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Ontario Swine Research Review 2006

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Food Safety and Pork Quality

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Prevalence of *Salmonella* in Different Age Groups of Pigs

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Background

Salmonellosis is the most important food safety issue associated with pork in North America. Most studies have examined the level of infection at slaughter or if they have evaluated prevalence at the farm they have concentrated their efforts on market weight animals. In previous studies we have shown that approximately 40% of finisher herds test positive for Salmonella, and the prevalence is often high in herds that one might least expect. It is possible that in herds with very good hygiene procedures pigs become infected at a later stage than in herds with less stringent sanitation. Knowing when pigs become infected and when they are most likely to be shedding bacteria has a great impact on designing control and monitoring programs.

Objectives

The objective of this study was to determine if the prevalence of *Salmonella* changes with age through the grower-finisher period.

Results

Of 20 farms tested, all had finisher pigs on-site and 17 also had nursery pigs. Fourteen farms tested positive for *Salmonella* in the finisher barn. Eleven farms tested positive in the nursery and 10/17 tested positive in both the nursery and finisher phase. Increasing pig size resulted in a decreased risk of *Salmonella* and increasing the number of pigs per pen increased the risk of *Salmonella*.

Take Home Message

The highest shedding of *Salmonella* occurred at the end of the nursery phase and there was a gradual decrease in the prevalence of *Salmonella* throughout the finisher period. These results suggest that on most farms pigs are entering the grow-finish barn already carrying Salmonella so that thorough clean-up and tough biosecurity procedures at the grower stage will not be effective in reducing the problem on these farms. This means that intervention strategies for *Salmonella* should concentrate on the young grower pig or the nursery pig. It also reveals that when monitoring a population for the presence of *Salmonella,* sampling animals close to slaughter weight may underestimate the prevalence at the farm level and within a given farm.

Financial support was provided by the Ontario Ministry of Agriculture Food and Rural Affairs Food Safety Program, the University of Guelph-OMAFRA Animal Research Program and Ontario Pork.

Antimicrobial Resistance to *Salmonella* in Ontario Swine Herds

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Background

Salmonella continues to be an important foodborne pathogen in Canada and the USA, particularly serovars demonstrating multiple antimicrobial resistance (AMR). A major concern associated with *Salmonella* prevalence on pig farms is the increasing incidence of multi-drug resistant serovars, particularly *Salmonella* Typhimurium DT104. Surveillance of bacterial resistance in swine is important in understanding the relative significance of this source of bacteria to the human population.

Objective

To describe antimicrobial resistance patterns of *Salmonella* spp. isolated from healthy pigs on Ontario farms.

Results

Thirty-eight (23.2%) of the isolates (16 farms) had no antimicrobial resistance. Of 164 isolates, 126 (76.8%) of those were resistant to 1 to 11 of the remaining antimicrobials including tetracycline (T) (65.2%), streptomycin (S) (65.2%), sulfisoxazole (Su) (64%), ampicillin (A) (61%), spectinomycin (Sp) (59.1%), chloramphenicol (C) (58%), florfenicol (57.3%), amoxicillin and clavulanic acid (Ac) (55.5%), kanamycin (K) (14%), neomycin (N) (14%), nitrofurantoin (Nit) (1.2%). Resistance to these antimicrobals at the farm level is shown in figure 1.

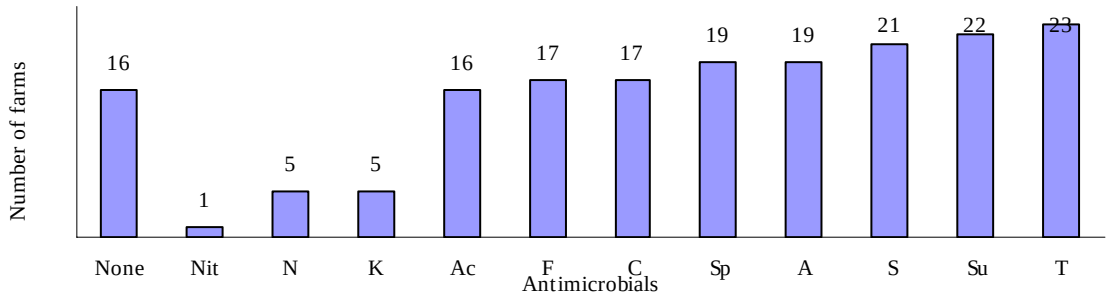

Figure 1: Distribution of resistance of *Salmonella* spp. on 37 Ontario swine farms.

Take Home Message

Salmonella are present on many farms and in some cases may not pose a problem but the industry should pay particular attention to the presence of multiple drug resistant *Salmonella* Typhimurium as this organism is a serious public health risk.

Financial support was provided from Ontario Pork, OMAFRA Food Safety Program, and the Public Health Agency of Canada.

Ontario Data Analysis Project – Ten Years of Benchmarking

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Background

Results from the Ontario Data Analysis Project (ODAP) have been used as a means of benchmarking in the swine industry. This is a compilation of farm level production and financial data from Ontario farrow-to-finish producers. These participants are full-time farmers and most rely on family members to fill additional labour needs.

Objectives

- Overview of Ontario hog farm production and financial performance
- Assess individual benchmarks to determine if they suggest profitability at the farm level

Results

i) Benchmarking

- Productivity and financial improvements have been achieved over time

	1995	2004
Average # of Sows	133	217
Weaned/litter	9.1	9.6
Finishing Weight (kg live)	104.2	113.6
Days to Market	177	166
Pork/sow (kg)	1420	1766
Farm assets/sow	$7,555	$12,554
Farm equity/sow	$4,791	$8,379

ii) Profitability at the farm level

- A combination of factors affect financial success
- Separating farms on a profit/pig produced basis showed that high profit/pig farms reported higher revenue and lower expenses per pig. This resulted in $24.41/pig additional profit for the high profit group.

	Profit Group		
	High	Low	Difference
Revenue/pig produced	$158.77	$148.59	$10.18
Expenses/pig produced	$126.66	$140.89	($14.23)
Profit/pig produced	$32.11	$7.70	$24.41

Take Home Message

- Farms are larger and more productive in 2004 compared to 1995
- Farm size is not an indicator of financial success
- Cost control and revenue optimization are important drivers of profitability

Special thanks to Agriculture & Agri-Food Canada for funding this project. Recognition and appreciation is extended to the farms that participated.

Effect of Group Gestational Housing on Sow Fertility

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Background

The most common housing system for breeding and gestating sows in North America is individual stalls, which allow for ease of mating and for individual feeding as well as lower space requirements per sow. However, there is a public perception that sow gestation crates are not "welfare-friendly" and a future consumer demand may be that pregnant sows be housed in groups. There is some concern that grouping of sows after breeding can result in reduced fertility because of the stress of fighting when sows are mixed.

Objectives

To determine if the day post-breeding of grouping sows has an effect on fertility as measured by farrowing rate and litter size.

Results

Table 1**:** Reproductive performance of sows when mixed at various stages of gestation. Control sows were housed in gestation stalls for the duration of pregnancy.

Mix day post-breeding	Pregnancy rate (%)	Farrowing rate (%)	Total litter size
Controls	103/126 (81.8)	74/93 (79.6)	11.31 ±3.54
2	69/86 (80.2)	53/67 (79.1)	11.06 ±3.68
7	67/83 (80.7)	51/64(79.7)	11.24 ±3.42
14	67/89 (75.3)	53/71 (74.7)	11.23 ±3.33
21	73/88 (82.9)	57/70 (81.4)	11.19 ±3.42
28	70/85 (82.4)	52/67 (77.6)	11.13 ±2.66
Total	449/557 (80.6)	340/432 (78.7)	11.20 ±3.35

Take Home Message

There was a tendency for lower fertility when sows were mixed at 14 days post-breeding but this is not a statistically significant difference. This difference might be amplified if overcrowding or other stresses are imposed at the time of mixing.

Support was received from the University of Guelph-OMAFRA Animal Research Program and the National Pork Producers Council.

Do Boars Need Higher Levels of Certain Nutrients?

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Background

Boar nutrition has long been neglected but with the emergence of swine AI studs, boar health and fertility has become extremely important. There is limited research regarding the impact of increased levels of micronutrients in boar rations but there is general acceptance in the industry of nutrient rich supplements for working boars with the hope that these vitamin and mineral packages will improve boar performance and result in better quality semen.

Objective

To determine if boars being fed a top dress of vitamins, minerals and fatty acids would produce better semen than boars being fed a standard dry sow ration.

Results

The treatment group (15 boars) received 200g of PRO 22:SIX® (a vitamin and mineral top-dress) supplement in addition to their regular ration three days a week. PRO 22:SIX® contained: crude protein 26%, crude fat 13.5%, crude fiber 3.9%, calcium1.8%, phosphorus 1.3%, selenium 0.3 mg/kg vitamin A 64000 lU/kg, vitamin D 4850 lU/kg, vitamin E 3078 lU/kg. Boars were collected on a weekly basis and semen assessed using a Computer-Assisted Semen Analysis (CASA) procedure. No differences in semen volume, concentration, motility or abnormalities were found between the treatment group and the control group. It was beyond the scope of this trial to determine if fertility was enhanced by the treatment. This would have necessitated a large breeding trial.

Take Home Message

Based on semen analysis there did not appear to be an advantage to supplementing a standard ration with extra micronutrients.

Support was provided by East-Man Feeds, the University of Guelph-OMAFRA Animal Research Program and Minitube, Canada.

Sperm-mediated gene transfer (SMGT)

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Background

Understanding the function of genes require ability to study their expression not only in vitro but in the whole animal. While production of transgenic mice is a routine approach and used extensively to understand function of novel genes, the similar technology is too expensive and labour intensive to use routinely in the livestock. Sperm mediated gene transfer (SMGT) is an alternative technique using natural ability of spermatozoa as a vector to transfer DNA into the oocytes. However, this, cheap, easy and highly efficient (30-80% in pigs) technique is very controversial and have not yet been replicated. If reproducibility of SMGT can be increased it would be an ideal method for adding transgenes to the genomes of pigs. Various aspects of the interaction of sperm with exogenous DNA need to be better understood to improve efficiency of this method.

Objectives

The objectives of this study are to better understand factors affecting efficiency of sperm DNA interaction and to develop effective methods for selection of high quality donor boars for SMGT.

Methods and procedures

Sperm was collected from 20 boars and evaluated for sperm quality, DNA binding ability, motility (CASA) and viability (PI/ SYBR 14) after DNA binding. DNA treated semen was inseminated using intrauterine artificial insemination method into synchronized sows (Yorkshire breed) and oocytes were recovered after 2 days through embryo flushing using 54 sows and 3 donor boars.

Results

- The percentage of motile and progressive motile sperm were drastically decreased after 24 hr incubation with DNA in all 20 boars ($p<0.05$) compare to viability (Table 1). While this seems to confirm our initial hypothesis, further studies demonstrated that the cause of decrease motility was washing step, which is used to remove inhibitory semen factors.

Table 1. Average of motile sperm and progressive motile sperm after incubation with DNA

Parameters	Motile sperm		Progressive motile sperm		Viability
Treatments	DNA/washing	control	DNA/washing	control	DNA/washing
Percentage (%)	86.46	46.59	67.72	14.25	73.49

- From embryo experiment, it was found that although DNA treated demonstrates decreased motility, exogenous DNA binding to sperm does not affect fertilization. However, no GFP transgenic embryos were detected which indicates that despite unaffected ability of sperm to fertilize, the DNA is not delivered to oocyte (Table 2).

Take Home Messages: While DNA treatments of sperm decreased sperm motility and progressive motility, it seems to be caused by washing step, and it does not inhibit production of fertilized oocytes. Further studies, would allow to better understand natural sperm barriers against foreign DNA transfer and to improve reproducibility of SMGT.

Financial support from NSERC-CRD, OMAF and Ontario Pork is appreciated.

Developing Gene Silencing Technology for Pigs

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Background

One of the major goals of functional genomics is to understand the function of genes. One of the ways to achieve this is to up/down regulate the expression of the gene and examine the resulting phenotype. RNA interference is a recent and very promising technique by which genes can be efficiently down regulated. Successful applications of RNAi have been demonstrated mainly in human and mice but could be valuable in studying the function of genes and producing economically beneficial phenotypes in livestock. The presence and efficiency of RNAi in porcine cells was tested by attempting to silence endogenous myostatin. Loss-of-function of myostatin leads to 20% increase in lean meat as in double-muscled breeds of cattle. It is anticipated that this phenotype can be generated in pigs.

Objective

- To determine whether RNAi is present in porcine cells.
- To evaluate possible side effects associated with RNAi.

Methods

Five short hairpin RNA constructs (shRNAs) were introduced into porcine fibroblast cells to determine their efficiencies in silencing endogenous myostatin. Real Time RT-PCR was used to compare the relative expression of myostatin in targeted cells to cells containing the scrambled shRNA control in which myostatin levels should not be affected. The expression of OAS1, an interferon gene, was examined in the targeted, control and mock-transfected cells to determine whether induction of the interferon response, a known side effect of RNAi, occurred.

Results

Highly efficient silencing (up to 97%) of myostatin mRNA was detected, with various efficiencies among different constructs. Unexpectedly, all shRNA constructs induced interferon response, which upon further examination was determined to be generated by plasmid backbone, and seemingly not related to shRNA expression or silencing of myostatin. Methylating CpG islands in the plasmid appear to decrease the level of interferon induction.

Take home message

These results demonstrate, for the first time, that highly efficient RNAi in porcine cells can be used to silence endogenous myostatin. This knowledge could be valuable for studying the function of other genes in the porcine genome and to produce economically beneficial double muscled phenotypes *in vivo*. However, further work must be performed to improve efficiency and decrease undesirable side effects.

This work was supported by Ontario Pork, OMAF and NSERC CRD.

The Effects of Compensatory Growth on Production Efficiency, Carcass Traits, and Pork Quality

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Background
There is evidence (Therkildsen et al., 2002) that restrictive feeding in the growing phase can stimulate compensatory gain during the finishing period while improving feed utilization, efficiency of protein synthesis and tenderness.

Objectives:
This trial was designed to further investigate the performance and economic ramifications of limit feeding during the growing phase. The effects of feeding strategy (full versus limit feeding in the growing phase) and gender (gilt versus barrow) on growth performance (average daily gain, feed intake and feed efficiency), carcass and meat quality and economic returns are being investigated.

Experimental Procedures:
One hundred and eight feeder pigs (30 kg - average weight) were randomly assigned to eighteen pens with six pigs per pen. A specific gender (gilt or barrow) and feeding strategy was assigned to each pen to provide three replications (pens) of each treatment combination. Control pens were fed a grower diet *ad libitum* until the pigs were marketed at 110 kilograms BW. A second group of pigs (six pens) were limited to 85% of control feed intake until 60 kg followed by *ad libitum* feeding until market while the third group was limited to 70% of control intake during the growing phase.

Results to date:

1) Daily gains (kg) were similar ($P \geq 0.50$) for each feeding strategy (full, 85% and 70%) with similar ($P > 0.25$) days to market.
2) Total feed intake was 7.5% less for 70% restricted group (versus control) with an improved F/G (2.6 vs. 2.4; SE=0.03; P=0.0001).
3) Evaluations of meat quality characteristics are in progress.

Reference:
Therkildsen, M., B. Riis, A. Karlsson, P. Ertbjerg, P. P. Purslow, M. Dall Aaslyng, and N. Oksbjerg. 2002. Compensatory growth response in pigs, muscle protein turn-over and meat texture: effects of restriction/realimentation period. Anim. Sci. 75:367-377.

The authors would like to thank Ontario Pork and the Ontario Ministry of Agriculture, Food and Rural Affairs (OMAFRA) for their financial support of this project.

The Use of Distiller's Dried Grains with Solubles (DDGS) in Feeder Pig Diets

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Background

The use of DDGS, as an alternative feed ingredient in pig diets, has been successfully investigated in the United States. However, very little research has been completed in Ontario using product generated from Commercial Alcohols Inc.'s plant in Chatham. Since variation in product quality can exist from one plant to the next, feeding trial initiatives were needed to quantify the economic ramifications of feeding DDGS (Chatham) to swine herds in Ontario.

Objective

The project evaluated the effects of feeding Chatham co-product (DDGS) to pigs based on measurements of growth, feed intake, economic returns and carcass quality.

Experimental Procedures

After a three week adjustment period, ninety-six pigs (33.2 ± 5.8 kg) began the trial on July 13th, 2004. Each pen (3 barrows and 3 gilts) was randomly assigned to one of three grower diets until they were 70 kilograms body weight (BW). They were then fed an assigned finisher diet until they were marketed ($\geq$ 110 kg BW) by pen.

The following dietary treatments were formulated and fed:

1) Grain corn, SBM and premix. A grower diet (0.83 % lysine) was fed until the pigs were 70 kg (per pen) followed by a finisher diet (0.69 % lysine) until they were marketed.
2) Similar diets and feeding strategy to control group. However a 10 percent inclusion rate of DDGS was added to replace some of the SBM as a protein source. To achieve similar levels of lysine an increased protein (CP) level was needed in the grower (19.1%) and finisher (16.8%) diets.
3) Similar diets and feeding strategy to control group. However 20 percent DDGS was added to replace a greater amount of SBM as a protein source. An increased protein level was again needed in the grower (20.5%) and finisher (18.2%) diets to achieve the required lysine levels.

Results and take home message

- When diets were balanced to a constant lysine level (growing and finishing phase) – similar growth rate, feed intake and efficiency estimates were obtained for diets containing: 0, 10 or 20 percent DDGS.
- In this trial, the costs of gain were similar for each DDGS inclusion rate. However due to similar feed efficiencies, costs of gain were strongly related to ingredient costs. Therefore producers are advised to incorporate DDGS when this co-product is favorably priced relative to corn and soybean meal.

The authors would like to thank the Innovation and Risk Management Branch (OMAFRA), Commercial Alcohols Inc. and OMAFRA for their financial and technical assistance.

Creation of a Molecular Catalog for the Swine Parotid Gland using Omics Techniques

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Background

One of the major goals of functional genomics is the creation of catalogs of molecular parts for each tissue. Recent paradigm shift with wide spread use of Omics technologies in model organisms to create such molecular catalogs, have not yet been applied systematically in the livestock. Application of Omics technologies was tested with parotid glands to develop a data collection, analysis, and integration pipeline. It is well known of the important role that saliva plays in reducing disease susceptibility, promoting overall oral health and improving feed digestibility. It has also been shown of the potential for porcine salivary glands to serve as models for human diseases such as Sjögren's Syndrome and radiation-induced salivary gland injury. To further our understanding in such areas, several researchers have sought to investigate the genes that are being expressed in salivary glands and proteins present in saliva. Unfortunately none of the studies to date have focused specifically on cataloguing which genes and proteins are being expressed in the parotid gland, the largest of the salivary glands.

Objectives

1) Identify the compliment of genes and proteins expressed in the swine parotid gland.
2) Integrate both sets of Omic data, and attempt to identify affected classes of molecules as well as biochemical pathways. Human homologs for porcine molecules will be gathered to increase the overall annotation of Omic datasets.

Methods

Transcriptome profiling has been performed using Affymetrix Porcine GeneChips (6 control vs. 6 transgenic), along with Real Time RT-PCR for validation. Proteomic profiling has been performed using Isotope Tagging for Relative and Absolute Quantification (iTRAQ) (4 controls vs. 4 transgenic).

	Identified
Transcripts	11,494
Proteins	395

Results

The genes and proteins have been classified into Gene Ontology (GO) categories (cellular component, biological process, and molecular function). We are currently integrating gene and protein data into a single database.

Take Home Message

- Affymetrix GeneChips and iTRAQ are excellent tools for the high-throughput identification of transcripts and proteins as well as the evaluation of differential expression.
- This is the first time such comprehensive catalog of genes and proteins has been produced for any porcine tissue. This type of molecular index will further our understanding of the parotid glands physiology, role saliva plays in reducing disease susceptibility, promoting overall oral health and feed digestion.

Our work is supported by the Advanced Foods and Materials Network.

Gene Defects that Increase Susceptibility of Pigs to Infectious Diseases

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Background

Innate resistance to various infections depends in part on the amounts and functions of several collagenous lectin proteins that bind to the surfaces of invading micro-organisms. These lectins, such as mannan-binding lectins (MBL), ficolins and lung surfactant proteins (SP), are especially important before development of specific immune responses. Several mutations in the human MBL gene are now known to affect resistance to various common infections. Previous work in our laboratory has demonstrated that MBL-A and ficolin alpha are important bacteria-binding proteins in the blood of pigs. Because susceptibility to disease varies widely among pigs exposed to a particular infection, we have looked for genetic defects that might explain impaired innate resistance to various infections in some young pigs.

Objectives

- Characterize the role of major collagenous lectins in resistance of young pigs to various economically important infections
- Identify gene defects that impair this resistance.

Results

The normal pig mbl-1 and mbl-2 genes supply the MBL-A and MBL-C proteins that are produced in the liver and circulate in the blood. A defect in the pig mbl-1 gene was discovered and a genetic test for this was developed. The mbl-1 defect was more frequently found in pigs culled with various common infections. Low MBL-C producers were more frequently sick. Several defects were identified in the pig mbl-2 gene, and some of these explain why liver production of MBL-C is highly variable among pigs. Several mutations were also discovered in the pig SP-A, ficolin alpha and ficolin beta genes, and their functional significance is being studied. Genetic tests are being developed to enable us to determine which of the MBL, ficolin and SP-A mutations have the most effect on disease resistance in homozygous or heterozygous carriers.

Take Home Message

A substantial proportion of pigs carry gene defects that might interfere with the function or supply of several innate resistance proteins. Genetic tests are being developed for determining the association between these defects and the severity of various economically important infections. Our tests might be used in marker-assisted selection programs to identify breeders that do not have the defects.

Financial support: Natural Sciences and Engineering Research Council of Canada (NSERC), NSERC Canadian Research Network on Bacterial Pathogens of Swine,

Ontario Ministry of Agriculture and Food, Ontario Pork, Ontario Veterinary College Graduate Fellowship and CIHR Fellowship.

Application and Field Validation of a PCR Assay for the Detection of *Mycoplasma hyopneumoniae* from Swine Lung Tissue Samples

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Background

M. hyopneumoniae cause enzootic pneumonia, which is one of the most common, economically significant diseases of pigs. Due to the slow growth of *M. hyopneumoniae* and the overgrowth of other mycoplasmas (e.g., *Mycoplasma hyorhinis*), culture isolation of this organism is difficult. Fluorescence antibody (FA) test has been the major antigen identification assay till PCR tests were developed recently.

Objective

To adapt, optimize and validate a PCR assay for the detection of *M. hyopneumoniae* in swine lung tissue.

Results

A PCR assay was optimized for the detection of *M. hyopneumoniae* in swine lung tissue. The detection limit of the assay was 0.18 cfu/g of lung sample spiked with *M. hyopneumoniae*. There was no non-specific reaction when testing other mycoplasma and bacteria.

In field validation, from February 2000 to October 2004, 426 pigs from 220 cases were examined for *M. hyopneumoniae* infection by *M. hyopneumoniae* PCR and FA test. At the specimen level, there were 103 pig lungs (24.2%) positive in the PCR test, 69 pig lungs (16.2%) positive in the FA test. At the case level, lungs from 66 cases (30%) were positive with the PCR test; 46 cases (20.9%) were positive with the FA test. Agreement between FAT and PCR was moderate (kappa was 0.654 at the specimen level and 0.668 at the case level). Bayesian modeling, a 'non-gold standard' method that incorporates prior scientific information, was used to determine the test sensitivities, specificities and prevalence and their uncertainties. The diagnostic sensitivity of the PCR was determined to be 97.3% and 97.0%, the diagnostic specificity 93.0% and 92.2% at the specimen and case level, respectively.

Take Home Message

A PCR assay has been optimized and validated for the detection of *M. hyopneumoniae* from lung tissue samples. The assay is sensitive and specific, and has been implemented in the routine diagnosis at the AHL.

Financial support was received from Ontario Pork and the Ontario Ministry of Agriculture, Food and Rural Affairs.

Rapid detection of *Brachyspira hyodysenteriae* and *Brachyspira pilosicoli* in porcine fecal and intestinal tissue samples using real-time PCR

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Background

The spirochetes *Brachyspira hyodysenteriae* and *Brachyspira pilosicoli* are the etiological agents of swine dysentery and swine intestinal spirochetosis, respectively. Traditional detection and identification of *B. hyodysenteriae* and *B. pilosicoli* is very difficult because of the fastidious nature and slow growth of these spirochetes, and it is difficult to distinguish among *B. hyodysenteriae*, *B. pilosicoli* and other non-pathogenic intestinal spirochetes such as *B. innocens*. In addition, there is little information on the prevalence of either spirochete in North American pig herds

Objectives

- To develop rapid real-time PCR assays for the detection of *B. hyodysenteriae* and *B. pilosicoli* directly from fecal and intestinal tissue samples.
- To determine the prevalence of *B. hyodysenteriae* and *B. pilosicoli* in fecal samples collected from Ontario swine herds and in fecal or intestinal tissues submitted to the Animal Health Laboratory (AHL) from cases of porcine enteric disease.

Results

- Two rapid real-time PCR assays were developed, which can detect *B. hyodysenteriae* and *B. pilosicoli* specifically from fecal and intestinal samples directly. The detection limit of the *B. hyodysenteriae* real-time PCR was 10^3 cells/0.2g of feces. The detection limit of *B. pilosicoli* real-time PCR was 10^4 cells/0.2g feces.
- In 2004, among 105 Ontario swine herds, one (0.9%) was positive for *B. hyodysenteriae* and two (1.9%) were positive for *B. pilosicoli* tested by the real-time PCR assays.
- In 2004, fecal or intestinal tissues submitted from 39 clinical cases of porcine enteric disease were tested using the real-time PCR assays. Two cases (5.1%) were positive for *B. hyodysenteriae*, and four (10.3%) for *B. pilosicoli*.

Take Home Message

- Rapid, sensitive and specific real-time PCR assays were developed for the detection of *B. hyodysenteriae* and *B. pilosicoli* directly from fecal and tissue samples, and the services are available at the AHL.
- The prevalence of *B. hyodysenteriae* and *B. pilosicoli* infection in Ontario swine herds is low.
- The prevalence of *B. hyodysenteriae* and *B. pilosicoli* infection in pigs with enteric disease is higher and should be considered in the differential diagnosis.

Financial support was received from Ontario Pork and the Ontario Ministry of Agriculture, Food and Rural Affairs. We thank Drs. Marie Archambault, Gaylan Josephson, John Prescott and Grant Maxie for technical advice.

Molecular Epidemiology of Antimicrobial Resistance in *Escherichia coli* from Pigs in Ontario

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Background

Antimicrobial resistance is a major problem in human and veterinary medicine but our understanding of its epidemiology is only partial. The phenomenon is complicated by the ability of bacteria to transmit resistance genes from one to another. In addition, resistance genes are frequently linked and transferred together between bacteria. Transfer of resistance genes can also occur between bacteria of the normal flora and bacterial pathogens. Therefore, molecular characterization of the genes responsible for antimicrobial resistance and the study of their distribution in bacterial populations is a key to a better understanding of antimicrobial resistance epidemiology.

Objectives

- To assess the distribution of resistance genes in *Escherichia coli* (normal flora and pathogens) from swine in Ontario.
- To identify possible associations between resistance genes, and between virulence (ability to cause disease) and antimicrobial resistance.

Results

Major differences exist in the distribution of resistance genes between *E. coli* from the normal flora and pathogenic *E. coli* from swine. Associations between antimicrobial resistance genes and between resistance and virulence genes are present in *E. coli* from swine. The most important associations are due to the co-location of resistance and virulence genes on single plasmids (extra-chromosomal genetic element potentially transferable from bacterium to another). Some tetracycline, sulfonamide, and spectinomycin/streptomycin resistance genes are linked together with toxin genes. The major chloramphenicol resistance gene still remaining in porcine *E. coli* is physically linked to other antimicrobial resistance genes and to virulence genes.

Take Home Message

Use of one antimicrobial agent may not only select for resistance to other antimicrobial agents (including to those not used anymore). It could potentially also select for virulence genes and therefore for pathogenic organisms.

Financial support was received from Ontario Pork, the Ontario ministry of Agriculture and Food, and the Public Health Agency of Canada.

Antimicrobial Resistance Testing of *Campylobacter coli* from Ontario pigs

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Background

Campylobacter infection is a very common cause of food borne illness in humans. Generally the disease is self-limiting but in severe cases antibiotics are used. The most commonly used types of drugs to treat human disease include fluoroquinolones and macrolides. *Campylobacter coli* are present in the feces of all pigs and do not appear to cause disease in pigs. There are various methods that can be used to measure resistance and it is generally unclear which type of test is best.

Objectives

To determine if antimicrobial resistance is present in *Campylobacter coli* isolated from Ontario pigs and whether an E-Test would give similar results to the standard agar dilution test.

Results

The overall prevalence of resistance to one or more antimicrobials among the isolates was 99%. Although multiple resistance was detected by both tests, none of the isolates were resistant to all of the 11 antimicrobials tested. The E-test and agar dilution method produced comparable results when testing antimicrobial susceptibility for most drugs, major discrepancies were observed when testing for nalidixic acid and ampicillin. There were almost no isolates that were resistant to fluoroquinolones.

Take Home Message

Campylobacteriosis is a disease of public health significance. Although the pork industry contributes to this problem in only a minor way, it is important that the *Campylobacter* isolates from pigs do not become resistant to the drugs commonly used to treat human infection. Fortunately at present there is almost no resistance to fluoroquinolones, which is in sharp contrast to reports from Europe where resistance has become common.

Financial support was received from OMAFRA, Ontario Pork and the University of Guelph-OMAFRA Animal Research Program. Veterinary Laboratory Services performed culturing and agar dilution testing.

Tetracycline Use and Selection of Virulent Enterotoxigenic *Escherichia coli* (ETEC)

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Background

A new O149 ETEC variant has recently emerged in swine in Ontario and is apparently associated with an increased severity of post-weaning diarrhea. A plasmid (pTENT2) carrying a tetracycline resistance gene and several virulence genes not previously known to occur in ETEC from pigs in Ontario is present in this new ETEC variant. This plasmid can be transferred at low frequency between laboratory strains of bacteria in the laboratory, but nothing is know about its transferability *in vivo*. It is likely that the use of tetracycline in swine may select for the persistence of the new ETEC variant. In addition, tetracycline may also select for other bacteria, which have received the pTENT2 plasmid from the new ETEC variant and therefore select for the spread of virulence genes in bacterial populations and for the creation of new potential pathogens.

Objectives

- Develop a multiplex PCR for rapid identification of the new virulent ETEC variant present in pigs in Ontario.
- Study the effect of the new combined virulence-resistance plasmid pTENT2 of ETEC on severity of post-weaning diarrhea in pigs.
- Study the effect of tetracycline use on persistence of the new virulent ETEC variant in pigs.

Results

A new multiplex PCR for the identification of pTENT2 positive ETEC has been developed and validated. We have demonstrated that the transfer of pTENT2 between wild-type *E. coli* strains is highly repressed *in vitro*. ETEC strains devoid of the pTENT2 plasmid have been prepared for comparative experiments on the role of pTENT2 in virulence *in vivo*. We are currently developing a real time PCR for the direct detection of O149 ETEC strains in clinical samples.

Take Home Message

New diagnostic tools for the rapid identification of the new porcine ETEC-variant recently emerged in Ontario are available. Further tools are under development for its direct detection in clinical samples.

Financial support was received from Ontario Pork, the Ontario ministry of Agriculture and Food.

The Increase in Prevalence of a New Strain of Swine Influenza in Ontario

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Background

Previous studies of Canadian pig farms have determined that swine influenza subtype H1N1 is widespread but other subtypes have been only sporadically reported. A study of 80 herds in Ontario in 2003 found no serological evidence of subtype H3N2. In the spring of 2005 acute outbreaks of swine influenza occurred in Ontario and subtype H3N2 was isolated from some of the affected herds.

Objectives

To determine the serological prevalence of swine influenza virus H3N2 in the Ontario pig population 6 months after the initial reports of outbreaks and to determine if there was serological evidence that the disease had existed in the province prior to the spring outbreak of clinical disease.

Results

Fifty farms were studied in 2005, however, sera from the previous year was only available for 47 of these farms. In 2004, 11 of 47 herds (23%) were classified as positive for swine influenza H1N1 and 3 herds (6%) were considered positive for swine influenza H3N2. In 2005 the number of herds positive to H1N1 and H3N2 were 18 (36%) and 22 (44%), respectively. Across all herds, the prevalence of serologically positive pigs for H1N1 was 13% in 2004 and 15% in 2005 and for H3N2 the prevalence of positive pigs was 7% in 2004 and 23% in 2005. A herd was classified as influenza positive if the number of apparently positive pigs exceeded two.

Take Home Message

This study clearly demonstrates how rapidly H3N2 influenza was able to spread throughout the provincial pig population. Herds with high levels of biosecurity and no history of pig introductions did become infected. The spread of this "new" influenza virus in the naïve pig population in Ontario resulted in outbreaks of respiratory disease that caused high levels of morbidity but low mortality for the most part. However, it is unclear how much the spread of this virus contributed to the clinical expression of other viral diseases such as porcine reproductive and respiratory syndrome and porcine circovirus type 2-associated disease which both appeared to increase during this same time period.

Financial support was provided by the Ontario Ministry of Health and Long Term Care as well as Pfizer Animal Health

The effect of feeding grains naturally-contaminated with *Fusarium* mycotoxins on production, reproduction and serum chemistry of late-gestating and lactating first parturition sows and the efficacy of a polymeric glucomannan adsorbent in preventing these effects.

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Background

The feeding of diets naturally-contaminated with *Fusarium* mycotoxins to pigs can cause immunosuppression and can reduce feed intake, body weight gain and hepatic protein synthesis. No differences in feed intake have been seen in sows fed diets contaminated with 3.3 mg/kg DON during gestation and lactation, but the loss of body weight was significantly higher when compared to controls. Little information is available regarding the effect of feeding diets contaminated with a combination of *Fusarium* mycotoxins to first parturition sows.

Objectives

To asses the effect of feeding diets naturally-contaminated diets with *Fusarium* mycotoxins on feed intake, body weight changes, serum chemistry, stillbirth rate, born alive rate, weight of piglets at birth and at weaning, and weaning to estrus interval in first parturition sows.

Materials and Methods

36 Yorkshire first-parturition sows were utilized. Diets included: (1) control (2) contaminated (5.5 ppm DON, 0.5 ppm 15-acetyl DON and 0.3 ppm zearalenone), and contaminated + GMA (5.7 ppm DON, 0.5 ppm 15-acetyl DON and 0.3 ppm zearalenone). Twelve sows were assigned to each diet.

Results

Sows did not have reduced feed intake in late gestation, but average daily gain was reduced. The was prevented by the feeding of GMA. No differences in serum chemistry were observed. There was no effect of diets on piglet body weight at birth. Stillbirth rate of piglets born of sows fed contaminated diets was higher than piglets born of sows fed contaminated grains + GMA. Feed intake was reduced and body weight losses were observed in sows fed both contaminated diets in the lactation period. Total serum protein and serum globulin concentrations were reduced in sows fed contaminated diet but not in sows fed contaminated grains + GMA. Total serum urea was reduced in sows fed the contaminated diet and contaminated diet + GMA. A strong trend toward longer weaning to estrus interval was observed in sows the contaminated diets.

Take Home Message

The feeding of diets naturally-contaminated with *Fusarium* mycotoxins to sows results in adverse effects on production, reproduction and serum chemistry. Dietary supplementation with a polymeric glucomannan adsorbent prevented many of these effects.

These studies were supported, in part, by the Ontario Ministry of Agriculture, Food and Rural Affairs and by Alltech Canada, Guelph, Ontario.

Ontario Data Analysis Project – Ten Years of Benchmarking

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Background

Results from the Ontario Data Analysis Project (ODAP) have been used as a means of benchmarking in the swine industry. This is a compilation of farm level production and financial data from Ontario farrow-to-finish producers. These participants are full-time farmers and most rely on family members to fill additional labour needs.

Objectives

- Overview of Ontario hog farm production and financial performance
- Assess individual benchmarks to determine if they suggest profitability at the farm level

Results

i) Benchmarking
- Productivity and financial improvements have been achieved over time

	1995	2004
Average # of Sows	133	217
Weaned/litter	9.1	9.6
Finishing Weight (kg live)	104.2	113.6
Days to Market	177	166
Pork/sow (kg)	1420	1766
Farm assets/sow	$7,555	$12,554
Farm equity/sow	$4,791	$8,379

ii) Profitability at the farm level
- A combination of factors affect financial success
- Separating farms on a profit/pig produced basis showed that high profit/pig farms reported higher revenue and lower expenses per pig. This resulted in $24.41/pig additional profit for the high profit group.

	Profit Group		
	High	Low	Difference
Revenue/pig produced	$158.77	$148.59	$10.18
Expenses/pig produced	$126.66	$140.89	($14.23)
Profit/pig produced	$32.11	$7.70	$24.41

Take Home Message

- Farms are larger and more productive in 2004 compared to 1995
- Farm size is not an indicator of financial success
- Cost control and revenue optimization are important drivers of profitability

Special thanks to Agriculture & Agri-Food Canada for funding this project. Recognition and appreciation is extended to the farms that participated.

Walking the Pens Reduces Fear Response at the Farm and Improves Handling of Market Hogs at the Packing Plant

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Background

Reducing stress during handling and transport at slaughter is important for ensuring good meat quality and animal welfare. Calm, low-stress handling depends on a combination of good facility design, experience and technique of the handlers and the response of pigs to handling. Pig behaviour during handling is thought to depend in large part on their previous experience with humans. One practical recommendation for getting pigs used to moving around people is to "walk the pens" in the finishing barn, but there is little known about how often pens need to be walked to improve handling.

Objective

To determine how walking the pens one, two, or three times per week, or not at all, affects fear response and improves handling during loading and unloading at the farm and at the packing plant.

Methods

Data were collected on 1900 market hogs on two commercial farms. Pens of 15 to 24 pigs were walked zero, one, two or three times per week during the final 12 weeks of finishing. Walking the pens involved a handler with a pig board walking purposefully through the pen, spending an average of 40 ± 0.39 seconds there. Responses to humans were monitored in the home pen weekly. Handling behaviour was measured at the farm during shipping and in a crowd pen and single-file chute at the packing plant

Results

- At both farms, pigs in all walked pens showed a reduction in escape behaviour over time, but pigs in pens walked two or three times per week were significantly less fearful than pigs in pens walked only once per week. ($P < 0.05$).
- During loading no significant treatment effects were observed. ($P>0.10$).
- At the packing plant different treatment effects were found for each farm. Pen-walked pigs from Farm 1 showed a significant reduction in jamming at the chute entrance ($P < 0.05$), while pen-walked pigs from Farm 2 took less time to move through the crowd pen ($P < 0.05$) compared to pigs whose pens had not been walked.

Take Home Message

Pigs whose pens were walked two or three times per week showed the least escape behaviour to humans in their home pens. Walking the pens as little as one time per week showed a positive effect on handling at the packing plant.

We appreciate the financial support from the Ontario Pork, NSERC and the OMAF-Animal Research Program at the University of Guelph

Analyzing Livestock Farm Odour Using an Intelligent Electronic Nose

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Background

Odour emissions from livestock operations have raised significant public concerns about environment conditions and human health. Odour control technologies are greatly impeded by the lack of science-based approaches to assess the effects of odour. With the current state of technology, the most commonly used methods to measure odour from livestock facilities is human sniffing methods, but they are very expensive and difficult to overcome the analyst's personal bias. Therefore, automatic objective odour evaluating technologies are desirable.

Objective

To develop a portable electronic nose for objectively measuring livestock farm odour, and to adequately model and analyze livestock farm odour.

Methods and Results

A portable intelligent electronic nose prototype which can be used both in the laboratory and for field studies was developed to analyze and predict the strength of livestock farm odour. The prototype consists of 14 gas sensors that were especially selected for measuring odorous components from livestock farms. An expert system called "Odour Expert" was developed to support researchers' and farmers' decision making about odour control strategies for livestock operations. "Odour Expert" incorporates both conventional analysis methods and artificial intelligent methods. It is capable of taking odour measurement from livestock farms, and giving expert advice on what control actions to take in order to efficiently reduce odour.

Downwind odour measurements were conducted at 14 Ontario livestock farms in 2004. The data gathered by the electronic nose were correlated with the readings from human. The analysis of the data shows that using the electronic nose to predict livestock farm odour yields more accurate and consistent results.

A neural network based multi-component, multi-factor analytical odour model was proposed to adequately model livestock farm odours. A structural learning with forgetting algorithm was proposed for contribution analysis of odorous gas components and factors to the perception of odour. Contribution analysis would allow identification of significant odour components and major contributing factors, thus improving the efficiency in developing odour measurement and reduction technologies. The effectiveness of the proposed approach is demonstrated by using a livestock farm odour database.

Take-home Message

The developed electronic nose proved its capability of producing an objective reliable output, lower operation cost, and greater consistency in odour measurement.

Financial support was received from Ontario Pork and OMAF

Gene Expression in the Parotid Gland of the Enviropig™ Breed and Conventional Yorkshire Pigs

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Background

The Enviropig™ breed of pigs, by virtue of its capability to synthesize in the parotid glands phytase enzyme that enables the utilize phytate phosphorus more efficiently than conventional pigs can provide economic and environmental benefits not achieved with conventional pigs. The challenge is to determine without a doubt that the new breed is substantially equivalent to conventional pigs and is safe for human food consumption.

Objective

The objective of this study was to assess gene expression in the parotid glands of enviropigs and conventional pigs by microarray analysis and real-time PCR to determine whether there were any substantial differences.

Methods and Procedures

Gene expression in parotid tissue from six growing phase Enviropig™ and six grower phase Yorkshire pigs was assessed by microarray analysis using Affymetrix Porcine Genome Genechips.

Results

Detection calls based on the raw data showed that over the twelve chips processed 9687 transcripts were present, 252 were marginal and 13,964 were absent. Twenty-seven transcripts were up-regulated and 41 were down regulated in the Enviropig™ parotids. Examples of genes that were unregulated included secreted phosphoprotein-1 (+5.8), transcribed locus similar to olfactomedin 4 precursor (+5.3) and several down regulated proteins were cytochrome P_{450} 2B22 (-3.8), transcribed locus similar to putative 6-16 protein (-2.8). Greater than half of the transcripts that exhibited significant fold changes (≥1.5, p-value of ≤0.05) were transcribed loci of unknown function.

Take Home Message

The interpretation of the microarray data is premature since we do not know the impact, if any, of the regulation of the genes listed. Two aspects are the lack of knowledge of the functions of the transcribed loci, and the possible variation in the relationship between mRNA production and protein synthesis for a specific locus. The underlying issue is swine health, and studies are ongoing to assess the comparative health of Enviropig™ and conventional Arkell pigs. To the present there is no difference in the reproductive characteristics of Enviropigs™ and Yorkshire pigs.

This research was primarily supported by the Advanced Foods and Biomaterials Network with tissues recovered from pigs obtained through the OMAFRA program supported by funding from Ontario Pork and the MaRS Landing funded research program.

Hematology and Blood Biochemistry of Enviropig™

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Background

The Enviropig™ breed of pigs, synthesize phytase that is secreted in the saliva enabling the utilize phytate phosphorus more efficiently than conventional pigs thereby providing economic and environmental benefits not achieved with conventional pigs. An unanswered question was whether phytase production has an effect of health of the pigs.

Objective

Hematology and biochemistry of animal blood is a well documented standard method for assessing animal health. Therefore these parameters were assessed on blood samples collected from Enviropigs™ (EP) and conventional Yorkshire (YK) pigs to assess health implications.

Methods and Procedures

A total of 17 hematology and 25 biochemistry parameters were determined on fresh blood samples to assess the health of forty eight pigs (24 EP and 24 Yorkshire [YK]) comprised of 12 boars and 12 gilts of each breed were used in the trial, with blood collected at 54.6 ± 0.7 and 55.7 ± 0.5, 90.6 ± 0.7 and 91.7 ± 0.5 and 125.6 ± 0.7 and 126.7 ± 0.5 days of age for weanling, growing and finishing phases, respectively. The experimental design was 2 x 2 factorial combinations of breed and gender. Blood samples were collected from the intraorbital sinus.

Results

There were either no significant differences in the assayed parameters across the treatments ($P<0.05$), but if there were differences, the values were within published normal ranges.

Take Home Message

These data indicate no appreciable biological differences between the EP and YK blood hematology and biochemistry.

This research was financially supported by Ontario Pork, MaRS Landing, Food System Biotechnology Center and the Ontario Ministry of Agriculture, Food and Rural Affairs.

Antimicrobial Resistance to *Salmonella* in Ontario Swine Herds

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Background

Salmonella continues to be an important foodborne pathogen in Canada and the USA, particularly serovars demonstrating multiple antimicrobial resistance (AMR). A major concern associated with *Salmonella* prevalence on pig farms is the increasing incidence of multi-drug resistant serovars, particularly *Salmonella* Typhimurium DT104. Surveillance of bacterial resistance in swine is important in understanding the relative significance of this source of bacteria to the human population.

Objective

To describe antimicrobial resistance patterns of *Salmonella* spp. isolated from healthy pigs on Ontario farms.

Results

Thirty-eight (23.2%) of the isolates (16 farms) had no antimicrobial resistance. Of 164 isolates, 126 (76.8%) of those were resistant to 1 to 11 of the remaining antimicrobials including tetracycline (T) (65.2%), streptomycin (S) (65.2%), sulfisoxazole (Su) (64%), ampicillin (A) (61%), spectinomycin (Sp) (59.1%), chloramphenicol (C) (58%), florfenicol (57.3%), amoxicillin and clavulanic acid (Ac) (55.5%), kanamycin (K) (14%), neomycin (N) (14%), nitrofurantoin (Nit) (1.2%). Resistance to these antimicrobals at the farm level is shown in figure 1.

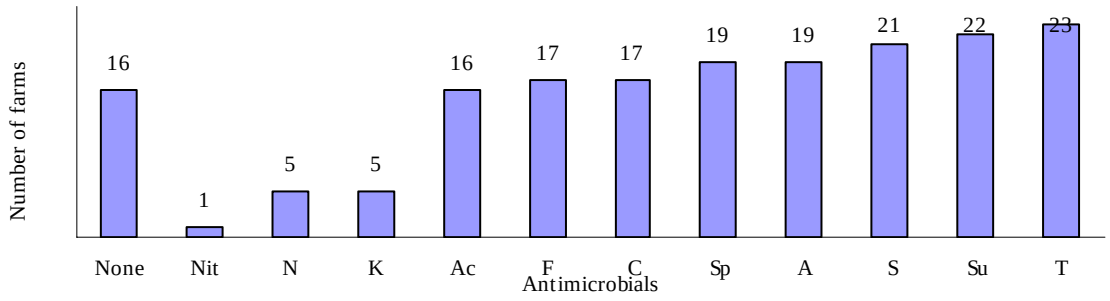

Figure 1: Distribution of resistance of *Salmonella* spp. on 37 Ontario swine farms.

Take Home Message

Salmonella are present on many farms and in some cases may not pose a problem but the industry should pay particular attention to the presence of multiple drug resistant *Salmonella* Typhimurium as this organism is a serious public health risk.

Financial support was provided from Ontario Pork, OMAFRA Food Safety Program, and the Public Health Agency of Canada.