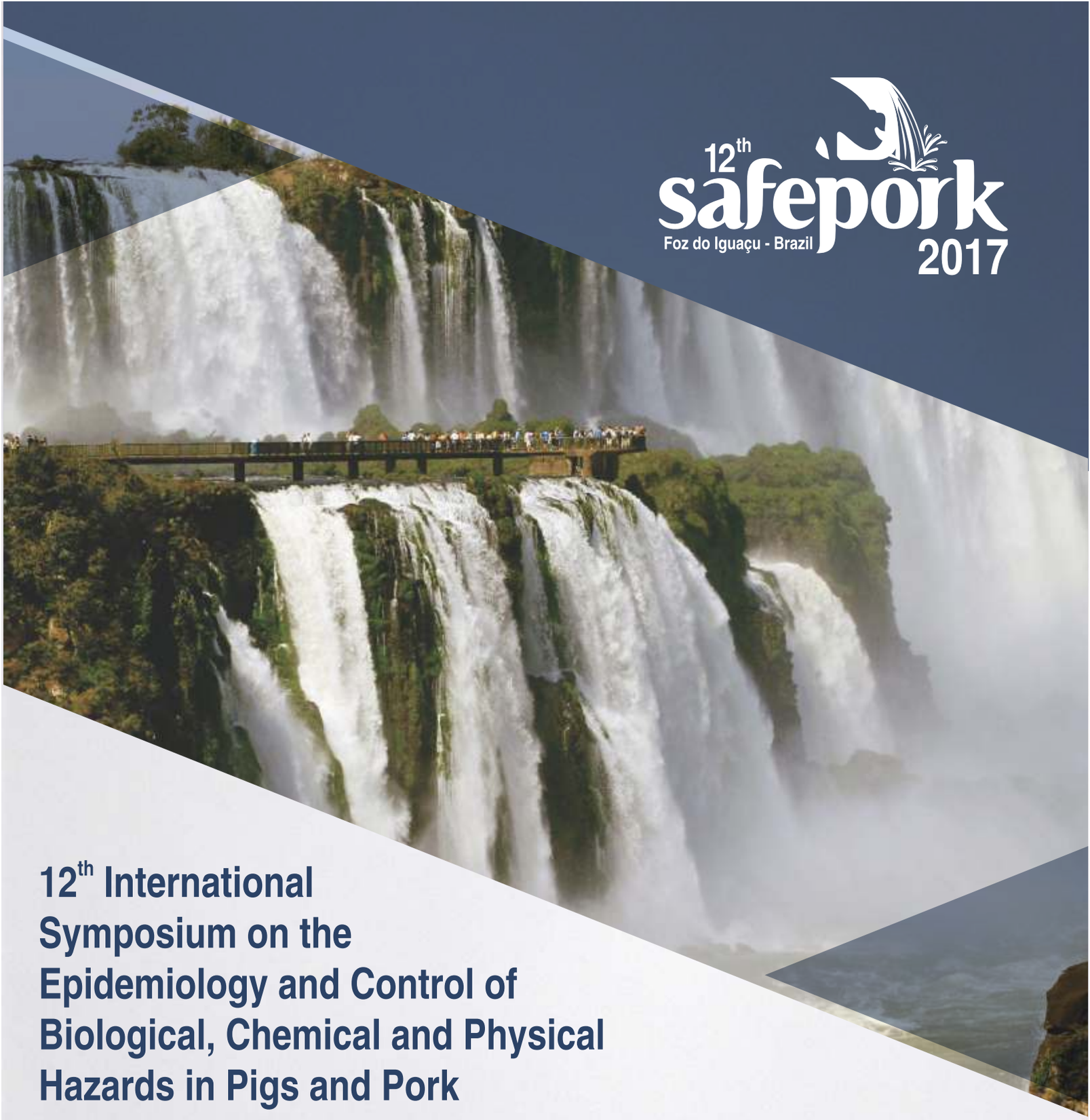

12th safepork 2017
Foz do Iguaçu - Brazil

12th International Symposium on the Epidemiology and Control of Biological, Chemical and Physical Hazards in Pigs and Pork

Proceedings Book

Foz do Iguaçu - Brazil
August 21-24, 2017

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Embrapa Suínos e Aves
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12th International Symposium on the Epidemiology and Control of Biological, Chemical and Physical Hazards in Pigs and Pork

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IDEXX

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Presentation

Welcome to Foz do Iguaçu - Brazil!

After the completion of eleven Safepork conferences: Ames, Iowa (USA), - Copenhagen, Denmark - Washington DC (USA) - Leipzig (DE) - Crete (GR) - Sonoma Valley (California, USA) - Verona, Italy - Quebec City, Canada - Maastricht, Netherland - Portland, Maine (USA), and Porto (Portugal,) the 12th conference will be held in Iguassu Falls (Foz do Iguaçu, Brazil) in 2017.

The meeting will be organized in a partnership among Embrapa Suínos e Aves, the Brazilian Pig Veterinary Society (Abraves) and the Federal University of Rio Grande do Sul.

Pork is the most important source of animal protein in the world, and Brazil is one of the important players in the international market. Brazil´s performance in the last decade has been positive with an increase in production and exportation. In 2015, Brazil ranked as fourth producer and exporter of pork in the world. It is estimated that the pork production chain employs directly and indirectly about 835,000 people in Brazil. Brazilian pork is the result of an intensive and technological process that involves modern genetics, housing, management and feeding. For Brazil, hosting Safepork 2017 is also an opportunity to bring to South America an active and participative conference for all the players in this sector (producers, technicians, industry, policy makers and researchers), who can exchange knowledge and experience, fortifying relationships and discussing effective measures to improve food safety, from an integrated perspective. Additional to the Safepork 2017 conference, all the participants will have the pleasure and opportunity to visit the Iguassu Falls. The magnificent spectacle of these 275 individual falls has awed tourists, locals and indigenous inhabitants for centuries. They originate from the Iguassu River and are located on the border of Brazil and Argentina. In fact, the Iguassu Falls are what divides the river of the same name into its upper and lower portions, a fact that has given rise to several myths and legends as to their origin. Nowadays, the Iguassu Falls are owned by the two Unesco World Heritage Sites: the Iguazú National Park in Argentina and the Iguaçu National Park in Brazil. Thank you for joining us at Safepork2017 in Iguassu Falls. We are very glad to receive you.

Organization Committee

Program

August 21st

PRE-CONFERENCE WORKSHOP

Workshop 1

Modernization of Meat Inspection

Room: Alegro 1

Prof. Dr. Lis Alban
Chief Scientist Danish Agriculture & Food Council - DAFC
Adjunct Professor at the University of Copenhagen - Denmark

Dr. Elenita Ruttscheidt Albuquerque
Brazilian Federal Meat Inspection Service - SIPOA/DIPOA

Modernization of meat inspection is on the agenda in several countries. Resources are scarce and the challenges plenty, so cost-effective ways of inspection are sought for. Moreover, pork is traded internationally. Would it make sense to have similar inspection regimes in place all over the world? - Or do the production systems and perceived risks differ too much between countries? Should focus rather be on a harmonisation of the outcome of inspection more than the way it is performed? - while remembering that the main objective is to ensure that the meat, which reaches the consumer, is safe and wholesome. Moreover, we must not forget that inspection is also made to ensure early detection of animal health and welfare problems. So, how can we undertake meat inspection in way, where all three aims are met? According to the EU Meat inspection Regulation from 2014, meat inspection of swine should be visual-only, unless other information points to a need for traditional inspection involving incisions and palpations. To which extent has visual-only inspection been implemented? What is the experience, pros and cons? And what have the reactions from trade partners, consumers and workers' union been? How is modernization of meat inspection being interpreted in countries such as Brazil, Chile and the US? - Are the challenges the same or do they differ due to historical/cultural issues? The concept of Food Chain Information is in use in the EU. How meaningful is this concept? And are similar concepts being used on the American continents? Which solutions to challenges have been found? And what are the future developments and next challenges within meat inspection of swine? These questions will be addressed in detail during this workshop, where representatives from industry, academia and veterinary authorities from various countries will share their experience, so we can all learn how to optimise meat inspection.

09:00 am	**Welcome**
09:10 am	**Introduction to the workshop by Lis Alban** (DAFC)
09:20 am	**Status for Brazil** - Elenita Ruttscheidt Albuquerque (SIPOA/DIPOA)
09:40 am	**Status for Colombia** - Annette Hjorth (Independent consultant)
10:00 am	**Status for the Netherlands** - Derk Oorburg (VionFood)
10:20 am	**Status for Germany** - Nina Langkabel (Freie Univ. Berlin, Germany)

10:40 am Coffee Break

11:00 am	**Status for Denmark** - Lis Alban (DAFC)
11:20 am	**Status for Portugal** - Madalena Viera Pinto (Univ. Trás-os-Montes e Alto Douro, Portugal), Paulo Carneiro (Direção Geral de Alimentação e Veterinária - DGAV) and Susana Santos (DGAV)

11:40 am	**Status for US** - Melanie Abley (US Food Standard and Inspection Service)
12:00 pm	**Status for Australia** - David Hamilton (South Australian Research & Development Institute) presented by Nina Langkabel

12:20 pm Lunch

13:20 pm	**Establishment and maintenance of a Trichinella Negligible Risk Compartment** - Dan Kovich (Nat. Pork Prod. Council)
13:40 pm	**A new concept for pre-harvest monitoring** - Patrik Buholzer (Thermo Fisher Scientific)
14:00 pm	**Risk-based monitoring of residues of antimicrobials in pig meat** - Lis Alban
14:20 pm	**Feed-back of information from meat inspection to producers** - Derk Oorburg
14:40 pm	**Panel discussions about the status for modernization of meat inspection** - facilitator Lis Alban
15:20 pm	**Summing up: Where are we heading?** - Cláudia Valéria Cordeiro (Department of Inspection of Products Animal Origin - DIPOA/MAPA)

Workshop 2

Innovation in the pork chain: new technologies in diagnostics, data and monitoring

Room: Alegro 2

Prof. Dr. Alasdair J. C. Cook

Head of Department of Veterinary Epidemiology & Public Health
School of Veterinary Medicine - Faculty of Health & Medical Sciences University of Surrey - United Kingdom

Prof. Dr. Diana Meemken

Professor of Herd Health Management
Martin-Luther-University Halle-Wittenberg
Institute of Agricultural and Nutritional Sciences
D-06120 Halle (Saale)

Meat inspection started in the early 19th century as an important improvement measure with respect to food safety and public health and has been a success story until recently. Due to ante and post-mortem meat inspection - both can be labelled as end product controls - "animals not fit for slaughter" and "meat not fit for consumption" were sorted out. The core preconditions of the functionality of this system is the detectability of lesions due to disease such as tuberculosis and cysticercosis. However, with the BSE crises and the rise of salmonellosis it became obvious that the traditional meat inspection alone and the applied methods like inspection, palpation and incision as end product controls are not addressing the risks of today in a sufficient way - especially with respect to subclinical zoonoses like salmonellosis, toxoplasmosis and yersioniosis. Furthermore, the optimization of animal health and animal welfare quality of food animals are increasing social demands which cannot be addressed by the traditional meat inspection in a sufficient way only by sorting out animals and carcasses. One of the most important changes is the deeper understanding of the food chain and that the risks and demands of today can only be addressed by including the whole food chain into a process control. This workshop will give insights into innovative "detection and monitoring methods" as well as new systems for the information exchange along the food chain which is an indispensable requirement for a continuous process along all stages of the food chain improving food safety, animal health and animal welfare.

09:00 am	**Welcome**
09:10 am	**Introduction: Presenting the aims of the workshop** - Prof. Dr. Alasdair J. C. Cook
09:20 am	**Teamwork with all workshop attendees regarding "The structure of the pork chain and the stakeholders**
09:40 am	**Teamwork with all workshop attendees regarding "Considering the issues, risks, perceptions and values at each stage"**

10:20 am Coffee Break

Case presentation as examples for innovations along the food chain

10:50 am **Post-mortem meat inspection assisted by 3D camera** - Dr. Lara Blömke

11:10 am **Serological herd profiles gained by meat juice multiserology** - Dr. Diana Meemken

11:30 am **Blockchain: a digitised approach to transaction management** - Dr. Alasdair Cook

12:00 pm Lunch

13:10 pm **Teamwork in four groups: Innovations initiated by a) farmers and their vets, b) food operators and/or retailers, c) official veterinarians, and d) consumersbusiness**

14:30 pm T**ea/coffee and prepare summary of teamwork**

15:00 pm **Present teamwork summaries; Discussion and conclusion**

August 22nd - Morning

Conference

Room Vivace

08:00 am **Registration**

08:30 am **Open Ceremony**

Dr. Janice Ciacci Zanella

(Director of the Embrapa Swine and Poultry; Researcher in Swine Health)

Pork production chain: importance and challenges faced

09:15 am **Keynote: Toxoplasma gondii and the role of pork**

Dr. Sara Monteiro Pires

Senior Researcher - Risk Benefit Research Group, Division of Diet, Disease Prevention and Toxicology, National Food Institute - Technical University of Denmark

ORAL SESSION

Epidemiology of foodborne pathogens and zoonotic diseases in the pork production chain

10:15 am **Genomic characterization of Staphylococcus aureus at the swine-human interface** (Peter Davies; University of Minnesota, USA)

10:30 am **Taenia solium cysticercosis in the unprocessed pork supply chain in Nairobi and environs, Kenya** (James Akoko; International Livestock Research Institute, Tanzania)

10:45 am **Diversity of Yersinia enterocolitica population in a slaughterhouse between 2009 and 2010 and discrimination ability of MLVA compared to PFGE** (Pierre Raymond, Hygiene and Quality of Poultry and Pig Products Unit, University of Bretagne-Loire, Ploufragan, France)

11:00 am Coffee Break

11:30 am	**National prevalence of *Salmonella* spp. in pork slaughterhouses under Federal Inspection in Brazil, 2014/2015** (Anna Carolina Massaro Brasileiro; Universidade Federal de Minas Gerais, Brazil)
11:45 am	**Prevalence and associated risk factors of *Salmonella* spp. on the pork production chain in Córdoba, Argentina** (Juan Pablo Vico; IRNASUS-CONICET, Universidad Católica de Córdoba, Argentine)
12:00 pm	**Dynamic of excretion and immune response of experimentally infected pigs with monophasic variant of *Salmonella* Typhimurium serovar 1,4,[5],12:i:** (Maria Bellen Cevallos Almeida; Hygiene and Quality of Poultry and Pig Products Unit, University of Bretagne-Loire, Ploufragan, France)
12:15 pm	**Evaluation of the colonizing ability on IPEC-J2 cells and of the pathogenicity on Caco-2 cells of the 3 major French pig *Salmonella* serovars** (Annaelle Kerouanton; French Agency for Food, Environmental and Occupational Health & Safety, France)

12:30 pm Lunch

August 22nd - Afternoon

02:00 pm	**Keynote: Resilience in the pork supply chain from the food safety perspective -** Prof. Dr. Lis Alban (Chief Scientist Danish Agriculture & Food Council - DAFC; Adjunct Professor at the University of Copenhagen - Denmark)

ORAL SESSION

Surveillance and control of foodborne pathogens at pre-harvest and post-harvest level

03:00 pm	**Evaluating correlations in *Salmonella* serotypes in swine in four dataset** (Annette O'Connor, Iowa State University, USA)
03:15 pm	**Revisiting the role of pig-serology in the context of *Salmonella* control programs in countries with high prevalence of infection - a preliminar study** (Raul C. Mainar-Jaime; Universidad de Zaragoza, Spain)
03:30 pm	**Results from a study of the effect of enhanced cleaning and disinfection on *Salmonella* prevalence in finisher pig buildings** (Richard Smith; Animal and Plant Health Agency, UK)
03:45 pm	**New innovative feeding strategy for reduction of *Salmonella* in swine** (Janekke Allaart; Trouw Nutrition Research and Development, Netherlands)

04:00 pm Coffee Break

04:30 pm	**Maternal vaccination as an effective *Salmonella* reduction strategy** (Richard Smith; Animal and Plant Health Agency, UK)
04:45 pm	***Salmonella* Typhimurium fecal shedding following *Salmonella* Choleraesuis-Typhimurium vaccination via drinking water and challenge** (Jessica Seate; Boehringer Ingelheim Animal Health, USA)
05:00 pm	**Influence of different vaccination strategies against *Salmonella* Typhimurium in pig farms on the number of carriers in ileocecal lymph nodes** (Linda Peeters, Faculty of Veterinary Medicine, Ghent University, Belgium)

05:15 pm	**Vaccination against Lawsonia intracellularis decreases shedding of *Salmonella* enterica serovar Typhimurium co-infected pigs and changes host gut microbiome** (Fernando Leite; University of Minnesota, USA)
05:30 pm	**Development and evaluation of a novel orally administered inactive subunit vaccine to control foodborne pathogens** (Sherry Layton, Vetanco, Argentine)

05:45 pm GUIDED STRETCHING & WALKING
Foyer Vivace

August 23rd - Morning

08:30 am	**Keynote: Transmission of antimicrobial resistance from pigs to humans: trues and lies** Prof. Dr. Luca Guardabassi - Professor of Clinical Microbiology at Ross University School of Veterinary Medicine in St Kitts, West Indies)

ORAL SESSION

Antimicrobials in swine production, antimicrobial resistance, alternative strategies to antimicrobial use

09:30 am	**Human health implications of MRSA CC398 in Denmark** (Jan Dahl; Danish Agriculture & Food Council, Denmark)
09:45 am	**MRSA in breeding pigs in Germany 2015** (Bernd-Alois Tenhagen; Bundesinstitut für Risikobewertung, Germany)
10:00 am	**Genotypic characterization of a monophasic variant of *Salmonella enterica* serotype Typhimurium in swine in USA Midwest** (Peter Davies; University of Minnesota, USA)
10:15 am	***Escherichia coli* resistance and gut microbiota profile in pigs raised with different antimicrobial administration in feed** (Caroline Pissetti; Departamento de Medicina Veterinária Preventiva, Universidade Federal do Rio Grande do Sul, Brazil)

10:30 am Coffee break

11:15 am	**In vitro assay for antimicrobial interaction evaluation and risk assessment of antimicrobials in anaerobic digestion of swine manure** (Ricardo Steinmetz; Embrapa Swine and Poultry, Concórdia, Brazil)
11:30 am	**Alternatives to antimicrobial treatment in weaners - the veterinary practitioner's solution** (Frede Keller; DanVet, Denmark)
11:45 am	**Efficient waterlines cleaning protocols in post-weaning rooms: a new way to reduce antibiotic consumption?** (Mily Leblanc-Maridor; LUNAM Université, Oniris, Nantes-Atlantic College of Veterinary Medicine and Food Science and Engineering, France)
12:00 pm	**Natural feed additives as alternative to in-feed medication** (Antonia Tacconi; Biomin Holding GmbH, Austria)

12:30 pm Lunch

August 23rd - Afternoon

02:00 pm	**Keynote: The use of risk assessment to support control of *Salmonella* in pork** Prof. Dr. Maarten Nauta (Senior Researcher - Research Group for Risk-Benefit, National Food Institute, Technical University of Denmark)

ORAL SESSION

Risk Assessment and risk communication in food safety

03:00 pm	**A cost-benefit assessment of *Salmonella* control strategies in pig herds within the United Kingdom** (Richard Smith; Animal and Plant Health Agency, UK)
03:15 pm	**Using serological monitoring, internet-based feedback and on-farm auditing to improve Toxoplasma gondii control at Dutch pig farms** (Derk OOrburg; Vion Food, Netherlands)
03:30 pm	**Application of qualitative risk assessment to prioritize hazard in pork products in Brazil** (Eduardo Freitas Costa; EPILAB, Faculdade de Veterinária, Universidade Federal do Rio Grande do Sul, Brazil)
03:45 pm	**A spatial entry assessment model for incursion of exotic swine diseases into the European Union** (Richard Smith; Animal and Plant Health Agency, UK)

04:00 pm Coffee Break

ORAL SESSION

Slaughter process and Meat Inspection: quality, hygiene and safety

04:45 pm	**Control of *Salmonella* environmental contamination during pig transport and lairage: a realistic project?** (Mily Leblanc-Maridor; LUNAM Université, Oniris, Nantes-Atlantic College of Veterinary Medicine and Food Science and Engineering, France)
05:00 pm	**Association between slaughter practices and the distribution of *Salmonella*, ESBL/AmpCproducing Escherichia coli and hygiene indicator bacteria on pig carcasses after slaughter** (Wauter Biasino; Ghent University, Belgium)
05:15 pm	**The harmonization of sanitary decision criteria for vertebral osteomyelitis in pig carcasses** (Maria Madalena Vieira-Pinto; Universidade Trás-os-Montes e Alto Douro, Portugal)
05:30 pm	**Guidelines for farmers, transporters and official veterinarians to assess the "Fitness for Transport and Slaughter" of slaughter pigs** (Diana Meemken; Martin-Luther-University Halle- Wittenberg, Germany)
05:45 pm	**Automated assessment of animal welfare indicators in pigs at slaughter** (Lara Bloemke; Tierarztliche Hochschule, Hannover, Germany)

7:30 pm SAMBA PARTY AT "PRAÇA DE ENTRETENIMENTO"
Underground

August 24th - Morning

Discussion Forum

Food safety: commercial, economic, social and political perspective

Room Vivace

08:30 to 08:35 am	**Opening** - Dr. Marcelo Miele (Embrapa Swine and Poultry)
08:35 to 08:50 am	**Panel 1: Pig production costs in major global players and biosecurity and medicines costs impacts** - Dr. Marcelo Miele
08:50 to 09:10 am	**Panel 2: Global pork market, trade barriers and customers challenging Brazilian industry** - Dr. Rui Eduardo Saldanha Vargas (Brazilian Animal Protein Association, ABPA)
09:10 to 09:30 am	**Comments and discussion on Panels 1 and 2**
09:30 to 09:50 am	**Panel 3: Technological trends in pork food safety and their costs impacts** - Dr. Marcos H. Rostagno (Elanco Knowledge Solutions)
09:50 to 10:10 am	**How to prevent presence of residues in pig meat - on-farm actions and monitoring to check for compliance -** Lis Alban and Frede Keller (DAFC; Practitioner)
10:10 to 10:30 am	**Comments and discussion on Panels 3 and 4**

10:30 to 11:00 am Coffee break

11 am to 12 pm	**Questions for the key speakers: Where are we heading?** Moderator: Dr. Marcelo Miele (Embrapa Swine and Poultry) Comments from the plenary and discussion

12:00 pm Closing Ceremony

Summary

Posters

12th safepork
Foz do Iguaçu - Brazil
2017

Keynote

PORK PRODUCTION CHAIN: IMPORTANCE AND CHALLENGES FACED

Janice Reis Ciacci Zanella

Embrapa Suínos e Aves

Brazil is doing well in agriculture. We have availability of grains, abundant natural resources and diversity in the broad national territory, herd health and biosecurity stand out from the other livestock producing countries. There are funding for agricultural research, technology, technical assistance and extension. We have a sophisticated and diversified integration model. Therefore, Brazilian agriculture is efficient and competitors must observe closely.

Swine production in Brazil ranks fourth in the world for pork production and export. It produces 3,643 million tons and exports 555 thousand tons annually. In 2015 there were increases in domestic production by about 5% and 9% of exports. National Pork annual consumption per capita is 15.1 kg, still considered low compared to other meats such as poultry (43 kg) and bovine (39 kg) (CIAS, 2016).

The rapid and constant evolution of new technologies and management tools makes swine farming a very dynamic activity. Consequently, increasing consumer demands for animal welfare and sustainability coupled with labor shortages pose major challenges (CIAS, 2016). Sustainability translates into balancing the economic, social and environmental tripods. It should account for investments, costs, employment generation, social responsibility and citizenship, preservation and management of natural resources, renewable energy, among others, respectively. It is to look at a systemic view of the whole. This concept of agribusiness was already defined in 1957 by John Davis and Ray Goldberg from Harvard University as *"the sum total of all operations involved in the manufacture and distribution of farm supplies; production operations on the farm; and the storage, processing and distribution of farm commodities and items made from them"*. And it involves from scientific research to the marketing of food, fiber and energy (Davis and Goldberg, 1957).

Embrapa Swine and Poultry within a Embrapa's institutional program called "Agropensa" has sought to anticipate and follow trends that influence all the links of the productive chains of swine and poultry (CIAS, 2016). This vision of the future of the technological development of the Brazilian agriculture of 2014 - 2034 seeks, in addition to anticipating trends, but ensuring the permanent adjustment of the priorities of research and technology transfer, with a view to innovation.

The importance of swine farming for Brazil lies in several aspects. Among them that the swine industry is an organized segment of the Brazilian agribusiness, being a model for other chains and even other countries. It is a long chain, involve a complex of relationships and interrelationships with other activities (corn, soybean meal, vitamins, minerals, animal health, transport, buildings, machinery and equipment, advanced genetics, etc.), with a large multiplier effect of income and employment. Furthermore, it provides the internal supply of high quality protein. National swine production has experienced strong growth in recent years and is showing a trend towards expansion. Brazil is an important player in food production, especially meat, with a projection to expand by 35% in pork production, 23% in beef and 34% in chicken meat by 2025 (Ministério da Agricultura, 2015). Pig farming contributes to the economic development

of Brazil and more effectively in the regions where they operate, with important role in job creation, in support of family farming and the generation of income and revenue for the country (Filho, 2013).

Nevertheless, the big challenge is to feed 9 billion people worldwide by 2050 in a sustainable way, maintaining efficiency and competitiveness. Studies point out that population growth, increased urban population and world income will raise the demand for animal protein. Technology will be a great ally to multidisciplinary teams working in a collaborative, innovative and competent manner (FEEDINFO, 2013).

Due to the dynamism and interrelationship with other chains, swine production suffers from constant and profound changes with several threats that afflict the sector. The occurrence of climate change, the emergence in recent decades of diseases, mainly the zoonotic ones due to the concentration of production, urbanization, changes on the virulence of pathogens as well as the antimicrobial resistance (AMR). The advance of the globalization of markets has brought increasing volatility in the prices of grains and meat. There has never been such a great interdependence among nations. Protectionism, subsidies, requirements for disease and/or pathogen control, as well as control of residues or contaminants and requirements in environment and animal welfare are the result of this dynamism. This is aside from the rising wave of activism that strongly influences consumer opinion.

In the social and human field, the lack of skilled labor and the pursuit for better working conditions require the automation of processes at all levels of the chain. It's challenging and urgent the need to qualify the producers to improve business management, in particular to the family subsistence production, to improve income when accessing local markets and also reduce the risks to industrial production (FEEDINFO, 2013).

The constant search for increasing sustainability and competitiveness has as one of the main pillars the quality of products generated and animal health. Actors should seek the incorporation of good practices, both for the production and for the safety of foods. Important public policies should guarantee the improvement of the Brazilian product. Currently, Embrapa Swine and Poultry researches to review and modernize inspection systems for pork and poultry, as well as projects aimed at reducing the occurrence of foodborne diseases and mitigation of the risk of diseases that affect not only the herds, but the final consumer, be it Brazilian or the importing countries.

Another action of the Embrapa's team occurs in the surveillance, mainly in support of the Agriculture Ministry (MAPA) and accredited laboratories in the validation and development of techniques for diseases diagnosis and research in support of animal health surveillance. All this research effort is carried out together with industry and government to guarantee Brazil a sanitary standard of excellence, allowing exporting our products to dozens of countries in all continents.

In animal health, in addition to emerging diseases, a challenge is the increased attention to safe food production. According to the UN, WHO, FAO and OIE the high levels of antimicrobial resistance or drug resistance (AMR) seen in the world today are the result of overuse and misuse of antibiotics and other antimicrobial agents in humans, animals and crops, as well as the spread of residues of these drugs in soil, tilth and water. Within the broader context of AMR, antibiotic resistance is considered the largest and most urgent global risk requiring national and international attention. On the other hand, effective and affordable antibiotics are so vital to protect animal health and welfare and good veterinary medicine as they are to human health. Responsible and

prudent use, good practices and implementation of established standards and guidelines should be followed (ONU, 2016).

In welfare of pigs, there is a tendency both internal and external in demanding minimum standards of animal welfare in the productive chains. This includes everything from handling in farms such as housing systems (gestations stalls for sows) and automation of production systems such as boarding and transport procedures, stunning systems and euthanasia (CIAS, 2016).

Genomic tools, gene edition and genetic selection will get animals more efficient, disease resistant and with better meat quality, with reduced fat, including odor reduction. The selection of animal genetics focusing on niches and specific markets will also be trend. Genomics through nutrigenomics will also be a useful tool to better express the potential action of biologically active substances in food and its effects on animal health. Other advances in nutrition may include the use of enzymes and intestinal modulators as probiotics and prebiotics. These advances bring much effect in improving digestibility as targeting the reduction or elimination of the use of antibiotic growth promoters and for prophylactic use.

For greater sustainability of swine industry, it will be necessary to create more rigorous environmental regulations, based on technical criteria validated by agricultural research. Thus, there will be a need to adopt water management technologies and to treat and recycle the waste generated by these activities: manure and litter, dead animal carcasses, slaughter waste, among others (CIAS, 2016).

The expansion of Brazilian pig farming will also require a greater integration of this chain with other agricultural and agroindustrial production systems through the use of animal production residues as fertilizers for the production of grains, forage and biomass (integrated crop-livestock-forest systems or ICLF). Alternatively, for the generation of co-products with higher benefit (added value), such as organomineral fertilizers, energy and biofuels, among others. In addition, the adoption of computing, remote sensing and information technology for the automation of equipment, practices and processes used in the environmental management of swine and poultry farming will be increasing. (CIAS, 2016). Sustainable production should be concerned with environmental impact. The global increase in temperature, the search for greenhouse gas (GHG) mitigation practices, the reuse and reduction of water use, biofertilizers and biogas are topics that will require more affordable technology (CIAS, 2016).

In summary, the lecture will address the main trends and the challenges for Brazilian pork production, as well as to discuss the role of agricultural research in this context, aiming to support the swine industry.

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TOXOPLASMA GONDII AND THE ROLE OF PORK

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Background

Toxoplasma gondii is an important zoonotic pathogen. Humans can acquire *T. gondii* infection through foodborne or environmental exposure, and recent estimates suggest that more than 10 million human cases of toxoplasmosis occur globally every year[1]. Infections in humans can be post-natal (i.e. acquired toxoplasmosis) or vertical (i.e. congenital toxoplasmosis). Because congenital toxoplasmosis is considered particularly problematic due to the severe health effects it can cause in children since birth and the possibility of fetal death, its public health impact has been more extensively studied than acquired toxoplasmosis, where infection is usually associated with mild flu-like symptoms. However, several newer studies suggest that in some cases ocular disease and severe syndromes such as psychiatric disorders may develop as a result of infection[2].

The recent "Initiative to Estimate the Global Burden of Foodborne Diseases" of the World Health Organization provided crucial evidence to raise awareness on the importance of toxoplasmosis by estimating the disease burden in terms of incidence, mortality and disability adjusted life years (DALYs). This study ranked toxoplasmosis as number 13th among 31 foodborne diseases globally, also demonstrating regional differences, with e.g. a relative higher importance of toxoplasmosis in the Americas than in Europe[1]. While crucial to guide public health policy at the global level, regional disease burden estimates are insufficient to direct intervention strategies that are targeted to the reduction of incidence in the population. To identify and implement effective interventions to reduce the burden of toxoplasmosis, risk managers need knowledge on both the local disease burden and the contribution of the most important sources and transmission routes of infection in each population in that area.

Investigating the role of different sources and transmission routes

Cats and wild felids are the only definite hosts of the parasite, but virtually all warm-blooded animals can act as intermediate hosts, and most species can be carriers of tissue cysts of *T. gondii*. *T. gondii* has been isolated from most livestock species such as pigs, cattle, sheep, poultry, as well as wildlife and game. Like many other foodborne hazards, *T. gondii* can be transmitted to humans through consumption of contaminated foods but also by other routes: through water, soil, or air; by direct contact between people, or by contact between people and animals. The relative importance of exposure from a contaminated environment versus consumption of meat or other foods is unclear.

The process of partitioning the human disease burden of a foodborne infection to specific sources is known as *source attribution*, where the term source includes reservoirs (e.g. animal reservoirs like pigs, cattle, pets) and vehicles (e.g. food products like pork or beef). A variety of methods to attribute foodborne diseases to sources are available, including approaches based on analysis of data of occurrence of the pathogen in sources and humans, epidemiological studies, intervention studies, and expert elicitations. Each of these methods presents advantages and limitations, and the usefulness of each depends on the public health questions being addressed and on characteristics and distribution of the hazard.

Source attribution methods have been extensively used to investigate the contribution of food and animal sources for several diseases, e.g. salmonellosis, campylobacteriosis, and listeriosis. Measuring the proportion of *Salmonella* infections that is attributable to different sources has proven particularly useful in several countries and regions, with Denmark pioneering the *One Health* efforts to guide food-safety interventions based on scientific evidence. Relying on knowledge on the most important sources of salmonellosis in the country in different time periods, policy-makers have implemented and revised *Salmonella* control programmes in broiler, layer and pig production with great success in terms of reducing the burden of disease. But application of source attribution methods for other zoonotic pathogens is often more challenging, which can be due to the characteristics of the pathogen or due to lack of data. In the case of absence of quantitative data, expert elicitations are crucial to fill in gaps and combine knowledge from multiple sources and experts.

Source attribution of toxoplasmosis: what do we know?

Source attribution of toxoplasmosis is particularly challenging due to lack of data, and very few studies conducted so far. To overcome this challenge, WHO's Initiative to estimate the global burden of foodborne disease included a large expert elicitation study to assess the contribution of sources for several diseases, including toxoplasmosis. This study estimated that between 42 and 61% of acquired toxoplasmosis cases globally are due to foodborne transmission, with other important routes being water (11-27%) and soil (18-38%)[3]. The next step of the source attribution process is to measure the contribution of specific sources within these major transmission routes, which would ideally be based on data on prevalence, contamination and exposure of/to each source.

Opsteegh et al.[4] measured the relative contribution of three meat types for infection with *T. gondii* in the Netherlands. The authors used a comparative risk assessment approach and concluded that 70% of meat-related infections were due to consumption of beef products, 14% due to sheep meat, and that 11% were attributable to pork products. A case-control study in the United States supported these estimates by finding that the leading foodborne risks associated with toxoplasmosis were eating raw ground beef, rare lamb or processed meats produced and consumed without heat treatment[5].

To our knowledge, these are the only data-driven published studies on source attribution of toxoplasmosis. Several other studies investigated the prevalence of *T. gondii* in different sources, including meats, and others have estimated the risk of disease through consumption of one specific food type, but have not compared these with the risk of other routes.

So what do we know about the role of pork?

Consumption of pork and pork products has historically been attributed an important role for toxoplasmosis. An expert elicitation ranked this food-pathogen combination second among the top 10 most important in the US[6], and several epidemiological and prevalence studies have focused on pigs and associated products as an important source. However, more recent studies indicate that other food products, particularly beef and lamb, may play a more important role. In addition, increasing evidence shows that prevalence in pigs is decreasing[7] worldwide. This is likely to be associated with the intensification of pig production globally - implying indoor production.

Several factors may explain a lower contribution of pork for human toxoplasmosis. On one hand, *T. gondii* seroprevalence in many animal hosts (as in humans) increases with age[8], and market pigs are relatively young when compared with other livestock. Sows have reportedly a higher prevalence, but their meat is mostly used for processed pork products (such as sausages, salami), and this processing involves high saline content which eliminates viable stages of *T. gondii* in the meat[9]. However, it has also been shown e.g. in studies in the Netherlands[10] and Denmark[8] that outdoor pigs have a higher prevalence than indoor/intensive-production animals, and the increasing demand for organic and free range animal products may represent an increased risk for the consumer unless the meat is thoroughly cooked prior to consumption.

What are we doing to address knowledge gaps?

In the Nordic countries, efforts have been joined to conduct the first large study of source attribution of toxoplasmosis. In this regional initiative, the lack of data on national level is compensated for, enabling an estimation of the relative contribution of the different sources for infection. We are developing a methodological framework that can be applied by each country to produce evidence for risk management, including prioritization of food safety strategies. We expect that this approach will be useful derive national and regional source attribution estimates for toxoplasmosis, identify differences between countries, and help understanding the reasons for such differences.

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RESILIENCE IN THE PORK SUPPLY CHAIN FROM THE FOOD SAFETY PERSPECTIVE

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Summary

Challenges in food production are plenty, while resources are scarce. In the interest of the consumer as well as the producer, it is of utmost importance to prevent unwanted events from happening. This may be done through focus on resilience in the pig and pork supply production in relation to food safety. In this paper, resilience - and its counterpart vulnerability - is introduced and discussed specifically for food safety. It is noted that to manage unwanted events, focus must be on effective handling on the event itself. But focus should also be on the patterns of events - including the trends in society which may be leading to increased or decreased risks. To gain a more comprehendsive understanding of these patterns, an analysis of the entire structure of the production system is necessary. Through an analysis of these three levels - event, patterns and structure - and their complex interrelationships, targeted prevention activeties may be identified and put in place to raise the system's long-term resilience. Hereby, the risk to consumers and producers can be reduced to an acceptable level. Such risk-mitigating activities may consist of surveillance, own check systems, HACCP, risk analysis, and legislation including use of private standards.

Definition of resilience and vulnerability

Resilience may be defined in different ways depending on the context. In this paper, the definition used in general systems theory is applied: the capacity of a system to absorb disturbance and reorganize while undergoing change so as to still retain essentially the same function, structure, identity, and feedbacks. Moreover, in this paper resilience is not just seen as a response to an acute event but also as the ability of the system to prevent events from happening in the future.

In pig and pork production, resilience may be interpreted as the capacity of the value chain to recover after unwanted events such as zoonotic disease outbreaks in the pig population or feed/food contamination. These events may lead to perturbations of the system and requirements for withdrawal of the product. Subsequent negative effects may be seen on the income of the Food Business Operator (FBO i.e. the farmer, the abattoir, the meat processor, the retailer) due to the necessary handling of the event and loss of consumer confidence which will lead to a lower future market share.

Vulnerability may be interpreted as the contrary of resilience. Vulnerability analysis is a process that defines, identifies, and classifies the weak points and links (vulnerabilities) in a network or production chain. In this process, uncertainty can - and should - be taken into account, so we can prepare for the unexpected. In this way, the value chains will be able to constantly deliver the values demanded by the consumer: plenty of safe and nutritious food at an affordable price.

An increased focus on resilience and vulnerability is also important to secure employment and income - and hereby for the sustainability of the entire value chain.

Unwanted events require response

We tend to focus on individual events and not the trends and deeper structures leading to them. For example during an outbreak of Salmonella, the event may be detected through an increase in the number of human cases. This imposes a response by the authorities and the supply chain. Once the source of infection has been identified, targeted actions are taken, such as withdrawal of the product from the market, whereby costs are incurred to the industry. Hence, the whole system of food safety regulations and public and private governance in place are part of resilience, because it allows reacting to these events and make sure that public health and animal health are protected. However, if focus is only on the event itself, resources are spent on responding and future outbreaks may not be prevented appropriately.

Patterns of events provide understanding

Contrary to events, which may be interpreted as a here-and-now picture of a single incident, patterns represent a series of events. This implies using surveillance/ monitoring data to address location, timing, frequency and size of events and change in these. This includes analysing the causes of the individual events - and by discerning commonalities get an idea of how prevention may be initiated.

In line, change in consumers' behaviour and purchase patterns should be foreseen and dealt with. An example is the move towards a higher demand for outdoor-produced pork, which changes the risk patterns and puts new requirements on food safety measures/prevention methods in the food supply chain.

Trends outside the value chain may also influence the patterns of events. Climatic changes may result in an expansion of the habitat of an insect or a parasite leading to an increase in the disease burden of the animals that form part of the supply chain. Likewise, demographic changes may imply establishment of urban populations in areas previously free from human activities, which may lead to a higher probability of introduction of pathogens into the food supply chain. This emphasizes that food supply chains are nested in a social-ecological context that is unpredictable from a mere production chain perspective and demands a broader approach.

Analysis of the structure reveals identification of solutions

To get a more comprehensive understanding of what is leading to unwanted events, it is important to analyse the structure of the supply chain. Hence, an identification of the vulnerabilities during the chain could help to identify weak points and links. This implies focus on the identification of the factors, which influence the probability of the unwanted event as well as factors impacting on the size of the consequences. Moreover, the capacity to respond when needed should be assessed to understand what to do to solve a specific issue. Finally, the time dimension - including changes in the system over time - is important to elucidate.

Ways of undertaking such analyses include 1) simple sketches of the production including risk pathways, and causal loop diagrams 2) clinical trials and risk factor studies (e.g. for contamination during processing) to bring about information about the size of the individual effects and 3), risk assessment including simulation or modelling as well as economic analysis. Such activities can generate knowledge about what to do in the most cost-effective way. The work should be made in an iterative way, preferably in a group of people with different qualifications, and in collaboration with the relevant actors and stakeholders to get the best-suited overview of the system. Here, it should be remembered that the selection of stakeholders will impact on the scope of the analysis and the understanding of what effectiveness means.

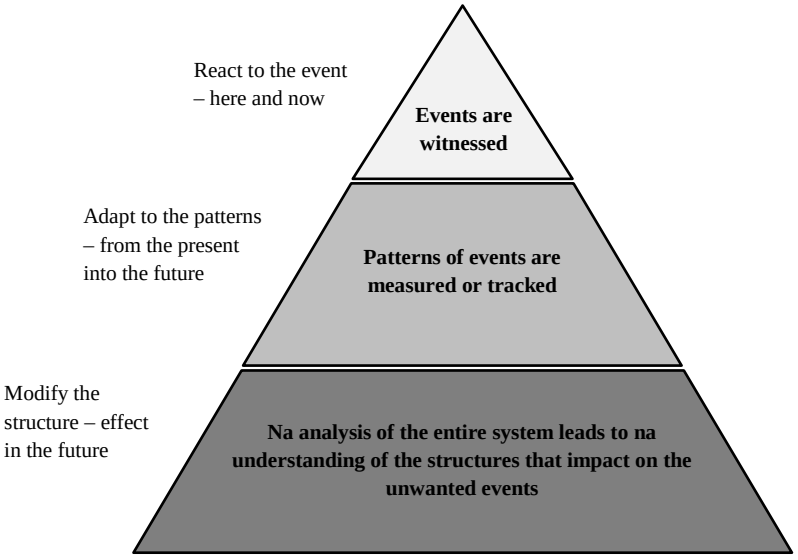

Figure 1. Description of how events, patterns and structure of a supply chain may be interrelated (adapted after Anderson & Johnson, 1997).

An analysis of the value chain with a focus on the vulnerabilities can forecast the effectiveness of proposed countermeasures and evaluate their actual effectiveness after they are put into use. For food safety, such measures include good manufacturing practices (GMP), own check, HACCP, preparedness, and targeted surveillance activeties. Other tools may consist of private standards, which are voluntary in principle, but which have to be complied with, if the FBO want to be part of the quality label represented by the private standard.

Example 1: Antimicrobial resistance

Increase in antimicrobial (AM) resistance constitutes an unwanted food safety event. Limitation on the use of AM in pigs may be considered a timely reaction intended to lower the negative long-term effect on resistance development in humans. But without a deeper focus on what is causing the need for treatment, negative side effects may be seen e.g. animal welfare issues related to the lack of treatment of ill animals. Moreover, production may be less effective, if use of AM is limited unnecessarily. Finally, the effect of the action taken (to improve human health) may be lower than expected, because the AM use in humans is maintaining the resistance levels observed in human pathogens and indicators.

Another approach may be to focus on the need for treatment of livestock, which is often related to pig-specific pathogens. This implies that an analysis is undertaken to elucidate which conditions are associated with AM use. Then, focus can be directed towards prevention of these conditions including development of cheap and easy-to-use vaccines, as well as focus on management including age and weight at weaning and type of feed. These interventions may help to maintain herd health even in the presence of pathogens. Moreover, both zoonotic and non-zoonotic pathogens may be kept at bay, if external biosecurity is high. Likewise, spreading of infection within a herd may be kept to a tolerable level, if internal biosecurity is high. In this case, specific use of AM may be allowed as a timely reaction to an acute health issue - and the potential negative impact on human and animal health due to resistance development is kept low due to prudent use of AM.

Example 2: Residues in pork

Residues are unwanted from a consumer point of view and may lead to a requirement for withdrawals on the local market and to export bans on sensitive markets. Residues may consist of AM arising from lack of compliance of withdrawal times after treatment with AM. But residues may also consist of illegal substances such as hormones or of environmental polluters such as dioxin. Actions are warranted to keep the prevalence of residues of AM at a low level - and to keep the other kind of residues away from the supply chain. An apparently effective instrument would be to operate with high fines in case of non-compliance. However, monitoring for residues is expensive as there is a plethora of residues to look for in feed and pork. Therefore, only a limited proportion of the feed batches and carcasses are tested officially leading to low surveillance system sensitivity. A fining system may therefore not be very effective.

Instead an analysis along the supply chain of what may cause presence of residues in pork should be undertaken. This may show that no illegal substances or environmental polluters are found, and that penicillin is the AM most commonly found in monitoring. Moreover, it may be revealed that sows apparently have a higher probability than finishing pigs. Then, interviews with affected farmers may disclose that sows were sent to slaughter before the withdrawal period was over due to a mistake arising from inadequate marking, poor record-keeping and lack of communication. A discussion with producers may reveal that for finishing pigs, it is easy to comply with the withdrawal times, because the date of slaughter is known. Hence, the vulnerable part of the system is the sow production, where time of slaughter is not pre-planned.

As a solution, several actions can be taken: 1) tools to mark treated sows can be developed, 2) a requirement for own check related to farmers' AM treatment can be put into the private standard governing pig production and 3) regular campaigns may be planned targeted pig producers informing them about the consequences of not complying with withdrawal periods. By use of these initiatives, the system becomes more resilient in the long run as it prevents the event from occurring.

Discussion

It is only natural that we try to simplify systems to get an overview of cause and effect. However, the scientific approach of A leading to B while maybe incorporating the effect of C as a confounder may not correctly reflect the systems we are operating with. Therefore, predictions of effect of risk mitigation may be wrong, wasted and misleading. In the more complex cases, multi-collinearity is often present and should not be ignored as it in fact may reveal the correct relationships to react on. This can be done by using explorative and analytic techniques such as adaptive Bayesian network analysis, factor analysis, advanced causal loop diagrams, and risk assessments. In all cases, uncertainty should be taken into account to help prepare for the unexpected.

Vulnerability and resilience in food safety may not be interpreted the same way all over the world due to different contexts related to diversities in risk perception, production systems, and economic abilities. Still, we may be able to learn from sharing results and experience - enabling identification of other ways of addressing a food safety threat than the one commonly used in an area or country. By the end of the day, this could result in acceptance of equivalence of approaches used in other countries easing trade for the benefit of consumers, producers and society as a whole.

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TRANSMISSION OF ANTIMICROBIAL RESISTANCE FROM PIGS TO HUMANS: TRUES AND LIES

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Through the years, use of antimicrobials in livestock has been the subject of an endless debate about the appropriateness of using these important medicines in the veterinary sector. This is a highly controversial topic involving ethical issues on animal welfare and human health, as well as economic interests by the pharmaceutical industry, the food industry and various professional categories, including farmers, veterinarians, pharmacists and researchers. As a consequence of all these factors, the debate has been often vigorous but not always scientifically unbiased. The aim of this lecture is to review the state-of-the-art on transmission of antimicrobial resistance (AMR) from pigs to humans with particular emphasis on specific risks that were overestimated by the scientific community in the past.

The human health risks associated with consumption of pork contaminated with resistant bacteria are highly dependent on the bacterial species and the type of AMR involved. Historically, the main risks have been associated with foodborne pathogens such as *Salmonella* and *Campylobacter*. However, the public health burden attributable to AMR in these species is limited, since infections are generally self-limiting and, in most cases, managed without antimicrobial therapy. Moreover, resistance to clinically-relevant antimicrobial agents is generally low among both porcine and human clinical isolates, although there are significant geographical differences.

Pigs are also a reservoir of other types of resistant bacteria that may colonize the human body without causing disease, transfer AMR genes to the commensal microbiota, and eventually cause opportunistic infections by taking advantage of specific host factors such as immunosuppression, altered microbiota or breached integumentary barriers. Vancomycin-resistant enterococci (VRE) belong to this category. The risk of foodborne transmission of VRE was highly debated during the 1990s in relation to the use of avoparcin in livestock, which was banned in the EU in 1997. However, the use of highly discriminatory typing methods based on DNA sequencing has shown that the VRE lineages causing infections in humans are not epidemiologically related to those occurring in pigs and other types of livestock. Moreover, the countries where VRE are a frequent cause of human infections (e.g. USA) have never used avoparcin in livestock but have extensively used vancomycin and other glycopeptides in human medicine, suggesting that human use of these antibiotics is the main driver for the spread of VRE.

In recent years, methicillin-resistant *Staphylococcus aureus* (MRSA) and extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli* are the most important multidrug-resistant bacteria that have emerged in pig production. These bacteria are, by definition, resistant to cephalosporins, which are first-line agents in the therapy of severe *E. coli* and *S. aureus* infections, and therefore resistance has a considerable impact on morbidity, mortality and healthcare costs. Human infections caused by LA-MRSA clearly originate from pigs but their public health impact is negligible in countries with high MRSA prevalence, and their widespread occurrence in countries with high pig production and low MRSA prevalence in the human population has not resulted in an increase of the overall mortality rate due to *S. aureus* infections, which is

mainly impacted by methicillin-susceptible strains. In these countries, the main impact of LA-MRSA is economic since their spread among farm workers has increased the costs associated with active surveillance and decolonization, and represents a potential threat to the sustainability of the national 'search and destroy' control policies.

Notably, the risk that ESBL-producing *E coli* are transmitted by food is higher than for LA-MRSA, since this route of transmission is unusual and largely unknown for *S. aureus*. Furthermore, while LA-MRSA have limited ability to transfer methicillin resistance to other human pathogenic *S. aureus* lineages, ESBL-encoding genes spread by horizontal transfer of plasmids that can readily be exchanged between *E. coli* of animal and human origins. Consequently, foodborne transmission of ESBL-producing *E coli* is more insidious and difficult to assess and control compared to LA-MRSA, and the actual burden of human infections attributable to ESBL-producing *E. coli* of animal origin remains poorly assessed. Based on recent whole genome sequencing studies, such burden appears to be smaller than previously predicted by the scientific community, as confirmed by the low prevalence of the predominant ESBL type in pigs (CTX-M-1) among human clinical isolates and by the structural differences observed between the plasmid vectors carrying this resistance determinant in human and pig populations within a defined geographical area. Various modelling studies have estimated that the contribution of pork to human exposure to ESBL-producing *E. coli* is significantly lower than for poultry meat and beef.

Other multidrug-resistant bacteria responsible for treatment failure and high mortality in human medicine, such as carbapenem- and colistin-resistant Enterobacteriaceae, are increasingly reported in livestock in some Asian countries but these bacteria are much less frequent in pig production systems in the rest of the world, and display significant differences in comparison with those isolated from human patients. In general, remarkable geographical differences exist in the prevalence of AMR resistance and such differences often, not always, reflect national patterns of antimicrobial usage. Accordingly, the type and extent of the interventions needed for prevention and control of AMR are not the same for all countries. Each country should define specific objectives based on the national context, and develop an adequate action plan that is able to accomplish them in line with the available resources. Establishing national One Health surveillance programs is a good starting point to understand the complex interactions between antimicrobial usage and the occurrence of AMR, but adequate resources should also be allocated to ensure efficient communication and education of antimicrobial prescribers and users, effective infection prevention, and optimization of antimicrobial use, which in turn requires investment for updating and implementing national guidelines on prudent antimicrobial use as well as for developing new medicines, diagnostic tools, vaccines and interventions.

THE USE OF RISK ASSESSMENT TO SUPPORT CONTROL OF *SALMONELLA* IN PORK

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Introduction

Salmonella is one of the major zoonotic foodborne pathogens worldwide and pork products have been identified among the main sources of salmonellosis in humans. In some countries, like Brazil, relatively high prevalences may be found in pig herds and among carcasses at slaughter (Schwarz et al. 2010). In Denmark, a contingency plan has been in place but nevertheless domestic pork is still estimated to be the most important food source for salmonellosis, as it is in the EU as a whole (Anonymous 2015). Therefore, there is a continued focus on the identification of effective intervention measures in the pig and pork production chain. In this keynote, an overview will be given of some research projects that have been performed to study the potentials of interventions in the pork production chain. The specific objective in these projects was to estimate their effectivity in terms of reduction of the public health risk of salmonellosis. The results illustrate how a quantitative microbiological risk assessments (QMRAs) approach can be applied to support the control of *Salmonella* in pork.

QMRA for *Salmonella* in pork

Quantitative microbiological risk assessment (QMRA) is an approach to integrate scientific data and knowledge with food processing information, in a transparent and logical structure. It offers a decision support tool for food safety risk managers by an assessment of the public health burden associated to microbial hazards and food products and a platform for comparative evaluation of different options for intervention and control. In so-called "farm to fork" risk assessments, the effectivity of intervention measures at the farm, during transport, slaughter and processing, and the stages beyond that, can be quantified and compared in terms of expected health risk reduction. This is particularly useful for a zoonotic disease like salmonellosis from pork, which may be transmitted from the life animal, via the production chain, to the consumer. Until now, several authors have published QMRAs related to *Salmonella* in pigs and pork (e.g. Bollaerts et al. 2009, Corbellini et al. 2017). A major one is the "farm to fork" risk assessment performed for EFSA (Snary et al. 2016), which is based on a combination of well-developed models for different parts of the pork production chain, applied to different EU Member States. From this QMRA, it is concluded that interventions are best focused on reducing the level of *Salmonella* in the feces, preventing fecal contamination of the carcass or on reducing the level of contamination at the end of processing.

The slaughter process

One challenge of a QMRA for *Salmonella* in pork is the description of the slaughter process of pigs, where the intestinal content of the animals is generally

considered the main source of carcass contamination. A quantitative description of this process is needed to translate prevalence and concentrations of *Salmonella* into exposure, and to assess the effects of interventions reducing them (Swart et al. 2016a). Here, a complication is that the *Salmonella* that ends up on the chilled carcass after slaughter can originate from the hide and from feces, and this feces can be of the animal itself and from other animals (Nauta et al. 2013). As shown by Smid et al. (2012), the house flora in slaughter environment is also an important source of *Salmonella*. Interestingly, even the meat inspection itself can contribute to cross-contamination between carcasses, be it with low levels of *Salmonella* (De Freitas Costa et al. 2016). Due to the variation in slaughter practices between slaughterhouses and the variation between pigs, the importance of the different sources and their impact on the contamination of the chilled carcasses is highly variable and hard to predict. Therefore, alternative approaches have been taken to study the impact of the slaughter process on pig carcass contamination and to assess the effects of potential interventions during this process.

The use of fecal indicators during slaughter

A Danish project (DECONT) aimed to study carcass contamination and the potential effectivity of decontamination of pig carcasses during slaughter. In this project, samples were taken from 2822 pigs in five different slaughterhouses and quantitative samples were taken for fecal indicator bacteria (*E. coli*) and *Salmonella*. The hypothesis was tested that *Salmonella* contamination of carcasses originates from the animal itself and could be predicted from the fecal carriage of *Salmonella* and the fecal contamination of carcasses, as predicted from *E. coli* data in animal feces and hygiene performance of the slaughterhouse. This hypothesis could not be confirmed (Nauta et al. 2013), which suggests that other factors than hygiene performance may affect the *Salmonella* status. Another indicator, *Enterobacteriaceae*, was used in Brazil (Corbellini et al. 2016). Although a statistically significant association between the log of the *Enterobacteriaceae* count and the *Salmonella* occurrence was found, the variation in *Salmonella* contamination per day was a dominant factor, which made the *Enterobacteriaceae* as indicator for hygienic failure unsuitable as indicator for *Salmonella* contamination.

The consumer phase

Another special challenge in "farm to fork" models is the consumer phase, where the consumers buy, transport, store and prepare their pork products. The transfer, growth and survival of *Salmonella* during this phase are difficult to predict due to a large variation of pork products, a large variation between consumers and scarcity of data. Also, consumers cannot be enforced to comply with regulatory hygiene standards or storage conditions. Yet, the consumer phase is the stage where exposure occurs and therefore it is of crucial importance for the assessment of the human health risk. Models targeted at specific products (like meatballs, pork cuts and fermented sausages) and specific populations have been developed (e.g. Møller et al. 2015, Swart et al. 2016b), but may not be generally applicable for all pork products consumed. Therefore, an alternative generic approach was developed that strongly simplifies the consumer phase and is based on an epidemiological estimate of incidence of salmonellosis (Duarte et al. 2016). It allows us to estimate the effects of interventions that modify the concentrations on the carcass in terms of relative risk reduction for the consumer.

Impacts on human health risk

Using this approach, Duarte et al. (2016) were able to assess the risk reducing effects of different experimental carcass decontamination scenarios in the DECONT project. An interesting finding was that it is important to not only estimate the mean effect of decontamination in terms of log reduction obtained, but that an estimate in the variation of that effect is at least as important. In general, a larger variation in the effect will lead to a reduced efficiency of carcass decontamination. Hence, the most effective decontamination strategy is not only effective in terms of mean log reduction, it also shows little variation in its effect, in particular for decontamination methods with mean effects around 1 log reduction, like lactic acid treatment. For heat treatment with mean log reductions between 2 and 3, the effect of variation was less pronounced.

Towards risk-based criteria

The same model principle was applied by Bollerslev et al. (2017a and 2017b), who studied the feasibility of using either enterococci or *E.coli* as an indicator for the presence of higher concentrations of *Salmonella* on pig meat. The prevalence of *Salmonella* was positively correlated to the concentration of the indicator. A positive association was also found between the concentration of *Salmonella* and the concentration of the indicator, but only for *Salmonella* positive samples. More specifically, the objective of these studies was to develop an approach that could make it possible to define microbiological limits for a bacterial indicator that is associated with an increased risk of salmonellosis, due to bacterial growth in the fresh meat chain or improper hygiene at the slaughterhouse. It was estimated that the majority of salmonellosis cases, caused by the consumption of pork in Denmark, is caused by the small fraction of pork products that has enterococci concentrations above 5 log CFU/g. The results obtained can be used to evaluate the potential effect of setting different microbiological limits on the risk of salmonellosis and consequently they may be used for the definition of a risk-based microbiological limit for enterococci and development of a process hygiene criterion in cutting plants and retail butcher shops. At slaughter, *Salmonella* may continuously be brought to the slaughter line by intestinal carriage of pigs. Therefore, the ability to control fecal contamination through good slaughter hygiene management is crucial. The risk model allows us to associate a hygiene level measured by *E. coli* to a possible *Salmonella* consumer risk. In this way a more risk-based approach for setting criteria in slaughterhouse HACCP programs has been developed.

Discussion

These results show that quantitative microbiological risk assessment allows an evaluation of the effect of control measures to reduce *Salmonella* in pork, in terms of reduced risk of salmonellosis. Its strengths are that it allows a transparent comparison of interventions and control measures in different parts of the chain, and can translate their effectivity in terms of relative risk reduction for the consumers. Both are of major importance for food safety risk managers. Challenges are the variation of processing practices and their potential effects on the transfer and survival of *Salmonella*, as well as the uncertain impact of the simplifying assumptions that have to be made when a QMRA model is constructed. Further research to meet these challenges will further strengthen the importance of QMRA as a tool to practically support decision making about microbial safety in the pork production chain.

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Epidemiology of foodborne pathogens and zoonotic diseases in the pork production chain

GENOMIC CHARACTERIZATION OF *STAPHYLOCOCCUS AUREUS* AT THE SWINE-HUMAN INTERFACE

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Background

The epidemiology of *S. aureus* in swine held little interest until the ST398 lineage of MRSA was found to be prevalent in pigs and pig farmers in the Netherlands in 2004 (Voss et al. 2005). ST398 MRSA have since been detected in multiple livestock species and in many countries (EFSA, 2009; Smith and Pearson, 2011), while genetically distinct variants of ST398 *S. aureus* occur in some human populations independent of livestock reservoirs (Carrel et al., 2017). Furthermore, other genotypes of MRSA can occur in pigs, particularly ST9 MRSA in Asia, and ST5 MRSA in North America (Chuang and Huang, 2015; Frana et al. 2013). In the USA, methicillin susceptible variants of the ST398, ST9 and ST5 lineages are widespread in commercial swine, yet MRSA variants appear to occur at relatively low prevalence (Sun, et al., 2015). Despite common exposure to, and colonization of, swine workers by livestock associated *S. aureus*, significant clinical infections appear to be uncommon in occupationally exposed people. However, invasive and even fatal infections are reported at relatively low incidence in some countries, and medically compromised people appear to be at particular risk, even in the absence of animal contact (Larsen et al., 2017). There is evidence that ST398 MRSA of livestock origin are less transmissible among humans than MRSA of human origin. Also, genomic studies typically have indicated that livestock associated MRSA (both ST398 and ST5) lack most virulence factors that occur in human clinical isolates (Schijffelen et al. 2010; Price et al. 2012; Hau et al, 2015). However, to date there has been little genomic characterization of methicillin susceptible *S. aureus* (MSSA) that are prevalent in swine populations. The purpose of this study was to describe the occurrence of virulence factors and antibiotic resistance genes in *S. aureus* isolates from pigs and swine veterinarians in the USA.

Material and methods

S. aureus isolates from growing pigs (n=30) or swine veterinarians (46) included ST9 (n=47), ST398 (19), ST5 (9), and ST72 (1). Isolates were analyzed using next generation sequencing and completed chromosome and plasmid sequences were annotated with gene and protein information using Prokka. Genes encoding virulence factors and antibiotic resistance were detected using only the filtered read-set and SRST2 (0.1.8) software based on published databases for sequence typing, virulence factors and antibiotic resistance genes, and genes detected were visualized using heat maps (presence/absence).

Results and discussion

No systematic differences were evident between the isolates from pigs and veterinarians, consistent with interspecies transmission without host adaptation. Among 173 putative virulence genes examined, 42 genes (including enterotoxins, Panton-Valentine leukocidin or toxic shock syndrome toxin genes) were not detected in any of the isolates. All isolates harbored 77 genes belonging to 6 functional groups with roles in cell attachment, iron regulation and cytotoxin production. The remaining 54 virulence genes were variably distributed and clustered by sequence type, with ST398 isolates having fewer virulence genes than ST9 and ST5 isolates. Twenty-four resistance genes were detected, also associated with sequence type. Overall, each MLST lineage carried distinct sets of putative virulence factors, and profiles of antibiotic resistance genes also differed among sequence types. However, apart from SCCmec elements including *mecA*, MRSA and MSSA isolates displayed similar profiles within MLST lineages.

Conclusions

The findings indicate that these 3 lineages that are prevalent in swine in the USA have evolved somewhat independently in this species, suggesting a limited effect of horizontal gene transfer of virulence genes and antibiotic resistance among different sequence types.

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TAENIA SOLIUM CYSTICERCOSIS IN THE UNPROCESSED PORK SUPPLY CHAIN IN NAIROBI AND ENVIRONS, KENYA

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The zoonotic parasite, Taenia solium, is a serious public health threat in countries where it is endemic. The larval stage of this parasite is responsible for porcine cysticercosis and neurocysticercosis in humans, which is one of the leading causes of seizures and epilepsy in developing countries. While documented studies have only been conducted in western areas of Kenya, other areas, including Nairobi, have not been investigated to fully understand the epidemiology of the parasite. Seven hundred blood samples were collected from randomly selected pigs presented for slaughter at the largest porcine abattoir supplying unprocessed pork to butcheries within Nairobi city and its surroundings. The samples were tested using an antigen ELISA to determine the prevalence of cysticercosis. Information regarding the pigs' age, sex and source was obtained from the traders and pork destinations recorded.

The abattoir received pigs from eleven different counties during the study period. Cysticercosis was detected in pigs from 7 of these counties, with adjusted (for diagnostic sensitivity/specificity) sero-prevalence ranging from 2.6% to 29.1%. The overall adjusted sero-prevalence was estimated as 5.9% (95% CI: 3.9-8.3). Post-mortem inspection by incision and palpation conducted by the government meat inspectors did not detect cysticercosis or any other condition that would have warranted condemnation during the study duration. Therefore, all the carcasses suspected to contain infective T.solium cysts based on the Ag- ELISA either entered into the food chains of Nairobi (70%), or Nairobi's neighboring counties (30%).

The detection of Sero-positive pigs in 7 out of the 11 counties is an indication that cysticercosis may be widespread in Kenya. Risk mapping is recommended to identify high-risk counties and consequently risk based meat inspection instituted by strengthening of routine meat inspection through periodic carcass dissection and serological methods. A comprehensive One Health approach control strategy is recommended.

DIVERSITY OF *YERSINIA ENTEROCOLITICA* POPULATION IN A SLAUGHTERHOUSE BETWEEN 2009 AND 2010 AND DISCRIMINATION ABILITY OF MLVA COMPARED TO PFGE

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Abstract

Yersiniosis is a human disease mainly due to the ingestion of raw or undercooked pork meat contaminated mostly with *Yersinia enterocolitica* (*Ye*). In France, 74.3% of pig batches at slaughterhouses carried pathogenic *Ye*. Among them, biotype 4 (BT4) and biotype 3 (BT3) were often recovered. PFGE is one of the most used methods to type *Ye*, with the restriction enzymes, *Xba*I and *Not*I. Nevertheless, MLVA method based on the diversity of six loci tends to replace PFGE; this method showed a higher discriminatory power in others studies. We investigated the genetic diversity of *Ye* strains isolated in 2009 and in 2010 in one pig slaughterhouse in France and compared the ability of MLVA and PFGE to discriminate the strains.

During these two years, 335 isolates were collected from pigs. The BT4 represented 88.4% of the strains (296/335) and the BT3 only 11.6% (39/335). PFGE using *Xba*I enzyme allowed the identification of 12 *Xba*I-PFGE types and among them only one was common to the both surveys. Because the Simpson's Index shows a low genetic diversity 31 BT4 strains and 39 BT3 strains were typed using MLVA. For BT3 strains, MLVA had the same index of diversity than PFGE (DI=0.472). In contrary, the index of diversity was significantly higher with MLVA (DI=0.871) than with PFGE (DI=0.665) for BT4 strains.

Our study revealed that the population of *Ye* in pig varied over the time. The comparison of the both typing methods indicated that MLVA has a better discriminatory power than *Xba*I-PFGE method for BT4 strains but not for BT3 strains.

Introduction

Yersinia enterocolitica is one of the most relevant biological hazards in the European Union, with yersiniosis being the third most frequently reported foodborne bacterial zoonosis after campylobacteriosis and salmonellosis. The average incidence of human yersiniosis in the European Union in 2015 was 2.2 cases per 100,000 populations (EFSA and ECDC 2016). Yersiniosis cases reported in 2015 in the European Union were mostly due to *Y. enterocolitica*, and especially to biotype 4 (BT4) (EFSA and ECDC 2016). In France most of the clinical cases are largely due to BT4 strains (Le Guern et al. 2016).

Pigs are considered to be the most important reservoir of *Y. enterocolitica*. This species can be divided into six biotypes and two groups according to their pathogenicity, biotype (BT) 1A which one is considered as "nonpathogenic", and BT1B, BT2, BT3, BT4 and BT5 that are known to be pathogenic. To better appreciate the risk that these strains represent to humans, it is very important to characterize them.

Currently, to study the genetic diversity of *Y. enterocolitica*, the most common genotyping method used to discriminate between *Y. enterocolitica* strains is Restriction Fragment Length Polymorphism-Pulsed-Field Gel Electrophoresis (RFLP-PFGE). In other studies, many different restriction enzymes have been used. The most frequent restriction enzymes are *Not*I and *Xba*I (Fredriksson-Ahomaa, Stolle, and Korkeala 2006). Researchers have observed limited diversity among BT4, even among strains of different geographical origin with RFLP-PFGE typing (Fredriksson-Ahomaa et al. 2003; Fredriksson-Ahomaa et al. 2006). That's why other typing method more discriminatory are needed

In this context, the MultiLocus Variable-number tandem repeats (VNTR) Analysis (MLVA) have been developed. This method is increasingly used in subtyping *Y. enterocolitica* strains. This technique consists of the detection of six VNTRs. Several studies showed a high discriminatory power of the MLVA methods (Gierczynski et al. 2007; Sihvonen et al. 2011).

The aim of the study was to evaluate the diversity of *Y. enterocolitica* strains isolated from two different years in one pig slaughterhouse and to evaluate the two typing method *Xba*I-RFLP-PFGE and MLVA.

Materiel and methods

Bacterial strains and biotype

Strains used in this study were collected in one French pig's slaughterhouses during two surveys. A total of 304 strains were isolated in 2009 (Fondrevez et al. 2010) and 31 in 2010-2011 (Fondrevez et al. 2014). The biochemical assays used to biotype *Yersinia enterocolitica* strains was carried out as described in the ISO 10273:2003 method.

Restriction fragment length polymorphism-pulsed-field gel electrophoresis (RFLP-PFGE)

RFLP-PFGE was done using *Xba*I (XbaI-PFGE) restriction enzymes (Roche, Boulogne-Billancourt, France). Strains were sub-cultured on Plate Count Agar (PCA) at 30°C for 24h. Bacterial suspension in TE buffer (0.01 M Tris-EDTA buffer, pH 8.0) were adjusted to an optical density (600 nm) of 1.5 and mixed with 1% agarose for the plug preparation. Plugs were incubated for 48h at 50°C in lysis solution (Na2EDTA 0,5M, pH9, N-lauryl-Sarcosyl 1%, proteinase K 1 mg/ml). A total of five washes with TE buffer were used to remove excess reagents and DNA was then digested with 40U of *Xba*I at 37°C for 4 hours. The electrophoresis conditions had an initial switch time of 1.5s, with final switch time of 18.0s, for 25h. Electrophoretic patterns were compared using BioNumerics software (Applied Math, Sint-Martens-Latem, Belgium). The Simpson's index (DI) was determined as described by Hunter and Gaston (1988) to assess the diversity of the populations.

Multi-locus variable number tandem repeat (MLVA)

MLVA was performed using the technique developed by Gierczynski et al. (2007) and improved by Sihvonen et al. (2011). Six Variable Number Tandem Repeat (VNTR) were considered; they are coded V2A, V4, V5, V6, V7 and V9. We used the same

primers as Gierczynski et al. (2007). The six VNTR were amplified in two distinct multiplex PCRs. The first one amplified the VNTRs V2A, V4 and V6 with the forward primers labelled respectively by 6-FAM, HEX and Cy3 fluorescent Dye. The second amplified the VNTRs V5, V7 and V9 with the forward primers labelled respectively by 6-FAM, HEX and Cy3 fluorescent Dye.

The multiplex PCRs were performed with QIAGEN Multiplex PCR kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions in a total volume of 25 µl. The PCR conditions were the same as those described by Sihvonen et al. (2011). The two PCR products of each strain were diluted to 1/100 in sterile water, and run separately in a capillary electrophoresis with an ABI 3130 DNA Analyzer (Applied Biosystems, Foster City, CA) using D (DS-30) fragment analysis chemistry according to the manufacturer's instructions. The Geneflo™ 625 ROX labelled (EurX, Gdańsk, Poland) was used as an internal size standard. Electrophoretic patterns were analyzed using BioNumerics 7.6 software (Applied Math, Sint-Martens-Latem, Belgium). The Simpson's index (DI) was determined to assess the diversity of the populations.

Results

Among the 335 *Y. enterocolitica* strains isolated from one french slaughterhouse, the majority of the strains were from BT4 (88.4%). BT3 was less abundant (11.6%) and was only recovered in 2009 probably because there were many more strains isolated in 2009 (304 strains) than in 2010 (31 strains) in this slaughterhouse.

The 335 strains were distributed in 12 *Xba*I-PFGE profiles coded X1 to X12 with a DI of 0.647. We obtained eight *Xba*I-PFGE profiles (X1 to X8) in 2009 and five in 2010 (X1 and X9 to X12) with only X1 common over the two years (Figure1).

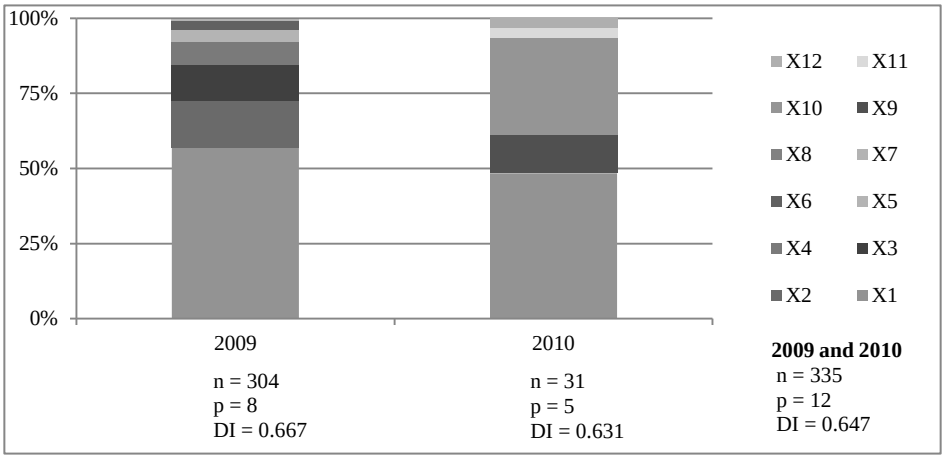

Figure 1. XbaI-PFGE profile (X1 to X12) distribution between the two years. The Simpson's Index (DI) and the number of *Xba*I-PFGE profile (p) and the number of strains (n) are indicated for 2009, 2010 and for the two years.

The *Xba*I-PFGE shows also a low discriminatory power among years with a DI of 0.661 in 2009 and 0.631 in 2010. Moreover, BT4 and BT3 strains showed three similar profiles (X3, X8 and X10). So another typing method is necessary.

We evaluated the discriminatory power of MLVA compared to PFGE on 70 strains including 31 BT4 strains and 39 BT3 strains. We obtained a total of 16 MLVA

genotypes, and no one was common between BT4 and BT3. The BT4 strains were distributed in 13 MLVA genotypes (M1 to M13) and the BT3 strains were distributed in three MLVA genotypes (coded M14 to M16), (Figure 2).

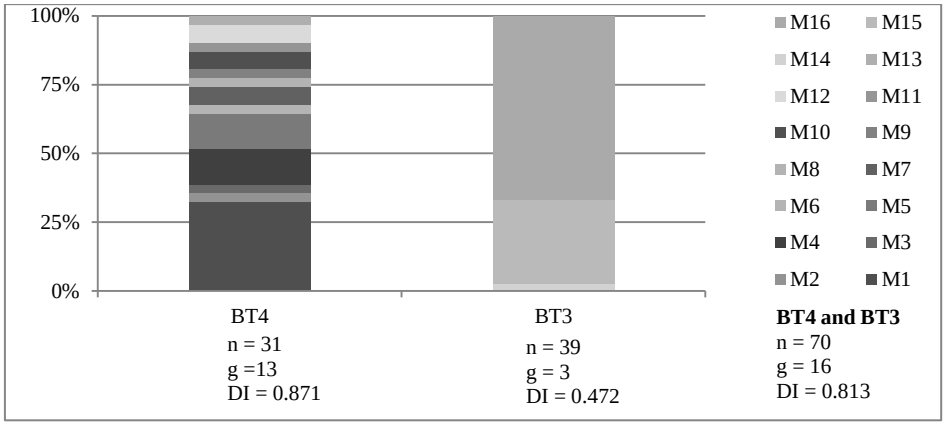

Figure 2. MLVA genotype (M1 to M16) distribution among BT4 strains isolated in 2010 and among BT3 strains isolated in 2009. The Simpson's Index (DI) and the number of strains (n) and MLVA genotype (g) are indicated for BT4, BT3 and the combination BT4 and BT3.

The MLVA compared to *Xba*I-PFGE on 70 strains presents a significantly higher DI which is respectively 0.813 and 0.647 (Table 1). For the BT4 strain the DI was equal to 0.871 and significantly higher than the DI obtained from PFGE (DI=0.665). For the BT3 strains the DI was identical for the two methods (DI=0.472).

Table 1. Simpson's index (DI) of the RFLP-PFGE and MLVA on the 70 strains studied in MLVA, and the confidence interval at 95% ($CI^{95\%}$).

	Number of strains	RFLP-PFGE		MLVA	
		DI	$CI^{95\%}$	DI	$CI^{95\%}$
Total strains	70	0.647	[0.596 ; 0.699]	0.813	[0.745 ; 0.881]
BT4	31	0.665	[0.559 ; 0.770]	0.871	[0.785 ; 0.957]
BT3	39	0.472	[0.354 ; 0.591]	0.472	[0.354 ; 0.591]

Discussion

This study showed that BT4 strains are mainly recovered in pig slaughterhouses, as in many other studies (Van Damme, Habib, and De Zutter 2010). The RFLP-PFGE analysis revealed the presence of an identical *XbaI*-PFGE profile over the two years. This profile is perhaps strongly associated with the French pork industry. The others PFGE profiles were different over the two years suggesting that the population colonizing the pig seems to be changing over time.

This study revealed that MLVA shows a better discriminatory power than RFLP-PFGE for the BT4 strains. This result is in accordance to those of Sihvonen et al. (2011). MLVA typing methods seems to be a better tool for discriminatory of *Y. enterocolitica* BT4 than RFLP-PFGE using *Xba*I enzyme. For the BT3, we observed that MLVA and RFLP-PFGE had exactly the same discriminatory power. This result is not in agreement with a study of Wang et al. (2012) that revealed in MLVA a great

heterogeneity between BT3 strains isolated during a 20 year period. Our study was carried out on 39 strains isolated during the year 2009 which may explain our result.

On the other hand, we used only one enzyme (*Xba*I). It would be interesting to compare our MLVA results with the combination of RFLP-PFGE profiles issued from digestion with other restriction enzyme, which may give a better discriminatory power (Fredriksson-Ahomaa, Stolle, and Korkeala 2006)

Conclusion

This study revealed that the population of *Y. enterocolitica* colonizing the pig seems to be changing over time. It also showed that MLVA typing present a better discriminatory power than RFLP-PFGE using *Xba*I restriction enzyme for BT4 strains.

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NATIONAL PREVALENCE OF *SALMONELLA* SPP. IN PORK SLAUGHTERHOUSES UNDER FEDERAL INSPECTION IN BRAZIL, 2014/2015

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Introduction

Meat consumption is frequently associated with foodborne diseases. The onset of such occurrences may be due to failures on animal health surveillance or during meat processing. Many efforts are made in the industry to minimize any kind of meat contamination which can affect human health. *Salmonella* has an important role in the public health and economy, once it is recognized as one of the most important zoonosis (Valero *et al*., 2014). In Brazil, 90% of reported cases involving foodborne pathogens, in which the pathogens were identified, from 2007 to 2016, were caused by *Salmonella* spp. (Brazil, 2016).

Brazilian pork meat has an important representativeness in the global market, since the country is currently the 4th largest pork producer and exporter in the world (ABPA, 2015). In 2014 and 2015 the official veterinary service, under the Ministry of Agriculture, Livestock and Food Supply (MAPA), conducted a national baseline survey, sampling establishments under Federal Inspection Service (SIF) to estimate the prevalence of *Salmonella* spp. on pork carcasses at the pre-chilling and at the post-chilling phases (Brazil, 2014).

The present study aims to estimate the prevalence of *Salmonella* spp. in Brazil during the years of 2014 and 2015 by quantifying levels of the pathogen in pork carcasses and by identifying which sizes of establishments were the most involved in the contamination through data analysis.

Material and methods

In order to estimate the prevalence in slaughterhouses, a two-level sampling (establishments and carcasses) was used, in which their respective sample weights were implemented to increase the external validity of the data. For the definition of the sampling plan, abattoirs under federal inspection had the following classification according to their slaughter capacity: Small (S), for up to 200 pigs slaughtered per day; Medium (M), for 201 to 700 pigs slaughtered per day; Large (L), for 701 to 1,800 pigs slaughtered per day and Very Large (VL), for 1,801 or more slaughtered pigs per day. The purpose of the classification of establishments was to have a proportionality between the number of samples to be collected in relation to the magnitude of the production, which were: S = 4; M = 8; L = 12 and VL = 16. Two samples were collected after random selection of two half-carcasses from 76 slaughterhouses, one before chilling (BC) and another 12 hours after chilling (AC). Samples were collected on carcasses using swabs, aseptically, in a total area of 400 cm², using sponges at standard points such as belly, jowl, ham and loin (Brazil, 2014).

The study was conducted from October/2014 to June/2015. During the sample collection period there were approximately 128 establishments slaughtering over 32 million pigs.

A total of 1.487 samples were analyzed for *Salmonella* spp. in official laboratories (LANAGRO) using ISO methodology (ISO, 2002).

Data were stored in spreadsheets and georeferencing of the establishments was performed through the TerraView 4.2.1 program (São José dos Campos, SP: INPE, 2012), after verification and adjustments. Moreover, the establishments were labeled according to their respective markets, in which they could be exporters or not. The program used for analysis was Stata 12.0 (Stata Statistical Software: Release 12. College Station, TX: StataCorp LP).

Results and discussion

The results obtained were 10.00% (CI 7.50-13.22) of BC carcasses positive for *Salmonella* spp.. Establishments classified by size as M were the major responsible ones, with a prevalence of 18.51% (CI 9.27-33.56) and there was a marginally significant difference ($p = 0.051$) between this category of abattoirs in relation to the others, regarding positivity (BC). Establishments limited for national market (NM) had an observed prevalence of 17.43% (CI 12.00-24.63), while in those qualified for international market (IM) the prevalence was 9.05% (CI 6.39-12.66).

For the AC carcasses, the prevalence was 4.58% (CI 3.13- 6.65) and there was not a statistical significant difference of positiviness between the other categories. In establishments for NM the prevalence was 12.25% (CI 7.75-18.81) and for IM was 3.57% (CI 2.15-5.89).

According to (Arguello *et al*., 2012), *Salmonella* contamination is particularly higher in many points of the slaughtering process in the pork production chain in Spain, including the conditions of animal transportation, holding pens, and several points of the slaughtering line, due to the high number of animals raised in different systems and regions. A Brazilian study in Santa Catarina state has shown similar results about the chance of *Salmonella* positiveness in the slaughterhouses being bigger, it is relative to the finishing step that is responsible for enhancing *Salmonella*-transmission and the high number of carries responsible for the delivery of pig batches to slaughterhouses (Kich *et al*., 2011).

The georeferencing allowed a better visualization of the distribution of these establishments in Brazil (Figure 1), strengthening the need of actions regarding epidemiological surveillance. In Brazil, 51.56% (66/128) of pork slaughterhouses under federal inspection commercialize their products only to national market (NM) and 48.44% (62/128) are approved by MAPA to national and international market (IM), attending several countries in the world.

Conclusion

It was possible to establish the association between sample positivity and the geographical location of the occurrence, as well as the size of the abattoirs with their respective markets served. The determination of this scenario allows MAPA to perform actions aimed at risk mitigation with a greater assertiveness.

The information and knowledge acquired may support further investigations and evaluation of surveillance programs developed by the official veterinary service to guarantee food safety.

Figure 1. Spatial distribution of pork slaughterhouses under federal inspection in Brazil. Blue: Internal market establishments; Red: External market establishments.

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PREVALENCE AND ASSOCIATED RISK FACTORS OF SALMONELLA SPP. ON THE PORK PRODUCTION CHAIN IN CÓRDOBA, ARGENTINA

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Abstract

Salmonella is considered a major pathogen in Public Health. Córdoba is an important region for the production of pork. In this study we estimated the prevalence and risk factors associated with *Salmonella* at different stages of pig production chain (farms, slaughterhouses and pork sausages industries). The observed prevalence of farms in this study was higher than reported by EU. The main Risk Factors could be linked to the lack of good manufacturing practices in the whole pork chain in the region.

Introduction

Salmonella is considered a major pathogen in Public Health. Pork and its meat products were identified as an important source for salmonellosis in humans (Pires *et al*., 2011). Córdoba is an important region for the pork production chain in Argentina, representing 24% of the country's total pork production (INTA, 2015). Most of the pork meat is destined to pork products, with fresh pork sausage (here so called "chorizo") being the main selling product. In Argentina, slaughterhouses and pork sausages industries are classified in different categories based on differences in hygienic-sanitary requirements (hereinafter referred to as National, Provincial and Municipal categories). A slaughterhouse or pork sausages industry with a National category are able to sale their meat products among the whole country, while those with Provincial or Municipal category can only sale their products inside their jurisdiction. That implies that different governmental authorities are the responsible for applying control and monitoring programs, resulting in diverse criteria for hygienic-sanitary standards. The aim of this study was to estimate the *Salmonella* prevalence and risk factors associated with the different stages of pork production chain, including farm, slaughterhouse and "chorizo" at retail markets. This is the first study among the whole pork production chain in Córdoba.

Materials and methods

This study was carried out from July of 2013 to June of 2017. 580 samples of pig mesenteric lymph nodes (MNL) from different farms were collected. Moreover, 300 samples of environmental and carcasses swabs were obtained from 4 slaughterhouses (2 National and 2 Provincial; in Córdoba there is not municipal slaughterhouses) and 655 samples from sausages ("chorizo") from different retailers were analyzed according to ISO 6579:2002 to estimate the prevalence of *Salmonella* spp.

For the assessment of the associated risk factors linked to *Salmonella* prevalence, an epidemiological questionnaire was carried out in farms. For this purpose, a hygienic-sanitary checklist for slaughterhouses and pork sausages industries were used. All data were corrected and categorical variables were created for farm questionnaires and checklist. SPSS was used for data analysis, crosstabs analysis was chosen for Risk Factors estimations.

Results

The observed prevalence of *Salmonella* at different stages of pork production chain in Córdoba is shown in Table 1.

Two variables related to pre-slaughter practices were associated with infection of *Salmonella* at farms: distance from farm to slaughterhouses ($p<0.01$) and lairage time in pens ($p<0.01$). Moreover, slaughterhouses with "Provincial" category and sausages factories with Provincial and Municipal categories were also associated with higher prevalence of *Salmonella* (p=0.02, p=0.02 and p<0.01 respectively) (Table 2).

Table 1. *Salmonella* prevalence among the pork production chain in the region.

Stage	Prevalence (%)	95%CI
Farms	41.55	37.54-45.56
Slaughterhouse	23.70	17.36-30.04
Pork Sausage (“chorizo”)	22.50	19.16-25.84

Table 2. Main Risk Factors associated with *Salmonella* prevalence at different stages on pork production chain.

Stage	Variable	OR	95%CI	*p*
Farm	Distance from farm to slaughterhouse			
	< 120 km	1		
	> 120 km	5.93	3.35-10.5	0.01
	Lairage			
	< 12 horas	1		
	> 12 horas	2,24	1.36-3.68	<0.01
Slaughterhouse	Category			
	National	1		
	Provincial	1.87	1.04-4.16	0.02
Pork Sausage (Chorizo)	Category			
	National	1		
	Provincial	3.06	1.74-5.44	0.02
	Municipal	3.22	1.84-5.76	<0.01

Discussion

There are few studies reporting *Salmonella* prevalence in our country, so the importance of this pathogen in the region is not well established. The observed prevalence in Córdoba would be comparable to a similar study carried out in other provinces of Argentina (44.1%) in which 193 MLN samples were analyzed (Ibar *et al.*, 2009). Furthermore, in another study, a *Salmonella* cluster was detected in pig farms located in the southern region of Córdoba (Parada *et al.*, 2017). This epidemiological study shows that the prevalence of *Salmonella* is closely related to several factors, mainly associated with inadequate pre-slaughter practices at the slaughterhouse and with biosafety practices at the farms. Moreover, other risk factors associated with farms like the absence of rodent control, and low frequency of silo cleaning was significantly related to deficiencies in good manufacturing practices on farms (Sánchez I., 2016).

To our knowledge, there are no official reported data about the prevalence of *Salmonella* at slaughterhouses or pork products at retail markets in the country, so this may be the first epidemiological study among the whole pork production chain in the region. When our results were compared with studies from other countries, the *Salmonella* prevalence at slaughterhouses was similar that observed in Vietnam (22%) but higher than reported in Naples (15.9%), Parma (10.9%) and Rio de Janeiro (10.5%) (Yokomo *et al*., 2016; Piras *et al*., 2014; Bonardi *et al*., 2013; Cabral *et al*., 2016). The *Salmonella* prevalence in pork sausage ("chorizo") at retail markets was consistent to other study in Brazil, with a similar pork production chain (26%) (Cabral *et al*., 2014).

Slaughterhouses and pork industries included in the "Provincial" category had higher risk of isolate *Salmonella* positive samples (OR=1.87 [95%IC=1.04-4.16] and OR=3.06 [95%IC=1.16-15.40] respectively). The provincial category may be related with the lack of adequate sanitary standards, less official controls and inadequate microbiological controls compared to those with national category. Other factors in this study, like the absence of microbiological monitoring for *Salmonella* or the inefficient implementation of plague control at Provincial slaughterhouses seems to be linked with the higher prevalence observed.

These results highlighted the need to improve the hygienic-sanitary requirements through the whole pork production chain in Córdoba, Argentina, to reduce *Salmonella* risk to Public Health.

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DYNAMIC OF EXCRETION AND IMMUNE RESPONSE OF EXPERIMENTALLY INFECTED PIGS WITH MONOPHASIC VARIANT OF *SALMONELLA* TYPHIMURIUM SEROVAR 1,4[5], 12:i:-

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Key words: monophasic *S.* Typhimurium, pigs, excretion, immunity, antibodies, interferon.

Introduction

In recent years *Salmonella* enterica serovar Typhimurium 1,4[5],12:i:- (monophasic variant of *S.* Typhimurium or vmST) was focused as a major zoonotic problem because produce human gastroenteritis outbreaks in many countries. Human disease caused by this serovar is considered as emergent in European Union countries and actually is ranked as the third most reported serotype after *S.* Enteritidis and *S.* Typhimurium (EFSA, 2016; Fernandes et al., 2016).

Swine (pig and pork products) was reported as reservoir of vmST. In pig production systems, *Salmonella* colonization in the pig intestine, and subsequent excretion, determine the possible contamination of the meat for human consumption. Extensive information about colonization and immune response in pigs are available for *S.* Typhimurium but not for vmST.

Thus, the aim of this work was to study the dynamic of excretion and immunelogical response of pigs after inoculation with a vmST, using an experimental Specific Pathogen Free (SPF) pig model. Excretion was followed twice a week by numeration of *Salmonella.* Moreover, from blood serum, the antibody presence was measured by ELISA assay to assess the humoral response in pigs (Osterberg & Wallgren, 2008) and the mesure of interferon Gamma (INF-γ) was used as marker of innate immunity for *Salmonella* infection (Huang et al., 2011; Uthe et al., 2009).

Materials and methods

The trial was conducted on 32 SPF piglets, born into ANSES Ploufragan protectted animal house. Piglets aged of 5 weeks were placed in hermetic and separated experimental animal houses. Inoculated experimental herd of 24 piglets was placed in three separate rooms (8 piglets per room), each one containing 2 pens. Four piglets were placed in each pen. The three groups of 8 pigs (G1, G2 and G3) were slaughtered at different ages, were followed respectively during 21, 49 and 84 days post inoculation (p.i.). Control herd of 8 pigs was kept in 2 separate rooms.

Pigs were monitored daily for surveillance of clinical manifestations and body temperature control. Food consumption and body weight were reported weekly.

A field strain of vmST was used for the experiment. In order to easily enumerate the strain in fecal samples, it was transformed in strain resistant to Rifampicin (SRif). At seven weeks of age, piglets were orally inoculated with 10 ml of a solution containing a SRif bacterial solution of 10^8 CFU per ml. Control herd received only 10 ml of Tryptone Salt Broth. Fecal samples were taken twice a week, directly from the animal rectum. Blood samples were taken from the jugular vein once a week, beginning at day 7 p.i. for G3 and at day 3 p.i. for G1 and G2.

Fecal samples were diluted 1: 10 with Buffered Peptone Water (BPW). 5 ml of this dilution (10^{-1}) were taken and serials dilutions were performed in Salt Tryptone Broth tubes until dilution 10-^{4}. 1 ml of 10^{-1} dilution was seeded in 3 plates of Xylose Lysine Desoxicholate agar supplemented with Rifampicin (XLD+Rif). 100 µl of dilutions 10-^{2} and 10-^{4} were also plated in XLD+ Rif. Plates were incubated at 37 ° C for 24 hours. Typical black colonies of *Salmonella* were counted and expressed in Log10 CFU/g. When enumeration was negative, a *Salmonella* detection protocol was performed following NF-U47-102.

For antibody screening, IDEXX Swine *Salmonella* Ab Test® (IDDEX, Montpellier, France) was used. Samples with OD% values equal to or greater than 15 % (S/P=0.375) were considered as positive.

Interferon γ response was measured in serum samples, using Porcine IFN γ ELISA Kit ® (Thermo Fisher Scientific, Villebon-sur-Yvette, France), according to the manufacturer instruction. Results were expressed in pg/ml and they were compared with LLD (Lower Limit of Detection) calculated for each plaque.

Pigs were euthanized and autopsied at 21 days (8 inoculated pigs and 2 controls), at 49 days (8 inoculated pigs and 2 controls) and 84 days (8 inoculated pigs and 4 controls). We took in aseptic conditions tonsils, mesenteric lymph nodes, and different parts of the intestinal content: duodenum, jejunum, ileum and caecum. All samples were analyzed following the microbiological protocol describe above for the feces.

Statistical analysis

To establish the excretion kinetic we compared the excretion levels of pigs each day after inoculation (24 pigs during 21 days, 16 pigs during 49 days and 8 pigs during 84 days). Area Under Curve (AUC) was measured for individual excretion for all pigs until day 21 post inoculation (p.i.). AUC obtained were compared with non-parametric method (Kruskal-Wallis test). Pearson's correlations ($p<0, 05$) were performed between the results of pigs excretion and the antibody and interferon response.

Results

Excretion in feces

All groups of inoculated pigs had shed vmST continuously during respectively 21, 49 and 84 days p.i. with daily variations of excretion (Figure 1).

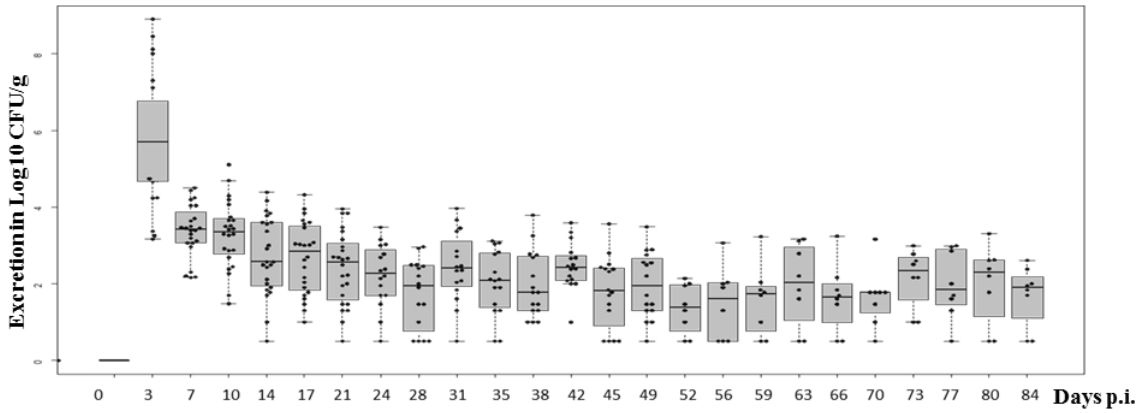

Figure 1. Blox pot representing the excretion kinetic for all pigs during 84 days (in Log10 CFU/g).

After a peak of excretion just post inoculation (5.8 ± 1.6 log10 CFU / g at day 3), the mean amount excreted decreased significantly (2.4 log 10CFU/g at day 7). The lower amount was detected at day 53 (1.4 ± 0.7 log10 CFU / g).

Comparison of AUC (Area under curve) measured for each pigs using Kruskal-Wallis test did not show a significant difference between the 32 pigs ($p > 0.05$) for excretion in feces from day 0 to day 21. Inside G1 and G2 we did not find significant differences between pigs ($p => 0.05$). However in G3, we found significant difference in pig's excretion levels ($p < 0.001$). Control group remained negative for *Salmonella* during all the trial.

Immune response

Kinetics of individual seroconversion

Antibody response started at day 7 p.i. with variability among experimental groups and pigs. 100% of pigs from Group 2 and 3 were seroconverted at day 49 p.i. and remained positives until the end of experiment (Figure 2). A positive correlation was found between excretion of vmST in feces and serological results with Pearson test p=-0.29

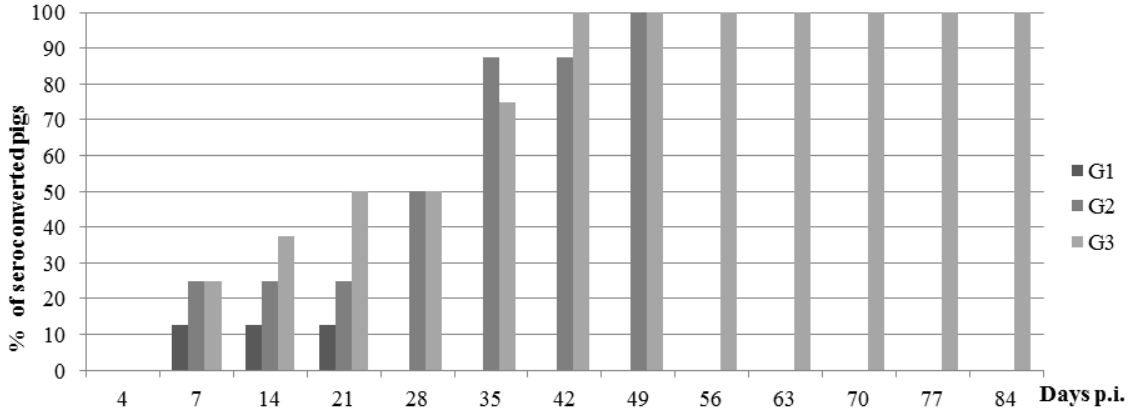

Figure 2. Frequency (%) of seropositive pigs during 84 days.

Interferon γ level in serum

50 % of pigs showed IFN γ levels higher than 50 pg/ ml at day 3 p.i. (Figure 3). These levels decreased at day 7, and became lower than 15 pg /ml in 80% of pigs. At day 14, IFN γ were not detectable. Positive correlation between IFN γ and excretion levels was found at day 3.

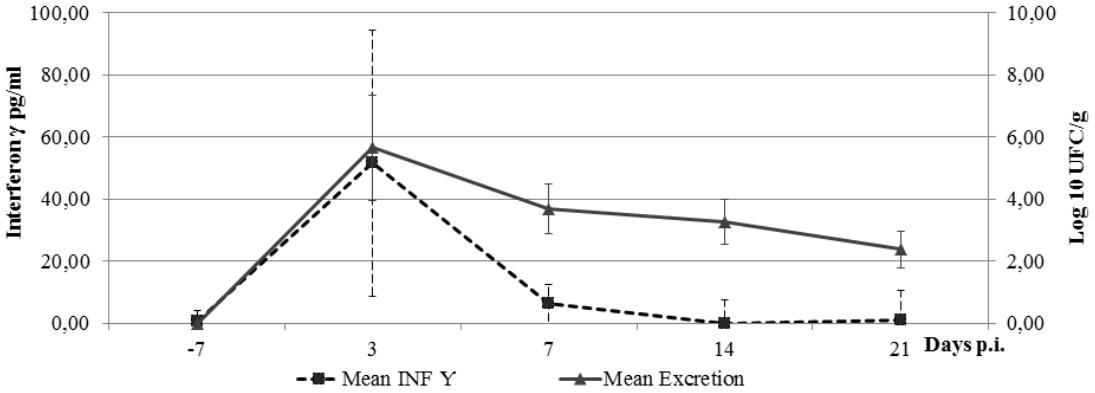

Figure 3. Interferon γ average and excretion levels in pigs artificially contaminated with *Salmonella* during 21 days. (Group 1 and 2).

Conclusion

To our knowledge this work is the first description of excretion dynamic and immunological response of experimental pigs after infection with a monophasic variant of *Salmonella* Typhimurium strain. Fecal shedding of the 24 pigs used was persistent and continuous during 84 days. At the autopsies, the highest contamination was evidenced in tonsils and the lowest in mesenteric lymphatic nodes, for all pigs of the three experimental groups. Concerning antibody response, seroconversion begins at day 7, and all pigs followed during 49 and 84 days seroconverted at day 49 post-inoculation. The highest levels of INF-γ were highlighted 3 days after inoculation with the monophasic variant of *S.* Typhimurium.

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EVALUATION OF THE COLONIZING ABILITY ON IPEC-J2 CELLS AND OF THE PATHOGENICITY ON CACO-2 CELLS OF THE 3 MAJOR FRENCH PIG *SALMONELLA* SEROVARS

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Key words: Salmonella, in vitro assays, colonization, IPEC-J2, pathogenicity, Caco-2.

Introduction

Salmonella enterica subspecies *enterica* is recognized as the etiological agent of Salmonellosis, a zoonotic disease transmitted in humans through contaminated food. In 2015, *Salmonella s. p.* was reported as the second bacterial agent responsible of gastro-enteritis in the European Union (EFSA, 2016). Pig consumption is considered as a major source of human *Salmonella* infections (Bonardi, 2017). Salmonella subspecies can be divided in 1530 serovars based on different epitopes and surfaces antigens. Associations exist between serovars and livestopchk species. In Pigs, in France, *Salmonella* Typhimurium, Derby and recently the monophasic variant of *S.* Typhimurium (vmST) are the most frequently isolated serovars (Denis *et al.*, 2013; Kerouanton et al., 2013). The presence of monophasic variant of *S.* Typhimurium is also described in pigs in Europe (Bonardi et al. 2016).

To assess their colonization ability in pigs and their pathogenicity in humans, we tested *in vitro* a panel of 15 strains of these three serovars on respectively non-transformed cell line IPEC-J2 derived from porcine jejunal epithelial (Shierack et al., 2006) and human colon adenocarcinoma Caco-2 cells.

Materials and methods

Strains

Five strains per serovar have been studied. The have been isolated from pigs between 2007 and 2014, from feces, lymph nodes or carcass swabs (Table 1).

Table 1. *Salmonella* strains used for the study.

Serovar	Strain number	Isolation year	Origin
S. Derby	07CR553	2007	Lymph nodes of conventional slaughtered pig
	07CR179	2007	Lymph nodes of conventional slaughtered pig
	07CR223	2007	Lymph nodes of conventional slaughtered pig
	S12AK059	2012	Carcass swab of conventional slaughtered pig
	S12AK118	2012	Fecal sample of conventional slaughtered pig

Serovar	Strain number	Isolation year	Origin
S. Typhimurium	07CR073	2007	Lymph nodes conventional pig at slaughter
	07CR095	2007	Lymph nodes conventional pig at slaughter
	08MD138	2008	Fecal sample of conventional breeding pig
	08MD869	2008	Fecal sample of conventional slaughtered pig
	S12AK086	2012	Fecal sample of conventional slaughtered pig
Monophasic variant of *S.* Typhimurium	S14CH63	2014	Fecal sample of pig at farm
	S12AK050	2012	Fecal sample of conventional slaughtered pig
	S12AK070	2012	Carcass swab of organic slaughtered pig
	S12AK100	2012	Fecal sample of organic slaughtered pig
	S12AK107	2012	Fecal sample of conventional slaughtered pig

Assays

Intestinal cells, IPEC-J2 or Caco-2, were seeded in DMEM medium at 2.10^5 cells per well in a 24 well-plate. Then, 2.10^7 bacteria (m.o.i. of 200) of each strain were added in two wells, one to numerate adhesive and invasive bacteria and the other, to numerate invasive bacteria. Cells were incubated for 3h at 37°C with 5% CO_2 to allow bacterial adhesion and invasion. To determine the adherence, cells were washed three times with PBS solution and then the monolayer was lysed with 0.1% triton X-100 for 15 minutes at 37°C, and total bacteria (intracellular and adherent bacteria) were enumerated by serial dilutions in Tryptone salt (TS) by plating on PCA agar plates.

To determine invasion, the monolayer was washed three times in PBS solution, and 1ml of DMEM containing gentamicin at 480 mg/L was added to kill extracellular bacteria. After 2-hour incubation, the monolayer was washed twice in PBS solution and lyzed with 0. 1% Triton X-100 as described above. After serial dilution in TS, released intracellular bacteria were enumerated by the colony count method on PCA agar plates.

Invasion ability (Invasion percentage) was expressed as the percentage of the inoculum surviving the gentamicin treatment, and adherent bacteria (Adhesion percentage) were expressed as the total number of bacteria counted without antibiotic treatment relative to the initial inoculum. Three to four technical replicates were performed for each experiment.

Statistical analyses

Statistical analyses have been performed in the R software (R version 3.2.4). The significance of the differences observed was tested using Kruskal-Wallis test and Mann-Withney test. The differences were found to be significant when the test results gave $p \leq 0.05$.

Results

On IPEC-J2 cells, significant difference was observed between serovars for adhesion ($p=0.002$). *S.* Derby and monophasic variant of *S.* Typhimurium had the highest capacity of adhesion (56.41% and 33.80%, respectively) compared to *S.* Typhimurium (24.36%). The invasion varied from 0.69 % to 18.58 % with a mean of

4.81% (Figure 1). The highest values of invasion are obtained for monophasic variant strains but in mean for this serovar the difference is not significant with others serovars (p=0.07).

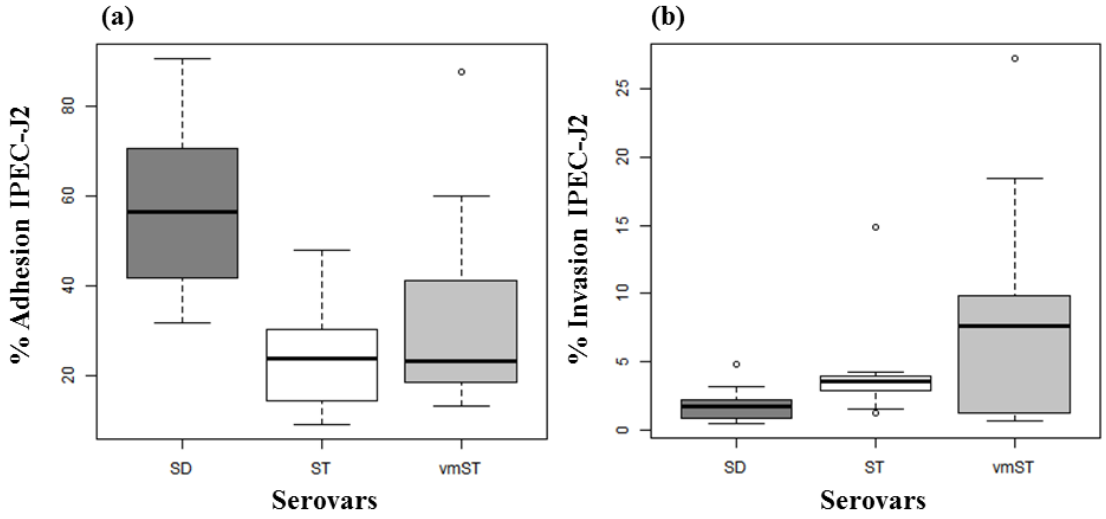

Figure 1. Box-Plot representing the adhesion percentage (a) and the invasion percentage (b) on IPEC-J2 cells according to serovars (SD = S. Derby; ST= S. Typhimurium; vmST = monophasic variant of S. Typhimurium).

In a hierarchical classification analysis based on IPEC-J2 invasion results, 4 strains particularly invasive clustered, three of them are variant monophasic of *S.* Typhimurium and one is a *S.* Typhimurium. The hierarchical classification analysis based on IPEC-J2 adhesion and invasion gave three clusters. None cluster is link to a specific serovar, each cluster contained 2 or 3 different serovars.

On Caco-2 cells, monophasic variant of *S.* Typhimurium showed the highest capacity of adhesion (46.17%) and *S.* Derby the lowest (26.28%), but there was no significant difference between the three serovars (p=0.15). Even if invasion was low for all strains (1.91% in mean), a significant difference was observed between serovars (p=0.008), and, surprisingly, even if it no so often associated with human cases than the two others serovars, *S.* Derby showing the highest capacity of invasion (2.39%).

Hierarchical classification analysis based on Caco-2 adhesion and invasion highlighted four clusters. A cluster contained 2 monophasic strains (the most adhesive strains, with respectively 69.02 and 74.58% adhesion) but in the 3 others clusters the 3 serovars studied were represented (Figure 2).

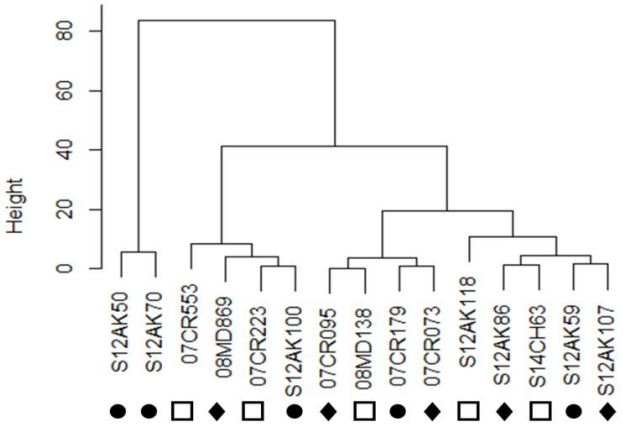

Figure 2. Dendrogram repre-senting the hierarchical clas-sification of strains of the ba-sis of caco-2 adhesion% and invasion% results (S. Derby □; S. Typhimurium ♦; mo-nophasic variant of S. Typhi-murium ●).

Conclusion

Our results didn't allow us to clearly differentiate the serovars on the basis of their ability to adhere and invade IPEC-J2 or Caco-2 cells. However, monophasic variant of *S.* Typhimurium strains showed a good ability to adhere to those pig or human cells, and to invade pig cells, which could explain its importance in pig colonization and human infections, in France. However, in Caco-2 model, *S.* Derby strains showed the highest invasion ability. To complete this study, the virulence of these 15 strains will be evaluated using *Galleria mellonella* pathogenesis model.

Acknowledgments

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SEROPREVALENCE OF HEPATITIS E VIRUS (HEV) IN DOMESTIC NON-COMMERCIAL PIGS REARED IN SMALL-SCALE FARMS AND WILD BOAR IN SOUTH OF BRAZIL

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Abstract

Hepatitis E is a zoonotic emerging disease distributed worldwide. The domestic swine and wild boars (*Sus scrofa*) are known as important reservoirs of HEV although HEV infections have been detected in other animal species. The southern region of Brazil has the largest swine productions in the country, ranging from highly-specialized commercial swine productions to small-scale non-commercial pig farms. The small-scale farms allow interactions between wild boars and domestic pigs, when occasionally pathogens transmission can occur between these populations. The aim of this study was to determine HEV seroprevalence in non-commercial domestic pigs and wild boars from two southern Brazilian states (RS: Rio Grande do Sul; SC: Santa Catarina), and discuss if the consumption of raw or undercooked meat from these animals is a potential risk to public health. Animals from RS and SC States were sampled. Serum was harvested from wild boar hunted between 2012 and 2016, and from non-commercial small-scale pig farms in 2014. Overall 249 wild boars (56 from RS and 193 from SC) and 382 pigs (261 from RS and 121 from SC) were tested to detect anti-HEV IgG antibodies using a commercial HEV antibody ELISA kit (Thermo fisher), specific for swine. Overall difference was observed (P<0.0001) regarding HEV seroprevalence between wild boar 4.42% (n=249) and non-commercial domestic pigs 46.60% (n=382). In relation to wild boars samples, higher seroprevalence for Hepatitis E was observed in RS (14.29%; n=56) and lower in SC (1.55%; n=193; P<0.0004). In relation to pigs, RS had also higher seroprevalence (53.26%; n=261) than SC (32.23%; n=121; P<0.0002). Although interactions between wild boar and non-commercial domestic pigs are known to occur, the lowest antibody detection in wild boar suggest that these contact may not be sufficient to explain seroprevalence in studied populations. Our results indicate that non-commercial pigs are a more likely source of infection for the human population than wild boar.

Introduction

Hepatitis E is a zoonotic emerging disease distributed worldwide. The domestic swine (*Sus scrofa domestica*) and wild boars (*Sus scrofa scrofa*) are known to be an important reservoirs of HEV although infections have been detected in other animal species.

The southern region of Brazil has largest swine productions in the country, ranging from highly specialized commercial swine productions to small-scale non-commercial pig farms. Non-commercial pig farms are intended for subsistence food for

low-income households, where animals are slaughtered under precarious conditions, handled and consumed, often without the minimum safety care.

On the other hand, currently wild boar populations are present in all five Brazilian political regions, with the major concentration on the South and Southeast, where estimations of population density ranges from 0.22 to 22.3 individuals km-². Despite the increasing dispersion and density of this exotic invasive specie in several regions of the country, little is known about the health condition and the risks these animals pose to public health. Since the Brazilian Institute of Environment and Renewable Natural Resources (IBAMA) authorized population control (NI 03/2013) by hunters legally authorized, the collaboration of hunters allowed to access biological samples of wild boar populations for epidemiological studies. Then, recently the health monitoring became part of ongoing surveillance performed with collaboration of volunteers hunters legally authorized to kill wild boar for population control purposes.

The fragility of the facilities of small-scale pig farms often allow interactions between wild boars and domestic pigs, when occasionally pathogens transmission can occur between these populations. Though no study directly related HEV prevalence in pig farms to contact with wild boars, they may play a potential role in the swine HEV epidemiology in free-ranged pig production units. In our knowledge, to date, no study have been performed to evaluate the occurrence of HEV antibodies in non-commercial pigs reared in small-scale farms and free living wild boar from Santa Catarina and Rio Grande do Sul States, which could be a source for human infection.

The aim of this study was determine HEV seroprevalence in non-commercial domestic pigs and wild boars from two southern Brazilian states considering the potential risk to public health by consumption of their raw or undercooked meat and meat products.

Material and methods

Considering logistical limitations to access samples from target populations the serological study based on convenience sampling were performed.

Wild boar sampling

Blood samples were collect immediately after hunting by puncture in the cavernous sinus or heart, by exsanguination (cervical major veins) or from the thoracic cavity. Following, samples were placed in sterile tubes transported in cooler box to the laboratory for centrifugation, and stored at −20 ° C for serological analysis. Overall 249 wild boar samples, 56 from Rio Grande do Sul State (RS) and 193 from Santa Catarina State (SC) were obtained.

Since wild boar were killed for population control purposes, according Brazilian Agency for Environmental Protection (IN 03/2013 – IBAMA), no ethical approval was needed.

Domestic pig sampling

Serum samples from non-commercial pig farms previously harvested in 2014 on the context of official surveillance program to substantiate freedom from classical swine fever (CSF) were used. From the serum bank, we select regions with presence of wild boar according survey performed by official veterinary service in 2014. From each

municipality with wild boar were selected up to three pig non-commercial farms and serum of up to three pigs/farm were randomly sampled. A total of 382 samples from domestic pigs were obtained, 261 from RS and 121 from SC. Overall, we sampled 30 and 87 municipalities and 53 and 131 small-scale pig farms in SC and RS, respectively (Figure 1).

HEV-specific antibodies (HEV-Ab) were detected by a commercially available ELISA (PrioCHECK_ HEV Ab porcine) specific for swine, according to the manufacturer instructions. For comparison of the prevalence between different groups, Fisher's exact test was applied. Differences were considered significant at $P \leq 0.05$.

Results and discussion

Overall there was a significant effect ($p < 0.01$) of population category on the HEV seroprevalence, being 4.42% for wild boars and 46,60% non-commercial pigs. Samples from RS presented higher seroprevalence for HEV, both in wild boars and pigs compared to SC, 14.29% versus 1.55% for wild boars and 53.26% Versus 32.23% for pigs, respectively, differing significantly ($p \leq 0.05$) by Fisher's exact test (Table 1). There was no significant effect of gender within each population category evaluated. Although interactions between wild boar and non-commercial domestic pigs are known to occur, the lowest antibody detection in wild boar suggest that these contact may not be sufficient to explain seroprevalence of studied populations. The marketing and distribution of wild boar meat and meat products originated from the hunt is banned in Brazil but the consumption of boar meat from population control is a common practice among hunters and their families. Our study showed that HEV seroprevalence in domestic non-commercial pigs are as prevalent in SC and RS as in other countries (4). We also detected that HEV circulates in wild boar populations in low seroprevalcence differing of some countries where wild boar plays roles as HEV reservoir. Since the sanitary status of wild population can indicate the environmental health, including sympatric livestock and wildlife, ecological factors should be considered to understand the HEV seroprevalence of wild boars. The wild boar samples from RS were collected in regions of fields with few crops and vegetation scarcity favoring their carnivorous habits. The consumption of carcasses from dead animals in the field, both wild and livestock, is a common alimentary habit of wild boar in RS. In contrast, SC wild boar sera were obtained predominantly in forest areas characterized by abundant fauna and flora, and with large plantations in the surroundings. These environmental conditions can influence alimentary habits of wild boar and consequently their exposition to pathogens such as HEV, which is known to be a multispecies virus. Our results suggest that non-commercial pigs can play a more important role as an HEV reservoir than wild boars, and that non-commercial pigs are a more likely source of infection for the human population. However, HEV is also present in wild boar populations and variations in prevalence may occur according to the natural characteristics of the regions where they live.

Conclusion

Our results suggest that non-commercial pigs can play a more important role as a HEV reservoir than wild boars. Our results suggest that the non-commercial pigs can play a more important role as a HEV reservoir than wild boars. The non-commercial pigs are a more likely source of infection for the human population but also the risk on the consumption of wild boar meat exist and cannot be neglected.

Table 1. HEV seroprevalence in domestic non-commercial pigs collected in 2014 and wild boar collected between 2012 and 2016 in Santa Catarina (SC) and Rio Grande Sul (RS).

Animal category	Number tested/ positive serum samples	Seroprevalence %	95% CI		Fisher's P-value
Total swine	382/178	46.60	41.6	51.6	<0.0001
Total wild boar	249/11	4.42	1.8	7.0	
SC domestic non-commercial pigs	121/39	32.23	23.9	40.6	0.0002
RS domestic non-commercial pigs	261/139	53.26	47.2	59.3	
SC Wild boar	193/3	1.55	0.0	3.3	0.0004
RS wild boar	56/8	14.29	5.0	23.5	

HEV – hepatitis E virus; CI – confidence interval,; SC – Santa Catarina State; RS – Rio Grande do Sul State

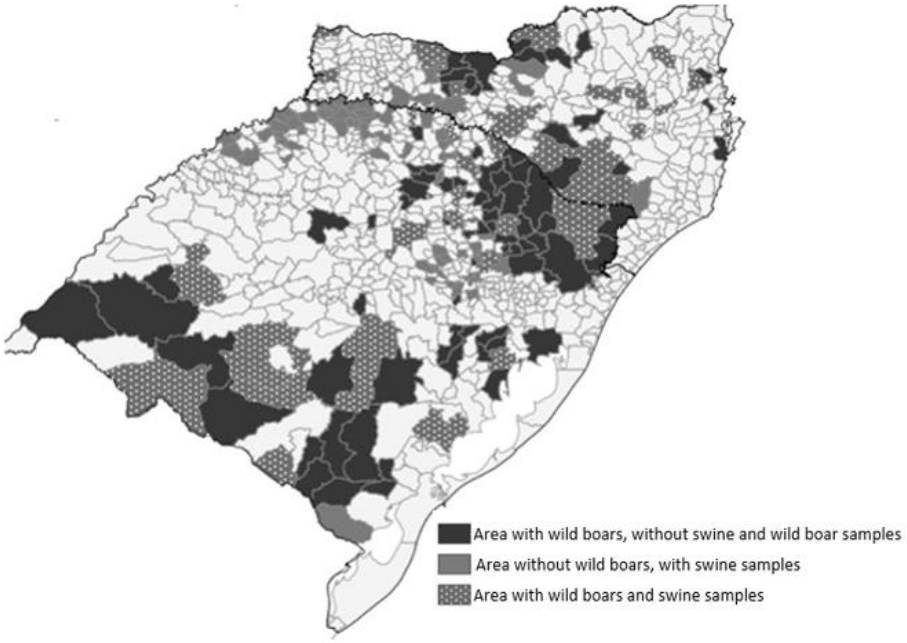

Figure 1. Distribution of municipalities with records of wild boar, and with or without swine serum samples.

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SEROPREVALENCE OF *BRUCELLA* SPP, *LEPSTOSPIRA* SPP AND *TOXOPLASMA GONDII* IN WILD BOARD (*SUS SCROFA*) FROM SOUTHERN BRAZIL

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Abstract

The wild boar (*Sus scrofa*), exotic invasive specie, is currently distributed in many Brazilian states, including Santa Catarina (SC) and Rio Grande do Sul (RS). Since the wild pigs are susceptible to zoonotic pathogens as *Leptospira* spp, *Toxoplasma gondii* and *Brucella* spp, their large populations, movements and dispersion can spread diseases, being a potential transmission source to humans, livestock and other sylvatic sympatric species. Despite its importance are there few information about sanitary status of this wild populations and their impact for human and livestock health in Brazil. Objective this work was to investigate the presence of antibodies to *Brucella* spp, *Toxoplasma gondii* and *Leptospira* spp in blood samples of wild boar legally hunted for population control in SC and RS states. From January 2014 to July 2016, 193 samples were tested to antibodies against *Brucella* spp (buffered acidified plate agglutination test-BAPA) and *Leptospira* spp (micro agglutination test-MAT). Of these 193, 149 were tested to *Toxoplasma gondii* antibodies (HAI test). Overall, serological results showed negativity to *Brucella* spp and low prevalence for *Toxoplasma gondii* (2,7%). On the other hand, the seroprevalence of *Leptospira* spp was 6,74% with significantly greater percentage of positivity in RS (12,5%) compared to SC (3,88%). The most prevalent serovars ware Icterohaemorragiae and Pomona, with titers ranging from 1:400 to 1:12,800 in RS. Icterohaemorragiae was the most prevalent serovar in SC but with lowest titres. Seroprevalence of these pathogens in wild population indicate the environmental health, including sympatric livestock and wildlife. Ecological factors should be considered to understand the role of wild boars in the cycle of these diseases. Our results indicate that hunters and consumers of wild boar meat of must be aware about zoonotic risk in carcass handling and meat intake.

Introduction

The European wild boar (*Sus scrofa*) is an exotic animal to the Brazilian fauna. Currently the invasive specie is distributed in many Brazilian states, including Santa Catarina (SC) and Rio Grande do Sul (RS). Since the wild pigs are susceptible to zoonotic pathogens as *Leptospira* spp, *Toxoplasma gondii* and *Brucella* spp, their large populations, movements and dispersion can spread diseases, being a potential transmission source to humans, livestock and other sylvatic sympatric species. Despite the importance of the subject there is little information about sanitary status of this wild population and their impact for human and livestock health in Brazil.

Brucellosis is a zoonotic infection caused by the bacterial genus *Brucella*. It affects domestic and wild mammal species and it is usually transmitted from animals to humans by ingestion through infected food products, direct contact with infected animal or contaminated environment.

Leptospirosis is a zoonosis caused by pathogenic spirochetes of the genus *Leptospira*. The bacteria can be found worldwide in soil and water. Leptospirosis is commonly associated with rodents in settings of poor sanitation, agricultural occupations and activities involving fresh water, mud, or soil exposure. Human infection can occur through direct contact with infected animals or by exposure to water or soil contaminated by urine of infected animals.

Toxoplasmosis is a zoonotic infection caused by *Toxoplasma gondii* protozoan capable of infecting an unusually wide range of hosts. Domestic and wild felidae are considered the definitive hosts, while the other species are considered intermediate hosts as people and other warm-blooded animals. The main routes of transmission, both in the definitive and intermediates hosts, are the ingestion of tissues of infected animals or ingestion of food contaminated with infective oocysts or by transplacental route.

Objective of this work was to investigate the presence of antibodies against to *Brucella* spp, *Toxoplasma gondii* and *Leptospira* spp in blood/serum samples of wild boar legally hunted for population control in SC and RS states.

Material and methods

From January to July 2016 legally authorized hunters harvested blood samples from 193 wild boars. Immediately after the death, blood were collected from the heart or thoracic cavity, and transported to the Embrapa laboratory. Serum was recovered by centrifugation and frozen at -20°C until analysis. The 193 samples were tested to detect antibodies against *Brucella* spp with buffered acidified plate agglutination test (BAPA), and with micro agglutination test (MAT) to detect antibodies against *Leptospira* serovars: Autumnalis, Ballum, Bratislava, Grippotyphosa, Hardjo, Icterohemorragiae, Pomona, Tarassovi and Wolffi. Titres ≥ 1:100 were considered reagent. Of those 193 sera, 149 was assayed using qualitative and semi-quantitative indirect hemagglutination assay (IHA) to *Toxoplasma gondii* antibodies. A titre 1:64 is considered positive. Fisher Exact Test was used to evaluate the effect of Federative Unit, gender and age on the seroprevalence. The age was based on the live weight, juvenile with less than 40kg and adult with 40kg or more. Results were evaluated by the FREQ procedure of the SAS software.

Results and discussion

All 193 samples tested for antibodies to *Brucella* spp were negative. Little studies have been devoted to the investigation about wild boar brucellosis in Brazil. According a study in 2010, samples from captive wild boar from the same area and others regions of the country, also not presented positivity to antibodies to *Brucella* spp. The low prevalence also occurs in domestic pigs in Brazil 0.0% to 0.34%.

The examination of 149 wild boars serum samples for *Toxoplasma gondii* revealed only four seropositive individuals, representing the prevalence of 2.7% (Table 1). The results show a low prevalence of the parasite, compared with, Central Italy (8.1% to 14%), Austria (19%) and USA (18%). The same occurs when compared to the seroprevalence of *T. gondii* in Brazilian domestic pigs from 4% to 54.1% in different regions or compared with European data that range from 8.1% to 38.4%. Our results suggest low or rare contact of wild boar populations with environments and infected animals.

In this study, overall the 6.7% prevalence of *Leptospira* spp antibodies in wild boars were detected. An expressive percentage of positivity (12.5%) for *Leptospira* spp in RS samples were detected comparing to SC (3.88%) (Table 1). Five samples from SC were positive for Leptospira spp (Table 2), from these, four were positive for the serovar Icterohaemorrhagiae, one reacted to serovar Grippotyphosa and one to Pomona serovar, being the Suidae the main reservoir of these last serovars. The low seroprevalence to *Leptospira* spp found in SC wild boar (3,88%) may be associated the region where the samples of SC were collected, that is characterized by forested areas with abundant and diverse fauna and flora, as well as crops and pig production in the vicinity of forest areas. These conditions can reduce exposure to the agent since wild boars would feed on seeds, roots and crops, decreasing contact with potentially infected animals and carcasses.

From the RS samples were found 12.5% reagents to *Leptospira* spp (Table 2), with higher titres than those found in SC wild boars. These titres indicate late infection and/or that the wild boar population has been persistently exposed to the pathogens. The most prevalent serovar was Icterohaemorragiae, but the titles for the Pomona serovar were the highest, followed by the three reagents to the Autumnalis serovar. There was only one reagent to the serovar Grippotyphosa and one to Hardjo in RS samples.

The RS samples were collected in a region of in Pampa biome, characterized by fields with grasses great diversity of sylvatic animals, few crops and abundance of livestock, escpecially sheep and bovine creations. The wild boars are omnivorous and scarcity of vegetation favors carnivorous habits, increasing the chance of contact and ingestion of carcasses and potentially infected animals. Thus, in addition to possible contaminated environmental sources, the highest seroprevalence for *Leptospira* spp found in RS may be related to a scarce supply of food by local vegetation favoring them contact and feeding of infected sympatric dead animals, as sheeps, capybaras (*Hydrochoerus hydrochaeris*) or other abundant species in that region.

Serovar Icterohaemorragiae is generally associated with direct or indirect contact with rodents and Suidae are natural reservoirs of Pomona while Autumnalis serovar reagents probably were results of the contact with canines, goats and sheep that are the natural reservatories. Overall, six samples were reagent for two or more serovars. The reactions to multiple serovars were more frequent in RS samples. The coinfection by different serovars may be associated to expositions to different sources of infection and/or reservoirs or even to infections by multiple serovars from the same animal or environmental source but other studies must be performed to elucidate these questions

The reactive samples to *T. gondii* and *Leptospira* spp were not significantly associated with gender or age. Seroprevalence of these pathogens in wild population indicate the environmental health, including sympatric livestock and wildlife. Ecological factors should be considered to understand the role of wild boars in the cycle of these diseases.

Conclusions

Our results showed that there are no *Brucella* spp circultation in the studied population but the seroprevalcence of *Toxoplasma gondii*, although low, showed the circulation of the pathogen pointing the boar as a potential source of infection to animal and human.

The seroprevalence of *Leptopira* spp and high titers to serovares tested, especially from the RS samples, indicate that wild boar were persistent exposit to the agents. These results raise questions relevant to both, livestock, wildlife and public health.

Hunters and consumers of wild boar meat should be aware of the risk in carcass management and consumption of boar meat and meat products, raw or poorly cooked.

Table 1. Apparent serorevalence of Leptospira spp and Toxoplasma gondii in RS and SC boars.

Agent	State	Apparent prevalence	95% CI	
Toxoplasma gondii	RS/SC	2,7%	0,1%	5,3%
Leptospira spp	RS/SC	6,7%	3,2%	10,3%
Toxoplasma gondii	RS	3,3%	0,0%	6,9%
Toxoplasma gondii	SC	1,75%	0,0%	5,3%
Leptospira spp	RS	12,5%	4,3%	20,7%
Leptospira spp	SC	3,1%	0,1%	6,1%

Table 2. *Leptospira* species and serovars antibodies detected in wild boar from SC and RS.

State	*Leptospira interrogans* Serovars				
	Autumnalis	**Grippotyphosa**	**Hardjo**	**Icterohaemorrhagiae**	**Pomona**
RS				1:400	1:3200
	1:200			1:400	1:400
	1:1600			1:1600	1:12800
		1:400			
				1:200	
	1:400			1:800	1:6400
				1:200	
			1:200	1:400	1:3200
SC				1:100	
				1:100	
		1:800		1:200	
				1:100	
					1:200

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Surveillance and control of foodborne pathogens at pre-harvest and post-harvest level

EVALUATING CORRELATIONS IN SALMONELLA SEROTYPES IN SWINE IN FOUR LONGITUDINAL DATASET

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S. enterica serovars surveillance program have been established for many years in the United State of America (USA). Data from long running surveillance programs provides the opportunity to compare prevalence of serotypes within and across surveillance programs, observed patterns and generate hypothesis. To this end, the aim of this project was to estimate the correlation between changes in the yearly changes in serotypes proportions in concurrent years and lagged years from swine, beef and avian longitudinal datasets: (The Iowa State University Veterinary Diagnostic Laboratory (VDL), The National Antimicrobial Resistance Monitoring System (NARMS animal-based isolates only), compared to data from The Centers for Disease Control (CDC) Laboratory-based Enteric Disease Surveillance (LEDS) Program. The lagged correlations were as follows: a) 1-year lag with the animal data preceding the human data and b) The correlation across a 2-year lag with the human data preceding the animal data. We calculated correlations and corresponding intervals for the following comparisons

- CDC-LEDS correlation with ISU VDL swine, avian and bovine data-sets.
- CDC-LEDS correlation with NARMS-USDA swine, avian and bovine data-sets.
- CDC-LEDS correlation with NARMS-FDA retail pork, poultry and beef data-sets.

Using the 10 most common serotypes found in swine based on the ISU database, some of the correlations observed included: 1) positive correlations between concurrent yearly proportional in changes in S. enterica 4,[5],12:i:- in swine VDL submissions, bovine VDL submissions, NARMS bovine submissions with the LEDS yearly proportional changes and 2) positive correlations for concurrent yearly proportions changes S. Anatum between LEDS and the NARMS swine dataset. No correlations where found for the lagged correlations.

REVISITING THE ROLE OF PIG SEROLOGY IN THE CONTEXT OF *SALMONELLA* CONTROL PROGRAMS IN COUNTRIES WITH HIGH PREVALENCE OF INFECTION - A PRELIMINARY STUDY

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Abstract

In this study, we assess whether on-farm serology may be useful for predicting *Salmonella* shedding at slaughter. Serology on serum samples collected 60 and 90 on fattening and three days before slaughter predicted somewhat shedding at slaughter with no significant differences among them. Pigs with higher OD% values at these point times would have higher risk of shedding when arriving to slaughter. On-farm serology may help to predict to some extent the risk of *Salmonella* shedding at slaughter in seropositive fattening units, which may allow for prompt on-farm and slaughter interventions to mitigate the risk of shedding when pigs arrive to slaughter.

Introduction

Most *Salmonella* national control programs in Europe have been based on the categorization of pig herds according to risk levels based on serological results, but most of them have not reported of any overall significant success on *Salmonella* infection reduction in fattening pigs or on the number of human cases attributable to pigs or pork. The United Kingdom suspended its meat juice testing for *Salmonella* antibodies in 2012 and moved towards an "on-farm risk assessment" approach based on a scoring system[1]. Belgium discontinued its serological program in 2015 but kept advisory veterinarians on the field (Brossé, 2015), and Germany, although still keeps its initial program, has not detected a significant reduction of category III farms (Blaha, 2017). The limited diagnostic accuracy of the tests used, the small number of animals sampled, and the likely lack of herd representativeness of the samples used, could have played a part in the wrong *Salmonella* characterization of a pig herd and the misperception of its true *Salmonella* risk. Spain, a country of high *Salmonella* prevalence and without any control program yet, may require a different strategy to tackle this problem.

Salmonella shedders are an important source of slaughter and carcass contamination (Argüello *et al*., 2013), thus from a pig farm´s perspective, a major goal to address should be the reduction of the number of pigs shedding *Salmonella* that arrive to the slaughterhouse. Focusing on the prevention of *Salmonella* shedding at slaughter may be far more important than focusing on detection of seropositivity at this stage. Pigs shedding *Salmonella* at slaughter seroconverted earlier during the fattening period

[1] http://www.pigprogress.net/Health-Diseases/Health/2012/6/UK-New-direction-for-Zoonoses-National-Control-Programme-ZNCP-PP008961W/

than non-shedder pigs (Casanova-Higes *et al*., 2016), therefore serology may help to predict the risk of shedding at slaughter.

Material and methods

The pigs considered for this preliminary study belonged to 6 control groups (batches A to F), of approximately 50 animals, included in previous studies carried out between 2010 and 2016 in a small fattening unit (N≈110) for other purposes. The farm was in NE of Spain and was known to be *Salmonella* positive. Pigs had been individually identified and only those that had been blood sampled at 30 (30d), 60 (60d), 90 (90d) days in the fattening unit and within 3 days before slaughter (BS), and for which a minimum of 25g of fecal (FEC) samples were collected at slaughter, were considered for this study. In addition, 25g of mesenteric lymph nodes (MLN) were also collected at slaughter from these pigs to assess their infection status. For serological analysis the HerdCheck Swine *Salmonella* ELISA (IDEXX Laboratories, ME, USA) was used. Bacteriology on FEC and MLN samples was performed according to the EN ISO 6579:2002/A1:2007.

Median OD% values and their 95%CI were estimated for each sampling time and for each batch of pigs. Overall estimates of prevalence of shedding (FEC+) and infection (MLN+) at slaughter were also calculated for each batch. The relationship between log-transformed OD% values at each sampling time (30d, 60d, 90d and BS) and shedding at slaughter was assessed by logistic regression analysis after adjusting by batch. When a significant association was found, Receiver Operating Characteristic (ROC) curves were constructed and the area under the curve (AUC) estimated for the ELISA. Estimates of probability of shedding *Salmonella* were calculated from logistic regression equations. Statistical analyses were performed with STATA software (STATA, StataCorp, L.P., USA).

Results

A total of 233 (76.1%) control pigs met the inclusion criteria and were included in the study. The number of sampled pigs varied among batches (Table 1). A total of 101 (43.3%; 95%CI: 36.9, 49.8) pigs shed *Salmonella* spp. at slaughter and in 97 (41.8%; 95%CI: 35.4, 48.2) the bacterium was isolated from MLN.

Table 1. Proportion of slaughter pigs shedding *Salmonella* and infected with *Salmonella* for each batch of pigs analyzed.

	Batch (no. of pigs)					
	A (25)	**B (28)**	**C (49)**	**D (48)**	**E (41)**	**F (42)**
% of shedders at slaughter (95%CI)	60 (39.4, 80.6)	21.4 (5.2, 37.6)	69.4 (66, 82.8)	75 (62.3, 87.7)	9.7 (0.3, 19.2)	14.3 (3.2, 25.3)
% of infected pigs at slaughter (95%CI)	76 (58, 94)	18.5 (2.8, 34.2)	40.8 (26.5, 55.1)	68.7 (55.1, 82.3)	7.3 (0, 15.3)	40.5 (25,55.9)

Serological results differed among batches (Figure 1). The OD% values for pigs from batches B and C remained quite low for all sampling times. In contrast, for batches A, D, and F, OD% values increased significantly after first sampling on day 30. For batch E, OD% values remained similar along the fattening period with some increase in

the last sampling. No relationship was observed between ELISA results and shedding at slaughter when serum samples were collected on day 30 on fattening (*P*=0.79), but a positive significant relationship was found between OD% values and shedding at slaughter for samplings on days 60, 90 and BS (*P*-values <0.01).

Figure 2 depicts the ROC curves for the ELISA test with regard to shedding at slaughter when performed at 60 and 90 days on fattening and BS. No differences were observed regarding AUC among these three sampling times (AUC≈0.83). Since batches B and C presented very low OD% values along the entire fattening phase (median OD%≤10), a further ROC analysis was performed excluding them. Results remained similar, although the AUC increased somewhat (AUC≈0.87).

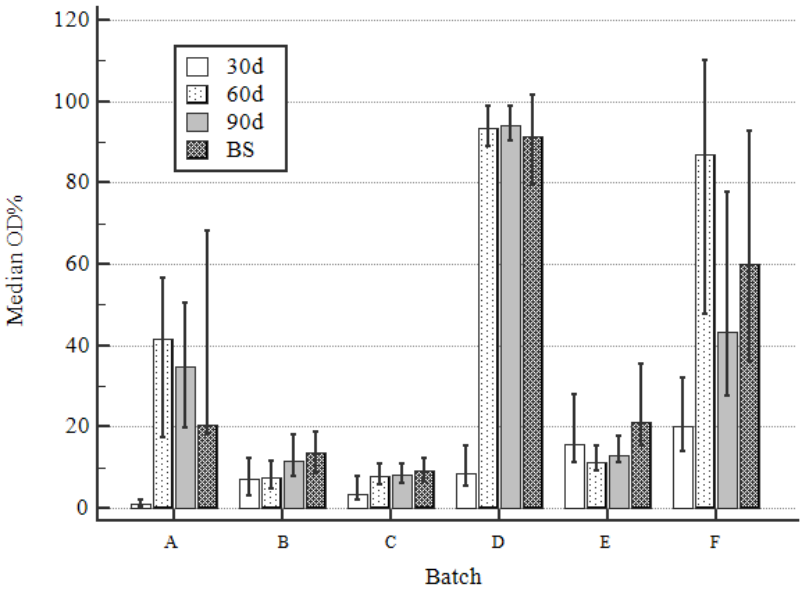

Figure 1. Median OD% values and their 95%CI for pig serum samples collected on day 30 (30d), 60 (60d), 90 (90d) on fattening and before slaughter (BS) for six batches of pigs (A to F).

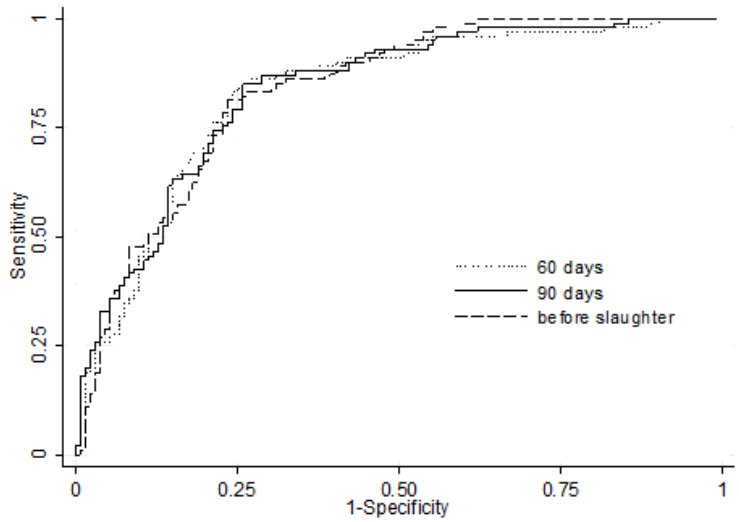

Figure 2. Receiver Operating Characteristic (ROC) curves estimated for prediction of shedding when an ELISA test was used on serum samples collected at 60 and 90 days on fattening, and before slaughter.

Estimates of the probability of shedding *Salmonella* spp. at slaughter with regard to OD% values for a pig sampled on day 90 on fattening from batch A are shown in Figure 3. When all batches were considered in the logistic regression analysis the probability of shedding *Salmonella* spp. for a pig showing an OD%=10 was as high as 43%. This risk increased significantly up to 65% for an OD%=40. When only batches A, D, E and F were considered these probabilities were 39.7% and 66% respectively.

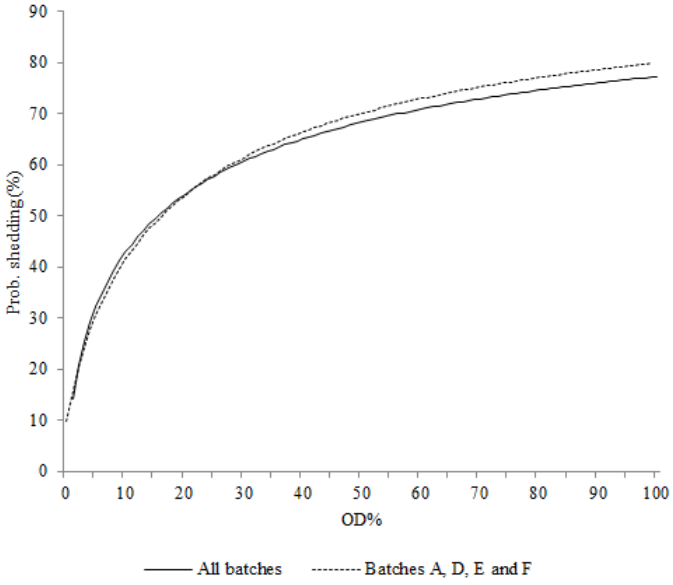

Figure 3. Estimated probability of shedding *Salmonella* at slaughter for a pig sampled on day 90 from batch A. Estimates are calculated for all the batches together (All batches) and also for those batches that showed increasing OD% values along the fattening period (A, D, E and F).

Discussion

In this study, serological results showed different on-farm pig *Salmonella* exposure experiences among batches, representing the expected variability for an infection like this one. In batch B, and particularly in C, pigs would have been infected at the end of the fattening period, i.e. within the last 10-15 days BS, with no time to develop detectable IgGs (Nielsen *et al*., 1995). The use of on-farm serology at any point time during the fattening period would have been useless in these two batches. In contrast, pigs from batches A, D, E and F experienced a significant increase of OD% values after day 30, which was compatible with pigs being exposed to the bacterium. Pigs from batch E showed OD% values relatively low and fairly constant along the fattening period. Thus, to explore the potential that serology may have on predicting *Salmonella* shedding at slaughter considering this variability of scenarios, this study initially took all the batches into account.

On-farm serology at the beginning of the fattening period, i.e. on day 30, was useless for the objective of predicting shedding at slaughter. This was an expected result as many pigs on day 30 may not have getting in contact with *Salmonella* yet, and some seropositive pigs at this time may have become seronegative BS (van der Wolf *et al*., 2001). However, a significant correlation was observed between serology on days 60 and 90 on fattening or BS and shedding at slaughter.

Analyzing pig serum in any of the last three sampling times yielded similar results as indicated by their corresponding AUC. Pigs with higher OD% values at these point times would have higher risk of shedding when arriving to slaughter (Figure 3). Estimates of the risk of shedding when batches B and C were not considered seemed to be somewhat more realistic since pigs from these batches were likely infected close to the date of slaughter, thus serology would yield biased results towards increasing the risk of shedding for pig showing low OD% values. Preventing infection during transport and lairage would surely improve the ability of on-farm serology to predict shedding at slaughter.

These results suggest that, for infections occurring time ahead of the slaughter date (between 15 and 45 days before), on-farm serology may help to determine to some extent the risk of *Salmonella* shedding at slaughter. For this purpose, a representative sample of pigs should be selected from the batch of interest. On-farm serology could be performed at any time after 60 days on fattening and until 2-3 days before slaughter, but as sooner is the serum analyzed, longer is the time available to respond to the risk. Thus, when shedding is likely, on-farm interventions could be promptly scheduled to try to mitigate the probability of shedding when pigs arrive to slaughter, and additional interventions could be implemented during transport or at slaughter. This approach would also allow for a more precise serological characterization of the pig farms through weighted averages of seroprevalence, as a representative sample of the pigs in the farm would be collected. Farms presenting low OD% values on day 90 would be expected to remain so for the rest of the fattening period if nothing wrong happens during the time remaining until slaughter. Special care on truck and lairage cleaning and disinfection procedures should be considered for these herds to prevent late infections and further shedding. A large-scale study to confirm the potential of this approach to reduce *Salmonella* shedding at slaughter is warranted.

Acknowledgements

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RESULTS FROM A STUDY OF THE EFFECT OF ENHANCED CLEANING AND DISINFECTION ON *SALMONELLA* PREVALENCE IN FINISHER PIG BUILDINGS

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Introduction

Salmonella is the second most commonly reported zoonotic gastrointestinal pathogen in the European Union [1]. Although the majority of foodborne outbreaks have been linked to the consumption of eggs and egg products (44.0%), a substantial proportion originate from pork and pork products (9.3%) [1]. Biosecurity measures correctly implemented on farm are important to reducing *Salmonella* carriage in live pigs and consequently the number of *Salmonella* contaminated carcasses entering the food chain. Cleaning and disinfection (C&D) of pig pens is considered an essential part of any successful on-farm *Salmonella* control regimen [2,3]. Environmental *Salmonella* contamination increases the risk of *Salmonella* shedding in newly introduced batches of pigs [4]. The ability of disinfectants to eliminate *Salmonella* is influenced by the type of disinfectant chosen and its concentration, and may be severely compromised by the presence of organic matter [5,6]. Different types of disinfectant are commercially available, such as quaternary ammonium compounds (QAC) products containing glutaraldehyde or formaldehyde, peroxygen or peracetic acid based compounds, iodine based compounds or chlorocresols. Their effectiveness against *Salmonella* varies greatly, as demonstrated in several in *vitro* and on farm studies [5,6,7]. Currently, disinfectants intended for veterinary use may be assessed for efficacy using methods which do not use the matrices commonly found on farms, and therefore the efficacy of a disinfectant in field conditions can be overestimated. Within this study, a C&D regimen consisting of disinfectants of known efficacy and following a rigorous standardised procedure was compared to farmers' routine C&D procedures on *Salmonella* contaminated pig holdings in the UK. The effectiveness of C&D procedures was evaluated by the reduction in Total Bacterial count (TBC), Enterobacteriaceae and *Salmonella* contamination.

Method

Ten farms were enrolled, all of which produced finished bacon pigs; operated the study buildings using an all-in/all-out programme; and had a previous pen faecal prevalence of *Salmonella* of over 20%. In each of the farms, two buildings housing finishing pigs at the same stage, with similar size and management practices, were selected. One building was randomly assigned as the intervention building, and the other building served as control. Four sampling visits were carried out to each of the study farms. The first visit (pre-C&D) was carried out when the first batch of pigs was

close to slaughter. The second visit (post-C&D) was carried out when the buildings had been cleaned and disinfected and were still empty. The third visit was carried out 2 to 3 weeks after the second batch of pigs had been housed in the study buildings (post-restocking). The fourth visit was carried out when the second batch of pigs was close (2 to 3 weeks) to slaughter. At each visit, one pooled faecal sample was collected for each 50 pigs housed in a pen. Furthermore, 10 individual faecal samples were collected from the floor in up to 6 randomly selected pens for each building. At the second visit (post-C&D), feeders, drinkers and floors of the empty buildings were swabbed with hand-held gauze swabs. All intervention buildings were cleaned and disinfected by trained contractors, according to a protocol comprising a series of steps (removal of faeces, foaming, washing, disinfecting and cleaning portable equipment). All intervention buildings were disinfected using GPC8 (Evans Vanodine International Plc, Preston, UK) at Defra-approved concentration (1:35 ratio) and boot dips were refilled with FAM30 1:90 (Evans Vanodine). The procedures and products used in the control buildings were those usually employed by the farmer, and therefore differed from farm-to-farm. The association between building type (intervention or control) and the shedding of *Salmonella* by pigs was assessed using generalised linear mixed models (GLMM) in R version 3.2.4 using the *lme4* package. Sample type and age group of pigs sampled, season and whether the sample came from an intervention building or a control, were included in the model as *a priori* variables. The farmer C&D practices were recorded and a forwards stepwise selection process was used to identify variables that were significantly associated with *Salmonella* prevalence. When analysing the Enterobacteriaceae and total bacterial counts, statistical analyses were carried out with STATA® software (StataCorp, Texas, USA). The model tested both Enterobacteriaceae and TBC counts as two separate outcomes, and included the farm identifier as a random effect to account for the non-independence of samples from the same farm.

Results

Salmonella was isolated from all buildings at the pre-C&D visit, apart from the control buildings in farms 222C and 225C. At the post-C&D visit, *Salmonella* was isolated only in farm 228C in the intervention building, and in farms 221C, 229C and 230C in the control buildings. At the post- restocking visit, *Salmonella* was isolated from all buildings in all farms, except for the intervention building in farm 225C. At the pre-slaughter visit, *Salmonella* was isolated from all buildings in all farms, except for farm 222C. The samples from intervention buildings were significantly less likely to be positive for *Salmonella* than the control buildings after C&D and at the pre-slaughter visit (both p=0.004) (Figure 1). However, there was no difference in the likelihood of samples from the intervention and control buildings being positive for *Salmonella* at the post-restocking visit (p=0.119).

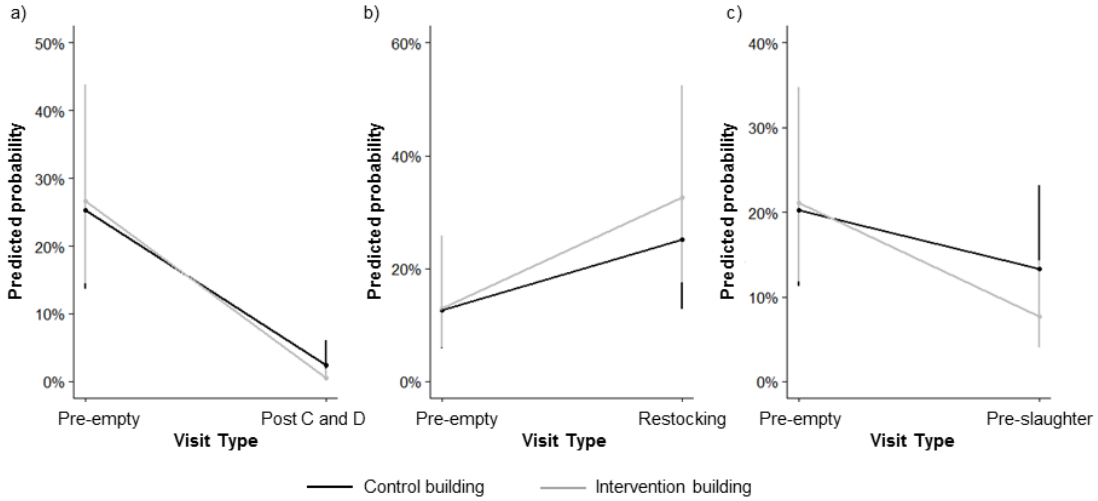

Figure 1. Plot showing the interaction effect of intervention and visit type on the predicted probability of a sample being positive for *Salmonella* (with 95% confidence interval error bars). Samples used in this analysis were: a) the pre-C&D and post-C&D visits only; b) the pre-C&D and post-restocking visits only; c) the pre-C&D and pre-slaughter visits only.

TBC counts were significantly lower in intervention buildings in samples from floors, drinkers and feeders ($p<0.001$) after C&D but only in drinker samples ($p=0.003$) for Enterobacteriaceae (Figure 2).

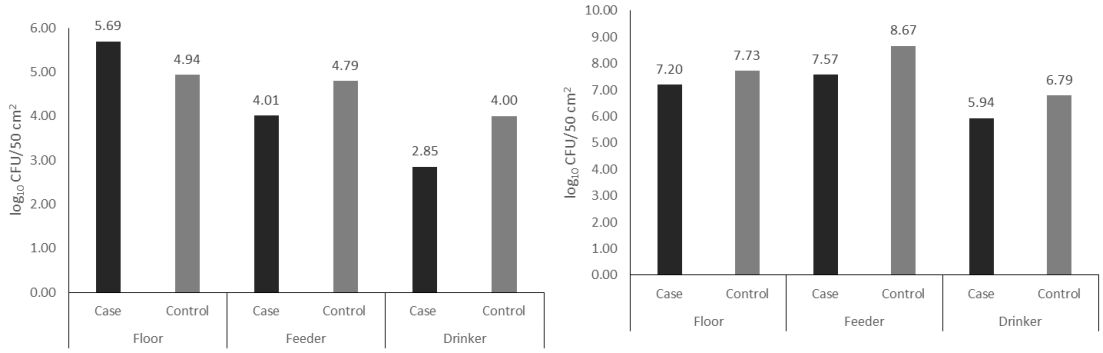

Figure 2. Mean Enterobacteriaceae (A) and total bacterial counts (B) in intervention (case) and control buildings from samples from floors, feeders and drinkers on 10 farms.

The results of the multivariable analysis assessing the farmer's methods of C&D showed how thorough cleaning and disinfection of ledges, beams, vents and ceilings and allowing 3-10 days downtime between batches was an effective measure to reduce the likelihood of residual *Salmonella* contamination. Leaving pens empty for longer period (2-3 weeks) appeared to be a significant risk and this may reflect a less intensive management system on these farms. Leaving a pen to dry after cleaning for 3-4 days was a risk factor when compared to 1-2 days. Other significant risk factors included changing feed between visits, coughing present in the pigs, the use of treatments between visits, whereas improvements to wildlife control and harbourage was identified as a significant protective factor. These individual factors appeared to explain the difference between the results from the intervention and control buildings and may highlight the key differences between the cleaning protocols.

Salmonella contamination was observed post-C&D in the control buildings of four farms. Three of these farms used glutaraldehyde and QAC products, but at a dilution rate (1:200) which was far higher than the recommended dilution (1:49). Over-diluting disinfectants is a common reason for disinfection failure [2]. The fourth control farm with residual post C&D contamination used an iodine-based disinfectant. In this farm, the disinfectant was used at a lower dilution rate (1:50) than the recommended rate (1:90). However, iodine-based compounds have been demonstrated to be less effective than aldehydes, especially in the presence of organic matter. This was confirmed in this study by the results of the multivariable analysis that showed that the iodine-based product used on this farm was significantly more likely to result in residual *Salmonella* contamination. Whilst there was no difference in the *Salmonella* prevalence between intervention and control buildings at the post-restocking visit, a significant difference was observed at the pre-slaughter visit, where pigs housed in the intervention buildings had a significantly lower prevalence. Samples collected during the summer were more likely to be positive for *Salmonella* and the highest *Salmonella* prevalence is observed on farms in these summer months, and this can be attributed to the fact that the higher temperature represents a stress factor for the pigs and it can result in higher shedding rates [8].

Conclusion

The study found that buildings that were cleaned by the intervention method, using Defra recommended concentrations, were more likely to be *Salmonella* free post-C&D and this reduced the probability of samples from them being positive pre-slaughter, which demonstrates the effectiveness of appropriate disinfection programmes aimed at eliminating *Salmonella*. The study also highlighted key risk and protective C&D procedures that can influence the likelihood of *Salmonella* contamination. Due to the high prevalence of infection in replacement breeding and weaned pigs, elimination of *Salmonella* from pig holdings is unlikely to be possible in most countries. C&D is a useful measure to reduce the proportion of infected pigs prior to slaughter, but is only one of many combinations of measures needed to minimise *Salmonella* contamination of pig meat. Abstract adapted from Martelli *et al* [9], where further details of the study are presented.

Acknowledgements

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NEW INNOVATIVE FEEDING STRATEGY FOR REDUCTION OF *SALMONELLA* IN SWINE

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Introduction

Salmonella sp. are a leading cause of gastro-intestinal disease in humans with tens of millions of human cases worldwide every year. Pork is an important food vehicle of Salmonellosis, resulting in fever, abdominal cramps, diarrhea, vomiting, and occasionally death. Reduction of Salmonella in the feed-to-food chain is key to reduce the number of human Salmonellosis cases. Furthermore, it has frequently been demonstrated that the use of antimicrobial agents in food animals favors the development of resistance among foodborne pathogens like Salmonella spp. (WHO, 2012). Therefore, there is an urgent need for new antibacterial strategies to reduce Salmonellosis, since antimicrobial resistance is on the rise. Due to their bacteriostatic and bacteriolytic properties, organic acids are frequently used to control *Salmonella* in the feed production process. Organic acids increase stomach barrier function by reducing stomach pH and destabilize bacterial membrane function resulting in bacterial cell death. New strategies focus on the reduction of *Salmonella* in the intestinal tract by competitive binding of bacteria, and support of intestinal function to reduce colonization. Natural food and feed compounds such as processed grains and fungal extracts are new innovative ingredients that can have high potential in combination with organic acids. Mannobiose obtained by mannanase-hydolysation of copra meal has binding properties to bacteria as well as immunomodulatory properties. Edible mushroom extracts have currently been a hot topic and many prophylactic and therapeutic properties are described, including antimicrobial and immunomodulatory. This research evaluates the ability of hydrolyzed copra meal and rye overgrown with the mycelium of *Agaricus Subrufescens* to reduce *Salmonella* colonization in pigs and compares its efficacy to other currently used products.

Materials and methods

Twenty compounds were evaluated on their ability to bind *Salmonella* typhimurium in an *in vitro* adhesion assay. Besides hydrolyzed copra meal and rye overgrown with mycelium, commercially available compounds were tested including yeast cell wall products, deactivated yeast cells, yeast fermented products, yeast derived products, yeast cell product, gluco-mannans, gluco-oligosaccharides (GOS), mannan-oligosaccharides (MOS), xylo-oligosaccharides (XOS), chitosan-oligosaccharids (COS), 1,3-1,6-beta-D-glucan, and specific dietary fibre abstract.

The compounds were suspended in PBS to a final concentration of 1 % and centrifuged for 5 minutes at 460 g at 20ºC. The supernatant was transferred to a 96 wells plate (MicrolonF) to coat the wells and incubated overnight at 4ºC. The plates were washed with PBS, blocked with 1 % bovine serum albumin and incubated for 1 hour. Subsequently the plates were washed with PBS and *Salmonella typhimurium* culture (OD600 of 0.02) was added to the plates and incubated for 30 minutes and

washed again with PBS. Than growth medium was added to the plate and the plate was incubated at 37ºC. Optical density was read continuously at a wavelength of 600 nm using a microplate reader (Spectramax) and time to onset OD600 0.5 was determined. Well performing components were tested a second time and an average growth time relative to the control group value was determined for all components after two assays.

Efficacy of hydrolyzed copra meal and rye overgrown with mycelium was evaluated in a *Salmonella* typhimurium (ST) challenge pig study. Efficacy was compared to efficacy of the best performing candidate in the *in vitro* assay and fat coated butyrate, a frequently used anti-Salmonella strategy in poultry which alters Salmonella virulence properties and reduces colonization in pigs (Boyen et al., 2008). All four components were combined with a blend of formic and lactic acid and fed from weaning until the end of the study. The acid blend was mixed in the feed at 4 kg/ton; fat coated butyrate at 6 kg/ton; the hydrolyzed copra-meal at 1 kg/ton; the rye overgrown with mycelium at 2 kg/ton; and the mannan-oligosaccharides at 2 kg/ton.

Forty pigs were housed individually directly after weaning and equally divided over five different treatment groups: a control group, a group receiving the acid blend with fat coated butyrate, a group receiving the acid blend with hydrolyzed copra meal, a group receiving the acid blend with rye overgrown with mycelium and a group receiving the acid blend with mannan-oligosaccharides. Pigs of all treatment groups got infected with 10^9 CFU *Salmonella typhimurium* in a feed matrix which was applied from ten days after weaning for seven consecutive days. Body temperature, fecal consistency scores, performance and Salmonella fecal shedding were evaluated.

Results

Three of the best-performing compounds: hydrolyzed copra meal, rye overgrown with mycelium performed and MOS (mannan-oligosaccharides) were further evaluated in the Salmonella infection model. Mannan-oligosaccharides performed better than gluco-mannans or oligosaccharides from other sources. Other well-performing compounds were 1,3-1,6-beta-D-glucan and dietary fibre extract. Binding capacity of yeast products was very variable.

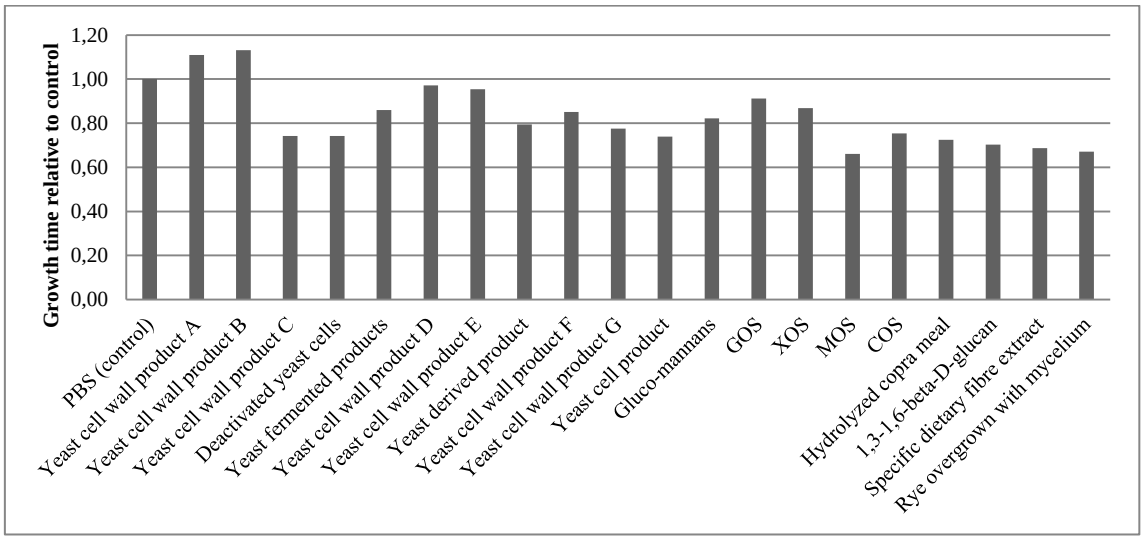

Figure 1. Growth time of bacteria to OD600 after adhesion to substrate (relative to control).

Pigs from the *in vivo* experiment got a slight fever during the first days after infection, which was not influenced by the different treatments. A peak in Salmonella shedding occurred during day 2- 4 after infection. The combination of the acid blend with hydrolyzed copra meal as well as with rye overgrown with mycelium significantly ($p<0.05$) reduced this peak in shedding (figure 2). The acid blend combined with mannan-oligosaccharides showed a numerical reduction; however, this was not significant. The combination of the acid blend with fat coated butyrate did not show a clear effect on Salmonella peak shedding. Pigs receiving rye overgrown with mycelium showed the lowest fecal consistency scores, however these did not significantly differ from the control group (figure 3).

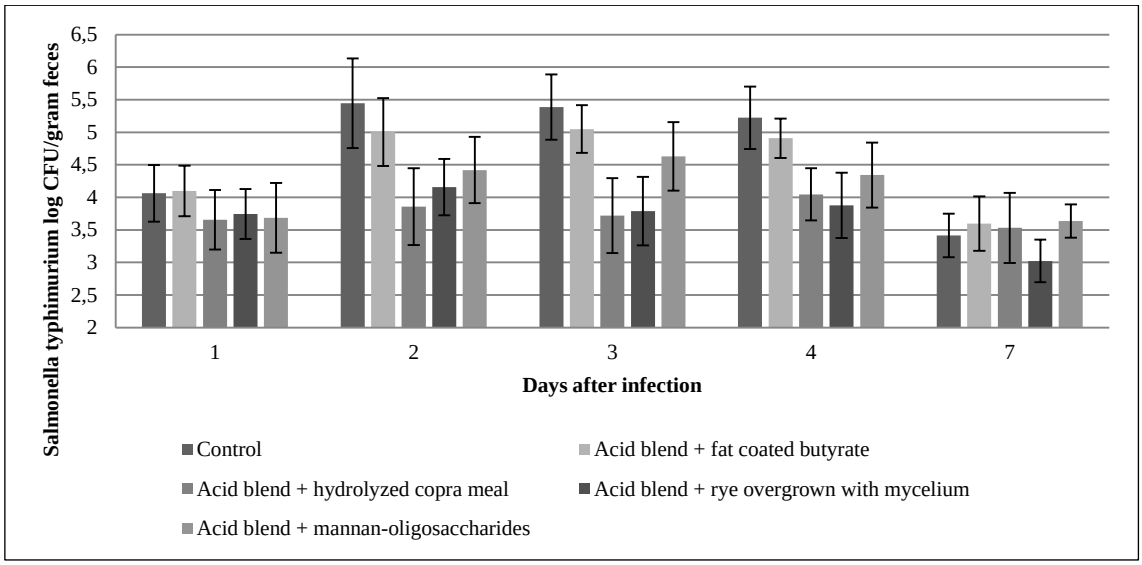

Figure 2. Fecal *Salmonella typhimurium* shedding during infection (n=8).

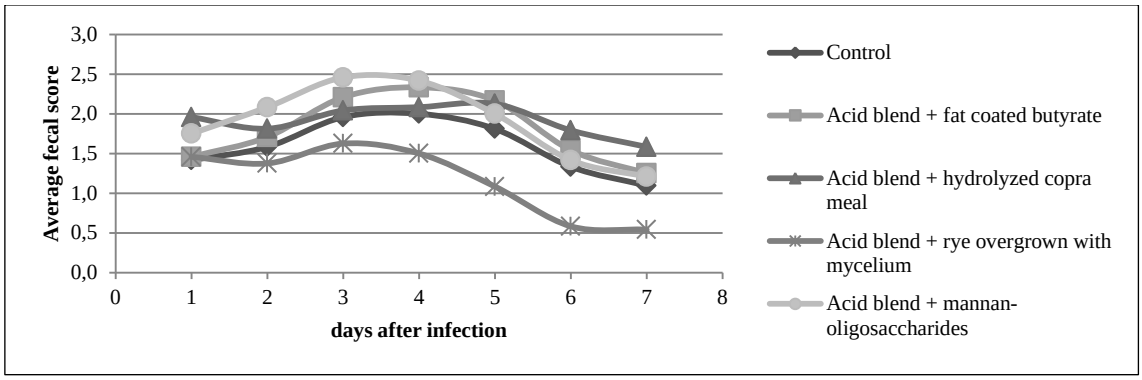

Figure 3. Average fecal consistency scores of piglets during infection (0 = normal feces; 1= flat feces; 2= diarrhea; 3= watery diarrhea)

Average daily gain and feed efficacy tended to improve compared to the control group when feeding rye overgrown with mycelium during the week of infection ($p<0.10$; figure 4).

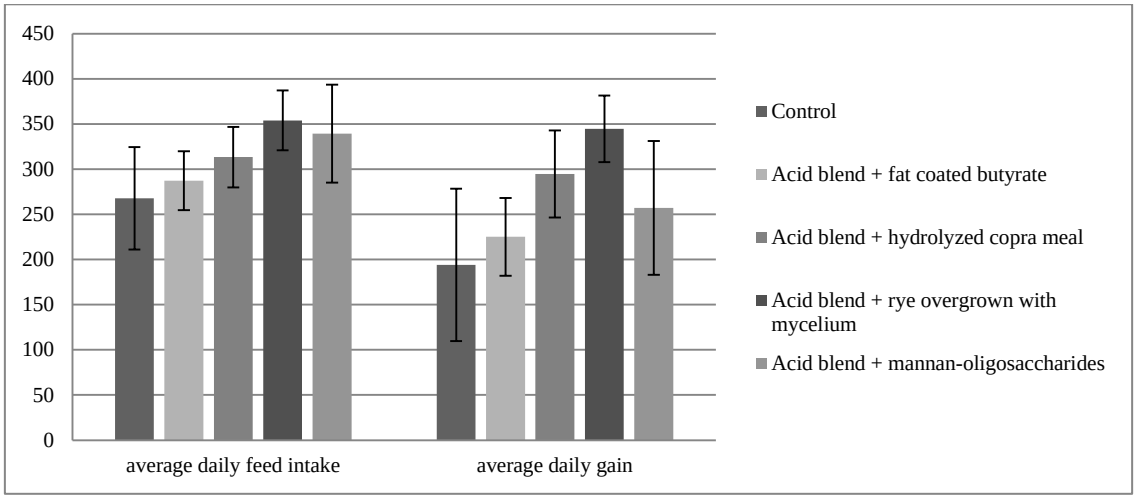

Figure 4. Performance of piglets during infection.

Discussion and conclusion

Both hydrolyzed copra meal and rye overgrown with mycelium performed well in the adhesion assay. Also in the *in vivo* assay, both hydrolyzed copra meal and rye overgrown with mycelium showed a significant reduction in Salmonella shedding in pigs. The variability in binding capacity of yeast products in the adhesion assay may dependent on cell wall structure, strain diversity, structural diversity, structural surroundings, and non-specific interactions (Ganner et al., 2013). Yeast-derived 1,3-1,6-beta-D-glucan and mannan-oligosaccharides which seem to be responsible for the binding of yeast products to *Salmonella typhimurium* indeed strongly did bind the bacterium in the adhesion assay. Mannan-oligosaccharides performed better than gluco-mannans or other oligosaccharides in the adhesion assay and previously proved to be effective in a field study (Andrés-Barranco et al., 2015). However, in this study, infection dose of pigs was higher and number of animals was lower, which may be the reason that the product only numerically reduced Salmonella shedding *in vivo*.

Many previous performed studies have shown a reduction in Salmonella shedding after the use of butyrate (de Ridder et al., 2013; Lynch et al., 2017; Walia et al., 2016). Butyrate did not significantly reduce colonization in this study, possibly because the infection dose of Salmonella used in this study was higher than in previous studies using seeder birds or field data instead of direct infection with a high inoculation dose. Therefore, it seems that both hydrolyzed copra meal and rye overgrown with mycelium have a stronger effect on Salmonella shedding than currently used anti-Salmonella strategies and therefore are promising building blocks of new strategies to control *Salmonella* in the swine production chain.

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MATERNAL VACCINATION AS AN EFFECTIVE *SALMONELLA* REDUCTION STRATEGY

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Introduction

Salmonella is a widespread pathogen that can infect a variety of animals, including man. Pork is considered, after eggs, the major source of infection in humans in the EU, with *S.* Typhimurium (ST), including monophasic strains (mST; *S.* 1,4,[5],12:i- and *S.* 1,4,12:i-) being frequently implicated [1]. Reducing the prevalence of infected pigs on-farm contributes to minimising contamination of pig meat and edible offal for human consumption, as the slaughter process alone cannot cope with high levels of contamination. The persistent and frequently asymptomatic nature of porcine *Salmonella* infection, the organism's colonisation of farm pests, such as rodents and wild birds, and ability to survive in the environment, means that effective control generally requires multiple measures [2]. It is generally accepted that vaccination can play a role in reducing the prevalence of *Salmonella* in pigs and could assist other on-farm control measures by helping to prevent *Salmonella* colonizing the gut and reducing the subsequent shedding and development of a carrier state [3]. Several vaccines for *Salmonella* have been developed; from inactivated bacterins to elicit a humoral response, to live or adjuvanted vaccines that can stimulate cell-mediated immunity. A recent study examined the vaccination of sows in three farms with follow-up of the breeding and rearing animals for up to two years after the initial pre-vaccination visit [4]. The study provided evidence for sustained reductions of ST and mST-shedding among pigs up to slaughter age, although it was based on an uncontrolled observational field study. This project was tasked with investigating the effectiveness of sow vaccination with a live *Salmonella* Typhimurium vaccine by comparing results from eight indoor farrow-to-finish herds that were vaccinated and eight similar control herds.

Material and methods

Farms were selected based on the following inclusion criteria: (i) indoor breeder-finisher enterprise, (ii) Herd size of 100-600 sows, (iii) a recent occurrence of ST or mST, (iv) presence of ST or mST in finishing pigs, (v) sows free of significant clinical disease which may have affected the efficacy of the vaccine. Herds were randomised into vaccinated (n=8) and non-vaccinated control groups (n=8). Herds were followed for approximately 69 weeks after the start of the trial, with four sampling visits. Sows were vaccinated with a live attenuated vaccine by subcutaneous injection (Salmoporc STM, IDT Biologika GmbH, Dessau-Rosslau, Germany). Vaccine was administered to pre-partum sows (6 weeks and 3 weeks ante-partum) with a single booster dose three weeks before each subsequent farrowing. The first dose was given to the first batch of

sows in week 1, with sampling visits taking place prior to vaccination (week 0); at a point when half of the progeny were estimated to originate from vaccinated sows (week 21); when all of the finishers were from vaccinated sows (week 55); and a final sampling visit three to four months after visit 3 (week 69).

A target of sixty individual floor faeces samples were collected at each visit from each of the following pig stages: weaners, growers, and finishers, providing a 95% probability of detection per group, assuming a 5% prevalence. In addition, pooled pen faeces samples (one or two pools per pen according to the number of pigs in the pen) were taken from the following pig stages: gestation, farrowing, weaners, growers, finishers and a combination of dry sows, gilts and boars. For each pig stage, up to a maximum of 20 pooled samples were collected per building and 60 per pig stage to ensure effective detection of *Salmonella* prevalence and diversity of serovars across the farm. In addition, wildlife and environmental samples (wildlife faeces, pooled water, etc) were collected at each visit. Material was cultured for *Salmonella*, using a modification of the ISO 6579:2002 (Annex D) method, as described previously [5]. Briefly, all pooled faeces samples (approximately 25 g) and swabs were pre-enriched in 225 ml BPW at 37ºC for 18 h followed by enrichment in Modified Semi-Solid Rappaport-Vassiliadis medium (MRSV) for 24h and 48h at 41.5ºC then plating on Rambach agar which was incubated for 24h at 37°C. Sub-samples (2 g) of individual pig faeces samples were pre-enriched in 20 ml BPW and cultured as above. For *Salmonella*-positive individual faeces samples, a subset from each farm, building and epidemiological group sampled was subjected to a semi-quantitative enumeration procedure by creating a decimal dilution series in BPW immediately before pre-enrichment. A selection (all isolates from pooled samples and individual samples that was cultured semi-quantitatively) of *Salmonella* isolates were serotyped using standard methodology.

A mixed-effects logistic regression model was used to assess the effect of vaccination, to examine the association between time from the start of vaccination and the odds of a sample being *Salmonella*-positive. The *a priori* variables were pig stage from which the sample was collected (named pig type), sample type (individual or pooled) and sampling season (winter (Dec-Feb), spring (Mar-May), summer (Jun-Aug) and autumn (Sep-Nov)). The farm study identifier was added as a random effect to account for the non-independence of sample results from the same farm. An interaction term, including visit number and experimental group (vaccine or control), was added. Two outcomes were tested in the model: presence of all *Salmonella* or presence of only serovars of public health concern (ST/mST). All analyses were performed in Stata 12 (StataCorp, College Station, Texas, USA).

Results

A total of 22,246 samples (9,747 pooled faeces samples, 10,905 individual faeces samples and 1,594 environmental samples) were collected from farm visits conducted between April 2014 and May 2016. The initial visit (visit 1) results demonstrated a similar high prevalence of *Salmonella* from faeces samples in both vaccine and control groups; 30.8% vs 36.2% of pooled samples, 19.1% vs 21.9% of individual samples, and 34.6% vs 53.0% of environmental samples, for vaccine and control groups respectively (Table 1). Clinical problems associated with *Salmonella* infections were reported from six vaccine and three control farms respectively at visit 1.

Table 1. Results from the pooled and individual faecal samples and environmental samples collected for the evaluation of the protection against *Salmonella* Typhimurium and its monophasic variants conferred by a vaccine administered to sows on eight pig herds and compared to eight control farms. *Salmonella* vaccination commenced between the first and second visit (N: total number of samples.)

***Salmonella*-positive**												
Visit	Pooled samples				Individual samples				Environmental samples			
	Vaccine		Control		Vaccine		Control		Vaccine		Control	
	N	%	N	%	N	%	N	%	N	%	N	%
1	1,297	30.8	1,169	36.2	1,430	19.1	1,062	21.9	238	34.6	160	53.0
2	1,268	28.2	1,240	32.0	1,429	20.0	1,382	26.9	201	29.2	159	47.4
3	1,279	26.1	1,178	31.4	1,394	20.6	1,360	26.8	188	31.3	228	40.6
4	1,288	19.8	1,028	41.0	1,423	13.4	1,425	32.0	208	21.2	212	42.8
***S.* Typhimurium and monophasic variants -positive**												
Visit	Pooled samples				Individual samples				Environmental samples			
	Vaccine		Control		Vaccine		Control		Vaccine		Control	
	N	%	N	%	N	%	N	%	N	%	N	%
1	1,297	26.6	1,169	31.3	1,430	17.8	1,062	21.7	238	30.1	160	46.3
2	1,268	26.4	1,240	26.0	1,429	19.5	1,382	23.2	201	28.7	159	40.8
3	1,279	24.2	1,178	27.8	1,394	18.7	1,360	23.8	188	27.2	228	36.6
4	1,288	19.1	1,028	34.3	1,423	13.2	1,425	27.0	208	20.6	212	35.8

At the second and third visits, following the start of the vaccination programme, reduction in prevalence of *Salmonella* and ST/mST was not apparent in control farms. However, vaccine farms showed sustained reduction of *Salmonella* prevalence up to the final visit. In addition, a higher proportion of vaccine farms (5 farms out of 6) resolved clinical salmonellosis than control farms (2 of 3). The effect of vaccination was not consistent on all farms; in one farm prevalence increased at visit 2 and this rise was sustained up to the final visit for both pooled samples and individual samples. Another vaccine farm showed only a slight reduction after vaccination, with a similar sample prevalence observed at visits 2 and 3 to that at visit 1. The results of *Salmonella* enumeration in positive faecal samples showed no apparent significant effect of vaccination.

The mixed-effects models showed a significantly decreased odds ratio of *Salmonella*-positive (Odds Ratio (OR) = 0.73, $p<0.001$) and ST/mST-positive samples (OR = 0.71, $P<0.001$) for vaccine farms in comparison to control farms and that samples collected at visit 2 where at significantly lower odds for both outcomes than at the first visit. Examining the interaction between the experimental groups and visit number showed there was a significantly decreased odds of *Salmonella*-positive (OR = 0.51, $p<0.001$) or ST/mST-positive (OR = 0.61, $p<0.001$) at visit 4 for vaccine farms only. The inclusion of the *a priori* variables accounted for a significantly increased odds of isolation of *Salmonella* and ST/mST in pooled samples. There was a significantly increased odds of isolation in summer of *Salmonella* and ST/mST and an increase in spring and autumn for ST/mST-positive when compared with winter. Finally, the model showed significantly increased odds of *Salmonella*-positive and ST/mST-positive samples for all pig group types (except boars) and significantly reduced odds for farrowing groups, when compared against the gestation group.

Conclusion

The significant results of the mixed-effects model have demonstrated that the strategy of maternal vaccination against ST is able to reduce, in a substantial proportion of treated farms, both faecal and environmental prevalence of *Salmonella* in farrow-to-finish pig herds. Although a beneficial association between vaccination and *Salmonella* reduction was observed, vaccination strategies alone were not sufficient to eliminate infection and vaccines should preferably be applied to uninfected animals on a preventative basis rather than in the face in infection [6].

Vaccinal protection of sows is particularly relevant in farrow-to-finish pig herds where breeders and finishers are housed in the same environment and weaned pigs present a continuous source of environmental contamination with ST or mST. Once all sows were vaccinated a reduction in *Salmonella* prevalence was observed in all stages of pig production, and mainly in finishers, hence reducing the *Salmonella* burden before slaughter. Previous findings have also shown that pigs born from vaccinated sows have reduced *Salmonella* faecal shedding and that the effect on environmental contamination and re-cycling of infection is also important [4].

However, the *Salmonella* prevalence reduction observed in the vaccinated farms was not observed in all herds, and this is consistent with other studies. De Ridder et al [7] observed response variability after oral vaccinated of piglets with the same product on three farrow-to-finish pig herds. In our study, vaccination did not have a marked effect on two herds which had clinical salmonellosis reported before the start of vaccination, which may have represented a recent outbreak caused by a new strain and presenting an overwhelming challenge for the vaccine within the timescale of the study. Our results provide evidence that maternal vaccination on a farrow-to-finish pig herd was a suitable ST/ mST reduction strategy and helped to control clinical salmonellosis. *Salmonella* vaccines therefore have the potential to reduce prevalence of *Salmonella* in pigs and result in a reduction of human cases.

Acknowledgements

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SALMONELLA TYPHIMURIUM FECAL SHEEDING FOLLOWING SALMONELLA CHOLERAESUIS-TYPHIMURIUM VACCINATION VIA DRINKING WATER AND SUBSEQUENT CHALLENGE

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Introduction

Salmonella typhimurium (ST) is a primary cause of enteritis and subclinical production losses in growing or finishing swine. Due to the zoonotic potential, intervention programs for ST have been established attempting to reduce carcass contamination. The objective of this study was to evaluate Salmonella fecal shedding of pigs vaccinated with a commercial, avirulent live culture (ALC) Salmonella Choleraesuis-Typhimurium vaccine when challenged with virulent ST.

Materials and methods

Eight litters of two-week-old pigs were blocked by litter and 3 pigs/litter assigned to treatment groups; ALC vaccine group (n=24) and a placebo group (n=24). Both treatments were administered through the drinking water. Pigs were housed by treatment during the vaccination phase to avoid unintentional exposure of ALC vaccine to the placebo group and were re-penned individually with treatments comingled in the same room for the challenge phase. Four weeks after treatment, all pigs were challenged intranasally with 2mL of virulent ST (4x108 CFU/dose). Fecal samples were collected daily for 14 days post-challenge (DPC) then three times weekly until 84DPC. Fecal samples were tested via modified enrichment culture (lower detection limit ~4000CFU/gram).

Results

During the 12-week challenge phase, the mean number of positive samples/pig was 26.2 (placebo) and 15.9 (vaccine) which was a significant improvement. All placebo pigs were positive from 3DPC to 6DPC, and ≥78.3% of placebo pigs continued to shed until 31DPC; then at least 47.8% of placebo pigs shed from 33DPC to 49DPC. For Vaccine pigs, 82.6% were positive the day after challenge which steadily declined to ≤13.0% from 47DPC through the end of the study.

Discussion

The vaccine group had significantly reduced shedding within two to six weeks post-challenge while several pigs in the placebo group continually shed through the challenge phase. Preliminary data suggests that the use of an ALC Salmonella Choleraesuis-Typhimurium vaccine clinically reduces fecal shedding in pigs post-challenge.

INFLUENCE OF DIFFERENT VACCINATION STRATEGIES AGAINST *SALMONELLA* TYPHIMURIUM IN PIG FARMS ON THE NUMBER OF CARRIERS IN ILEOCECAL LYMPH NODES

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Introduction

Persistent *Salmonella* Typhimurium (ST) infections in pigs are characterized by chronic colonization of the lymphoid tissue and constitute a major source of human Salmonellosis. The present study investigated to which extend different vaccination strategies against ST reduce the number of carriers in ileocecal lymph nodes.

Materials and methods

Five different vaccination strategies were tested on three Belgian pig farms: 1. vaccination of sows; 2. vaccination of sows and piglets; 3. vaccination of sows and fatteners; 4. vaccination of piglets; 5. vaccination of fatteners. A comparison was made with a non-vaccinated control group (group 6). Each vaccination strategy was implemented in each farm. An attenuated vaccine (Salmoporc®, IDT Biologika) was applied twice with an interval of three weeks (sows and fatteners: subcutaneously, piglets: orally). Ileocecal lymph nodes were collected in the slaughterhouse and tested for the presence of *Salmonella* according to ISO 6579:2002. *Salmonella* isolates were classically serotyped (slide agglutination) or mPCR for identification of *Salmonella* genus and ST was used. To distinguish field and vaccine strains, ST isolates were tested with the "IDT *Salmonella* Diagnostikum®" kit, based on the growth requirements of the auxotrophic vaccine strain.

Results

In total, 1098 lymph nodes were collected (farm 1: 576, farm 2: 74, farm 3: 448). The overall percentage of ST field strain positive lymph nodes was low in all three farms: 3% in farm 1 and 2 and 8% in farm 3. The percentages ST field strain positive lymph nodes per treatment group (1-6) on farms 1, 2 and 3 were respectively: 1-1-0-8-0-6, 0-0-10-0-4-0 and 4-2-12-14-9- 2. Isolates from 7 lymph nodes (0.6%) originating from vaccinated pigs (groups 2-3-4-5) tested positive for the vaccine strain.

Table 1. *Salmonella* Typhimurium field strain positive lymph nodes per treatment group per farm.

Group	Farm 1			Farm 2			Farm 3		
	# analyzed	# positive	% positive	# analyzed	# positive	% positive	# analyzed	# positive	% positive
1	78	1	1%	19	0	0%	69	3	4%
2	74	1	1%	10	0	0%	95	2	2%
3	112	0	0%	10	1	10%	85	10	12%
4	115	9	8%	1	0	0%	78	11	14%
5	93	0	0%	27	1	4%	74	7	9%
6	104	6	6%	7	0	0%	47	1	2%
Total	576	17	3%	74	2	3%	448	34	8%

Conclusion

At farms with a relatively low prevalence of ST field strain carriers in ileocecal lymph nodes, vaccination does not seem to be the measure to be advocated to further reduce this prevalence.

VACCINATION AGAINST *LAWSONIA INTRACELLULARIS* DECREASES SHEDDING OF *SALMONELLA ENTERICA* SEROVAR TYPHIMURIUM IN CO-INFECTED PIGS AND CHANGES THE HOST GUT MICROBIOME

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Introduction

Salmonella enterica is a leading cause of foodborne illness in the world. Attribution studies have suggested pork as a major source for human salmonellosis in different countries (Mughini-Gras et al., 2013, Pires et al., 2014). In the United States, efforts to reduce the incidence of salmonellosis have mainly remained ineffective, the incidence of salmonellosis has not been reduced appreciably since 1996 (Boore et al., 2015). There is a consensus in the field that there is a lack of on farm cost-effective strategies to reduce the prevalence of salmonellosis (Dickson & Hurd, 2013). The source of *S. enterica* that contaminates pork products are the animals themselves (Dickson & Hurd, 2013) and novel intervention strategies are much needed.

Like *S. enterica*, *Lawsonia intracellularis* is a common porcine intestinal pathogen and is prevalent in pig production sites worldwide with prevalence ranging from 48 to 100% in different swine producing countries (Dors et al., 2015). In the United States, it has been estimated that *L. intracellularis* is present in more than 90% of swine farms (Armbruster et al., 2007). The last study conducted by the USDA found that *S. enterica* was present in 52.6% of swine productions sites (USDA, 2009), thus it is reasonable to assume that co-infection with both pathogens occurs frequently. *L. intracellularis* causes porcine proliferative enteropathy (PPE). This disease more commonly occurs in post-weaned pigs and leads to decreased weight gain, diarrhea and is often subclinical. Transmission of this organism also occurs by the fecal oral route and lesions are marked by a thickening of the mucosa of the ileum and colon (Lawson & Gebhart, 2000).

The association between *L. intracellularis* infection and increased shedding of *Salmonella* was first demonstrated by Beloeil et al., 2004, who performed an epidemiological study and found a significant association between seroconversion to *L. intracellularis* and increased prevalence of pigs shedding *S. enterica*. In this study, we hypothesized that vaccination against *L. intracellularis* would decrease shedding of *S. enterica* in co-infected animals.

Materials and methods

Animals and experimental design

In this study a total of five treatment groups were used. The treatment groups were: 1) challenged with *S.* Typhimurium alone, 2) challenged with both *S.* Typhimurium and *L. intracellularis*, 3) challenged with *S.* Typhimurium and vaccinated against *L. intracellularis*, 4) challenged with both *S.* Typhimurium and *L. intracellularis* and vaccinated against *L. intracellularis*, and 5) a non-infected control. Each treatment group was comprised of 9 pigs housed in three separate isolation rooms with three

animals per room. The non-challenge control group was comprised of 6 animals divided among two rooms with three animals per room. These rooms were distributed between two isolation buildings. The groups that were vaccinated against *L. intracellularis* received the single dose oral live attenuated vaccine Enterisol Ileitis (Boehringer Ingelheim) at three weeks of age. Twenty-one days post vaccination, animals were challenged with a pure culture of *L. intracellularis* (2×10^9 organisms per pig) (strain PHE/MN1-00). One week post *L. intracellularis* challenge, pigs were challenged orally with *S.* Typhimurium (strain 798) (1×10^8 organisms per pig). Fecal samples from pigs were obtained on the day of challenge and two days post *S.* Typhimurium challenge and weekly thereafter until 49 days post infection.

Salmonella quantification

To quantify the amount of *Salmonella* shed in feces of pigs, a most probable number (MPN) enrichment method was used as in Borewicz et al., 2015. Briefly, one gram of feces was suspended in 9 ml tetrathionate broth (TTB) and incubated at 41°C for 48 hours. One hundred µl was then transferred to 900 µl Rappaport-Vassiliadis R10 broth and incubated for 24 hours at 41°C. Cultures were then inoculated on to XLT4 agar plates containing 100µg/ml of nalidixic acid (NA) to quantify the challenge strain which is NA resistant. A duplicate inoculation was performed on XLT4 agar without antibiotic to quantify any other potential *Salmonella* strains the animals could harbor. Colonies with typical *Salmonella* morphology were confirmed by PCR using primers specific for the gene *invA* (Singer et al., 2006).

DNA extraction and 16S sequencing

For microbiome analysis, DNA was extracted from fecal samples using the MoBio PowerSoil DNA extraction kit. DNA concentration was measured by Nanodrop. The V1-V3 region of the 16SrRNA gene was amplified following a dual indexing approach as described in Gohl et al., 2016. Quality filtered sequences were analyzed with QIIME (Caporaso et. al, 2010).

Statistical analysis

To test for differences in MPN between treatments over time, a linear mixed model was used with $\log_{10}$ (MPN) as the response, treatment, day, and the treatment/day interaction as fixed effects, barn as a fixed block effect, and pen, pig, and day within pen as random effects. Reported are least square means for treatment by day, and pairwise comparisons between treatments for each day, with p-values corrected for multiple comparisons.

Results and discussion

L. intracellularis vaccination reduces *S.* Typhimurium shedding in co-infected animals

No animals had detectable levels of the challenge strain prior to challenge and detection levels of *Salmonella* were similar in XLT4 with *S.* Typhimurium. The greatest difference in shedding level between groups was found at 7 days post infection. At this time point, the co-challenged non-vaccinated group shed 2.94 $\log_{10}$ *S.* Typhimurium

organisms per gram of feces while the vaccinated co-challenged group shed 0.82 log_{10} *S.* Typhimurium organisms per gram of feces (p=0.003) (Figure 1). The co-challenged vaccinated group also shed significantly less *S.* Typhimurium then the singly infected *S.* Typhimurium group which shed 2.44 Log_{10} *S.* Typhimurium organisms per gram (p=0.03). *L. intracellularis* vaccination did not have a significant impact on *S.* Typhimurium shedding when animals were singly infected with *S.* Typhimurium.

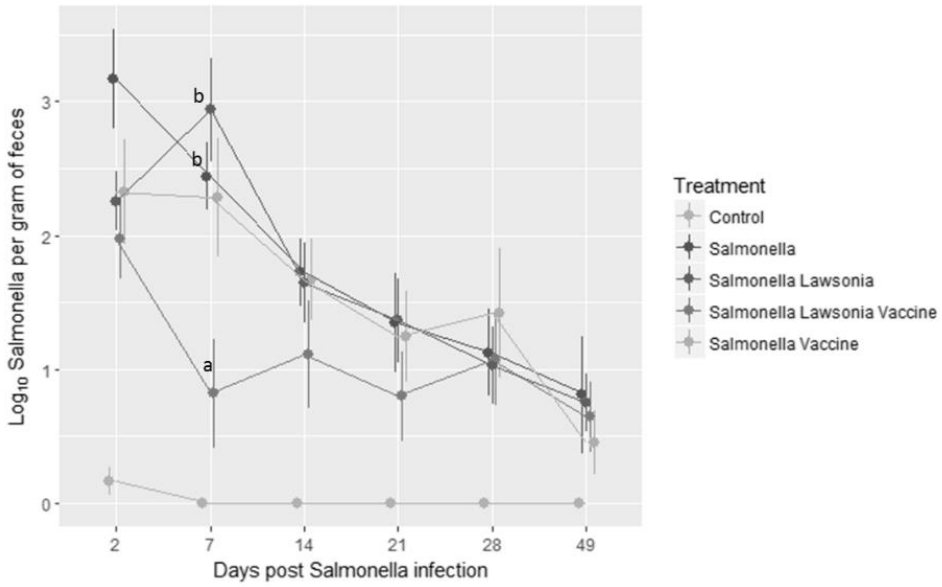

Figure 1. Fecal shedding of *Salmonella enterica* serovar Typhimurium measured by the MPN method. Line graph of *S. Typhimurium* shedding by group in different time points. Significant differences between treatment groups are designated by different letters ($P < 0.05$).

L. intracellularis vaccination alters the microbiome in co-infected animals

To investigate microbiome differences between treatment groups, beta diversity analysis was performed using the weighted UniFrac distance which measures dissimilarity in microbiomes based on their phylogenetic composition (Lozupone et al., 2007). Investigating the 7 day post infection timepoint, different treatment groups had significant differences in their microbiome community structure (ANOSIM $p<0.05$). The co-infected vaccinated group clustered apart from all other treatment groups. Again, this effect was dependent on an animal receiving both *S.* Typhimurium and *L. intracellularis* as well as prior *L. intracellularis* vaccination (Figure 2).

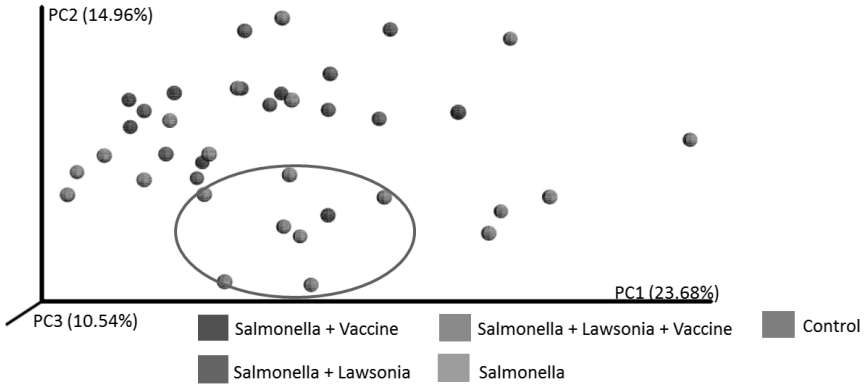

Figure 2. Beta diversity analysis, Principal coordinate analysis plot of weighted UniFrac distance among different treatment groups at 7 days post *S.* Typhimurium infection (ANOSIM $p < 0.05$).

Conclusion

Vaccination against *L. intracellularis* significantly reduced *S.* Typhimuirium shedding ($p<0.05$) in co-infected animals in comparison to the co-infected group without vaccination and the group challenged with *S.* Typhimuirium alone. Significant differences in beta diversity were found (ANOSIM $p < 0.05$) and the co-challenged vaccinated group had a distinct community structure form other groups demonstrating that co-challenge and vaccination lead to different changes in the microbiome compared to single or dual infection and vaccination without challenge. These results indicate that vaccination against *L. intracellularis* impacts the microbiome and reduces shedding of *S.* Typhimurium in co-infected animals. This evidence suggests that *L. intracellularis* vaccination may be used as novel tool to aid in the control of *Salmonella* on swine farms as well as an alternative to reduce the need for antibiotic treatment of pigs and improve food safety.

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DEVELOPMENT AND EVALUATION OF A NOVEL ORALLY ADMINISTERED SUBUNIT VACCINE TO CONTROL FOODBORNE PATHOGENS

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Introduction

Development of vaccines for effective control of foodborne pathogens and infection represents an important development in reducing public health risk. Advancements in the area of biotechnology have increased innovative potential and allow new technologies to be used as a promising control strategy for alternatives to antibiotics. We have been working to create a novel vaccine platform that incorporates a subunit/epitope sequence, common for all *E. coli* strains (broad spectrum), into an inactivated orally administered vaccine platform that protects against infection and disease by inducing mucosal immunity.

The mucous membranes constitute the major portal of entry for infectious agents and include membranes of the nasal, respiratory, gastrointestinal, and genitourinary tract; as well as the occular conjunctiva, the inner ear and the ducts of all exocrine glands. Collectively they cover more than $400m^2$ in humans, compared to only $2m^2$ of skin, and serve as the first line of defense against infection at the entry points for a variety of pathogens (Ogra et al., 2001). The gastrointestinal system is the largest lymphoid organ in the body containing an estimated 70% to 80% of the body's immunoglobulin–producing cells (Kaul 1999). 80% of all the activated B cells in the body are located at the mucosal tissues (Brandtzaeg et al., 1989) In fact the only way to contract an infection other than the mucosal portal of entry is through blood-borne vectors or damage to epithelial surfaces.

Despite its important role, currently only a handful of vaccines specifically target this area of the immune system despite strong evidence that a robust mucosal response can effectively prevent systemic infections (Ogra et al., 2001). Increasing evidence has indicated that mucosal vaccination can induce both systemic and local mucosal immunity, while systemic immunization generally fails to elicit strong mucosal immunity (Valosky et al., 2005). Also, the concept of a common mucosal immune system predicts that induction of immunity at one mucosal surface, such as the gut, can provide immunity at another mucosal surface, such as the lung (Cerkinsky et al., 1995) providing a necessary link for immunity transfer throughout mucosal surfaces. Mucosal immunity may prove to be the link in fighting a complex infection in which systemic and local immunity are necessary in preventing the spread and transmission of infectious disease and foodborne pathogens.

Pathogenic *E. coli* infections, or colibacillosis, is one of the most prevalent diseases affecting the global swine industry (Fairbrother J. et al., 2005). Enterotoxigenic *Escherichia coli* (ETEC) is a major cause of illness and death in neonatal and recently weaned pigs in some cases young pigs can lose up to 40% of their body weight and in severe cases mortality can reach 100% (USDA 2002). Colibacillosis not only has a direct economic impact on producers it also represents a potential human transmission route of foodborne illness. Common treatment options often include incorporation of antibiotics to control and limit spread of the disease; however, the disease is becoming

increasingly more difficult to treat due to acquired antibiotic resistance. Moreover, consumer pressure and changing government regulations may limit or omit the use of antibiotics necessitating the need for alternative intervention strategies.

Our vaccine development, in which a single vaccine, can simultaneously and effectively control all or the majority of serotypes/strains that make up the 150-200 serotypes that represent the *E. coli* family of pathogens (broad spectrum) and provide protection in multiple species (swine, poultry, bovine, fish, humans) potentially represents practical biotechnological progress as an alternative intervention strategy in controlling diseases and foodborne pathogens.

Materials and methods

Development of subunit orally administered inactive vaccine against E. coli spp. (Biotech Vac E. Coli)

Briefly vaccine construction was as follows: a synthetic, antigenic, epitope, genetic sequence common for *E. coli spp*. was inserted by direct ligation into a *Bacillus subtilis* expression plasmid. The genetic sequence was put under the control of an IPTG inducible promoter present on the expression plasmid and inserted into the multiple cloning site of the plasmid for Open Reading Frame expression. The modified expression plasmid was then transfected into *E. coli* TOPO 1 cells for confirmation of gene insert and multiplication of the plasmid. The multiplied confirmed plasmid was then isolated, concentrated and transfected into the vector *Bacillus subtilis* VBTSLL11™, a proprietary *Bacillus* strain selected specifically for use in the Biotech Vac platform. Once plasmid insertion was confirmed by colony PCR, DNA sequencing was performed to confirm correct genetic sequence and protein expression was quantified by SDS-PAGE western blotting. This newly constructed and verified *Bacillus* strain was used to manufacture the antigenic *E. coli* subunit. The bacteria were grown under normal Bacillus culture conditions in Tryptic Soy Broth (TSB) at 37°C, after 4 hours of growth the culture was induced with 1mM of Isopropyl β-D-1-thiogalactopyranoside (IPTG) followed by an additional 5 hours of growth. Once fermentation was complete the culture was inactivated and added to an encapsulation media for incorporation of the epitopes into micro-particles for oral delivery. Subunit concentration for each 2.0ml dose of vaccine is approximately 500ng.

Challenge with *Escherichia coli*

Two wild-type field isolates (VBTEcoli1-2) of Enterotoxigenic *E. coli* (ETEC) originally isolated from swine farms in Argentina were grown individually to log phase, combined, serially diluted, and enumerated by spectrophotometric density and comparison to a previously generated standard curve.

These strains were diluted to approximately 10^8cfu/ml for challenge by oral gavage at a dose of 2.0ml/pig for 2 consecutive days.

Vaccination study 1

In the first challenge trial, two groups of 3 day old piglets at a commercial production farm were assigned to one of two experimental groups, the piglet and sows in these two groups were isolated from the rest of the commercial farm. A non-treated

control group (n=13) that received 2.0ml of saline by oral gavage on days 3 and 14 of life or a treated group (n=12) that received 2.0ml of Biotech Vac E. coli vaccine by oral gavage on days 3 and 14 of life. All piglets in both groups were challenge with 2.0ml of ETEC *E. coli* by oral gavage (challenge preparation described above) on days 17 and 18 of life. Piglets were observed for 1 week following challenge and presence or absence of diarrhea was recorded daily. Additionally, individual weight gain was calculated. During the course of the experiment, neither the piglets nor the lactating sows received antibiotic treatment and antibiotics were not present in the commercial feed.

Vaccination study 2

In the second challenge trial, thirty 28 day old pigs from a commercial production farm were randomly assigned to either a non-treated control group or a Biotech Vac E. coli vaccine treated group (n=15/group), transferred to adjacent weaning boxes isolated from the rest of the commercial farm and allowed to acclimate for 5 days. Following the acclimation period (day 33 of life), pigs were either given by oral gavage 2.0ml saline (control group) or 2.0ml Biotech Vac E.coli. and subsequently administered the same treatment 10 days (day 43 of life) following the first administration. Three and four days following the second treatment administration (day 44 & 47 of life), all pigs in both groups were challenged with 2.0ml of ETEC E. coli by oral gavage (challenge preparation described above). Pigs in both groups were observed for 10 days following challenge and presence or absence and type of diarrhea was recorded daily. Pigs were fed standard commercial diets containing no antibiotics.

Results

Results from the 1st challenge trial done in newborn piglets under standard commercial production conditions showed that on day 20 48 hours after trial 100% (13/13) of the piglets in the control non-treated group had developed clinical signs of diarrhea consistent with Colibacillosis with the diarrhea continuing for 72 hours. While the piglets that were vaccinated with Biotech Vac E. Coli, exhibited no clinical signs and did not develop diarrheas throughout the 1 week observation period. Additionally, piglets in the Biotech Vac E. coli treated group had a slightly increased total weight gain and daily body weight gain when compared to the non-treated controls; 46.4kg vs 45.6kg respectively (total weight gain) and 1.93kg vs 1.90kg respectively (daily body weight gain). No mortality was observed in either experimental group.

In the 2nd challenge trial, conducted in weanling pigs, results demonstrated significant reductions in the percentage, severity and duration of ETEC associated diarrheas in the group vaccinated with Biotech Vac E. coli when compared to the non-treated control group under standard commercial production conditions. In the group treated with the vaccine 4 days after challenge 20% (3/15) of the pigs exhibited diarrheas lasting 1 day and 6.7% (1/15) of the pigs had mild diarrhea that lasted 3 days. Also, in the group receiving the vaccine, 1 pig exhibited severe diarrhea (perineal congestion) and ultimately was diagnosed with pneumonia. While in the non-treated control group:

- Day 3 post-challenge: 20% (2/15) of the pigs began with diarrhea.
- Day 4 post-challenge: 73% (11/15) of the pigs were present with diarrhea ranging in severity and consistency.
- Day 5 post-challenge: 73% (11/15) of the pigs were still present with diarrhea.

- Day 6 post-challenge: 20% (2/15) of the pigs continued to exhibit mild diarrhea.
- No mortality was observed in either experimental group.

Conclusions

Results of 2 separate ETEC *E. coli* challenge trials in commercial newborn piglets and weanling pigs that received two doses of Biotech Vac E. coli demonstrated: significant reductions of clinical symptoms associated with *E. coli*, significant reductions in the severity of *E. coli* associated diarrheas and a reduction in time in which the clinical signs and diarrheas persisted. These preliminary results suggest that our inactivated orally administered subunit vaccine platform offers a promising alternative for the control of infections and pathogens associated with foodborne diseases.

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A SUDDEN INCREASE IN SALMONELLA - HOW THE SURVEILLANCE SYSTEM REACTED: A DANISH EXPERIENCE

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Introduction

Denmark has had a serological surveillance of finisher herds since January 1995, and the current surveillance of Salmonella on carcasses has been in place since 2001. Both surveillances are parts of the Danish Action Plan for Salmonella in pigs and pork. Variations in the Salmonella prevalence are evaluated, and if a variation is exceptional, much effort is done to try to find the reason for the variation, to correct it and to learn from it.

In January 2016 there were an alarming high number of positive samples in the carcass surveillance, the highest monthly prevalence seen since the beginning of the surveillance. The serological surveillance showed only a slight and not alarming increase. However, the serological surveillance stayed at an increased level and in May it reached the highest value seen since the beginning of the serological surveillance. But that was with a delay of more than 3 months, compared to the increase in carcass prevalence.

A thorough evaluation of results was carried out during the first 6 months of 2016. The results from the serological surveillance of slaughter pigs and the bacteriological surveillance of pork were supplemented with porcine caecal samples from a national project. To get enough data a further 301 caecal samples were analyzed by the slaughterhouses.

Materials

In the surveillance of Salmonella on pig carcasses swab samples are taken from the carcasses 12 hours after chilling. Each carcass is sampled 4 places, 100 cm^2 each: on the hind leg near the tail, near the sternum, on the jowl and on the mid-back region. On each slaughterhouse 5 carcasses are sampled on each slaughter day and the samples are analyzed as one pooled sample providing one result per slaughter day. The results are evaluated daily at the slaughterhouses and once a month by Danish Agriculture and Food Council and by the Danish Veterinary and Food Administration.

In the serological surveillance a 10 g meat sample is taken from the neck muscle of randomly selected carcasses at the slaughterhouse, the samples are frozen and sent to the laboratory. The meat juice is analyzed for specific Salmonella antibodies by a mix-Elisa. The results are transferred to the Zoonosis Register owned by the authorities, where they are merged with the herd specific numbers and information sent from the slaughterhouses. The results are evaluated once a month.

National projects outline plans for caecal samples taken at the slaughterhouses. These samples are among others analyzed for Salmonella in accordance with NMKL Method no 187, 2nd Ed., 2016, and reported to the Danish Veterinary and Food Administration. The results are evaluated yearly.

Results

Carcass swabs

During January 2016 the slaughterhouses reported, that they found an alarming increase in the number of Salmonella positive samples from carcasses, Figure 1.

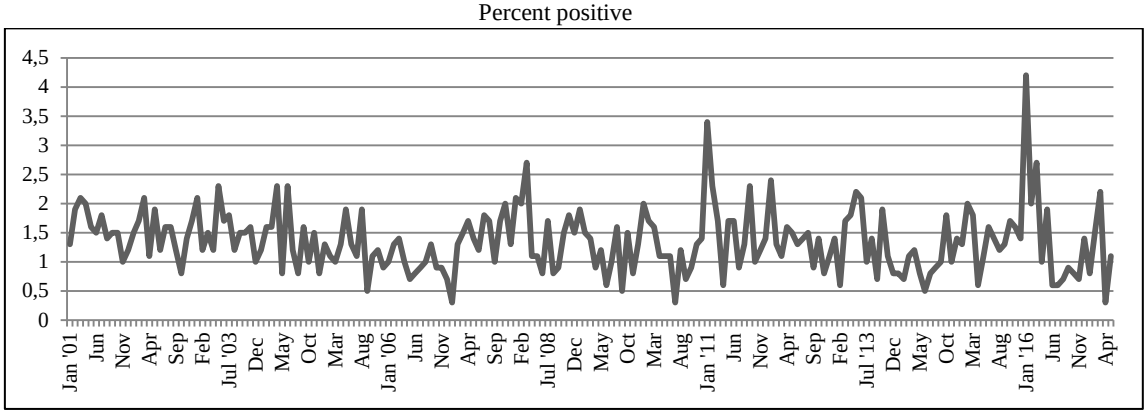

Figure 1. Salmonella on fresh pig carcasses (bacteriology).

The slaughterhouses could find reasons for some of the positive samples, but not for all of them. They reacted immediately by comprehensive sampling schemes and initiatives along the slaughter lines. This effort led to a decrease already the following month, and with minor fluctuations this decrease was maintained for the rest of the year.

From October 2015 to March 2016 a shift was seen in the prevalence of serotypes. In this period the predominant serotype was Typhimurium including the monophasic with a prevalence of 53 percent compared to a prevalence of 41 percent in the time leading up to October 2015. This shift has maintained up to the writing of this paper, data not shown.

Serological surveillance

The serological surveillance showed only a slight and not alarming increase in January 2016. However, the serological surveillance stayed at an increased level and in May it reached the highest value seen since the beginning of the surveillance. After May the prevalence of serologically positive samples declined to the level before the increase, Figure 2.

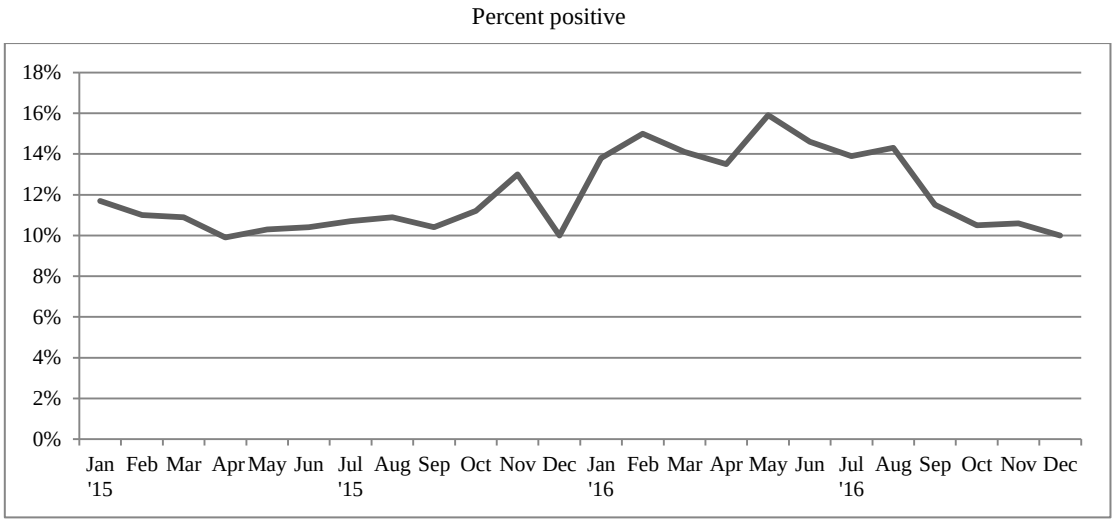

Figure 2. Increase in meat juice prevalence (serology).

Caecal samples

The first evaluations of the caecal samples showed no explanation for the increase in the positive carcass swabs at the slaughterhouses. The prevalence of almost 36 percent in March is uncertain as it is based on only 14 samples, Figure 3.

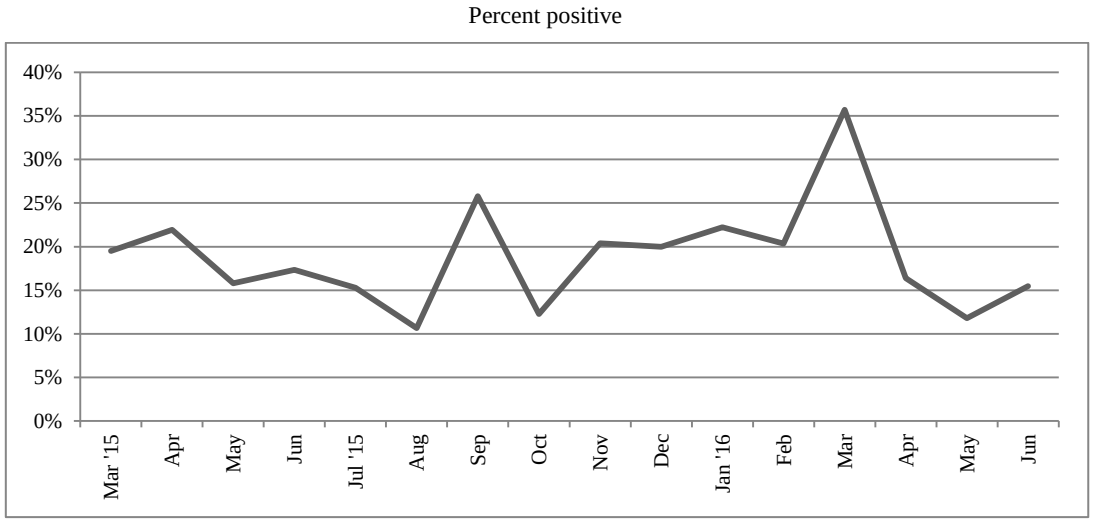

Figure 3. Salmonella positive caecal samples.

However, a more detailed evaluation of the results from the caecal samples revealed a transient increase in the prevalence of Typhimurium and monophasic in the finisher production from November 2015 till March 2016, Figure 4.

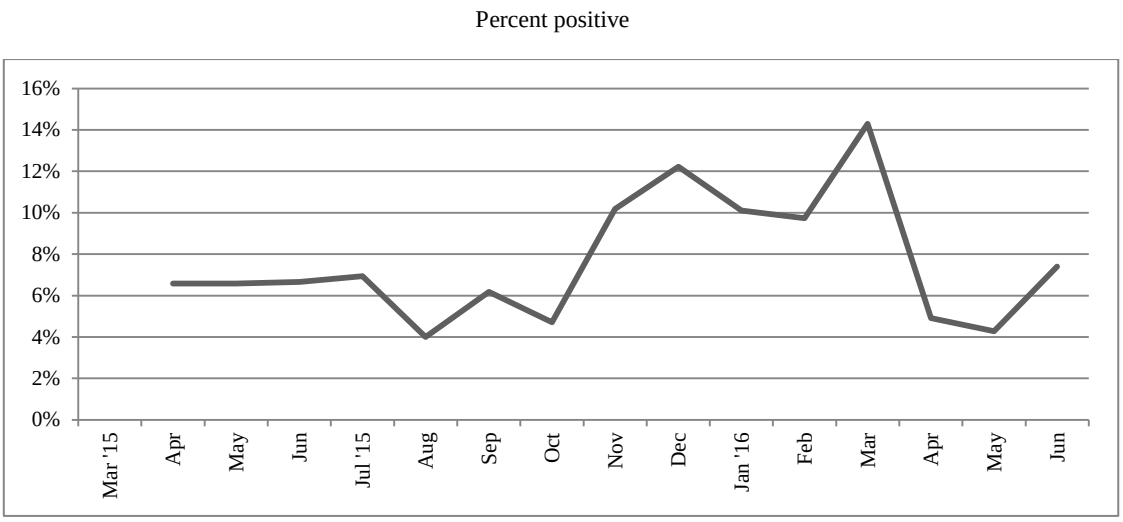

Figure 4. Positive caecal samples – Typhimurium and monofasic.

Antibiotic treatment

An extraction and analysis of data from the Danish Vetstat database revealed, that there had been an increase of about 10 percent in the use of antibiotics for treatment of gastrointestinal diseases in slaughter pigs in the months December 2015 and January 2016, Figure 5.

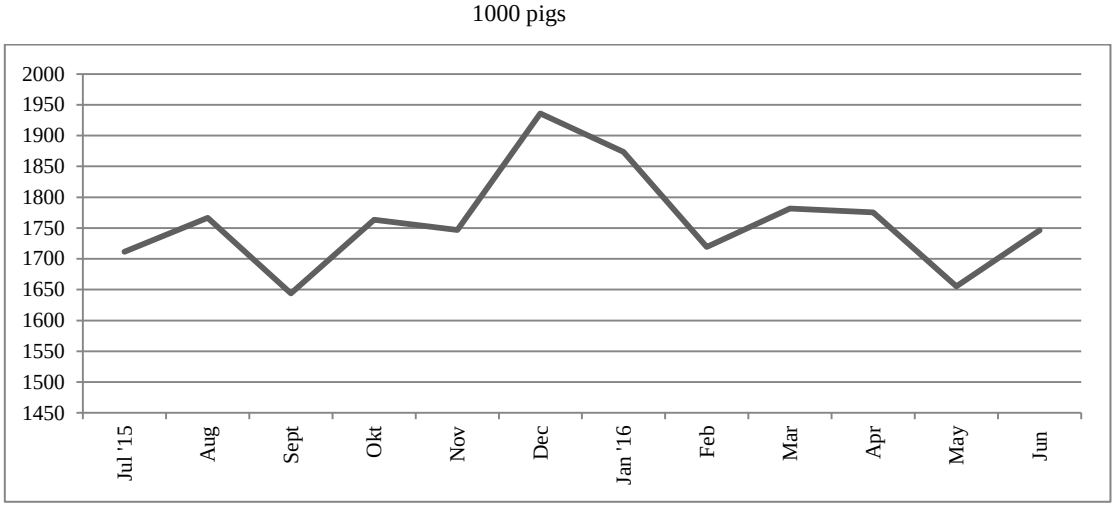

Figure 5. The number of 70 kg pigs treated with antibiotic for gastrointestinal diseases per month.

Discussion

The dramatic increase in the number of positive swab samples of carcasses appeared in January 2016. However, already in November 2015 a shift in the prevalence of serotypes started, whereby Typhimurium and monophasic became the predominant serotypes. The reason why the dramatic increase did not happen until January was not identified, but it may have been due to an increase in gastrointestinal disorders among the slaughter pigs. As the results of the swab samples are evaluated daily, it makes it possible for the slaughter houses to react promptly on an increase.

The serological surveillance is excellent to distinguish between low and high prevalent herds, but the test has several times proven to be unstable, so an increase always calls for considerations. However, all control procedures at the laboratory showed that the test performed within acceptable range. Therefore the increase in the prevalence of positive serological samples was considered to be real. However, as the serological prevalence is only calculated and evaluated once a month, there is some delay in the reaction to an increase. As there was no evident reason for the increase, it was decided to see, if the caecal samples could provide an explanation.

The increase in caecal samples positive with Typhimurium or monophasic started in November 2015, which is concurrent with the shift in serotypes from the carcass swabs. This support that the distribution of serotypes found at carcass swabs reflects the distribution of serotypes from the primary production.

When the increase in the prevalence of Typhimurium and monophasic was found in the caecal samples pig producers, veterinarians and feed companies were asked, if they had observed anything unusual. Some pig producers and veterinarians answered that they had observed diarrhea in slaughter pigs due to feed of poor quality, while others did not share this observation. The data from the Danish Vetstat database support the hypothesis that there had been gastrointestinal problems among the slaughter pigs around the turn of the year 2015. This may have led to loose faecal contents, which is a known challenge to slaughter hygiene.

Conclusions

This experience taught us that while investigations of signs of increase in Salmonella prevalence in the primary production take months, the surveillance at the slaughterhouses is able to react swiftly on an increase, when the input of Salmonella unexpectedly overloads the slaughter hygiene. This is of greatest importance to food safety, and thanks to the prompt reaction at the slaughterhouses, the Salmonella increase had no impact on the number of human cases of Salmonella infection in the beginning of the year.

A STUDY OF THE EFFECT OF MOVEMENT OF OUTDOOR PIGS TO A NEW SITE ON *SALMONELLA* PREVALENCE

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Introduction

Outdoor breeding of pigs in Great Britain constitutes a substantial proportion of the British pig industry, with at least 40% of the breeding herd kept outdoors. Outdoor production is viewed as more welfare friendly and forms a break between cereal crops. Outdoor pig production has a tendency for high *Salmonella* seroprevalence at slaughter [1], with evidence for a wide diversity of resident and transient infections on farm, often showing some overlap with local environmental and wildlife isolates [2]. Outdoor pigs may be at increased risk of infection due to: the lack of a controlled environment that can be cleaned and disinfected between batches of pigs; less control over exposure of pigs to factors such as cold and heat stress; and increased exposure to *Salmonella* through difficulties in applying biosecurity for personnel and vehicles as well as increased contact with the environment and wildlife [2]. Anecdotal information has shown that moving outdoor pigs to new land is usually followed by an improvement in herd health and productivity. It is believed that outdoor herds in the UK typically move site every 2-3 years. The aim of this longitudinal study was to investigate the effect on the occurrence of *Salmonella* of moving pigs to new land and the sustainability of any improvements over a one year period.

Material and methods

Nine farms participated in the study and these consisted of five breeder farms, three finishers and one farrow-to-finish farm. The farms were visited four times over a 12 month period. The first sampling visit was shortly before a change of site, followed by three visits, each four months apart, to evaluate the change in prevalence and distribution of *Salmonella*. Over 200 pooled and individual faecal samples were collected from pigs at each farm visit to estimate the prevalence in the different pig stages present and identify the *Salmonella* serovars present, with some additional samples from the environment (wildlife faeces, waste runoff, etc). *Salmonella* was isolated according to ISO6579 Annex D and serotyping carried out for positive isolates. Up to 40 positive individual faecal samples were enumerated using a dilution/ enrichment method to estimate the concentration of *Salmonella* present per gram of faeces. At the initial visit, a questionnaire was completed with the farmer to collect farm information on the original location and pig health and management. At each subsequent visit, a short form was completed to detail the new site (at visit 2) and identify any changes to farm management since the last visit, and other general observations that might have influenced the *Salmonella* results.

A mixed-effects model, accounting for farm clustering, was generated to assess the effect of visit number on *Salmonella* presence. *A priori* variables for sample type and seasonality (sinusoidal quarterly components) were included, and a forwards stepwise selection used to identify other differences between the farms, significantly associated with *Salmonella*, which needed to be accounted for. All analyses were completed in Stata 12 (Statacorp, College Station, Texas, USA).

Results

A total of 8,549 samples were collected from the farm visits, with 92% being pooled or individual faecal samples from pigs, and 8% being environmental or faeces samples from wildlife or cattle. *Salmonella* was most frequently detected in pooled pig faeces samples (33.2%) as well as from samples from run-off and pooled water (47.3%), rodent faeces (40.0%) and wild bird faeces (37.3%). From the pooled faecal samples, up to 13 different serovars were detected at each farm visit (mean 5.5), with 37 different serovars detected in total. The most prevalent serovars were the two mST variants: *S*.4,12:i:- (210 isolates) and *S*. 4,5,12:i:- (200 isolates), followed by *S*. Derby (148) and S. Rissen (124). Amongst the individual samples, 1-10 serovars were detected per visit (mean 3.8), with 25 different serovars detected in total. The most prevalent serovars were similar to those from the pooled samples. The number of serovars detected across samples at each visit varied, with an average of 6.2 separate serovars detected at the baseline visit, 4.8 at visit 2, 5.8 at visit 3 and 6.9 at visit 4.

The prevalence of both *Salmonella* spp. and serovars of public health importance (ST, mST or *S*. Enteritidis (SE)) were lower on average after the move to the new site, which increased at visits 3 and 4 but remained lower than at visit 1 (Table 1). Some variability was shown in the results from individual farms, but a year after the move, six farms maintained a prevalence lower than at the original visit.

Table 1. Summary of sample results from the visits to nine outdoor pigs herds.

Visit	Nº. samples	Nº. *Salmonella* positive	Per cent positive	No. ST/ mST/ SE positive	Per cent positive
visit 1	2,562	758	29.6%	282	11.0%
visit 2	1,947	330	16.9%	124	6.4%
visit 3	2,031	436	21.5%	181	8.9%
visit 4	2,009	526	26.2%	150	7.5%

An average of 15 *Salmonella*-positive individual samples were selected for enumeration from each farm visit (range 1-40). Estimates of numbers of salmonellae ranged between <1 to 10^5-10^6 cfu/g. Samples with the highest *Salmonella* level were only found at visit 1, and the number of samples with levels of 10^4-10^5 increased from zero at visit 2 to 1 at visit 3 and 2 at visit 4. However, a comparison of these results indicated that they did not differ widely between visits and the proportion of samples with levels over 10^2/g was small (~5%).

A mixed-effects model (Table 2) showed that the *Salmonella* prevalence at visits 2 and 3 was significantly lower than before the move but no significant reduction was observed at the final visit, after accounting for a number of significant factors included in the model. The final model included three variables additional to the *a priori* variables for sample type and seasonality. These were: coughing in the sampled group,

which was a risk factor; Glasser's Disease diagnosed in the herd since the last visit, which was protective; and the use of tent or kennel accommodation for the sampled group, which was a risk when compared with arcs.

Table 2. Results from a mixed-effects model, determined by stepwise selection, assessing the effect of visit number on *Salmonella* prevalence on nine outdoor pig farms (n=8,548). Levels included for missing values or not applicable results have been omitted from the table.

Variable	Level	Odds Ratio	P-value	95% Conf. Interval	
Visit	1	1.00			
	2	0.41	<0.001	0.34	0.51
	3	0.74	0.025	0.57	0.96
	4	1.18	0.083	0.98	1.42
Sample type	Individual	1.00			
	Pooled	3.05	<0.001	2.71	3.44
Sampled area	Gestation	1.00			
	Farrowing	0.19	<0.001	0.16	0.23
	Weaners	0.48	<0.001	0.33	0.69
	Growers	0.49	0.001	0.32	0.75
	Finishers	0.27	<0.001	0.20	0.36
	Gilts	0.65	0.030	0.44	0.96
	Maiden Gilts	0.44	0.252	0.11	1.80
	Dry Sows	0.31	<0.001	0.24	0.41
	Environmental	1.12	0.784	0.50	2.49
Sinusoidal quarterly cycle	Sin	0.82	0.001	0.73	0.92
	Cos	0.98	0.702	0.86	1.11
Coughing in sampled group	Yes	4.01	<0.001	2.78	5.78
	No	1.00			
Clinical Glasser's disease present on farm	Yes	1.00			
	No	2.04	<0.001	1.68	2.48
Pig Accommodation	Arc	1.00			
	Hut	1.04	0.777	0.79	1.38
	Kennel	7.73	<0.001	2.45	24.43
	Lairage building	1.68	0.089	0.92	3.03
	Tent	3.82	<0.001	2.59	5.61

Two farms showed an increase in prevalence from the pooled samples after the site move but no information collected by the questionnaire or discussion with the farmers could explain this difference. The results for the individual faecal samples were more prone to fluctuations with greater numbers of farms showing prevalence increases at these stages, but smaller numbers of positive individual samples may have been associated with greater sample variability. Some variation in the results may reflect seasonality, as farms were sampled at different times of the year and heavy rain or higher temperatures, potentially causing heat stress in the pigs, have been linked to

Salmonella shedding [3]. Although seasonality was accounted for in the risk factor model, it would not have reflected local weather conditions.

The multivariable risk factor model identified only a small number of factors that were significantly associated with *Salmonella* prevalence. As expected, the *a priori* variables showed that pooled faecal samples were at greater risk of being positive than individual samples, and samples from farrowing, weaner, grower, finisher, dry sow and gilt areas of the farm were all at lower risk compared to those from gestation areas. This may be related to greater movement and mixing of sows after weaning and during service procedures, together with higher stocking densities in more muddy paddocks and the use of floor feeding. Coughing detected in the sampled pigs was a risk factor and positive associations between *Salmonella* presence and pneumonia have been shown by previous studies [4]. This may be due to the effect of one pathogen challenging the immune system and facilitating the infection by another, or may be due to *Salmonella* and respiratory conditions sharing similar risk factors, such as the use of straw-based housing and continuous flow production [5; 6]. However, the presence of Glasser's disease in the herd at a specific sampling visit was protective which was counterintuitive. This may have been due to random chance or it may have been a proxy for the effect of pig management factors used to control Glasser's disease, such as use of quarantine and improved cleaning and disinfection, that would also help protect against *Salmonella*. Pigs using kennels and tents were shown to be at greater risk than those using arcs, which may be due to larger groups of younger pigs typically using this type of accommodation. Additionally, solid structures like arcs may be easier to clean between batches, whereas tents may remain contaminated and spread infection to new batches.

Conclusion

The prevalence of *Salmonella* was lower following movement of pig herds to a new site and, while it increased throughout the subsequent study, it remained on average lower at the end of the sampling period (a year later). The risk factor model showed that the odds of a sample being positive was reduced by more than 50% after movement to the new site and by 25% at the third visit, both of which were statistically significant. The diversity of serovars detected after the site movement decreased, which supports this conclusion and results suggest that the land was less contaminated before the pigs were moved to the new site but after a year the site was at a similar level of contamination to that previously. These findings, along with the high prevalence detected from samples from run-off and pooled water, and wild birds and rodents may highlight the greater risk of infection via environmental sources for outdoor pigs.

The findings provide important evidence that more frequent site moves may help reduce *Salmonella* prevalence in outdoor herds. Additionally, *Salmonella* is a useful indicator bacterium, highlighting areas of poor control for other pathogens transmitted by faeces, and so the improvements may also have impact on reducing the occurrence of other diseases. It should be noted that moving site would incur a cost to the farmer and new land is not always available. This was a small trial of nine outdoors farm and so corroboration of the findings in other trials would be beneficial, as well as an investigation of any potential cumulative benefit if herds were followed over a series of annual moves. Abstract adapted from Smith *et al* [7], where further details of the study are presented.

Acknowledgements

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SURVEY OF *SALMONELLA* SPP. WITHIN A SWINE PRODUCTION COMPANY TRANSITIONING TO ANTIBIOTIC FREE

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Introduction

As farms remove antibiotics from grow finish diets increases in Salmonella prevalence have resulted. Identification of Salmonella spp. early on can minimize future disease. The objective of this study is to determine the prevalence of Salmonella spp. in a swine production system through cross-sectional surveillance of wean-to-finish sites.

Materials and methods

The 24 wean-to-finish sites sampled pigs ages 10, 15, and 20 weeks for surveillance. Per site, 20 random pig serum samples were collected and tested by ELISA for Salmonella exposure. Per barn, 6 pens were tested using an EnviroBootieTM pair, which were individually sent for Salmonella culture. Twelve necropsies on pigs 6-9 weeks of age exhibiting clinical signs were submitted. Samples were sent to ISU-VDL and BI Health Management Center (Ames, IA).

Results

Antibodies to Salmonella were detected in 67.6% of the pigs sampled. The highest prevalence of Salmonella antibodies was in the 20-week age group with 91% positive. Pigs at 10 and 15 weeks of age had Salmonella positive samples 44% and 70% of the time, respectively. EnviroBootieTM Salmonella cultures returned 6.2% positive of the pigs sampled. Culture positive prevalence was highest in the 15-week age group with 9% positive. Tissue diagnostics for Salmonella spp. identified clinical salmonellosis in 1 of the 12 pigs submitted.

Conclusion

The variation in prevalence among set age groups in the initial surveillance concluded that secondary testing was essential. *Salmonella spp.* diagnostics revealed 1 pig with clinical salmonellosis, but high percentage of exposure in the herd. This raises a large concern within a swine production company as it questions routine hygiene, biosecurity, management practices and subclinical disease. Vaccination may reduce overall disease status within the herd, however, it is important to have all-inclusive management and biosecurity protocols in place to minimize risk and help in overall reduction of exposure.

VACCINATION WITH ENTERISOL® SALMONELLA T/C REDUCES SALMONELLA ENTERICA COLONIZATION OF ILEOCECAL LYMPH NODES IN GROWING PIGS

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Introduction

Salmonellosis in pigs may have both production and food safety impacts. In this study, lymph node colonization was compared between a new, bivalent Salmonella vaccine and a baseline vaccine program.

Materials and methods

Pigs were placed into a Salmonella positive wean to finish barn, 288 pens and ~6600 pigs. Pigs were vaccinated with Enterisol Salmonella T/C® (44 pens, 1100 pigs), or a baseline vaccination program (remaining pigs). Pigs selected for harvest were placed by treatment onto two separate semitrailers (one/group). Swab samples were collected from each treatment at loading (3/group), the common loading chute (1/chute) and transport trailers (8/trailer) prior to loading, and lairage pens prior to pig arrival (four/pen). Pigs were harvested by treatment after a cleaning of facilities. Baseline pigs were harvested two hours after arrival; vaccinated pigs two hours later. Blood (171 Enterisol, 166 control) was collected after stunning, lymph nodes (153 Enterisol Salmonella T/C, 137 control), removal of viscera sets, chilled diaphragm (172 Enterisol Salmonella T/C, 171 control) and carcass swabs (342; Speci-sponge) the following day. Lymph nodes were frozen on dry ice and held for concurrent semi-quantitative culture. Serum and meat juice samples were tested by ELISA (Idexx). Swabs and lymph nodes were cultured via selective enrichment at ISU-VDL.

Results

All farm pre-load samples and transport samples were culture negative. All lairage samples (7/7) and one carcass swab were positive. Enterisol pigs were positive for Salmonella enterica at a significantly lower rate than baseline pigs (12% vs 63%, $p<0.0001$). Enterisol pigs had a higher seroprevalence rate in meat juice (75% vs 42%, $p<0.001$) but not serum (98% vs 95%, $p=0.26$). There was a two log reduction in CFU in positive vaccinated vs control lymph nodes (103 vs 105).

Conclusion

Vaccination with Enterisol Salmonella T/C® significantly reduced colonization of pigs with Salmonella enterica species compared to baseline control vaccination.

INFLUENCE OF DIFFERENT VACCINATION STRATEGIES AGAINST *SALMONELLA* TYPHIMURIUM IN PIGS FARMS ON *SALMONELLA* SEROLOGY AT SLAUGHTER AGE

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Introduction

Serology is a widely used tool in *Salmonella* monitoring programs in the pig industry. Vaccination might be an effective measure to control *Salmonella* infections at farm level, but may affect the herd's serological status. The present study investigated the effect of different vaccination strategies against *Salmonella* Typhimurium in pigs on *Salmonella* serology at slaughter age.

Materials and methods

Five different vaccination strategies were tested on three Belgian pig farms: 1. vaccination of sows; 2. vaccination of sows and piglets; 3. vaccination of sows and fatteners; 4. vaccination of piglets; 5. vaccination of fatteners. A comparison was made with a non-vaccinated control group (group 6). Each vaccination strategy was implemented in each farm. An attenuated vaccine (Salmoporc®, IDT Biologika) was applied twice with an interval of three weeks (sows and fatteners: subcutaneously, piglets: orally). Blood samples of 10 fattening pigs/group/farm were collected at slaughter. Sera were analyzed by ELISA (IDEXX Swine *Salmonella* Ab Test) and sample-to-positive-ratios (S/P-ratio) were assessed using the cut-off value: S/P≥0.25=positive.

Results

Of all samples across all groups, 158 samples (88%) were positive. On farm 1, the S/P-ratios of the vaccinated fatteners (group 3 and 5, means: 2.13 and 3.49) were significantly ($p<0.05$) higher compared to those of the control group (mean: 1.24). On farm 2, no significant differences between the S/P-ratios of groups 1-2-3-4-5 and the control group were detected. On farm 3, the S/P-ratios of the vaccinated pigs in groups 2-3-4-5 (means: 0.92, 1.72, 1.79, 0.95) were significantly ($p<0.05$) higher compared to the S/P-ratios of the control group (mean: 0.45). In none of the farms, a significant difference between the S/P-ratios of the pigs in group 1 and the control group was detected.

Table 1. Average S/P-ratios and 95% confidence interval per treatment group per farm (10 samples/ group/farm).

Group	Farm 1		Farm 2		Farm 3	
	Average S/P-ratio	**95% CI-interval**	**Average S/P-ratio**	**95% CI-interval**	**Average S/P-ratio**	**95% CI-interval**
1	0.37	0.20-0.54	0.82	0.30-1.35	0.62	0.24-0.99
2	1.92	0.83-3.00	0.75	0.55-0.96	0,92*	0.56-1.29
3	2.13*	1.52-2.74	1.63	1.24-2.02	1,72*	1.09-2.34
4	2.51	1.47-3.56	1.15	0.48-1.82	1,79*	1.24-2.34
5	3.49*	1.86-5.11	1.47	1.02-1.93	0,95*	0.50-1.40
6	1.24	0.10-2.38	1.53	0.83-2.24	0,45	-0.08-0.98

* indicates a significant difference ($p<0.05$) with the S/P-ratios of the control group of the farm.

Conclusion

Vaccination of piglets and fatteners, but not sows, against *Salmonella* Typhimurium can result in increased S/P-ratios with potential implications for serology-based *Salmonella* monitoring programs in slaughter pigs.

EVALUATING CHANGES IN SALMONELLA SEROVARS ASSOCIATED WITH SWINE OVER THE PAST 20 YEARS

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The aim of this project was to described changes in Salmonella serotypes prevalence over the past 20 years in the datasets from Iowa State University Veterinary Diagnostic Laboratory (ISU VDL), The National Antimicrobial Resistance Monitoring System (NARMS animal based isolates only), and the CDC Laboratory-based Enteric Disease Surveillance (LEDS) Program. We calculated the proportion with the numerator being the count of the serovar and the denominator is the total count of serovars. Linear regression was then conducted with the yearly proportion change regressed on year to obtain an estimate of the change in proportion for the given serotype over the years within each data set i.e. the slope of the regression line. Based on the slope of the line, S. Typhimurium, S. Derby, S. Heidelberg, where associated with decreases in proportion over the years. S. Typhimurium also appears to be decreasing in other data sets: NARMS-FDA retail, NARMS-USDA and CDC-LEDS data-sets. For other serotypes that appear to be decreasing in prevalence in swine submitted to the ISU VDL, the patterns were less consistent. For S. Derby, the decrease was observed in the ISU VDL, NARMS-S, and CDC-LEDS, but the prevalence appears to be increasing for S. Derby in NARMS-R (based on only 27 isolates). S. Heidelberg appeared to have a common pattern of decreasing across the data sets. Over time, S. serovar 4,[5],12:i:, S. Infantis and S. Johannesburg were associated with increases in proportion in ISU-VDL swine. It is interesting to note that scale of the increase observed in S. serovar 4,[5],12:i:. Expressed as a percentage, the change is around 2 % (95% CIs [1.02, 2.98]) each year, which is a rapid change.

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Antimicrobials in swine production, antimicrobial resistance, alternative strategies to antimicrobial use

HUMAN HEALTH IMPLICATIONS OF MRSA CC398 IN DENMARK

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Introduction

Methicillin-resistant Staphylococcus Aureus CC-398 (MRSA CC398) was first isolated from humans in Denmark in 2003 and from pigs in 2006 (anonymous, 2007-2015). Since then it has spread in the pig population, and a screening from 2016 found MRSA CC398 in 88 % of the screened slaughter pig herds, indicating that the majority of Danish pig herds are positive.

However, the human health consequences are less obvious, since the epidemiology and implications of antimicrobial resistance in staphylococci causing human infections is not straight forward.

Based on surveillance data from Denmark and the Netherlands, published studies from other European countires and a model developed by the European Centre for Disease control (anonymous, 2009), the human health consequences for the Danish population is evaluated.

Method

Based on data extracted form databases from the Danish national healt institute (Statens Serum Institut) (anonymous, 2017) and from the DANMAP-reports (anonymous, 2007-2015), number of MRSA CC398-carriers, MRSA-infections and MRSA-bacteremias are calculated year by year, and compared to the herd prevalence (anonymous, 2007-2015, anonymous 2016).

Number of bacteremia-cases is used as input to the model developed by ECDC (anonymous 2009), calculating the extra number of deaths and extra hospital days attributed to MRSA CC 398-bacteremia.

Number of carriers in the Danish human population was estimated using data from an unpublished Danish study on carriage of MRSA CC398 in people working with pigs and household members, combined with data from Statistics Denmark (anonymous, 2017b).

Results

Based on the above mentioned sources, Alban et al, 2017, estimated the number of human carriers of MRSA CC398 to be 10,600 in 2014, based on 69% positive herds. If all herds were positive, they estimated the number of carriers to be 15,100. In the general population without contact to pigs, they estimated that 5,600 humans were carriers of MRSA CC398, or 0.1% of the general population (population of Denmark 5.5 million), if all pig herds were positive.

Figure 1 shows the number of humans with a clinical infection of MRSA CC398 without pig contact and the herd prevalence (anonymous, 2007-2015, anonymous, 2016).

The incidence of clinical cases in the human population without pig contact is directly proportional to the herd prevalence. In 2011 there were 1.1 clinical cases for each percent infected herds, in 2014 it was 1.06 and in 2016 it was 1.1. Assuming a 100 % infected herds, by extrapolation it is estimated, that total number of clinical cases will be 110. Or an annual incidence of 2 out of 100,000 Danes without pig contact. Number of incident cases of clinical infection with MRSA CC398 amongst persons with pig contact was 120 in 2016.

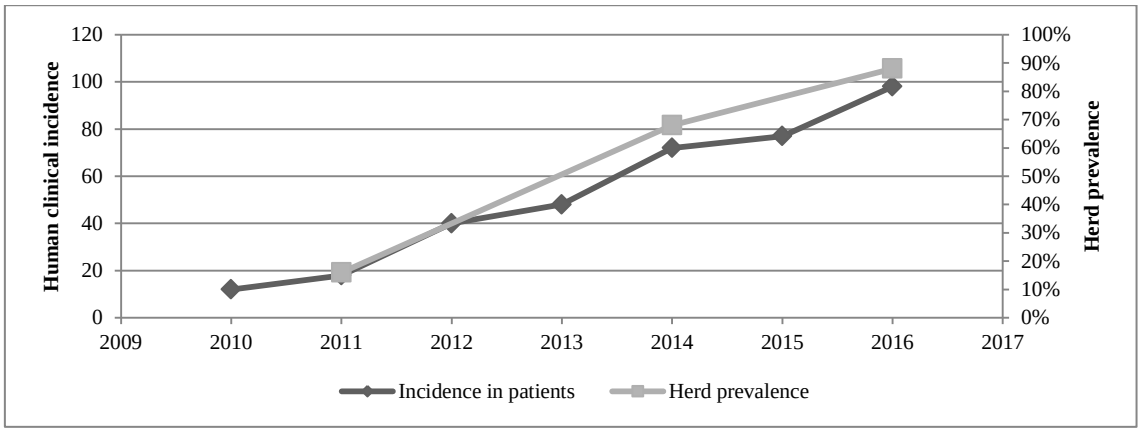

Figure 1. Incident cases of clinical infections of MRSA CC398 in Danish patients without pig contact and the MRSA CC398 herd-prevalence.

The majority of these infections are soft tissue infections, wound infections and eczemas. Bacteremias are considered the most severe Staphylococcal infection. Statens Serum Institute (anonymous, 2017) publishes each year the number of bacteremia's caused by MSSA, MRSA and MRSA CC398. Figure 2 shows the annual number of bacteremias caused by MSSA, MRSA other than CC398 and MRSA CC398 in Denmark. MRSA CC398 constituted between 0.15 % and 0.3 % of bacteremias caused by Staphylococcus aureus from 2014 to 2016 (7 in 2014, 3 in 2015 and 7 in 2016), and between 10 and 18 % of MRSA bacteremias. An annual incidence between 0.05 and 0.1 out of 100.000 Danes.

30 day mortality for 2014 to 2016, estimated as number of deaths within 30 days after a positive blood culture was 23 % for MSSA, 20 % for MRSA and 24 % for MRSA CC 398. In total 4 patients died within 30 days after a positive blood culture for MRSA CC398 in the 3 years from 2014 to 2016 (anonymous 2007-2015, anonymous 2017). All patients had severe underlying disease and had the typical age profile for patients dying after Staphyloccus aureus-bacterimia (median age for patients dying within 30 days after S. aureus bacteremia is close to 80 years in Denmark (anonymous 2015)).

The model developed by ECDC (anonymous, 2009) estimates that 9.8 % of bacteremias caused by MRSA will have a fatal outcome because of antimicrobial resistance, based on Cosgrove, 2003. Given an average number of MRSA CC398 bacteremias over the last three years of (7+3+7)/3=5.6, the extra annual deaths attributed to the antimicrobial resistance is 9.8 % times 5.6, or 0.6 per year. Based on

the Danish estimates for mortality after MRSA-bacteremias, there are no extra deaths, because there is no additional mortality associated with bacteremias caused by MRSA in Denmark, compared to MSSA-bacteremia.

ECDC estimates that there is an increased length of hospital-stay of 8 days for patients with MRSA-infections. With 5.6 cases of MRSA CC398-bacteremias each year, there will be an extra 45 bed-days in Danish hospitals due to bacteremia caused by MRSA CC398.

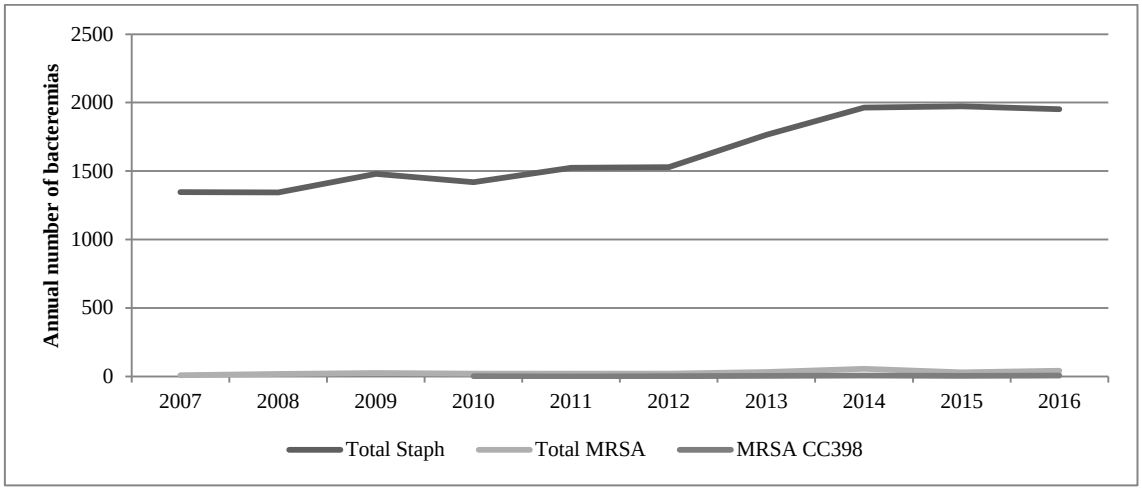

Figure 2. Annual cases of bacteremias caused by Staphylococcus aureus, MRSA and MRSA CC398.

Discussion and conclusion

Estimating the human health consequences of antimicrobial resistance in zoonotic pathogens is not straight forward. Estimates are highly influenced by the assumptions. ECDC estimates the excess mortality and morbidity that is related to antimicrobial resistance, assuming that the patients would have been infected by staphylococci irrespective of resistance. This assumption is critical for the evaluation of the human health consequences. Several studies have found that staphylococci are transmitted from pigs to humans, irrespective of resistance (Armand-Lefevre, 2005, Oppliger, 2012). Sun (2015) found that US swine veterinarians carried pig related staphylococci to the same extent as Dutch swine veterinarians (Verkade, 2013). In the USA the majority of pig related staphylococci were MSSA, and in the Netherlands it was MRSA, reflecting differences in MRSA prevalence in pig herds in the two countries.

So it is probable, that if pigs contribute to the total load of staphylococci in humans, then it is not dependent on antimicrobial resistance, making it justifiable to estimate the health consequences using the ECDC-model.

Human health consequences should not only be evaluated at the present time, but also future consequences should be taken into consideration. The human population can be divided into three groups: People working with or handling live pigs (group 1), house hold members living with people working with or handling live pigs (group 2) and the rest of society (group 3).

After the MRSA CC398 is spread to all herds, group 1 and 2 will stabilize. Further increases in the human population will be because of further spread in group 3. Figure 1 indicates no increased spread in group 3, except for the spread explained by the increase in herd prevalence.

Data from Germany (van Alen, 2016) found that MRSA CC398 increased in human patients from 2005 to 2011, and then remained stable. In the Netherlands the level of MRSA CC398 in the human population has remained stable since 2010 (anonymous, 2016b).

In conclusion epidemiological evidence suggests limited spread in group 3, and no indication of increased spread in group 3 after MRSA CC398 has spread to most of the animal population at risk.

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MRSA IN BREEDING PIGS IN GERMANY IN 2015

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Introduction

Methicillin resistant *Staphylococcus aureus* has been known to be prevalent in the pig production for nearly 15 years now (Meemken et al., 2010). In 2008 a survey carried out in the EU determined a high prevalence of MRSA in herds of breeding pigs also in Germany (EFSA, 2010). Likewise, MRSA were identified in Germany in herds of fattening pigs (Alt et al., 2011), pigs at slaughter (Tenhagen et al., 2009), on carcasses (Beneke et al., 2011) and in meat from pigs at retail (BVL, 2013). The current investigation was carried out to determine the current prevalence of MRSA in herds of breeding pigs, analyse patterns in the type of MRSA isolated from pigs and determine differences between the MRSA observed in units only housing sows and those housing weaned piglets. In previous studies it could be shown, that the prevalence of MRSA is higher in weaned piglets than in sows but little is known about differences in the types of MRSA that can be isolated in the different units.

Material and methods

Boot swab samples were collected in pig herds producing piglets for fattening purposes. Samples from herds were distributed across Germany based on the respective share of sows in the respective province ("Land"). A sample size of 384 samples was targeted. Within the herds two boot swab samples were collected: One sample was collected in the area where pregnant sows were housed, the other one was collected in the premises, where weaned piglets were housed. Samples were collected by the local veterinary authorities and submitted to the regional state laboratories for testing for MRSA.

Laboratory procedures

Boot swab samples were tested using a double selective enrichment method as previously described for environmental samples (Kraushaar et al., 2016). Briefly, samples were incubated with Mueller-Hinton broth containing 6% of NaCl and 1 ml of this broth after 18 to 24 hours was transferred to 9 ml of tryptic soy broth containing 50mg of aztreonam and 3.5mg of cefoxitin. 0.05ml of this broth were then plated on selective agar plates.

One colony from each positive sample was submitted to the National Reference Laboratory for coagulase positive staphylococci incl. *S. aureus* at the Federal Institute for Risk Assessment (BfR), where it was confirmed as MRSA using a multiplex PCR targeting the *nuc* gene for species identification, and the *mec* gene for methicillin-resistance (Kraushaar et al., 2016).

Data analysis: Only herds were included where samples were collected in both parts of the herd (i.e. sow and weaner premises) and where in case of positive results isolates were available for further characterization.

In pig herds agreement of samples and relative sensitivity of sow and weaner samples were calculated using the combined result of both samples as a reference. Results from the two samples were combined as a herd status. If any of the two samples was positive, the herd was considered positive, otherwise negative. Calculation of specificity was not done as this definition of a positive herd excluded false positive results.

Results

Data on 337 sow herds were finally included in the analysis. The overall prevalence of MRSA in the herds was 47.5 % (160/337). The observed MRSA prevalence was lower in sow stables (25.8 %, 87/337) than in the weaner premises (41.2 %, 139/337). MRSA were found in both, the sow stable and weaner premises in 66 herds (19.6 %). Agreement between the two samples was limited. Neither samples from sows nor those from weaners identified all positive herds. Relative sensitivity when compared to the combined results of the two samples was higher for the weaner samples than for the sow samples (86.9 vs 54.4 %).

From each positive sample, one isolate was further characterised. Overall 226 isolates were available for typing. Most of the MRSA- isolates (80.9 %) were from *spa*-types t011 and t034. This was similar in the sow and the weaner units (78.1 and 82.8 %, respectively). However, in the weaner units, slightly more MRSA of *spa*-type t011 were observed (50.4 % vs. 42.5 % in the sow herds). Eighteen other *spa*-types that could be assigned to the livestock associated clonal complex CC398 were identified and constituted 14.6 % of all MRSA-isolates were from. Eleven different additional CC398 associated types were seen in weaners, ten in sows. Seven isolates (4.5%) were from *spa*-types that could not be assigned to the CC398. However, all of those could be assigned to CC9: 6/7 t1430, 1/7 t15199. Type t1430 was seen in both, sows and weaners, with 3 isolates each, t15199 only in weaners.

With respect to *spa*-types of the isolates agreement between sow and piglet samples was limited. Only in 48 of the total of 160 positive farms (30.0 %), the same *spa*-type was identified in the sow stable and the weaner stable. In 39 of those 48 herds (81 %) both samples harboured either t011 or t034. In the remaining 18 herds with both samples positive the *spa*-type differed between the two parts of the herd.

Discussion

Overall, prevalence of MRSA in sow herds was similar to the prevalence determined during the EU-Baseline survey in 2008 (EFSA, 2009). However, the sampling methods differed and therefore comparison of the prevalences is not fully valid. The EU baseline survey included only sow premises and was based on dust samples. Purposeful inclusion of the weaner units in our study may have increased the prevalence as the proportion of positive samples was substantially higher in the weaner units than in the sow units. This agrees with previous studies on MRSA in pigs that found weaned piglets to have the highest prevalence of MRSA (Broens et al., 2010; Bangerter et al., 2016). The reason for this difference is not fully clear. However, frequently piglets from different sows are mixed in the weaner units and bacteria of

different farrowing crates may therefore contribute to the bacterial population in the weaner units. Additionally use of antimicrobials is frequent in weaners. Use of antimicrobials may foster detection of MRSA in the groups. As neither sampling weaners nor sows identified all positive herds, a combination of samples will be required for optimal sensitivity. Moreover, it is likely, that including more samples from other parts of the farm may further increase sensitivity.

Diversity of *spa*-types was substantial. However, most isolates were from *spa*-types that had already been identified in previous studies on pigs and other livestock in Germany. As only one isolate per positive sample and unit was typed, variability is probably even larger than observed here. In line with that, *spa*-types did not always agree between sows and weaners, indicating the presence of several *spa*-types in the same herd. However, most isolates could still be assigned to the clonal complex CC 398, the so-called livestock associated MRSA (LA-MRSA) and within that complex to the *spa*-types t011 and t034. This indicates persistence of this type of MRSA in the population over time. Other CC398 *spa*-types may indicate further evolution of MRSA in the pig population which is underlined by the frequent identification of new *spa*-types that can still be assigned to CC398 the MLST-based clonal complex that is most widely spread in the German farm animal population.

The proportion of isolates not assignable to CC398 was low and all of these isolates belonged to a *spa*-type related to the clonal complex CC9, that has frequently observed in isolates from poultry in Germany (Vossenkuhl et al., 2014; Kraushaar et al., 2016) . This type differs from most CC398 isolates by frequent resistance to ciprofloxacin, a feature that is less frequently seen in CC398 isolates. Whether these isolates originate from poultry farms or are also fully established in the pig population is not known.

In terms of food safety, MRSA positive herds constitute a permanent source of these bacteria in the food chain and although appropriate slaughter hygiene can limit the spread to carcasses, it is well established that MRSA can also be found on pork and be introduced in the households of consumers. Moreover, people working on pig farms are frequently carriers of the bacteria and may introduce them into the healthcare system.

Currently there is no specific strategy in place to contain MRSA in the pig population in central Europe. Therefore all efforts should be made to control the introduction of livestock associated MRSA into the healthcare system.

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GENOTYPIC CHARACTERIZATION OF A MONOPHASIC VARIANT OF *SALMONELLA ENTERICA* SEROTYPE TYPHIMURIUM IN SWINE IN USA MIDWEST

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Abstract

Background

Non-typhoidal *Salmonella enterica* are a major human foodborne pathogens worldwide (Kirk et al., 2015). Salmonella enterica is divided into six subspecies and approximately 2,500 serotypes according to the Kauffmann-White scheme (Brenner et al., 2000). Serotype 4,5,12:i:-, a monophasic variant of *S.* Typhimurium lacking the 2nd phase flagellar antigen. *Salmonella* 4,5,12:i:- has emerged globally in both humans and animals (Echeita et al., 2001; Switt et al., 2009; Centers for Disease Control and Prevention (CDC), 2013) and pork products and pigs are implicated as important sources of human infections (Mossong et al., 2007; Hauser et al., 2010).

Distinct clades of *S.* 4,5,12:i:- of apparently separate *S.* Typhimurium ancestry were previously characterized in Europe and USA, (Soyer et al., 2009). The existence of distinct clades was supported by different phenotypic antimicrobial resistance patterns: USA isolates were generally susceptible to antimicrobials while European isolates demonstrated multidrug resistance to tetracycline, ampicillin, sulphonamides and streptomycin (Mossong et al., 2007; Switt et al., 2009; Hauser et al., 2010).

Numerous European *S.* 4,5,12:i:- strains have been genotypically characterized, but to date the genotypic characteristics and ancestry of USA strains is not well documented. Whole genome sequencing (WGS) was used to characterize and compare *S.* 4,5,12:i:- collected from swine in the USA Midwest with *S.* Typhimurium from this region and with *S.* 4,5,12:i:- isolates from other locations in the USA and Europe.

Methods

Maximum likelihood phylogenetic trees were constructed using the WGS reads of *S.* 4,5,12:i:- collected from swine in the Midwest with *S.* Typhimurium collected from livestock in the Midwest and with *S.* 4,5,12:i:- collected from different sources (i.e. human, animal and environment) in Europe and USA. The presence of resistance genes was determined using multiple BLAST searches.

Results

In the first part of the analysis, we found that the *S.* 4,5,12:i:- collected from swine in the Midwest were located in a distinct clade from the majority of the *S.* Typhimurium collected from livestock in the Midwest. In the second part of the analysis, we found

that *S.* 4,5,12:i:- isolates from Europe and USA were located in two main clades, regardless of their origin. In addition, the majority of the isolates collected during 2014-2016 (84%), including all swine samples, belonged to an "emerging" clade. Most (80%) of the isolates in this clade demonstrated multidrug resistance including resistance to tetracycline, ampicillin, sulphonamides and streptomycin.

Conclusions

This study results suggest that the *S.* 4,5,12:i:- found in swine in the USA Midwest did not originate from livestock *S.* Typhimurium isolates in the USA Midwest. This local clade belongs to a globally multidrug resistance clade. The occurrence of multidrug resistance to multiple antimicrobial classes in these isolates is of concern.

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ESCHERICHIA COLI RESISTANCE AND GUT MICROBIOTA PROFILE IN PIGS RAISED WITH DIFFERENT ANTIMICROBIAL ADMINISTRATION IN FEED

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Abstract

Antimicrobials have been widely used in veterinary medicine for disease treatment, disease prevention, and growth promotion. Although, the mechanisms about in-feed additives use are still not completely understood, it is accepted that its use improves feed efficiency by reducing the microbial load in the intestinal tract, thereby reducing the pressure on the immune system and increasing energy availability for the animal. However, disruption to commensal bacterial communities by antibiotics in some cases can increase the gut colonization by pathogenic bacteria. In this sense, the aim of this study was to evaluate the *Escherichia coli* antibiotic resistance and the gut microbiota profile from pigs raised in Brazilian farms with different in-feed antimicrobials protocols. Pigs from four farms with distinct antibiotic usage, including one farm that used no antibiotics, were followed from weaning to finishing, and the frequency of antimicrobial resistance and gut bacterial profile by 16S rRNA gene sequencing were evaluated. The gut microbial community structure was the same among all groups of pigs despite different antibiotic use on the farms; however, the antimicrobial resistance profiles of *E. coli* isolates were different between groups. One farm administered seven antibiotics at different times, and *E. coli* isolates from these pigs showed higher frequency of resistance and multidrug resistance as compared with samples from the farm that did not administer in-feed antimicrobials. The phenotypes included resistance to drugs considered critically important antimicrobial agents in veterinary medicine (ampicillin, ciprofloxacin, florfenicol, sulfonamide and tetracycline) as well as one highly important antibiotic in human medicine (colistin). Resistant *E. coli* strains were screened for the presence of the *mcr-1* gene by PCR. The colistin-resistant strains were positive for the presence of the *mcr-1* gene. These results suggest that although different antibiotic uses on-farm might not impact microbial community structure, it does impact bacterial functions, namely antibiotic resistance. Our results show that prudent use of antimicrobials is important for decreasing selective pressure for antibiotic resistance gene evolution.

Introduction

Antimicrobials have been widely used in veterinary medicine for therapeutic and non-therapeutic purposes. The non-therapeutic use can affect the gut microbiome balance, and may increase the gut colonization by pathogenic and antimicrobial resistant

bacteria. In this sense, meat products can act as vehicles of resistant strains (Chantziaras et al., 2014; Boeckel et al., 2015).

In order to study the effect of continuous in-feed antimicrobial administration on bacterial resistance, many studies used *Escherichia coli* as a biological indicator. It's known that the resistance profile of *E. coli* strains may vary according to in-feed antimicrobial administration protocol (Chantziaras et al., 2014; EFSA, 2015). This administration can also have an effect on the gut microbiota (Looft at al., 2012; Kim et al., 2012; Fleury et al., 2016). However, it is not clear if the gut microbiota structure in pigs from in-feed antimicrobial free farms is different compared to pigs grown in farms, where antimicrobials are used massively. In order to contribute to this knowledge, the aim of this study was to describe the gut microbiota and *E. coli* phenotypic resistance profile in pigs grown in farms adopting different in-feed antimicrobial administration protocols.

Material and methods

Gut microbiota and *E. coli* antimicrobial resistance profile were investigated in four groups of swine submitted to different in-feed antimicrobial administration protocols from 32 to 140 days old. The study was conducted at Embrapa Swine and Poultry (Brazil) and the farms were located at the same region.

Swine groups

- **G1:** Piglets from a very small farrow to finishing operation (462 finished pigs per year), without in-feed antimicrobials administration since 2008.
- **G2:** A subgroup of G1, submitted to three ten-day pulses of in-feed antimicrobial administration: colistin (120 ppm) at the nursery; doxycycline (200 ppm) and tiamulin (120 ppm) at the growing phase; and florfenicol (120 ppm) at the finishing phase.
- **G3:** Piglets from a small farrow to finishing operation (1,100 finished pigs per year). In-feed antimicrobials were used continuously: colistin (133 ppm) from farrow to nursery; halquinol (4000 ppm) at the growing phase; and lincomycin (733 ppm) at the finishing phase.
- **G4:** Piglets from a medium farrow to finishing operation (28,800 finished pigs per year). In-feed antimicrobials were used continuously: florfenicol (60 ppm) and norfloxacin (200 ppm) in nursery (first week), colistin (200 ppm) and neomycin (200 ppm) until end of nursery stage; halquinol (120 ppm) and tiamulin (150 ppm) at growing stage; tylosin (80 ppm) at finishing stage.

Sampling

Six piglets from three different litters were identified and sampled at four time points: nursery (days 32 and 47 of age); growing (day 63 of age); and finishing (ten days before slaughter) stages. Two producing cycles in each farm were sampled, totaling 183 feces samples.

Microbiota analysis

For this analysis, feces samples from four animals in one sampling cycle were used (n = 64). The fecal DNA was extracted by Power Fecal kit (MoBio Laboratories). The V1-V3 region of bacterial 16S rRNA gene was amplified and sequenced in the MiSeq plataform (Illumina). Sequence data analysis was performed with *mothur* software package version 1.34.4. Statistical analysis for taxonomic differences was conducted using the STAMP software. T-test with Storey FDR multiple comparison correction was used to detect significant differences in taxonomic distribution between samples, with the unclassified reads retained in the analyses.

Isolation and antimicrobial susceptibility test of *Escherichia coli*

Escherichia coli was isolated on Eosin Methilene Blue Agar (EMB) and one isolate per animal was tested for susceptibility against ten different antimicrobial agents: ampicillin (10 µg); cefotaxime (30 µg); ceftazidime (30 µg); ciprofloxacin (5 µg); chloramphenicol (30 µg); florfenicol (30 µg); gentamicin (10 µg); nalidixic acid (30 µg); sulfonamide (300 µg); and tetracycline (30 µg), according to CLSI (VET01-S2 and M100S documents). Antimicrobial resistance frequencies in the groups were compared by the chi-square test ($\chi 2$) with 95% of confidence. In addition, Marascuilo procedure was performed to test the differences between categories. For colistin, the Minimal Inhibitory Concentration (MIC) was determined. All isolates were also screened for the presence of *mcr-1* gene by PCR (Liu et al., 2016).

Results and discussion

Microbiota analysis produced a total of 853,301 reads with an average of 488 bases (280 to 582). From them, 164,351 reads were used for assessing bacterial diversity. Among the OTUs 99.16% were identifiable, and the majority was assigned to the phyla *Firmicutes* (65.43%) and *Bacterioidetes* (27.98). These groups have been identified as part of the gut microbiota in pigs (Looft et al., 2012; Kim et al., 2015; Mach et al., 2015). At the genus level, *Prevotella* and *Ocillibacter* followed by *Bacteroides* and *Lachnospiracea* were the most frequent groups. *Prevotella*, which belongs to the phylum *Bacteroidetes*, is the most frequent genus in pig gut microbiota (Looft et al., 2012; Mach et al., 2015). This genus has been associated with feed fermentation and provision of energy to the host (Lamandella et al., 2011). *Oscillibacter*, which belongs to the phylum *Firmicutes*, ranged from 17.64 to 19.92% of all classifiable genera identified in the four pig groups. *Ocillibacter* has been reported as a low frequent genus in the gut microbiota of adult pigs. On the contrary, this genus together with *Bacteroides* may predominate in piglets, due to their ability of utilization of oligosaccharides present in the milk (Park et al., 2014; Mach et al., 2015). Overall, no significant difference on the gut microbiota profile was detected among the four pig groups or between growth stages.

As in other studies, *Escherichia*, which belongs to the phylum *Proteobacteria*, was amongst the least common genera of the gut microbiota. Despite this fact, *E. coli* has been adopted as an antimicrobial resistance indicator in animals (EFSA, 2015). Evaluation of antimicrobial susceptibility test was carried out in 183 *E. coli* isolates (Table 1). Overall, 7.65% were susceptible to all antimicrobials tested and 78.14% were considered multidrug-resistant (MDR) (resistant to ≥ 3 antimicrobial classes). Among the pig groups, G4 presented the highest frequency of MDR strains (22.95%), followed

by G3, G2 and G1 (21.3%, 19.7% and 14.2%, respectively). Significant differences between groups were observed in the resistance to ampicillin, ciprofloxacin, chloramphenicol, florfenicol, sulfonamide and tetracycline ($P < 0.05$).

Table 1. Percentage of antimicrobial resistant *Escherichia coli* strains from pigs subjected to different in-feed antimicrobial administration protocols.

Antimicrobial	Group 1	Group 2	Group 3	Group 4	*P value*
Ampicillin	32.61[a]	63.83[a,b]	86.96[b]	77.27[a,b]	0.001
Cefotaxime	8.70	6.38	2.17	2.27	0.389
Ceftazidime	0.00	4.26	0.00	0.00	0.119
Chloramphenicol	34.78[a]	48.94	67.39	84.09[b]	< 0.001
Ciprofloxacin	10.87[a]	23.40	30.43	43.18[b]	0.006
Florfenicol	28.26[a]	34.04	52.17	84.09[b]	< 0.001
Gentamicin	8.70	19.15	21.74	6.82	0.102
Nalidixic acid	60.87	59.57	54.35	75.00	0.217
Sulfonamide	52.17[a]	68.09	69.57	84.09[b]	0.014
Tetracycline	67.39[a]	70.21[c]	78.26	97.73[b,d]	0.002

Different letters demonstrate differences between groups by Marascuilo procedure.

Regarding colistin, most *E. coli* strains displaying MICs 8≥ ug.mL^{-1} belonged to G3 and G4 (Table 2). Among the resistant strains, *mcr-1* gene was detected in 77.5% of them. This is a matter of concern, since this gene may be located in plasmids and be transferred among bacteria, decreasing the colistin efficacy in controlling swine diseases. Moreover, the hazard for humans has been pointed out, because this drug is considered the last option for human treatment (Liu et al., 2016).

Table 2. Distribution of *Escherichia coli* strains from pigs subjected to different in-feed antimicrobial administration protocols, according the Minimum Inhibitory Concentrations (MIC) of colistin.

Groups	***E. coli* (n)**	**Colistin MIC (µg/mL)**			
		2.0	**4.0**	**8.0**	**16.0**
G1	46	22	22	1	1
G2	47	27	18	2	0
G3	46	13	10	19	4
G4	44	14	17	12	1
Total	**183**	**76**	**67**	**34**	**6**

Conclusion

These results suggest that although different antibiotic uses on-farm might not impact microbial community structure, it does impact bacterial functions, namely antibiotic resistance. Our results show that prudent use of antimicrobials is important for decreasing selective pressure for antibiotic resistance gene evolution.

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IN VITRO ASSAY FOR ANTIMICROBIAL INTERACTION EVALUATION AND RISK ASSESSMENT OF ANTIMICROBIALS IN ANAEROBIC DIGESTION OF SWINE MANURE

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Introduction

The energy demand increase and recent new regulation for biomethane in Brazil have aroused new interests and perspectives for biogas from livestock wastes, especially swine manure. Brazil is the largest producer of animal protein and has perspective to keep growing in the next years, but to achieve this goal good practices of livestock waste management are required. The anaerobic digestion has become a common practice to treat the manure and also reduce production costs through energy recovery (Cherubini et al. 2015).

On the other hand, the use of veterinary drugs in livestock production and the occurrence in manure can lead to a number of concerns about interference in biogas production. The knowledge about inhibition effect and persistence of antimicrobials compounds in anaerobic digesters is important to take decision about the technology selection for manure treatment and to evaluate the environmental risk assessment in swine manure management.

Antibiotics are globally used in food-production animals as therapeutically (typically higher doses and administered individually or as group-treatment) to treat specific diseases and subtherapeutically (typically lower doses) for disease or infection prevention (Venglovsky, Sasakova, and Placha 2009). It is known that a significant fraction (30 to 90% - (Sarmah, Meyer, and Boxall 2006) of the antibiotics are excreted in the parent form or its metabolites (Schlüsener, Von Arb, and Bester 2006). In the excreted material, some metabolites are still biologically active and some inactive metabolites can potentially be transformed back to the bioactive parent compound under relatively mild conditions (Zhou et al. 2012).

Once tetracyclines have an antimicrobial wide spectrum, they are commonly used to treat infections in livestock. Unfortunately, the persistence of tetracyclines compounds in the manure can cause environment impact and influence the natural processes of biological treatment of waste (Prado, Ochoa, and Amrane 2009). Interference by veterinary drugs in the anaerobic digestion are diverse, such as excessive foaming in the reactor, decline in biogas productivity, accumulation of organic acids and imbalances in microbiology community (Shimada et al. 2011). For example, tetracycline in concentrations higher than 9 mg/L can reduce the methane production more than 50% in digestion of swine manure (Álvarez et al. 2010) and oxytetracycline reduce in 27% the biogas yield in manure from calves medicated for 5 days with 22 mg/kg/day (Arikan et al. 2006). Besides, the anaerobic digestion promotes the degradation of the antibiotic compounds and could mitigate the environmental

impact.

This work presents a proposal of laboratory procedure for anaerobic toxicity assay integrated with biodegradability assay, based on standard methods (ISO 13641, ISO 11734 and VDI 4630).

Material and methods

The antibiotics standards used in this study were purchased from Sigma-Aldrich (Germany) VETRANAL™ quality, as follows: tetracycline hydrochloride, chlortetracycline hydrochloride, oxytetracycline hydrochloride and methacycline hydrochloride. As inhibitory control substance was used 3,5-dichlorophenol, also from Sigma-Aldrich (Germany).

The assays were performed according to VDI 4630 (2006). Batch experiments were performed in 250 mL glass reactors connected to 500 mL glass eudiometers. The reactors were prepared with 200 g (4.5 gVS) of the acclimated inoculum [mixture of anaerobic sludge from swine manure, UASB sludge from food industry and cow manure, prepared according Steinmetz et al. (2014)] and added 1 gVS of microcrystalline cellulose (20 µ, Sigma, Germany). To evaluate the antibiotic interference, 4 levels of concentration (x, $x.10^1$, $x.10^2$, $x.10^3$) of each tetracycline standard were spiked. The reactors were then sealed and stored at 37 °C until the establishment of stationary daily gas production rate (< 1% of the total amount produced). The production of gas was quantified on a daily basis by displacement of the sealant liquid level (DIN 38414-8, 1985) in the eudiometers. The dried biogas volume was determined by subtracting the water content based in the water vapour pressure, according to VDI recommendations. The dried biogas volume was then normalized to standard temperature and pressure i.e., 273 K and 1013 hPa, respectively. Specific biogas yield of each sample was estimated as the total biogas produced divided by the respective sample VS content. The results were normalized to the total biogas produced from negative controls prepared with inoculum only. All experiments were performed in triplicate.

The biogas inhibition was determined according ISO 13641-1 (2003) using the cumulative biogas after 72 h of incubation and using the maximal biogas production. The inhibition factor was estimated by application of the equation bellow:

$$I\,[\%] = \left(1 - \frac{V_t}{V_c}\right).100$$

where *I* is the percentage inhibition, *Vt* is the biogas produced with the antibiotic in the selected time and *Vc* is the biogas produced in the control at the same time. The 10% and 50% inhibition concentration (IC_{10} and IC_{50}) was estimated by regression analysis by plotting *I* against the logarithm of antibiotic concentrations.

The residual tetracycline content was evaluated by LC-MS/MS in a sample pool by mixture of 3 repetitions after de BMP assay (30 days). The sample preparation was done using SPE according Groth et al. (2015). The analysis was done by liquid chromatographic system Surveyor Plus coupled to mass spectrometer TSQ Quantum Access Max, both from Thermo Scientific (USA). The tetracycline separation was done using column Acclaim TM 120 C18 (150 mm x 4.6 mm, 5 µm - Thermo Scientific, EUA). The injection volume was 10 µL. The elution gradient had two phase: eluent A) formic acid 0.1% (v/v) in ultrapure water; eluent B) formic acid 0.1% (v/v) in acetonitrile; following the mixture system: 0-2 min isocratic 95% A and 5% B; 2-3 min

gradient to 25% of B; 3-7 min isocratic 25% of B; 7-12 min gradient to 50% of B; 12-15 min isocratic 50% of B; 15-16 min gradient to 100% of B; 16-18 min isocratic to 100% of B; 18-18,5 min gradient to 5% of B; 18,5-22 min isocratic to 5% of B, in 1.0 mL/min flow rate. Detection was preceded by electrospray ionization in positive mode. The quantification of tetracyclines was done by signal evaluation in "Selected Reaction Monitoring" mode. The precursor ions evaluated was (m/z) 461.1, 445.1, 479.1, 443.1 e 445.1 for oxytetracycline, tetracycline, chlortetracycline and doxycycline, respectively. The secondary ions evaluated were: m/z 426.3 (17 eV) and 443.3 (11 eV) for oxytetracycline; m/z 410.3 (18 eV) and 427.4 (11 eV) for tetracycline; m/z 462.3 (16 eV) and 444.3 (19 eV) for chlortetracycline and m/z 201.1 (33 eV) and 426.3 (13 eV) for methacycline.

Results and discussion

All treatments show low changes in pH (start test was 7.73 ± 0.04; after 30 days was 7.54 ± 0.07), confirming no limitations of alkalinity and the possible inhibitory effect are not linked to rapid changes in pH. The standard inhibiting substance 3,5-dichlorophenol of 150 mg/L presented 28 ± 6% inhibition after 72 h which is in accordance with ISO 13641-1(2003) for valid inhibition assays (> 20%).

The Figure 1a shows the cumulative biogas production profile versus time, for the anaerobic digestion assays in the presence of tetracycline. There is a tendency of reduction in biogas production by increasing the concentration of antibiotics added. Tests with addition of tetracycline and chlortetracycline had similar profile, characterized by time increasing in the adaptation phase in the early days (from 1.2 days in the control to approximately 4 days in the highest concentration of antibiotic). Figure 1b shows the regression model obtained by the inhibition effects observed after 72 h and for the maximal biogas production (30 days). The same data evaluation was applied for the others antibiotics compounds. Table 1 show the estimative of IC_{10} and IC_{50} for biogas production at 72 hours of incubation and for the biogas yield. The IC_{10} represent the minimum quantified level of inhibition.

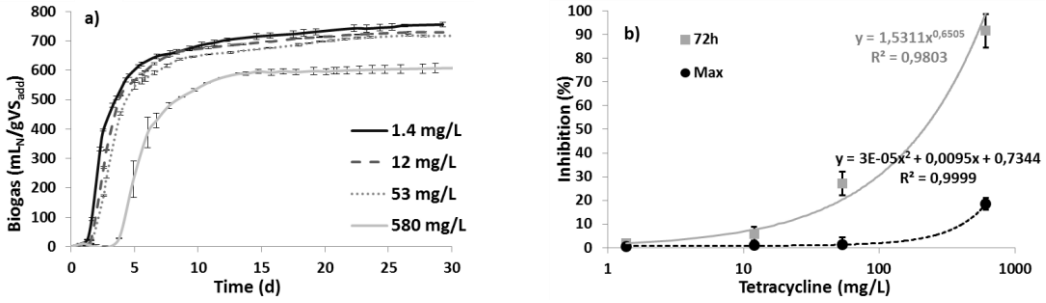

Figure 1. a) Cumulative biogas production profile from microcrystalline cellulose in the presence of variable concentrations of tetracycline; b) Regression equations for biogas inhibition.

Table 1. Estimation of IC_{10} and IC_{50} on biogas yield and tetracycline's compounds reduction after anaerobic assays.

Compound	IC_{10} mg/L	IC_{50} mg/L	Reduction* %
Tetracycline			
I_{72h}	19	219(207-231)	
I_{max}	414	1370(1339-1401)	46.0 - 96.5
Chlortetracycline			
I_{72h}	27	193(103-283)	
I_{max}	372	>> 2000	82.0 - 98.4
Oxytetracycline			
I_{72h}	68	495(361-629)	
I_{max}	287	1908(1354-2446)	76.6 - 98.7
Methacycline			
I_{72h}	18	142(75-208)	
I_{max}	127	>> 2000	57.1 - 97.6

Confidence interval in parenthesis is 95% based in standard error of regression.
*Reduction of initial concentration after 30 days of incubation.

In this case, by comparison of the IC_{50} at 72 h, highest toxicity was observed for methacycline, in sequence chlortetracycline, tetracycline and finally oxytetracycline. But, according to the confidence interval is only possible to confirm that the toxicity of oxytetracycline was lower than the others. The IC_{50} in biogas yield was significant in comparison between the initial gas productions in 72 h. In this case, tetracycline and oxytetracycline demonstrate similar level of inhibition. Similar profile has also identified in the methane production. The concentration of methane found in the tests were 55 ± 5% for tetracycline, 54 ± 5% chlortetracycline, 55 ± 4% oxytetracycline and 56 ± 4% methacycline. Therefore, based on the results from Table 1 it is possible to occur of acute inhibition in digester in full scale. For example, in pig manure occurrence of tetracycline 98,8 mg/L, oxytetracycline 354 mg/L, chlortetracycline 764.4 mg/L and methacycline 5.43 mg/L have been reported (Zhao, Dong, and Wang 2010; Pan et al. 2011; Chen et al. 2012).

On the other hand, the analysis of the residual concentration of veterinary drugs after the batch anaerobic assays indicates high degradation. In some experiments it was observed reduction of antibiotics over than 98%. These values agree with the data observed by Tong et al. (2012), that found reduction of 88.6-91.6% for tetracycline and 97.7-98.2% for chlortetracycline and by Turker et al. (2013) observed decrease of 55 to 70% for oxytetracycline.

Conclusions

The IC levels founded by the in vitro assay showed that is possible to occur of acute inhibition in anaerobic digestion of swine manure in full scale. The general inhibition effect in biogas production follows the order: methacycline > tetracycline ~ chlortetracycline > oxytetracycline. However, anaerobic digestion has been shown to be effective for the degradation of the compounds evaluated in the study and could help to reduce the environmental impact of veterinary drugs in the manure.

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ALTERNATIVES TO ANTIMICROBIAL TREATMENT IN WEANERS – THE VETERINARY PRACTITIONER'S SOLUTION

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One way of lowering the need for antimicrobial treatment is to ensure that piglets are healthy and strong at weaning. This implies that the weaning weight should be 6-7 kg. Unfortunately, many piglets only weigh 3-5 kg at weaning, which predisposes them to disease including diarrhea, because they are not necessarily ready for a soy-based diet at weaning.

This abstract is based on a veterinary practitioner's experience. The presentation will show how a change of the sow feeding during lactating will improve milk production, and through this increase the weaning weight of the piglets, and hereby lower the need for treatment of diarrhea post-weaning.

Nowadays, sows are fed twice a day with a cheap kind of feed with low energy, lowly digestible and high on soya. This should be replaced by feeding four times a day in the farrowing unit. Two phases of feeding should be used; Phase 1 lasting from 3 days before farrowing to 8 days after with a high-energy diet, highly digestible proteins and a low amount of soya. Phase 2 lasting from day 8 until weaning, where the ordinary feed is enriched with energy and soya. Hereby, the sows' milk production is increased substantially. The weaned piglets will be more resilient and have a much lower probability of developing diarrhea compared to piglets weaned at a lower weight.

This presentation will share the experience obtained with this feeding regime in more than 100 Danish sow herds, and show that a sow can wean 12-14 piglets on average, whereby the need for cross-fostering is reduced.

The disadvantages of this system are that the farmer needs to feed four times a day and be willing to spend more money on the sow's feed. However, the extra costs are compensated by lower costs for weaner feed.

EFFICIENT WATERLINES CLEANING PROTOCOLS IN POST - WEANING ROOMS: A NEW WAY TO REDUCE ANTIBIOTIC CONSUMPTION?

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Abstract

In this study, we have chosen a sensitive period, the weaning period, to evaluate in pig farms the effects of different mechanical and chemical waterlines cleaning protocols similar to those used in poultry farms. The experiment has been set up during the down period in two post-weaning rooms with two different protocols. They combined the mechanical action of draining, one detergent (either alkaline or enzymatic), another draining state, and finally one acid used at an antibacterial concentration. To follow the bacteriological quality of water during protocols, we have counted the total flora at 22°C and 37°C in water. Before and after the experiment, cotton swabs were applied into the pipes to evaluate the biofilm. Bacterial concentration in water increased along the pipelines: total flora was higher at watering place than at the entry of the building. Both protocols combining mechanical and chemical procedures reduced total flora, improved water quality and cleanliness of pipes. Our results show that waterlines cleaning protocols used in poultry farms can be transferred in post-weaning rooms. By reducing water's total flora and the formation of biofilms, they could be part of the health prevention measures for troubles which are linked to a poor water quality.

Introduction

Drinking water is an essential nutrient for animals. Indeed, when the physiological animal's requirements are not satisfied, performances can decrease and/or diseases may appear, both having an economical impact for pig or poultry productions (Gogny and Debrueker, 1999).

To guarantee the best quality of water from the source to the animal troughs, it's important to be aware that water quality can be adversely affected by the formation of biofilms in distribution systems, which represent persistent reservoir for potentially pathogenic bacteria (Wingender and Flemming, 2011). Biofilm may clog water pipe and filter, and thus, restrict water flow, which can lead to poor herd or flock performances (Fairchild and Ritz, 2009). In addition, the presence of biofilm in water distribution systems makes disinfection difficult or it can decrease the efficacy of oral treatments administered to the animals like vaccines, antibiotics or nutritional factors (Chazarenc, 2010).

For many criteria regarding water quality, poultry producers are more aware than pig farmers. The differences in their practices concern the monitoring of water consumption and the maintenance of water pipes, including cleaning measures to eliminate the biofilm (Brilland *et al*., 2016). In pig husbandry, weaning is a critical

management period since piglets become exposed to social, environmental as well as nutritional changes which might be regarded as stressful events. Digestive disorders are the main health problem and could be linked with unadapt water quality. In this study, we have chosen this sensitive period to evaluate in pig farms the effects of different mechanical and chemical waterlines cleaning protocols, similar to those used in poultry farms.

Material and methods

Selection of the farms

The inclusion's criteria for the farms were first to be equipped with a specific system for waterlines in the post-weaning rooms (dual water circuit with a treated water circuit connected to a metering pump and a clean water circuit), then to have a recurrent problem of digestive disorders on their piglets during the post-weaning period. We selected for this study three commercial farrow-to-finish farms from one production company located in the West of France, an area with a high pig industry profile.

Waterlines cleaning protocols

Two different waterline cleaning protocols commonly used in poultry farms have been tested in parallel during the sanitary break in two post-weaning rooms. They combined the mechanical action of draining, one detergent (either alkaline or enzymatic), another draining state and finally one acid used at an antibacterial concentration (Table 1). The mechanical action of flushing water under pressure in the pipes is necessary to pull off the biofilm in order to increase the efficiency of the disinfection.

Table 1. Waterline cleaning protocols.

Protocole 1: Post-weaning room 1	**Protocole 2: Post-weaning room 2**
Mechanical action: line flushing	
Sanolin®: alkaline detergent (potassium hydroxyde) 45 minutes at 1%	Sanozym®: enzymatic detergent (protease, amylase) 45 minutes at 1%
Mechanical action: line flushing	
Sanocidex®: acid (peracetic acid 5%, hydrogen peroxid 14.5%)1 heure à 2%	
Mechanical action: line flushing	

Experimental design

Prior to set up the experiment, a drain valve has been added at the end of each water pipeline of each post-weaning room. This terminal drain valve was necessary for water line purges. In order to have an efficient mechanical action, the pressure regulator of the waterline system was set at three bars. There were four steps for the line flushing procedure : adjusting the pressure reducer to reach 3 bars, opening the drain valve to purge one volume of water, closing the drain valve and opening all the water troughs of the room to purge one volume of water. A metering pump was used to dispense each product in the treated water circuit.

In each farm, the two waterline cleaning protocols were set up at the same time in two post-weaning rooms, the day before the entrance of the piglets of the same batch in the room (weaning time).

Sampling and bacteriological analyses

To follow the bacteriological quality of water during protocols, we counted the total flora at 22ºC and 37°C in the water at different time and locations on the water line system and evaluated the biofilm before and after the experiment with cotton swabs.

The first water analysis was done before the experiment directly on the water coming from drilling before the metering pump and the treated water circuit. This bacteriological analysis was considered as a starting point to indicate the quality of the water in each farm before any protocols. Then to evaluate the bacterial concentration along the pipelines, the water quality have been checked directly at the watering place, in the troughs before the protocol, after the mechanical action and at the end of the protocol. In parallel, to evaluate the cleanliness of the pipes, water quality was checked with cotton swabs in the pipes of the troughs before and after the experiment. All the samples were stored at 4°C and analysed within 4 hours after sampling.

Five hundred-milliliter sterilized collection bottles that contained sodium thiosulfate to neutralize residual chlorine (IDEXX Labs) were used to collect the water samples. Sterile cotton swabs (Copan®) for polyvinylchloride surface or sterile nylon swabs for stainless surface were used and dipped in 25 milliliter of sterile buffer phosphate diluent. Ten-fold serial dilutions of each sample were made with phosphate buffer solution.

The enumeration of the mesophilic and aerobic total flora was done following standard plating technique. One milliliter of each sample (water or swabbed solution) was directly plated on the petrifilm (3M™ Petrifilm™) and one milliliter of each serial dilution was plated on PCA plates (Plate Count Agar) in duplicate and incubated for 48h respectively at 22ºC and 37ºC.

Results and discussion

In the three selected farms, the initial water quality (before the metering pump) was really different (Tables 2 and 3). Farm A presented a total flora at 37°C above 100 Colony Forming Unit (CFU)/mL of water, which is higher that the recommendations of the OIE for animal drinking water quality (10 CFU/mL) (Table 2).

Bacterial concentration in water increased along the pipelines: total flora was higher at watering place than at the entry of the building before the metering pump for all farms (Tables 2 and 3).

Table 2. Evolution of the total flora at 37ºC in the water and in the pipelines along the water system of each farm before and after the two different waterline cleaning protocols.

		Total flora at 37°C (CFU/ml)					
		Before the metering pump	Water analysis (CFU/ml)			Cleanliness of the pipelines (CFU/swab)	
			Water at watering place (troughs)			Water pipes of the troughs	
			Before protocol	After mechanical action	After protocol	Before protocol	After protocol
Farm A	*PW1*	>100	356	19	29	660	<100
	PW2		312	412	9	60	<100
Farm B	*PW1*	<10	17.000	63.000	1.000	2.800	10
	PW2		13.000	340.000	800	20.000	180
Farm C	*PW1*	10	6.000	410	110	540.000	60
	PW2		60.000	180	7	5.300	30

For some rooms, the number of CFU in the water at the watering place increased after the line flushing compare to the initial state before the protocol (Tables 2 and 3), which underline the mechanical effect of line flushing on the biofilm. Even when the water supplies are clean, biofilm formation can still occur (Momba et al., 1998) and the biofilm growth can be impacted by temperature or flow of water (Silhan et al., 2006).

In the post-weaning rooms, just after weaning, the temperature is often around 28-30°C with a slight flow of water to start with. Moreover these biofilms can harbor pathogens (Wingender and Flemming, 2011). The mechanical action of flushing water under pressure is an essential step, necessary to pull off the biofilm, which became available for the disinfectant.

Both protocols combining mechanical and chemical procedures reduced total floral (22°C and 37°C), improved water quality and cleanliness of pipes (Tables 2 and 3).

Table 3. Evolution of the total flora at 22ºC in the water and in the pipelines along the water.

		Total flora at 22°C (CFU/ml)					
		Before the metering pump	Water analysis (CFU/ml)			Cleanliness of the pipelines (CFU/swab)	
			Water at/in the troughs			Water pipes of the troughs	
			Before protocol	After mechanical action	After protocol	Before protocol	After protocol
Farm A	*PW1*	77	548	116	34	360	<100
	PW2		95	456	5	70	<100
Farm B	*PW1*	<10	27.000	380.000	3.000	2.500	10
	PW2		110.000	780.000	160	20.000	20
Farm C	*PW1*	16	6.100	450	92	10.000	10
	PW2		150.000	990	3	2.300	<10

Conclusion

Our study confirmed that waterlines cleaning protocols used in poultry farms can be transferred easily in post-weaning rooms. The setting up of these cleaning protocols in the water system requires only a drain valve and a pressure reducer for the mechanical action and eventually the add of a metering pump (really common now in most of the pig farms). By reducing water's total flora and the formation of biofilms, these waterlines cleaning protocols could be part of the health prevention measures for troubles which are linked to a poor water quality. The improvement of water management could be also used to reduce antibiotic consumption especially during this period. It would be interesting to measure the re-contamination of water flowing in pipes in order to adapt protocols mixing optimization of water quality for animals and convenience for farmers.

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NATURAL FEED ADDITIVES AS ALTERNATIVE TO IN-FEED MEDICATION

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Introduction

The use of antibiotics in livestock production is very common and widespread. The reason for their use can be very diverse. Examples are the treatment of clinically sick animals, the improvement of growth performance, as well as the prevention of common bacterial infections. However, the extensive use of such antimicrobials raised concerns of increasing the incidence of resistant pathogenic bacteria, which has a negative impact not only on livestock production, but also on human health. In the last years, many different substances have been investigated as suitable alternatives to the use of antibiotics as growth promoting agents and as prophylactic substances. Organic acids, blends of such acids and phytogenic feed additives have been accepted as a possible alternatives with distinct mode of actions. Organic acids are strong antimicrobial substances proven to inhibit the growth of pathogenic bacteria in the gut and support a balanced gut microbiota (Suiryanrayna and Ramana, 2015), while selected phytogenic feed additives including essential oils proved to be able to reduce inflammation and oxidative stress in animals, improving herewith the nutrient digestibility. In this study the efficacy of an organic acid based feed additive (OA) alone and in combination with a phytogenic feed additive (PFA) as natural alternative to in-feed antibiotics on the performance of nursery pigs was evaluated.

Materials and methods

480 weaned piglets [PIC 280 x 1050 (average body weight 6.22 ± 1.4 kg, age 22 days)] were randomly assigned to 4 different treatments (12 pens per treatment and 10 animals per pen). Pigs were fed 2 phases of experimental corn-soybean meal-dry whey based diets (Phase 1: from day 0 to 8; Phase 2: from day 9 to 21). Diets were formulated to contain 4.35 and 4.10 g SID lysine/MCal ME for phase 1 and 2, respectively. Dietary treatments were: 1) basal diet with no additive (NC), 2) basal diet with 50 ppm carbadox in phase 1, and 50 ppm neomycin plus 50 ppm oxytetracycline in phase 2 (PC), 3) basal diet with 50 ppm carbadox in phase 1, and 1000 ppm OA in phase 2 (COA) and 4) basal diet with 1000 ppm OA and 125 ppm of PFA in both phases (OAEO). Body weight and feed intake were measured weekly. Average daily weight gain was calculated. Data were subject to statistical analyses using a mixed model. Weight block was used as the random effect, and multiple comparisons were evaluated using the t-test method.

Results and discussion

Body weight (BW) for PC group (11.19 kg, P=0.001) at the end of the trial was greater than NC (10.53 kg) but it did not differ from COA (11.03 kg, P=0.382) and OAEO (10.85 kg, P=0.074). BW difference between OAEO and NC (P=0.100) was not significant. Average daily weight gain (ADWG) for the total trial duration was higher in

PC (226 g/d) and COA (215 g/d) compared to NC (193 g/d, $P \leq 0.024$), but did not differ from OAEO (210 g/d, $P \geq 0.101$). Average daily feed intake (ADFI) did not differ among groups ($P=0.242$). Results are summarized in Table 1.

Table1. Effect of in-feed antibiotics (PC), an organic acid based product (COA), and a combination of the organic acid based product with a phytogenic feed additive (OAEO) on growth performance parameters.

Overall period (days 1 to 21)			
	BWG kg/animal	**ADFI g/animal/day**	**ADWG g/animal/day**
NC	10.53[a]	267	193[a]
PC	11.19[b]	281	226[b]
COA	11.03[b]	279	215[b]
OAEO	10.85[a,b]	272	210[a,b]

[a,b] values with different superscripts differ significantly.

The use of antibiotics in animal production is a common procedure. However, this practice has increased the emergence of resistant pathogenic bacteria. The transmission of antimicrobial resistance between animals, environment and humans increased the pressure to find alternatives to the use of in-feed antibiotics used as growth promoters. The exact mode of action of antibiotics used at sub-therapeutic levels has not yet been clearly described in literature. However, it is clear that it can both positively influence the animals' microbiota and exert and anti-inflammatory effect in the gut (Niewold, 2007; Lin, 2014). Organic acid based products and their combination with essential oils can be a powerful tool to be applied in a program that aims to reduce the usage of antibiotics as growth promoters. In fact, organic acids have a strong antibacterial efficacy and can be used to reduce pathogen pressure in swine production (Dibner and Buttin, 2002). Phytogenic feed additives have different properties depending on the substances used. Selected phytogenic substances can exert anti-oxidative and anti-inflammatory effects and are even able to improve digestibility (Windisch *et al.*, 2008; Hafeez *et al.*, 2015). Independently from their mode of action, alternatives to antibiotic growth promoters should aim to increase performance of the animals. The results of this experiment showed that it is possible to reduce or replace in-feed antibiotics with natural alternatives.

Conclusion

The reduction or elimination of the use of antibiotic growth promoters can be very difficult. When seeking for alternatives, a holistic approach needs to be considered. Solutions should be designed according to producers needs and biosecurity has to be one of the main factors to be taken into consideration. Organic acid based products and phytogenic feed additives can be considered as successful alternatives to antibiotics used at sub-therapeutic levels.

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EFFECT OF THE ADDITION OF PROTECTED SODIUM BUTYRATE TO THE FEED ON *SALMONELLA* SPP. INFECTION DYNAMICS IN FATTENING PIGS

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Abstract

The effectiveness of a new form of sodium butyrate protected with sodium salt of coconut fatty acid distillate for the control of *Salmonella* infection in fattening pigs was assessed. A dose of 3 kg/T of this product was added to the basal diet of a group of pigs for the whole fattening period while another group within the same fattening unit remained without treatment. A significant reduction in the number of infected pigs (4% *vs*. 61%; $P<0.01$) and in the median OD% values (19.4 *vs*. 55.9) at slaughter were observed in the pigs under treatment compared to the controls. Also, a significant association between high OD% values and *Salmonella* shedding and infection was detected. The use of this form of protected sodium butyrate may be useful to reduce *Salmonella* shedding and infection in slaughter pigs.

Introduction

In the pig industry, the use of antimicrobials may have favored the selection for antimicrobial resistance (AR) (Davies *et al*., 2010). The emergence of AR has prompted European Health Authorities to reconsider their use for meat production, reinforcing the search for alternative products for the control of enteric infections in pigs and triggering new EU antibiotics regulations.

Organic acids (OA) might be beneficial for the productive performance of fattening pigs (Partanen *et al.,* 1999), but they are also known for their *in vitro* capacity to inhibit the growth and proliferation of Gram-negative pathogens such as *Salmonella* spp. (Gantois *et al*., 2006). Therefore, OA are seen as an alternative to antibiotics to reduce the burden of pig salmonellosis.

Results on the effectiveness of OA for the control of pig salmonellosis are variable (Creus *et al.*, 2007; Walia *et al*., 2016), which is likely associated to different study designs or the use of different acids, blends, or doses. There is a clear need for more research on the use of OA for *Salmonella* reduction in fattening pigs to get a better idea of the effectiveness of the different types of OA available in the market. In particular, on new forms of OA (i.e. encapsulated or protected OA) that may act on the more distal part of the gastrointestinal tract (Piva *et al*., 2007). The effectiveness of protected sodium butyrate (PSB) in reducing *Salmonella* has been shown previously in chicken (Fernández-Rubio *et al*., 2009). Thus, a field trial was carried out to assess the effect of the addition of PBS to the feed on *Salmonella* infection dynamics in fattening pigs from an area of high *Salmonella* infection prevalence.

Material and methods

This study was carried out in a small (8 pens, ≈110 pigs) commercial *Salmonella*-infected fattening unit located in Spain. A new form of sodium butyrate protected with sodium salt of coconut fatty acid distillate (DICOSAN+, Norel SA, Spain) was added to the feed (3 kg/T) in 4 randomly selected pens (approx. 50 animals, treatment group - TG). The remaining 4 pens were fed with the same regular diet without the treatment (control group -CG). The treatment with the protected sodium butyrate (PSB) was initiated after finishing the in-feed antibiotic treatment.

Serum samples from all pigs were collected after 30 (beginning of the treatment with PSB), 60 and 90 days on the fattening unit, and within three days before slaughter, to check for the presence of antibodies against *Salmonella* spp. On-farm fecal samples (OFFS) were collected along with the blood from 25 pigs per group, after spontaneous defecation. At slaughter, fecal (FSS) and mesenteric lymph nodes (MLN) samples from all pigs were collected after evisceration. Bacteriology from individual fecal samples and mesenteric lymph nodes (MLN) was performed following the EN ISO 6579:2002/A1:2007. For serology an indirect ELISA (HerdCheck Swine *Salmonella*, IDEXX Laboratories, ME, USA) was used and three cut-off values considered (%OD ≥10, ≥20 y ≥40).

Fisher exact test was used to assess statistical differences between the CG and the TG regarding the proportion of *Salmonella* shedders at different sampling times, and infection prevalence (proportion of MLN-positive pigs) at slaughter in each trial individually. A one-tailed *P*-value ≤0.05 was considered for significance. A repeated measures analysis was used to estimate differences in median OD% values in each group after taking into account sampling times and the interaction treatment*time. Statistical analyses were performed with STATA software (STATA, StataCorp, L.P., USA).

Results

Both groups showed a large proportion of shedders on the first sampling, but somewhat higher for the CG. The proportion of shedders decreased significantly in the following samplings in both groups and virtually no significant differences between them were observed along the fattening period (Figure 1). However, at slaughter, the proportion of infected pigs (MLN+) was significantly higher for the CG compared to the TG (61% vs. 4%; *P*<0.01).

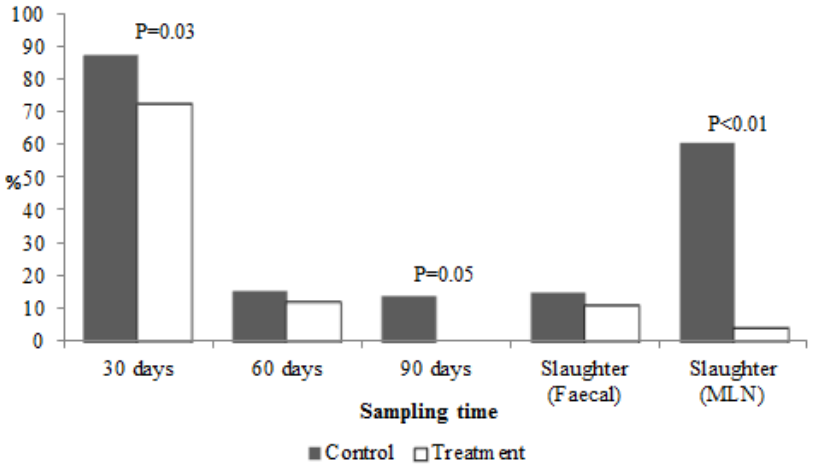

Figure 1. Summary results of bacteriology on fecal samples for the control (CG) and treatment (TG) groups at different sampling times.

The median OD% value for both groups was similar at 30 days, but in subsequent samplings median OD% values remained significantly lower for the treatment group (Figure 2). A significant overall interaction between treatment and time was observed. For the TG, median OD% rose significantly from the first (OD%=14.9) to the second sampling (OD%=29.5), but they started to decrease after that. Median OD% values at 90 days on fattening and at slaughter were similar to the median OD% value found for the first sampling on day 30 (14.9 and 19.4, respectively). However, in the CG an overall increasing trend of median OD% values was observed, from 20.2 on day 30 to 55.9% at slaughter. Differences in seroprevalence were also observed after 60 days on fattening when a cut-off value ≥40% was used. These differences remained significant in subsequent samplings for the 20% and 40% cut-off values as well.

It was observed a positive relationship between serology and shedding and serology and infection (MLN+). A seropositive pig (i.e. OD%≥40) had 9 times (OR= 9.2; 95%CI: 1.9, 45.3; *P*= 0.003) higher risk of shedding *Salmonella* at slaughter and 4 times (OR= 4.1; 95%CI: 1.4, 12.4; *P*=0.003) higher risk of being infected.

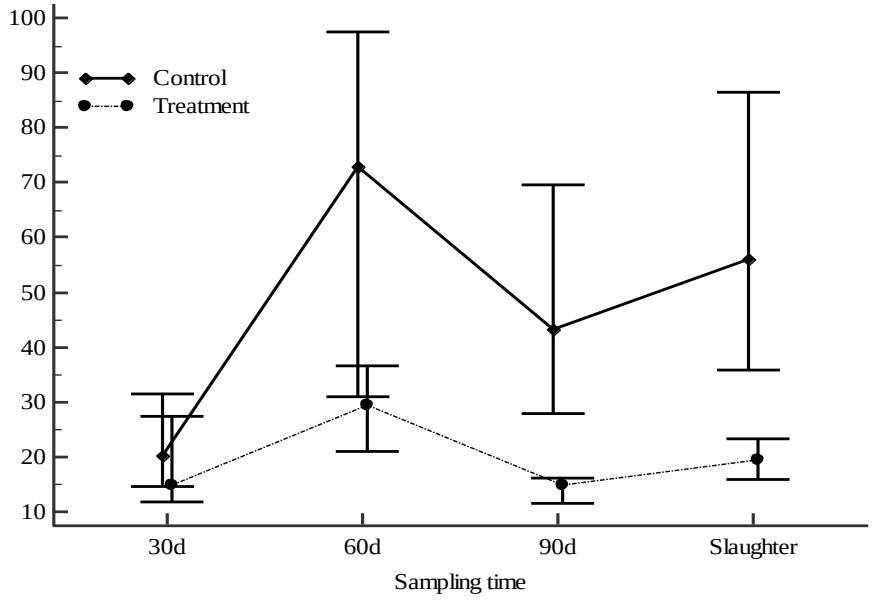

Figure 2. Median OD% values and their corresponding 95%CI for the control and treatment group at each sampling time.

Discussion

A very large percentage of pigs were shedding *Salmonella* on the first sampling after 30 days on fattening in both groups. This result seemed to be related to an early exposure of the farm unit to *Salmonella*. Further samplings showed a significant reduction in the level of shedding in both groups. This drop of *Salmonella* shedding along the fattening period seemed to be likely related to the adaptation of the pigs to the unit environment.

Given the large number of pigs shedding *Salmonella* on day 30 in both groups, a large number of infected (MLN+) pigs at slaughter was expected. Although this was true for the CG, as 60.7% of pigs resulted infected, it was not observed in the TG (4.3%). This finding suggested a protective effect of DICOSAN+ against *Salmonella* infection despite the high level of exposure to *Salmonella* of these pigs at the beginning of the fattening period. Serological results would further support this conclusion. After

an initial increase of median OD% values in the TG, they significantly decreased over time despite the presence of *Salmonella* in the farm environment. The OD% values in the TG only increased from the first to the second sampling, suggesting that some time under treatment is required (probably a minimum of 3-4 weeks) in order for the product to be effective. On the contrary, in the CG, OD% values increased significantly from day 30 to slaughter, with a median OD% value at slaughter much higher than the maximum cut-off value usually considered for deeming a pig as seropositive (i.e. 40%).

The low number of pigs in both groups that were shedding at slaughter was an unexpected result and it may be the consequence of the short period of transport and lairage. Transport to slaughter and lairage are usually factors that prompt infected pigs to shed *Salmonella* due to the stress that they produce on the animals (Scherer *et al*., 2008). In this case, the transport lasted only for half an hour and the lairage was short as well (≈ 2 hours), which may have prevented high levels of stress in these pigs. In addition, the level of cleanliness of the lairage area was high as new facilities had been built for this slaughterhouse, which likely contributes to impair the transmission of the pathogen between pigs (Berends *et al.*, 1997).

Conclusion

A significant reduction in the number of infected pigs and in OD% values were observed in the pigs under treatment, and high OD% values were positively associated to both *Salmonella* shedding and infection. Therefore, the use of DICOSAN+ at 3kg/T would appear as a potential strategy to reduce *Salmonella* shedding and infection in slaughter pigs when used during the whole fattening period.

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COMBINATION OF ESSENTIAL OILS AND ANTIBIOTICS AGAINST *STREPTOCOCCUS SUIS*: A PRELIMINARY STUDY

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Introduction

Streptococcus suis is a major pathogen in the pig industry, associated with a wide variety of pigs diseases, such as meningitis, arthritis, bronchopneumonia, endocarditis, polyserositis and septicaemia. In addition, it is a zoonotic agent causing severe infections in people related with infected pigs or pork-derived products. The control of the disease is based on the antimicrobial therapy and sanitary measures, since there are not available commercial vaccines yet.

Relatively high levels of resistance have been detected in *S. suis* species against antimicrobials of frequent use in swine herds. Different strategies to reduce the use of antimicrobials have been proposed, natural products with antimicrobial effects and its use combined with antibiotics can be an attractive alternative. Unlike antibiotics, the complex composition of the essential oils (EO) allows them to develop a great diversity of mechanisms of action, emphasizing the alteration of permeability and bacterial survival. Previous studies by our research group (de Aguiar *et. al*. 2017) showed the effectiveness of four essential oil against *S. suis*. The objective of this study was to evaluate the possible *in vitro* effect of the combination of penicillin (P) and trimethoprim-sulfamethoxazole (SXT) with cinnamon, oregano, common thyme and red thyme essential oils, against five *S. suis* field isolates resistant to P or SXT.

Materials and methods

The four commercial EOs (purity ≥ 98%) were purchased from Aromium® (Barcelona, Spain). A total of eight *S. suis* isolates from diseased pigs resistant to P and/or SXT from the Culture Collection of the Animal Health Department (University of Cordoba, Spain) were analyzed. The test was carried out in 96 wells microtiter plates, mixing different concentrations of each antimicrobial and EO, using the Checkerboard method. Both products were diluted in Brain-Heart Infusion Broth to concentrations twice or three times the expected MIC. The combined products was mixed with the equal volume of bacterial suspension (106 CFU/mL). Then, the plate was incubated at 35°C for 20-24 h under aerobic conditions. Every assay were carried out in duplicate. From the best combination that inhibited the bacterial growth, the fractional inhibitory concentration index (FIC_{index}) was was calculated to determine possible synergic effect [FIC_{index} = antimicrobial agent FIC (minimum inhibitory concentration (MIC) of antimicrobial agent combined/antimicrobial agent MIC alone) + essential oil FIC (essential oil MIC combined/ essential oil MIC alone)]. According to EUCAST (2000), a synergic effect was consider when $FIC_{index} \leq 0.5$; Additive >0.5-1; Indifferent >1 <2; or antagonist when $FIC_{index} \geq 2$.

Results

The combined effect of the antimicrobial agents with EOs expressed by FIC_{index} (Table 1), did not present any antagonism. The combination of penicillin-EOs showed additive effect in 50% of cases, with better results for P-oregano and P- common thyme (additive effect in 3/5 strains). For P-cinnamon and P-red thyme, an additive effect was observed in 2/5 isolates. All other cases of penicillin-EOs were indifferent. On the other hand, the combination of SXT-cinnamon and SXT-common thyme showed an additive effect in 2/5 strains, while for SXT- red thyme this effect was observed in 1/5 isolates. An indifferent effect was observed with SXT-oregano for all the strains. A reduction of the MIC values for penicillin (2-16 fold), SXT (2-256 fold) and EOs (below the cytotoxicity concentration described by other authors) were also observed.

Table 1. The fractional inhibitory concentration index (FIC_{index}) of conventional antibiotics and EOs in combination.

Penicillin				
Strains	**Cinnamon**	**Oregano**	**Common thyme**	**Red thyme**
123/11	0,56	0,56	1,25	1,13
3144	0,56	0,63	0,63	0,75
226/03	1,25	1,25	0,56	1,03
6217	1,25	1,25	0,63	1,00
CO 73H	1,25	1,00	1,01	1,01
Trimethoprim-sulfamethoxazole				
123/11	1,13	1,25	1,25	1,25
3144	1,00	1,25	1,25	1,25
10/06	1,50	1,25	0,51	1,25
6217	0,75	1,25	1,25	0,75
8010	1,25	1,25	0,50	1,50

Additive effect in bold.

Conclusion

The results of this work point out the potential of combination therapy for the control of resistant isolates of *S. suis* obtained from pigs. Further studies are necessary to obtain synergic combinations of antimicrobials and essential oils and *in vivo* safety for the therapeutic use of OEs.

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SALMONELLA CLINICAL ISOLATES FROM BRAZILIAN PIG HERDS: GENETIC RELATIONSHIP AND ANTIBIOTIC RESISTANCE PROFILING

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Abstract

In Brazil, since 2011 clinical cases of salmonellosis has been increasing substantially. Nevertheless, few information is available about the antimicrobial profile, distribution, serotypes and genetic relationship among the strains. The objectives of this study were: to identify the *Salmonella* serotypes, to characterize the *in vitro* antimicrobial resistance profiles and to determine the genetic relationship of clinical isolates in Brazil. During 2016, clinical isolates of *Salmonella* (111) from nine States were sent to Embrapa Swine and Poultry for complementary analysis. First, isolates were serotyped by Kauffmann White Scheme. In parallel, the strains were tested against fifteen antimicrobials by disk diffusion method and genotyping was performed by Pulsed Field Gel Electrophoresis (PFGE) using the *XbaI* restriction enzyme. As expected, the main serovars found were Typhimurium and Choleraesuis. Four strains showed resistance to only one antimicrobial and 76.5% (85/111) were considered multi-resistant. The highest level of resistance was found against to tetracycline. More than 80% of the strains were susceptible to fosfomycin, lincomycin/spectinomycin and norfloxacin. It was possible to identify one major Choleraesuis clonal group present in different Brazilian States. Further, several small clonal groups were obtained for Typhimurium. In conclusion, clinical salmonellosis caused by Typhimurium and Choleraesuis is endemic in pig production areas and the majority of the strains are multi-resistant.

Introduction

Enteric diseases are a big concern for pig production due the economic impact resulting of poor animal performance. Among all the bacteria that are involved in enteric disorders, *Salmonella* is always present and plays an important role as pathogen and reservoir of antimicrobial resistance genes. The major *Salmonella* serovars associated with clinical disease in pigs are related with pathogenic features. Septicemic salmonellosis is usually caused by host-restricted serovar Choleraesuis and severe enteritis due to the ubiquitous serovar Typhimurium (Mastroeni et al., 2006).

Until 2011 few clinical cases of salmonellosis were reported in Brazil, indicating that the problem used to occur less frequently than other countries. However, at beginning of 2013 the number of salmonellosis outbreaks has been increasing

substantially, mainly in the most important swine production states (Vanucci et al., 2014). Also, a study by Santos et al. (2016) has reported an occurrence of *S.* Choleraesuis in outbreaks with respiratory and circulatory disorders during 2013 to 2015 in Brazil.

In order to understand the epidemiology of this disease, the phenotypes and clonal relationships investigation between strains from different areas can provide valuable information (Zhao et al., 2007). Furthermore, to determine the antimicrobial resistance profile is important in the clinical perspective and helps to understand the epidemiological context. In Brazil, there are still a limited published data relative to *Salmonella* clinical isolates characterization. In view of that, the objectives of this study were: to identify the serotypes, to determine the antimicrobial resistance profiles and to relate the isolates by pulsed-field gel electrophoresis (PFGE).

Material and methods

During 2016, clinical isolates of *Salmonella* provided from three specialized diagnostic laboratories were sent to Embrapa Swine and Poultry for phenotypic and genotypic characterization. Initially the isolates were serotyped according Kauffmann White Scheme by slide agglutination. Strains without phase 2 flagellar antigen expression after two tries of phase inversion were considered monophasic.

Antimicrobial resistance profile was determined against fifteen antimicrobials by disk-diffusion method according to Clinical and Laboratory Standards Institute, document VET 01-S2 and M100 (CLSI, 2013, CLSI 2016). In particular, disc zone diameters determined for colistin (resistance $\leq$ 8mm and susceptibility $\geq$ 11mm) has been according with Maalej et al, 2011 and Rodríguez et al, 2004. The following antimicrobial were analyzed: ceftiofur (CEF) 30µg; ciprofloxacin (CIP) 5µg; colistin (COL) 10µg; doxycycline (DOX) 30µg; enrofloxacin (ENR) 5µg; streptomycin (STR) 10µg; florfenicol (FFC) 30µg; fosfomycin (FOS) 200µg; gentamicin (GEN) 10µg; lincomycin/spectinomycin (LSC) 109µg; marbofloxacin (MAR) 5µg; neomycin (NEO) 30µg; norfloxacin (NOR) 10µg; sulfamethoxazole-trimethoprim (SXT) 25µg; and tetracycline (TET) 30µg.

The isolates relationship was investigated by macrorestriction analysis with *Xba*I. The technique was performed based on PulseNet protocol (www.cdc.gov/pulsenet/pdf/ecoli-shigella-salmonella-pfge-protocol-508c.pdf). The DNA fragments were separated by pulsed-field gel electrophoresis (PFGE) using CHEF-DR III (BioRad). Whole cell DNA of *S.* Braenderup H9812 digested with *Xba*I served as size marker. Macrorestriction profiles (pulsotypes) were analyzed using BioNumerics software package, version 3.0. The similarities were determined by Dice correlation coefficient, with a maximal position tolerance of 1.7% (Carriço et al., 2005). Pulsotypes were clustered the unweighted pair group method with arithmetic averages (UPGMA).

Results and discussion

Studied clinical cases were diagnosticated in nine States of Brazil from 2011 to 2016 as follow: Santa Catarina/n=37, Minas Gerais/n=35, Rio Grande do Sul/n=11, São Paulo/n=10, Paraná/n=8, Mato Grosso/n=3, Mato Grosso do Sul/n=1, Goiás/n=1 and Distrito Federal/n=1. Out of 111 strains, 64 were from septicemic and 45 from enteric cases (two strains lacking information). The serotypes distribution were: Typhimurium 60/111 (monophasic 34/60; Typhimurium 26/60); Choleraesuis 40/111; Rissen 5/111;

and a single isolate of Heidelberg, Panama, Derby, Grupo D, Anatum, and Bovins-morbificans.

From 111 tested *Salmonella* sp. only four strains showed resistance to one antimicrobial. In the other hand, 85 (76.5%) isolates were resistant to three or more antimicrobial classes and classified as multi resistant according to Schwarz et al. (2010). The highest frequency of resistant isolates was found against tetracycline (90,99%), followed by gentamicin (77,47%), doxycycline (76.57%) and florfenicol (74.77%) as summarized in Figure 1. In contrast, more than 80% of the strains were susceptible to fosfomycin and lincomycin/spectinomycin. Comparable results were found by Vannucci et al (2014) and Santos et al. (2016) in *Salmonella* Choleraesuis isolates from Brazilian clinical outbreaks.

Specifically for Choleraesuis and Typhimurium confirmed serovars, it was possible to get PFGE pulsotypes from 90 isolates. The results had shown one major Choleraesuis clonal group (pulsotype C1- Figure 2) in 35/38 isolates, conversely for Typhimurium (including the monophasic ones) it was found 16 pulsotypes. Choleraesuis pulsotype C1 was wide distributed in the pig production area encompassing six States: MG, SP, PR, GO, RS and SC. Besides that, 14 pulsotype C1 isolates showed the same resistance profile [DoxStrpFfcGenTet] and more 10 isolates contained the same basic profile with others additional antibiotics.

As expected the serovar Typhimurium has presented a large genotype and phenotype variability. It was obtained several small clonal groups from 52 isolates, the biggest one with 13 isolates and a single profile in 8 isolates. Likewise, 43 resistance profiles were determined for 60 Typhimurium isolates, the major clonal group presented 9 profile in 13 isolates. The serovar Typhimurium is considered ubiquitous and wide spread in Brazilian pig farms (Kich et al. 2011), in view of that is logical to found more genetic differences among the isolates. Also, the heterogeneity resistance profiles for Typhimurium may be attributed to the different antimicrobial exposures that the microorganisms are submitted to animal husbandry environments (Mathew et al., 2007).

Conclusion

Clinical salmonellosis is endemic in pig production areas, the main involved serovars are Typhimurium and Choleraesuis. One big clonal group of Choleraesuis and several small groups of Typhimurium are widely distributed in Brazil. The *Salmonella* strains involved in these cases present high level of antimicrobial multi resistance.

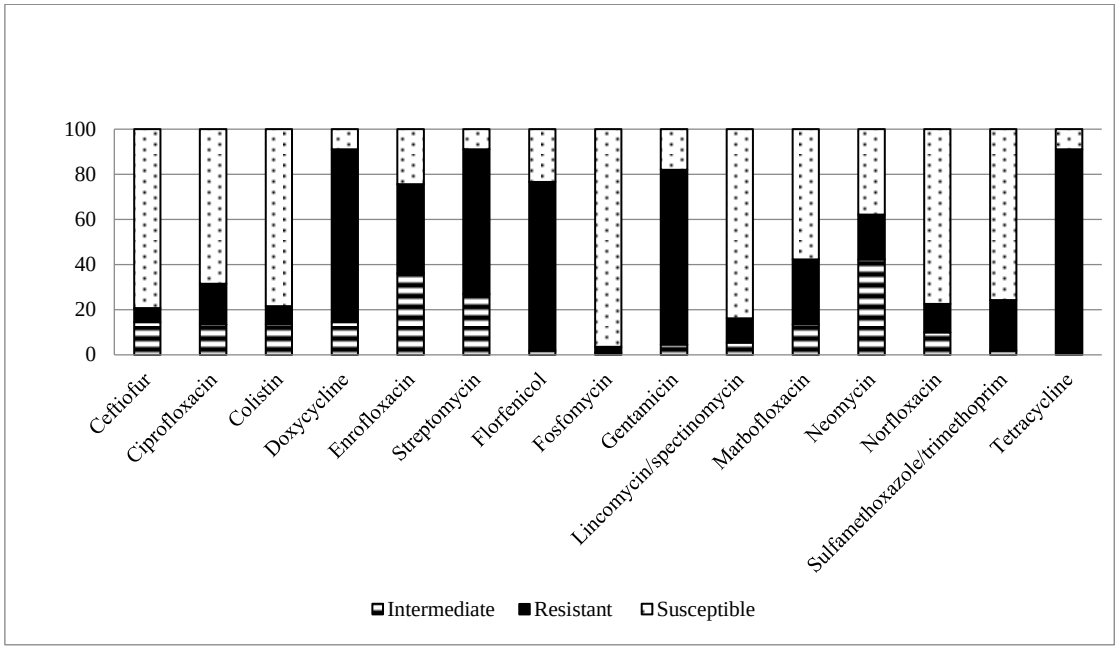

Figure 1. Percentage of *in vitro* antimicrobial resistance of 111 *Salmonella* clinical isolates.

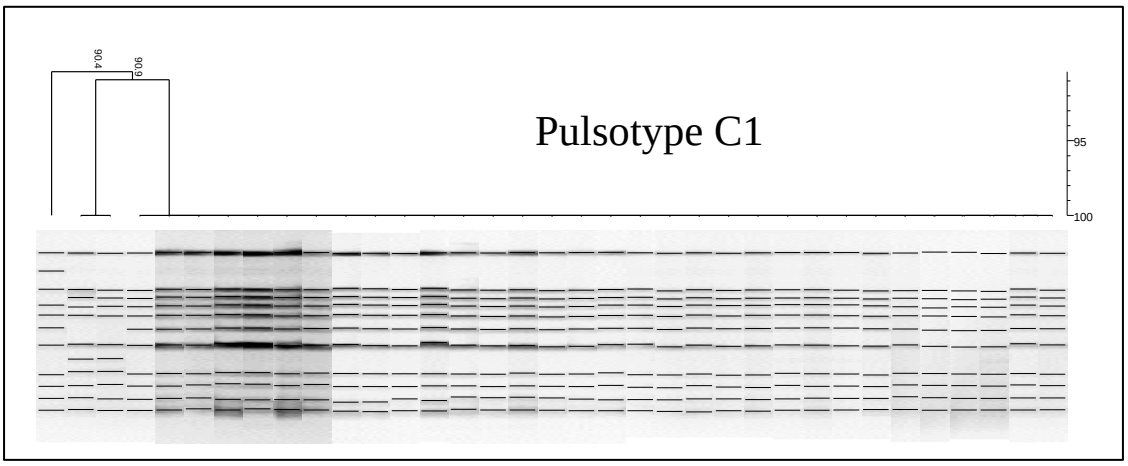

Figure 2. PFGE dendogram of *S.* Choleraesuis isolates from Brazilian clinical septicemic cases

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DETECTION OF *SALMONELLA* HEIDELBERG RESISTANT TO COLISTIN IN THE INTESTINAL CONTENT OF PIGS AT SLAUGHTER

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Abstract

S*almonella* Heidelberg has increasingly been reported as cause of human salmonellosis worldwide. In Brazil, *S.* Heidelberg has been reported in poultry but it is infrequently isolated from pigs. Here, we describe the isolation of *S.* Heidelberg resistant to colistin from slaughter pigs. Five pigs and their carcasses belonging to a same slaughter batch in ten consecutive days were sampled for fragment of intestine in the ileocecal region and sponges rubbed on the carcass surface (400 cm^2) before chilling. *Salmonella* detection was performed according to the ISO 6579:2002. Intestinal content was also subjected to *Salmonella* enumeration by a miniaturized Most Probable Number (MPN) protocol. *Salmonella* isolates were characterized by antimicrobial resistance by the disk diffusion test, the minimum inhibitory concentration to colistin determination and to gene *mcr*-1 investigation by PCR. *Salmonella* was isolated from the intestinal content of 64% (32/50) of the pigs, in amounts that varied from 2.7 to >1,400 MPN/g. *Salmonella* Heidelberg was the most frequent serovar identified in the intestinal content samples (20/50; 40%), and this serovar was present in eight of the ten pig batches sampled. At the prechill, *Salmonella* was isolated from 8% of carcasses, and *S.* Heidelberg was not detected. *Salmonella* Heidelberg strains were resistant against ampicillin (n=9), tetracycline (n=8), sulfonamide (n=8) and gentamicin (n=5). Nine multi-drug resistant strains were detected; among them four strains were positive for the gene *mcr*-1. In these strains the MIC value was 8 $\mu g.mL^{-1}$, while in the strains without the *mcr*-1 gene it ranged from 2 $\mu g.mL^{-1}$ to 4 $\mu g.mL^{-1}$. Therefore, humans in contact with carrier pigs or their environment may be exposed to *S.* Heidelberg, including strains harboring the gene *mcr*-1.

Introduction

Salmonella enterica figures amongst the most important foodborne transmitted bacteria, and pork has been reported as a vehicle in outbreaks worldwide (EFSA, 2015). *Salmonella* strains isolated from pig carcasses are originated from the intestinal content of *Salmonella*-carrier pigs or are acquired by the contact with residual contamination of the slaughterhouse environment (BUNCIC et al., 2011). Therefore, the delivery of pig batches with a large number of animals excreting *Salmonella* represents a burden to the slaughtering process, in terms of bacterial load and serovar diversity. In Brazil, the high prevalence of *Salmonella* carrier pigs at slaughter has been reported, demonstrating that this may be a relevant factor in the *Salmonella* control in pork (KICH et al., 2011; SILVA et al., 2012).

The *Salmonella* serovars vary in prevalence overtime and according to the region. Recently, *Salmonella* Heidelberg has increasingly been reported in poultry and as cause of human salmonellosis (COLLA et al., 2012; CDC, 2016; GIERALTOWSLI et al., 2016). This serovar has also been pointed out as more invasive and carrying multi-drug resistance (MDR), which represents a further concern for human and animal health (CDC, 2016; GIERALTOWSLI et al., 2016). In swine, reports of *S*. Heidelberg are still rare, being *S*. Typhimurium and *S*. Derby among the most isolated serovars worldwide (KICH et al., 2011; DENIS et al, 2013). In this study, we describe the isolation of *Salmonella* Heidelberg in pig batches processed in a slaughter plant from Southern Brazil and their antimicrobial resistance profile.

Material and methods

Five slaughtered pigs and their carcasses belonging to a same slaughter batch in ten consecutive days were sampled for: *i*. blood collected at bleeding; *ii*. sponges (Nasco®) rubbed on the carcass surface (400 cm^2) after bleeding and before chilling; *iii*. fragment from the ileocecal region of intestine.

Serum samples were tested by the ELISA-Typhimurium test as previously described (KICH et al., 2007). The cut-off point of the test was the OD 0.169 (KICH et al., 2016). Sponges were individually suspended in 30 mL of buffered peptone water 1%, and 5 mL of suspension was used for Total Aerobic Mesophilic (TAM) enumeration in Plate Count Agar (PCA) (DOWNES et al., 2011). The remaining suspension volume was used for *Salmonella* detection according to the ISO 6579:2002. Intestinal content (25 g) was suspended in 225 mL buffered peptone water 1% and subjected to *Salmonella* detection (ISO 6579:2002). It was also subjected to *Salmonella* enumeration by a miniaturized Most Probable Number (MPN) protocol (PAVIC et al., 2010). *Salmonella* isolates were serotyped at the Fundação Instituto Oswaldo Cruz (FIOCRUZ).

Antimicrobial resistance profiling was determined against twelve antimicrobials by disk-diffusion test method performed and interpreted by Clinicial and Laboratory Standards Institute, document M100 (CLSI, 2016). The following antimicrobials disks were tested: ampicillin (10µg), azithromycin (15µg), ceftiofur (30µg), cefotaxime (30µg), ceftazidime (30µg), cefotriaxone (30µg), cefoxitin (30µg), ciprofloxacin (5µg), gentamicin (10µg), meropenem (10µg), sulfonamide (300µg) and tetracycline (30µg). Furthermore, the Minimum Inhibitory Concentration (MIC) to colistin was also determined (EUCAST, 2016). *Escherichia coli* ATCC® 25922 was used for quality control purpose. These isolates were screened for the presence of *mcr*-1 gene by PCR with primers CLR5-F (5′-CGGTCAGTCCGTTTGTTC-3′) and CLR5-R (5′-CTTGGTCGGTCTGTAGGG-3′) (LIU et al., 2016).

The TAM results were calculated as CFU.cm^{-2} and transformed into log10 for the analysis. Means of log10 CFU.cm^{-2} TAM were compared by the Tukey test (p=0.05) of the SPSS software.

Results and discussion

All 50 sampled pigs were positive in the ELISA-Typhimurium test demonstrating that they had been exposed to *Salmonella* on farm. A positive result in the ELISA-test cannot be interpreted as an active infection at slaughter; however, seropositive slaughter batches have higher chances of including pigs excreting *Salmonella* at slaughter (KICH et al., 2007). In this study, *Salmonella* was isolated from the intestinal content of 64% (32/50) of the seropositive pigs, in amounts that varied from 2.7 to >1,400 MPN/g, indicating that the burden of *Salmonella*-carrier pigs to the slaughter processing may be very high. Thus, hygienic measures are of utmost importance to avoid the contact of the carcass with the intestinal content and the environment contaminated with feces.

The carcasses sampled after bleeding presented TAM average of 3.28±0.53 log cfu.cm^{-2} and *Salmonella* was isolated from 16% (8/50) of them. At the pre-chill step, the same carcasses presented a TAM average of 1.84±0.49 log cfu.cm^{2}, representing a logarithmic reduction ranging from 0.64 to 2.35 log cfu.cm^{-2}. Moreover, the frequency of *Salmonella*-positive carcasses decreases to 8% (4/50). Slaughter steps, such as singeing, can decrease the superficial microbial contamination, while others represent a hazard of adding bacteria to the surface of the carcass (BUNCIC et al., 2011). Since the monitoring of TAM reflects the change on contamination throughout the processing, the logarithmic reduction achieved demonstrated that all batches were processed in accordance with hygiene standards. As a result, the number of *Salmonella*-positive carcasses detected after bleeding was decrease by the half, despite the high load of *Salmonella* present in the intestinal content.

Salmonella Heidelberg was the most frequent serovar identified in the intestinal content samples (20/50; 40%), and this serovar was present in eight of the ten batches. In spite of that, *S.* Heidelberg was detected neither in carcasses after bleeding nor at the prechilling, which indicate that the slaughter process might have been able to control the contamination by this serovar. *Salmonella* Heidelberg has infrequently been reported in pigs and pork (KICH et al., 2011; EFSA, 2015). On the other hand, this serovar has been increasingly prevalent in poultry and humans (COLLA et al., 2012; FOLSTER et al, 2012; GIERALTOWSKI et al., 2016; PALMEIRA et al., 2016). Noteworthy is that *S.* Heidelberg has been associated with invasive human infections and high mortality rates (CRUMP et al., 2011). Moreover, a *S.* Heidelberg outbreak in humans in close contact with cattle was reported (CDC, 2016), demonstrating that this is another possible transmission route to humans. Antimicrobial resistance in *S.* Heidelberg is of particular concern, since MDR strains have been implicated in human outbreaks (GIERALTOWSKI et al., 2016). In this sense, the 20 *Salmonella* strains tested were fully susceptible to azithromycin, cephalosporin (ceftiofur, cefotaxime, ceftazidime, cefotriaxone, and cefoxitin), ciprofloxacin and meropenem, antimicrobials considered as highly critical to human treatment (WHO, 2012). Resistance was detected against ampicillin (n=9), tetracycline (n=8), sulphonamide (n=8), gentamicin (n=5). A total of nine MDR strains (resistant to ≥3 antimicrobial classes) were identified. Four MDR strains presenting a profile including all the four aforementioned antimicrobials were also positive for the gene *mcr*-1, demonstrating that they were resistant to colistin. In all resistant strains the MIC value was 8 μg.mL^{-1}, while in the susceptible strains it ranged from 2 μg.mL^{-1} to 4 μg.mL^{-1}.The presence of the gene *mcr*-1 in *Salmonella* is a concern, since colistin is considered the last resource for treatment of MDR bacteria in humans and the gene is usually located in transferable genetic elements (LIU et al., 2016).

Conclusion

Although not detected in carcasses in this study, the identification of *S.* Heidelberg in the intestinal content demonstrated that this serovar is circulating in pigs and might eventually also be found in pork. Therefore, humans in contact with carrier pigs or their environment may be exposed to *S.* Heidelberg, including to strains harboring the gene *mcr*-1.

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INDUCTION OF RESISTANT MUTANTS OF *SALMONELLA* TYPHIMURIUM UNDER ENROFLOXACIN AND NATURAL ALTERNATIVES FOR CONTROL IN PIGS

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Introduction

The increase in bacterial resistance to Enrofloxacin (ENR) in recent years has been associated with the selective growth of single-step mutant strains resistant to the frequent dose in use, based on the Minimum Inhibitory Concentration (MIC). Because of that, it is supported the need to establish optimal dosage intervals taking into account the minimum concentration of antibiotic capable of inhibiting the growth of pre-existing or first pass mutant strains: Mutant Prevention Concentration (MPC). Therefore, it is of importance to maintain the antimicrobial and molecular monitoring of quinolones and to search for new alternatives, such as the use of essential oils for the control of salmonellosis.

The objectives of the present study were to induce the emergence of mutant strains of *S.* Typhimurium with reduced sensitivity to ENR, molecular characterization of the Quinolone Resistance Determining Region (QRDR) and to determine the MPC of ENR. Moreover, in order to examine the molecular basis of resistance to this fluoroquinolone, single-step induced strains selected from sub-MPC plaques for point mutations in the QRDR of the *gyrA*, *gyrB*, *parC* and *parE* genes were investigated.

In addition, the antimicrobial activity of EOs of cinnamon, oregano, red thyme and common thyme was evaluated against the strains obtained after being overexposured to ENR.

Material and methods

Bacterial strains: Two clinical strains susceptible to ENR of *S.* Typhimurium, isolated from clinical cases of pigs, and the reference strain ATCC 14028 were subjected to study.

Antibiotic: Enrofloxacin (HPLC 98% purity) of Sigma-Aldrich Co. (San Luis, Missouri, USA) has been used for this study.

Essential oils: Cinnamon *(Cinnamomum zeylanicum)* (cinnamaldehyde, linalool, eugenol); oregano (*Origanum vulgare*) (carvacrol, thymol, *γ*- terpinene); common thyme (*Thymus vulgaris*) (Thymol, *p*-cymene, linalool) and red thyme (*Thymus zygis*) (Thymol, *p*-cymene, *γ*-terpinene, linalool) were utilized, all of them of natural origin an provided by Aromium S.L., Barcelona-Spain.

Enrofloxacin susceptibility test: The methods of microbroth dilution and agar dilution were used to determine the MIC of ENR, according to CLSI guidelines (CLSI, 2015). Microdilution method was performed in microtiter plates, containing twofold dilutions of ENR. The agar dilution method consisted in incorporating the ENR into the

agar, containing each plate different concentrations of ENR in double dilution together with the bacterial inoculum until reach a final concentration of 5x105 CFU/mL.

Enrofloxacin resistance-inducing test and determination of MPC: To establish MPC, a bacterial inoculum of ≥1010 CFU/mL was used. This inoculum was cultured for 5 days on Müller Hinton agar plates supplemented with ENR (1x to 64x fold MIC) to identify the mutant strains and to determine the MPC. From plates supplemented with an enrofloxacin concentration ≥ 1x MIC, possible single-step mutants were randomly selected, cultured on enrofloxacin free agar plates for three serial passage, serotyped and then stored at -70 ºC.

DNA sequencing of QRDR: In order to examine the molecular basis of resistance to this fluoroquinolone, single-step induced strains selected from sub-MPC plaques for point mutations in the resistance-determining region to quinolones (QRDR) of the *gyrA, gyrB, parC* and *parE* genes were investigated.

Susceptibility test of EOs: The original strains and a selection of the strains obtained after induction were put together with the EOs by means of microdilution technique. This step was performed in microtiter plates, containing twofold dilutions of each EO tested and a bacterial inoculum with a final concentration of $5x10^5$ CFU/mL. The MIC of each test was determined as the lowest concentration of EO that prevents the visible growth of the bacteria in wells.

Results

The three strains of *S.* Typhimurium used in this study showed a MIC of 0.0625 μg/mL in test of microbroth dilution and agar dilution. This way, the concentration of EOs necessary to prevent the total growth of the bacterial population was in the three cases 4 times the MIC (MPC = 0.25 μg/mL).

In all assays, strains with reduced susceptibility to enrofloxacin (MPC/MIC = 4) were obtained, although none of them reached the point of resistance.

Characterization of QRDR region of the original strains and the strains obtained in the induction showed the wild type (*wt)* of the genes *gyrA, gyrB, parC* and *parE*. However, none of them presented genetic mutations.

The EOs of cinnamon, oregano, red thyme and common thyme demonstrated a strong antimicrobial activity against *S.* Typhimurium strains with reduced susceptibility to enrofloxacin (MIC 312.5 - 625 μg/mL).

Conclusions

The reduction observed in the susceptibility to ENR of the tested strains, without isolated mutations in the target genes of the QRDR region, supports the importance of other mechanisms described for the development of quinolone resistance (alterations in external membrane and the active efflux pump).

The selective growth of strains with reduced susceptibility to concentrations of ENR higher than MIC, highlights the importance of MPC in the determination of dosage regimens.

The results of this research show that EOs of cinnamon, oregano, common thyme and red thyme are able to control the growth of strains with reduced susceptibility to ENR arising by exposure to sub-MPC doses. These AE and/or their combination with

ENR would prevent the selective growth of resistant bacterial subpopulation and, in consequence, therapeutic failure.

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ANTIMICROBIAL RESISTANCE PROFILE OF *TRUEPERELLA PYOGENES* ASSOCIATED WITH SLAUGHTERHOUSE CONDEMNATIONS OF PIGS IN SPAIN

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Introduction

Trueperella pyogenes is an oportunistic pathogen involved in miscellaneous pyogenic infections in pigs and ruminants, including metritis, udder lesions, abscesses, pneumonia, arthritis, endocarditis, lymphadenitis and osteomyelitis. This microorganism has been related to located or generalized piogranulomatous lesions in different body locations, which are responsible for total or partial condemnations at slaughterhouses (Lara et al., 2011; Cardoso-Toset et al., 2015).

Nowadays, the antimicrobial therapy is the most important tool to control this disease, and the selection of the best drug is a critical point to avoid the development of antimicrobial resistances (Sheldon et al., 2004). However, studies in this field are scarce and the *in vitro* susceptibility of *T. pyogenes* isolated from swine against some antimicrobial has been poorly described in the literature. The determination of the resistance profile of *T. pyogenes*, by standard methods, to antimicrobials commonly used in swine livestock will contribute to the selection of adequate control measurements.

Objective

The objective of this research was to carry out an *in vitro* study to determine the resistance profile of *T. pyogenes* isolated from pigs against 11 antimicrobials commonly used in swine livestock.

Material and methods

A total of 182 *T. pyogenes* strains were isolated from healthy pigs sacrificed at different slaughterhouses in Spain, 88 belonged to animals raised in intensive systems and 94 to animals raised in free-range ones. Following the broth microdilution test (CLSI 2013, 2015) double serial dilutions of penicillin (P), amoxicillin (AMX), ceftiofur (EFT), apramycin (APR), gentamicin (CN), neomycin (N), streptomycin (S), enrofloxacin (ENR), oxytetracycline (OT), tylosin (TIL) and trimethoprim-sulfamethoxazole (SXT) were prepared in Müller-Hinton (MH) broth. All the antimicrobial agents were purchased from Sigma-Aldrich (Madrid, Spain). For every antibiotic, double serial dilution (from 0.06 μg/mL to 64 μg/mL) was mixed with an equal volume of bacterial suspension ($5x10^5$ CFU/mL). Test were carried out in a 96 wells plate and conducted in duplicate. Positive growth controls and negative ones were

included. The *Streptococcus pneumoniae* ATCC 49619 was included as quality control. After incubation at 37°C for 16-20 h, the Minimum Inhibitory Concentration (MIC) was determined as the lowest concentration of antimicrobial able to inhibit the visible bacterial growth in wells. MIC50 and MIC90 were also determined for every antimicrobial.

Results and discussion

According to cut-off points established for other Gram-positive bacteria, between 96.15% and 100% of isolates (MIC_{90} 0.06 µg/mL-1 µg/mL) were susceptible to ceftiofur, gentamycin, penicillin and amoxicillin. No differences were observed between isolates obtained from intensive or free-ranged pigs in regard to MIC_{50} and MIC_{90} values obtained with penicillin, amoxicillin, ceftiofur and apramycin. An important percentage of isolates were resistant to oxytetracycline (68.14%), profile which was higher in strains obtained from pigs reared in intensive system (81.82%) than in the ones obtained from free-ranged pigs (55.32%). Moreover, intermediate susceptibility or resistant isolates to trimethoprim-sulfamethoxazole (82.96% and 12.09%, respectively) were detected.

Conclusion

MIC_{90} values obtained for the strains under study show that beta-lactams, gentamycin and ceftiofur could be selected for empirical treatment of swine *T. pyogenes* infections. MIC_{90} of apramycin, neomycin, streptomycin and tylosin, for which cut-off points are not available, varied between 4 µg/mL and 64 µg/mL. All these results provide relevant information concerning the behaviour of *T. pyogenes* against different antimicrobials, as well as being particularly useful to propose cut-off points.

Table 1. Frequency distribution (%) of MIC of selected antimicrobials against 182 *Trueperella pyogenes* isolates with antimicrobial susceptibility cut-off points (CLSI, 2013).

Antimicrobial agent	% of isolates with MIC (µg/mL)											MIC_{50}	MIC_{90}
	≤0.06	0.125	0.25	0.5	1	2	4	8	16	32	≥64		
Penicillin	98.90	0.55	0.55	0	0	0	0	0	0	0	0	≤0.06	≤0.06
Amoxicillin	91.22	4.39	4.39	0	0	0	0	0	0	0	0	≤0.06	≤0.06
Ceftiofur	7.14	9.34	25.27	32.97	21.43	0	0	1.10	0.55	0	2.20	0.5	1
Apramycin	0	0	0	0	12.08	58.23	25.27	3.87	0	0.55	0	2	4
Gentamicin	3.30	7.69	26.92	32.97	19.78	8.24	0.55	0.55	0	0	0	0.5	1
Neomycin	0	0	1.10	1.10	2.20	12.64	43.41	18.68	12.08	8.79	0	4	16
Streptomycin	1.65	3.85	10.99	12.64	8.79	2.20	8.24	26.37	18.68	6.04	0.55	8	16
Enrofloxacin	2.20	2.75	13.74	45.60	29.12	6.59	0	0	0	0	0	0.5	1
Oxytetracycline	2.20	7.14	6.59	6.04	3.30	6.59	19.24	33.52	12.08	3.30	0	4	16
Tylosin	39.01	10.44	7.69	4.39	3.85	0.55	0.55	1.10	1.10	3.30	28.02	0.25	≥64
T-Sulfametox.	0.55	1.10	3.30	28.57	31.32	15.38	7.69	1.65	3.85	5.49	1.10	1	16

MIC_{50}: 50% of 182 = 91; MIC_{90}: 90% of 182 = 164. The cut-off points of the resistance profile are showed with single (susceptible) and double (resistant) vertical lines. Straight lines correspond to specific animal-species data, and zig-zag lines, to data taken from human (CLSI, 2013).

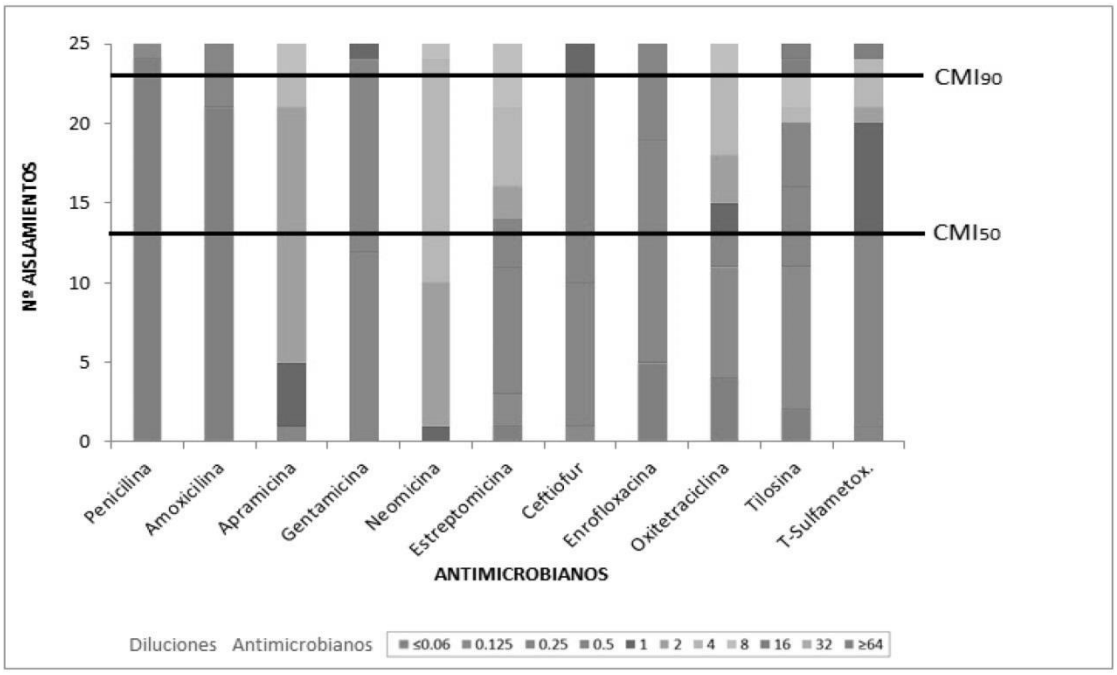

Figure 1. MIC values of selected antimicrobials against *T. pyogenes.* MIC_{50} and MIC_{90} (µg/mL) in horizontal bars.

DIETARY STRATEGIES AS ONE OF THE PILLARS TO REDUCE ANTIBIOTIC USE IN SWINE

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The Dutch government and various stakeholders in the industry set a joint target to reduce prophylactic and therapeutic antibiotic use by 70% in 2015 compared to the reference year 2009. The use of anti-microbial growth promoters was already banned in the European Union in 2006. Various biosecurity measures and specific vaccination programs were implemented in the last decade to reduce the risk of animals being exposed to pathogens and to raise the level of disease resistance. These strategies were complemented with dietary measures to improve hygiene of feed and water and to support homeostasis and stability in the gastrointestinal tract contributing to disease resistance and resilience. The Netherlands succeeded in reducing antibiotic use by 58% in swine production in 2015 compared to 2009. Although the target of 70% was not completely reached, it is encouraging that the reduced use of antibiotics resulted in a reduction in anti-microbial resistance (MARAN, 2015).

A variety of learnings and best practices can be adopted in other markets and areas where programs to reduce antibiotics are being developed and implemented. It is important to emphasis that implementation of Hazard Analysis and Critical Control Points (HACCP) based quality assurance systems has contributed significantly to systematically work on improving animal health and performance. Suitable housing conditions, proper climate control, a well developed feeding and vaccination strategy, and strict biosecurity measures form the basis of a proper farm management. In case of disease occurrence, veterinarians should perform proper diagnostics and investigate risk factors for disease occurrence, before antibiotics are used. Subsequently reduction of risk factors for disease occurrence by improvement of farm management should lead to prevention of disease outbreaks instead of preventive use of antibiotics.

The sow has a significant impact on growth and survival of piglets and development of disease resistance and resilience (Funkhouser and Bordenstein, 2013). In early life, colostrum intake is key to neonatal intestinal development and survival. Growth factors in colostrum are important for the tremendously development of the gastro-intestinal tract during the first days of life. Other important factors in colostrum are steroids, vitamins, enzymes and immunoglobulins. Piglets with low birth weights have a higher than average neonatal mortality rate. However, if intake of colostrum in these piglets is sufficient, mortality rate drops dramatically. Therefore, nutritional strategies influencing mammary gland development and colostrum yield are of importance and currently under further investigation (Decaluwe et al., 2014; Farmer et al., 2016).

Moreover, the transfer of the microbiota from the sow to its offspring plays an important role in development of immune competence of piglets, growth and survival rate. Rapid and large changes in abundance, composition and diversity characterize the development of the microbiota in the gastrointestinal tract of neonatal piglets (Bauer et al., 2006). The composition of the microbiota is initially influenced by the vaginal flora of the sow and subsequently by colostrum intake and environmental influences including fecal composition of sow feces. Changes in the composition of the microbiota

not only have short term effects on health and growth but also will affect later life disease resistance and performance. Oral antibiotic treatment of sows has a significant effect on the microbiota composition in the intestinal tract of their offspring and can lead to impairment of intestinal integrity after weaning (de Greeff et al., 2015). Also direct antibiotic treatment of piglets may lead to permanent changes in their microbiota with similar long-lasting effect (Zang et al., 2014).

Piglet performance later in life is determined for 30% by birth weight, for 30% by the weaning weight, and for 70% by the weight at 14 days after weaning, which shows the importance of the weaning process (Paredes et al., 2012). After weaning, environmental stress leads to reduction of blood flow to the intestinal tract and a drop in feed intake and immunity, which reduce intestinal integrity and disturb microbial balance in the intestinal tract. Creep feed intake during the sucking period stimulates early postweaning feed intake and performance (Bruininkx et al., 2002). Specific feeding measures like increasing prebiotic fiber in milk replacer during the suckling period increase gut maturation before weaning resulting in a higher intestinal weight, more cell proliferation and increased metabolic fermentation in the intestinal tract and therefore may help the piglet through the transition period (de Greeff, 2016).

High quality and highly digestible proteins prevent diarrhea in young piglets after weaning. An overload of undigested protein entering the distal intestinal tract may lead to excessive putrefactive fermentation and induce undesired changes in microbiota composition in the hindgut. Lowering the crude protein content, but keeping the essential amino acid levels adequate by using purified amino acids, is a well-established nutritional strategy to create feeds with a lower potential for diarrhea (Wellock et al., 2008). Atrophy of intestinal villi in the first week post-weaning causes an impairment in digestion and absorptive capacity of dietary fat (Price et al., 2013). The fat digestion process is dependent on hydrolysis of fats by lipase and micelle formation by bile salts, which is a more complex process compared to the hydrolysis of starch. This is the main reason why a high starch diet instead of a high fat diet can improve piglet performance post-weaning. Thermal processing can enhance the digestion of starch, which may further benefit the piglet in the first week post-weaning. Thereafter, the benefit of processing starch usually disappears. Providing more structure to the diet, by inclusion of larger particles by coarser milling, can promote the peristalsis of secretions in the digestive system, which can help prevent gastrointestinal infections (Flis et al., 2014).

Organic acids and short and medium chain fatty acids are the most common applied feed additives to reduce antibiotic use and are also applied via the drinking water in critical transition phases such as weaning and after transport or relocation. These additives contribute to feed and water hygiene, support the function of the stomach as microbial barrier, support the digestibility of protein, and control microbial activity in the proximal gastrointestinal tract (FEFANA, 2014). In addition, specific non-digestible oligosaccharides functioning as receptor analogue can reduce the risk of colonization of the large intestine by pathogens such as *Salmonella* and *E.coli* (Adewole et al., 2016; Baurhoo et al., 2007; Ganner et al., 2013; Jahanian et al., 2015).

Besides the intervention route of modulation of microbiota, it is possible to strengthen the mucosal barrier with feed additives. Blood plasma supports barrier function in piglets and reduces mucosal permeability and inflammation (Bosque et al., 2016). Butyrate promotes mucus secretion, stimulates turnover of enterocytes and has anti-inflammatory properties (Berni Canini et al., 2014). Additives such as β-glucans from fungi, plant extracts containing specific phenolic compounds, specific prebiotic sugars and some probiotic strains have immune-modulatory effects, of which some have

shown benefits in infection challenge studies with piglets (Rop et al., 2009; Chaucheryas-Durand et al., 2010; Gaggia et al., 2010). All these measures may contribute toward the host defense system. Combining additives with a different mode of action enhances support of the small intestinal microbiota and mucosal barrier function.

Overall, well-targeted dietary intervention strategies form an important pillar to reduce the use of antibiotics in swine, whilst maintaining performance and product quality. Therefore, the global feed industry can play a major role by adopting new insights and novel technologies in feed formulations and feed additives. However, all stakeholders in the chain need to cooperate and work at high speed to win the battle against antimicrobial resistance.

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ANTIMICROBIAL RESISTANCE PROFILING OF *SALMONELLA ENTERICA* DISTINCT SEROTYPES ISOLATED FROM PORK IN SÃO PAULO

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Introduction

Salmonellosis still is one of the most important worldwide zoonosis due to its high endemicity, mortality, and difficulty in control (Stevens et al., 2009). In the São Paulo city, different realities regarding good production practices and quality control of animal products coexists, especially when considering points of direct consumer sales. The aim of this study was to evaluate the antimicrobial resistance profiles of *Salmonella enterica* distinct serotypes isolated from pork in São Paulo.

Material and methods

A total of 60 *Salmonella enterica* strains were studied. The strains were isolated from pork cuts sold in butchers and small markets distributed among five regions of São Paulo city during the period of 2013 to 2016. The strains were submitted to antimicrobial resistance profiling by broth microdilution technique according to CLSI (2016) using Sensititre® standard susceptibility MIC plate EUVSEC (TREK Diagnostic Systems/Thermo Fisher Scientific). The strains were also serotyped through slide agglutination (Popoff, 1998). The association among strains origin, serotype and resistance profile was assessed through cluster analysis using Bionumerics 7.6 (Applied Maths NV).

Results

The most prevalent identified serotypes were Typhimurium (33.3%), London (26.7%) and Brandenburg (10.0%), followed by Schwaezengrund (8.3%), Derby (8.3%), Infantis (6.7%) and Javiana (6.7%). Only three markets were positive for more than one serotype. All strains were resistant to azithromycin and 98.3% to sulfamethoxazole; 55% of strains were classified as multiresistant (Tables 1 and 2). Resistance profiles cluster analysis enabled differentiation of two main groups: one group (A) comprising 28 strains presenting resistance to four to eight antimicrobial classes, and the other (group B) composed mostly by strains resistant to only two antimicrobial classes (Figure 1).

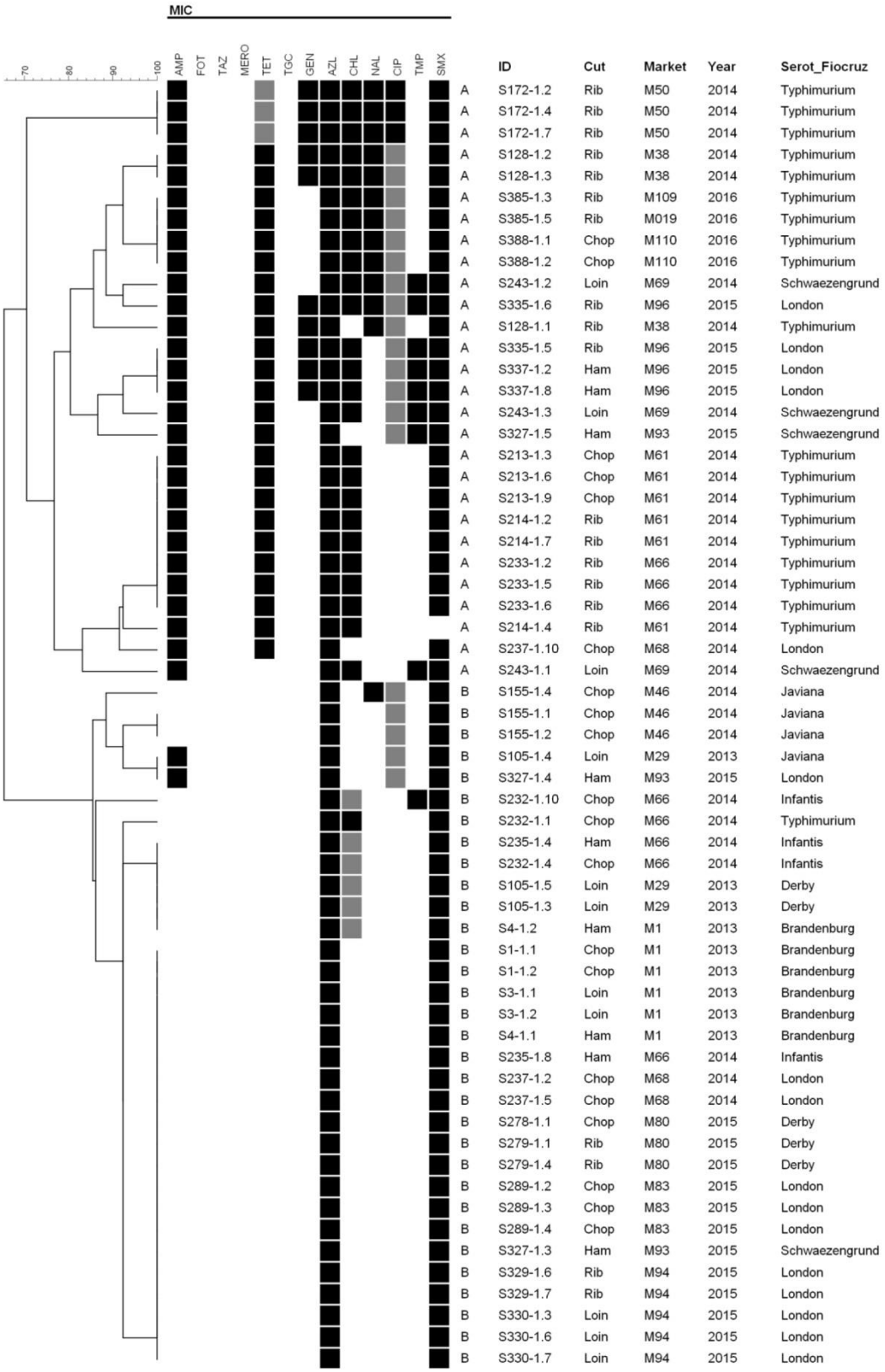
Table 1. MIC range, MIC_{50}, MIC_{90}, resistance rates and applied breakpoints against tested antibiotics.

Antimicrobial	MIC range[1]	MIC_{50}	MIC_{90}	Resistance N (%)	MIC breakpoints		
					S	I	R
Ampicillin	1 – 64	4	> 64	30 (50,0)	≤ 8	16	≥ 32
Cefotaxime	0,25 - 4	≤ 0,25	0,5	0	≤ 1	2	≥ 4
Ceftazidime	0,5 – 8	≤ 0,5	1	0	≤ 4	8	≥ 16
Colistin	1 – 16	≤ 1	≤ 1	1 (1,7)	-	-	≥ 4
Meropenem	0,03 – 16	≤ 0,03	≤ 0,03	0	≤ 1	2	≥ 4
Tetracycline	2 – 64	4	> 64	24 (40,0)	≤ 4	8	≥ 16
Tigecycline *	0,25 – 8	≤ 0,25	0,5	0	≤ 2	4	≥ 8
Gentamicin	0,5 – 32	1	> 32	10 (16,7)	≤ 4	8	≥ 16
Azithromycin	2 – 64	> 64	> 64	60 (100)	≤ 16	-	≥ 32
Chloramphenicol	8 – 128	16	> 128	25 (41,7)	≤ 8	16	≥ 32
Nalidixic acid	4 – 128	≤ 4	> 128	13 (21,7)	≤ 16	-	≥ 32
Ciprofloxacin	0,015 – 8	0,03	0,5	3 (5,0)	≤ 0,06	0,12 – 0,5	≥ 1
Trimethoprim	0,25 – 32	0,5	> 32	9 (15,0)	≤ 8	-	≥ 16
Sulfamethoxazole	8 - 1024	> 1024	> 1024	59 (98,3)	≤ 256	-	≥ 512

1 Sensititre™ EUVSEC MIC plate antimicrobials range. * Tigecycline breakpoint retrieved from FDA document NDA 21-821/S-016.

Table 2. Strain distribution according to resistance profile and isolation year.

Resistance profile	2013	2014	2015	2016	Total
≤ 2 classes	8	7	12	-	27
3 - 4 classes	1	4	1	-	6
≥ 5 classes	-	18	5	4	27
Total	9	29	18	4	60



	ID	Cut	Market	Year	Serot_Fiocruz
A	S172-1.2	Rib	M50	2014	Typhimurium
A	S172-1.4	Rib	M50	2014	Typhimurium
A	S172-1.7	Rib	M50	2014	Typhimurium
A	S128-1.2	Rib	M38	2014	Typhimurium
A	S128-1.3	Rib	M38	2014	Typhimurium
A	S385-1.3	Rib	M109	2016	Typhimurium
A	S385-1.5	Rib	M019	2016	Typhimurium
A	S388-1.1	Chop	M110	2016	Typhimurium
A	S388-1.2	Chop	M110	2016	Typhimurium
A	S243-1.2	Loin	M69	2014	Schwaezengrund
A	S335-1.6	Rib	M96	2015	London
A	S128-1.1	Rib	M38	2014	Typhimurium
A	S335-1.5	Rib	M96	2015	London
A	S337-1.2	Ham	M96	2015	London
A	S337-1.8	Ham	M96	2015	London
A	S243-1.3	Loin	M69	2014	Schwaezengrund
A	S327-1.5	Ham	M93	2015	Schwaezengrund
A	S213-1.3	Chop	M61	2014	Typhimurium
A	S213-1.6	Chop	M61	2014	Typhimurium
A	S213-1.9	Chop	M61	2014	Typhimurium
A	S214-1.2	Rib	M61	2014	Typhimurium
A	S214-1.7	Rib	M61	2014	Typhimurium
A	S233-1.2	Rib	M66	2014	Typhimurium
A	S233-1.5	Rib	M66	2014	Typhimurium
A	S233-1.6	Rib	M66	2014	Typhimurium
A	S214-1.4	Rib	M61	2014	Typhimurium
A	S237-1.10	Chop	M68	2014	London
A	S243-1.1	Loin	M69	2014	Schwaezengrund
B	S155-1.4	Chop	M46	2014	Javiana
B	S155-1.1	Chop	M46	2014	Javiana
B	S155-1.2	Chop	M46	2014	Javiana
B	S105-1.4	Loin	M29	2013	Javiana
B	S327-1.4	Ham	M93	2015	London
B	S232-1.10	Chop	M66	2014	Infantis
B	S232-1.1	Chop	M66	2014	Typhimurium
B	S235-1.4	Ham	M66	2014	Infantis
B	S232-1.4	Chop	M66	2014	Infantis
B	S105-1.5	Loin	M29	2013	Derby
B	S105-1.3	Loin	M29	2013	Derby
B	S4-1.2	Ham	M1	2013	Brandenburg
B	S1-1.1	Chop	M1	2013	Brandenburg
B	S1-1.2	Chop	M1	2013	Brandenburg
B	S3-1.1	Loin	M1	2013	Brandenburg
B	S3-1.2	Loin	M1	2013	Brandenburg
B	S4-1.1	Ham	M1	2013	Brandenburg
B	S235-1.8	Ham	M66	2014	Infantis
B	S237-1.2	Chop	M68	2014	London
B	S237-1.5	Chop	M68	2014	London
B	S278-1.1	Chop	M80	2015	Derby
B	S279-1.1	Rib	M80	2015	Derby
B	S279-1.4	Rib	M80	2015	Derby
B	S289-1.2	Chop	M83	2015	London
B	S289-1.3	Chop	M83	2015	London
B	S289-1.4	Chop	M83	2015	London
B	S327-1.3	Ham	M93	2015	Schwaezengrund
B	S329-1.6	Rib	M94	2015	London
B	S329-1.7	Rib	M94	2015	London
B	S330-1.3	Loin	M94	2015	London
B	S330-1.6	Loin	M94	2015	London
B	S330-1.7	Loin	M94	2015	London

Figure 1. Dendrogram showing the relationship among the *Salmonella* serotypes resistance profiles.

The multiresistant group A comprised strains of serotypes Typhimurium, London and Schwaezengrund, while the Brandenburg, Derby, Infantis and Javiana serotypes were associated to strains with increased sensitivity profile (group B). No further relation between strains resistance profiles and origin, including isolation year, was observed.

The multiresistant strains were characterized as resistant to ampicillin, chloramphenicol and tetracycline, and presented variable resistance to gentamicin, nalidixic acid, trimethoprim-sulfamethoxazole and ciprofloxacin. The high resistance rates to antimicrobials traditionally indicated for human salmonellosis treatment and especially azithromycin, demand attention to the multiresistance dissemination in pork and its risks and implications to public health.

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Risk assessment and risk communication in food safety

A COST-BENEFIT ASSESSMENT OF *SALMONELLA*-CONTROL STRATEGIES IN PIG HERDS WITHIN THE UNITED KINGDOM

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Introduction

Pork and pork products are considered to be a major source of human salmonellosis in the United Kingdom (UK). Despite a number of control programmes implemented within the UK, such as the Zoonoses National Control Programme (ZNCP), the prevalence of *Salmonella* in the UK slaughter pig population remains over 20% [1]. In particular, *S.* Typhimurium (including monophasic variants) continues to be a predominant serotype in humans, which is known to be most commonly found in pigs [2]. Therefore, to identify potential control measures that could reduce the number of human *Salmonella* cases it is necessary to understand the farm-to-consumption chain for pork and pork products. However, implementing control measures across the whole pig industry and food-chain would be a costly large scale project. It is therefore important to assess whether the benefit of implementing the control measures justifies the cost.

Cost-benefit analysis (CBA) is a standard method employed in a number of fields to assess whether a change in a process will produce a beneficial result and/or in a situation where a number of changes are proposed, to predict which change would produce the biggest benefit. CBAs are increasingly being employed in the field of epidemiology and food safety, for example to assess which control measures should be implemented within the food-to-consumption pathway. In these cases two main inputs are needed; a mathematical model to determine the effectiveness of the control measure (e.g. the reduction in number of cases of *Salmonella*) and an estimate of the costs, and savings made, of implementing the intervention. One of the most commonly used metrics to report results of a CBA is the 'benefit-cost ratio' (BCR), which gives an indication of how much benefit is obtained for each unit of cost, with a BCR>1 indicating that the benefits outweigh the costs.

Previous work has identified numerous risk factors as potential drivers of *Salmonella* transmission on pig farms; such as types of flooring, feed types and composition, and annual number of pig deliveries [3, 4]. However, results from previous CBA's were not promising in terms of the overall cost-benefit to the European Union (EU) for *Salmonella* control at the stage of the live animal, whereby interventions produced BCRs considerably less than 1, both for the UK and worldwide [5-8]. One study estimated the BCR for the UK to be greater than one under a number of scenarios investigated [5]. While the positive cost-benefit appears to be due to the relatively high prevalence of *Salmonella* infection in UK pigs compared to the rest of the EU, it is worth investigating further.

The aim of this study was to conduct a cost-benefit analysis for *Salmonella* control measures on pig farms, utilising recent data from studies on UK pig farms to estimate whether any interventions would produce a positive cost-benefit to the industry and if not what would be the predicted change in cost to achieve it.

Materials and methods

To determine the cost-benefit of an intervention, *i*, a simple benefit-cost ratio, *BCR(i)* was used

$$BCR(i) = \frac{S(i) + B(i)}{C(i)}$$

where *C(i)* was the total cost of intervention *i* to the UK pig industry (£/year), *S(i)* the total saving (£/year) made from a reduction in human cases of *Salmonella* attributable to domestic pig meat consumption and *B(i)* the saving to the industry (£/year) from intervention *i*. It is clear from the equation, that if $S(i) + B(i) \geq C(i)$ then $BCR(i) \geq 1$, resulting in a net gain to the UK economy. The cost to the industry (*C(i)* £/year) for intervention *i* was given by $C(i)=c_{pp}(i)*N_T$, where $c_{pp}(i)$ is the cost per pig (£) for intervention *i* and N_T is the total number of pigs slaughtered in the UK per year, assumed to be 10.23 million. The total economic productivity saving as a result of implementing intervention *i*, *S(i)*, was given by $S(i)=c_H*H(i)$, where c_H is the cost (£) per human case of *Salmonella,* estimated to be £503.34, and *H(i)* is the annual number of human cases prevented by intervention *i*. The saving to the industry, *B(i)*, is given by $B(i)=b(i)*W(i)$, where *b(i)* is the savings per pig by preventing infection from intervention *i*, estimated to be £1.22, and *W(i)* the annual number of pig cases prevented by intervention *i*. By substituting these values into the BCR equation, rearranging and setting BCR(i)=1, we can obtain an equation for the cost per pig to get zero cost-benefit; $c_{pp0}(i)= (c_H H(i)+b(i)*W(i)) / N_T$.

Within this study, we assessed five interventions; vaccination, movement to an outdoor breeding unit, improved cleaning and disinfection practices, fermented liquid feed (wet feed) and use of organic acids in feed. Whenever there was uncertainty about parameterisation we erred towards a 'best-case scenario', so the results of the CBA are an upper estimate of how cost-effective we expect the interventions to be based on the available data. The model used here to obtain subsequent effectiveness estimates along the farm-to-consumption chain was a quantitative microbiological risk assessment (QMRA) previously developed for the European Food Safety Authority (EFSA) by a European consortium including APHA, RIVM and Food DTU [9, 10]. For each scenario tested, the QMRA is modified to simulate the effect of the intervention and estimates are obtained for the resulting percentage change in national pig prevalence at the point of leaving the farm, $p_w(i)$, and prevalence of human cases, $p_h(i)$. As such, the total number of prevented pig cases per intervention, is given by $W(i)= W_0*p_w(i)$, where W_0 is the number of pig cases predicted by the QMRA in the absence of the intervention. The number of prevented human cases per intervention is given by $H(i) =H_0*p_h(i)$, where H_0 is the total number of domestic pig meat-attributable human cases of *Salmonella*. Note that while $p_h(i)$ is estimated using the QMRA, H_0 is estimated from data according to the equation, $H_0=N_h*U_f*p_{sa}*p_{uk}$, where N_h is the total number of reported UK human cases of *Salmonella, *U_f is the under-reporting factor, i.e. ratio of community to laboratory confirmed cases, p_{sa} is the proportion of human *Salmonella* cases attributed to pigs and p_{uk} is the proportion of UK-produced pig meat that enters the UK food chain, as opposed to being exported to another country. The costs were either provided by the companies conducting the intervention studies or estimated from the literature. The global parameter estimates are shown in Table1.

Results

The results of the CBA analysis (Table 1) suggest that none of the on-farm interventions were predicted to achieve a net gain to the UK economy (i.e. BCR >1). The intervention with the highest BCR was the addition of organic acids to pig feed. Analysis of what the cost per pig would need to be in order to achieve a BCR=1, suggests that all interventions would need to be considerably cheaper; e.g. the cost of the vaccine would need to reduce from £0.60 to £0.39 per pig.

Table 1. Cost-benefit results, showing QMRA results of % reduction in slaughter pig prevalence and reduction in human cases, estimates of the intervention cost per pig and financial savings to pig productivity and human illness, along with the resulting BCR and the estimated cost per pig necessary to achieve a BCR equal to 1.

Intervention	CBA Model Inputs						Results	
	Reduction in national slaughter pig prevalence (%), $p_w(i)$	Cost per pig, £ per year, $c_{pp}(i)$	Cost of nationwide implementation, £'000 per year, $C(i)$	Benefit to pig productivity, £'000 per year, $B(i)$	Reduction in human cases per year, $H(i)$	Human illness savings, £'000 per year, $S(i)$	Benefit-Cost Ratio, BCR(i)	Cost per pig to achieve BCR =1, £ per year $c_{pp0}(i)$
Wet feed	58.8*	£1.16	£9,645k	£1,260k	6,086	£3,063k	0.448	£0.423
Organic acids	94.6*	£0.80	£8,184k	£2,502k	9,171	£4,616k	0.870	£0.696
Vaccination	48.8	£0.60	£6,179k	£1,290k	5,321	£2,678k	0.642	£0.388
Cleaning & disinfection	28.9	£5.21	£53,298k	£764k	3,244	£1,633k	0.0450	£0.2343
Movement to outdoor breeding unit	40.1	£0.75	£7,673k	£1,059k	4,745	£2,388k	0.449	£0.337

*These reductions are a result of the APHA QMRA farm model, rather than extrapolated from an experimental study.

Discussion

There are large uncertainties associated with how representative the experimental intervention studies are if implemented on a national level, not least because of the relatively small sample size. For example, the organic acids intervention, which assumes that pigs will not shed more than 10^4 cfu/g, gives a high reduction in national slaughter pig prevalence, but is very much a best case estimate and would benefit from further UK studies that could demonstrate a product can achieve this in practice. Also, while there are clearly costs associated with outdoor units, it is possible that the land may be used for other sources of income (e.g. sugar beet or cereal crops), which would in turn lower the costs calculated previously. If further investigation can prove that there is a link between *Salmonella* infection and Key Performance Indicators then this will impact the BCR by increasing the financial benefit to the farmer by preventing cases of *Salmonella*. There are around 10,000 pig farms in the UK, although the vast majority of production comes from a much smaller number of large scale industrial farms. Thus, another possible way to improve the benefit-cost ratio of interventions would be to make them risk-based, e.g. to target intervention measures at a sub-set of farms, thus reducing the scale (and cost) of the operation required by the UK pig industry to reduce human risk. Such a risk-based approach could target larger farms or use *Salmonella* monitoring data to target farms with a high prevalence of infection, which could also act as an incentive for improvement. Consequently, the investigation of a risk-based intervention programme on human Salmonellosis due to the consumption of pork/pork products would be beneficial.

There are other factors not considered in this analysis that may provide additional benefits, such as the interventions being effective against other foodborne and pig diseases and the effect of combining multiple interventions. Intervention measures further down the farm-to-consumption chain, e.g. slaughterhouse interventions such as anal bunging, more thorough scalding, double singeing or improved slaughter hygiene to reduce cross-contamination, might incur a lower cost as there are fewer slaughterhouses than pig farms to implement interventions and less opportunities further down the chain for re-contamination or cross-contamination of carcasses to occur. However, such interventions would have no benefit to pig productivity so could well be less cost-effective.

Conclusion

Even under the best case scenarios (full implementation of effective intervention across the UK), the estimated cost of implementing all interventions exceeds the estimated financial benefit to pig productivity and human health. However, there are factors other than simple cost, e.g. Government targets to reduce foodborne illness, trade, societal pressure due to fear of contaminated meat products, which may encourage the desire for implementation of control measures. The analysis here would help in the determination of which interventions would be the most beneficial.

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USING SEROLOGICAL MONITORING, INTERNET-BASED FEEDBACK AND ON-FARM AUDITING TO IMPROVE TOXOPLASMA GONDII CONTROL AT DUTCH PIG FARMS

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Background and objectives

Toxoplasma gondii is a relevant foodborne pathogen due to its human disease burden. In the Netherlands, pork is estimated to contribute to 11% of the meatborne T. gondii infections. The European Food Safety Authority advised to perform serological testing of pigs and on farm audits on risk factors for T. gondii infection.

Materials and methods

The true within-herd seroprevalence of T. gondii was estimated for pig farms using longitudinal data from Dutch abattoirs. Farms were ranked based on the estimated within-herd seroprevalence. Selected 'high risk' farms were audited. An internet-application was developed to report results back to the farm and increase awareness of relevant risks.

Results

Between 1 and >700 blood samples were collected from >3,500 farms. The estimated within-herd seroprevalence (preliminary results) of the top-ranked farms ranged from 15% to 50%. Final analyses are currently ongoing. Relevant risk factors were found to be present on farms with higher seroprevalences.

Discussion and conclusions

Serological screening of pig herds for T. gondii lead to the identification of herds in which typical risk factors for T. gondii infections are present. Effort to improve biosecurity were undertaken to reduce the seroprevalence. On farm audits and self-assessment tools are helpful in increasing the awareness of biosecurity. Changing farm management to reduce the exposure of pigs to T. gondii may reduce the human disease burden.

APPLICATION OF QUALITATIVE RISK ASSESSMENT TO PRIORITIZE HAZARDS IN PORK PRODUCTS IN BRAZIL

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Introduction

The main objective of carcass and meat inspection is to promote animal and public health by controlling and detecting pathogens. (1) Procedures of inspection of pig carcasses in Brazil are based on macroscopic examinations (incisions and palpations) attempting to identify potential hazards to animal and human health, mainly related to classic zoonotic diseases. This inspection structure is based on evidence related to the high relevance of parasites in which transmission and maintenance are strictly linked to the low level of biosecurity measures applied in the farms. (2)

Until 2007 approximately 60% of Brazilian pork production could be characterized as industrial, adhering to vertical integration systems in which companies have control of management practices, nutrition, technical assistance, increasing the size of herds and complexity of the chain. (3) If in one hand the industrial production reduces the release of parasites and classic zoonosis, on the other hand, microscopic hazards with no clinical signs or macroscopic lesions are arising.

There is a distance between the structure of industrial pig herds in Brazil and the procedures of inspection of pig carcasses, bringing some skepticism about how suitable the inspection procedures are in promoting public health. To attempt for this new reality, adequacy of a monitoring system must be based in a rational and transparent way. (4)

Risk assessment (RA) is a step of risk analysis being usually referred between the risk management and risk communication. In food safety context RA can be defined as a process of scientific considerations and data collection in a structured way following the production flow assisting the understanding of risks and adverse effects resultant of exposure to hazards present in food. Thus RA aids risk managers to apply strategies to promote public health, taking into account different epidemiological realities and complex relationship between different steps of production and its uncertainties. (5)

In this sense, this work aims to describe the application of a qualitative risk assessment to prioritize hazards in Brazilian industrial pork production and highlight the adequacy of the current inspection of pig carcasses in Brazil.

Material and methods

The model adopted was the one from *Codex Alimentarius* (CAC/GL 2007) (6) with four steps: I) hazard identification; II) hazard characterization; III) exposure assessment and IV) risk characterization. The interaction between different dimensions in this model followed the matrix in Table 1.

Table 1. Qualitative matrix used to interact with the different dimensions in the model.

	2º Dimension				
1º Dimension	**1**	**2**	**3**	**4**	**5**
1	1	1	1	1	1
2	2	2	2	2	3
3	2	2	3	4	4
4	3	3	4	4	5
5	3	4	4	5	5

A systematic review approach was used in hazard identification step using the keywords: ("bacterial agents OR viral agents OR fungal agents OR parasitic agents") AND ("swine OR pork OR pig"). Searches were done in January 2015 in Portuguese and English in PubMed, ScienceDirect, ISI and Web of Science. Abstracts of events such as SafePork and IPVS and hazards already described in Brazilian legislation were also included.

To be considered as relevant each hazard should answer YES to the following three questions: 1) Can the hazard be responsible for an infection/intoxication in human beings through the pork consumption?; 2) Is the hazard present in Brazilian industrial herds?; 3) Is the hazard introduced by slaughter and dressing activities?

Hazard characterization was done using information about pathogenicity and magnitude of the adverse effects of each hazard. Exposure assessment was the probability of ingestion of the hazards by pork consumption and takes into account the whole production. The conceptual model considers the interaction between the level of presence of the hazard in the herds/animals (called "initial presence") and probabilities of reduction and amplification along the process. Hence, exposure assessment was modeled sequentially as: initial presence*amplification*reduction of the hazard. A baseline scenario was modeled to non-processed pork and two different scenarios regarding cooked and fermented products were also made.

Risk characterization is the interaction between exposure and hazard characterization and refers to the probability of occurrence (i.e. exposure x pathogenicity) of foodborne disease by pork consumption associated with the adverse effects, and was described in five levels: 1) risk is very low; 2) risk is low; 3) risk is moderate; 4) risk is high; 5) risk is very high.

Results and discussion

One hundred twenty four hazards were indentified of which 88 were excluded because the transmission does not occur through pork meat. Of 36 remaining, 14 were excluded for not being present in the population in the past 20 years and two were included because of the potential contamination during slaughter processing. At the end, 24 hazards were included: 66.6% were bacterial, 20.8% parasites, 12.5% toxins or virus.

There were no hazards characterized as very high risk [5] and *Salmonella* sp. was classified as high risk [4]. *Salmonella* figured as the highest hazard among those identified for non-processed pork. Parasites had risk characterized as very low [1] or low [2] in non-processed pork but there are uncertainties regarding the pathogenicity and adverse effects of these hazards. The risk rank of hazards for non-possessed pork (baseline scenario) is demonstrated in Figure 1.

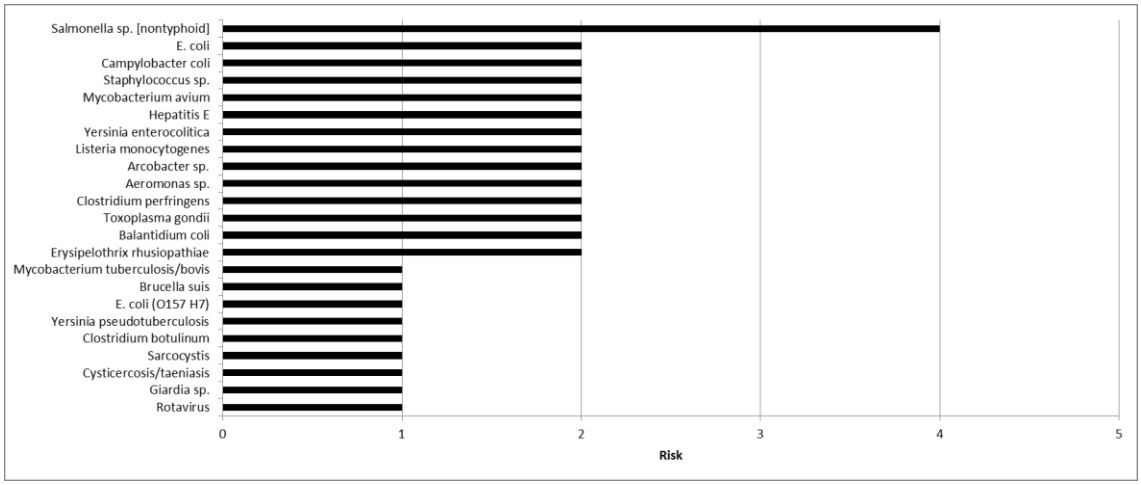

Figure 1. Risk characterization of the identified hazards related to the consumption of non-processed pork.

For cooked products, the thermal resistance of both spores of *Clostridium perfringens* and Ochratoxin A (OTA) increased their risks to high [4]. OTA is only accounted in the model when kidneys are included in the product (i.e. scenario of cooked products), and uncertainties associated to OTA are very high, including for the presence in Brazilian herds, pathogenicity, and adverse effects. Therefore, more information is needed to an accurate characterization of risk for this hazard. Furthermore, the heat reduces the risk of *Salmonella* sp. to moderate [3] in these products.

For fermented products, none hazards were classified as very high risk [5], and *Salmonella* sp. was classified as high [4], followed by *Staphylococcus aureus*, *Escherichia coli*, *Clostridium perfringens* and *Campylobacter coli* with moderate risk [3]. The effect of bacterial reduction in fermented products (by pH variation or water activity reduction) can be faced as conservative and according to several authors more than inhibition of bacterial growth, there is an actual reduction in the number of cells. Anyway, considering fermentation, *Salmonella* sp. was kept in high risk [4].

Regarding the model sensitivity, the initial presence of the hazards had the highest impact in the overall risk characterization. When the prevalence is increased the risk profile shifts from bacterial to parasitic, and the lasts reach high risk [4] and *Clostridium botulinum* reaches very high risk [5] for non processed pork.

Conclusion

The hazards characterized as high risk in non-processed, cooked and fermented pork are mainly bacterial, with a complex cycle, and rarely affecting clinically pigs with no macroscopic lesions. In this sense, macroscopic examinations of carcasses are no longer adequate to account for the main zoonotic hazards in Brazilian industrial pork production bringing the need to quantitative modeling, to identify critical points to control microscopic hazards keeping then in strict statistical control and applying corrective procedures when necessary.

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A SPATIAL ENTRY ASSESSMENT MODEL FOR INCURSION OF EXOTIC SWINE DISEASES INTO THE EUROPEAN UNION

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Introduction

The threat of incursion of exotic animal pathogens into the European Union (EU) Member States (MS) via transmission routes such as human travel and trade of live animals and their products is dynamic and needs to be continually re-assessed. Here, we present a quantitative spatial entry assessment model for assessing the risk of incursion of exotic pig diseases such as Classical Swine Fever (CSF) into the EU.

Methods

Overview: Import risk assessments generally follow the World Organisation for Animal Health OIE risk assessment framework process [1], comprising a release (or entry) assessment, an exposure assessment and a consequence assessment. Here we describe a model, which builds on one developed previously for introduction of bat-borne viruses [2], to estimate the risk of entry to the EU using classical swine fever (CSF) as a case study. Routes considered include live animal trade, legal trade of meat products and illegal trade of meat via air freight, marine cargo containers or aircraft passenger luggage. Figure 1 shows the generic model framework. It is designed to utilise input data such as pathogen incidence and animal demographics to derive an estimate for country level pathogen prevalence by animal species. This is then combined with data on trade products and pathogen survival rates to estimate the risk of introduction to EU Member States. The framework is designed to be generic and applicable for any pathogen.

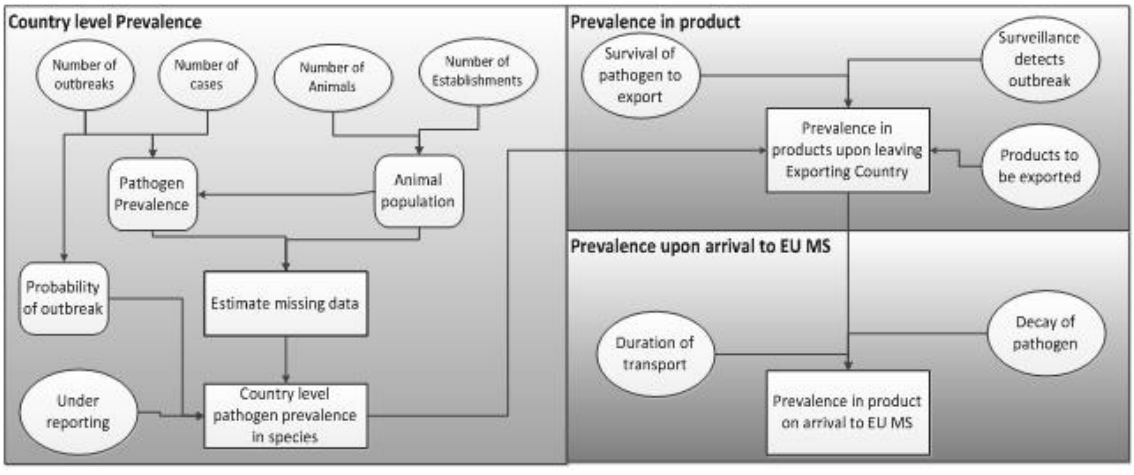

Figure 1. Model Framework, describing the processes simulated in the release assessment.

OIE input data: Data from the OIE on animal demographics and pathogen prevalence were used to estimate the prevalence of CSF in pigs at the point of export, at the country level; this included estimating pathogen prevalence for countries with no, or limited data. For all 180 countries in the OIE databases over the years 1996-2014, data were obtained for the number of reported outbreaks and cases by country, *k*, species, *s*, and year, *y*, $N_{ob}(k,s,y)$ and $N_{case}(k,s,y)$ respectively. For the period 2004-2014 these data were obtained from the annual reports on the World Animal Health Information System (WAHIS) section of the OIE website [3]. For the period 1996-2004 these data were obtained from Handistatus II [4]. Data on the total number of animals, $N_{animal}(k,s)$, and the number of 'animal establishments', $N_{ebl}(k,s)$, by country and species, were also obtained, as defined in the country annual reports in the WAHIS section of the OIE website for 2014; this is essentially equivalent to farms.

Country level CSF prevalence: To obtain an estimate of the annual number of cases of CSF in pigs in country *k*, $N_{country}(k)$, an estimate for the observed number of cases, $N_{caseEst}(k)$ was multiplied by an under-reporting factor, $U_f(k)$ and the probability of an outbreak occurring, $P_{ob0}(k)$

$$N_{country}(k)=N_{caseEst}(k)*U_f(k)*P_{ob0}(k).$$

The estimate for number of cases was based on the historical OIE data. For countries where data were missing an estimate was derived based on the number of cases in the same geographical region [5]. The under reporting factor, $U_f(k)$, was used to account for potentially unreported cases that could be a risk for disease transmission in an import risk assessment.

Prevalence at export: The number of infected pigs at export, $N_{exp}(k)$, was estimated by multiplying $N_{country}(k)$ by the probability a random infected pig will still be infected when exported, $P_{surv}(k)$, and the proportion of cases expected to occur before detection of the outbreak, $P_{det}(k)$

$$N_{exp}(k)=N_{country}(k)*P_{surv}(k)*P_{det}(k).$$

Countries were defined as either *sporadic* or *continuous*, based on the annual frequency of CSF outbreaks; countries with more than one outbreak every 2 years were considered to be continuous, $P_{det}(k)=1$, while for sporadic countries it was assumed that the outbreak would be detected and a ban on exports implemented, thus $P_{det}(k)=0.1$, based on data from a CSF outbreak in the Netherlands. $P_{surv}(k)$ was estimated by dividing the average duration of clinical signs of CSF, assumed to be 20 days, by the number of days in the year, 365.

Legal trade: Annual EU import data on live animals and meat products were obtained from the *comext* database in Eurostat [6]. For live animals, the prevalence of the pathogen is assumed to be equivalent to the prevalence at export in the export country, as described previously. For meat products, the prevalence is predicted by estimating the number of animals contributing to the annual quantity of meat and then using the prevalence of the pathogen in the export country. Reduction of the pathogen due to processing effects and natural decay during travel time is also taken into account.

Illegal trade: The model considers illegal importation of pig products into the EU via legal shipments of maritime containers, air freight and commercial air passenger luggage; illegal smuggling operations were not considered. The number of containers, freight and air passengers were obtained from Eurostat databases [6]. The proportion of containers, freight and passenger luggage that contain illegal meat was estimated based on aggregated data obtained from UK border force (unpublished data). The prevalence of the pathogen was assumed to be equivalent to the prevalence in the exporting

country, but with $P_{det}(k)$=1 for both sporadic and continuous countries, as we don't expect illegal activities to cease once an outbreak has been detected.

Outputs: As well as estimates for CSF prevalence at export, the model consisted of three outputs; 1) annual number of CSF infected live animals entering the EU, 2) annual volume of CSF contaminated pig products entering the EU, 3) annual volume of illegal CSF contaminated pig products entering the MS. Here we rank the results by route and present the relative ranking.

Results

From Figure 2 it can be seen that the model predicted the countries at highest risk of CSF in pigs at export are in Central and South America and South East Asia, as would be expected from the raw OIE data. Estimates are also provided for countries where data are missing, e.g. in Africa, and allows for very low probabilities of future occurrence in countries currently officially free of CSF.

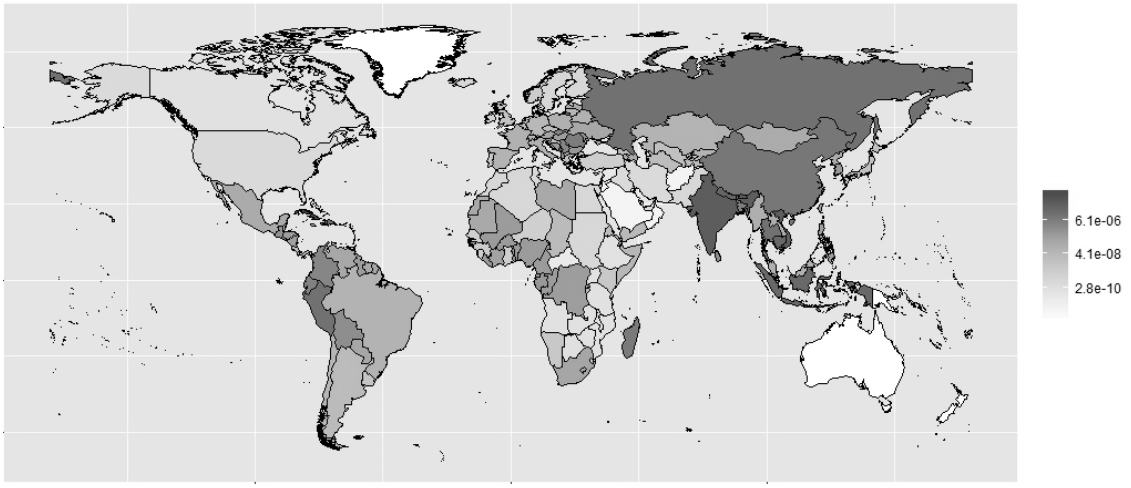

Figure 2. Model predicted country prevalence of CSF in pigs at export.

From Table 1 it can be seen that the relative ranking of EU MSs for entry of CSF differs between routes; e.g. Great Britain has lower risk than other MSs for import from legal trade products, but has the highest risk from illegal trade products. Note a low ranking does not necessarily imply low risk.

Table 1. Relative ranking of EU MSs for risk of introduction of CSF via three routes; live animal imports, legal trade and illegal trade of pig products.

MS	Live animals	Legal trade	Illegal trade	MS	Live animals	Legal trade	Illegal trade
AUT	4	13	9	**HUN**	1	7	19
BEL	11	17	7	**IRL**	22	20	22
BGR	21	8	25	**ITA**	9	1	6
CYP	25	27	24	**LTU**	12	21	21
CZE	14	2	12	**LUX**	18	26	16
DEU	5	4	3	**LVA**	20	23	23
DNK	23	19	13	**MLT**	28	28	18

MS	Live animals	Legal trade	Illegal trade	MS	Live animals	Legal trade	Illegal trade
ESP	15	3	4	**NLD**	8	11	5
EST	24	25	17	**POL**	3	6	14
FIN	27	24	8	**PRT**	6	14	10
FRA	17	5	2	**ROU**	2	9	26
GBR	19	12	1	**SVK**	7	16	20
GRC	16	10	15	**SVN**	13	18	27
HRV	10	15	28	**SWE**	26	22	11

Discussion

The model described here uses information from global datasets to give a quantitative estimate of the prevalence in pigs at the point of export in all countries of the world and the subsequent risk of introduction of CSF to EU MSs. To make use of these data and cover the broad scope of introduction to the EU, the model cannot be particularly complex, which does lead to quite large uncertainty surrounding the absolute values of the model outputs. However, the strength of the model lies in the relative risks between MSs and routes. The model can be updated with new data as they become available and so can be used as an early warning system, to highlight a change in risk due to factors such as changing trade patterns.

The results of the assessment described here suggest that the EU MSs at highest risk of incursion of CSF differ depending on the route of introduction. The risk is influenced by volume imported and prevalence of CSF in the import country. Live animal imports to EU MSs predominantly occur within the EU only, while imports of pig products are more widespread. The model assumes that illegal meat imports could come from any country of the world, so while the legal trade depends specifically on reported data on pig products, the illegal trade is influenced by total volumes of trade of maritime cargo, air freight and commercial air passengers. It should be noted that, due to lack of data, the model does not include other routes of illegal trade, such as being smuggled across borders, and thus could underestimate the risk from illegal trade in some geographical areas. The model also does not account for differences in border controls upon arrival to EU MSs.

The different scales of the routes should be remembered when considering these results; the live animal route considers the introduction of an infected animal while the other routes consider contaminated pig products. It is likely that the risk of onward spread from an infected live pig will differ from that of a contaminated meat product and the risk from a legally imported meat product may differ from that from an illegally imported product. Thus, the results from this release assessment are not the whole story, but will provide a useful input for an exposure assessment to assess the risk of onward transmission within individual MSs and the EU as a whole.

This spatial assessment model is capable of assessing the risk of disease incursion for other pathogens and thus can provide a framework to compare the relative risk between multiple pathogens and countries for different livestock species. These outputs can help drive surveillance activities, by indicating which pathogens are most likely to enter the EU, by which route and into which MS.

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APPLICATION OF MICROBIAL RISK ASSESSMENT IN BRAZIL: OPPORTUNITIES FROM THE INDUSTRY TO THE GOVERNMENT

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Introduction

To investigate the public health risks of microbial in food, quantitative microbial risk assessment (QMRA) is a useful approach and a good alternative when surveillance data are sparse, and for management decisions. The goal of QMRA is assess the probability of the disease occurrence given the ingestion of a contaminated food and its consequences, and when the risk is estimated, control measures can be applied and its impact on disease assessed. Consequently, this method supports the promotion of public health by the authorities.

QMRA is now well recognized as a risk management decision-support tools, and a well-designed risk assessment provides the means to evaluate and compare the effects of different control measures on public health. Moreover, the application of QMRA is not restricted only to the government decision-makers. Since this tool can be used to validate the impact of the HACCP measure on the public-health, the industry can also make use of this tool to help the manager to decide whether to change the process accordingly with the safety objectives. In this sense, the impact of a given critical control point can be evaluated using risk assessment approach because it allows describing the changes in microbiological numbers along the food-processing chain from the farm to the consumer using mathematical models.

Recently, this method (qualitatively and quantitatively) was applied to ranking hazards that can be present in pork meat as well as to assess the effect of meat inspection practices on the contamination of the pig carcasses. These are practical examples of application of microbial risk assessment in our region and they also illustrate the potential of this approach in helping decision makers in both public and private sector.

It was reported in the literature that few quantitative microbial risk assessment have been performed in developing countries, (1) as is the case of Brazil. The objective of this study was to discuss the using of QMRA in Brazil and its potential regarding its use as a tool for decision-making.

Material and methods

A systematic review was made with the main objective to assess how many articles on QMRA performed in Brazil have been published in peer reviewed Journals. The search engines used were PubMed and ScienceDirect and included original articles (not reviews) published in English language from 2007 to date. Only QMRA performed in Brazil were selected. The search strategy used the combination of the following keywords: "quantitative microbial risk assessment" or "microbial risk assessment" or "quantitative risk assessment" and "food" and "Brazil". The data collected from the studies were basically the type of food and hazard involved.

In order to improve the discussion regarding the application of this method in the current Brazilian situation, a "reference search" was made using the same keywords ("quantitative microbial risk assessment" or "microbial risk assessment" or "quantitative risk assessment" and "food") and period in the EFSA (European Food Safety Authority) Journal. Hence, we discuss the perspective of the application of this tool by the Brazilian authorities.

Results and discussion

The searching strategy resulted in 178 publications, of which nine articles described QMRA performed in Brazil in food (n=7) and water (n=2). Publication in peer review journal started only in 2013. QMRA were done in food of animal origin in three published papers, in which the risk was estimated for food of pork origin in two of them. Details of the published QMRA performed in food are described in Table 1.

Table 1. Quantitative Microbial Risk Assessment in food performed in Brazil and published in peer review journals from 2007 to 2017.

Type of food	Origin	Pathogen	Year	Authors
Salad crops irrigated with effluents	Vegetal	*Escherichia coli*	2013	Pavione et al. (2)
Fresh sausage	Animal	*Salmonella* spp.	2013	Mürmann et al. (3)
Oyster	Animal	*Vibrio parahaemolyticus*	2014	Sobrinho et al. (4)
Ready-to-eat leafy vegetables	Vegetal	*Salmonella* spp. *and Listeria* spp.	2014	Sant'Ana et al.(5)
Leaf green	Vegetal	*Salmonella* spp.	2017	Pavione et al. (2)
Cheese	Animal	*Staphylococcus* enterotoxin	2017	Nunes and Caldas (6)
Fermented sausage	Animal	*Salmonella*	2017	Corbellini et al. (7)

Considering that quantitative microbial risk assessment applied to evaluate the risk of diseases caused by the exposure to pathogens in food started in the middle nineties, there is evidence that this tool is still overlooked in Brazilian reality. That is because only in 2013 the first two published articles describing QMRA usage in Brazil were found in the search along with the low number publications. Santos et al. (8) pointed that the lack of training opportunities and a formal organization to conduct risk assessment are some limitation for the use of this tool within National Veterinary Services in Brazil, for example.

In contrast, the search made in the EFSA journal resulted in 107 publications of which at least 33 of them described the use of risk assessment to help authorities in the process of decision-making. EFSA is an agency that works as risk assessor to promote, for example, food safety and animal health and welfare, and it was possible to observe that there are many risk assessment developed to cover all these aspects. Among these publications, there are several reports describing scientific opinions on the risks estimated by risk assessments, guidance to harmonize procedures, requirements for a risk assessment on antimicrobial resistance, etc.

This fact clearly denote that there is a formal organization to conduct risk assessment to aid authorities to take decision in Europe, in contrast to what happens Brazil. This situation i.e., the absence of a formal organization might be one of the reasons to the lack of opportunities for the using of risk assessment in large scale to help the process of decision-making on food safety for both private and public sectors in Brazil.

Conclusion

Risk assessment is a tool largely used to promote animal health and public health. In Brazil, there are still few publications describing risk assessment on food safety, which might be related with the lack of formal organization to promote its use in large scale by the authorities or the lack of formal training.

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QUALITATIVE RISK ASSESSMENT OF ANIMAL MEAL APPLIED TO SWINE PRODUCTION

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Abstract

There are gaps in the pig production chain, particularly as regards the possible destinations of dead animals on farms. The production of animal meal presents itself as an alternative for the recycling of biological waste. The objective of this study was realize a qualitative microbiological risk assessment on animal meal in order to provide subsidies to indicate possible hazards and risks associated with the use of meal produced from dead pigs on farms. The microorganism *Salmonella* was the main hazard reported. The scenario tree presented 15 scenarios for contamination and recontamination of animal meal. For the first scenario was defined release and exposure risk levels as moderate, was obtained as the risk level of the occurrence moderate. The risk level of the consequence determined as low together with the level of occurrence obtained previously resulted in the final risk level low. For the second scenario defined release risk level as high and the exposure risk level as moderate, we obtained as moderate risk level of occurrence. The risk level of the consequence determined as high related to the level of occurrence previously obtained resulted in the final risk level high. From the information and scenarios considered, it was not possible to indicate the production of animal meal as a probable destination for dead animals on pig farms.

Introduction

The necessity for food in the world and the rapid growth of the human population has generated greater demand in the national and international markets. Brazil ranks fourth in exports and third in pork production, registering 3.643 million tons of pork in 2015 (ABPA, 2016). There are gaps in the pig production chain, particularly as regards the possible destinations of dead animals on farms. Production of animal meal presents as an alternative for the recycling of biological waste. The lack of technical and scientific information about animal meal produced from dead animals compromises decision making it necessary to analyze the processes to identify risks associated with the presence of hazards and consequences that could arise from the adoption of the process. The aim of this study was realize a qualitative microbiological risk assessment on animal meal in order to provide subsidies to indicate possible hazards and risks associated with the use of meal produced from dead pigs on farms.

Material and methods

In order to identify the biological hazards, it was firstly pointed out that relevant microorganisms to swine farming could survive in the animal meal production process, according to methodology by OIE (2010). From this information, a systematic review protocol was initiated according to Bucher et al. (2012) considering the animal meal contamination. Different studies attributed the higher microbiological risk to the stage

following the end of industrialization process (mainly regarding the risk of recontamination). From this information, a systematic review was conducted to determine which microorganisms could be recontaminated from animal meal after cooling.

The effectiveness of animal meal production in preparation of a microbiologically acceptable end product was considered in the risk assessment. Thereby, the risk of contamination and recontamination would be subsequent to industrialization. The risk assessment was structured considering the following steps: I) Release assessment, where the stages responsible for contamination of the animal meal were indicated; II) Exposure assessment, analyzing presence of microorganisms in the animal meal from the recontamination (considering factors that contribute to spread hazard such as inactivation, survival, prevalence and incidence in the Brazilian herd; III) Consequence assessment, checking presence of hazard in animal meal may generate health and economic problems for both public health and animal health (represented by the percentage of associated mortality, zoonotic potential of the disease and level of economic impact generated by the occurrence in the country); IV) Risk estimation, considering the risk levels from previous evaluations (stages I and II) to determine risk levels of the possible scenarios using the risk and maturity matrices, since for each of the two scenarios evaluated were level of risk (insignificant, very low, low, moderate and high).

A scenario tree was constructed with aim of identifying critical points of contamination and animal meal contamination. From the scenarios found, we chose to evaluate the worst and the intermediate scenarios to verify the risk involved in steps considered.

Results and discussion

The microorganism *Salmonella* was considered the main contaminant of both raw materials and products and by-products used in animal feed, followed by mycotoxins (Ochratoxin, Fumonisin and Aflatoxin) present in grains and cereals used as a basis for diets (Kosicki et al., 2016). Other microorganisms mentioned in the studies were *Clostridium* and *Listeria*, capable of contaminating grains and environment because they are ubiquitous (Maciorowski et al., 2007). *Salmonella* was the only microorganism with records in scientific articles that evaluated animal meal therefore it was considered the main microbiological hazard.

The possibility of introduction was reported from absence of this microorganism in industrialization process (up to the digester). In the following steps such as handling, packaging, storage and transport of animal meal at room temperature, there were risks of contamination and recontamination by microorganisms that were distributed in the industry environment. It is important to consider that these studies were carried out in greases that used as raw material by-products generated from slaughtering under inspection of animal products (federal, state or municipal) (Larsen et al., 2014; Maciorowski et al., 2007; Melo et al., 2011; Moura et al., 2014; Pellegrini et al., 2015). In the European Union, Larsen et al. (2014) reported a decrease in the presence of *Salmonella* from 0.7% to 0.5% in the diet and from 2.9% to 0.6% in meat and bone meal between 2002 and 2010. Albuquerque et al. (1999) when evaluating raw materials, grease surfaces and final product found eight positive samples in bone meal (61.53%), three in meat meal (50%) and one in blood meal (33.33%), demonstrating the importance of *Salmonella* in the animal meal production cycle and its capacity to

contaminate and recontaminate products. According to the systematic review, the process of animal meal production performed adequately presents a low risk of contamination and recontamination by *Salmonella*. However, recontamination may generate several scenarios for introducing the microorganism into animal meal, since this bacterium may be present in any equipment, especially in the subsequent stages to digester (silos and transporters) due to dust and presence of biofilms (Vestby et al., 2009).

The scenario tree presented 15 possible scenarios of contamination and recontamination of animal meal, so, it was decided to analyze two scenarios. The first scenario (intermediary) considered to use as raw material the by-products from the slaughter destined to production of animal meal, being contamination by *Salmonella* spp. from transport vehicle due to lack of cleaning and hygiene. The final product would be used as fertilizer (Figure 1). Defining release and exposure risk levels as moderate, we obtained as the risk level of the occurrence moderate. The risk level of the consequence determined as low together with the level of occurrence obtained previously resulted in the final risk level low.

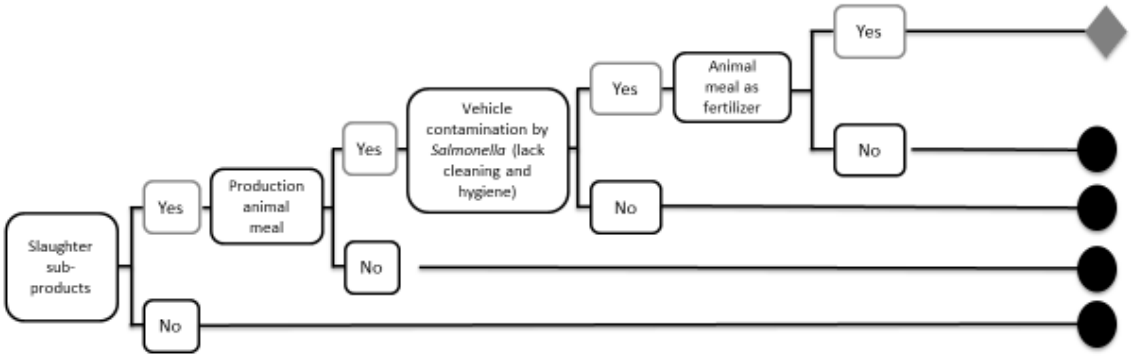

Figure 1. The first scenario (intermediary) of contamination and recontamination of animal meal.

Second scenario (worst) considered to use as raw material the by-products from the slaughter destined to the production of animal meal, being the recontamination by *Salmonella* spp. would occur by biofilms presence in silos due to the high contamination of industry environment and lack of adequate cleaning. *Salmonella* would spread by the property with low prevalence from use of animal meal in animal feed (Figure 2). Defining release risk level as high and the exposure risk level as moderate, we obtained as moderate risk level of occurrence. The risk level of the consequence determined as high related to the level of occurrence previously obtained resulted in the final risk level high.

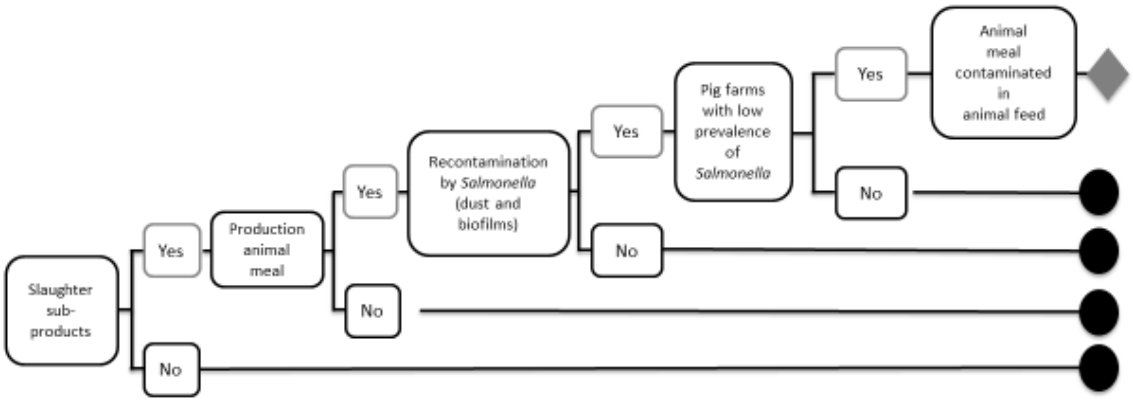

Figure 2. The second scenario (worst) of contamination and recontamination of animal meal.

Conclusion

Salmonella was the main hazard reported. The scenario tree presented 15 scenarios for contamination and recontamination of the animal meal. If the industrialization process were adequate, considering time/temperature, the risk of contamination would be insignificant. The presence of contamination in equipment due to presence of dust and biofilms may contribute to the recontamination of the sterilized meal. The storage place and poor hygiene of the vehicles also contribute. The first scenario (intermediary) considered to use as raw material the by-products from the slaughter destined to production of animal meal, being contamination by *Salmonella* spp. from transport vehicle due to lack of cleaning and hygiene. The final product would be used as fertilizer. When setting the release and exposure risk levels both as moderate and the risk level of the consequence as low, the final risk level is obtained as low.

The second scenario (worst) considered to use as raw material the by-products from the slaughter destined to the production of animal meal, *Salmonella* spp. recontamination in industry environment (by biofilms presence and dust) and the use of animal meal in animal feed. Defining release risk level as high and exposure as moderate, the level of the occurrence was moderate. This level allied to the high consequence risk level resulted in the high end risk level. From the information and scenarios considered, it was not possible to indicate the production of animal meal as a probable destination for dead animals on pig farms.

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IN VITRO CHARACTERIZATION OF THE ABILITY OF *YERSINIA ENTEROCOLITICA* BT4 TO COLONIZE PIGS AND STAINLESS STEEL SURFACES

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Abstract

Yersiniosis is, after campylobacteriosis and salmonellosis, the third most frequently reported zoonosis in Europe. Humans become infected with *Y. enterocolitica* through the consumption of undercooked pork and raw food having been in contact with contaminated surfaces. Pigs, the main reservoir for human pathogenic strains, do not develop clinical signs. In France and worldwide, biotype 4 (BT4) is the biotype the most frequently isolated from both pigs and clinical yersiniosis. In this study, a collection of 26 pathogenic BT4 strains isolated from pig tonsils was used to investigate their ability to adhere and invade intestinal pig cells (IPEC-J2) and to adhere to abiotic surfaces (stainless steel coupons) using two *in vitro* tests. Regression analysis was performed between data sets obtained from IPECJ2 cells assays *versus* stainless steel assays.

All BT4 strains were able to adhere and invade IPEC-J2 cells. However, the results showed heterogeneity between strains with respect to their ability to adhere to IPECJ2 cells, with a percentage of adhesion varying from 9% to more than 90%. The BT4 population displayed a more homogeneous ability to invade IPECJ2 cells with percentages varying from 10% to 26%. The BT4 strains displayed a great ability to adhere to the stainless steel surface, percentage of adhesion varying from 0.3% to 4.2%. No correlation was observed between IPEC-J2 cell adhesion, cell invasion and adhesion to the stainless steel surface ($R^2 < 0.02$). In conclusion, these results reflect the ability of the different BT4 strains to colonize the intestinal tract of pigs and to contaminate the stainless steel surfaces of the food processing environment.

Introduction

Yersiniosis is, after campylobacteriosis and salmonellosis, the third most frequently reported zoonosis in Europe (EFSA and ECDC 2016). Humans become infected with *Y. enterocolitica* through the consumption of undercooked pork meat and raw food having been in contact with contaminated surfaces (Bottone 1999). The ability of *Y. enterocolitica* to infect humans may depend on their capability to colonize pigs and to develop biofilm on conventional materials used in food industries. In France and worldwide, biotype 4 (BT4) is the biotype the most frequently isolated from both pigs and clinical yersiniosis (Fondrevez et al. 2014; Le Guern et al. 2016). In this study, a collection of 26 pathogenic BT4 strains isolated from pig tonsils were investigated for their ability to adhere and invade intestinal pig cells (IPEC-J2) and to adhere to abiotic surfaces (stainless steel coupons) using two *in vitro* tests.

Material and methods

IPEC-J2 cells assay

A suspension of 2.10^7 CFU *Y. enterocolitica* bacteria were added to the 2.10^5 Porcine IPEC-J2 cells, then the plates were incubated at 37°C in the presence of 5% CO_2. Three hours following infection, the cells were washed extensively with PBS (Phosphate Buffered Saline). The total number of adherent bacteria was determined by cell lysis using 0.1% Triton X-100 and plating on a bacterial agar medium. Bacterial uptake was assessed by adding 100µg of gentamicin per well. After two hours of incubation. The percentage of bacteria that survived killing was determined after plating on a bacterial agar medium. For each strain, the relative level of bacterial adhesion and uptake was determined by calculating the number of CFU relative to the total number of bacteria introduced into cells.

Stainless steel assay

The INOX coupons were contaminated by immersing them in a saline solution (0.9%) containing 10^6 CFU of *Y. enterocolitica* bacteria during 6h at 12°C. Non-adherent cells were removed by successive soaking in sterile saline solution (0.9%). Adherent bacteria were detached from the coupon by a 40s-vortex and a 2 min-sonication steps. The adherent bacteria were then enumerated after plating on a bacterial agar medium. For each strain, the relative level of bacterial adhesion was determined by calculating the ratio of adherent cells to the total number of bacteria in the immersion suspension.

Statistical analysis

Regression analysis was performed between data sets obtained from IPEC-J2 cells assays *versus* stainless steel assays by using logiciel R (R Development Core Team, 2015).

Results

All BT4 strains were able to adhere and invade IPEC-J2 cells. However, the results showed heterogeneity between strains with respect to their ability to adhere to IPEC-J2 cells, with a percentage of adhesion varying from 9% to more than 90%. The BT4 population displayed a more homogeneous ability to invade IPEC-J2 cells with percentages varying from 10% to 26%. The BT4 strains displayed a great ability to adhere to the stainless steel surface, with a percentage of adhesion varying from 0.3% to 4.2%.

No correlation was observed between IPEC-J2 cell adhesion, cell invasion and adhesion to the stainless steel surface ($R^2 < 0.02$).

Discussion

Y. enterocolitica has been reported to colonize pigs. The ability of *Y. enterocolitica* BT4 strains to adhere and invade epithelial cells is crucial for host colonization. The 26 strains studied were able to adhere and enter the intestinal porcine cells *in vitro*. This observation is in accordance with the fact that pigs are considered a reservoir for human pathogenic BT4 strains (Fondrevez et al. 2014; Le Guern et al. 2016). In IPEC-J2 cells assay, the adhesion profile was different from the invasion profile. The percentage of adhesion reached about 90% *versus* a maximal percentage of invasion of only 26%, regardless of the strain tested. These results may support the fact that *Y. enterorocilita* BT4 is able to colonize pork asymptomatically. *In vivo*, pigs do not develop clinical signs, but they do carry pathogenic *Y. enterocolitica* in their oral cavity and excrete the bacterium in their feces (Thibodeau et al. 1999).

Various bacteria including food spoilage bacteria and pathogens can form biofilms on different food processing surfaces, leading to potential food contamination or spoilage. All the *Y. enterocolitica* BT4 strains exhibited the ability to adhere to the stainless steel surface. This observation is in concordance with the study of Allan *and al*. which reported the fact that like *L. monocytogenes* and *Salmonella*, *Y. enterocolitica* strains are able to form biofilm on stainless steel surface (Allan, Yan, and Kornacki 2004).

Some cellular components, like the protein OmpR, seem to be involved in adhesion-invasion capacities and biofilm formation (Raczkowska et al. 2011). Pleiotropic effect of a component can be highlighted by demonstrating a correlation between IPEC-J2 cell adhesion, cell invasion and adhesion to the stainless steel surface at the cell level. In the present study, no correlation between IPEC-J2 cell adhesion, cell invasion and adhesion to the stainless steel surface was observed, for the 26 strains studied.

Conclusion

These results reflect the ability of the different BT4 strains to colonize the intestinal tract of pigs and to contaminate the stainless steel surfaces of the food processing environment.

Acknowledgements

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Slaughter process and Meat Inspection: quality, hygiene and safety

CONTROL OF *SALMONELLA* ENVIRONMENTAL CONTAMINATION DURING PIG TRANSPORT AND LAIRAGE: A REALISTIC PROJECT?

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Abstract

This study aims at investigating *Salmonella* environmental contamination of trucks and lairage pens and evaluating the efficiency of an improved cleaning and disinfection protocol to reduce *Salmonella* environmental contamination.

During four days, the lairage of two French pig slaughterhouses were sampled twice a day when pigs were present and once at the end of the week after the cleaning protocol. In parallel, six trucks per day were randomly selected and sampled at their arrival and after the cleaning procedure. The samples consisted of floor surface swabbing. *Salmonella* occurrence, level of contamination and serotypes were determined. The efficiency of different cleaning and disinfection procedures on the presence of *Salmonella* was also estimated.

Salmonella was isolated in 97.7% of the lairage samples when pigs were present (contamination levels >104UFC/m^2) and in 65% of the truck samples (contamination levels from <10 to >104UFC/m^2). An improved cleaning and disinfection procedure reduced efficiently the occurrence and the level of contamination in the trucks (almost 100%) compared to a simple wash with cold water (no effect), more partially in the lairage.

This study showed the importance of a good cleaning and disinfection protocol to decrease the level of contamination or eliminate the bacteria in the trucks used for the transport of pigs.

Introduction

Salmonella is a leading cause of foodborne illness worldwide and the consumption of pork meat is a major source for human infection (EFSA 2014). *Salmonella* is commonly carried in the intestinal tracts of a wide range of animals, including livestock animals. The organism may also be transmitted through direct contact with infected animals or humans or faecally contaminated environments.

In a recent survey in UK, *Salmonella* was isolated from 30.5% of individual pig caecal contents at slaughter (Powell et al., 2016). Pigs get infected through oral intake of *Salmonella* and they can carry this bacterium asymptomatically in their tonsils, gut and gut-associated lymphoid tissue for months resulting in so called *Salmonella* carriers. During periods of stress, re-excretion may occur. In this way, carriers are a permanent potential source of infection for other animals, including humans. Stress

factors can occur during the fattening period, but also prior to slaughter, for instance during transport to the slaughterhouse or during the stay in the lairage. Evidence supporting non negligible transmission rates during transport may be found in the literature (Ferrer Savall et al., 2016). Up to 20% of uninfected pigs within a batch may become infected during transport and lairage (Hurd et al., 2002). After 2 or 6 hours of combined transport and lairage, the number of animals excreting *Salmonella* has more than doubled (Hurd et al., 2001). *Salmonella* can be isolated from initially healthy pig faeces after 2 hours exposure of the pigs to a contaminated environment (Boughton et al., 2007).

Cleaning and disinfection of pig pens at lairage is important and considered as an essential part of any successful on-farm *Salmonella* control (Andres and Davies, 2015). What about the protocols used for trucks and lairage? Are they sufficient to eliminate or to reduce *Salmonella* environmental contamination (De Busser et al., 2013)?

This study aims at investigating *Salmonella* environmental contamination of trucks and pens of lairage in two French pig slaughterhouses. In parallel, we evaluate the efficiency of an improved cleaning and disinfection protocol to reduce *Salmonella* environmental contamination.

Material and methods

Sampling and experimental design

During four successive worked days, the floor of the pens of lairage of two pig slaughterhouses (coded S1 and S2) were sampled twice when pigs were present and once at the end of the week after the cleaning protocols. In parallel, in each slaughterhouse, six trucks per day were randomly selected and floor environmental samples were collected at their arrival at the slaughterhouse (transport duration between 30 and 180 min) and after the cleaning procedures. All samples were analyzed within four hours after collection.

Standardization of the swab sampling method

Each sample consisted in a total swabbing of 2 m^2 of floor surface. A swab was used on 4 sites of 50 cm^2 using an iron frame to standardize the method.

Two samples per pen or per truck has been done each time, and one sample after the cleaning and disinfection procedures.

In parallel, general information concerning the transport and the lairage conditions were recorded (duration or waiting time, origin of the herds or process of the batches, characteristics of the trucks: type, presence of sawdust… or identification of the pens, cleanliness level, localization of the animals, time of sampling).

Salmonella detection and semi-quantification

Swab samples were diluted in 150 mL of peptone buffered water and homogenized 1 min in a stomacher.The detection of *Salmonella* in the sample was done according the ISO 6579 reference method and the semi-quantification by using the Miniaturized Most Probable Number technique as described in part 2 of the ISO 6579 reference method. Two isolates per positive samples were serotyped according the Kaufmann-White classification (Popoff, 2001).

Results and discussion

Salmonella environmental contamination

Salmonella was isolated in 65 % of all the truck samples, with respectively 50% and 81% for the trucks of slaughterhouses S1 and S2. For the different sampling days, the contamination of positive trucks varied from 25% to 83%; with respectively from 25% to 67% for S1 trucks and from 80 to 83% for S2 trucks. Previous French study showed that 47% of the trucks were positive (Rossel et al., 2002).

In our survey, 97.7% of all the pen lairage samples were positive in *Salmonella*. Previous French study showed that 41% of the pens were positive (Rossel et al., 2002) This high prevalence is close to those found in the literature (Hurd et al., 2002; De Busser et al., 2007; Ferrer Savall et al., 2016)The high levels of contamination of the holding pens point out that it is a major source of contamination of healthy pigs arriving at the slaughterhouse.

The amount of *Salmonella* in the environment was highly variable (from 0 to >104 CFU/m^2) and varied between two different samples for a given pen or truck or between pens or trucks at the same sampling time (Figure 1). This variation is probably due to the transport and lairage conditions that were heterogeneous. In this study, we had different herds, different duration of transport and different waiting times in the lairage. Moreover, some trucks drivers used or not sawdust and we noticed less occurrence of positive samples in the trucks with sawdust.

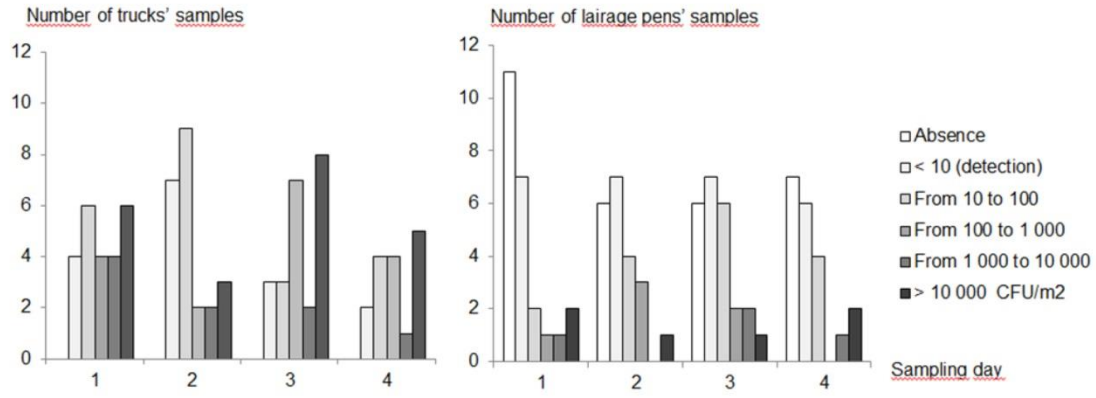

Figure 1. Level of Salmonella contamination for trucks' and lairage pens' samples (S1 and S2) during the four successive days of sampling.

When the animals were in the pens, environmental samples were positive with a high variability of the contamination level (from less than 10 to more than 10^4 CFU/m^2). Nevertheless, there was no difference between the four days of sampling. This variability could be due to differences in the excretion of the pigs; some with intermittent excretion of *Salmonella* and/or re excretion due to stress. A study exploring the level of *Salmonella* excretion of the pigs in the trucks or in the pens would allow to see if there is a correlation between the level of *Salmonella* excreted by pigs and the contamination level of the environment.

Evaluation of the cleaning and disinfection procedures

An improved cleaning and disinfection procedure reduced efficiently the occurrence and the level of contamination in the trucks compared to a simple wash with cold water: 97.5% of trucks were negative after the cleaning and disinfection protocol and none with a simple wash with cold water. We observed a reduction of more than 10^4 log UFC/m^2 of the contamination level with the cleaning and disinfection procedure.

The improved cleaning and disinfection procedure reduced also *Salmonella* contamination in the lairage; 73% of the holding pens were negative after the cleaning and disinfection protocol with still a reduction of the contamination level of more than 10^4 log UFC/m^2.

Conclusion

No *Salmonella* have been detected after the cleaning and disinfection protocol in 97.5% of the trucks showing that good measures of hygiene (cleaning with hot water and disinfection) between two transports of animals allow the elimination of *Salmonella* in the trucks used for the transport. The high levels of contamination of the holding pens point out that it is a major source of contamination of healthy pigs arriving at slaughterhouse. The waiting period in the lairage of at least two hours contains a substantial risk for slaughter pigs to become infected with *Salmonella* as the cleaning and disinfection procedures are still not satisfactory in the field. Moreover, in lairage, a good protocol is not always sufficient to eliminate the bacteria. It could be interesting to go further in this investigation by looking for each data at the general information, the type of floor (concrete, partial, presence of cracks and crevices), the type of disinfectant chosen and its concentration, the presence of organic matter…to understand why an apparent good cleaning and disinfection protocol is not always sufficient to eliminate the substantial risk for slaughter pigs in the lairage and find different solutions.

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ASSOCIATION BETWEEN SLAUGHTER PRACTICES AND THE DISTRIBUTION OF *SALMONELLA*, ESBL/AMPC-PRODUCING *ESCHERICHIA COLI* AND HYGIENE INDICATOR BACTERIA ON PIG CARCASSES AFTER SLAUGHTER

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Introduction

Pigs are well-known asymptomatic carriers of foodborne pathogens, which may contaminate pork carcasses during slaughter [1]. Several pig body parts (e.g. the oral cavity, the palatine tonsils and the gastro-intestinal tract) are natural reservoirs of bacteria, including important human pathogens such as *Salmonella* [2,3]. The contamination level of a pig carcass is generally expressed as one value for the whole carcass. However, contamination levels may vary between different carcass areas.

The aim of the study

The aim of this research was to map the distribution of *Salmonella*, ESBL/AmpC-producing *E. coli* and hygiene indicator bacteria on pig carcasses and estimate associations between slaughter practices and carcass contamination.

Material and methods

Seven Belgian pig slaughterhouses were visited two times to sample carcasses before chilling. Each visit, the following 9 carcass areas (100 cm^2) from 5 randomly selected carcasses were swabbed using cellulose sponges: head (nose bridge and ears), foreleg, elbow, throat, sternum, belly, ham, pelvic duct and loin. All samples were analyzed for the presence of *Salmonella* by pre-enrichment in Buffered Peptone Water, enrichment Modified Semi-solid Rappaport Vassiliadis and plating on Xylose Lysine Deoxycholate agar. The total aerobic bacteria, *Enterobacteriaceae* and *E. coli* were quantified by direct plating on Plate Count Agar, Violet Red Bile Glucose agar and Tryptone Bile agar with X-Glucuronide, respectively. The presence of ESBL/AmpC-producing *E. coli* was investigated by plating on Tryptone Bile agar with X-Glucuronide supplemented with cefotaxime. Additionally, specific slaughter practices were recorded for each sampled carcass to identify risk factors for microbial contamination.

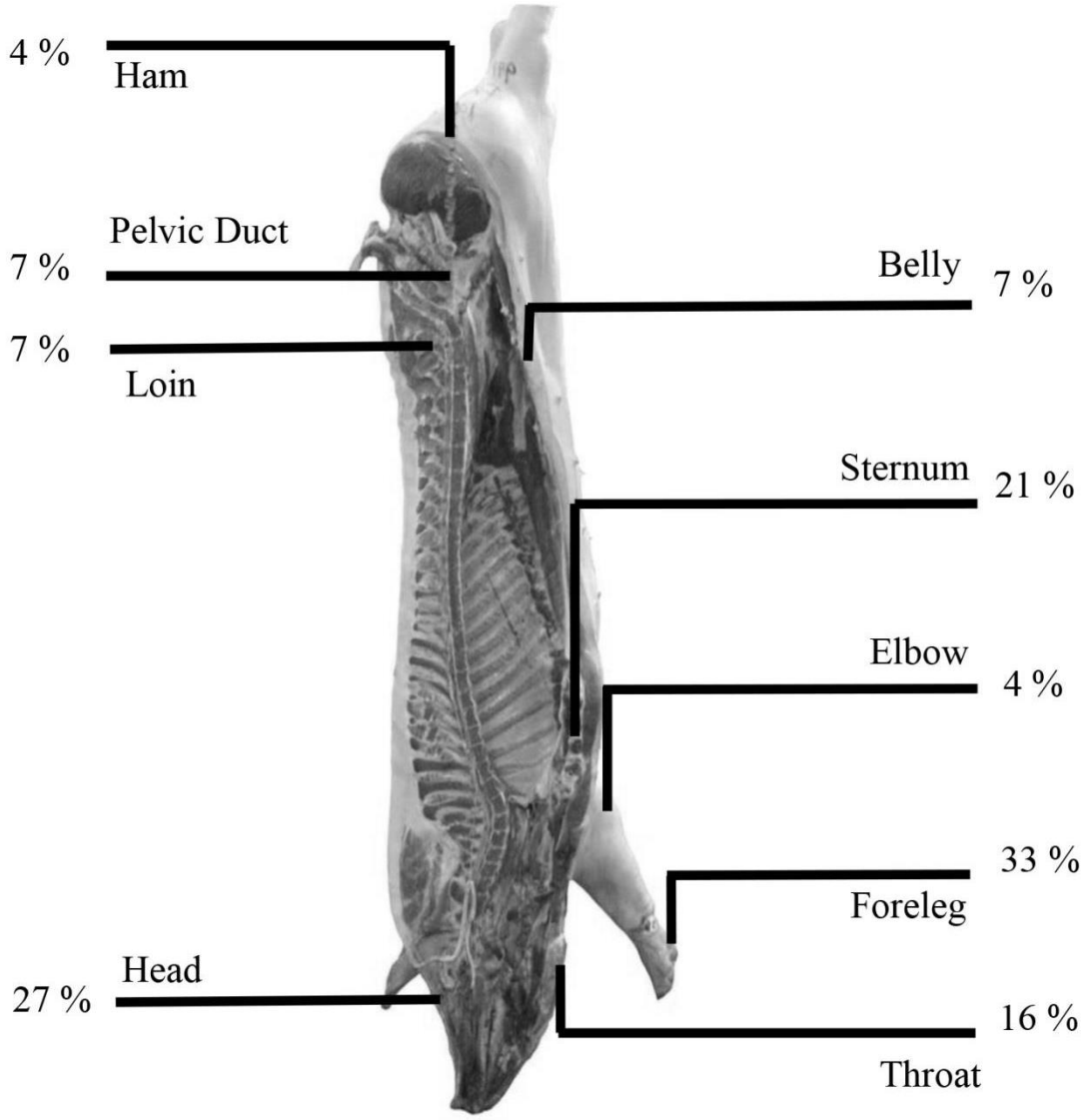

Figure 1. Qualitative analysis of *Salmonella* on pig carcasses before chilling.

Results and discussion

Overall, the average total aerobic count varied between 3.1 (loin, pelvic duct, ham) and 4.4 log10 CFU/cm^2 (foreleg). Median *Enterobacteriaceae* numbers of all samples collected in the 7 slaughterhouses ranged from values under the detection limit (ham) to 1.65 log10 CFU/cm^2 (foreleg). Median *E. coli* numbers varied between values under the detection limit (elbow, ham, loin, pelvic duct) and 1.14 log10 CFU/cm^2 (foreleg). *Salmonella* was recovered from 4% (ham and elbow) to 33% (foreleg) of the samples. In total, 53% of the carcasses were contaminated with *Salmonella*, varying between slaughterhouses from 0% to 100%. ESBL/AmpC-producing *E. coli* was found in 1% (loin) to 23% (head) of the swabs.

The number of *Enterobacteriaceae* and presence of *Salmonella* were higher on the sternum, elbow and foreleg of carcasses from which tonsils ($p<0.05$) or intestines ($p>0.05$) were incised. *Enterobacteriaceae* and *Salmonella* were lower at the belly/sternum when an automated belly/sternum opener was used instead of knifes and when knifes were disinfected prior to opening the belly/sternum compared to the use of non-disinfected knifes ($p>0.05$).

Conclusion

Large variations in contamination levels between different carcass areas were observed. Certain slaughter procedures are significantly associated with the contamination levels of particular carcass areas, which indicates the possibility to implement specific adaptations to improve the microbiological quality of pork carcasses and the need for risk categorization of pork cuts along the production chain.

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THE HARMONIZATION OF SANITARY DECISION CRITERIA FOR VERTEBRAL OSTEOMYELITIS IN PIG CARCASSES

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Introduction

Vertebral osteomyelitis (VO), a typically suppurative lesion, is the main cause of pig carcass condemnation during *post mortem* inspection in Portugal, being responsible, in 2015, for total condemnation of 0.1% of the slaughtered finishing pigs.

Sanitary decision of VO cases taken by official veterinarians should be based on Regulation (EC) N.º 854/2004 which defines that meat must be declared unfit for human consumption if it derives from animals affected by a generalized disease, such as septicaemia, pyaemia, toxaemia or viraemia. So, considering that presence of VO in pigs is indicative of pyaemia, it seems logical to declar VO cases unfit for human consumption. However, if the pyaemia has ended, is it then necessary to condemn the entire carcass?

For the cases not recognised in the live animal during *ante mortem* inspection, pyaemia may be detected during *post mortem* inspection by revealing suppuration, with or without abscesses, and haemorrhagic infarcts in different parts like the lung, mediastinum, pleural cavity, spleen, kidneys, joints and muscle. Those cases, with multiple suppurative findings, obviously require total condemnation. On the opposite, VO cases associated with prior pyaemia and no indication of current systemic changes, the judgment of meat inspection could be condemnation only of the affected parts. Nevertheless, in Portugal many of these VO cases are totally condemned in an undifferentiated way, because of the perceived risk related to pyaemia and due to the lack of objective criteria to support an alternative sanitary decision.

The objectives of this study were 1) to assess the need for total condemnation of carcasses with VO based on the health risk for the consumer and 2) to define harmonized criteria for the condemnation of these cases based upon objective macroscopic parameters, scientific-based. This would lead to avoidance of unnecessary condemnation of affected carcasses that do not represent a risk to the consumers.

Material and methods

During 17 weeks in the winter 2015/16, meat inspection of 211,159 pigs was undertaken in one Portuguese abattoir. All VO cases were declared unfit for human consumption by the official veterinarians. From those, 40 VO cases were selected at random and analysed in order to assess the need to apply this sanitary decision.

Based on Regulation (EC) N.º 854/2004, Gracey *et al*. (1999) and Ninios (2014) the following objective macroscopic parameters were used as criteria to classify VO cases with respect to need for condemnation:

Total condemnation

- **Generalized (G) cases:**
  - ✓ **G1** - ≥1 Acute VO lesion;
  - ✓ **G2** - 1 or 2 Chronic VO lesions in contiguous vertebrae with additional suppurative lesions in other parts of the carcass and/or respective viscera;
  - ✓ **G3** - ≥ 2 Chronic VO lesions in separated vertebrae;
  - ✓ **G4** - ≥ 3 Chronic VO lesions in contiguous vertebrae.
- Extensive carcass contamination with purulent exudate.

Partial condemnation

- **Localized (L) cases:**
  - ✓ **L1** - 1 Chronic VO lesion without additional suppurative lesions in other parts of the carcass and/or respective viscera;
  - ✓ **L2** - 2 Chronic VO lesions in contiguous vertebrae without additional suppurative lesions in other parts of the carcass and/or respective viscera.

In order to support the aforementioned criteria, VO lesions were classified as acute or chronic, based on the following objective macroscopic characteristics:

- **Acute:** Shiny and moist lesions with, sometimes, congested areas Evident bone destruction not circumscribed by adjacent remodelling tissue; presence of fluid purulent exudate;
- **Chronic:** Moderate bone destruction circumscribed by remodelling tissue; thickened exudate.

To evaluate the effectiveness of these macroscopic criteria, the same lesion was submitted to a histopathological analysis. For that, the column fragment with the VO lesion was cut and stored in a container with formalin (10%) for further evaluation. During *post mortem* inspection, each carcass and respective viscera were evaluated being registered the local (Cervical, Thoracic, Lumbar, Sacral, Coccygeal) number and contiguity of affected vertebrae, presence of perivertebral abscesses and other suppurative lesions beyond VO (muscle, joints, tail, lung…) in order to apply harmonizing criteria for the condemnation.

In order to evaluate the risk to the consumer related to the presence of pyaemia, paired samples of VO purulent exudate and muscle (diaphragm) were aseptically collected in sterile containers and sent to the laboratory under refrigerated conditions for further microbiological analysis. The presence of the same bacteria in both samples was considered as indicative of pyaemia related to VO. Additionally, from each batch of VO cases provenance, a sample of diaphragmatic muscle was collected from one carcass fit for consumption (with no lesions) to be used as controls in the microbiological analysis.

At the laboratory, microbiological analyses were carried out according to standard techniques targeting the main etiological agents responsible for these lesions: *Staphylococcus, Trueperella pyogenes, Streptococcus and Pasteurella*. Presumptive *Staphylococcus* spp. isolates (Coccus Gram +, DNase and catalase +) were identified by molecular methods (multiplex PCR 16S rDNA, *mecA, nuc*).

For histopathology examination, specimens were fixed in 10% neutral-buffered formaldehyde and routinely processed for histological examination, embedded in paraffin wax and sectioned at 3 μm. One section was stained with HE (haematoxylin - eosin) and the other with Gram coloration. Histopathological classification of VO lesions was based in the following criteria:

- **Acute:**
  - ✓ **Osseous changes:** Osteonecrosis; Irregular contours and fragmentation of bone trabeculae, with bone sequester formation; There are intramedullary granulocyte infiltrates and fibrin exudates; Reduced or complete lack of haemopoiesis.
  - ✓ **Soft tissue changes:** necrosis;
  - ✓ **Inflammatory infiltrate pattern:** neutrophilic granulocyte infiltrate diffuse.

- **Chronic:**
  - ✓ **Osseous changes:** Bone neogenesis, medullary space tissue with granulation tissue formation;
  - ✓ **Soft tissue changes:** Granulation tissue formation and fibrosis;
  - ✓ **Inflammatory infiltrate pattern:** Lymphocyte/macrophage/plasma cell infiltrate, with a few neutrophilic granulocytes.

The statistical significance of the association between macroscopic and histopathological classification of VO lesions was assess by Phi test for paired samples, using IBM SPSS Statistics® 20 software. Phi test was also applied in order to evaluate the association between pyaemia and the macroscopic parameters used to classify VO cases. Differences were considered significant at $P < 0.05$.

Results

From the 211.159 pigs slaughtered during this study period, 240 carcasses (0.11%) were totally condemned. From those, all VO cases (n=152) were declared unfit for human consumption by the official veterinarians, representing 0.08% of the slaughtered pigs and the main cause of total condemnation (152/240; 63%).

From the 40 randomly selected VO cases, using the objective macroscopic parameters pre-determined, 20 were classified as chronic and 20 as acute. Results from histopathological analyses revealed a highly significant association (Fi=0.,804, p<0.001) with the macroscopic results allowing to validate the proposal for VO classification in acute and chronic during *post mortem* inspection contributing to its harmonisation.

In this study, lesions indicative of VO were more commonly observed in only one location (35/40, 87.5%) than in two or more different locations (5/40, 12.5%). From the cases found in only one location, the majority were (F=2.19, p<0.05) observed in the thoracic vertebrae (n=19).

Additionally, *post mortem* inspection of the carcass and viscera allowed to identify other suppurative lesions beyond VO. In the 40 VO cases, the majority (23/40, 57.5%) did not present additionally suppurative lesions observed during *post mortem* inspection. The remaining 17 (42.5%) presented additionally diverse suppurative lesions (Table 1). Pleuropneumonia was found in 12 cases. Suppurative exudate or abscesses were not found in any of these cases, and therefore, these lesions were not considered as related to VO but rather an independent finding of *post mortem* inspection reflecting

that chronic pleuritis is the most common finding in *post mortem* inspection of finishing pigs.

Microbiological analysis revealed 13 (32.5%) positive pyaemic cases (presence of the same bacteria in both samples: muscle and VO), No bacteria were isolated from the 36 control muscle samples.

Microbiological results showed that *T. pyogenes* (32) was the most frequently isolated bacteria from both VO and muscle samples, followed by *Streptococcus* spp. (16) and *S. aureus* (6).

Table 1 resumes the main results reached in this study.

Table 1. Sanitary decision and judgment criteria of VO cases, characteristics of lesions and presence of pyaemia.

Judgment criteria			VO lesion		Additional suppurative lesions	N	Microbiology	
SD	**Criteria reference**		**A/C**	**Nº and contiguity**			**Pyaemia (N)**	**Bacteria isolated**
Total condemnation	G1	Generalized process	A	V1	One muscular abscess	2	2	*T. pyogenes, S. aureus*
	G1		A	V1	Two muscular abscesses	1	0	n.i.
	G1		A	V1	Joint injury	1	1	*Streptococcus* spp.
	G1		A	V1	One muscular abscess and tail injury	1	1	*T. pyogenes*
	G1		A	V1	No lesions	4	1	*Streptococcus* spp.
	G1		A	V2 contiguous	One muscular abscess	1	0	n.i.
	G1		A	V2 contiguous	Two muscular abscesses	1	0	n.i.
	G1		A	V2 contiguous	Joint injury	2	1	*Streptococcus* spp.
	G1		A	V2 contiguous	No lesions	3	3	*T. pyogenes*
	G1		A	V2 separated	Joint injury	1	0	n.i.
	G1		A	V2 separated	No lesions	1	1	*T. pyogenes*
	G1		A	V3 contiguous	Two muscular abscesses	1	1	*T. pyogenes*
	G1		A	V3 separated	No lesions	1	1	*S. aureus*
			Subtotal G1			20	12	-
	G2		C	V1 contiguous	One muscular abscess	2	0	n.i.
	G2		C	V1 contiguous	Two muscular abscesses	1	0	n.i.
	G2		C	V1 contiguous	One abscess and joint injury	1	0	n.i.
	G2		C	V2 contiguous	Two muscular abscesses and tail injury	1	0	n.i.
			Subtotal G2			5	0	-
	G3		C	V2 separated	Tail injury	1	1	*T. pyogenes*
	G3		C	V3 separated	No lesion	1	0	n.i.
			Subtotal G3			2	1	-
	G4		C	V3 contiguous	No lesion	1	0	n.i.
	Subtotal generalized					28	13	-
	Extensive carcass contamination with purulent exudate		C	V1 contiguous	No lesion	1	0	n.i.
Partial condemnatio	L1	Localized process	C	V1 contiguous	No lesions	8	0	n.i.
	L2		C	V2 contiguous	No lesions	3	0	n.i.
	Subtotal localized processes					11	0	-
	TOTAL					**40**	**13**	**-**

A/C – Acute/Chronic; **SD** – Sanitary decision; **Criteria reference** – Reference used for judgment criteria: G1 – Generalized1, G2 – Generalized2, G3 – Generalized3, G4 – Generalized4, L1 – Localized 1, L2 – Localized2; **V1, V2, V3** – One, two and tree affected vertebrae with osteomyelitis, respectively; **n.i.** – No bacterial isolation

The data presented in Table 1 shows that both 13 active pyaemia cases were classified as generalized (totally condemned), being this association very significant (Fi=0.4543; p<0.01). Also, acute cases revealed a very significant association (Fi=0.6547; p<0,01) with pyaemia in opposite to the other macroscopic parameters used to classify VO cases that did not shown any association.

Using the criteria proposal to classify VO cases with respect to need for condemnation, 11 carcases out of 40 (27.5%) could have been spared from total condemnation. Those were chronic cases that matched 100% with HP evaluation, and did not reveal any signs of pyaemia, meaning that the criteria used to judge VO cases represent no risk to the consumer concerning to the presence of pyaemia.

Conclusions

Total condemnation of VO cases are mainly due to the perceived risk related to pyaemia, which is one of the criteria defined in the Regulation (EC) No. 854/2004 to declare meat unfit for consumption. Based on this it was important to understand and objectively detect, under meat inspection conditions, the cases of VO that are not related to pyaemia and could be spared from a total condemnation. In this study, the harmonized criteria proposal for condemnation of VO cases, defined by objective macroscopic parameters, allowed to allocate them in generalized (pyaemia) and localized cases leading, respectively, to total and partial condemnation. Under this harmonised protocol, classification of VO in acute or chronic was determinant, especially in single cases. The highly significant association found between macroscopic and histopathologic results allows to validate the proposal for VO classification in acute and chronic during *post mortem* inspection that strengthens this harmonized protocol.

The application of the proposed objective criteria to support sanitary decisions for VO, could have spared the rejection of 27.5% of the affected carcases, not constituting any risk to consumers concerning to the presence of pyaemia and fitting the objective of Regulation (EC) No. 854/2004. Also, using these harmonized criteria, an approximate loss of €1,309 could have been avoided, considering a production cost of €109/pig with approximately 107 kg live weight (SIP, 2015). This result is of extremely importance in Portugal since VO it is the main cause of condemnation of finishing pigs at slaughterhouse (In 2015, 4.267 carcasses were totally condemned by VO, corresponding to 0.1% of the slaughtered pigs).

Considering the microorganisms isolated, the majority (*T. pyogenes* and *Streptococcus*) should be considered as a potential occupational risk for slaughterhouse worker. Only *S. aureus*, isolated from two cases, should be considered as potential foodborne pathogen and a potential risk to consumers, if carcases reach to the market under conditions that favour the production of enterotoxins.

As final remark we would like to underline the importance of VO lesion in pork production chain that requires a multidisciplinary approach in order to mitigate the economic loss at the slaughterhouse level that, according to the authors, should include the revision of condemnation judgment during *post mortem* inspection. In fact, the inexistence of objective criteria to classify VO lesions may favour some subjectivity judgments concerning to carcasses condemnation, leading to unnecessary economic losses and to uncomfortable and inconvenient perceptions by food business operators concerning to official veterinarians, that must be avoid.

These results allows to define objective macroscopic criteria, scientific based, to support the harmonization of sanitary decision procedures associated with VO cases, reducing economic losses for industry without compromising food safety and public health.

GUIDELINES FOR FARMERS, TRANSPORTERS AND OFFICIAL VETERINARIANS TO ASSESS THE "FITNESS FOR TRANSPORT AND SLAUGHTER" OF SLAUGHTER PIGS

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Introduction

Legislative provisions related to animal transport and ante-mortem meat inspection of farm animals exist both at national and at European level. The Regulation (EC) No. 1/2005 characterizes the transport criteria that are to be met with regard to the animals' health and welfare.

In accordance with this regulation it is prohibited to transport animals in a way that is likely to cause injury or undue suffering to them. It is also prohibited to transport injured animals or animals with physiological weaknesses or pathological conditions. Furthermore, animals that are only able to move with pain or with assistance or animals that have open wounds or severe organ prolapses are not allowed to be transported. This also applies to pregnant animals in advanced stages of gestation (>90%) or neonatal animals (<7 days of life).

The European Regulation (EC) No. 854/2004 defines specific exclusion criteria regarding the decision "fit for slaughter", which is to be verified during the official ante-mortem inspection by the official veterinarian. That means that animals are "not fit for slaughter for human consumption", if they have severe physiological or functional disorders.

And although these regulations are in force, there are disagreements regarding the decisions on the "fitness for transport" or the "fitness for slaughter" between farmers, transporters, official veterinarians and also official veterinarians among themselves.

Material and methods

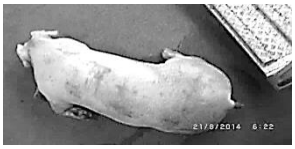
In order to standardize these official decisions with high animal welfare relevance regarding "fitness for transport" and "fitness for slaughter" as well as to give farmers and transporters assistance to prevent the delivery of animals that are "not fit for transport" and/or "not fit for slaughter", written guidelines and practical illustrations of the pathological alterations were developed in cooperation between the University of Veterinary Medicine Hannover, the Food and Veterinary Authority of the District of Cloppenburg as well as the Pig Health Service of the Agricultural Chamber of the Federal State of Lower in Germay.

Results

The developed and validated guidelines contain practise-oriented cases exemplified by images and symptom descriptions such as cases with joint diseases, injuries or organ prolapses in different degrees of severity. For each case a tentative

diagnosis as well as a decision with regard to the assessment of the "fitness for transport" and the "fitness for slaughter" is given using a traffic light system (red, yellow, green). Furthermore, the guideline proposes precise instructions for concrete activities like reporting an offence to the Responsible Authority in the framework of the legislative to prevent animal cruelty (Figure 1, 2, 3).

Case 1:



Symptom description:

- Poor general condition
- Inability to stand or walk
- Necrosis of the tail

Tentative diagnosis:

- Ascending bacterial infection caused by the necrosis of the tail
- Abscesses in the spinal canal as cause for the inability to stand
- Sepsis which leads to the poor general condition

Decisions:

fit for transport		"No" due to inability to stand or walk
fit for slaughter		"No" due to the poor general condition and the risk of sepsis and multiple abscesses
Further instruction	!	Report of an offence regarding "Animal Cruelty"

Figure 1. Pig with tail necrosis and inability to stand.

Case 2:

Symptom description:

- Intense bulge with superficial wound
- >50% of the space between abdominal wall and floor is involved
- Good general condition

Tentative diagnosis:

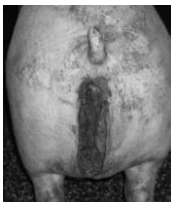
- Intense umbilical hernia

Decisions:

fit for Transport		"No" due to severe organ prolapse
fit for Slaughter		"Yes", logistic slaughter: • at the end of the day due to a high risk of accidental incising of the intestines
Further instruction	!	Report of an offence regarding "Animal Cruelty"

Figure 2. Pig with umbilical hernia.

Case 3:



Symptom description:

- Wound with beginning granulation and fecal contamination
- Except for signs of pain, good general condition

Tentative diagnosis:

- Incisional laceration wound not older than 12 hours

Decisions:

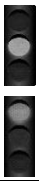
fit for transport		"Yes" with restrictions: • Separation of injured pig during transport to avoid attacks from other pigs "No" if separation of injured pig has not been applied
fit for slaughter		"Yes", logistic slaughter: • Separation during lairage • Early slaughter to reduce pain of the injured animal
Further instruction	!	• Unseparated transport: report of an offence Lead the farmer and/ or transporter to find and eliminate the cause for the incisional laceration

Figure 3. Pig with incisional laceration.

Discussion and conclusion

Although even images and symptom descriptions are not able to reflect a complete and realistic presentation of a case, if they are used as typical examples they are quite instructive tools within a guideline. By teaching this guideline to all relevant stakeholders along the food chain like farmers, transporters and official veterinarians, the majority of disagreements can be prevented as well as the still occurring deliveries of animals to slaughterhouses that are "not fit for transport" and/or "not fit for slaughter".

AUTOMATED ASSESSMENT OF ANIMAL WELFARE INDICATORS IN PIGS AT SLAUGHTER

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Introduction

During the last years, animal welfare has received special attention in German animal husbandries. Based on the German Protection of Animals Act, § 11 Section 8, the farmer has to assess and evaluate animal welfare indicators directly on the animal within the legally required self-monitoring. Via this self-monitoring, the demands of § 2, German Protection of Animals Act (for instance adequate feed, care, and accommodation appropriate to the species) should be complied. In the official explanatory statement this is commented as follows: "The aim of animal-based self-monitoring is to provide an assessment of the animal's well-being based on appropriate indicators such as foot pad health, mortality rates or organ findings at the abattoir, and to plan and implement improvement measures if necessary." The assessment of animal based welfare indicators at the slaughterhouse represents a particular feasible tool for the retrospective evaluation of the animal's living conditions due to large animal numbers and the realization of comparative observations. However, if assessed by individual observers, the amount of work and the subjectivity of the results represent major problems. In order to guarantee consistent, comparable and objective results, food pad condition as welfare indicator is assessed at most larger poultry slaughterhouses in Germany via camera-based monitoring-systems by now. The question arose if such a system can be developed and implemented for pigs as well. Therefore, the aim of this study was to test a camera-based rating system for the automated and standardized assessment and documentation of animal welfare indicators in pigs.

Material and methods

To realize the mentioned aim, a pilot study on the automated assessment of welfare indicators in pigs at slaughter was initiated in summer 2015. Main emphasis was laid on the indicators ear lesions, tail lesions and swellings in joints, based on the recommendations of another pilot study on findings at slaughter (QS-Project "Befunddaten in der Schweineschlachtung"). On the participating abattoir, approximately 4,500 pigs are slaughtered daily. The monitoring system (CLK GmbH, Altenberge, Germany) consists of five cameras, taking pictures of the hind legs, the back, and the head and two lateral pictures per pig, respectively, against a blue background. The lateral pictures are important for the identification of the pigs for the distinct classification. The system was installed after the processes of bleeding, scalding, de-bristling and flame-scarfing. A schematic side-view of the system is represented in Figure 1.

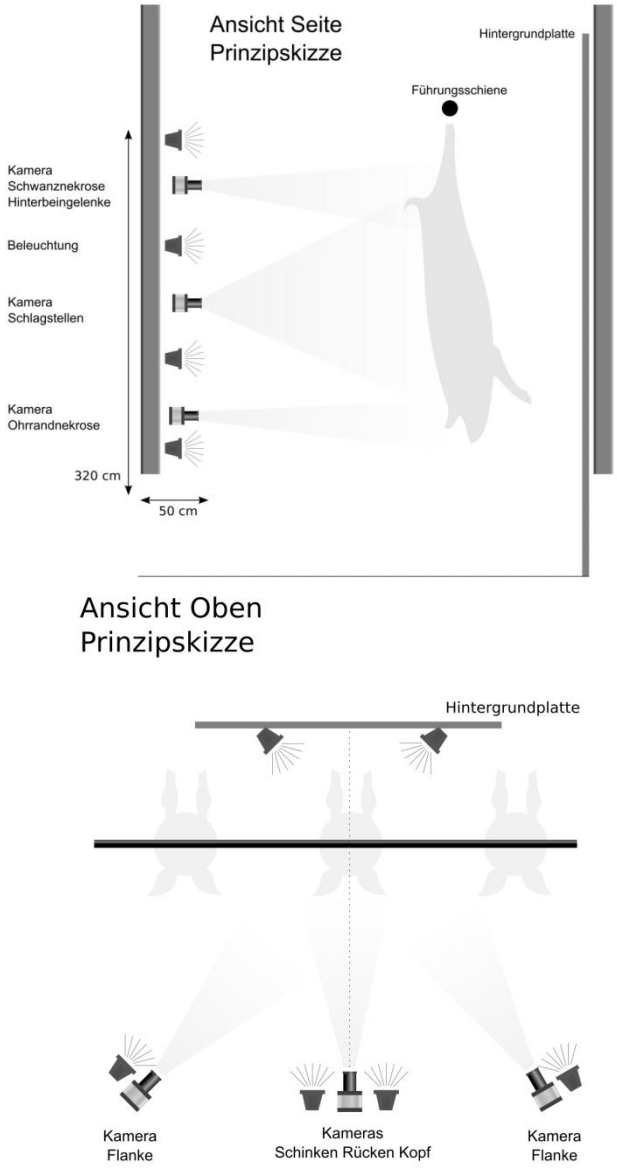

Figure 1. Side view of the automated rating system (CLK GmbH).

In the developing phase, the system was optimized with regard to the individual assignment of the pictures and findings to each animal. After calibration and parallel assessments by experts, specifications for the defined indicators and the development of respective algorithms, findings were detected automatically. After the developing phase, the system was able to detect the welfare indicators ear lesion and tail lesions and to mark noticeable problems with red circles (Figure 2).

Moreover, joint changes were detected, for example swellings. Thresholds for the swellings of joints are planned to be defined in a subsequent research project.

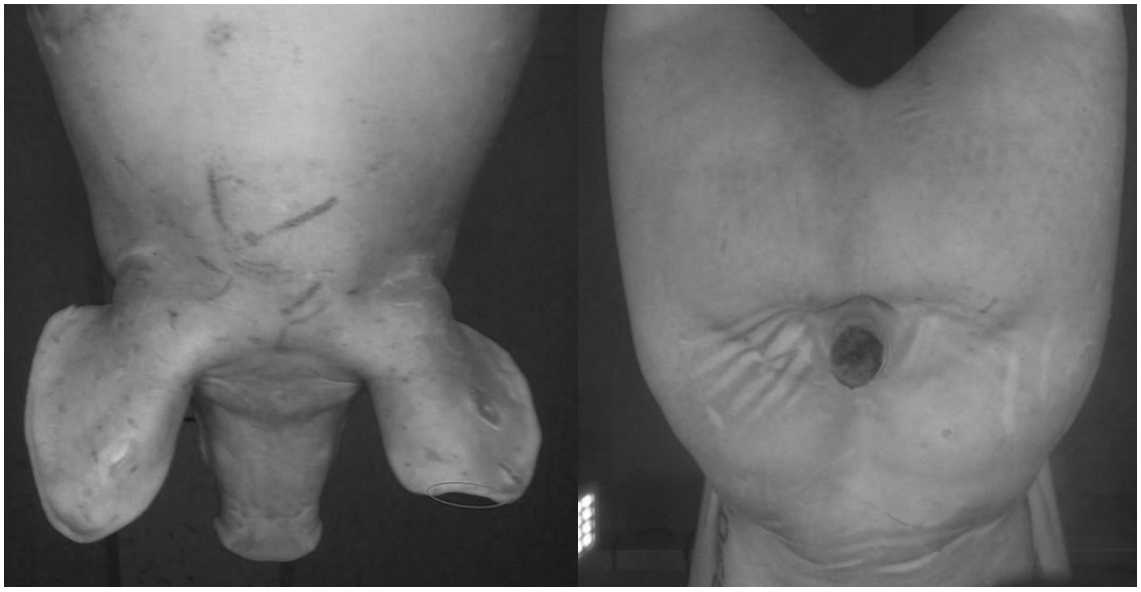

Figure 2. Marking of the indicators "ear lesion" and "tail lesion" by the automated rating system (Picture: CLK GmbH).

After the intensive developing phase, sensitivity (true positive rate) and specificity (true negative rate) were calculated based on a comparison of pictures. Moreover, a comparison of the assessed findings by the system and by individual experts took place on several dates. The system runs on every slaughter day and monitors tail and ear lesions. Pictures are saved for further evaluation and documentation. The rate of non-evaluable pictures, for instance based on carcasses hanging askew, is determined as avoid-rate.

Results

Sensitivity and specificity were calculated at three test days as documented in Table 1.

Table. 1. Sensitivity and specificity of the indicators ear and tail lesions based on pictures from three test days.

	Sensitivity / specificity
	Indicator ear lesions
Day 1 (n= 732)	75.0% / 97.2%
Day 2 (n= 1,033)	80.0% / 94.4%
Day 3 (n= 869)	80.0% / 96.7%
	Indicator tail lesions
Day 1 (n= 815)	80.0% / 99.1%
Day 2 (n=1,016)	72.7% / 100.0%
Day 3 (n=853)	92.3% / 99.3%

Based on the manual comparisons of findings of the system and the experts' findings, the settings of the system were adopted. Due to the lesions detected by the system, various evaluations can be performed, for example a comparison of data based

on single slaughter dates, as documented in Table 2. Within six days, the prevalence of ear lesions varied between 8.6% and 15.5%, and of tail lesions between 0.6% and 1.3%.

Table 2. Prevalence of ear and tail lesions on six consecutive slaughtering days (n= number of evaluated pictures).

Prevalence	*Indicator ear lesions*	*Indicator tail lesions*
Day 1 (n= 4,489)	9.1%	1.3%
Day 2 (n= 4,206)	10.9%	0.6%
Day 3 (n=4,226)	9.5%	0.8%
Day 4 (n= 3,697)	15.5%	1.0%
Day 5 (n= 4,251)	8.6%	1.3%
Day 6 (n= 4,193)	11.4%	1.3%
Total (n= 25,062)	10.7%	1.1%

The avoid-rates in the considered period differed daily, too. For the indicator ear lesions they varied between 4.3% and 6.7%, and for tail lesions between 1.9% and 2.9%. This rate should be further reduced.

Conclusion

The automated assessment system was implemented successfully for the welfare indicators ear and tail lesions at the test slaughterhouse. The system is very suitable for the daily use. As shown for the joint lesions, besides the technical development, the definitions of indicators and thresholds are of special importance. With regard to the amount of assessed data, a huge variety of different analyses is possible. The system is open for further indicators, e.g. indicators of inappropriate chasing. After the previous operating experience with the automated recording system, the prevalence of ear and tail lesions differs considerably depending on the animal batches, and origins, respectively. For comparison, the system should be installed in several abattoirs, and, moreover, the thresholds, especially for lesions and swellings in the joints, have to be discussed critically and to be standardized, in the best case.

Acknowledgements

This study is financially supported by the QS-Wissenschaftsfonds. Moreover, we thank our cooperating partners CLK GmbH, Westfleisch SCE mbH, Fleischhof Rasting GmbH, ISN-Projekt GmbH and Referat 71: Veterinär- u. Lebensmittelüberwachung der Stadt Gelsenkirchen for the very constructive collaboration.

ANALYSIS OF MICROBIOLOGICAL TESTS RESULTS FOR PORK AND PORK PRODUCTS PRODUCED IN THE CENTRAL REGION OF THE RUSSIAN FEDERATION IN 2012-2016

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The FGBI "ARRIAH" annually performs tests of raw food materials and ready-to-eat food products manufactured in some Oblasts of the RF Central Region in the framework of National laboratory veterinary monitoring of banned and harmful substance residues in live animals, products of animal origin and feed in the Russian Federation territory. The study was aimed at analysis of the results of microbiological tests of pork and pork products produced in the Vladimir, Ivanovo and Kostroma Oblasts in 2012-2016. During this period 3,878 tests were carried out including 1,062 tests of pork (27.38%); 293 tests of offal (7.56%); 1,264 tests of pork fat (32.59%), 1,259 tests of pork preparations (32.46%). It was demonstrated that 5.57% of samples of raw meat and food products were non compliant with microbiological criteria laid down in hygienic standards. Therewith, the proportion of samples that were noncompliant with sanitary and hygienic requirements (total viable count and coliforms) and safety criteria (detection of Listeria monocytogenes and Salmonella) increased from 1.63% in 2012 г. up to 14.89% in 2016. Noncompliance with sanitary and hygienic criteria indicates problems in sanitary control and HASPP system absence at pork- and porcine product-producing establishments. Analysis of porcine products monitoring with microbiological tests (detection Listeria monocytogenes and Salmonella bacteria) for 5 years demonstrated an increase in their contamination with pathogens: Salmonella spp. detection rate increased from 2% in 2012 up to 5.7% in 2016; L. monocytogenes contamination detection rate increased from 0.66% in 2012 up to 14.77% in 2016. Increase in pork contamination with Salmonella correlates with data on Salmonella epidemic situation in pigs in the Russian Federation (number of infected settlements and diseased animals also increased) whereas increase in Listeria contamination does not correlate with data on Listeria freedom of the RF pig establishments.

EFFECT OF PLUCK SET REMOVAL TECHNIQUES DURING SLAUGHTER ON PIG CARCASS CONTAMINATION WITH HYGIENE INDICATOR BACTERIA, ESBL/AMPC-PRODUCING *E. COLI*, *SALMONELLA* AND *YERSINIA ENTEROCOLITICA*

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Introduction

Pigs are asymptomatic carriers of pathogenic and antibiotic resistant bacteria, which may contaminate pig carcasses during slaughter [1]. Especially opening the oral cavity during pluck set (i.e. lungs, heart, liver, and tongue) removal is a potential risk for spreading bacteria over the carcass [2, 3].

Purpose

The aim of this research was to compare carcass contamination between pigs of which the pluck set was removed following standard procedures and pigs of which the pluck set was alternatively removed (leaving tongue and highly contaminated tonsils inside the unopened oral cavity).

Methods

From each of 12 pig batches, 20 carcasses (ten slaughtered normally and ten slaughtered alternatively) were sampled after pluck set removal by swabbing the elbow, throat and sternum (100 cm² each) in two Belgian slaughterhouses. Samples were analyzed to quantify total aerobic bacteria, *Enterobacteriaceae* and *E. coli* by direct plating on Plate Count Agar, Violet Red Bile Glucose agar and Tryptone Bile agar with X-Glucuronide, respectively. The presence of ESBL/AmpC-producing *E. coli* was investigated by plating on Tryptone Bile agar with X-Glucuronide supplemented with cefotaxime. Further, qualitative analysis was performed for *Salmonella* using pre-enrichment in Buffered Peptone Water, enrichment on Modified Semi-solid Rappaport Vassiliadis and plating on Xylose Lysine Deoxycholate agar. *Yersinia enterocolitica* was isolated after enrichment on Phosphate Buffered Saline medium, KOH treatment and plating on Cefsulodin Irgasan Novobiocin agar.

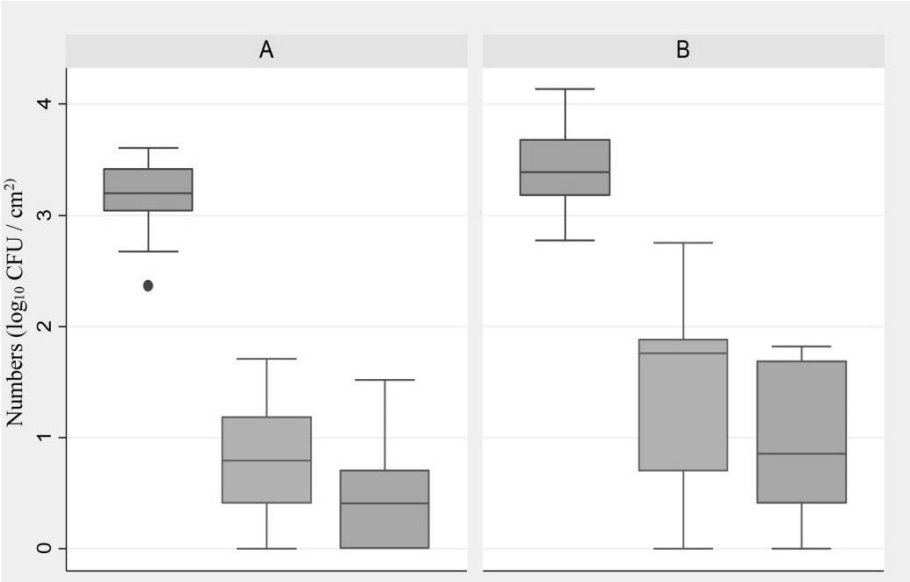

Figure 1. Quantitative analysis of hygiene indicator bacteria on the sternum.

Comparison between 10 alternatively slaughtered pigs (A) and 10 pigs slaughtered according to standard procedures (B), all originating from the same batch. Total aerobic count = blue ; *Enterobacteriaceae* = red; *E. coli* = green.

Results

Average total aerobic counts for throat samples ranged between batches from 2.1 to 3.8 log10 CFU/cm^2 with mean reductions up to 0.6 log10 CFU/cm^2 when using the alternative method. Median throat *Enterobacteriaceae* and *E. coli* numbers varied between batches from 0.6 to 2.8 log10 CFU/cm^2 and 0.4 to 2.3 log10 CFU/cm^2, respectively, with maximal mean reductions of 1.0 log10 when applying alternative pluck set removal. The proportions of *Salmonella* and *Y. enterocolitica* positive throat samples were equal for both slaughtering methods and pathogens (1.7%). The presence of ESBL/AmpC-producing *E. coli* on throat samples diverged from 5% (normally slaughtered) to 14% (alternatively slaughtered). Similar results were seen for other carcass areas.

Conclusion

The alternative pluck set removal method, requiring only minimal adaptations in the slaughterhouse, may contribute to improve the microbial quality of pig carcasses.

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SENSITIVITY OF SELECTED ORGAN DISSECTION TO DIAGNOSE *TAENIA SOLIUM* CYSTICERCOSIS IN PIGS FROM ENDEMIC AREAS

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Introduction

Taenia solium, also known as the pork tapeworm, is a neglected zoonotic parasite which is endemic in many developing countries, including Zambia. The tapeworm causes two disease conditions in humans: (1) taeniosis, which is the intestinal tapeworm infection, obtained after consumtion of raw/undercooked infected pork; and (2) cysticercosis, which is the metacestode larval stage infection, obtained after ingestion of tapeworm eggs. A human tapeworm carrier can excrete high numbers of eggs with the stool (100 000 eggs per day) and is thus an important source of environmental contamination. The transmission of cysticercosis is thus enhanced with poor sanitation and the lack of clean drinking water. After ingestion of the eggs, oncospheres hatch in the intestine and disseminate to several body tissues, including the central nervous system. Infection of the central nervous system with cysticerci is called neurocysticercosis, which is a major cause of acquired epilepsy worldwide.

Pigs are the intermediate host of *T. solium* and may get cysticercosis after ingestion of human stool infected with *T. solium* eggs from a tapeworm carrier. In pigs, cysticerci are primarily located in muscle tissues and the brain. Full carcass dissection is considered as the gold standard diagnostic method to detect cysticerci in pigs. Full carcass dissection additionally provides information on cysticercus abundance, status, and distribution in different organs and muscles, but has the drawback of being time-consuming, laborious and costly. Therefore, the aim of this study was to evaluate the effectiveness of alternatives to full carcass dissection, the alternatives being (1) the dissection of a selected number of organs/muscle groups, (2) tongue palpation, and (3) serological detection by Ag-ELISA.

Material and methods

Sixty-eight randomly selected pigs of slaughter age were fully dissected in three villages in Katete District (Eastern Province of Zambia). Muscle tissues/organs were excised from one carcass half of each pig, together with the complete head, heart, tongue, neck, diaphragm, psoas muscles, spleen, kidneys, lungs, liver, eyes and brain.

Slices of maximum 0.5 cm thick were made and cysticerci were enumerated. Cysticercus counts and stages (viable, degenerated or calcified) were recorded for each location. Cysticercus counts for the muscles of the half carcass were doubled and added to the counts of the different organs to obtain the total number of cysticerci per carcass. If no cysticerci were found in the first half of the carcass, then the other half of the carcass was dissected. Collected cysticerci were identified morphologically and confirmed using PCR-RFLP.

Tongue examination/palpation was performed before slaughter. Pigs were considered positive for cysticercosis if cyst-like nodules were either seen or felt after gently pulling the tongue with a mutton cloth.

Blood was collected immediately after exanguation and serum samples were stored at -20°C until analysis. All sera were analysed with the B158/B60 antigen detecting ELISA (Ag-ELISA).

Results

Carcass dissections

In total, *T. solium* cysticerci were detected in 38 pigs (56%; 95% confidence interval (CI): 44-67%). In 42% of positive carcasses, less than 10 cysticerci were detected, whereas 8 carcasses (21%) had over 100 cysticerci. Viable *T. solium* cysticerci were found in 22 carcasses.

Table 1. Effectiveness of selected organ/tissue dissection for the detection of T. solium cysticercosis in 38 *T. solium* infected pigs. The numbers represent the number of *T. solium* positive pigs per organ/muscle group and all possible 2x2 combinations.

	Heart	Tongue	Diaphragm	Masseter	Psoas
Heart	14				
Tongue	21	17			
Diaphragm	22	25	17		
Masseter	19	21	21	13	
Psoas	16	19	19	16	10

When considering different predilection sites, cysticerci were mostly found in the diaphragm, and tongue, followed by the heart, masseter, and psoas muscle (Table 1). When only dissecting the diaphragm or tongue, 17 out of 38 *T. solium* positive animals (45%; 95% CI: 29-61%) would be detected. If only two of these selected tissues would be dissected, the highest sensitivity was observed when combining tongue and diaphragm, which were able to detect 25 out of 38 positive pigs (66%; 95% CI 49-80%). This combination lead to the detection of 17 out of 22 pigs that was infected with more than ten cysticerci (77%; 95% CI 55-92%; Table 2). The combination of heart, diaphragm and tongue was able to detect 27 out of 38 *T. solium* positive animals (71%; 95% CI 54-85%) and 18 out of 22 pigs with more than ten *T. solium* cysticerci (82%; 95% CI 60-95%).

Table 2. Effectiveness of selected organ/tissue dissection for the detection of *T. solium* cysticercosis in 22 *T. solium* infected pigs with more than 10 cysticerci. The numbers represent the number of *T. solium* positive pigs per organ/muscle group and all possible 2x2 combinations.

	Heart	**Tongue**	**Diaphragm**	**Masseter**	**Psoas**
Heart	12				
Tongue	15	13			
Diaphragm	16	17	12		
Masseter	16	16	16	12	
Psoas	14	15	14	15	10

Tongue palpation

Only four cysticercosis positive pigs (4/38, 11% [95% CI 4-26%]) were detected using *ante mortem* tongue palpation. All these pigs had viable cysts, and three out of four tongue palpation positive pigs had more than 10 viable cysts.

Serology

A total of 36 pigs (53%) tested positive for *T. solium* cysticerci circulating antigens by Ag-ELISA. Using full carcass dissection as reference, the sensitivity and specificity of the Ag-ELISA for the detection of cysticercosis was 68% and 67%, respectively. The sensitivity of the Ag-ELISA reached 91% for detection of viable cysts only. Moreover, the ELISA detected all pigs that had 10 or more viable cysts (n = 13).

Conclusions

The present study shows that dissection of a selected number of organs underestimates the total number of *T. solium* positive pigs. When dissecting only two organs or tissues instead of the whole carcass, the highest sensitivity was obtained when using diaphragm and tongue. However, the sensitivity when combining both dissection results was only 66%. As the combination of heart, diaphragm and tongue was able to detect 82% of pigs with more than ten cysticerci, the combination of these three tissues might have an acceptable sensitivity to detect these pigs that represent a larger risk for public health.

As demonstrated before, tongue palpation was shown to have a very low sensitivity for detecting porcine cysticercosis. In contrast, the sensitivity of Ag-ELISA on serum samples to detect pigs with viable cysts was high.

REPORTS OF BRAZILIAN FEDERAL MEAT INSPECTION SYSTEM IN SWINE SLAUGHTERHOUSES

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Abstract

In the last decades the pig production improvement had impacted on zoonotic profile attributed to pork. This fact has created a need to modernize the meat inspection system traditionally applied, driving the focus to risks that really threaten food safety nowadays. This modernization is a trend in meat producer countries and European Union is ahead in this process. In this way, Brazil is assessing the new systems and building an own proposal which has been conducted under a national project linked by many coordinated actions. The first step of this work is to analyze the data of current inspection system. Then, the aim of this study is to evaluate the carcasses and viscera disposition reported by Brazilian Federal Meat Inspection. The analyzed database encompasses the reports of 114 slaughterhouses recorded from 2012 to 2014 into SIGSIF platform. The results were summarized by descriptive statistics on tables and figures using the SAS software. It was possible to show that the major "*post mortem*" causes of carcass/viscera trimming or condemnation were resulted from production diseases, as adherences (3.72%), pleurisy (0.85%), abscess (0.58%) and pneumonia (0.20%). Likewise for industrial process problems, as carcass contamination by evisceration leaking (1.8%) and traumatic lesions (1.57%). Zoonosis injuries condemnations were reported in very low frequency in a few slaughterhouses, usually once. Among the total of organs and carcass inspected, cysticercosis was registered in just 0.00092% in 25 slaughterhouses, endocarditis in 0.00193% (23 slaughterhouses), erysipelas in 0.0045% (38 slaughterhouses), sarcosporidiosis in 0.00051% (17 slaughterhouses) and tuberculosis in 0.000046% (12 slaughterhouses). Thus, the current meat inspection system is prepared to detect zoonosis that no longer occur or happen in very low frequency. In the other hand, the traditional *ante* and *post mortem* inspection methodologies are not able to detect the main current foodborne pathogens globally distributed as *Salmonella*.

Introduction

In the last decades the pig production improvement had impacted on zoonotic profile attributed to pork. This fact has created a need to modernize the meat inspection system traditionally applied, driving the focus to risks that really threaten food safety nowadays. This modernization is a trend in meat producer countries and European Union is ahead in this process.

In this way, Brazil is assessing the new systems and building an own proposal which has been conducted under a national project linked by many coordinated actions. The first step of this work is to analyze the data of current inspection system.

Then, the aim of this study is to evaluate the carcasses and viscera disposition reported by Brazilian Federal Meat Inspection.

Material and methods

Brazilian Federal Meat Inspection maintains and manages an Information System named SIGSIF, which allows recording a wide variety of data about slaughter, condemnation and trimming. Initially, data from 117 Federal Inspected swine slaughterhouses, with approximately 97.2 million pigs slaughtered from 2012 to 2014 were obtained from two Excel spreadsheets extracted from SIGSIF platform. One of them contained the data of total monthly slaughter by species and animal category and another containing the causes of condemnation or trimming per inspection line and respective quantities.

SAS software (2012) was used to make an exploratory statistical analysis aiming to evaluate the data consistence and to ensure the results robustness. Assisted by Federal Meat Inspection Service the analysis has started detecting and correcting (if possible) inconsistencies in the database, if the correction was not possible the data were discarded.

After the initial exploratory analysis the database encompasses the reports of 114 slaughterhouses (94,262,328 slaughtered pigs) recorded from 2012 to 2014 into SIGSIF platform. The results were summarized by descriptive statistics on tables and figures using the SAS software.

Results and discussion

The Brazilian condemnation/trimming in pigs slaughter for all causes is depicted in Figure 1 that shows the major percentages of condemnation/trimming, for example: 31.53% of the lungs, 15.24% of livers, 14.44% of kidneys and 10.2% of carcass. Contamination by evisceration leaking and other sources was one of the most important causes of condemnation/trimming, representing more than 60% of the causes for spleen, head, stomach, intestine and tongue. For carcass this cause represents 1.797% of the total of slaughtered pigs and approximately 18% of the all causes of condemnation/ trimming.

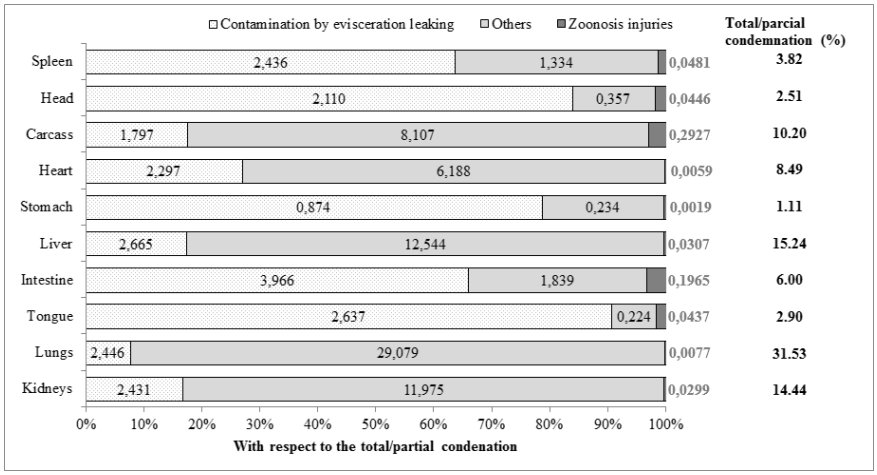

Figure 1. Percentage of total/partial condemnation by all causes and the contribution of contamination by evisceration leaking and zoonosis lesions in function to the body part.

By the other hand, zoonosis associated condemnations were reported in very low frequency, in a few slaughterhouses, usually once. Lymphadenitis is an exception in this context and represents the majority of this kind of condemnation, with 0.81% of the total of organs and carcass inspected (99% of all zoonosis associated lesions recorded), and 0.29% of the carcasses (Table 1). Among the total of organs and carcass inspected in others zoonosis associated lesions, erysipelas was recorded in just 0.0045% (38 slaughterhouses), endocarditis in 0.00193% (23 slaughterhouses), cysticercosis in 0.00092% (25 slaughterhouses), sarcosporidiosis in 0.00051% (17 slaughterhouses) and tuberculosis in 0.000046% (12 slaughterhouses).

It was possible to show that the major "*post mortem*" causes of carcass/viscera trimming or condemnation were resulted from production diseases, as adherences (3.72%), pleurisy (0.85%), abscess (0.58%) and pneumonia (0.20%). Likewise for industrial process problems, as carcass contamination by evisceration leaking (1.8%) and traumatic lesions (1.57%) (Figure 2).

Table 1. Quantity of zoonosis associated lesions condemnations reported among the total of organs and carcass inspected.

Zoonosis	Total condemnation	Number of positive slaughterhouses	Main body part condemned	Total condemnation on main body part
Cysticercosis	869 (0.00092%)	25	Heart	668 (0.00071%)
Endocarditis	1,815 (0.00193%)	23	Carcass	837 (0.00089%)
Erysipelas	4,269 (0.0045%)	38	Carcass	808 (0.00086%)
Lymphadenitis	760,643 (0.8069%)	75	Carcass	273,686 (0.2903%)
Sarcosporidiosis	482 (0.00051%)	17	Carcass	476 (0.00051%)
Tuberculosis	43 (0.000046%)	12	Carcass	22 (0.000022%)

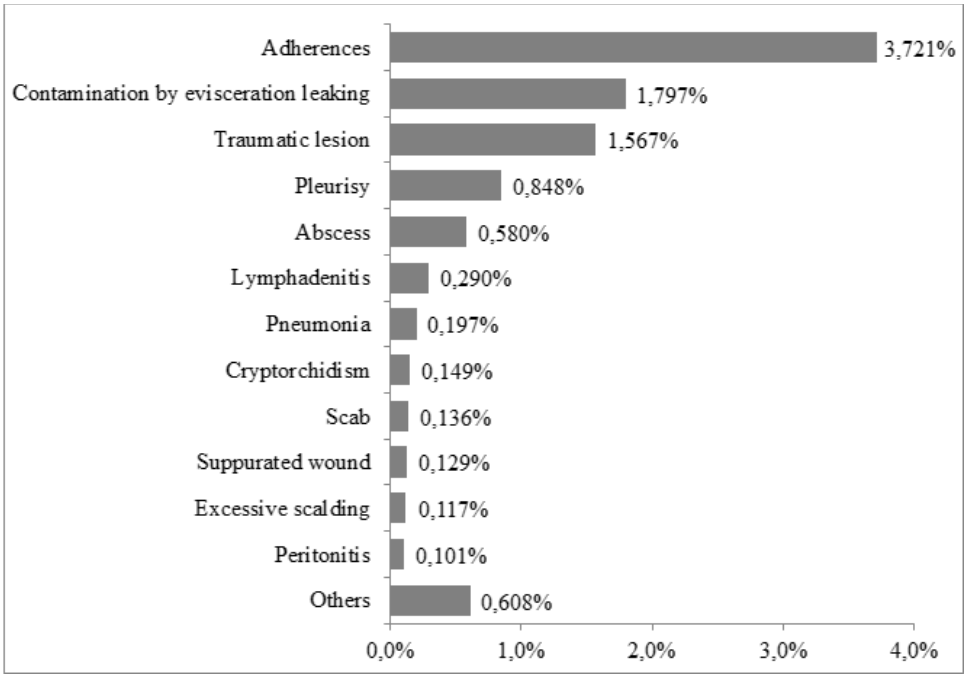

Figure 2. Main causes of carcass condemnations/trimming in pigs slaughtered in the Brazil from 2012 and 2014.

Back to the forties, Ribeiro (1951) reported that the principal cause of condemnation was tuberculosis follow by cysticercosis, that produce lesions easily detectable by the meat inspection service. This dramatic decline in both diagnosis to negligenciable level is a consist result of all systematic improvement in the Brazilian pig production chain. The worldwide evolution in biosecurity and good practices in pig farming has shifted the zoonotic profile attributed to pork. In face of that is necessary to adapt the meat inspection procedures to allow the detection of the current hazards in order to mitigate them.

Brazilian results are comparable to those obtained in Europe Community that, after an extensive study, changed the meat inspection procedures in 2014 (Official Journal of the European Union, 2014). EFSA (2011) had purposed that "risk reduction measures at slaughterhouse level are focused on prevention of microbial contamination through technology- and process hygiene-based measures (Good Manufacturing Practices (GMP)/Good Hygienic Practices (GHP) and Hazard Analysis and Critical Control Points (HACCP)), including omitting palpation/incision during post-mortem inspection in routine slaughter, as well as hazard reduction/inactivation meat treatments if necessary. At farm level, risk reduction measures are based on herd health programs, closed breeding pyramids and Hygienic Practices and Good Framing Practices".

Therefore, considering the role of meat business for Brazil is urgent to modernize many aspects of meat inspection system applied nowadays. These results are the base for a risk assessment study in order to identify the most relevant biological hazards in the context of Brazilian pork inspection. Wide studies nationally coordinated should be conducted to found out the real risks associated to specific country features. Differences in food animal production chain organization may result in distinct hazards. Furthermore, is essential to specify the production conditions that the modernized inspection procedures will be applied.

Conclusion

The current meat inspection system is not able to detect the main current foodborne pathogens globally distributed as *Salmonella.* Although the traditional *ante* and *post mortem* inspection methodologies was presented some effectiveness in detect zoonosis that happen in very low frequency, some of these procedures are intended to search for diseases that no longer occurs.

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