



# Silent superbug killers in a river near you

How factory farms contaminate public water courses on three continents

The health and wellbeing of animals, people and our planet are interdependent. Poor animal health and welfare in factory farming negatively affect food safety, our environment and climate. Ending factory farming will curb the rise of antimicrobial resistance (AMR) from farm animals and stop superbugs in their tracks. It will bring better animal health and welfare, healthier diets for people and a climate-safe and sustainable food system.

The United Nations is calling for an end to unsustainable agricultural practices and acknowledges that intensive farming carries high risk of disease outbreaks and fallout for public and environmental health. It recognises that antibiotics are used to mask poor conditions for farm animals, and calls for investment in sustainable, agroecological food systems<sup>i</sup>.

But we can only achieve a humane and sustainable food system if governments and industry take action to end factory farming. Ten years from now, to protect our animals, people and planet, the building of factory farms should have stopped. Existing factory farms should pay for the costs of their irresponsible practices, rather than communities paying the costs of poor public health and environmental pollution.

Greater attention should also be paid to factory farming's contribution to the climate crisis. While the focus is often on greenhouse gas emissions from beef, the scale and growth rate of factory farmed poultry and pigs is far higher. Pork and chicken produce 20-25 times more greenhouse gas emissions than soy products<sup>ii</sup>.

It won't be enough to use carbon-capture technology or on-farm solutions to mitigate carbon emissions. An overall reduction in meat production and consumption is required<sup>iii, iv</sup>. Changes to global temperatures through climate change also mean a greater risk of bacteria spreading and superbugs emerging<sup>v</sup>.

Governments and industry should support halving animal production and consumption across the world by 2040; they should embrace genuinely high welfare and sustainable food systems.

This means no more factory farms dependent on animal abuse – caging, painful mutilations and early weaning of young animals – where antibiotics are used to prevent sickness or promote growth.



**Image:** Collecting water samples near industrial farms, Thailand. Credit: World Animal Protection

Instead, it means predominantly plant-based diets, and a smaller number of farm animals in humane and sustainable systems where they lead good lives and antibiotics don't need to be used routinely.

Nothing less than a revolution in the way we produce and consume food is needed. A growing chorus of voices is calling for change and World Animal Protection is ensuring animal welfare is central to a food system that is sustainable, equitable and humane.

We call on the food industry and its funders, governments and intergovernmental organisations to recognise that our health and wellbeing depends on the wellbeing of all living things. Let's end factory farming for the sake of current and future generations, and work together for a humane and sustainable food system.

**Jacqueline Mills**  
Global head of farming  
World Animal Protection

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Road, London WC1X 8HB

## Silent superbug killers in a river near you: how factory farms contaminate public water courses on three continents

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**Cover image:** Mother pigs are kept in barren conditions with no enrichment on factory farms. Location undisclosed. Credit: World Animal Protection

# Introduction

## Creating a global health crisis – factory farming and antibiotic resistance genes

Water downstream from factory farms harbours an invisible threat to people's health which could eclipse the COVID-19 crisis. The threat? Antibiotic Resistance Genes (ARGs) which are driving antimicrobial resistance – the world's superbug crisis – projected to kill up to 10 million people annually by 2050<sup>vi</sup>.

Our 2020 research in four countries across three continents firmly links factory farms, where billions of animals are caged, mutilated and pumped full of antibiotics, to ARGs and superbugs. It is the first multi-country investigation into the problem and involves pig farming locations in Canada, Spain, Thailand and the USA.

Overall, our findings strongly suggest that factory farms are discharging resistance genes and superbugs into public waterways and the wider environment in all four countries sampled.

Already 700,000 people a year are dying from infections that cannot be treated with antibiotics. World Health Organisation (WHO) chief Tedros Ghebreyesus has warned that the superbug crisis is just as dangerous as the pandemic<sup>vii</sup>.

### Building antibiotic resistance

ARGs are the building blocks for superbugs; they create antimicrobial resistance, the resistance of common bacteria to antibiotics critically important to people. This means some antibiotics are already ineffective in some parts of the world; while in future, routine procedures like caesarean sections or cancer treatment will become dangerous worldwide<sup>viii</sup>.

Alarming, some ARGs found in our research convey resistance to antibiotics critically important to human health and are of most concern to the World Health Organisation. These antibiotics are the last line of defence to keep patients alive when other antibiotics have failed<sup>ix</sup>. Such antibiotics are needed because of the very nature of factory farming. They are used to prevent stressed animals getting sick in cruel confinement and so prop up a system of suffering for food production.

### Discharged through waste

Pig farms deliberately use very high levels of antibiotics. This is because pigs are one of the most intensively farmed species on the planet. Up to 90% of all antibiotics they receive are administered in the first 10 weeks of pigs' lives. Their use is associated with painful mutilations (especially surgical castration), early separation from mothers, barren and overcrowded environments and related gut and respiratory infections<sup>x</sup>.

Our sampling of water courses near to factory farms for this research shows that ARGs downstream from pig farms are different from those found in samples taken upstream, pointing to the likely discharge of ARGs in pig waste (manure and urine).

In Spain, our testing of groundwater near industrial pig farms revealed very high levels of ARGs; groundwater was used for human water supplies in some regions in the past. This study also revealed surface water from samples in Spain contained ARGs up to 200 times the baseline levels.

Testing in Thailand found superbugs resistant to third generation cephalosporins, fluoroquinolones, or colistin as well as co-trimoxazole, gentamicin, amikacin, trimethoprim-sulfamethoxazole or amoxicillin. This is the first finding of ARGs from pig farms in Central Thailand. Similar ARGs have been found near pig farms in other regions in Thailand in recent years.

Our research in Canada documents what we believe to be the first finding of ARGs in Manitoba that convey resistance to cephalosporins, fluoroquinolones, macrolides, and tetracycline. The results reflect similar findings from other provinces. Overuse of antibiotics is systemic in the pig industry. There is significant spill over, particularly of tetracycline, to public lake and river freshwater beaches, conservation areas, and to wildlife.

In the USA, our researchers found widespread evidence of ARGs conveying resistance to tetracycline and streptomycin. Most importantly, they also found ARGs conveying resistance to macrolides, cephalosporins, fluoroquinolone and possibly carbapenem antibiotics.



All but one sample was positive for tetracycline-resistance genes, consistent with available data documenting high sales and use of tetracycline across the US pig industry. Contamination both upstream and downstream was also found in areas with a high density of pig farms, strongly suggesting factory farms are discharging resistance genes and superbugs into public waterways and the wider environment. Our evidence also suggests airborne transfer of ARGs.

### International standards lacking

Waterways can be flash points for toxic pollutants and create reservoirs where superbugs accumulate and mix. However, there is no international standard describing the concentration at which superbugs in the environment become dangerous to people. Consequently, no one is held responsible, and farm discharge of antibiotics and superbugs into waterways are unmonitored.

This is despite the UN identifying superbug contamination as one of the most important emerging global environmental issues<sup>xi</sup>. Furthermore, most countries don't monitor antibiotic use directly on farm animals. And despite WHO recommendations it is common for farms to use antibiotics across groups of animals to prevent disease<sup>xii</sup>.

There is growing worldwide concern about the overuse of antibiotics in farming systems. Our 2020 survey across 15 countries found that 88% of people are concerned about superbugs from farm animals and want animals treated well and antibiotics used responsibly. And two-thirds of those surveyed said they would help test for pollution from farms to make big business accountable.



**Image:** Effluent discharge from farm in Thailand.  
**Credit:** World Animal Protection

### Safeguarding public health, ending factory farming

The problem must be addressed at its source – the wholesale dependence of factory farming on antibiotic overuse to cover up cruel, outdated practices. There is no other way of eradicating the problem. Livestock waste treatment technologies do not remove superbugs<sup>xiii</sup> and superbugs cannot be cleaned from waterways. Antibiotics should not be used across groups of animals to prevent disease or promote fast growth.

It is only possible to end wholesale dependence on antibiotics in animal farming by building the wellbeing and immunity of farmed animals. This means putting an end to the worst animal welfare abuses in factory farming, including use of cages, painful mutilations, early weaning of young animals, and use of high productivity breeds of animals. Farm animals in higher welfare systems have reduced stress and improved immunity and resilience to disease. This in turn, requires fewer antibiotics<sup>xiv, xv, xvi, xvii</sup>.

The world's retail, animal production and finance sectors, and governments and intergovernmental organisations must act now and globally to protect our animals, people and planet.

World Animal Protection's clear and far-reaching recommendations supported by our ARG research and investigations are featured on page 22.

# Determining ARG presence – study aim and methodology

ARGs are superbug building blocks. They drive common bacteria to become resistant to antibiotics that are important in helping people fight infections. This World Animal Protection study aimed to determine the presence of ARGs in animal waste discharged from industrial farms into public waterways or onto soil (or crops) in four countries. We also set out to gauge community impact and sentiment regarding the issue.

Water and sediment from public water courses connected to effluent discharges from 6-10 pig farms were tested in each of four countries (Canada, Spain, Thailand and the USA). Samples were collected on public land up and downstream of farm discharge<sup>xviii</sup>.

Surface soil or dust samples were also collected in Spain and Thailand by the 'bootie' method<sup>xix</sup>. This involved walking on public land beside the farms to collect samples to be analysed specifically for Methicillin Resistant *Staphylococcus aureus*

(MRSA), and the *mecA* gene. All samples were recorded and photographed into Epicollect5 – a mobile data gathering platform.

Samples were analysed for ARGs by national accredited laboratories using PCR (Polymerase Chain Reaction)<sup>xx</sup>. PCR does not identify the bacteria directly, but it does identify the antibiotic resistance genes that drive antimicrobial resistant bacteria (superbugs).

In Thailand, key bacteria were isolated and tested for resistance to a range of antibiotics first, then PCR analysis was done where results were negative. In Spain and the US, where there was a very high density of farms and ubiquitous contamination suspected, additional analysis to determine the up versus downstream sample concentration of ARGs was performed in some cases.



**Image:** Muddy water being tested downstream from a pig farm in Thailand. Credit: World Animal Protection

**Table 1 – ARGs found in public water /soil downstream to the farms and respective categories of World Health Organisation antibiotics to which they confer resistance. Critically important antimicrobials to human health<sup>xxi</sup> (highest priority, high priority and highly important categories)**

Country	ARGs found in the total samples (water / soil). Such ARGs confer resistance to...	WHO category - highest priority Antibiotics	WHO category - high priority OR highly important Antibiotics	Other findings / comments
Thailand	<i>bla<sub>SHV</sub></i> , <i>bla<sub>TEM</sub></i> , <i>bla<sub>CTX-M</sub></i> , and <i>bla<sub>VEB</sub></i>	cephalosporins (3+ generation) ciprofloxacin (a fluoroquinolone)	cephalosporins (generation 1,2)	Not resistant to carbapenems
	Resistant bacteria isolated.		gentamicin, co-trimoxazole, amikacin, trimethoprim-sulfamethoxazole	
	<i>mcr-1</i>	colistin		One farm
	<i>mecA</i>			gene may confer selection pressure for MRSA.
USA	<i>bla<sub>CMY</sub></i> , <i>bla<sub>CTX-M</sub></i> , and <i>bla<sub>TEM</sub></i>	cephalosporins (3+ generation)* ciprofloxacin (a fluoroquinolone)	penicillin, cephalosporins (generation 1,2) gentamicin	*Potential resistance to carbapenems – the last resort Reserve class antibiotics.
	<i>gyrA</i> (mutation <sup>^</sup> )	ciprofloxacin		<sup>^</sup> PCR positive does not guarantee mutation present
	<i>mphA</i>	macrolide		
	<i>strA</i> , <i>strB</i>		streptomycin	
	<i>tetA</i> , <i>tetB</i> , and <i>tetC</i>		tetracyclines	
Canada	<i>bla<sub>CMY</sub></i> , <i>bla<sub>CTX-M</sub></i>	cephalosporins (3+ generation)* ciprofloxacin (a fluoroquinolone)	penicillin, cephalosporins (generation 1,2)	*Potential resistance to carbapenems – the last resort Reserve class antibiotics.
	<i>gyrA</i> (mutation <sup>^</sup> )	ciprofloxacin		<sup>^</sup> PCR positive does not guarantee mutation present
	<i>mphA</i>	macrolides		
	<i>strA</i> , <i>strB</i>		streptomycin	
	<i>sulI</i>		sulphonamides	
	<i>tetA</i> , <i>tetB</i> , <i>tetC</i> <i>tetO</i> and <i>tetQ</i>		tetracyclines	
	<i>floR</i>		florfenicol	
Spain	#! <i>bla<sub>TEM</sub></i> , <i>bla<sub>CTX-M-32</sub></i> <i>bla<sub>OXA58</sub></i> ## were found in groundwater at very high concentrations in the three Catalonia evaluated locations. !found in very high levels in soil / dust near farms	cephalosporins (3+ generation) ciprofloxacin (a fluoroquinolone)		All ARGs found at high levels in surface water.  An increasing concentration of relevant ARGs was observed as the rivers Gallego and Cinca advanced downstream.
	# <i>qnrS</i>	ciprofloxacin		
	! <i>tetM</i>		tetracyclines	
	<i>sulI</i>		sulfonamides	



## Key antibiotic resistant bacteria and genes relevant to the study

The World Health Organisation identifies extended-spectrum  $\beta$ -lactamases (ESBLs)-producing Enterobacteriaceae (a family of gram negative bacteria including Salmonella, *E coli*, Klebsiella) as an emerging global threat. In recent years, the geographical distribution of ESBL-producing bacteria has also increased dramatically as has their transfer to different bacterial pathogens<sup>xxii</sup>. The prevalence of ESBL-producing bacteria in swine farms has been reported to range from approximately 10% to 45% and *E coli* was the major ESBL producer<sup>xxiii</sup>. ESBL genes identified at swine farms include *bla<sub>CTX-M</sub>* (most commonly) followed by a range of *bla<sub>CMY-2</sub>*, *bla<sub>TEM</sub>*, *bla<sub>SHV</sub>*, *bla<sub>OXA</sub>*, *bla<sub>KPC</sub>*, *bla<sub>DHA</sub>*, *bla<sub>VEB</sub>*. They may vary in prevalence and geographic location. It is expected that further *bla* genes will emerge in future. These genes can convey resistance to cephalosporin (third + generations), penicillin and carbapenem antibiotics as well as some fluoroquinolones (eg ciprofloxacin) and gentamicin. Carbapenems and some of the cephalosporin antibiotics are among the last resort ('reserve') antibiotics we have available to treat humans<sup>xxiv</sup>.

In addition, the *mcr1* gene is now found on every continent and conveys resistance to colistin. This is another highest priority category antibiotic often now prohibited or restricted for use in many countries, though enforcement is variable. Other genes such as *erm* or *mph* convey resistance to macrolides and *qnrS* or mutations in *gyrA* specifically to fluoroquinolones. Other genes which can convey widespread resistance to tetracycline, streptomycin and sulfonamides (eg various *tet*, *str* and *sul* genes respectively), were tested as they have been used almost everywhere for several decades by farming industries. These antibiotics are still classes of Critically Important Antimicrobials to human health.

The presence of the *mecA* gene isolated from nearby farm environments (from bootie collected dust/soil), demonstrates selection pressure in the environment for MRSA (Methicillin Resistant *Staphylococcus aureus*) which can be livestock associated. The amount of class 1 integron genes, a genetic element that can enable bacteria to acquire resistance used widely as a proxy for anthropogenic pollution, is also increasing<sup>xxv</sup>. It is important to note that resistance genes can be transferred to other types of bacteria through mobile elements such as bacteriophages, plasmids, and transposons. Any emergence of additional antibiotic bacterial resistance genes is concerning.



Image: Meeting local small-scale farmers in Thailand. Credit: World Animal Protection



# Country key findings summary

## Thailand – results summary report card

All ESBL producing bacteria isolated from discharges from 6/9 farms were resistant to cefotaxime (a third generation cephalosporin) and ciprofloxacin (a fluoroquinolone). Co-resistance to gentamicin and co-trimoxazole was also found. Non-ESBL bacteria from at least one sample downstream from each of 4/9 farms were also found resistant to one or both of the highest priority category antibiotics. These are third generation cephalosporins or ciprofloxacin plus either gentamicin, amikacin, trimethoprim-sulfamethoxazole or amoxicillin. Water discharge from one farm had *E coli* bacteria resistant to colistin which is now restricted for use in Thailand.

### The following ARGs were also detected by PCR downstream to the discharging farms:

- *bla<sub>SHV</sub>*, *bla<sub>TEM</sub>*, *bla<sub>CTXM</sub>*, and *bla<sub>VEB</sub>*; they convey resistance in this case to cephalosporins and usually to ciprofloxacin and gentamicin
- *mcr-1* gene was identified conveying resistance to colistin from one site
- The *mecA* gene was isolated within 150m from farm buildings at five farm sites, demonstrating selection pressure in the environment for potential MRSA. MRSA itself was not cultured.

This first report of environmental ARGs from pig farms in Central Thailand infers systemic regional use of third generation cephalosporins, fluoroquinolones and other antibiotics. Results are consistent with other regions studied. They also show little change since Thailand's national antimicrobial resistance (AMR) action plan was agreed. The colistin resistance gene finding (from one sample only) could represent legal use if currently prescribed by vet for injectable treatment. However, if used for herd therapy in feed or water then the use is illegal.

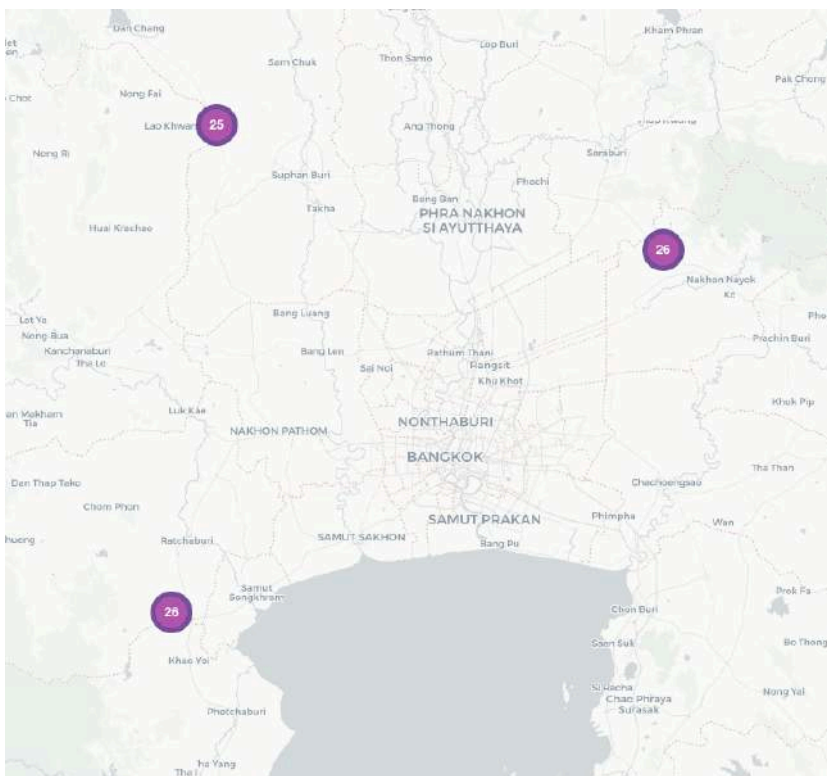


Image: Sampling locations in Thailand.

## Living with factory farms – Thailand

World Animal Protection researchers interviewed 18 local people in six communities. Some said they were too scared to speak out on how their health and ways of life as smallholder farmers have been affected by the big factory farms moving in. Others agreed that their comments could be used anonymously.

*“Since two farms have come to our community, everything has changed: the soil that once was good to grow plants has turned to rotten mud, and water that we could use and drink has become dirty and stinky.”*

Community member

*“I believe there must be drugs and disease in this canal... We don’t want such farms to locate near in the community because it creates a lot of impact to the agriculture and living. Why can’t we organise and do something with these farms?”*

Community member

*“Rice doesn’t grow the way it should when the farms release the water to the field. Some rice crops are damaged and some just die. Fish can’t also live in the pond, it’s actually the whole ecosystem in this area. I used to complain about this, but nothing has happened.”*

Smallholder

**Image:** Pollution from industrial farms impacts the local community in Thailand. Credit: World Animal Protection



## United States – results summary report card

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There was considerable ARG contamination in samples downstream and upstream of farms. There is a high density of pig farms in the area so the widespread contamination found strongly suggests factory farms are discharging resistance genes into public waterways.

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Ninety-two percent of samples (83/90) had positive PCR results for three or more resistance genes; the largest number of genes in single sample was 10. Around half of the samples were positive for three or more tetracycline-resistance genes, typically *tet<sub>A</sub>*, *tet<sub>B</sub>*, and *tet<sub>C</sub>*.

- ▣ Positive PCR results for resistance to streptomycin, an aminoglycoside were identified in 59/90 samples. *StrA* was predominantly found in downstream samples. Similarly, *strB* was found in 78 percent of downstream samples.
- ▣ Positive PCR results for the *gyrA* gene conferring possible resistance to fluoroquinolones were found in 31/90 samples. It was mostly detected in downstream samples from the target farm sites and only downstream in three sites. The process targeted the region in which a mutation of *gyrA* conferring resistance to fluoroquinolones is known to occur, though a PCR positive result is not a guarantee that this specific mutation was present.
- ▣ Genes conferring resistance to beta-lactam antibiotics, which include cephalosporins, and carbapenems, were identified in 23/90 samples. *Bla<sub>CTX-M</sub>* was most commonly found only in downstream samples. EBSL genes from a single downstream site included—*bla<sub>CMY</sub>*, *bla<sub>CTX-M</sub>*, and *bla<sub>TEM</sub>*.
- ▣ The *mphA* gene, conferring resistance to macrolides, was identified in 9/90 samples and found predominantly in samples taken downstream from target farm sites. The gene was found upstream at only two sites and only in soil samples. This indicated that the genes could have been carried by air rather than water.

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The results of this testing project are striking. They are supported by previous studies identifying antibiotic resistant bacteria and/or antibiotic resistance genes as widespread on farms, in manures, and in the environment near farms in the USA.

The frequency of finding genes that confer resistance to specific classes of antibiotics heavily used by or sold to the pork industry is critical in understanding the impact. Such frequency adds to the mounting evidence that routine farm use negatively affects public health by spreading antibiotic-resistance outside farms. Previous studies have confirmed a similar pattern in comparing ground water sampling downstream from farm sites. And the pattern has been associated with longstanding tetracycline use.

A separate study at monitoring wells and groundwater sites near two swine farms in North Carolina found *E coli* resistant to antibiotics approved for use in pig production. At the first site, 37% of *E coli* isolates were resistant to at least one antibiotic. Resistance to chlortetracycline, tetracycline, and sulfamethoxazole were identified. At the second site, 79% of *E coli* were resistant. Resistance was predominantly to tetracycline and chlortetracycline, and to ampicillin, streptomycin, chloramphenicol, sulfamethoxazole, trimethoprim, florfenicol, and neomycin<sup>xxvi</sup>.



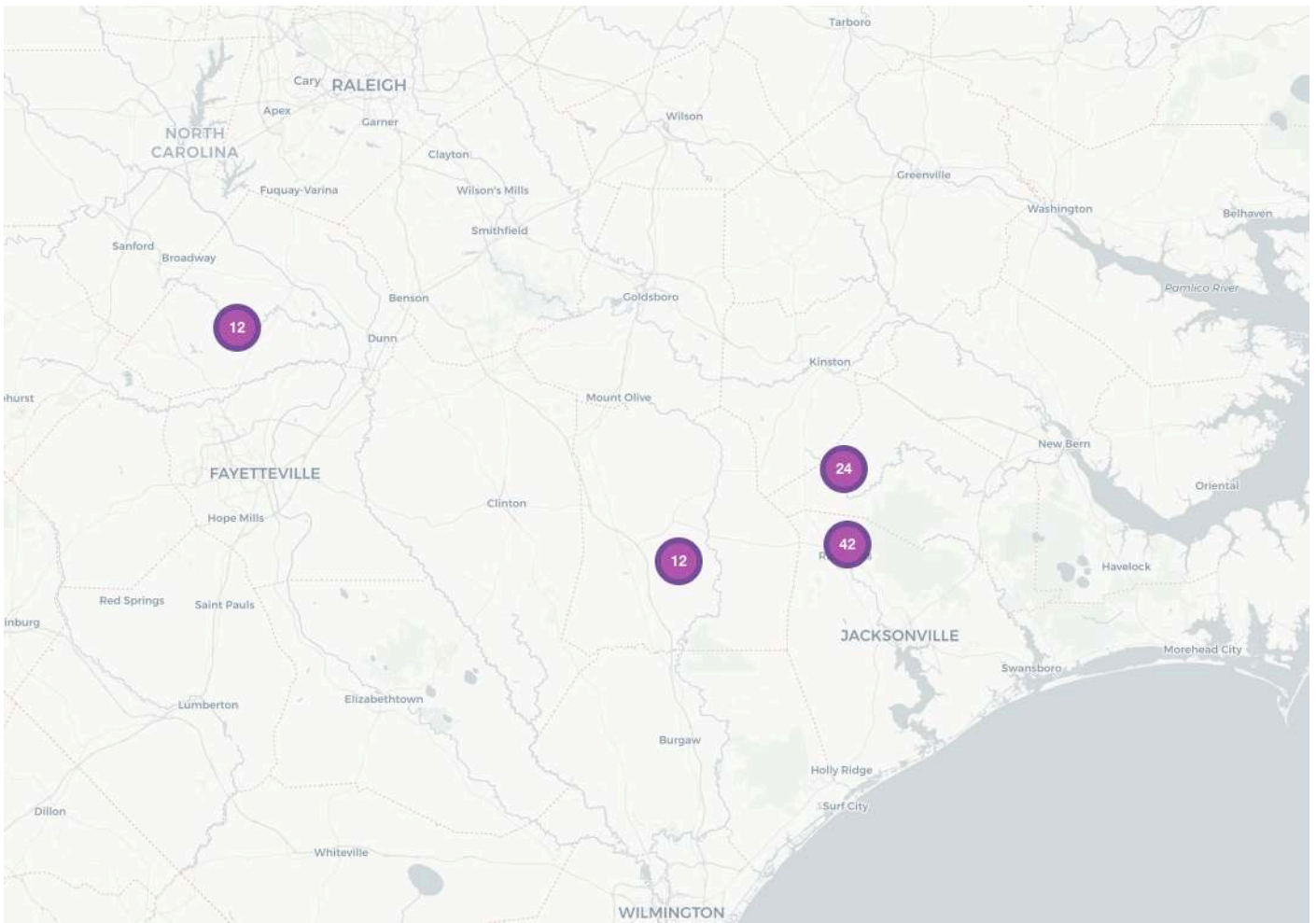


Image: Sampling locations in the USA.

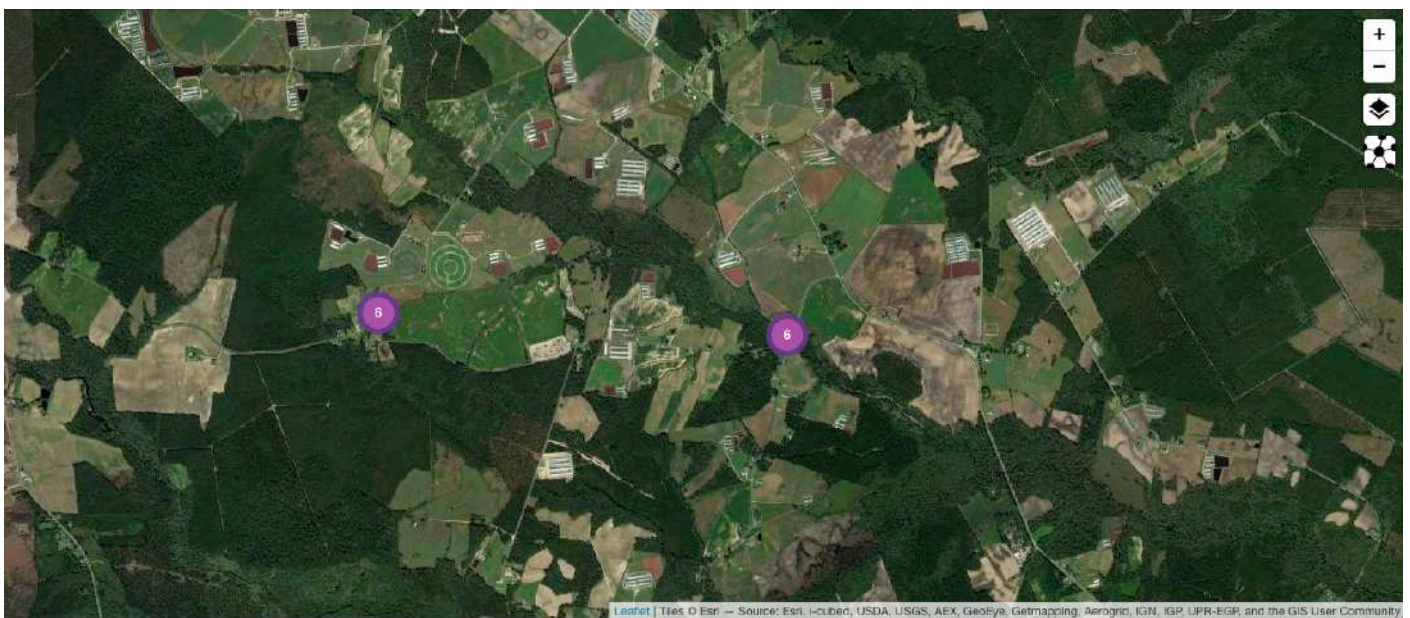


Image: Some US sampling sites in an area of high density of pig farms in North Carolina. Grey lines denote pig barns and beside them effluent ponds - showing many farms in a small area.

## Canada – results summary report card

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All samples (water or soil) had at least one of the target antibiotic resistance genes: *mphA*, *bla<sub>CTM</sub>*, *bla<sub>CTX-M</sub>*, *floR*, *gyrA*, *strA*, *strB*, *sul1*, *tetA*, *tetB*, *tetC*, *tetO* and *tetQ*.

- Nearly all samples (38/42) had positive PCR results for at least one of the tetracycline-resistant genes included in the analysis.
  - Positive PCR results for ARGs conveying resistance to streptomycin was identified in 36/42 samples. *StrA* was found predominantly in downstream samples.
  - Genes conferring resistance to beta-lactam antibiotics, which include cephalosporins and carbapenems, were identified from samples from all eight farm sampling locations. Such genes were only found in a downstream sample from one farm. Further analysis was not undertaken, and could show differences in concentration of genes between upstream and downstream samples.
  - The *mphA* gene, conferring resistance to macrolides, was identified in all eight farm sampling locations. Importantly, it was also present downstream of three farms.
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Our results align well with previous Canadian studies from other provinces that demonstrate a very similar range of genes from pig manure (slurry) and runoff from crops fertilised with it.<sup>xxvii</sup> Earlier studies show such genes (especially the *tet*, *sul*, *str* variants and *bla<sub>TEM</sub>*) have existed on farms and within the industry for well over a decade.<sup>xxviii, xxix</sup>

Resistance to tetracyclines was most frequently found in Canadian wild animals living on or near pig farms and from pigs on the same farms.<sup>xxx, xxxi, xxxii</sup> In Canada, more than 50% of antimicrobials are used for disease prevention<sup>xxxiii</sup> and around 80% of antibiotics are administered to pigs in their feed. Macrolides and tetracyclines represent two of the three most abundantly used classes of antibiotics used in pig feed.



**Image:** Dead pigs in a dumpster along a road near an industrial pig farm, Canada. Credit: [Planet in Peril – Where Science Gets Respect](#)

## Living with factory farms – Canada

World Animal Protection interviewed members of Hog Watch Manitoba.

**Janine**, one of the interviewees, reflected on how things have changed for the worse over the years.

*“There is so much misapplication of hog manure...I’ll show you pictures of the creek that runs through our land. When I moved here 40 years ago, it was a perfectly lovely creek, and now it’s choked with duckweed, showing all the excess phosphorus and nitrogen application...I would see three times the allowed rate being put on.*

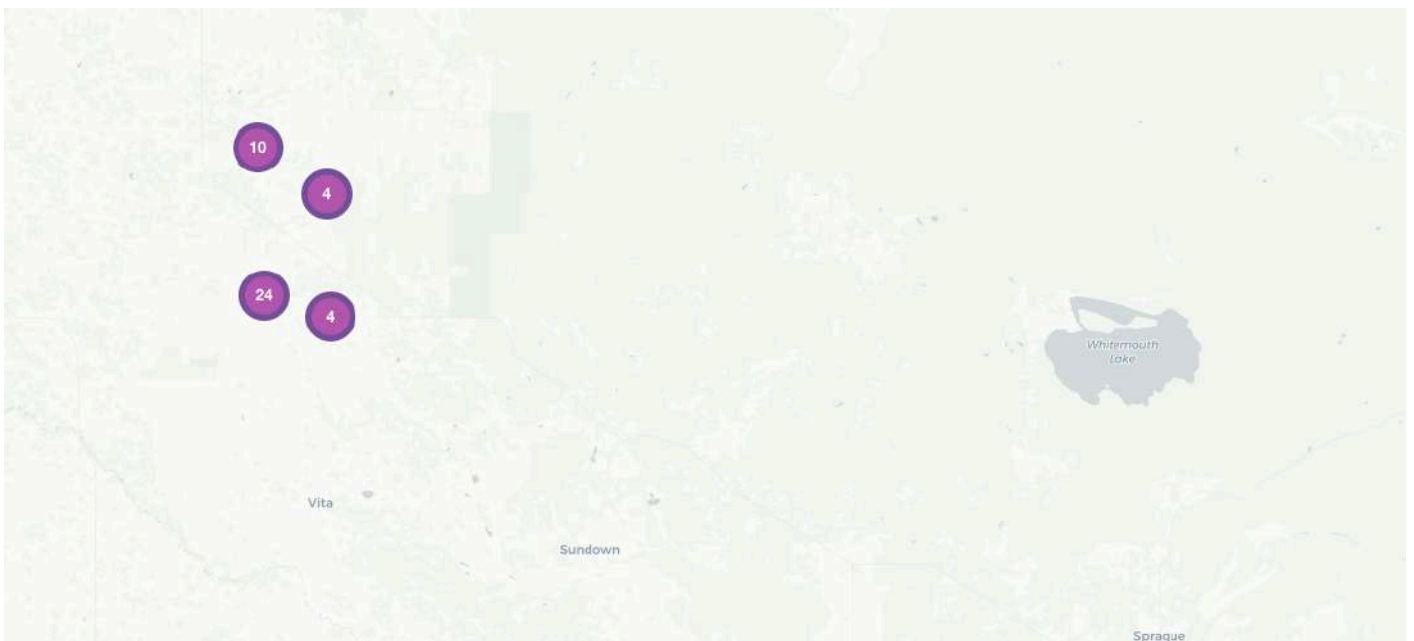
*The government is actively working for the industry as opposed to the greater good.”*

**Bill**, a local small-scale farmer, is impacted by practices of industrial farms.

*“To me, it’s such a shame to see pigs being raised in this industrial manner. And, of course, with the straw-based manure systems we have, that manure is collected, it’s solid, it’s spread on the fields, it can be spread anywhere on your land.*

*I think in terms of antibiotic use, animal welfare, animal health, and just the ethics of this whole industrial animal agriculture, I can hardly believe that a civilized society like ourselves can treat animals like this.”*

**Image:** Sampling locations around Manitoba, Canada.





## Spain – results summary report card

The most broadly contaminating ARG in public water tested was *tet<sub>M</sub>* conveying resistance to tetracyclines, while ARGs (*bla<sub>TEM</sub>*, *bla<sub>CTX-M-32</sub>*, *bla<sub>OXA-58</sub>*, *qnrS*, *sul1*) conveying resistance to cephalosporins, fluoroquinolones and sulfonamides were also prevalent in water samples. Testing in Spain included quantitative analysis of ARGs. Some levels in water downstream were more than five times higher than the baseline concentrations. Those differences reached levels up to 200 times higher than the baseline in specific sampling locations.

- ❖ *Bla<sub>TEM</sub>*, *bla<sub>CTX-M-32</sub>*, *qnrS*, *sul1*, *tet<sub>M</sub>* were detected in greater levels in the downstream water samples.
- ❖ An increasing concentration of relevant ARGs was observed as the rivers Gallego and Cinca advanced their courses downstream, especially for the gene *tet<sub>M</sub>*.
- ❖ The ARG results for sediment testing corresponded to the results of the most contaminated farm water testing. High levels were detected for the ARGs *bla<sub>TEM</sub>* and *tet<sub>M</sub>*.
- ❖ For the dust or soil samples tested by bootie samples, the ARG levels are well above what is expected from normal soils
- ❖ ARGs conferring resistance to fluoroquinolones (*qnrS*) and  $\beta$ -lactamases (*bla<sub>TEM</sub>*) were found from groundwater concentrations ( $\geq 1 \cdot 10^4$  copies/ $\mu$ L) in the three evaluated locations.

The genes found in water and sediment (particularly *bla<sub>TEM</sub>*, *tet<sub>M</sub>* and *qnrS*) are strongly associated with the use of pig slurry and soils fertilised with slurry in the studied regions, as confirmed in these regions.<sup>xxxiv</sup> This suggests a strong correlation between the presence of industrial pig farms and the high levels of ARGs. The results of bootie samples clearly show that pig farms are an important source of ARGs contaminating the surrounding environment.

Recent published studies reinforce our findings confirming many of the above genes found directly in pig slurry in Spain,<sup>xxxv</sup> especially from the Catalonia region. This area has one of the highest densities of pig farms in Europe.<sup>xxxvi</sup> Spanish studies also find a correlation of ARG levels with intensive pig farms and higher use of antibiotics.<sup>xxxvii</sup>



**Image:** Slurry containing pig manure dumped near a water course, Spain. Credit: Pueblos Vivos Cuenca

## Living with factory farms – Spain

World Animal Protection researchers interviewed 11 local people in two communities. A common thread was the fear of speaking out against a powerful industry.

**Amaia**, one of the interviewees, explained how life has changed since factory farms arrived in her area of Aragon, north-eastern Spain.

*“My parents have always had animals, as a child I lived in a house where there were chickens, rabbits and sometimes a pig. Everything was for our own consumption, because my parents combined working in the family fields with working elsewhere to earn a wage. In the town where we lived it had always been like that, and there were few people who were dedicated to extensive livestock farming.*

*The cattle farms were small and the animals went out to the meadows to graze (I speak of 30 years ago). Then everything changed. Larger and larger farms began to be built, with noises, smells, trucks, dust and dirt; and people no longer walked through those areas, nobody went near there.*

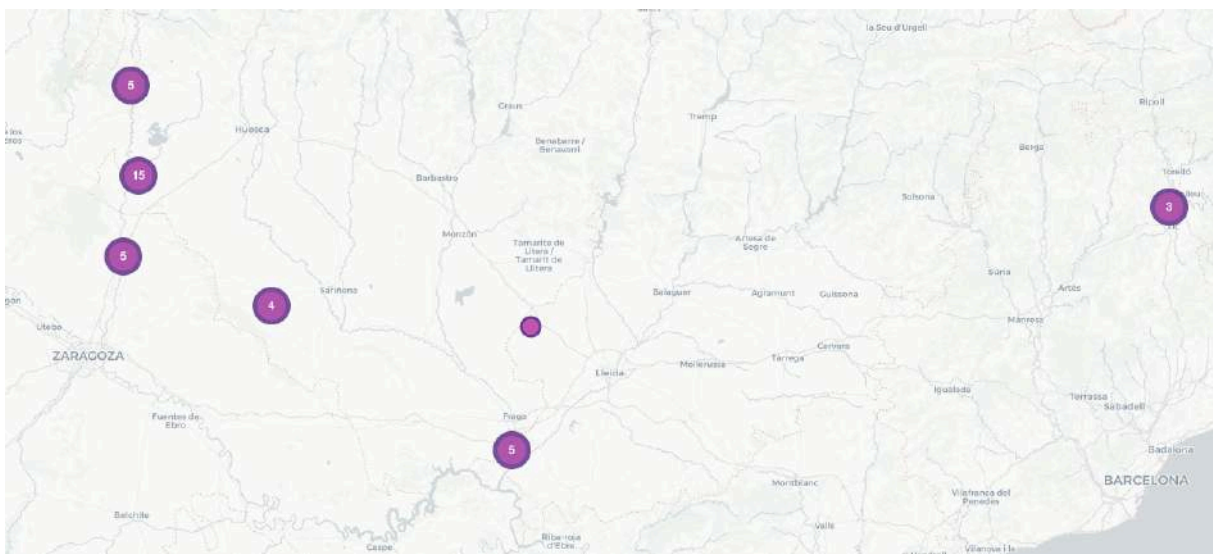
*The areas where the two pig farms are located are degraded, nobody comes to walk there, it smells very bad and the appearance of the area is very unpleasant: dust, dirt, noise... When they put the slurry in the fields no one can go near the farms as the smell is unbearable and lasts for several days.”*

**Rosa** who also lives in the Aragon region reported that her area had been badly affected by factory farming’s growth.

*“Without water there is no future, and here we don’t have any water to spare. These villages will not survive if you cannot open the windows, or be outside or walk, if you cannot drink the tap water, or if we lose our great attraction, which is the landscapes and tranquillity.*

*The meat lobby is very powerful, and the profit of a few companies is taking precedence over public health.”*

**Image:** Sampling locations in Spain.



Detailed methodology and country-by-country results are included in appendix 2.



Image: Global map of sampling locations.

## Enabling low welfare farming – antibiotic use

Factory farms squash billions of genetically identical animals into stressful, barren environments, with no access to outdoor space or natural light. Animals are often caged, with no room to turn around or lie down with their limbs, head or wings fully extended. This highly stressful and completely barren environment can lead to injuries and severe behavioural issues. These can include aggression or repetitive behaviour like cage-biting or chewing continuously on nothing until frothing at the mouth, feather pecking or cannibalism.

Antibiotics are used across groups to prevent stressed animals getting sick; they prop up a system of suffering for food production.

Pig farms deliberately use very high levels of antibiotics. This is because pigs are one of the most intensively farmed species on the planet. In some studies, up to 90% of antibiotics were administered in the first 10 weeks of pigs' lives. Their use is associated with painful mutilations (especially surgical castration) and related gut and respiratory infections<sup>xxxviii</sup>.

Larger farms use more antibiotics per kilogram of animal than smaller farms, and farms with pigs in the last fattening phase before slaughter use the most antibiotics, often to prevent disease<sup>xxxix</sup>.

Superbugs in retail pork samples – World Animal Protection research in 2018 and 2019 found superbugs critically important to humans in pork sampled from the shelves of large retailers in [Brazil](#), [Spain](#), [Thailand](#) and the [USA](#).



# Antibiotic overuse in farming and emerging superbugs

The UN, G20 and many world leaders recognise superbugs (antimicrobial resistance) as a global health emergency. They have called for comprehensive actions across medicine, the community and agriculture to tackle the problem. Yet, excessive use of antibiotics in animal farming continues.

Globally, around three quarters of all antibiotics are used in farming<sup>xl</sup>. And their overuse clearly facilitates the development of superbugs<sup>xl</sup>. These can then be spread via food, animals, manure, air, insects and the environment and pose major risks for people and public health.

Use of antibiotics in feed or water to promote fast growth of farm animals or to prevent disease across entire herds remains widespread in most countries. There is little or no national surveillance and reporting of antibiotic use and surveillance for superbugs. There are no internationally agreed maximum environmental safe levels or resistance indicators.

To date, 'antibiotics most critical for use in humans' have been the focus of discussion by the UN, public health bodies and the food industry. However, antimicrobial resistance is transferable between classes and types of antibiotics. Swapping to another antibiotic considered less important for humans, or replacing antibiotic use with a probiotic, herb or tightened hygiene and biosecurity are not the answers.

**Image:** Mother pigs (sows) are kept in barren conditions with no enrichment on a low welfare farm. Credit: World Animal Protection



# Environmental risks from factory farming

Superbugs and antibiotic resistance genes do not remain on the farm. Intensive animal production generates large quantities of animal waste, which is often spread on land for use as a fertiliser, or discharged into public water ways. It can also seep into groundwater.

Also, animals do not metabolise around 70% of the antibiotics administered to them, so antibiotics can transfer into animal waste. Bacteria can survive in untreated farm animal waste for 2-12 months<sup>xlii</sup>.

In the USA, other research has found that surface waters near factory farms have faecal bacteria concentrations exceeding federal and state recreational water quality values. In 112 of 185 water samples, enterococcus bacteria exceeded guidelines, with the highest concentration in a single sample roughly 150 times the standard level. The highest bacterial concentrations were found immediately downstream of where swine manure was sprayed on fields<sup>xliii</sup>.

Technology, including anaerobic digesters, is commonly used on pig farms. The digesters break down and treat pig manure to produce biogas for fuel on the farm and nutrient-rich sludge to apply to fields. However, anaerobic digesters do not remove antibiotics or superbugs, and can create favourable conditions for antibiotic resistance genes to develop and spread<sup>xliv</sup>.

This means that antibiotics or antibiotic resistance genes can pass from the farm into the environment, promoting the evolution of antibiotic resistant bacteria in soil and water<sup>xlv</sup>. This can extend to contamination of both surface water and groundwater with superbugs from manure sludge<sup>xlvi</sup>.

Waterways can act as reservoirs where superbugs accumulate. This is because they harbour discharge from agricultural runoff and human wastewater treatment plants<sup>xlvii</sup>.

Once in the environment, superbugs can reach humans in multiple ways. This ranges from recreation, water used for drinking and washing, consumption of fish and bivalves from contaminated water, and consumption of crop produce contaminated with surface water<sup>xlviii</sup>.

The problem also affects soil health. Manure sludge contaminated with superbugs can enter soil and alter the balance of bacteria. Once in the soil, superbugs can persist there even if there is no further contamination with antibiotics. Studies have shown higher levels of ARGs in soil where manure has been applied for up to six months following application. This suggests ARGs can accumulate in soil over time<sup>xlix</sup>.

Antibiotic resistant bacteria can also be found in the air surrounding livestock farms<sup>l</sup>. Flies and insects also have contact with livestock and manure, contract antibiotic resistant bacteria and transmit it to people. Research from Johns Hopkins University in the USA found that many houseflies near chicken operations carried antibiotic resistant bacteria strains<sup>li</sup>.

This is not just a problem for land-based farming. Up to 75% of antibiotics used in aquaculture may also be lost into the surrounding environment<sup>lii</sup>.

It is lesser known that heavy metals and disinfectants used in farming can develop resistance. Animal feed supplements include heavy metals, such as zinc oxide for pigs. Zinc oxide is expelled in pig manure when it is applied to fields, and this is another source of resistance in the environment. Disinfectants are widely used in cleaning farms or in foot baths for workers to prevent spread of disease. However, disinfectants can also drive antimicrobial resistance<sup>liii</sup>.



# Lack of monitoring of environmental antimicrobial resistance

Despite the environmental and public health risks, there is minimal surveillance or monitoring of antimicrobial resistance in the environment. A United Nations Food and Agriculture Organisation 2017-2018 global survey found of 78 countries surveyed, only 10 have regulations limiting the discharge of antimicrobial residues into the environment<sup>iv</sup>.

Studies have investigated antibiotic residues in rivers in different parts of the world and compared findings against the AMR Industry Alliance designated 'safe' levels<sup>v</sup>. However, monitoring of antimicrobial resistance itself is limited to smaller, ad hoc studies.

Switzerland has taken steps to monitor antibiotic residues in rivers and lakes<sup>vi</sup>, but most countries do not monitor either antibiotic residues or AMR.

This is despite the UN calling on countries to step up management of waste into water ways. The UN has expressed strong concern on the long-term risks of environmental AMR and the lack of monitoring in place<sup>vii</sup>.

**There remains no international standard that describes the concentration at which superbugs in the environment become dangerous to people. There are no agreed limits on superbug environmental contamination or any standardised international methodologies to monitor and track the problem.**

**Image:** The mother pigs on this Canadian farm live in groups, not cages. They have access to the outdoors. Credit: World Animal Protection







## Creating a humane and sustainable food system

The irresponsible use of antibiotics and unacceptable treatment of animals is causing increased concern locally, regionally and globally.

From 2022, the EU will prohibit all routine antibiotic use in farm animals including the use of antibiotics in animal feed and drinking water to prevent disease across groups. Antibiotics sales and use data will be collected. Live animals and animal products imported into the EU must not have been administered antibiotics to promote fast growth. It is imperative the new rules are enforced from 2022.

This development will bring the EU into line with Denmark, Finland, Sweden, Norway, Iceland and the Netherlands. These countries already have prohibitions on use of antibiotics to prevent disease in groups of animals.

The EU also has a target under its Farm to Fork policy to reduce sales of antibiotics for farm animals by 50% by 2030. It seems likely that further sales reductions will be required to meet the new requirements.

Reduction in antibiotic use can be achieved by improving animal welfare. If painful mutilations like tail docking and teeth clipping of pigs are not done, then antibiotics may not be used routinely to

prevent infection. In Finland, Sweden, Denmark, Netherlands and Thailand, ending tail cutting of piglets has reduced antibiotic use<sup>lviii, lvix</sup>.

Studies in Europe show that pigs in organic, high welfare systems have lower rates of antimicrobial resistance compared with pigs raised in conventional systems<sup>lx, lxi</sup>. Animals fed on pasture outside tend not to be given antibiotics routinely in their feed. A study in Belgium finds intensively farmed veal calves are given far higher amounts of antibiotics than less intensively reared beef cattle<sup>lxii</sup>.

The use of high welfare chicken breeds that grow more slowly allows for substantial reductions in antibiotic use compared with conventional intensive systems<sup>lxiii, lxiv</sup>.

Moving to more sustainable, high welfare food systems and lower animal production overall is critical in addressing the unsustainable overuse of antibiotics and farming and protecting public health and the environment. Reducing consumption of animal products and increasing consumption of plant-based foods is also vital<sup>lxv</sup>.

# Recommendations

World Animal Protection calls on the global retail sector, animal protein production sector, governments and intergovernmental organisations, and the global finance sector to act on our recommendations.

## Global food retail and animal protein production sectors should...

- **Meet [FARMS](#)** animal welfare requirements for production or procurement as a minimum. Develop an overarching animal welfare policy informed by the Five Domains<sup>lxvi</sup> model that leads to a Good Life<sup>lxvii</sup> for farmed animals.
- **Commit** to using antibiotics responsibly in farming: ending the routine use of antibiotics including to promote fast growth and to prevent disease across groups. Antibiotic use must be reduced by addressing underlying welfare issues, and not through pursuing 'antibiotic-free' or 'no antibiotics ever' or 'raised without antibiotics' policies or product lines. This can create a disincentive for producers to treat sick animals and harm animal welfare.
- **Increase** the proportion of plant-based protein options to support an average global reduction in meat production and consumption of 50% by 2040, and publicly document progress.
- **Publish** annual reports on progress towards implementing high welfare commitments in conjunction with antibiotic use data on supplier farms.

## Governments and intergovernmental organisations should...

- **Introduce** and enforce regulations in line with [FARMS](#) animal welfare requirements as a minimum.
- **Introduce** and enforce regulations ending the routine use of antibiotics including to promote fast growth and to prevent disease across groups.
- **Commit** to national surveillance and public reporting of antibiotic use at farm level in conjunction with reporting on welfare practices on farms.
- **Redirect** subsidies and financial incentives to high welfare systems that align with the Five Domains welfare framework and in support of an average global reduction in meat production and consumption of 50% by 2040.

## Financial investors in food systems should...

- **Require** companies to meet [FARMS](#) animal welfare requirements as a minimum.
- **Phase in** requirements for companies towards systems informed by the Five Domains welfare model that leads to a Good Life for farmed animals.
- **Require** companies to commit to using antibiotics responsibly in farming; ending the routine use of antibiotics including to promote fast growth and to prevent disease across groups. Antibiotic use must be reduced by addressing underlying welfare issues, and not through pursuing 'antibiotic-free' or 'no antibiotics ever' or 'raised without antibiotics' policies or product lines. This can create a disincentive for producers to treat sick animals and harm animal welfare.
- **Increase** the proportion of plant-based protein in the investment portfolio to support an average global reduction in meat production and consumption of 50% by 2040, and publicly document progress.
- **Influence** policy such as supporting regulations on corporate and financial services sustainability disclosures, frameworks to facilitate sustainable lending, due diligence processes, and antibiotic use.

**Image:** Free-range chickens raised for eggs can express natural behaviours on pasture. Credit: World Animal Protection



# Appendix 1 - A growing and worldwide concern

A World Animal Protection 2020 survey across 15 countries found that 88% of people are concerned about superbugs from farm animals and want animals treated well and antibiotics used responsibly.

Two-thirds of those surveyed said they would help test for pollution from farms to make big business accountable.

Table 2 - 2020 survey summary

<b>% in agreement, 1,000 participants per country</b>	<b>Canada</b>	<b>Spain</b>	<b>Thailand</b>	<b>USA</b>	<b>Global average of 15 countries<sup>lxviii</sup></b>
Believe overuse of antibiotics in farm animals is wrong	89	89	78	86	84
Concern over superbugs from farm animals	85	92	83	79	88
Believe antibiotics should only be used to treat sick animals	82	90	82	82	85
Believe governments should monitor and report on the use of antibiotics in farm animals	92	94	95	86	92
Believe governments should increase minimum welfare laws for farm animals	88	93	97	79	90
Prepared to help test for pollution from farms	63	75	81	56	65



# Appendix 2 - Study methodology and detailed results

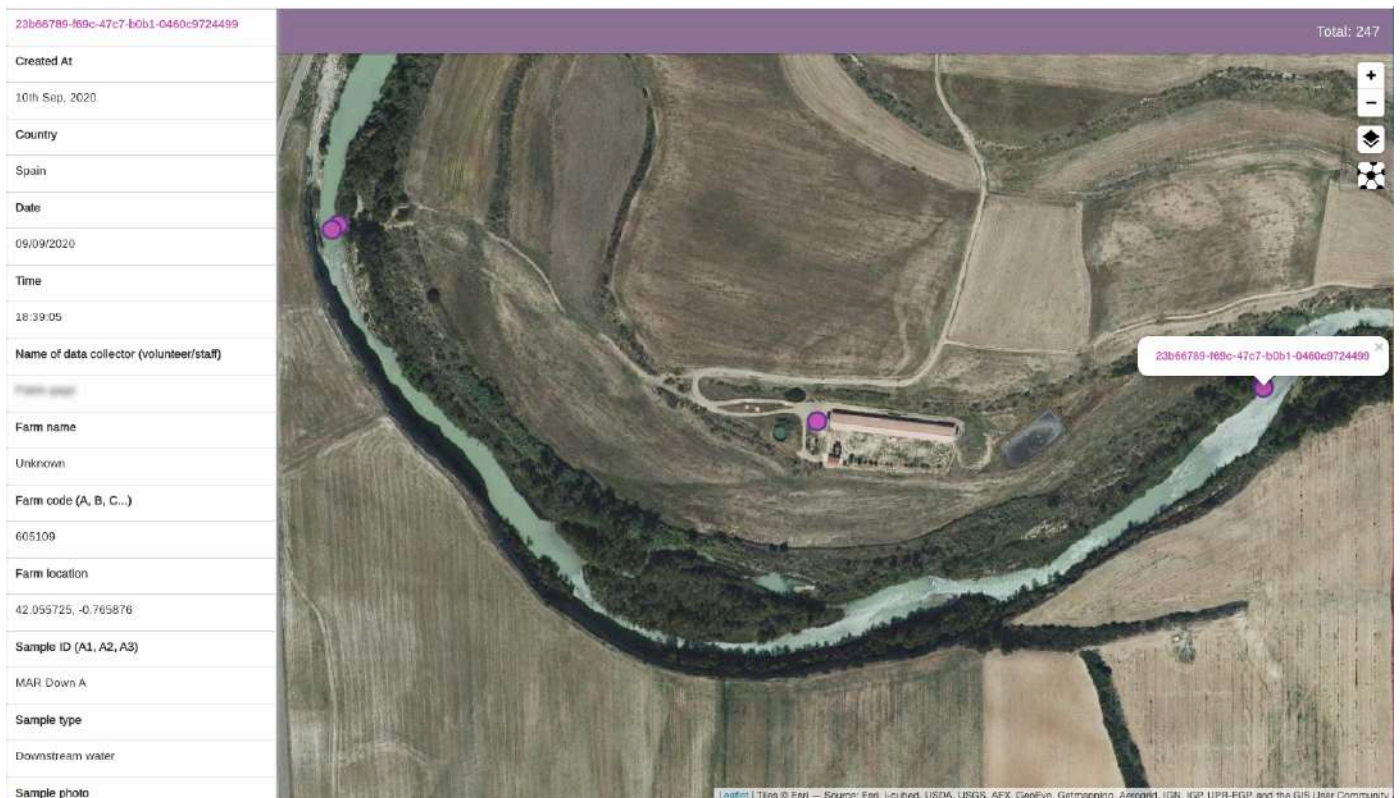
## Methodology

Water and sediment from public water courses connected to effluent discharges from 6–10 pig farms in each of four countries (Canada, Spain, Thailand, US) were tested. Samples were collected up and down stream of farm discharge.<sup>lxix</sup> In addition, surface soil or dust samples were collected by the 'bootie' method,<sup>lxx</sup> walking on public land within 150m adjacent to the farms for analysis specifically for the *mecA* gene in Spain and Thailand.

All samples were recorded within Epicollect5. Samples were analysed for ARGs (Antibiotic Resistance Genes) by national accredited laboratories using RT-PCR/qPCR looking for many of the key genes described previously.<sup>lxxi</sup> Bacteria were cultured and antibiotic sensitivity was conducted also in Thailand. Results were analysed for the presence of ARGs in downstream samples compared to upstream, and positive PCR by bootie sample or culture.

For Spain and the USA, quantification of relevant ARGs: a calibration curve generated by 10-fold dilutions of a plasmid vector was used as a standard. ARGs concentration in samples was analysed and calculated using the standard curve equation and the measured quantification cycle.

**Image:** This photo shows the sampling methodology of the study. The upstream water and sediment sampling is denoted by the far left purple markers. The far right markers denote downstream water and sediment sampling corresponding to the Epicollect code and details on the LHS. The middle marker adjacent to the rectangular farm barn and effluent pond is where the bootie sampling was done.



# 1. Thailand – results summary and interpretation

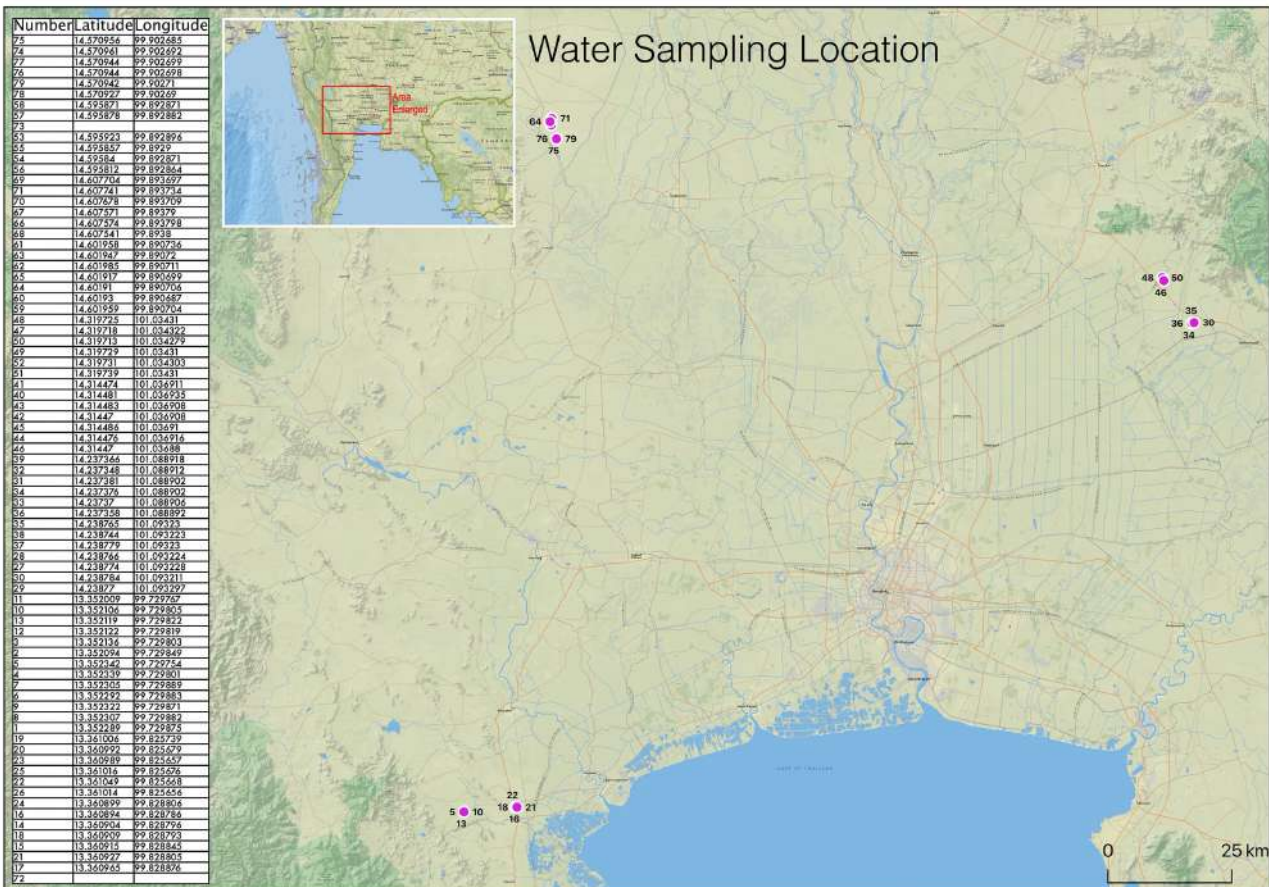
In August 2020, 77 environmental samples were taken up and downstream from nine medium or large industrial pig farms in central Thailand. Samples were batched for laboratory analysis (18 water, 18 sediment plus nine bootie samples, taken nearby to farm buildings). Bacterial culture and antibiotic sensitivity was performed. Where no bacteria were isolated, PCR testing was conducted for the ARGs: *bla*<sub>SHV</sub>, *bla*<sub>TEM</sub>, *bla*<sub>CTX-M</sub>, *bla*<sub>VEB</sub> or *mcr-1*, *mecA*.

Thirteen isolates of extended-spectrum  $\beta$ -lactamases (ESBLs) producing *E coli* and *Klebsiella* (eight *E coli* and five *K pneumoniae*) were present in water and sediment samples from six of the nine farms. These bacteria have ESBL genes which convey resistance to multiple antibiotics including some categorised as highest priority ‘critically important to human health’ by WHO (as bolded below). Additionally, 39 isolates of non-ESBL *E coli* and *Klebsiella spp* were cultured.

Specifically, all ESBL producing bacteria isolated from discharges from 6/9 farms were resistant to cefotaxime (a third generation cephalosporins) and ciprofloxacin (a fluoroquinolone). Co-resistance to gentamicin and co-trimoxizole was also found. Non-ESBL bacteria from at least one sample downstream from 4/9 farms were also found resistant to one or both of the highest priority category antibiotics: 3<sup>rd</sup> generation cephalosporins or ciprofloxacin plus either gentamicin, amikacin, trimethoprim, sulfamethoxazole or amoxicillin. Water discharge from one farm had *E coli* bacteria resistant to colistin which is now restricted for use in Thailand.

The following ARGs were also detected by PCR downstream of the discharging farms.

- ❖ *bla*<sub>SHV</sub>, *bla*<sub>TEM</sub>, *bla*<sub>CTX-M</sub>, and *bla*<sub>VEB</sub>; they convey resistance in this case to cephalosporins and commonly also to ciprofloxacin and gentamicin.
- ❖ *mcr-1* gene was identified conveying resistance to colistin in the one case above
- ❖ The *mecA* gene was isolated within 150m from farm buildings at five farm sites, demonstrating selection pressure in the environment for potential MRSA. MRSA itself was not cultured.



## Interpretation

This is the first report of environmental ARGs from pig farms in central Thailand. The bacterial resistance results also infer systemic use of third generation cephalosporins fluoroquinolones and other antibiotics by pig farms in this region. Findings reiterate culture and sensitivity results sampled in 2012 and 2015 from the pig industry in other regions,<sup>lxii, lxiii</sup> before the Thai national AMR action plan and revised feed regulations.

Our results suggest that such policy instruments have not effectively protected public waterways. The colistin resistance gene finding, from one farm only, could represent use which could be legal if currently prescribed by vet for injectable treatment. However, it could also mean illegal use if currently used for herd therapy in feed or water.

A 2020 study of farm use of antibiotics from north-east Thailand showed that medium-scale contract breeding farms (100-500 sows) used antibiotics mostly (94%) for disease prevention as advised by the parent companies. Of these, 41% used enrofloxacin (a fluoroquinolone) one of the limited types of antibiotics permitted. Other antibiotics allowed include gentamicin, amoxicillin, cephalexin, penicillin-streptomycin.

Interestingly, owners and managers of medium-sized farms in the study reported that pigs mostly experience gut diseases (particularly diarrhoea). They said they tried to protect them via vaccination (usually as piglets) and biosecurity fencing around the property. Furthermore, almost half of the farms surveyed (> 50 farms) received medicated feed. All farms belonging to one of the two contracting companies administered antibiotic medicated feed to their pigs. Sows received antibiotic injections after giving birth to prevent infections.<sup>lxiv</sup>

While the study compared the access, knowledge and education and practices of smaller and medium-sized farms, it did not compare the volumes of antibiotic use. Use of antibiotics to prevent disease may be a feature of contracts for large company pig farming in Thailand.



## 2. USA – results summary and interpretation

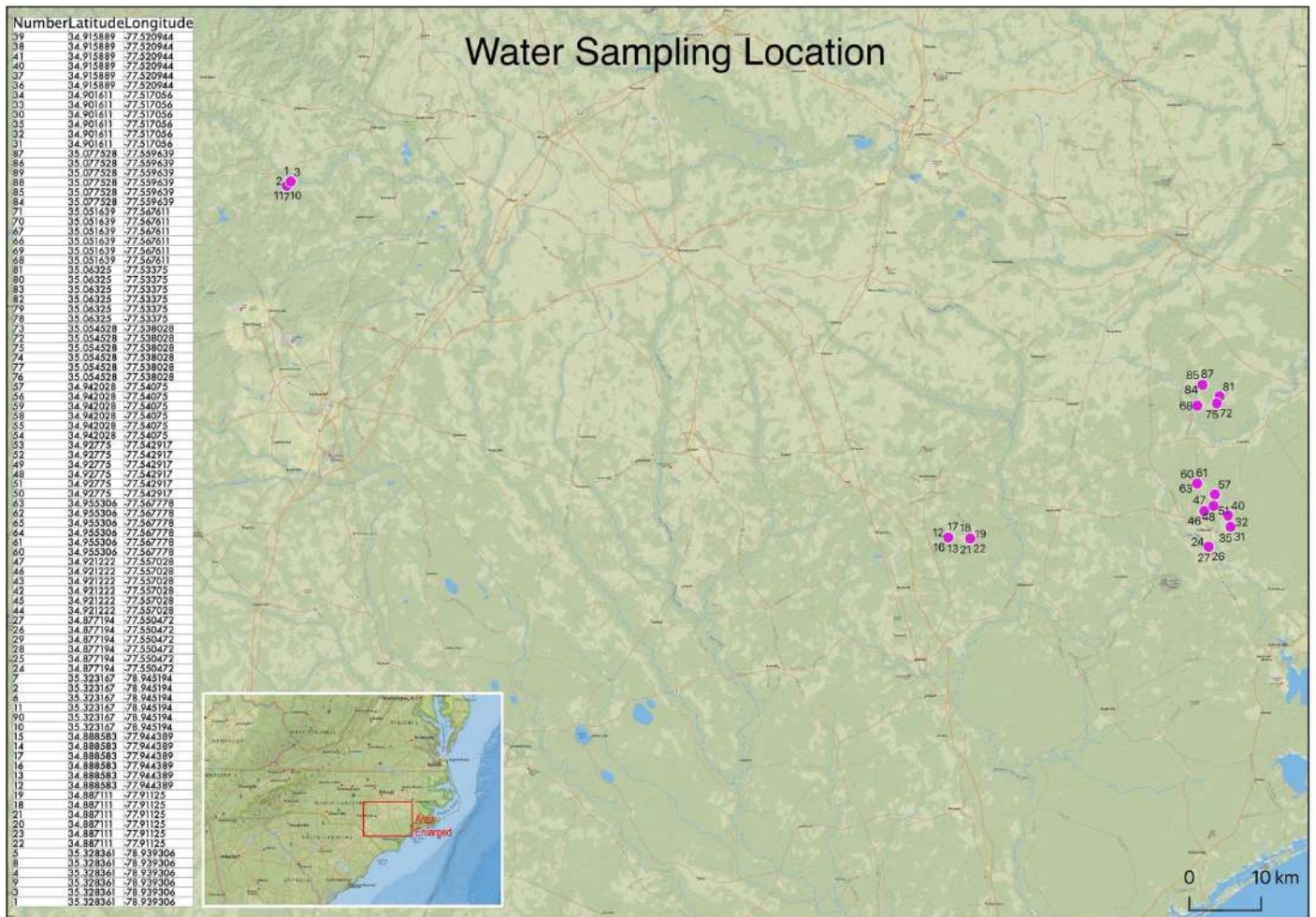
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In October 2020, 45 water samples and 45 soil samples were taken across eight sites in eastern North Carolina, both downstream and upstream of intensive confinement farms. These samples were then tested for the presence of 27 target ARGs.

Overall, the testing results indicate that all samples were positive for the presence of at least one of the target resistance genes. Given the high density of farms in the location, ARG presence in all samples is worth noting, as the upstream water is allegedly already contaminated. Ninety-two percent of samples (83/90) had positive PCR results for three or more resistance genes, with 10 the largest number of genes in a single sample.

Fifteen samples (17%) had positive PCR results for seven or more resistance genes. Seven resistance genes – *floB*, *gyrA*, *strB*, *sul1*, *tetA*, *tetB*, and *tetC* – were each found in at least one-third of all samples, with *tetA* found in nearly all (83/90) samples taken.

- Nearly all samples (89/90) had positive PCR results for at least one of the tetracycline-resistance genes included in the analysis. Roughly half of the samples were positive for three or more tetracycline-resistance genes, typically *tetA*, *tetB*, and *tetC*. Given the US pork industry's widespread use of tetracyclines (categorised as highly important by WHO)– it is unsurprising that tetracycline-resistance would be prolific in the surrounding environment. Information about tetracycline use is based on annual sales data and voluntary surveys.
- Positive PCR results for resistance to streptomycin, an aminoglycoside was identified in 59/90 samples. *StrA* was found less frequently, but where found it was predominantly in downstream samples. Similarly, *strB* was found in 78% of downstream samples compared to 45% of upstream samples.
- The *gyrA* mutation gene\* confers resistance to fluoroquinolones. PCR positives were found in 31/90 samples. It was most likely detected in downstream samples from the target farm sites and more soil samples had positive PCR for the gene than water. (\*additional testing would need to be done to confirm all positives).
- At three sites the *gyrA* mutation gene\* was identified PCR positive in only downstream samples
- Genes conferring resistance to beta-lactam antibiotics, which include penicillin, cephalosporins and carbapenems were identified in 23/90 samples. *bla<sub>CTX</sub>* and *bla<sub>CMY</sub>* were most commonly found. Samples from Site 2 had positive PCR results for two beta-lactam-resistance genes – *bla<sub>CMY</sub>*, and *bla<sub>CTX-M</sub>* – and samples from one site had three genes detected – *bla<sub>CMY</sub>*, *bla<sub>CTX-M</sub>*, and *bla<sub>TEM</sub>*.
- At five sites *bla<sub>CTX</sub>* was found only downstream samples (of the seven sites with both upstream and downstream samples).
- At three sites *bla<sub>CTX-M</sub>* was found only downstream.
- The *mphA* gene, conferring resistance to macrolides, was identified in 9/90 samples and found predominantly in samples taken downstream from target farm sites. The gene was found upstream at only two sites and only in soil samples, indicating that the genes could have been carried by air rather than water.



## Interpretation

The results of this testing project are striking. They are supported by previous studies identifying antibiotic resistant bacteria and/or antibiotic resistance genes as widespread on farms, in manures, and in the environment near farms in the USA. The frequent identification of genes conferring resistance to specific classes of antibiotics heavily used by the pork industry, increase evidence that routine use on farms is responsible for antibiotic resistance off farms and negatively affects public health.

Testing at monitoring wells and groundwater sites near two swine farms in North Carolina found *E. coli* resistant to antibiotics approved for use in pig production. At the first site, 37% of *E. coli* isolates were resistant to at least one antibiotic, with resistance to chlortetracycline, tetracycline, and sulfamethoxazole identified. At the second site, 79% of *E. coli* were resistant, with resistance predominantly to tetracycline and chlortetracycline, as well as to ampicillin, streptomycin, chloramphenicol, sulfamethoxazole, trimethoprim, florfenicol, and neomycin<sup>lxv</sup>.

A study of *Salmonella* spp isolated from manure and environmental samples in North Carolina after manure application on commercial swine farms found ARGs in the bacteria on plasmids - meaning they could be transferred to other bacteria. Five of the 14 plasmids were considered multidrug resistant. Salmonellae carried genes conferring resistance to sulfisoxazole, tetracyclines, and beta-lactams<sup>lxvii</sup>.

Another study identified ARGs conferring resistance to tetracycline in samples taken from groundwater and lagoons on two swine production sites. All eight target genes were found in the samples overall. However, samples taken from upstream wells for background control found none of the target genes at one site and only one target gene at the other<sup>lxviii</sup>.

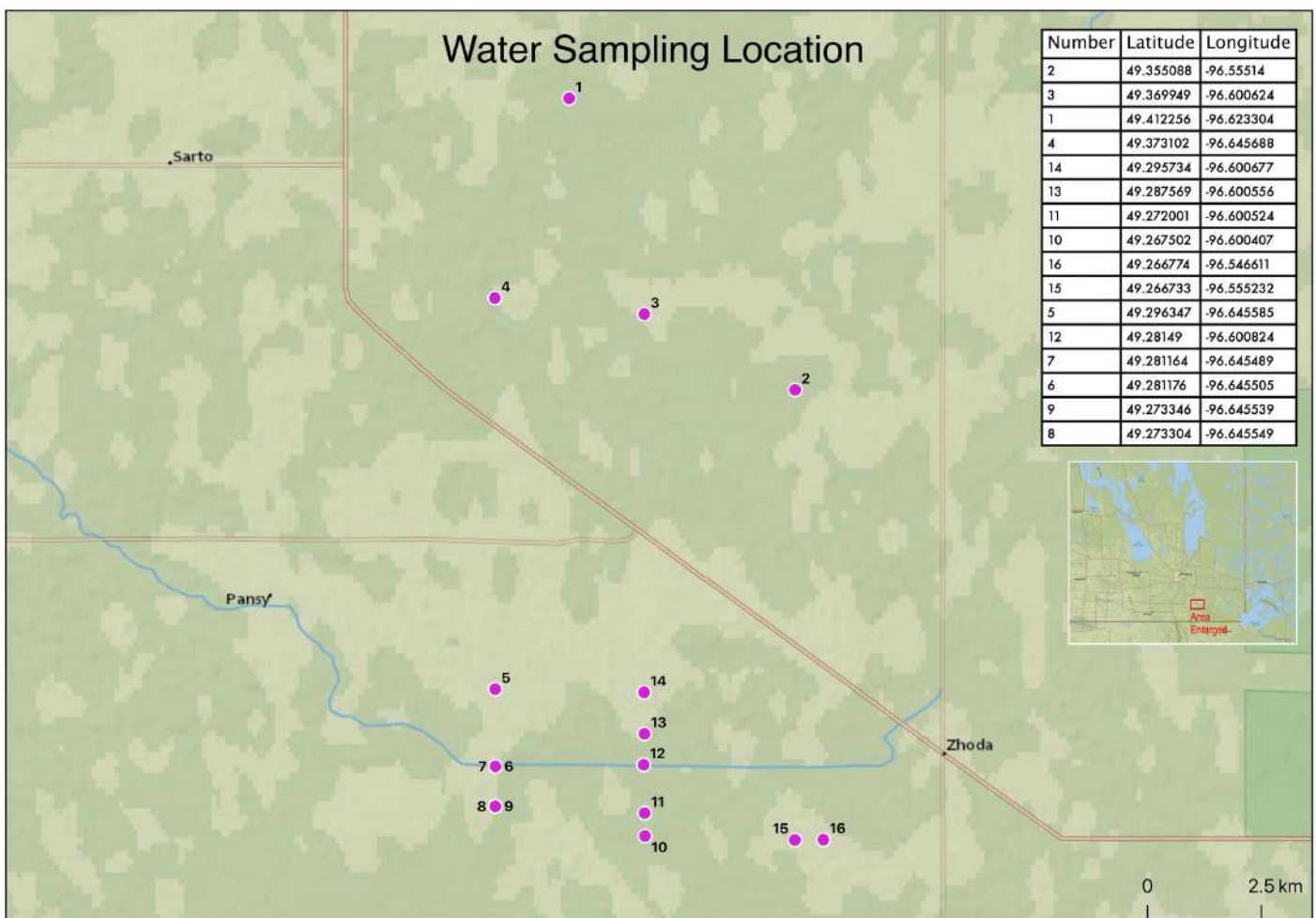
Yet another study sampled lagoons on three types of swine operations in the south-eastern USA. All samples taken from a sow (breeding) farm and all samples from a nursery farm were positive for tetracycline resistance genes (*tetA*) and macrolide resistance genes (*ermF*). Nearly all (90%) of samples from a finisher farm were positive for those same genes<sup>lxviii</sup>.

### 3. Canada – results summary and interpretation

The presence of ARGs was evaluated from 42 environmental samples (20 water plus 22 soil). The samples were collected in November 2020 from publicly accessible spaces upstream and downstream of eight industrial pig farms in the province of Manitoba. Testing for 27 target ARGs was conducted.

Overall, testing results indicate that all samples were positive for the presence of at least one of the target antibiotic resistance genes. The following range of resistance genes – *mphA*, *bla<sub>CTX-M</sub>*, *bla<sub>CTX-M</sub>*, *floR*, *gyrA*, *strA*, *strB*, *sul1*, *tet<sub>A</sub>*, *tet<sub>B</sub>*, *tet<sub>C</sub>*, *tet<sub>D</sub>* and *tet<sub>Q</sub>* – were found in the total water and soil samples.

- Nearly all samples (38/42) had positive PCR results for at least one of the tetracycline-resistance genes included in the analysis. More than three quarters of the samples were positive for three or more tetracycline-resistance genes, typically *tet<sub>A</sub>*, *tet<sub>B</sub>*, and *tet<sub>C</sub>*. Given the widespread use of tetracyclines (categorised as highly important by WHO) by the Canadian pork industry it is unsurprising that tetracycline-resistance would be prolific in the surrounding environment.
- Positive PCR results for resistance to streptomycin, an aminoglycoside (categorised as critically important by WHO) was identified in 36/42 samples. *StrA* was found less frequently than *strB*, but where found was predominantly in downstream samples.
- Genes conferring resistance to beta-lactam antibiotics, which include penicillin, cephalosporins,, and carbapenems, were identified in samples from all eight farm sampling locations. This included only in the downstream sample from one farm. *Bla<sub>CTX</sub>* and *bla<sub>CMY</sub>* were most commonly found, otherwise *bla<sub>CTX-M</sub>*.
- The *mphA* gene, conferring resistance to macrolides, was identified in all eight farm sampling locations. Importantly it was present additionally downstream to three farms.





## Interpretation

In Canada, around 80% of antibiotics are administered to pigs in their feed; macrolides and tetracyclines represent two of the three most abundantly used classes of antibiotics used in pig feed. More than half the antimicrobials given to pigs are for disease prevention (52%), while use for growth promotion is still extremely high (44% within the national industry).<sup>lxxxix</sup>

Our results align well with previous Canadian studies from other provinces that demonstrate a very similar range of genes from pig manure (slurry) and runoff from crops fertilised with it.<sup>lxxx</sup> Earlier studies show such genes (especially the *tet*, *sul*, *str* variants and *bla<sub>TEM</sub>*) have existed on farm and within the industry for well over a decade.<sup>lxxxi, lxxxii</sup>

Raising pigs (and poultry) and spreading manure are linked with contamination of surface and recreational water in Canada<sup>lxxxiii</sup>. A key study demonstrated that the percentage of land used for spreading liquid manure near southern Quebec beaches was highly correlated to resistant *E coli* contamination of such waters.<sup>lxxxiv</sup> While another study demonstrated that samples from four Canadian watersheds were resistant to 4–7 antibiotics including ampicillin, sulfisoxazole and tetracycline. There were overlapping resistance patterns from swine faecal samples and the Oldman River in Alberta.<sup>lxxxv</sup>

Many Canadians derive pleasure from these inland beaches and conservation areas. Wild animals not normally exposed to antimicrobial agents can also acquire antimicrobial agent-resistant bacteria. This happens through contact with people and domestic animals, and through the environment. A series of wildlife studies, near swine farms (in Ontario) were reported between 2009 to 2016. They all showed small mammals (mice, moles, shrews, racoons and other wildlife) with multi-drug-resistant *E coli*. Resistance to tetracyclines was the most frequently observed resistance in isolates recovered from wild animals living on or near pig farms and from pigs on the same farms.<sup>lxxxvi, lxxxvii, lxxxviii</sup>

Finally, more than 4 million people in Canada derive their drinking water from private wells. While we did not sample groundwater, the risks of contamination exist although apparently this has not been monitored recently.<sup>lxxxix</sup> Air pollution is another risk, with reports of almost 60% of Canadian meat pig barns harbouring air with genes resistant to colistin<sup>xc</sup>. In conclusion, in almost every province, ARGs have been found to derive from pig farms and contaminate the environment.

## 4. Spain – results summary and interpretation

In September 2020, 38 environmental samples were collected in the vicinity of pig farms located in the provinces of Aragon and Catalonia. These provinces are characterised by a large number of industrial pig farms. Catalonia has one of the highest densities of pig farms in Spain and within all of Europe.<sup>xci</sup>

In total, the presence of eight ARGs was evaluated from upstream and downstream water (16 water and 13 sediment samples); eight bootie samples from six separate farms plus a cluster of three farms. Analyses of three sites of ground water were also conducted. Farm sizes ranged from 1,200 to 7,200 pigs.

- ❖ *bla<sub>TEM</sub>*, *bla<sub>CTX-M-32</sub>*, *bla<sub>OXA58</sub>*, *qnrS*, *sul1*, *tet<sub>M</sub>* and 16S rRNA were detected in greater levels in the downstream water samples. All were present at relevant concentrations. Some ARGs were more than five times and in some cases up to 200 times more than the base concentration.
- ❖ *Sul1* and *int1* were found relevant in most samples and at two sites, the levels were indicative of significant contamination.
- ❖ *bla<sub>TEM</sub>*, *bla<sub>CTX-M-32</sub>*, *bla<sub>OXA58</sub>* – ARGs conveying resistance to cephalosporins at least as well as *qnrS*, conveying resistance to fluoroquinolones – were found at relevant levels at sites downstream from three farms. The most broadly contaminating ARG was *tet<sub>M</sub>* conveying resistance to tetracyclines. Such genes (*bla<sub>TEM</sub>*, *tet<sub>M</sub>*, *qnrS*) are strongly associated with the use of pig slurry and soils fertilised with slurry in the studied regions, as confirmed in these regions<sup>xci</sup>. This suggests a strong correlation between the presence of industrial pig farms and the high levels of ARGs.
- ❖ An increasing concentration of relevant ARGs was observed as the river Gallego advanced its course downstream. This was demonstrated by water samples tested from four farms located on the banks.
- ❖ Water samples from the cluster of farms located on the banks of the river Cinca also showed very high levels of ARGs, especially for the gene *tet<sub>M</sub>*. A very significant increase in ARGs was observed in the downstream samples. The ARG results for sediment testing corresponded to the results of the most contaminated farm water testing. High levels were detected for the ARGs *bla<sub>TEM</sub>* and *tet<sub>M</sub>* from the most contaminated samples (discharged from one farm).

Given the above results, the consultant also extended the PCR testing of surface dust, concluding:

‘The hypothesis of the impact of factory pig farms on the nearby environment in terms of ARG emissions is reinforced by analyses of surface dust collected in the vicinity of the farms (bootie samples). Most of the target ARGs were detected (see Table 3), with high levels for those quantified (Table 4). These levels are well above what is expected from normal soils and contamination from adjacent farms is clear. Given the high ARGs prevalences found, additional analyses were carried out for three specific genes (*int1*, *bla<sub>TEM</sub>*, *tet<sub>M</sub>*) to evaluate differences between farms.

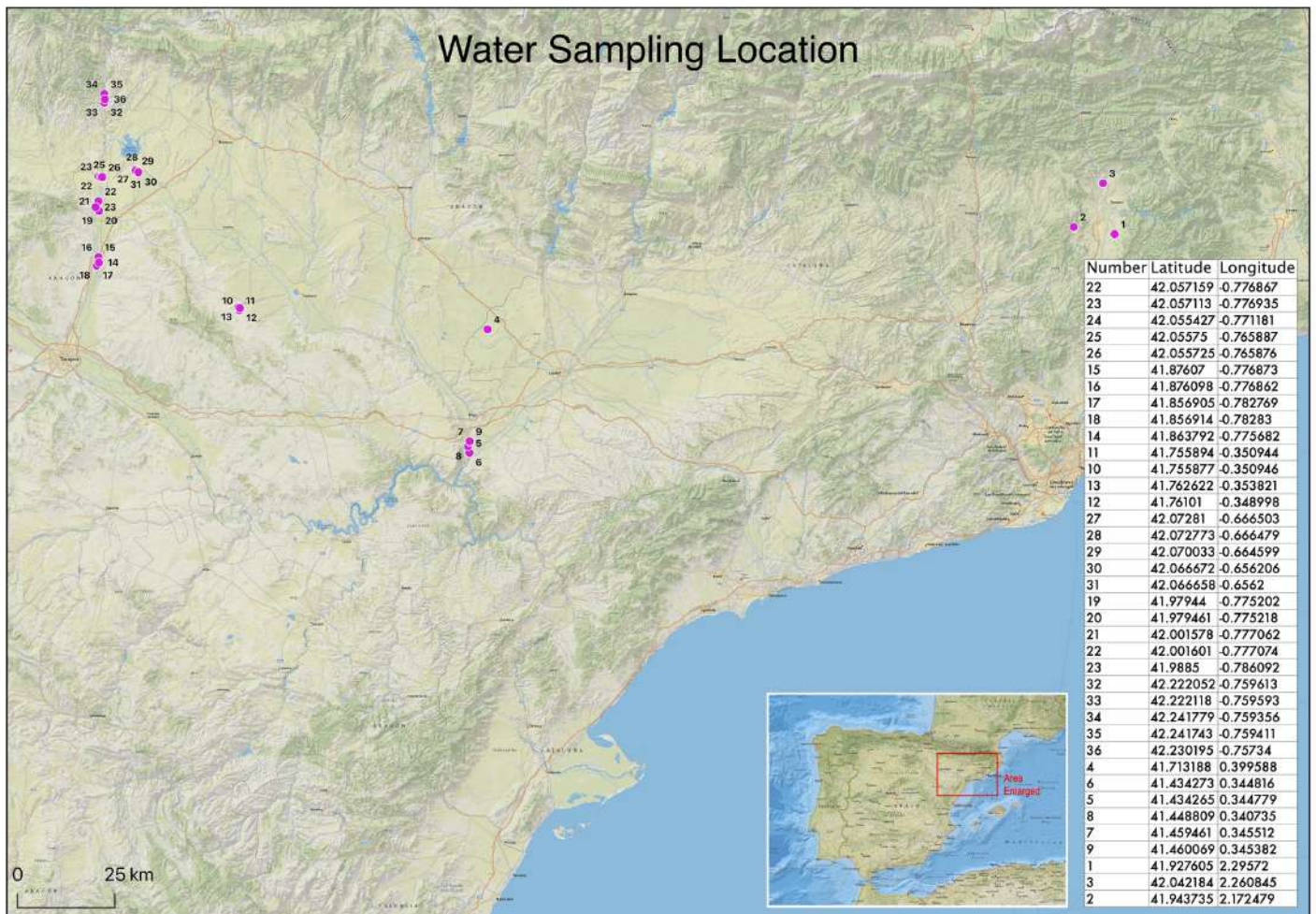
**Table 3 – ARG profiles from bootie samples**

Bootie	ARGs detected
POOL 1: 4 farms	<i>sul1</i> , <i>int1</i> , <i>bla<sub>TEM</sub></i> , <i>qnrS</i> , <i>bla<sub>CTX-M-32</sub></i> , <i>bla<sub>OXA58</sub></i> , <i>tet<sub>M</sub></i>
POOL 2: 4 farms	<i>sul1</i> , <i>int1</i> , <i>bla<sub>TEM</sub></i> , <i>qnrS</i> , <i>bla<sub>CTX-M-32</sub></i> , <i>bla<sub>OXA58</sub></i> , <i>tet<sub>M</sub></i>

Table 4 - levels of three specific genes.

Results in copies / grams extracted.

Bootie (analysis per farm sample)	<i>int1</i>	<i>bla<sub>TEM</sub></i>	<i>tet<sub>M</sub></i>
A	2.3E+07	5.5E+06	5.5E+05
B	2.3E+06	5.6E+06	1.1E+06
C	3.3E+08	1.2E+07	1.4E+07
D	2.3E+07	3.9E+06	9.1E+05
E	1.5E+07	3.6E+06	3.8E+05
F	3.5E+05	3.3E+06	4.1E+04
G	5.0E+09	5.8E+07	1.1E+09
H	2.5E+06	2.2E+06	7.7E+04





## Interpretation

The levels found were high for all the ARGs analysed, although those found from two farms (C and G) were striking, with levels several orders of magnitude higher than from the other farms. These levels are concerning from an environmental point of view. In conclusion, the bootie sample results clearly show that pig farms are an important source of ARGs to the surrounding environment.'

In addition, due to the rocky nature of the region and the presence of groundwater springs (historically used for drinking water) some groundwater testing was conducted. A seasonal survey of ARGs in three natural springs in Osona (Catalonia), an area with intensive livestock, was conducted. Sampling sites were selected based on their high nitrate concentrations,<sup>xciiii</sup> attributed to livestock waste fertilisation. Summarised results by the consultant:

'ARGs conferring resistance to fluoroquinolones (*qnrS*) and  $\beta$ -lactamases (*bla<sub>TEM</sub>*) were found at relevant concentrations ( $\geq 1 \cdot 10^4$  copies/ $\mu$ L) in the three evaluated spots. Moreover, the ARG *tet<sub>M</sub>*, that confers resistance to tetracyclines, was found in one sampling location ( $\geq 1 \cdot 10^3$  copies/ $\mu$ L). These levels are significant for groundwater, where lower concentrations were expected. The rest of the ARGs analysed were not detected or showed low levels (typically from 50 up to 300 copies/ $\mu$ L).

'Different factors including (I) high density of pig farms in the evaluated areas, (II) the high levels of nitrates detected in the same spots and (III) the fact that the ARGs found are closely related to antibiotics widely used for veterinary purposes, suggest that the presence of these ARGs in groundwater is linked to the livestock activities.

'This hypothesis is reinforced by the fact that the ARGs found at relevant levels are the predominant ones in samples of pig slurry and soils fertilised with slurry in studies carried out in the same area. The results are in agreement with those obtained in surface waters in the study of pig farms.'

Recent scientific studies reinforce our findings confirming many of the above genes found directly in pig slurry in Spain,<sup>xciiv</sup> especially from the Catalonia region.<sup>xciv</sup> Spanish studies also find a correlation of ARG levels with intensive pig farms and higher use of antibiotics.<sup>xcvi</sup>

# Appendix 3 - Defining FARMS and Five Domains model

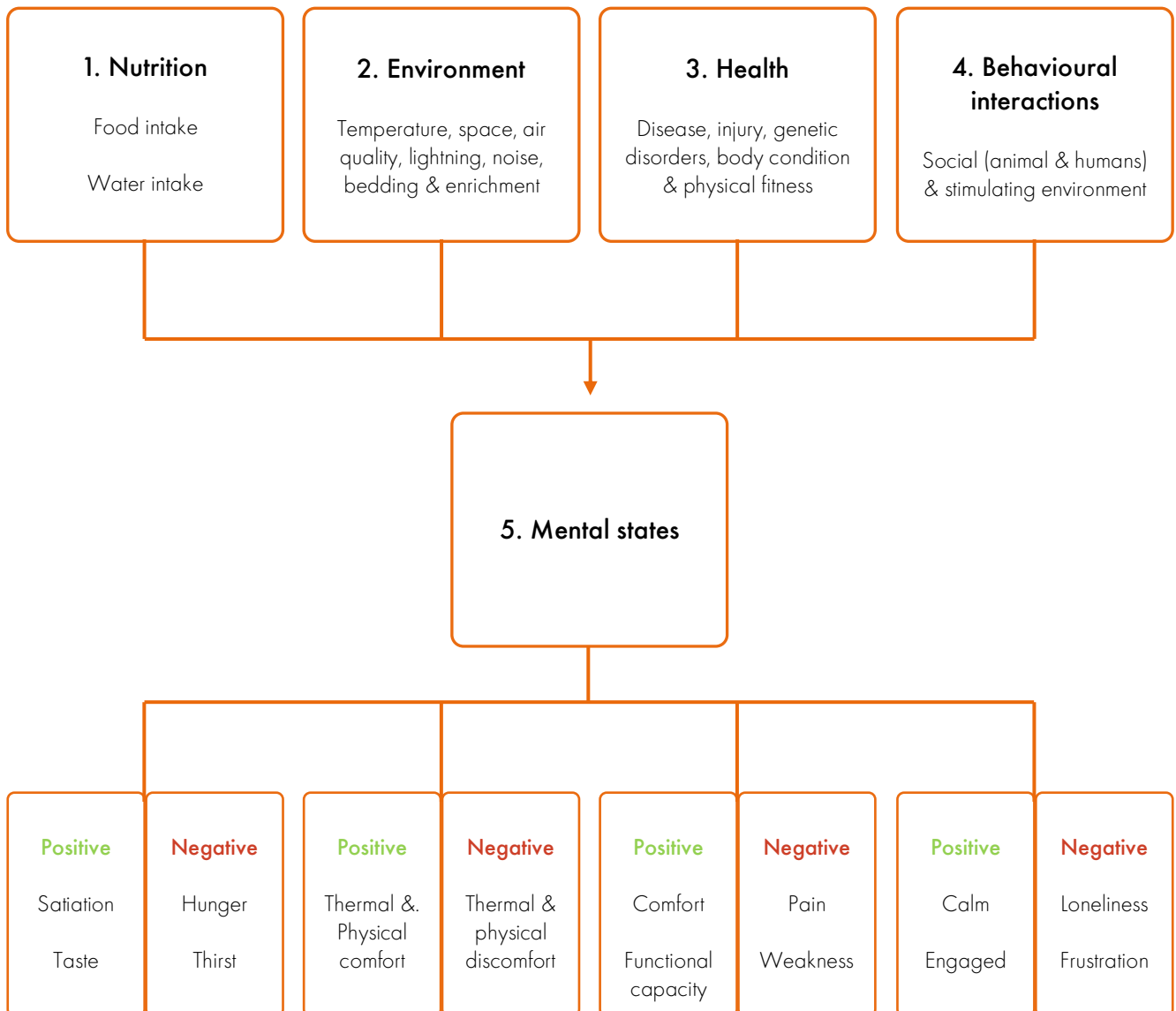
The Farm Animal Responsible Minimum Standards (FARMS) outlines minimum welfare standards for existing industrial farms, covering beef cattle, chickens raised for meat (broilers), dairy cattle, laying hens and pigs. They were developed by World Animal Protection, Compassion in World Farming and Humane Society International with reference to leading global frameworks and standards and the most recent science on behavioural needs of farm animals. FARMS outlines welfare risks and mitigation strategies to address those risks and specifically for [beef cattle](#), [broilers](#), [dairy cattle](#), [layer hens](#), [pigs](#).

Table 5 – Welfare risks and mitigation strategies as adapted from the [principles of the FARMS](#).

Welfare risks	Mitigation strategies	Farm animals included
Limitation on space – individual stalls, crates or cages restricting the movement of animals and overcrowded conditions increasing disease transmission and injurious contact with others	Increasing the space allowances (e.g. moving away from individual to group housing) and allowing animals to stand, stretch, turn around, sit, and/or lie down comfortably at the same time. Stocking densities low enough to prevent excessive temperatures and humidity; competition, stress, aggression, and abnormal behaviour; and to enable good litter management	Beef cattle, broilers, dairy cattle, layer hens, pigs
Barren and unsuitable environments – unstimulating environments leading to behavioural problems	Providing environmental enrichment (e.g., straw for pigs to manipulate, nest boxes for hens, perches and pecking materials for broilers and hens, brushes and chewable materials for dairy cows or calves) to stimulate positive emotional states	Beef cattle, broilers, dairy cattle, layer hens, pigs
Inappropriate diets – feeding diets that do not satisfy hunger	Adding bulk to high energy diets to help satisfy appetite	Beef cattle, dairy cattle, layer hens, pigs
Painful husbandry procedures – injurious husbandry procedures that cause pain	Alternatives to routine painful procedures (eg dehorning/disbudding, branding, castration, tail-docking, beak trimming) or effective pain relief	Beef cattle, dairy cattle, layer hens, pigs
Breeding and genetics – breeding for production traits that heighten anatomical or metabolic disorders	Re-aligning production-orientated genetic selection to include welfare traits	Beef cattle, broilers, dairy cattle, pigs
Animal specific welfare issues – lameness and mastitis for dairy cows and early weaning for pigs	Effective management programs in place to minimise mastitis (to < 25 cases/100 cows) and lameness (to < 10%)	Dairy cattle
	Piglets weaned at the age of 28 days or later.	Pigs
Transport	Minimise the distance and time taken for animal transportation, and animals slaughtered as close as possible to the farm to minimize the transport stress	Beef cattle, dairy cattle, layer hens, pigs
Slaughter	Animals handled, restrained, rendered unconscious until death, and slaughtered in the least distressing and most pain-free manner possible by trained and competent staff	Beef cattle, broilers, dairy cattle, layer hens, pigs
Compliance and transparency	Demonstrate compliance with the standards via annual third-party auditing and annual public reporting on progress.	Beef cattle, broilers, dairy cattle, layer hens, pigs

The Five Domains model is a more recent scientific framework designed to assess animal welfare<sup>xviii</sup>. The model consists of four physical/functional domains and the fifth domain, mental state. The fifth domain (mental state) represents the animal's experience of the four functional/physical domains and defines their welfare state. An animal cannot have an overall positive mental state in industrial farming. Humane and sustainable systems allow for a positive mental state.

Figure 1





# References

- i. United Nations Environment Programme and International Livestock Research Institute (2020). Preventing the Next Pandemic: Zoonotic diseases and how to break the chain of transmission. Nairobi, Kenya
- ii. Bailey R, Froggatt A, Wellesley L, 2014, Livestock – climate change’s forgotten sector / Global public opinion on meat and dairy consumption. Chatham House / The Royal Institute of International Affairs.
- iii. Bailey R, Froggatt A, Wellesley L. Livestock – Climate Change’s Forgotten Sector. *Clim Change*. :30.
- iv. FAO I. The State of Food Security and Nutrition in the World 2020: Transforming food systems for affordable healthy diets [Internet]. Rome, Italy: FAO, IFAD, UNICEF, WFP and WHO; 2020 [cited 2021 Jan 13]. 320 p. (The State of Food Security and Nutrition in the World (SOFI)). Available from: <http://www.fao.org/documents/card/en/c/ca9692en/>
- v. Rodríguez-Verdugo A, Lozano-Huntelman N, Cruz-Loya M, Savage V, Yeh P. Compounding Effects of Climate Warming and Antibiotic Resistance. *iScience*. 2020;23(4):101024. doi:10.1016/j.isci.2020.101024
- vi. Review on Antimicrobial Resistance. Antimicrobial resistance: tackling a crisis for the health and wealth of nations. Chaired by Jim O’Neill. 2014. Available from: [https://amr-review.org/sites/default/files/AMR%20Review%20Paper%20-%20Tackling%20a%20crisis%20for%20the%20health%20and%20wealth%20of%20nations\\_1.pdf](https://amr-review.org/sites/default/files/AMR%20Review%20Paper%20-%20Tackling%20a%20crisis%20for%20the%20health%20and%20wealth%20of%20nations_1.pdf)
- vii. WHO Director-General’s opening remarks at the media briefing on COVID-19, 20 Nov 2020, <https://www.who.int/director-general/speeches/detail/who-director-general-s-opening-remarks-at-the-media-briefing-on-covid-19-20-november-2020>
- viii. Review on Antimicrobial Resistance. Antimicrobial resistance: tackling a crisis for the health and wealth of nations. Chaired by Jim O’Neill. 2014. Available from: [https://amr-review.org/sites/default/files/AMR%20Review%20Paper%20-%20Tackling%20a%20crisis%20for%20the%20health%20and%20wealth%20of%20nations\\_1.pdf](https://amr-review.org/sites/default/files/AMR%20Review%20Paper%20-%20Tackling%20a%20crisis%20for%20the%20health%20and%20wealth%20of%20nations_1.pdf)
- ix. World Health Organisation list of Critically Important Antimicrobials for Human Medicine (6<sup>th</sup> Edition) released 2019 [https://www.who.int/foodsafety/areas\\_work/antimicrobial-resistance/cia/en/](https://www.who.int/foodsafety/areas_work/antimicrobial-resistance/cia/en/)
- x. Lekagul, A. Tangcharoensathien, V. Yeung, S. Patterns of antibiotic use in global pig production: A systematic review. *Veterinary and Animal Science*, Volume 7, 2019. <https://doi.org/10.1016/j.vas.2019.100058>
- xi. UNEP, Frontiers / 2017 Emerging Issues of Environmental Concern. United Nations Environment Programme, Nairobi
- xii. World Health Organisation, WHO Guidelines on use of medically important antimicrobials in food-producing animals, 2017, <https://apps.who.int/iris/bitstream/handle/10665/258970/9789241550130-eng.pdf;jsessionid=A54E0329CF76FEDFF283F2204ED430B0?sequence=1>
- xiii. He Y, Yuan Q, Mathieu J, et al, Antibiotic resistance genes from livestock waste: occurrence, dissemination and treatment, *npj Clean Water* 3, 4 (2020). <https://doi.org/10.1038/s41545-020-0051-0>
- xiv. Wageningen University and Research. Economics of antibiotic usage on Dutch farms. Wageningen Economic Research. 2019. Available from: <https://edepot.wur.nl/475403>
- xv. AVINED. Antibioticumgebruik Pluimveesector in 2019 en de Trends van Afgelopen Jaren. AVINED 2020. Available from: <https://www.avined.nl/sites/avined/files/2020-091-e0023-jaarrapport.pdf>
- xvi. Stygar A.; Chantziaras I.; Toppari I.; Maes D.; Niemi J. High biosecurity and welfare standards in fattening pig farms are associated with reduced antimicrobial use. *Animal*. 2020 Apr;1-9. doi:10.1017/S1751731120000828
- xvii. World Animal Protection. Sharing success – the global business case for higher welfare for pigs raised for meat. 2019. Available from: [https://www.worldanimalprotection.org/sites/default/files/media/int\\_files/sharing\\_success\\_-\\_gbc\\_pigs\\_raised\\_for\\_meat\\_final\\_moderate\\_size\\_pdf.pdf](https://www.worldanimalprotection.org/sites/default/files/media/int_files/sharing_success_-_gbc_pigs_raised_for_meat_final_moderate_size_pdf.pdf)
- xviii. Ng C, Gin KY-H. Monitoring Antimicrobial Resistance Dissemination in Aquatic Systems. *Water* [Internet]. 2019 Jan 11(1):71. Available from: <https://www.mdpi.com/2073-4441/11/1/71>
- xix. Schulz J, Friese A, Klees S, Tenhagen BA, Fetsch A, Rösler U, et al. Longitudinal Study of the Contamination of Air and of Soil Surfaces in the Vicinity of Pig Barns by Livestock-Associated Methicillin-Resistant *Staphylococcus aureus*. *Appl Environ Microbiol* [Internet]. 2012 Aug 15 [cited 2021 Feb 1];78(16):5666–71. Available from: <https://aem.asm.org/content/78/16/5666>
- xx. Li N, Liu C, Zhang Z, Li H, Song T, Liang T, et al. Research and Technological Advances Regarding the Study of the Spread of Antimicrobial Resistance Genes and Antimicrobial-Resistant Bacteria Related to Animal Husbandry. *Int J Environ Res Public Health* [Internet]. 2019 Jan 16(24):4896. Available from: <https://www.mdpi.com/1660-4601/16/24/4896>
- xxi. World Health Organisation list of Critically Important Antimicrobials for Human Medicine (6<sup>th</sup> Edition) released 2019 [https://www.who.int/foodsafety/areas\\_work/antimicrobial-resistance/cia/en/](https://www.who.int/foodsafety/areas_work/antimicrobial-resistance/cia/en/)
- xxii. Shinyoung Lee, Raies A. Mir, Si Hong Park, Donghyuk Kim, Hae-Yeong Kim, Raoul K. Boughton, J. Glenn Morris, Jr., and Kwangcheol C. Jeong. Prevalence of extended-spectrum  $\beta$ -lactamases in the local farm environment and livestock: challenges to mitigate antimicrobial resistance. *Critical Reviews in Microbiology*, 16(1). 2020. Pp. 1-14. <https://www.tandfonline.com/doi/pdf/10.1080/1040841X.2020.1715339>
- xxiii. Ibid.
- xxiv. World Health Organisation AWaRE Classification of antibiotics released 2019 [https://www.who.int/medicines/news/2019/WHO\\_releases2019AWaRe\\_classification\\_antibiotics/en/](https://www.who.int/medicines/news/2019/WHO_releases2019AWaRe_classification_antibiotics/en/)
- xxv. Gillings, M.R., et al., Using the class 1 integron-integrase gene as a proxy for anthropogenic pollution. *The Isme Journal*, 2014. **9**: p. 1269.
- xxvi. M.E. Anderson and M.D. Sobsey. *Detection and occurrence of antimicrobially resistant E. coli in groundwater on or near swine farms in eastern North Carolina*. *Water Science & Technology*, 54(3). 2006: 211-218.

- xxvii. Larouche É, Généreux M, Tremblay M-È, Rhouma M, Gasser M-O, Quessy S, et al. Impact of liquid hog manure applications on antibiotic resistance genes concentration in soil and drainage water in field crops. *Can J Microbiol* [Internet]. 2020 Apr 24 [cited 2021 Feb 10]; Available from: <https://cdnsiencepub.com/doi/obs/10.1139/cjm-2019-0343>
- xxviii. Rosengren L. Antimicrobial resistance of Salmonella, Escherichia coli and Campylobacter from pigs on-farm in Alberta and Saskatchewan Canada. 2007 [cited 2021 Feb 10]; Available from: <https://harvest.usask.ca/handle/10388/etid-09182007-095646>
- xxix. Associations between Antimicrobial Resistance Phenotypes, Antimicrobial Resistance Genes, and Virulence Genes of Fecal Escherichia coli Isolates from Healthy Grow-Finish Pigs | Applied and Environmental Microbiology [Internet]. [cited 2021 Feb 10]. Available from: <https://aem.asm.org/content/75/5/1373.full>
- xxx. Gosia K, Kozak, Patrick Boerlin, Nicol Janecko, Richard J. Reid-Smith, Claire Jardine Antimicrobial Resistance in *Escherichia coli* Isolates from Swine and Wild Small Mammals in the Proximity of Swine Farms and in Natural Environments in Ontario, Canada. *Applied and Environmental Microbiology* Jan 2009, 75 (3) 559-566; DOI: 10.1128/AEM.01821-08
- xxxi. Samantha E. Allen, Patrick Boerlin, Nicol Janecko, John S. Lumsden, Ian K. Barker, David L. Pearl, Richard J. Reid-Smith, Claire Jardine. "Antimicrobial Resistance in Generic *Escherichia coli* Isolates from Wild Small Mammals Living in Swine Farm, Residential, Landfill, and Natural Environments in Southern Ontario, Canada." *Public Health Microbiology*. 2011.
- xxxii. Bondo KJ, Pearl DL, Janecko N, Boerlin P, Reid-Smith RJ, Parmley J, et al. (2016) Epidemiology of Antimicrobial Resistance in *Escherichia coli* Isolates from Raccoons (*Procyon lotor*) and the Environment on Swine Farms and Conservation Areas in Southern Ontario. *PLoS ONE* 11(11): e0165303. <https://doi.org/10.1371/journal.pone.0165303>
- xxxiii. <https://www.canada.ca/content/dam/phac-aspc/documents/services/publications/drugs-health-products/canadian-antimicrobial-resistance-surveillance-system-2018-report-executive-summary/pub1-eng.pdf>
- xxxiv. Sanz C, Casado M, Navarro-Martin L, Tadić Đ, Parera J, Tugues J, et al. Antibiotic and antibiotic-resistant gene loads in swine slurries and their digestates: Implications for their use as fertilizers in agriculture. *Environ Res* [Internet]. 2020 Nov 24 [cited 2021 Feb 8];110513. Available from: <https://www.sciencedirect.com/science/article/pii/S0013935120314109>
- xxxv. Esperón F, Sacristán C, Carballo M, Torre A de la. Antimicrobial Resistance Genes in Animal Manure, Manure-Amended and Nonanthropogenically Impacted Soils in Spain. *Adv Biosci Biotechnol* [Internet]. 2018 Aug 28 [cited 2021 Feb 9];9(9):469–80. Available from: <http://www.scirp.org/Journal/Paperabs.aspx?paperid=87438>
- xxxvi. Sanz C, Casado M, Navarro-Martin L, Tadić Đ, Parera J, Tugues J, et al. Antibiotic and antibiotic-resistant gene loads in swine slurries and their digestates: Implications for their use as fertilizers in agriculture. *Environ Res* [Internet]. 2020 Nov 24 [cited 2021 Feb 8];110513. Available from: <https://www.sciencedirect.com/science/article/pii/S0013935120314109>
- xxxvii. Mencía-Ares O, Cabrera-Rubio R, Cobo-Díaz JF, Álvarez-Ordóñez A, Gómez-García M, Puente H, et al. Antimicrobial use and production system shape the fecal, environmental, and slurry resistomes of pig farms. *Microbiome* [Internet]. 2020 Nov 19 [cited 2021 Feb 8];8(1):164. Available from: <https://doi.org/10.1186/s40168-020-00941-7>
- xxxviii. Lekagul, A. Tangcharoensathien, V. Yeung, S. Patterns of antibiotic use in global pig production: A systematic review. *Veterinary and Animal Science*, Volume 7, 2019. <https://doi.org/10.1016/j.vas.2019.100058>
- xxxix. Lekagul, A. Tangcharoensathien, V. Yeung, S. Patterns of antibiotic use in global pig production: A systematic review. *Veterinary and Animal Science*, Volume 7, 2019. <https://doi.org/10.1016/j.vas.2019.100058>
- xl. World Economic Forum, Three-quarters of antibiotics are used on animals. Here's why that's a major problem, 24 Nov 2017, <https://www.weforum.org/agenda/2017/11/three-quarters-of-antibiotics-are-used-on-animals-heres-why-thats-a-major-problem>
- xli. Williams-Nguyen J, Sallach JB, Bartelt-Hunt S, Boxall AB, Durso LM, McLain JE, Singer RS, Snow DD, Zilles JL. *J Environ Qual*. Antibiotics and Antibiotic Resistance in Agroecosystems: State of the Science. 2016 Mar; 45(2):394-406
- xl.ii. Sobsey, M.D., L.A. Khatib, V.R. Hill, E. Alocilja, and S. Pillai. 2001. Pathogens in animal wastes and the impacts of waste management practices on their survival, transport and fate. White Paper Summaries. Available online [http://www.cals.ncsu.edu/waste\\_mgt/natlcenter/whitepapersummaries/pathogens.pdf](http://www.cals.ncsu.edu/waste_mgt/natlcenter/whitepapersummaries/pathogens.pdf) (verified 16 Feb. 2009)
- xl.iii. Christopher D. Heaney, Kevin Myers, Steve Wing, Devon Hall, Dothula Baron, Jill R. Stewart. *Source tracking swine fecal waste in surface water proximal to swine concentrated animal feeding operations*. *Science of the Total Environment*, 511. 2015. Pp. 676-683
- xl. iv. Cheng, D et al. (2019) Contribution of antibiotics to the fate of antibiotic resistance genes in anaerobic treatment processed of swine wastewater: a review. *Bioresource Technology*
- xl. v. Chee-Sanford, JC et al. "Fate and transport of antibiotic residues and antibiotic resistance genes following land application of manure waste." *Journal of Environmental Quality*, 38(3), 1086-1089 (2009). Retrieved January 13, 2017, from [http://www.ncbi.nlm.nih.gov/sites/entrez?Db=pubmed&Cmd=Retrieve&list\\_uids=19398507&dopt=abstractplus](http://www.ncbi.nlm.nih.gov/sites/entrez?Db=pubmed&Cmd=Retrieve&list_uids=19398507&dopt=abstractplus)
- xl. vi. U.S Centers for Disease Control and Prevention, the UK Science & Innovation Network, and the Wellcome Trust. *Initiatives for Addressing Antimicrobial Resistance in the Environment: Current Situation and Challenges*. 2018. <https://wellcome.ac.uk/sites/default/files/antimicrobial-resistance-environment-report.pdf>
- xl. vii. Maggie R. Williams, Robert D. Stedfeld, Xueping Guo, and Syed A. Hashsham. *Antimicrobial Resistance in the Environment*. *Water Environment Research*, 88(10). 2016. Pp. 1951-1967
- xl. viii. U.S Centers for Disease Control and Prevention, the UK Science & Innovation Network, and the Wellcome Trust. *Initiatives for Addressing Antimicrobial Resistance in the Environment: Current Situation and Challenges*. 2018. <https://wellcome.ac.uk/sites/default/files/antimicrobial-resistance-environment-report.pdf>
- xl. ix. US Centers for Disease Control and Prevention, Wellcome Trust, Initiatives for Addressing Antimicrobial Resistance in the Environment: Current Situation and Challenges. 2018. <https://wellcome.org/sites/default/files/antimicrobial-resistance-environment-report.pdf>
- l. de Rooij, M. M., Borlee, F., Smit, L. A., de Bruin, A., Janse, I., Heederik, D. J., & Wouters, I. M. (2016). Detection of *Coxiella burnetii* in ambient air after a large Q fever outbreak. *PLoS One*, 11(3). <https://doi.org/10.1371/journal.pone.0151281>

- li. Graham, JP, et al. "Antibiotic resistant enterococci and staphylococci isolated from flies collected near confined poultry feeding operations." *Science of the Total Environment*, 407(8):2701-10 (April 1, 2009). Retrieved January 13, 2017, from <http://www.ncbi.nlm.nih.gov/pubmed/19157515>
- lii. UNEP, *Frontiers / 2017 Emerging Issues of Environmental Concern*. United Nations Environment Programme, Nairobi
- liii. Singer AC, Shaw H, Rhodes V and Hart A (2016) Review of Antimicrobial Resistance in the Environment and Its Relevance to Environmental Regulators. *Front. Microbiol.* 7:1728. doi: 10.3389/fmicb.2016.01728 [Some common relevant disinfectants or biocides used on farms include: chlorhexidine, triclosan, and quaternium ammonium compounds and relevant heavy metals such as Pb, Cu, Zn, Cd have been used as animal growth promoters and nutritional supplements. The most relevant to swine production globally is Zinc oxide which will be banned for use as a veterinary product for diarrhoea management in 2022 in the EU but still retained for low dose use in animal feed animal feed though already prohibited in some member states. It is widely used globally.)
- liv. FAO, 2018, Monitoring global progress on addressing antimicrobial resistance: analysis report of the second round of results of ANTIMICROBIAL RESISTANCE country self-assessment survey 2018. <http://www.fao.org/3/co0486en/CA0486EN.pdf>
- lv. University of York, Antibiotics found in some of the world's rivers exceed 'safe' levels, global study finds, 27 May 2019, <https://www.york.ac.uk/news-and-events/news/2019/research/antibiotics-found-in-some-of-worlds-rivers/>
- lvi. Saam M, Kessel A, Waffler K, The Swiss recipe for containing antimicrobial resistance, *AMR Control / Overcoming global antimicrobial resistance*, <http://resistancecontrol.info/2018-frontpage/2018-2/the-swiss-recipe-for-containing-antimicrobial-resistance/>
- lvii. UNEP, United Nations Environment Assembly of UNDEP, third session, 4-6 December 2017, 3/4. Environment and health. [http://wedocs.unep.org/bitstream/handle/20.500.11822/30795/UNEA3\\_4EN.pdf?sequence=1&isAllowed=y](http://wedocs.unep.org/bitstream/handle/20.500.11822/30795/UNEA3_4EN.pdf?sequence=1&isAllowed=y)
- lviii. Stygar A.; Chantziaras I.; Toppari I.; Maes D.; Niemi J. High biosecurity and welfare standards in fattening pig farms are associated with reduced antimicrobial use. *Animal*. 2020 Apr;1-9. doi:10.1017/S1751731120000828
- lix. World Animal Protection. Sharing success - the global business case for higher welfare for pigs raised for meat. 2019. Available from: [https://www.worldanimalprotection.org/sites/default/files/media/int\\_files/sharing\\_success\\_-\\_gbc\\_pigs\\_raised\\_for\\_meat\\_final\\_moderate\\_size\\_pdf.pdf](https://www.worldanimalprotection.org/sites/default/files/media/int_files/sharing_success_-_gbc_pigs_raised_for_meat_final_moderate_size_pdf.pdf)
- lx. Österberg J.; Wingstrand A.; Nygaard Jensen A.; Kerouanton A.; Cibir V.; Barco L.; et al. Antibiotic Resistance in *Escherichia coli* from Pigs in Organic and Conventional Farming in Four European Countries. *PLoS One*. 2016 Jun;11(6):e0157049. Available from: doi:10.1371/journal.pone.0157049
- lxi. Kempf I.; Kerouanton A.; Bougeard S.; Nagard B.; Rose V.; Mourand G.; et al. *Campylobacter coli* in Organic and Conventional Pig Production in France and Sweden: Prevalence and Antimicrobial Resistance. *Front. Microbiol.* 2017 May; 8:955. Available from: doi: 10.3389/fmicb.2017.00955
- lxii. Catry B.; Dewulf J.; Maes D.; Pardon B.; Callens B.; Vanrobaeys M.; et al. Effect of antimicrobial consumption and production type of antibacterial resistance in the bovine respiratory and digestive tract. *PLoS One*. 2016 Jan;11(1):e0146488. Available from: <https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0146488#sec014>
- lxiii. Wageningen University and Research. Economics of antibiotic usage on Dutch farms. Wageningen Economic Research. 2019. Available from: <https://edepot.wur.nl/475403>
- lxiv. AVINED. Antibioticumgebruik Pluimveesector in 2019 en de Trends van Afgelopen Jaren. AVINED 2020. Available from: <https://www.avined.nl/sites/avined/files/2020-091-e0023-jaarrapport.pdf>
- lxv. Van Boeckel T.; Glennon E.; Chen D.; Gilbert M.; Robinson T.; Grenfell B.; et al. Reducing antimicrobial use in food animals. *Science* 2017; 357(6358): 1350-1352. Available from: doi:10.1126/science.aao1495
- lxvi. Mellor DJ (2017) *Operational details of the Five Domains Models and its key applications to the assessment and management of animal welfare*. *Animals* 7(8):60. doi:10.3390/ani7080060
- lxvii. Good Life: To define a 'good life' we look to opportunities for mostly positive experiences or welfare across the life of animals. Positive welfare includes comfort, pleasure, interest, vigour and confidence, feelings of satiation, calmness, opportunities to play and learn with freedom of choice. These are linked to inputs such as bedding and enrichment, abundant space, temperature zones, variable food presentation and formulation, nesting opportunities, positive social interactions, humane death, positive stockperson interactions, appropriate breeding/genetics and the highest level of veterinary care. Overall, animals have freedom of choice and have more of what they want in addition to what they need to function in terms of meat, milk or egg production.
- lxviii. The 15 countries included in the survey included: Australia, Brazil, Canada, Denmark, India, Indonesia, Kenya, Thailand, Mexico, Netherlands, New Zealand, Spain, Sweden, UK, US.
- lix. Ng C, Gin KY-H. Monitoring Antimicrobial Resistance Dissemination in Aquatic Systems. *Water* [Internet]. 2019 Jan 11(1):71. Available from: <https://www.mdpi.com/2073-4441/11/1/71>
- lxx. Schulz J, Friese A, Klees S, Tenhagen BA, Fetsch A, Rösler U, et al. Longitudinal Study of the Contamination of Air and of Soil Surfaces in the Vicinity of Pig Barns by Livestock-Associated Methicillin-Resistant *Staphylococcus aureus*. *Appl Environ Microbiol* [Internet]. 2012 Aug 15 [cited 2021 Feb 1];78(16):5666-71. Available from: <https://aem.asm.org/content/78/16/5666>
- lxxi. Li N, Liu C, Zhang Z, Li H, Song T, Liang T, et al. Research and Technological Advances Regarding the Study of the Spread of Antimicrobial Resistance Genes and Antimicrobial-Resistant Bacteria Related to Animal Husbandry. *Int J Environ Res Public Health* [Internet]. 2019 Jan 16(24):4896. Available from: <https://www.mdpi.com/1660-4601/16/24/4896>
- lxxii. Boonyasiri A, Tangkosal T, Seenama C, Saiyarin J, Tiengrim S, Thamlikitkul V. Prevalence of antibiotic resistant bacteria in healthy adults, foods, food animals, and the environment in selected areas in Thailand. *Pathog Glob Health* [Internet]. 2014 Jul 108(5):235-45. Available from: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4153825/>
- lxxiii. Nuangmek A, Rojanasthien S, Chotinun S, Yamsakul P, Tadee P, Thamlikitkul V, et al. Antimicrobial Resistance in ESBL-Producing *Escherichia coli* Isolated from Layer and Pig Farms in Thailand. *Acta Sci Vet* [Internet]. 2018 May 16 46(1):8. Available from: <https://www.seer.ufg.br/ActaScientiaeVeterinariae/article/view/81823>



- lxxiv. Hallenberg GS, Jiwakanon J, Angkitittrakul S, Kang-Air S, Osbjør K, Lunha K, et al. Antibiotic use in pig farms at different levels of intensification-Farmers' practices in northeastern Thailand. *PLoS One*. 2020;15(12):e0243099.
- lxxv. M.E. Anderson and M.D. Sobsey. *Detection and occurrence of antimicrobially resistant E. coli in groundwater on or near swine farms in eastern North Carolina*. *Water Science & Technology*, 54(3). 2006: 211-218.
- lxxvi. Suchawan Pornsukoom and Siddhartha Thakur. *Antimicrobial Resistance Determinants in Multiple Salmonella Serotypes following Isolation from the Commercial Swine Operation Environment After Manure Application*. *Applied and Environmental Microbiology*, 83(20). 2017: 1-14.
- lxxvii. J.C. Chee-Sanford, R.I. Aminov, I.J. Krapac, N. Garrigues-Jeanjean, and R.I. Mackie. *Occurrence and Diversity of Tetracycline Resistance Genes in Lagoons and Groundwater Underlying Two Swine Production Facilities*. *Appl. Environ Microbiol*, 67(4). 2001. Pp. 1494-1502.
- lxxviii. John P. Brooks, Ardeshir Adeli, and Michael R. McLaughling. *Microbial ecology, bacterial pathogens, and antibiotic resistance genes in swine manure wastewater as influenced by three swine management systems*. *Water Research*, 57. 2014: pp. 96-103.
- lxxix. <https://www.canada.ca/content/dam/phac-aspc/documents/services/publications/drugs-health-products/canadian-antimicrobial-resistance-surveillance-system-2018-report-executive-summary/pub1-eng.pdf>
- lxxx. Larouche É, Généreux M, Tremblay M-È, Rhouma M, Gasser M-O, Quessy S, et al. Impact of liquid hog manure applications on antibiotic resistance genes concentration in soil and drainage water in field crops. *Can J Microbiol* [Internet]. 2020 Apr 24 [cited 2021 Feb 10]; Available from: <https://cdnsiencepub.com/doi/abs/10.1139/cjm-2019-0343>
- lxxxi. Rosengren L. Antimicrobial resistance of Salmonella, Escherichia coli and Campylobacter from pigs on-farm in Alberta and Saskatchewan Canada. 2007 [cited 2021 Feb 10]; Available from: <https://harvest.usask.ca/handle/10388/etd-09182007-095646>
- lxxxii. Associations between Antimicrobial Resistance Phenotypes, Antimicrobial Resistance Genes, and Virulence Genes of Fecal Escherichia coli Isolates from Healthy Grow-Finish Pigs | *Applied and Environmental Microbiology* [Internet]. [cited 2021 Feb 10]. Available from: <https://aem.asm.org/content/75/5/1373.full>
- lxxxiii. Kadykalo S, Thomas J, Parmley EJ, Pintar K, Fleury M. Antimicrobial resistance of Salmonella and generic Escherichia coli isolated from surface water samples used for recreation and a source of drinking water in southwestern Ontario, Canada. *Zoonoses Public Health* [Internet]. 2020 [cited 2021 Feb 4];67(5):566-75. Available from: <https://onlinelibrary.wiley.com/doi/abs/10.1111/zph.12720>
- lxxxiv. Turgeon P, Michel P, Levallois P, et al. Agroenvironmental determinants associated with the presence of antimicrobial-resistant Escherichia coli in beach waters in Quebec, Canada. *Zoonoses Public Health*. 2011;58(6):432-439. doi:10.1111/j.1863-2378.2010.01386.x
- lxxxv. Jokinen, Cassandra & Koot, Jacqueline & Cole, Linda & Desruisseau, Andrea & Edge, T. & Khan, Izhar & Koning, Wendell & Lapen, D & Pintar, Katarina & Reid-Smith, Richard & Thomas, Janis & Topp, Edward & Wang, Lu Ya & Wilkes, G & Ziebell, Kim & van Bochove, Eric & Gannon, Victor. (2016). Jokinen et al. 2015.
- lxxxvi. Gosia K. Kozak, Patrick Boerlin, Nicol Janecko, Richard J. Reid-Smith, Claire Jardine Antimicrobial Resistance in *Escherichia coli* Isolates from Swine and Wild Small Mammals in the Proximity of Swine Farms and in Natural Environments in Ontario, Canada. *Applied and Environmental Microbiology* Jan 2009, 75 (3) 559-566; DOI: 10.1128/AEM.01821-08
- lxxxvii. Samantha E. Allen, Patrick Boerlin, Nicol Janecko, John S. Lumsden, Ian K. Barker, David L. Pearl, Richard J. Reid-Smith, Claire Jardine. "Antimicrobial Resistance in Generic *Escherichia coli* Isolates from Wild Small Mammals Living in Swine Farm, Residential, Landfill, and Natural Environments in Southern Ontario, Canada." *Public Health Microbiology*. 2011.
- lxxxviii. Bondo KJ, Pearl DL, Janecko N, Boerlin P, Reid-Smith RJ, Parmley J, et al. (2016) Epidemiology of Antimicrobial Resistance in *Escherichia coli* Isolates from Raccoons (*Procyon lotor*) and the Environment on Swine Farms and Conservation Areas in Southern Ontario. *PLoS ONE* 11(11): e0165303. <https://doi.org/10.1371/journal.pone.0165303>
- lxxxix. Brenda L. Coleman, Marie Louie, Marina I. Salvadori, Scott A. McEwen, Norman Neumann, Kristen Sible, Rebecca J. Irwin, Frances B. Jamieson, Danielle Daignault, Anna Majury, Shannon Braithwaite, Bryanne Crago, Allison J. McGeer. Contamination of Canadian private drinking water sources with antimicrobial resistant *Escherichia coli*. *Water Research*. Volume 47, Issue 9, 1 June 2013, Pages 3026-3036.
- xc. Pilote J, Létourneau V, Girard M, Duchaine C. Quantification of airborne dust, endotoxins, human pathogens and antibiotic and metal resistance genes in Eastern Canadian swine confinement buildings. *Aerobiologia* [Internet]. 2019 Jun 1 [cited 2021 Feb 10];35(2):283-96. Available from: <https://doi.org/10.1007/s10453-019-09562-6>
- xc. Vidal A, Aguirre L, Seminati C, Tello M, Redondo N, Martín M, et al. Antimicrobial Resistance Profiles and Characterization of *Escherichia coli* Strains from Cases of Neonatal Diarrhea in Spanish Pig Farms. *Vet Sci* [Internet]. 2020 Jun [cited 2021 Feb 8];7(2):48. Available from: <https://www.mdpi.com/2306-7381/7/2/48>
- xcii. Sanz C, Casado M, Navarro-Martin L, Tadić Đ, Parera J, Tugues J, et al. Antibiotic and antibiotic-resistant gene loads in swine slurries and their digestates: Implications for their use as fertilizers in agriculture. *Environ Res* [Internet]. 2020 Nov 24 [cited 2021 Feb 8];110513. Available from: <https://www.sciencedirect.com/science/article/pii/S0013935120314109>
- xciii. C. T. A. S. A. M. J. M.-P. N. Otero, "Monitoring groundwater nitrate attenuation in a regional system coupling hydrogeology with multi-isotopic methods: The case of Plana de Vic (Osona, Spain)," *Agriculture, Ecosystems and Environment*, vol. 133, pp. 103-113, 2009.
- xciv. Esperón F, Sacristán C, Carballo M, Torre A de la. Antimicrobial Resistance Genes in Animal Manure, Manure-Amended and Nonanthropogenically Impacted Soils in Spain. *Adv Biosci Biotechnol* [Internet]. 2018 Aug 28 [cited 2021 Feb 9];9(9):469-80. Available from: <http://www.scirp.org/Journal/Paperabs.aspx?paperid=87438>
- xcv. Sanz C, Casado M, Navarro-Martin L, Tadić Đ, Parera J, Tugues J, et al. Antibiotic and antibiotic-resistant gene loads in swine slurries and their digestates: Implications for their use as fertilizers in agriculture. *Environ Res* [Internet]. 2020 Nov 24 [cited 2021 Feb 8];110513. Available from: <https://www.sciencedirect.com/science/article/pii/S0013935120314109>
- xcvi. Mencía-Ares O, Cabrera-Rubio R, Cobo-Díaz JF, Álvarez-Ordóñez A, Gómez-García M, Puente H, et al. Antimicrobial use and production system shape the fecal, environmental, and slurry resistomes of pig farms. *Microbiome* [Internet]. 2020 Nov 19 [cited 2021 Feb 8];8(1):164. Available from: <https://doi.org/10.1186/s40168-020-00941-7>
- xcvii. Mellor D, Beausoleil N, Extending the "Five Domains" model for animal welfare assessment to incorporate positive welfare states. *Anim Welf*. 2015;24:241-53

## World Animal Protection

5th Floor

222 Grays Inn Road

London WC1X 8HB

UK

 +44 (0)20 7239 0500

 [info@worldanimalprotection.org](mailto:info@worldanimalprotection.org)

 W: [worldanimalprotection.org](http://worldanimalprotection.org)

 [/WorldAnimalProtectionInt](https://www.facebook.com/WorldAnimalProtectionInt)

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