogen survival in the environment. The initial outcome suggested that die-off (as impacted by temperature, humidity and rain) was complex to predict of survival. The same data was modelled under varying environmental conditions to predict die-off. Both the Gaussian plume and the MEDLI model regarded time as key for predicting withholding period. Modelling demonstrated a 2-log reduction after 24 hours. Both relative humidity and temperature with cool moist conditions played a role in die-off. Thus, such approaches could be used based on actual irrigation and meteorology parameters for a specific set of irrigation conditions to address application, crop and weather conditions to assist with predicting appropriate withholding periods for effluent irrigated crop.

6.4 Arcobacter and piggery effluent

The study of *Arcobacter* was undertaken and literature updated, because it is an emerging pathogen. *Arcobacter* is widely distributed in waters, its status of listing in the UNESCO "Global Water Pathogen Project" and its presence in high numbers in piggery effluent (Chinivasagam et al. 2007) indicates that there is a need to be cautious. *Arcobacter* occurs in higher numbers than *Campylobacter* and *Salmonella* in piggery effluent. The organism also can be used as an indication for disinfection studies. The previous APL study used *Arcobacter* as the organism of choice for the soil survival and run-off studies due to the high numbers consistently present in piggery effluent and the organisms' stability over *Campylobacter* in soil environments. This organism can be an organism of choice to address recent piggery effluent contamination rather than *E. coli* and is discussed further in the soil survival studies. A watching brief should be maintained on the pathogen status of this organism.

6.5 Pathogens survival in effluent treated soils

This is an important area and many studies using all types of manures place an importance on pathogen survival in effluent treated soils. Treated soil is one of the major pathways for the environmental transmission of pathogens, which can occur by direct contact to crop, via run-off to waterways and as a reservoir for those pathogens that can survive and perhaps multiply or even adapt. Global warming is likely to have an impact on food-borne pathogen ecology, such as their lifecycle in soils or waters. Their commensal or parasitic life in animals may be difficult to predict, with such changes likely driven by their mechanisms for evolution or adaptation (Carlin et al. 2010).

Manure amended soils can also act as an important reservoir for pathogens. The particular focus from the previous study was effluent irrigated pasture soil. This is common practice and quite possibly complicated where other livestock graze. Irrespective of these issues the continuous use of effluent in the same area is likely to impact on organisms that can adapt to soil survival. Environmental parameters such as soil moisture, pH and temperature can impact on pathogen survival in soil. *E. coli* is a common indicator organism used in most guidelines, has been isolated from environments with minimal or no animal activity (Byappanahalli and Fujioka 1998). *E. coli* has also demonstrated re-growth in tropical environments (Byappanahalli and Fujioka 1998). The soil survival studies (Blackall 2004) have also shown this to occur.

E. coli had already established in soils adjacent to piggeries possibly due to contact with effluent. *E. coli* was already present in the control study plots and did not show die-off as *Arcobacter* or *Campylobacter*. In some instances the *E. coli* populations never reached background levels due to the on-going presence of the organism, which was also influenced by rain (increased in levels). Thus, the organism's prior presence in soil around piggeries and the potential to re-grow makes the organism an unsuitable indicator to address compliance. In contrast, *Arcobacter* was not present in background (except once at low levels in an area with long history of cattle activity). *Arcobacter* reached background at shorter times than *E. coli* due to no environmental influence of prior populations. Thus, the use of Arcobacter is a better marker of recent piggery effluent exposure than *E. coli*.

6.6 Mobilisation of pathogens due to run-off

Both, heavy or on-going rainfall can contribute to mobilisation of pathogens through soils irrigated with piggery effluent – should the effluent contain pathogens. Mobilisation of pathogens can also occur under extreme weather conditions from effluent storage pond overflow, (a scenario simulated during the original studies), contributing to overland transport of pathogens. The simulation where effluent overtopping forced irrigation following a heavy rain event demonstrated run-off of pathogens is a possibility. Appropriately constructed and placed vegetative filter strips can contain pathogen movement. The guidance provided in the Australian guidelines (The National Environmental Guidelines for Indoor Piggeries 2018) for nutrient management and the summary of recent literature on pathogens provide guidance on vegetative filter strips to help manage pathogen run-off to sensitive areas. However, there is a need to consider the possible interference of established background populations in conforming to guidelines that may use *E. coli* as an indicator organism for run-off water. Thus, on-farm risk management protocols, that addresses the critical control point (or potential effluent over flow as a hazard) can address the management of such risks on an on-going basis.

6.7 The consequence of antibiotic usage and the development of antibiotic resistance organisms

This was addressed by studying common soil organisms *Pseudomonas* spp. and the *B. cereus* group. The antibiotics used were those commonly used by the industry at the time of the work. This work demonstrated that there was no statistically significant population distribution pattern from the organisms' source from both organic and conventional piggery farming environments, which had a history of on-going use of piggery effluent. Thus, the environment (or soil) was not a source of transfer as a result of continuous transfer of piggery effluent.

6.8 Updated guidelines that can contribute to addressing piggery effluent re-use

Quantitative Microbial Risk Assessment (QMRA) was reviewed highlighting the complexity involved and the applicability for its role in addressing risks for piggery effluent re-use. QMRA remains a potential approach but offers little practical advantages and requires complex input data. A survey of literature revealed there were no specific guidelines addressing piggery effluent re-use. One of the differences between the guidelines reviewed 20 years ago and the more recent guidelines is a general deviation of approach. Whilst the older guidelines adopted a more prescriptive approach, the more recent guidelines target a microbial risk management. Some of the end uses of the summarised guidelines are similar to what may occur with piggery effluent, for example, the irrigation of food crops to pasture. Both environmental and human risks including animal health are recognised.

Figure 16, provides a comprehensive summary that identifies direct and indirect risks to humans (via the environmental pathway) and the identification of critical control points as a means of containing or managing risks at various points through the re-use pathway. This flow diagram along with the table created with *E. coli* levels stipulated for various end uses can form the basis for proactively addressing and demonstrating risk management. The approaches suggested in this research summary are all drawn from guidelines and comments provided for reclaimed water as summarised. The comments provided in relevance to the EU guidelines on the use of an indicator such as *E. coli* or direct use of pathogens can address complex scenarios, should they arise in the future.

6.9 Other pathogens of emerging interest

Four pathogens have been included in this summary. *Clostridium difficile,* has been reported to be associated with neonatal pigs in Australia (Knight et al. 2015), though there is no conclusive evidence based on literature on the organism's zoonotic potential. Thus, *Clostridium difficile* remains a pathogen of uncertain significance and a watching brief should be maintained on this organism. *Leptospira* is a pathogen of significance as occupational risk and *Burkholderia pseudomallei* is a pathogen of low risk. For *leptospira* the literature indicates that the infection pathway is a direct connection between an urine from an infected pig and the human. This is evidence that the effluent pathway is not relevant for this organism. For *Burkholderia pseudomallei* there is no published evidence of any relevance of the effluent pathway. The widespread presence of this organism in sub-tropical and tropical environment means it would be difficult to connect the organism to the piggery effluent pathway. Both have been included due to public interest in these pathogens. *Pfiesteria piscicida* was part of the original study and has been updated with some Australian literature though the industry needs to be aware of the significance of this organism based on fish kills that have occurred in California following piggery waste spills to waters (Burkholder and Glasgow 1997).

6.10 Overall Summary

Almost 20 years ago, the food and water borne pathogens and their potential environmental pathways within a piggery were identified to addresses the various challenges that were likely to occur due to the re-use of piggery effluent as it occurs within Australian piggeries. These concerns are current today. Thus, both past Australian studies and the updated studies (presented in a literature review) provide a basis for addressing and managing some of these risks, in a factual and scientific manner to arrive at practical solutions.

7. Implications & Recommendations

This summary has provided outcomes of the previous two APL studies and updated literature on a range of studies undertaken at the time. It is recommended that this information be made available to the pig industry so as to have a better understanding of this area of research (i.e. pathogens and piggery effluent re-use).

In the absence of specific guidelines for addressing the pathogen risks, it is recommended that some of the approaches adopted across the summarised national and international guidelines be used as a basis to address pathogen risks linked to piggery effluent re-use. Most of the summarised guidelines have adopted a risk management approach. Such an approach can aid the proactive management of risks by identifying hazards as "critical control points" that can be monitored as an on-going risk management plan that can be implemented for a piggery.

To this end, a flow diagram and table have been included as a starting point. The risk management approach is a documented process that can be drawn up for each farm situation to aid proactive management of risks, based on an understanding of the various pathogen movement pathways. Such an approach can assist the pig industry demonstrate safe and sustainable use of a valuable water resource which also has industry benefits.

It is also recommended that the research undertaken by the pig industry be made available to the regulators who address such concerns to have an informed understanding, so that the various risks can be addressed in a comprehensive and collaborative manner.

8. Intellectual Property

None.

9. Technical Summary

Background

The concentration of animal farming adjacent to urban areas, soil and water environments can be impact farming or waste management practices. The pig industry needs a solid scientific basis to demonstrate safe and sustainable use of effluent. The environmental movement of food-safety pathogens is increasingly becoming an emerging area of concern. The significance of this issue was recognised nearly 20 years ago, and in response, the pig industry funded two projects, from 1998 - 2004. The two studies (a) "Pathogens and piggery effluent" (DAQ 60/1353) and (b) "Establishing guidelines for the safe application of piggery waste to pastures" (Project no. 1797) provided literature knowledge, research studies and explored approaches to address microbial risks. This work has been summarised and addressed in the context of both previous and recent literature, to provide a comprehensive summary on the environmental movement and management of food /water-borne / pathogens as a consequence of piggery effluent re-use.

Pathogens of concern in piggery effluent

The preliminary study identified the pathogens of significance following a review of literature, whereby pathogens chosen as high priority were subsequently quantified in piggery effluent. Key pathogens chosen as high priority were both *Salmonella* and *Campylobacter* (along with the indicator organism *E. coli*.) The food-safety focus identified 20 years ago remains relevant today, where there is an increase in the use of animal wastes in food agriculture. The Australian data remains comprehensive (and comparable) and thus can be used for purposes of developing guidelines or addressing risk management.

Pathogens, aerosols and human health

This is an important and sensitive area directly related to human health both on-farm and for communities adjacent to piggeries. Australian studies included testing within piggery housing to address concerns around effluent flushing and health of workers. Risks both in-shed and to residents at 500 m away from a spray irrigator (on the piggery) was within the allowable US EPA risk. It should be noted that food-borne pathogens are pathogens of the gastro intestinal tract and once inhaled need to be swallowed (at the infective dose) to initiate infection in humans (and this is what is imperative).

Pathogen survival in food crop, pasture, turf

The original studies were carried out when effluent from piggeries was commonly used to irrigate pasture. The modelling approaches used at the time predicted conditions that supported a 2-log reduction of bacteria after 24 hours of effluent application to leaf surfaces. This approach can be used to address withholding periods following effluent application to help manage risks. The review summarised studies linked to food-borne pathogen survival in water, soil, leaf surface and the potential for pathogens to internalise in leaf and root crop to be of a food-safety risk, following re-use of animal wastes.

Pathogens survival in effluent treated soils

Previous work was summarised detailing studies carried out across four piggeries to address pathogen die-off in effluent irrigated soil. The pathogen die-off time was longer in winter than summer. One of the key outcomes across the studies is that the commonly used indicator organism, *E. coli*, (for pathogen presence) was resident in soils in piggery environments with potential to re-grow. Thus, the organism's prior presence in soil around piggeries, as well as the potential to re-grow makes the organism an unsuitable indicator to address compliance in effluent irrigated soils.

Mobilisation of bacteria in effluent irrigated soils following potential heavy rainfall

Previous studies addressed pathogen run-off via a simulated condition related to effluent overfill (and effluent irrigation to contain overfill which is followed by heavy rain). The study included the comparison of the use of vegetative filter strips to manage run-off. Both *E. coli* and *Arcobacter* were collected in run-off under a simulated "heavy rain event", where the filter strips used failed to contain these organisms. The Australian guidelines (The National Environmental Guidelines for Indoor Piggeries 2018) for nutrient management and the summary of recent literature on pathogens provide guidance on vegetative filter strips to help manage pathogen run-off to sensitive areas.

Antimicrobial resistance from soils exposed to piggery effluent

The previous study was undertaken by testing common soil organisms against commonly used antibiotics for pigs, demonstrated that there were no population shifts in bacteria isolated from soil from organic and conventional farming environments. This is detailed in a manuscript entitled "Impact of antibiotics on fluorescent *Pseudomonas* group and *Bacillus cereus* group isolated from soils exposed to waste from conventional and organic pig farming" that is ready to submit for publication.

Updating guidelines and Quantitative Microbial Risk Assessment

Quantitative Microbial Risk Assessment for piggery effluent reuse remains a potential approach but is complex and offers little practical advantages. The updated guidelines summarised focus on a microbial risk management approach. This revised summary includes both national and international guidelines. The risk management approach demonstrated in some of the guidelines can be adopted for piggery effluent. A "flow diagram" that illustrates this approach along with a tentative table was created as a basis for discussion (and input). The risk management approach identifies hazards as "critical control points" that can be monitored as part of an on-going process for risk management.

Other organisms of interest

Arcobacter is widely distributed in waters, and due to its status of listing in the UNESCO "Global Water Pathogen Project" and its presence in high numbers in Australian piggery effluent. *Clostridium difficile* remains a pathogen of uncertain significance as there is no conclusive evidence based on literature on the organisms' zoonotic potential. A watching brief should be maintained for both organisms. *Leptospira* is a pathogen of significance as occupational risk and is based on direct contact with an infected pig and the effluent pathway is not relevant for this organism. *Burkholderia pseudomallei* is widely present in sub-tropical and tropical environment and there is no published evidence to the effluent pathway. The industry needs to be aware of *Pfiesteria piscicida as* fish kills have occurred in California due to piggery waste spills.

Overall summary

Almost 20 years ago, the food and water borne pathogens and their potential environmental pathways within a piggery were identified to addresses the various challenges that were likely to occur via the re-use of piggery effluent as it occurs within Australian piggeries. These concerns are current today. Thus, both past Australian studies and the updated studies (presented via a literature review) provide a basis for addressing and managing some of these risks, in a factual and scientific manner to arrive at practical solutions.

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11. Publications Arising

Tentative titles

- Impact of antibiotics on fluorescent *Pseudomonas* group and *Bacillus cereus* group isolated from soils exposed to waste from conventional and organic pig farming (previous APL study)
- Survival of *Campylobacter*, *Arcobacter* and *Escherichia coli* in soil following the application of piggery effluent to pasture (previous APL study)
- Re-using piggery effluent on-farm in a safe and sustainable manner in Australia: a risk management approach to address food and water-borne pathogen movement to the human food-chain (current review)