2016

[EMERGENCY MANAGEMENT]

The Emergency Management research theme focuses on emerging, re-emerging or endemic pathogens and pests in livestock and crops that require (or may require) an immediate and comprehensive response for containment that cannot be handled with typical resources. The theme is rooted in "One Health" at the interface of livestock, ecosystem and human health. The Emergency Management research theme has an emphasis on evaluating and mitigating the impact of emergencies on Ontario's agricultural sector and related public health through the lens of the core components of emergency management: prevention, preparedness, mitigation, response and recovery.

Contents

Submission number	**27092**		
Project Title	**Development of a benefit/cost financial management template for veterinary laboratory emergency preparedness**		
Key words	**Cost-benefit, financial management, disease outbreak**		
Lead Applicant	**Maria Spinato**		
Organization	**University of Guelph**		
Start Date	**2011-07-01**	**End Date**	**2012-12-31**

Objective

This financial template will calculate the real costs related to preparing for and conducting an emergency response at the diagnostic veterinary laboratory. The laboratory and its funding agency will use this management tool to evaluate the required investment and benefits of emergency preparedness. For example, rapid diagnostic turn-around will support a rapid field response that will mitigate the disease outbreak. These benefits will in turn be compared to the risks and costs of managing an emergency event (surge capacity expenses; cost of temporary/permanent loss of business continuity) in order to determine best outcomes and business practices.

Results Summary

This financial management template will assist veterinary diagnostic laboratories and their funding/regulatory agencies in determining the true financial costs of preparing for and responding to an emergency event such as a foreign animal disease outbreak, thereby facilitating the selection of best management practices. The template is easy to use, and incorporates most of the financial and human resources costs involved in emergency planning, surge capacity testing and business continuity planning. By conducting a temporal sensitivity analysis, managers can select and prepare an emergency management strategy that ensures business continuity and maintains the provincial capacity for passive animal disease surveillance, both during and following the outbreak.

Although veterinary diagnostic laboratories are eager to support provincial and federal animal health agencies' response during a foreign animal disease outbreak, most Canadian laboratories have little experience in managing the sudden and exponential increase in testing demand. This type of emergency can cause logistical challenges for both operational and human resource management. The ability of the laboratory to meet stakeholders' requirements for rapid and accurate diagnostic testing may become strained under these circumstances. The financial emergency management template developed by this project includes all of the fixed and variable cost calculations related to surge capacity and business continuity testing. The laboratory manager and funding agencies can use the template to conduct a sensitivity analysis and determine the costs of FAD testing, in addition to various business continuity/discontinuity strategies that have an impact on laboratory revenue and profit. These costs are summarized to derive a total financial cost of FAD testing on laboratory operations. Intangible costs such as loss of client goodwill and lack of passive disease surveillance are also highlighted. It is expected that laboratory managers and funding agencies will use this financial management tool to inform their decision-making process, and to support preparations for successful management of FAD testing.

Submission number	**27058**		
Project Title	**Understanding the effect of diagnostic misclassification bias on spatial surveillance methods ...**		
Key words	**Spacial statistics, epidemiology, disease outbreak, surveillance**		
Lead Applicant	**Olaf Berke**		
Organization	**University of Guelph**		
Start Date	**2011-09-01**	**End Date**	**2012-12-31**

Objectives

Spatial statistical methods are widely used in disease surveillance systems, but their performance is not well understood when it comes to diagnostic misclassification. How do false positive and false negative reports impact on critical information for disease control management systems?
With emerging diseases the quality of respective diagnostic tests (sensitivity and specificity) is generally unknown. This project will generally advance our understanding of disease surveillance methods and therefore improve emergency preparedness. This project will specifically address the question how misclassified farm health status information will impact statistics that (i) measure the range of infections spread, (ii) identify the location of disease hot spots, and (iii) identify potential risk factors of disease? This will be done by use of Monte Carlo simulation studies.
The results of this study will guide public health administrators in making informed decisions with respect to food animal disease control.

Results Summary

1. Current diagnostic tests and testing systems as, e.g., used for detection of influenza in swine will result in misleading conclusions at the population level.
2. Spatial epidemiological methods might falsely identify significant relations when applied to misclassified health status data.
3. Spatial epidemiological methods assume perfect diagnostic information and will overestimate the effect of potential causal relations in regression models under misclassification bias.

The key finding of this project is that diagnostic misclassification of cases and non-cases will result in data that informs epidemiologists and public health administrators poorly about the health status of populations and especially food animals.

Current diagnostic tests and testing systems as, e.g., used for detection of influenza in swine will result in misleading conclusions at the population level. Methods for geographic data analysis as investigated in this project are performing badly, when applied to biased diagnostic data. Since data analytical methods are applied under the assumption of valid and reliable diagnostic information the results from data analysis must be considered with extreme caution.
Specifically, the “Cuzick-Edwards test” used for the detection of disease clustering might identify disease clustering, when the true health status of farms is certainly not clustered.

Furthermore, “logistic regression models” used to identify predictors of disease and to estimate the effect of such predictors, will overestimate respective effects and indicate significance when this is not true. The same conclusion can be expected for other statistical tests, e.g. the spatial scan test for point data, i.e. such as farm locations. However, lack of software that allows necessary simulation studies, prohibited their performance evaluation.

Submission number	**27084**		
Project Title	**Emerging plant pathogens: Identification, biology, pathogenicity and integrated management of emerging pathogens infecting asparagus, cucumber and basil**		
Key words	**Plant pathogens, cucumber, basil, asparagus**		
Lead Applicant	**Mary Ruth McDonald**		
Organization	**University of Guelph**		
Start Date	**2011-05-01**	**End Date**	**2014-04-30**

Objectives

1. To identify, characterize and study the biology of new, emerging and the potentially more aggressive plant pathogens*: Phytophthora spp*., *Pseudoperonospora cubensis* and *Peronospora belbahrii* on asparagus, cucumber and basil, respectively, to improve pathogen monitoring and surveillance and pathogen control.
2. To evaluate reduced risk pesticides and biopesticides for their efficacy against the target plant pathogens, both in the greenhouse and in the field.
3. To improve and update Integrated Pest Management systems for downy mildew on cucumber.
4. To evaluate several cultivars and breeding lines of asparagus and basil for their resistance to *Phytophthora spp*. and *P. belbahrii*, respectively.

Results Summary

1. Emerging pathogens of asparagus (*Phytophthora asparagi*), basil (*Peronospora belbahrii*) and more aggressive strains of cucumber downy mildew (*Pseudoperonospora cubensis*) were studied. The pathogens were isolated, characterized using morphological and DNA fingerprinting methods. Ontario and Michigan *Ps. cubensis* populations are being characterized and compared.
   Basil cultivars were evaluated in the field and greenhouse for reaction to basil downy mildew. All cultivars were infected but cvs Lemon, Spice, Medinnette, Queenette Thai were less susceptible. None of these cultivars are commonly used by Ontario growers.
2. The Fungicides PRESIDIO, PRODUCT A, ZAMPRO, REVUS +Sylgard, RANMAN + Sylgard, CONFINE and the biofungicides: REGALIA, ORGANOCIDE,SONATA, TIMOREX GOLD, TIVANO, PREV-AM were evaluated on cvs Genovese and Sweet Basil for downy mildew control in the field between 2011and 2013. PRODUCT A and ZAMPRO were most effective, followed by REVUS and RANMAN. The biofungicides had some control at low disease. The same fungicides with exception of PRESIDIO were evaluated in the greenhouse. All fungicides performed similarly in 2012 (low disease). In 2013 (high disease) PRODUCT A and ZAMPRO were most effective, followed by RANMAN and REVUS.
3. Asparagus cultivars Millennium, Jersey Giant, Pacific Challenger, UC157, UG005 and UG020 were evaluated in the field and greenhouse for resistance to *Phytophthora asparagi*. All cultivars were susceptible but Pacific Challenger was less susceptible in the greenhouse and UG005 was less susceptible in the field. UG005 had the highest survival in 2013 after plants were inoculated in 2012.
4. The fungicides: PRODUCT A, ZAMPRO, RIDOMIL, ACTINOVATE, RANMAN, PHOSTROL, OXIDATE, PREVICUR-N, PREV-AM and SONATA were evaluated. Best control was achieved with PRODUCT A and ZAMPRO. The cultivars evaluated in 2012 in the greenhouse were transferred and evaluated in the field. In 2012 a field trial was established for cultivar and fungicide evaluations. Both trials were evaluated in 2012 and

2013. All cultivars were infected by Phytophthora asparagi, but UG005 and UG020 were less infected, and % survival (2013) was high for UG005. The fungicides evaluated in the greenhouse were tested in the field. PRODUCT A and ZAMPRO were most effective followed by PHOSTROL, RIDOMIL and RANMAM.

5. Cucumber downy mildew populations from Ontario and Michigan are being compared to see if populations from both sides are similar and possible implications in downy mildew initiation in cucumber fields and how the results of the study will impact/affect disease management strategies.

1. Basil Field
    a. Cultivars: 28 cultivars were evaluated at Simcoe Research Station (2011-2013) and 6 at a growers' field (2013) for downy mildew. All cultivars were susceptible. Lemon and Spice were less infected. Medinette, Queenette Thai and Purple Ruffles were moderately infected.
    Fungicides: PRESIDIO (292 mL/ha), PRODUCT A, 250 mL/ha), ZAMPRO (1L/ha), REVUS (584 mL/ha) +(Sylgard), RANMAN (210 mL/ha) + (Sylgard), CONFINE (5L/ha) and the biofungicides: REGALIA (0.25% v/v), ORGANOCIDE (15.6 mL/ha), SONATA (9.4 L/ha), TIMOREX GOLD (500 mL/ha), TIVANO (16L/ha), PREV-AM (3.9 mL/L) were evaluated on cvs Genovese and Sweet Basil for downy mildew control. PRODUCT A and ZAMPRO were most effective, followed by REVUS and RANMAN. Marketable yield was highest for PRODUCT A. The biofungicides had some control at low disease.
    Greenhouse
    b. Cultivars: Medinette, Sweet Basil, Genovese, Rubin, Spice and Queenette Thai were evaluated for basil downy mildew resistance in the greenhouse (2012-2013). All cultivars were susceptible. Spice, Medinette and Rubin were less infected.
    c. The same fungicides and biofungicides evaluated in field (except PRESIDIO), were evaluated in the greenhouse on cvs Genovese (2012-2013) and Sweet Basil (2012). All fungicides performed similarly in 2012 (low disease). In 2013 (high disease) PRODUCT A and ZAMPRO were most effective, followed by RANMAN and REVUS.
2. Asparagus
    a. *Phytophthora sp* was isolated from fields. Isolates were characterized, evaluated for virulence on asparagus spears and for temperature growth in the laboratory. No growth was recorded at 0 and 30ºC; maximum growth was at 25ºC. ITS sequencing of 16 isolates indicated *Phytophthora asparagi* as the asparagus *Phytophthora*.
    b. Cultivars: Pacific Challenger (resistant), UC 157 (susceptible), Millennium, Jersey Giant the breeding lines, UG 005 and UG 020 were evaluated in the greenhouse for *P. asparagi* resistance. In 2012 and 2013 Pacific Challenger was less susceptible in the greenhouse. The fungicides: PRODUCT A, (1400 mL/ha), ZAMPRO (1L/ha), RIDOMIL (25 kg/ha), ACTINOVATE (840 g/ha), RANMAN (0.44 L/ha), PHOSTROL (5.8 l/ha), OXIDATE (1L/100L), PREVICUR-N (1.5 mL/L), PREV-AM (391 mL/100L) and SONATA (9.4 L/ha) were evaluated. Best control was achieved with PRODUCT A and ZAMPRO.
    c. The cultivars evaluated in 2012 in the greenhouse were transferred and evaluated in the field. In 2012 a field trial was established for cultivar and fungicide evaluations. Both trials were evaluated in 2012 and 2013. All cultivars were infected by *Phytophthora asparagi*, but UG005 and UG020 were less infected, and % survival (2013) was high for UG005.
    d. The fungicides evaluated in the greenhouse were tested in the field. PRODUCT A and ZAMPRO were most effective followed by PHOSTROL, RIDOMIL and RANMAM.

3. Cucumber downy mildew
   Isolates (n=440) of *Pseudoperonospora cubensis* collected from Ontario fields were compared to Michigan isolates using DNA fingerprinting and population studies. Evaluations are ongoing.

Phytophthora asparagi (Asparagus) and *Peronospora belbahrii* were isolated, identified and characterized.

Population studies to characterize and compare *Pseudoperonospora cubensis* isolates from Michigan and Ontario are in progress.

Basil cultivars (28) were evaluated in the field and greenhouse for basil downy mildew reaction. All cultivars were infected but Lemon, Spice, Medinnette and Queenette Thai were less infected. Genovese and Sweet Basil, most grown in Ontario were very susceptible.

Fungicides and biofungicides were evaluated on cultivars Genovese and Sweet Basil. PRODUCT A and ZAMPRO were very effective, followed by REVUS and RANMAN. The biofungicides had some control at low disease. Highest marketable yield was obtained from treatments with Product A.

Asparagus cultivars and breeding lines were evaluated in the field and in the greenhouse for resistance to Phytophthora. All cultivars were susceptible, but Pacific Challenger was less infected in the greenhouse, and UG005 was less infected in the field. Fungicides and biofungicides were also evaluated. Best control was achieved with PRODUCT A and ZAMPRO, followed by PHOSTROL, RIDOMIL and RANMAM.

Submission number	**27118**		
Project Title	**Emerging and potentially zoonotic viruses in pigs and pork: identifying public health risks**		
Key words	**public health, pigs, disease transmission, HEV, rNoV, RV**		
Lead Applicant	**Scott McEwen**		
Organization	**University of Guelph**		
Start Date	**2011-05-01**	**End Date**	**2013-04-30**

Objectives

1. Summarize and synthesize the evidence of the public health risks of three selected emerging and potentially zoonotic viruses: hepatitis E virus (HEV), recombinant Norovirus (rNoV), and reassorted rotavirus (RV), using scoping review methods, followed by systematic review and where appropriate meta-analysis methodologies.
2. Estimate the prevalence of the three selected viruses (HEV, rNoV, RV) on retail pork chops and pork livers in Canada via a national field survey using the CIPARS retail sampling frame.
3. Estimate the prevalence of the three selected viruses on close-to-market swine using the CIPARS on-farm-swine sampling frame, and samples previously collected by the University of Guelph, to collect pen fecal samples for viral detection.
4. Create a risk profile for the virus for which description of the public health problem is most complete.

Results Summary

- We searched the literature and found that while pigs and pork are often blamed as the source of human Hepatitis E infection, the source most often reported with certainty was contaminated blood transfusions.
- We collected Canadian pork chops and pork livers from across Canada weekly for one year, and found a small percentage had HEV, comparable to foreign studies.
- We tested Canadian pigs in four different provinces and found a higher percentage of pigs have these viruses, compared with retail pork.
- We studied the genes of the HEV we found in retail pork and suspect that some of these viruses may have come from humans contaminating the pork during processing/handling.

There are only two reported cases of Hepatitis E virus causing illness in Canada. Their source of infection is unknown. The most commonly reported source for people with Hepatitis E, living in non-tropical areas, to have acquired their infection seems to be from contaminated blood transfusions.

We collected pork chops and pork livers from stores across Canada, and tested them for hepatitis E virus (HEV), norovirus (NoV) and rotavirus (RV). HEV was the virus that occurred most often (31/878 or 3.5% of samples), which is comparable with surveys done in other countries.
We found that HEV, NoV and RV occurred more often in samples from pigs on-farm than in retail pork samples. Some commonly used biosecurity measures (giving visitors boots and coveralls, or showering-in to the pig barn), were associated with reduced chances of finding HEV and NoV on-farm.

We propose that the greatest risk that pigs or pork pose to humans for getting HEV is as a reservoir or storehouse for the virus. The potential public health risk of zoonotic NoV remains hypothetical. The greatest impact of zoonotic or re-assortant RV may be the potential for reduced vaccine effectiveness against emergent strains.

Submission number	**27145**		
Project Title	**Development and applications of an amoeba-based pathogen-capture trap for detection of persisting and emerging microbial pathogens in farm and aquaculture**		
Key words	**Amoeba, surveillance, dairy, aquaculture**		
Lead Applicant	**Lucy Mutharia**		
Organization	**University of Guelph**		
Start Date	**2011-05-01**	**End Date**	**2014-04-30**

Objectives

The objectives of this study are to determine if free-living amoeba represent an appropriate tool for pathogen surveillance in dairy farms and aquaculture. By recovering FLA from these environments a knowledge of which pathogens reside within resident amoeba may improve our understanding of environmental reservoirs of pathogens and help explain their persistence. Once an understanding of the relationships between various resident FLA and environmental pathogens is better understood it is possible that FLA may represent a tool for pathogen surveillance. This project seeks to assess the efficacy of amoeba as a tool for pathogen surveillance by exploiting their ability to enrich for pathogens and select for virulent strains, increasing the effectiveness of molecular identification tools and providing advanced warning of persistence and emerging pathogens.

Results Summary

Amoeba feed on bacteria, but a few bacterial species can overcome amoeba defences, survive and replicate within amoebae. There are few studies linking amoeba in a specific settings (e.g. fisheries, dairy farms) with the bacteria that live within them. In this study we isolated amoeba from dairy and fishery samples and identified resident bacteria.

A problem with study of environmental amoeba is the contamination of cultures with fungi. In this study we developed protocols to prevent fungal growth.

Few studies have examined the long term persistence of bacteria in amoeba. We showed that only a few pathogenic bacteria can survive in amoeba cysts. Thus the diversity of truly amoeba resistant bacteria has been overestimated.

Isolation of environmental amoeba is made difficult by persistent fungal contamination. Fungal contamination limits the efficacy of the walk-out protocol for direct isolation of amoeba from soils and sediment samples. Chemical and pH-based decontamination treatments with or without forced encystation of the amoeba were developed to clear environmental samples of fungi, leaving viable amoeba. Using these protocols, 10 species of amoeba have been isolated from 16 samples to date. A 77% successful amoeba isolation rate from environmental samples was achieved using our methodologies. Amoebae were isolated from 6/11 (54.5%) sediment samples from aquaculture operations.

We showed that only a few bacteria are truly resistant based on their ability to survive within cysts versus transient survival in trophozoites. Survival within amoeba was amoeba species-specific with some species killing all examined bacteria and others hosting some bacteria. Future studies will examine persistence of animal and fish pathogens in different amoeba species. This gap in knowledge limits water management and conservation options in aquaculture, including recirculation systems

Submission number	**27063**		
Project Title	**Peri-weaning failure-to-thrive syndrome (PFTS) in pigs in Ontario**		
Key words	**Pigs, Peri-weaning failure-to-thrive syndrome**,		
Lead Applicant	**Robert Friendship**		
Organization	**University of Guelph**		
Start Date	**2011-05-01**	**End Date**	**2013-04-30**

Objectives

Evaluate association between gastritis in young pigs in Ontario and clinical cases of PFTS and identify potential associations between gastritis and clinical / management parameters. Characterize histologic features and anatomic distribution of gastritis and investigate potential infectious and non-infectious etiologies of gastritis, including speculative agents associated with PFTS (hemagglutinating encephalomyelitis virus (HEV), porcine cytomegalovirus) and agents associated with gastritis in other species (*Helicobacter spp*), with the eventual goal of diagnosis, prevention, and treatment of the condition. Establish the prevalence of PFTS in Ontario swine herds, and evaluate the potential economic impact of gastritis and PFTS to the swine industry. Correlate findings from Ontario cases with investigations taking place in western Canada (Harding) and the mid-west USA.

Results Summary

1. Peri-weaning failure to thrive syndrome is recognized across North America, but the prevalence does not appear to be increasing.
2. The etiology is still unknown and there may be multiple factors that result in newly weaned pigs not eating
3. The clinical signs of PFTS and the pathological lesions are the same as healthy pigs that have been fasted. Newly weaned pigs have a great deal of fat reserves and if they don't eat for several days they don't appear to be emaciated or weak and therefore are difficult to identify in a large nursery until the condition has progressed to a more severe state, making treatment difficult.
4. Early identification and special care to encourage feed intake is required to reverse the effects of PFTS.

Swine veterinarians were made aware of the clinical signs and post-mortem lesions of PFTS. The prevalence of the condition was established and shown to be widespread in North America. The syndrome did not appear to be increasing. The lesions and clinical signs were shown to be indistinguishable from pigs being fasted. Measuring blood ketones appeared to be a good method to detect pigs in the early stages of the disease.

Submission number	**UofG2011-1089**		
Project Title	**A functional prioritization tool for the prioritization of zoonotic diseases in Ontario**		
Key words	**Prioritization, zoonoses, MCDA, Conjoint analysis**		
Lead Applicant	**Jan Sargeant**		
Organization	**University of Guelph**		
Start Date	**2012-06-01**	**End Date**	**2014-05-30**

Abstract

Emergency Management requires the need to address priorities. Zoonotic diseases threaten the agri-food and rural sectors of Ontario, and are a potential threat to public health. There are currently no functional tools for prioritizing zoonotic diseases in Ontario due to the complexity and constraints associated with current prioritization methodologies. Further, traditional methods are ad-hoc and informal within many organizations, focusing on the priorities of one stakeholder (public health, animal health or the agri-food and rural industry), which often limits the utility of the tool.

We propose to build a functional prioritization tool for OMAFRA and other decision-makers to prioritize zoonotic diseases in Ontario. The tool will identify and prioritize existing and emerging zoonotic threats in Ontario thereby addressing the pre-event stages of Emergency Management (outbreak prevention and preparedness). Conjoint analysis and multi-criteria decision analysis will be used jointly to overcome limitations in current prioritization methodologies. The tool will allow OMAFRA decision-makers to consider input from agri-food and rural industry stakeholders and veterinary and medical professionals, thus providing a more egalitarian approach to the prioritization process. Additionally, the tool will allow stakeholders with different objectives to obtain a unique priority list on the strength of their preference for certain disease characteristics.

Objectives

1. Expand on the current knowledge of zoonoses in Ontario by conducting a scientific literature review and publishing a report on 63 emerging and established zoonoses as they relate to Ontario. This report will provide science-based inputs into the prioritization tool.
2. Build a functional prioritization tool for OMAFRA decision-makers to identify and prioritize zoonotic threats to Ontario; these include diseases of threat to the agri-food and rural sectors of Ontario (Campylobacteriosis, Salmonellosis, Listeriosis), to animal health (Q fever, Leptospirosis, Tularemia) and to public health (H1N1 influenza, Rabies, Lyme disease).
3. As the methods used and the program developed for the prioritization tool are transferable, the development and application of the tool to address disease prioritization in Ontario will provide a framework for a functional prioritization tool that can be used and applied to other prioritization objectives (for example, food safety, animal health and welfare, public health and research programs).

Project Results

The final disease prioritization Tool was presented to collaborators in May 2014 and to a team of OMAFRA managers in July 2014. The final Tool as well as the report on zoonotic diseases (Deliverables #1 and #2) were distributed to collaborators and OMAFRA managers during these meetings. The feedback so far has been positive and we hope that the OMAFRA managers will find the Tool useful in the prioritization of zoonotic diseases in Ontario.

Submission number	**UofG2011-1174**		
Project Title	**Prevalence and Severity of *Nosema cerane* in Ontario**		
Key words	**Nosema ceranae, honey bees, Ontario**		
Lead Applicant	**Ernesto Guzman**		
Organization	**University of Guelph**		
Start Date	**2012-05-15**	**End Date**	**2014-05-15**

Abstract

Nosema ceranae, a new species of the honeybee fungus *Nosema spp*., has been recently reported in Ontario, thus becoming an emergency issue for the province. Preliminary results have shown a potential association between colony mortality and high levels of Nosema disease. We know that *N. ceranae* exists in Ontario, but its prevalence, and infectivity to bees in the province is unknown. Without this knowledge it is not possible to assess the risks that this emergency pathogen possess to the beekeeping industry and will not be possible to design effective control strategies against this fungus in Ontario. Thus, this project is aimed at determining the prevalence, infection patterns and relationship of *Nosema ceranae* with bee mortality in the province. We will use molecular and fluorescent diagnostic techniques to positively identify, quantify, and assess the viability of the parasite in Ontario honeybee colonies. We will also associate colony mortality to *N. ceranae* infection levels.

Objectives

1. To establish bioassays to identify and assess the infectivity of *Nosema ceranae*
2. To determine the prevalence of *N. ceranae* and its infection patterns in Ontario
3. To measure the effect of *N. ceranae* on honey bee health

Results Summary

The following things were accomplished with this project: 1) The PCR technique to identify *Nosema* species was improved by incorporating a honey bee gene in a triplex reaction to insure that negative or positive results are accurate, by always detecting the bee gene. 2) The fluorescence staining protocol to assess *Nosema* viability was improved by adding extra steps in the spore purification and staining processes. Experiments were conducted to validate the repeatability and accuracy of assessing spore viability rates with fresh spores and inoculated bees. Results showed high repeatability (>90%) and a correlation between pre and post inoculation levels of spore viability. Therefore we concluded that the method reliably assesses the viability and infectivity of the spores. 3) The *Nosema* species, spore viability and infection levels of bee samples was determined for spring, summer and fall of 2013, as well as for spring and summer of 2014. Results showed that *N. ceranae* is the most prevalent *Nosema* species of honey bees in Ontario (91% vs 4% for *N. apis*) whereas mixed infections were less frequent than single infections (5%). Infection levels of colonies parasitized by N. *ceranae* were at least three times higher than those of colonies parasitized by *N. apis*, whereas mixed infections showed the highest spore counts. 4) *Nosema* spore viability and infection levels, as well as the proportion of infected bees are significantly higher during spring than in other seasons of the year. 5) The sampled colonies were assessed for population strength and food reserves. Results showed that low levels of *Nosema* infection do not seem to have a major negative effect on colony conditions, but very high levels (1.3-3 million spores per bee) significantly reduce bee populations and food reserves of colonies. 6) Experiments on survivorship of individual bees, with known infection levels, were conducted in the lab and it was found that *N. ceranae* reduces the length of life of bees by 16%. Experiments on bee survivorship in field colonies were also conducted and again, it was found that *Nosema* infections significantly reduce the life span of bees when infections are high. Infected bees lived 25% less than healthy bees. 7) A literature review on the effect of *Nosema* disease on bees was conducted and a preliminary economic analysis was done with

data of this project, value of hive products and information from the review. An equation was developed with this information to estimate economic losses caused by *Nosema* infections of honey bee colonies. The analysis revealed that infection levels of >750,000 spores per bee could cause a higher economic loss per hive than the cost of its treatment. This project is completed and this is the final report.

Submission number	**UofG2011-1217**		
Project Title	**Threat Assessment for the Spotted Winged Drosophila (SWD) in Southern Ontario**		
Key words	**Spotted winged drosophila, invasive species**		
Lead Applicant	**Jonathan Newman**		
Organization	**University of Guelph**		
Start Date	**2012-07-02**	**End Date**	**2015-06-30**

Abstract

The spotted winged drosophila (SWD) is potentially a very serious invasive pest species for southern Ontario. The first year of SWD in California, Washington and Oregon resulted in a $2.6 billion loss to the fruit industry. We propose to develop a threat assessment for SWD, specific to this region. We intend to integrate three kinds of data in developing this assessment: (1) capture data from OMAFRA's network of monitoring traps as well as trap data from bordering US States; (2) expansion, refinement and adaptation of a forecasting model; and (3) generation of new data for use in the development and refinement of a phenology model that may be used alone, or in conjunction with the forecasting model.

Objectives

We propose to conduct a threat assessment for SWD in southern Ontario. We will perform spatial and temporal analysis of monitoring data for southern Ontario and the surrounding US States. We will modify and refine a threat assessment model developed for the western United States and apply it to Ontario climates now and expected for the next 30 years. Finally we will develop a phenology model that can be used to assess and predict the number of generations per year that the SWD can complete in and when it establishes and how these predictions vary geographically.

Results Summary

Under ideal growing conditions, the spotted-wing drosophila is capable of doubling its population size in less than 4 days. Under its stable age distribution, only about 8% of the population exists as mature flies. This implies that trap monitoring of adults may be too late to be of much use. Ontario is at the greatest risk in Canada, compared to other fruit growing regions. This risk is similar too, and worse than, many other locations in the USA.

We determined the birth, death and development rates for the Ontario strain of the fly. We also determined the upper and lower thermal tolerances for development and reproduction. We combined the information into a mathematical model of the fly's population dynamics and used it to show that risk in Ontario, and in Canada more generally, is comparable to many locations in the US, but not as bad as some locations with more moderate climates than Ontario. We also used the model to show that climate change is likely to exacerbate the problem in Ontario but that biocontrol measures might be successful.

Spotted-wing Drosophila is like to remain a serious pest in Ontario and will perhaps get worse over the next 50 years due to the changing climate. We have developed tools that will be useful for further studies and for developing management protocols.

Submission number	**UofG2011-1031**		
Project Title	**Development of risk-based and consequence-based approaches to surveillance in swine populations using PRRS virus as a model**		
Key words	**Surveillance, PRRSV, disease-control, scenario-trees**		
Lead Applicant	**Zvonimir Poljak**		
Organization	**University of Guelph**		
Start Date	**2012-09-03**	**End Date**	**2015-09-04**

Abstract

Regional approach to control and elimination of porcine reproductive and respiratory syndrome virus (PRRSV) has recently grown in North America with respect to number of regions included, and their geographical distribution. One of the most critical issues in these projects is how to perform surveillance in the control zones. A targeted approach has a good potential to increase efficiency of surveillance, but no clear, scientifically-sound recommendations on how to do that are currently available. Our goal is to estimate "surveillance system sensitivity" and other measures of accuracy of alternative approaches to surveillance under two conditions: (1) after completion of elimination, (2) during regional control and elimination. Data for this project will be coming from the Niagara PRRSV project, and will be ultimately analyzed using stochastic scenario trees, and mathematical models. Although addressing endemic disease, the approach and expertise will be developed that could be used for substantiation of disease freedom, when resources need to be optimized; and after possible incursion of exotic diseases, when surveillance resources must be prioritized.

Objectives

1. To summarize epidemiological, biosecurity and diagnostic data from ongoing PRRS ARC&E projects in Ontario with specific purpose of informing design of targeted surveillance activities for PRRSV infection. By extension this information could also be used for design of surveillance for pathogens with similar transmission characteristics as PRRSV (ie. transmission through direct contacts via animal movement, indirect through mechanical vectors, fomites and vehicles, aerosol, and semen).
2. To estimate surveillance sensitivity, and other important parameters, of different approaches towards substantiation of freedom from PRRSV infection AFTER the elimination has been completed in PRRS ARC&E.
3. To estimate surveillance sensitivity, and other important parameters, of different approaches for ongoing surveillance of PRRSV infection in PRRS ARC&E project DURING the process of elimination.

Results Summary

Prevalence of active PRRSV circulation in Ontario herds during the study period varied regionally and over time, and was estimated to be between 17% and 48%, depending on the region. Importance of local spread and spread through transportation was determined to vary between different PRRSV genotypes. Connectivity through transportation linkages was determined to be a possible risk factor for 2 out of 3 investigated PRRSV genotypes. Local spread could have played some role for 1 out of 3 investigated genotypes.

Risk-based surveillance based solely on the biosecurity practices does not necessarily increase sensitivity of surveillance for detection of novel PRRSV genotypes. The approaches based on connectivity need to be incorporated as the next step.

The study described the demographics, biosecurity practices and ownership structure for swine sites located in different regions participating in PRRSV disease control projects in Ontario. For the

Niagara region, coordinated control efforts resulted in a 25% decrease in PRRS prevalence over the period of three years. Patterns of disease spread were also investigated and results showed no evidence for spatial dependence in the data. However, other visual representations, such as risk maps and cluster analysis, suggest the presence of high risk areas within regions, and those might be an important focus in situations where resource usage must be optimized. For disease investigations, proximity to another swine site that was positive with the same genotype was not identified as a significant risk factor. Being part of a transportation network that has at least one site positive for a specific genotype might increase the odds of a herd being positive for that genotype (particularly for G 1-8-4). Results showed that the importance of area spread and network connections were dependent on PRRSV genotype. The analyses of such networks separately and combined revealed that swine industry has high degree of contentedness through service providers. Network-related findings from this study should be considered to mitigate PRRS transmission risk, and results support the idea that interventions at the production system level would likely have the greatest impact on PRRS status of individual herds. Finally, the risk-based surveillance model developed in this work integrates the knowledge of the complex swine industry and characteristics of PRRSV transmission between herds to develop a comprehensive framework that could be used to test other hypotheses in the future regarding surveillance approaches for this and other emerging swine infectious diseases. When applied in a setting where herd type and biosecurity level were the only factors that informed targeted surveillance, the sensitivity of surveillance systems did not improve substantially. Other approaches based on connectivity of herds would have to be investigated.

Spatial dependence in the patterns of PRRS positivity were not detected for three regions in Ontario, even though defined spatial clusters could be found. An investigation of the occurrence of PRRS revealed that the importance of area spread and truck network connections were dependent on the virus genotype. Description of networks showed that the Ontario swine industry is highly connected through multiple service providers, which can represent a challenge for outbreak investigations and disease surveillance and control. Lastly, the development of two types of mathematical models (hybrid and agent-based) allowed for evaluation of herd- and regional-level control and surveillance strategies for PRRS. One of the take home messages from the herd-level model was that major PRRS outbreaks could occur in breeding herds long after the initial virus introduction, even in cases where the herd is naïve. The regional-level model suggested that contemporary approaches to implement risk-based surveillance based on site demographic characteristics (e.g. production type) do not appear to necessarily improve surveillance system sensitivity; which suggest novel strategies need to be explored to assure rapid detection of emerging PRRS virus strains.

Submission number	**UofG2011-1010**		
Project Title	**The design and analysis of experiments and observational studies on infectious disease spread in the livestock industries.**		
Key words	**infectious disease modelling; Bayesian statistics**		
Lead Applicant	**Rob Deardon**		
Organization	**University of Guelph**		
Start Date	**2012-09-01**	**End Date**	**2015-08-31**

Abstract

To control agricultural infectious diseases, it is vital to understand how they spread through populations and what factors lead to infection. Statistical models, preferably derived from high-quality observed data, such as the individual-level models (ILMs) of Deardon et al (2010), can aid greatly in such understanding. These ILMs can be spatial, contact network-based, and/or include numerous risk factors/covariates besides. They can also facilitate the design of experiments/observational studies for identifying key characteristics of disease systems.

The two overriding objectives of this project are concerned with:

1) Study Design – we plan to use ILM-based simulation studies to determine how best to design population-level and herd-level studies in which the aim is to understand underlying infection dynamics of disease systems;
2) Model Development – we plan to extend ILMs to enable the farm-level modelling of systems in which:
    a) There are multiple diseases/strains that can interact with each other;
    b) The time-varying infectiousness of individual farms is a key characteristic of the disease system in question, and can also depend upon individual-level covariates such as the number of animals on the farm;
    c) There is only partial information, and thus uncertainty, about underlying contact network structure.

Objectives

1. Develop an understanding about how best to design within-herd studies to extract maximum information about within-herd disease dynamics. (Theme 2)
2. Develop an understanding about how best to design population-level studies to extract maximum information about between farm/barn disease dynamics. (Theme 2)
3. Develop multivariate infectious disease models to enable the analysis of multiple diseases/multiple-strain disease at the farm level. (Theme 1)
4. Assess how uncertainty about potentially covariate-dependent time-varying infectiousness of individual farms can be best incorporated into analysis of epidemic data. (Theme 1)
5. Assess how uncertainty/partial information about underlying contact networks can be best incorporated into analysis of epidemic data. (Theme 1)

Results Summary

We have shown how we can design disease transmission experiments in animals, taking account of prior belief about expected results, to maximize the information the experiment gives us, and that, in some situations, very short experiments are optimal, meaning we do not have to run long and expensive transmission experiments in those situations.

We have shown the importance of devising models for analyzing data that take account of how the data was collected.

We have shown that it is possible to successfully model multi-strain disease systems with relatively simple models that are relatively quick to fit the data, as long as co-immunity mechanisms are strong.

We have shown the importance of accounting for time varying infectiousness in models, when the underlying infectiousness does indeed vary over time. We have also shown that it is possible to better estimate the nature of this time varying infectiousness when it is related to other factors; e.g., where the infectiousness of a farm as a whole depends upon the number and types of animals therein.

Finally, we have shown that, we can successfully estimate the characteristics of a simple disease system when the underlying contact network (e.g. trade routes) is unknown.

1. Designing transmission experiments. We have shown how we can design disease transmission experiments in animals, taking account of prior belief about expected results, to maximize the information the experiment gives us. We have shown that, in some situations, very short experiments are optimal, meaning we do not have to run long and expensive transmission experiments in those situations.
2. Disease surveillance. We have shown the importance of devising models for analyzing data that take account of how the data was collected. We have also shown that model estimated risk-based surveillance methods are going to be difficult to implement in practice in many situations. For example, if there is 30% rate of timely voluntary submission from farmers for disease testing in an epidemic, we have so-far found it hard to further mitigate the effect of swine influenza in a population of Ontario farms through additional, realistic model-predictive strategies.
3. Multi-strain system modelling. We have shown that it is possible to successfully model multi-strain disease systems with relatively simple models that are relatively quick to fit the data, as long as co-immunity mechanisms are strong.
4. Time varying infectious disease models. We have shown the importance of accounting for time varying infectiousness in models, when the underlying infectiousness does indeed vary over time. We have also shown that it is possible to better estimate the nature of this time varying infectiousness when it is related to other factors. A good example of this is how the infectiousness of a farm as a whole depends upon the number and types of animals therein. We have also applied these models successfully to data from the 2009 H1N1 influenza outbreak in Southern Ontario.
5. Network uncertainty. We have shown that, by using Bayesian data augmentation techniques, we can successfully estimate the characteristics of a simple disease system when the underlying contact network (e.g. trade routes) is unknown.

Submission number	**UofG2011-1205**		
Project Title	**Prevalence of Enteric Disease Agents in Ontario Commercial Rabbits: Zoonotic Potential and Impact on Animal Health**		
Key words	**rabbit, enteric disease, prevalence, risk**		
Lead Applicant	**Patricia V. Turner**		
Organization	**University of Guelph**		
Start Date	**2012-05-01**	**End Date**	**2016-08-31**

Abstract

The Ontario commercial rabbit industry produces an important alternative source of meat, and Ontario farms represent 32% of Canadian operations. Production losses from birth to weaning range up to 36%, largely from infectious enteric and respiratory diseases, and >60% of operations concurrently raise other food animal species, often in the same barns. There are few biosecurity practices in place within the industry and disease control efforts are made more challenging by industry practices including lairage prior to slaughter, transportation of live animals between provinces and to the U.S. for slaughter, and frequent sharing/sale of breeding stock. Further, off-label antimicrobial use for growth promotion and disease control is common. Preliminary industry disease surveys have identified pathogenic bacteria that may be infectious to humans and other agricultural species. In addition, newly emerging potentially zoonotic viruses have been identified in U.S. commercial rabbitries. This project will evaluate prevalence of common and emerging enteric disease agents (bacterial, parasitic, and viral), antimicrobial resistance, and potential risk to humans and other agricultural species. Our goals are to identity potential human and animal risks, improve production practices and human safety, and improve overall animal well-being.

Objectives

1. to survey the Ontario commercial rabbit industry for prevalence of viral, bacterial, and parasitic enteric disease agents from clinically healthy and affected rabbits. We will examine two age groups at two different times of the year;
2. to survey the Ontario commercial rabbit industry by questionnaire regarding on-farm euthanasia and deadstock disposal practices, antimicrobial use, and other livestock handling and management practices;
3. to characterize isolated pathogenic bacteria and evaluate for anti-microbial resistance and cross-species infection potential;
4. to develop and validate specific rabbit hepatitis E and astrovirus PCR assays for rapid, high throughput screening of rabbit tissue/fecal samples;
5. to characterize and compare the fecal microbiome from rabbits to evaluate changes in microbial shedding and resistance patterns; and
6. to disseminate information to producers to enhance herd productivity, animal health and well-being, and human health and safety

Results Summary

Our results show that of Ontario commercial rabbit farms tested, 48% were positive for astrovirus and 2 were positive for rotavirus, with no detection of rabbit HEV in 108 pooled samples from healthy rabbits. One sample from a group of commercial fryers submitted for post mortem analysis of a pneumonia outbreak were co-positive for rabbit rotavirus and hepatitis E virus. These results suggest that subclinical infection and shedding of rabbit astrovirus is relatively common in both fryers and does in Ontario commercial meat rabbits and there is no temporal difference in shedding patterns. Rotavirus was only noted on 1 farm with active diarrhea in fryers at the time of sampling and in a post mortem sample. These findings help to provide a better understanding of the prevalence of viral infections in commercial rabbit populations in Canada.

The results of our farm biosecurity survey indicate very few biosecurity practices being used routinely. Further, the AMR work in this project demonstrated that E. coli was present in at least one age group in all commercial rabbit farms, with 19% of positive samples demonstrating antimicrobial resistance to at least one class of antimicrobial agents. Salmonella spp. were identified in 5% of the commercial rabbitries, with *Salmonella kentucky* samples being resistant to several antimicrobials. These levels of antimicrobial resistance are consistent with or lower than levels in most other food animal species and suggest that Canadian meat rabbits do not pose a significant threat in terms of transmitting antimicrobial resistance to humans or other animals. However, isolation of Salmonella spp. in commercial meat rabbits raises some concerns regarding hygiene practices, cross-species bacterial contamination, and potential zoonoses, and supports the development of improved biosecurity practices on meat rabbit farms.

During the course of her research, J Kylie analyzed the results of an on-farm biosecurity survey and confirmed that these practices are poor for most Ontario commercial rabbitries. Antimicrobial resistance is moderate on rabbit farms and in-line with small ruminants. *Salmonella spp* were detected on 2 farms, likely related to transfer from other species. In addition, astrovirus is commonly isolated from commercial meat rabbit feces, although its exact role in enteric disease is unknown. Rotavirus was isolated from young rabbits with diarrhea, suggesting that it is only shed by sick rabbits. Hepatitis E was not isolated from the Ontario rabbit farms sampled in this study.

Finally, the microbiome work demonstrated significantly different types of flora in commercial meat rabbits compared with laboratory and pet rabbits, likely related to lower fibre in commercial meat rabbits. This also coincides with increased types of Gram negative bacteria in the gastrointestinal tract of commercial meat rabbits, although not necessarily known pathogenic species. How this changes intestinal mucosal immunity in meat rabbits is unknown at this time.

Submission number	**UofG2011-1177**		
Project Title	**Novel immunological compounds as an emergency response tool for control of avian influenza virus**		
Key words	**Avian influenza, control, immune enhancement**		
Lead Applicant	**Shayan Sharif**		
Organization	**University of Guelph**		
Start Date	**2012-07-02**	**End Date**	**2016-04-30**

Abstract

Control of avian influenza viruses (AIV) is of critical importance to maintenance of poultry and human health. However, there is a shortage of reliable and efficacious methods for containing AIV in chickens and disrupting its transmission from infected flocks to non-infected flocks or to humans. Although AIV vaccines are available, these vaccines have limited utility in an emergency (outbreak) situation. Moreover, after vaccination, it is difficult to differentiate vaccinated from infected birds (DIVA). Here, we will examine several microbial structural components, which induce potent anti-viral activities, to determine their efficacy to control AIV in chickens and disrupt its transmission to humans. Unlike vaccines, these novel compounds can elicit immunity within 24-48 hours of administration hence they can be used as an emergency response tool to contain an AIV outbreak. Moreover, treatment with these compounds does not interfere with DIVA. Finally, these compounds can be produced in a cost-effective manner.

Objectives

The overall objective of this study is to develop formulations of microbial products (namely PAMPs) that could be used as an emergency response tool for control of AIV during outbreaks. These products are known to induce innate anti-viral responses within a very short timeframe upon administration. Importantly, they can be produced in a cost-effective manner.

The specific aims of the study are to: 1- screen several PAMPs to identify the compounds that can control the in vitro replication of AIV, 2- investigate the effects of PAMPs against AIV infection in chickens, 3- optimize the dose and route of administration of PAMPs for enhanced efficacy against AIV replication and shedding, 4- assess the efficacy of PAMPs to control AIV in various emergency scenarios.

Results Summary

We have conducted a series of studies to screen and identify the innate immune stimulants with the highest ability of inducing antiviral responses in chickens. Two studies were conducted to address this objective, which are described below.

Macrophages play a critical role in immunity against viruses, including influenza viruses. The present study was designed to test the hypothesis that treatment of chicken macrophages with TLR ligands reduces avian influenza replication. Furthermore, we sought to study the expression of some of the key mediators involved in the TLR-mediated antiviral responses of macrophages. Chicken macrophages were treated with the TLR2, 3, 4, 7 and 21 ligands, Pam3CSK4, poly(I:C), LPS, R848 and CpG ODN, respectively, at different doses and time points pre- and post-H4N6 avian influenza virus (AIV) infection. The results revealed that pre-treatment of macrophages with Pam3CSK4, LPS and CpG ODN reduced the replication of AIV in chicken macrophages. In addition, the relative expression of genes involved in inflammatory and antiviral responses were quantified at 3, 8 and 18 hours post-treatment with the TLR2, 4 and 21 ligands. Pam3CSK4, LPS and CpG ODN increased the expression of interleukin (IL)-1β, interferon (IFN)-γ, IFN-β and interferon regulatory factor (IFR) 7. The expression of these genes correlated with the reduction of viral replication in macrophages. These results shed light on the process of immunity to AIV in chickens.

In a study, e employed an in ovo model to investigate the antiviral activities of TLR ligands. It was hypothesized that administration of TLR ligands in ovo at the appropriate dose and time can reduce AIV titer in embryonated chicken eggs. Moreover, the study aimed to determine the mechanisms involved in the TLR-mediated antiviral responses in the chorioallantoic membrane (CAM). Embryonated eggs (10-14 day old) were treated with TLR2, 4, 7, and 21 ligands using different doses and times pre- and post-AIV infection. The results revealed that treatment of embryonated chicken eggs with TLR ligands reduced AIV replication. Further analysis showed that TLR ligands induced interferon (IFN)-γ and IFN stimulatory genes in the CAM, which may have played a role in the reduction of the AIV titer. The timing and dose of TLR ligands administration had significant impacts on the outcome of the treated eggs. In conclusion, the present study demonstrated that the in ovo route may be employed to determine the antiviral characteristics of TLR ligands against AIV.

Submission number	**UofG2012-1355**		
Project Title	**The effects of in-feed zinc oxide on methicillin-resistant *Staphylococcus aureus* and *Staphylococcus hyicus* colonization in piglets post-weaning**		
Key words	**MRSA antibiotic-resistance zinc pigs**		
Lead Applicant	**Robert Friendship**		
Organization	**University of Guelph**		
Start Date	**2013-06-17**	**End Date**	**2014-06-16**

Abstract

High levels (>3000ppm) of zinc oxide are commonly used in starter pig rations for control of post-weaning colibacillosis. This strategy is particularly common in herds attempting to raise pigs without exposure to antibiotics. This practice might inadvertently be leading to the emergence of populations of swine pathogens that carry multi-drug resistance. Methicillin resistant *Staphylococcus aureus* carrying a zinc tolerance gene has been identified. Recently our research team has identified the zinc tolerance gene from methicillin-resistant *Staphyolococcus hyicus* isolated from clinical cases of exudative epidermitis (greasy pig disease). Our hypothesis is that zinc tolerance is closely tied to methicillin resistance and that the practice of feeding high levels of zinc oxide as an alternative to using an antibiotic to control E. coli is possibly causing selective pressure on bacterial populations of pathogenic bacteria like *S. hyicus* leading to disease that is difficult to treat. We will test this hypothesis by following two groups of pigs. One group fed a ration containing 3000ppm of zinc oxide and one group fed an identical ration without the zinc oxide supplement. Pigs will be closely monitored during the feeding trial for the presence of methicillin-resistant staphylococci and the presence of the zinc tolerance gene.

Objectives

The primary objective of this study is to determine whether the use of zinc oxide in swine feed affects the prevalence and antibiotic-resistance patterns *of S. aureus* and *S. hyicus* in pigs after weaning. We predict that zinc oxide inadvertently selects for multi-drug resistant staphylococci. This study will also investigate if the exposure to zinc oxide will increase the multi-drug resistance of these bacteria in addition to the acquisition of zinc tolerance as it is known that the zinc-resistance gene and other antibiotic-resistance genes are located within the same mobile genetic element. The secondary objective of this study is to determine the genetic similarity of antibiotic-resistance genes and the zinc-resistance gene between *S. aureus* and *S. hyicus*. We intend to establish a lineage of the transfer of these antimicrobial resistance genes in these staphylococcal species.

Results Summary

Staphylococcus aureus isolated from pigs often carries genes associated with both methicillin resistance and zinc resistance and that these resistance genes are linked together so that selective pressure for one results in the emergence of bacteria carry resistance to both.
The use of therapeutic levels of zinc oxide (>2000ppm in feed) causes selective pressure that results in the emergence of MRSA in weanling pigs in the absence of antibiotics

The commonness of a zinc and cadmium resistance gene (czrC) among methicillin-resistant *Staphylococcus aureus* (MRSA) of porcine origin is concerning as nursery pigs are frequently exposed to therapeutic doses of in-feed zinc oxide (ZnO). The objective of this investigation was to determine whether MRSA carriage in pigs is influenced by exposure to therapeutic doses of in-feed ZnO (3,000 mg/kg) when compared to the recommended dietary levels (100 mg/kg). A randomized-controlled trial was completed using 110 pigs that were naturally colonized with czrC-positive MRSA. The pigs were followed from birth to weaning (21 d), at which point they were randomized

into 8 pens and exposed to either a control feed (100 mg ZnO/kg feed; n=49 pigs) or a treatment feed (3,000 mg ZnO/kg feed; n=50 pigs); neither feed contained additional antimicrobials. MRSA carriage was monitored weekly in each group for 4 weeks post-weaning. The prevalence of MRSA was significantly higher in the treatment group at 1-week (OR=18.1; P=0.007) and 2-weeks (OR=3.01; P=0.015) post-weaning when compared to the control group, but there was no difference later in the nursery phase. Persistent MRSA carriage (testing positive ≥2 times post-weaning) was observed in 2% (1/49) of control pigs and 22% (11/50) of treated pigs (P=0.004). All recovered MRSA isolates (spa types t034 and t3075) carried czrC and showed uniform resistance to zinc. These findings demonstrate that the prevalence and persistence of MRSA in nursery pigs can be affected by the use of high levels of in-feed ZnO in the absence of antibiotics. In a second study we visited 30 Ontario pig farms of various sizes and types including 8 farms that used no antibiotics. Approximately half the farms were positive for MRSA, including all the farms that were antibiotic-free. All farms where MRSA were isolated used therapeutic levels (>2000 ppm) of zinc oxide in the starter feed. The MRSA found on these farms carried the zinc resistant gene as well as the methicillin resistant gene. These two genes are located on the same mobile genetic element and often are acquired together. The use of antibiotics was not found to be related to the presence of MRSA. The likely explanation for these findings is that the use of zinc oxide at high levels (typically to control post-weaning diarrhea) creates selective pressure so that the resistant staphylococci (MRSA) thrive. The use of zinc oxide is widespread because it is an effective therapeutic agent but this work suggests that alternative treatments to antibiotics such as zinc, can be the reason antimicrobial resistance persists in the absence of antibiotic pressure. The control of antimicrobial resistance is more complicated than the removal of antibiotics from pig feed.

Submission number	**UofG2012-1327**		
Project Title	**Assessment of the Distribution and Natural Enemies of the Brown Marmorated Stink Bug in Southern Ontario**		
Key words	**Brown marmorated stinkbug, survey**		
Lead Applicant	**Cynthia Scott-Dupree**		
Organization	**University of Guelph**		
Start Date	**2013-05-01**	**End Date**	**2015-04-30**

Abstract

Brown marmorated stink bug (BMSB) is an invasive pest native to subtropical and temperate areas in East Asia. Up to 300 hosts have been reported in the literature, including important fruit, vegetable and agronomic crops, and ornamental trees and shrubs. Where established, BMSB causes significant widespread economic losses in affected crops. Chemical controls have not been effective, and appear disruptive to established IPM programs in targeted crops. Zhu et al. (2012) forecasted the potential geographic spread of invasive BMSB based on climatic requirements and current distribution of this species in Asia, and suggested areas most at risk included north eastern North America. Since 2001, BMSB has been detected in 39 US states as single finds in private residences and as established populations. The first official records of BMSB in Ontario occurred in 2010 (Fogain and Graff, 2011). In August 2012, an established breeding population was confirmed in Hamilton, Ontario. Early detection is critical in mitigating potential damage. This project will assess the distribution and abundance of BMSB in southern Ontario; identify habitats suitable for BMSB build-up and associated agricultural areas at high risk for damage; and develop an inventory of BMSB natural enemies in southern Ontario.

Objectives

We propose to conduct a study on BMSB by:

1. Assessing the distribution and abundance of, and patterns of host use by BMSB in southern Ontario;
2. Identifying agricultural areas in southern Ontario at risk from BMSB impact; and
3. Developing an inventory of natural enemies of BMSB that exist in southern Ontario to determine whether endemic parasitoids and predators of *Pentatomidae* use BMSB as a resource. This will provide baseline data on the potential for augmentative biological control of BMSB in Canada.

To facilitate knowledge transfer on the status of BMSB in Ontario, we propose to:

4. Develop information for use in communications including websites (e.g., ontario.ca\stinkbug, stopBMSB.org), newsletters, tweets / blogs, conferences, online tools for IPM (e.g., CropIPM), outreach to traditional (i.e., grower) and non-traditional (e.g., homeowner, botanical gardens, pest control companies and tourism) stakeholder groups.

Results Summary

1. BMSB is an invasive insect pest, first found in Ontario in 2010, that has 170 North American plant hosts, including important fruit, vegetable and field crops, as well as landscape trees and shrubs.
2. BMSB in the northeaster US has been responsible for dramatic economic losses to a number of crops since it was first detected in Pennsylvania in the early 2000's.
3. BMSB is established in southern Ontario and steadily expanding its range into agricultural areas noted for growing many of the crops that are preferred by this invasive insect pest.

BMSB has now been either detected or established in 27 locations across southern Ontario. The presence of high populations of BMSB in urban areas, where homeowners are reporting damage to landscape plants, and garden fruits and vegetables, poses a risk of spillover into nearby agricultural areas. BMSB presence in proximity to tree fruit orchards and grape heightens grower concerns. Continued survey and identification of BMSB spread in Ontario is needed to facilitate response and ensure implementation of management strategies limiting crop damage. BMSB adults were observed exiting overwintering sites (urban homes) on May 10th. A mated female was collected at a Hamilton park May 29th (Laid eggs = June 13; Hatch = June 22). Numbers were low until late July, when 2nd and 3rd instar nymphs were observed on honeysuckle. BMSB were also observed on other landscape hosts including buckthorn, ash, Manitoba maple, locust, rose, catalpa, butterfly bush, dogwood, rose of Sharon, walnut and hosta. Based on reports to the Agriculture Information Contact Centre (AICC), BMSB began to move to overwintering sites in late September to late October. BMSB adults were found in pheromone traps at 9 agricultural sites (Beamsville-2, Essex, Hamilton/Hannon, Niagara-on-the-Lake, Smithville, St. David's, Waterdown-2) and at 4 urban sites (Hamilton, London, Newboro, Niagara Falls). Nymphs were trapped on a mixed fruit farm near St. David's. Signs of feeding injury were found at 2 apple farms in the Hamilton/Waterdown areas. Established breeding populations were found for the first time in London, St. Catharines and Windsor, following a series of confirmed homeowner finds. Homeowners reported damage to landscape and garden plants to the AICC. There have been 300 positive locations, with many others from "hot" areas that we know are positive (about 1600 emails and many phone calls). Of concern is the abundance and increasing number of sites in Niagara region along the corridor from Hamilton-Stoney Creek-Grimsby-Beamsville-St. Catharines- Niagara on the Lake due to the high density of tender fruit, grape and apples. In the north eastern US where these crops are grown high losses have occurred as a result if BMSB infestations. To date no BMSB have been found near or in corn and soybeans in southern Ontario. In 2014, 32 naturally-occurring *Pentatomidae* egg masses (614 individual eggs) were collected from host plants at 20 sites across southwestern Ontario. The egg masses were reared to obtain stink bug nymphs and/or parasitoids associated with the *Pentatomidae*. Emerged nymphs were identified to species based on their morphology and used to establish laboratory colonies. Emerged parasitoids were identified to Genus based on morphology and then preserved in 95% ethanol for molecular analysis. All samples are awaiting processing using molecular techniques to identify host and parasitoid DNA associated with each egg.

Submission number	**UofG2012-1292**		
Project Title	**Efficacy and cost-effectiveness of control strategies for newly emerging forms of swine dysentery**		
Key words	**swine dysentery, economics, modelling, brachyspira**		
Lead Applicant	**Zvonimir Poljak**		
Organization	**University of Guelph**		
Start Date	**2013-09-02**	**End Date**	**2015-08-28**

Abstract

The primary objective of the proposed research is to develop and parameterize a mathematical disease spread and control model for swine dysentery. Another objective is to develop economic model for different interventions and link the epidemiological and economic model. Probabilistic sensitivity analysis will be performed to identify factors that are most contributing to clinical efficacy and economic efficiency. This will be achieved by analyzing the field weekly mortality data from outbreaks in herds affected by this emerging pathogen. Then mathematical model of spread under baseline and common control conditions will be developed, and simulations will be performed. Data will then be analyzed for probabilistic sensitivity analysis for mortality and production parameters. Following this, an economic model will be developed. Inputs for the economic model will be sought from closeout production data before and after an outbreak, by eliciting expert opinion in a formal way, and by providing summary measures from meta-analysis. An economic model will be based on linear programing as the first step, and by developing framework for the Multiple Criteria Decision Making approach. A mathematical model of *Brachyspira* spread and economic model will be coupled during this process to reflect the dynamic nature of disease spread.

Objectives

Our objectives could be divided into two general themes. The first theme consists of specific objectives as they relate to this emerging disease. Our specific objectives are to:

1. Build and parameterize mathematical model of *Brachyspira hyodysenteriae* spread in a herd of growing pigs in the absence of any intervention, and additionally when common intervention strategies are applied.
2. Perform probabilistic sensitivity analysis to determine which factors contribute most to mortality, and to overall production losses.
3. Couple the mathematical model of disease spread with the economic model and determine which factors involved in disease ecology itself, or in the way that disease control measures are implemented would mostly contribute to the benefit-cost measures.

A more general objective is to establish mechanisms by which cost-efficiency of within-herd control measures should be studied for infectious diseases of swine. This will be achieved by collaboration within the University and internationally.

Results Summary

Records for injectable treatments could be used to study epidemiology of swine dysentery, under certain conditions. This was utilized in this project.

We found that linomycin was more likely to be used in population of pigs that grew during colder period of the year. This is in line with epidemiology of disease. Under simulation conditions, duration of infectiousness was the factor that was most correlated with the rate of mortality and could be a factor that we need to focus on in order to decrease mortality. Under simulation conditions, the most effective treatment was tiamulin, closely followed by lincomycin treatments.

Data from treatment records from 19 cohorts of a 1500-head grower-finisher barn were analyzed using descriptive statistics and Poisson regression to determine factors associated with rates of injectable treatments for tiamulin and lincomycin. Serial interval and reproductive numbers were extracted.

Treatment rates displayed marked seasonality. The highest rate of usage for lincomycin was in winter. Interestingly, winter was the season with the lowest usage for tiamulin. The serial interval was 17 d (sd=5.25) with variability observed among batches. In many cohorts the effective reproductive number did not exceed 1 and the highest estimate was 2.15. Average days to first treatment was 4.8 d which suggests that pigs were infected at time of entry.

In the deterministic model with parameter uncertainty, the strongest positive correlation with total SD mortality was the duration of clinical infectiousness for two of the models and the fraction of animals developing clinical disease for the third model. All three models showed a weak correlation between the duration of immunity and total mortality due to swine dysentery. Thus, interventions that target and influence the duration of infection in clinically-ill animals, and influence the fraction of animals that develop clinical disease should have the highest impact on mortality due to SD. Whereas interventions that influence the duration of immunity are unlikely to have an effect on SD mortality.

For the stochastic model with treatments involved, a compartmental model was developed to describe the infection and clinical disease in a population of pigs experiencing swine dysentery in the grower and finisher stages of production. Variations of this model described the progression of the disease in populations where clinical cases were treated with injectable lincomycin, tiamulin, and tylosin. In addition, disease progression was studied in populations with no treatment administered. Costs due to the burden of disease and production costs were incorporated in each variation of the model to determine the net loss in each model. The lincomycin model closely represented the shedding patterns of *Brachyspira* during the growing stage and the lincomycin treatment usage observed under field conditions, but only when the proportion of clinical morbidity was 5%. All model variations in this study did not yield the severe losses due to this disease as was seen from a historical perspective, except for the model that assumed that 25% of infectious cases develop clinical disease. Expected mortality due to SD for lincomycin (median=5, min=0, max=14 pigs), and tiamulin (median=5, min=0, max=12 pigs) were very similar, and numerically higher for tylosin (median=8, min=0, max=20 pigs). Under the assumptions used, the treatment that was expected to be most cost-effective was the application of tiamulin, which was influenced by the cost of treatment.

Seasonality was indicated for injectable treatments for lincomycin and tiamulin, both of which are suggestive of swine dysentery (SD). Lincomycin tretament rates were highest in the winter months. When adjusted for outliers, season showed no effect on tiamulin usage. The effective reproduction number estimated from treatment records was relatively low and, in many cohorts, did not exceed 1. The parameters from experimental studies were used in combination with dynamic models to replicate the progression of *Brachyspira* infection and clinical disease in individual pigs in a closed population. Strong positive correlation was seen with the duration of the infectious period in clinically affected animals and the fraction of animals that will develop clinical disease. Using parameters obtained from the literature, a stochastic model was developed that incorporated treatment effectiveness on average daily gain (ADG) and mortality and cost of treatment and production. The model output did not suggest severe losses would result from disease, except for the model variant that assumed high risk for clinical morbidity. The lincomycin treatment model variant was reflective of the results of the *Brachyspira* shedding patterns during the growing production stage. The most cost-effective treatment under simulation was tiamulin. Differences among effective treatments were low.

Submission number	**UofG2012-1329**		
Project Title	**Diseases threatening vegetable crops in Ontario**		
Key words	**Fusarium, Stemphyllium, carrots, onions, spinach**		
Lead Applicant	**Mary Ruth McDonald**		
Organization	**University of Guelph**		
Start Date	**2013-05-01**	**End Date**	**2016-04-30**

Abstract

Two new plant pathogens or pathogen groups have become an issue for growers of vegetable crops in Ontario. Stemphyllium blight, caused by the fungus, *Stemphyllium vesicarium*, was first identified on onions in 2009 and has become the most serious foliar disease of onions since then. This fungus also attacks the spears of asparagus and has recently become a serious problem.

Another new disease, Fusarium root rot, was recently identified on carrots in the Holland Marsh. A related disease, Fusarium wilt of spinach, has been present in Ontario for some time, and spinach is a good model crop for conducting research on root diseases. The proposed research includes surveys of carrot and onion fields in Ontario to determine how extensive these diseases are. More importantly, replicated field trials will be conducted to determine the most effective fungicides or biological controls for these diseases on onions, asparagus, carrots and spinach. Carrot and spinach cultivars will be screened for resistance or tolerance. Trials will be conducted with onions and asparagus to determine the best time to start the spray program and if *Stemphyllium* from onion will infect asparagus, and vice versa. Results will be communicated to growers.

Objectives

1. To survey southern Ontario for the incidence and severity of Stemphyllium on onion and Fusarium on carrot.
2. To determine if *Stemphyllium* spores from asparagus can infect onion and if *Stemphyllium* spores from onion can infect asparagus. Also, to determine if *Stemphyllium* from either crop can infect common weed species.
3. To conduct replicated field trials to determine the most effective crop protection materials (fungicides, biological controls and biorational materials) to control or suppress Stemphyllium on onions and asparagus and Fusarium on carrots and spinach.
4. To determine the optimum spray timing for the control of Stemphyllium on asparagus and onions, based on monitoring the microclimate and (for onions) spore trapping.
5. To identify cultivars of carrot and spinach with resistance or tolerance to these diseases.
6. To determine the optimum methods for managing the crop residue in asparagus to prevent the overwintering of Stemphyllium in asparagus fields.

Results Summary

Fusarium wilt of spinach can be managed by selecting tolerant cultivars and, when necessary, fumigation with chloropicrin.

Fusarium root rot of carrot was only found in some fields in the Holland Marsh. Only fumigation with moderate to high rates of fumigation reduced disease severity. Fungicide and biological controls were not effective.

Disease forecasting is very effective for managing Stemphylium on asparagus fern. The fungus from asparagus can infect onions, and vice versa.

Stemphylium blight of onion remains difficult to control. Fungicides are only moderately effective.

Fusarum wilt of spinach: Some spinach cultivars were less susceptible to the disease. However, under high disease pressure and high temperatures, none had below the economic threshold of 10%

disease. No fungicide or biological seed treatments reduced disease compared to the untreated check. Only fumigants and a high rate of nitrogen fertilizer reduced disease to an acceptable level. The biological controls, MustGrow and compost, were not effective.

Fusarium root rot of carrot: Some differences in susceptibility of carrot cultivars was identified but all were susceptible to the disease. No fungicides or biological controls were effective. Band fumigation with choloropicrin at moderate and high rates reduced disease incidence, but did not eradicate the pathogen from soil. Fusarium root rot was only found in some fields in the Holland Marsh and was not found in other carrot-producing regions of Ontario.

Stemphylium leaf blight of onion: Differences in cultivar susceptibility were identified, but these may be more related to cultivar maturity than to genetic resistance to the disease. The fungicides that were screened were only partially effective in reducing Stemphylium blight of onion. Disease forecasting reduced the number of fungicide applications from 10 to 6, but there were no differences in yield, suggesting that a grower would be best to not apply fungicide. Ascospores (sexual spores) of the fungus were found in high numbers early in the growing season. After about one month, conidia (the asexual spores) were caught in high numbers and the ascospores were no longer present.

Disease development appears to be associated with rainfall. In a year with limited rainfall, very few spores were found on traps and disease developed later in the season, even though information on leaf wetness suggested that the disease should be present on the leaves. Stemphylum leaf blight was found on all onions in Ontario, in the years that the survey was conducted (2013 and 2014). Stemphylium on asparagus: Spores of the Stemphylium fungus from asparagus could infect and cause disease on onions and vice versa. There were differences in aggressiveness among the isolates from both crops. Site specific disease forecasting, using the Tomcast program, was effective in controlling Stemphylium on asparagus fern with fewer fungicide sprays (4 instead of 6). Applying higher rates of fertilizer may also help to reduce disease severity

The plant disease, Stemphylium leaf blight of onion, is widely spread in onion production areas of Ontario, while Fusarium root rot of carrot has only been found in some fields in the Holland Marsh region. Fumigation was the most effective method of controlling Fusarium wilt of spinach and Fusarium root rot of carrot. High rates of slow release nitrogen may also control Fusarium wilt of spinach. No fungicides or biological controls were effective for control of either disease. Less susceptible cultivars of both crops were identified, but all were susceptible under high disease pressure. Site-specific disease forecasting using the Tomcast program was very effective for controlling Stemphylium on asparagus fern and reduced the number of fungicide sprays by 33%, from 6 to 4. Asparagus cv. Millenium was more susceptible than Jersey Giant. Stemphylium leaf blight of onion remains difficult to control. Disease forecasting for onion was less effective, possibly because none of the fungicides tested were very effective for the control of the disease. Disease forecasting reduced the number of fungicide sprays from 10 to 6, but this only provided about 25% reduction in disease severity. Spore trapping did not improve disease forecasting.

Submission number	**UofG2012-1381**		
Project Title	**Prevalence and strain identification of *Coxiella burnetii* on dairy goat farms and in associated wildlife**		
Key words	**Q fever, wildlife, *Coxiella burnetii***		
Lead Applicant	**Paula Menzies**		
Organization	**University of Guelph**		
Start Date	**2013-05-09**	**End Date**	**2015-04-30**

Abstract

Based on recent AHSI - OMAFRA research, infection due to *Coxiella burnetii* appears to be common in dairy goats (33 of 42 farms studied) and in the people that care for them (68.7% of people sampled). To control Q fever in humans, it is important to understand the epidemiology of the infection in non-livestock species that reside on and near the livestock premises and thus the risk that may be posed to the farm. Through selection of previously test positive farms (n=10), we will determine the prevalence and strain types of *C. burnetii* infection in wildlife species (e.g. rodents, raccoons, rabbits) trapped on these farms and in nearby non-agricultural areas. The prevalence and strains will be compared to those found in the goats, and non-livestock domestic species (e.g. cats, dogs) living on the affected farms. This will be done by PCR of fecal samples and / or vaginal swabs in the periparturient period and subsequent genotyping. This information will help to identify possible reservoirs of *C. burnetii*. Subsequent research regarding methods to eradicate infection from infected farms will use this information in developing strategies to be evaluated.

Objectives

1. To determine the prevalence of *C. burnetii* in wildlife species trapped on dairy goat farms and in nearby non-agricultural areas.
2. To determine strains and strain differences of *C. burnetii* in livestock species, non-livestock domestic species, and wildlife species on dairy goat farms and non-agricultural areas.

Results Summary

- *Coxiella burnetii* is most frequently shed by early postpartum goats from vaginal secretions, compared to milk and feces.
- The prevalence of *Coxiella burnetii* infection in wildlife is not different between those trapped on dairy goat farms and those trapped in natural areas in the same region.
- The strain of *C. burnetii* found in goats, was also found in some samples from dogs, cats, raccoons and eastern chipmunks.
- Control of *C. burnetii* infection in dairy goat farms should consider the risk of reinfection from wildlife reservoir species either found on farm or in nearby natural areas.

The objectives were to: 1) determine the prevalence of *C. burnetii* in wildlife and domestic animals on dairy goat farms and natural areas, 2) investigate the spatial prevalence of *C. burnetii* in southern Ontario, and 3) investigate the potential role of wildlife in the transmission dynamics of *C. burnetii*. If wildlife are acting as spillover hosts exposed to *C. burnetii* via infected livestock but not able to independently maintain the infection, then I predict that C. *burnetii* will occur only in wildlife living in close association with infected livestock. Alternatively, if wildlife are able to maintain *C. burnetii* infection independent of livestock, then I predict that *C. burnetii* will also occur in wildlife in natural areas. From April-August 2014, genital, fecal and milk samples were collected from goats on 16 Ontario farms. Feces and genital swabs were collected from other residents (19 cats, 4 chickens, 6 cows, 13 dogs, 5 horses, 2 pigs), and wildlife (167 deer mice, 20 house mice, 3 opossums, 86 raccoons, 3 red-backed voles, 14 red squirrels and 2 skunks) live-trapped on-farm and from 14 natural areas. *Coxiella burnetii* was detected by PCR in samples from 89.2% (404/453) of goats,

68.8% (33/48) of other farm animals, 64.7% (44/68) of wild animals sampled on farms, and 58.1% (165/284) of wild animals sampled in natural areas. *Coxiella burnetii* was detected at all study sites and the prevalence in wildlife was not statistically different between farms and adjacent natural areas, independent of site distances. These findings suggest that wildlife may form part of the *C. burnetii* reservoir in Ontario, Canada.

To date, there is no suggested sample type for the detection of *C. burnetii DNA.* The objectives of this study were to: 1) compare the prevalence of *C. burnetii* in different sample types from dairy goats and wildlife; and 2) assess the level of agreement among these sample types. Genital, fecal and milk samples were collected from 368 goats on 16 Ontario dairy goat farms, and fecal and genital samples were collected from 248 animals representing five wildlife species that were live-trapped on farms and 14 adjacent natural areas. It was determined that genital and fecal swab samples were the optimal sample types to use for the detection of *C. burnetii* DNA in deer mice, eastern chipmunks and raccoons, yielding the highest proportion positives. Genital swab, fecal swab and fecal material sample types were not significantly different from one another in detecting *C. burnetii* DNA in house mice and red squirrels. Genital swab sample yielded significantly higher proportion positives and thus, were determined the optimal sample type for detecting *C. burnetii* DNA in recently kidded dairy goats.

Coxiella burnetii infection is common in wildlife trapped on both dairy goat farms and in nearby naturalized areas. The prevalence in wildlife is not different between these two regions. This suggests that wildlife (raccoons, deer mice, eastern chipmunks) may be a potential reservoir for livestock infection. When measures are taken to control Q fever risk in dairy goat herds, veterinarians and producers should understand that the risk of reinfection may be substantial from wildlife. Eradication of wildlife from a farm would not likely change that risk as infection may be reintroduced from nearby natural areas. More work needs to be done with respect to the strain of *C burnetii* present in these wildlife species and if strain pathogenicity may be different.

Submission number	**UofG2012-1296**		
Project Title	**Optimal Design and Configuration of Detection Strategies for Foreign Pests and Diseases to Support Emergency Management in the Agricultural Sector**		
Key words	**detection methods, EAB, cost-effective**		
Lead Applicant	**Alfons Weersink**		
Organization	**University of Guelph**		
Start Date	**2013-06-02**	**End Date**	**2015-05-29**

Abstract

Pre-event and event components of emergency management for problems caused by animal and plant pests or diseases require understanding the extent of the outbreak. The underlying organisms are often cryptic and multiple choices exist for efforts assessing the spatial existence and intensity of the disease. These efforts involve different levels of resources and different expectations regarding the level of information received. Relatively cheap but imprecise search methods or more extensive, costly approaches with greater accuracy could be used to determine the extent of the outbreak. This work will provide a theoretical depiction of the problem regarding the means to obtain information on the extent of an outbreak associated with an animal or plant disease. The issue will be illustrated for an emerging insect problem for which good quality data is available (i.e. the Emerald Ash Borer). The study will help identify cost-effective information gathering strategies to deal with this present problem and also provide a knowledge base to better deal with future emergency management issues related to plant and animal pests and diseases in the agricultural sector.

Objectives

The purpose of this study is to enhance the decision-making capabilities of public policy decision makers facing a plant or animal pest and disease outbreak. The specific objectives are:

a) To develop a theoretical model highlighting the factors influencing the optimal choice of information gathering techniques on the spatial distribution and intensity of the pest and disease;
b) To identify emergency management planning best practice techniques addressing this issue in other jurisdictions;
c) To develop a decision-making tool for policy makers that determines the cost-effective approach to gather information on the pest and disease outbreak;
d) To evaluate the current range of detection choices for dealing with EAB;
e) To illustrate the approach for the optimal design and configuration of information gathering strategies for another potential, emerging pest and disease outbreak; and
f) To link up with other OMAFRA projects dealing with disease management to provide a comprehensive evaluation perspective.

Results Summary

The net benefits of the EAB detection methods are evaluated based on a qualitative analysis of their relative characteristics from the perspective of a municipal-level decision maker. Visual inspection is classified as low-cost due to its low equipment and training needs, and its information as low-value because it largely detects only late-stage infestations. Traps provide earlier warning of EAB presence, but not information on individual tree infestation. Because of the importance of early detection, it is considered here to provide more valuable information to a municipality than visual inspection. Trapping has equipment costs and is labour intensive, making it a higher cost option than visual inspection. Funnel traps to have produced superior results in some studies, but very inconvenient to deploy and store. Branch sampling and girdled trees provide specific information on

the infestation status of individual trees, at an earlier stage of infestation than other methods, but are more time intensive and require specialized training. Tree girdling, due to the loss of the tree, is classified as more costly here. Probability of false negatives affect the expected value of the trap, that is, the aggregate value of the information produced, including the possibility of suboptimal decisions. It is important to note that the positioning of detection methods in this framework is subjective to the needs and restrictions of the decision maker. In this case, the decision maker is a municipality that places a premium on early detection. As another example, a large-scale decision maker is not as likely to value highly specific information at the same premium as a municipality, placing branch sampling or a trap tree lower on the value axis. In contrast, an individual tree owner is likely to derive comparatively less value from a trap, which lacks the ability to confirm infestations of specific trees. Using a trap tree may produce very little value due to the resultant tree mortality. Visual inspection may provide higher value if performed accurately, but branch sampling may be the only effective option. The positioning of the detection methods suggests no unequivocally superior option, which would be the case if one of the detection methods was low cost/high value. Instead, there are trade-offs between the costs of obtaining the information versus the benefits from improved decision making. Thus, the actual choice of detection method requires an empirical analysis that could be guided by the conceptual framework developed. The research has now shifted to examine the cost-effective control strategies for PED in swine herds. A model that estimates weekly number of pigs by population cohort on the basis of assumed production parameters has been developed. While being used to examine PED impacts, the model can also be used to assess other diseases.

Submission number	**UofG2012-1365**		
Project Title	**Fate and Transport of Emerging Contaminants after Manure Application**		
Key words	**STEC, antibiotic resistance, anaerobic digestion**		
Lead Applicant	**Kari Dunfield**		
Organization	**University of Guelph**		
Start Date	**2013-06-01**	**End Date**	**2016-04-30**

Abstract

The prevalence of emerging pathogens such as non-O157 Shiga toxin producing *Escherichia coli* in livestock manure is poorly understood. Further, the fate of these pathogens in soil and tile water after land application of manure is unknown so the threat of contamination of water used for irrigation purposes exists. This project will assess the prevalence and fate of 6 non-O157 serovars of *E. coli* in dairy manure, and will compare their environmental persistence from fresh or anaerobically digested manure that is land applied at 2 different field sites. The results of this study will identify the potential threat of several emerging pathogens to animal and human health and give insight into the role of the environment as a potential reservoir of infectivity. Knowledge gained can be used to institute best management practices for livestock manure management, as well as to improve emergency management plans by providing insight into the risks raised by emerging pathogens of interest. This project will add a microbial analysis of emerging pathogens to two ongoing studies examining the environmental impacts of manure application.

Objectives

1. Assess the prevalence of 6 non-O157 Shiga toxin producing *Escherichia coli* serotypes (O26, O45, O103, O111, O121, O145) in fresh dairy cattle manure and in manure after anaerobic digestion.
2. Compare the prevalence of emerging pathogens (Obj. 1) to traditional human pathogens (i.e. *E. coli, Salmonella, Listeria monocytogenes*) and fecal indicators. (Funded through matching California Centre for Produce Safety – no funds from OMAFRA requested).
3. Determine the persistence of the pathogens of interest in soil and tile water after land application of either fresh manure or digestate.
4. Compare the persistence and transport of pathogens (Obj 3) to transport of nutrients and greenhouse gas emissions in the same field plots receiving either fresh manure or digestate. (Funded through AGGP and Dairy Farmers of Canada - no fund from OMAFRA requested).
5. Determine the prevalence of antibiotic resistance markers in raw manure, digestate, and soil/water samples after field application.

Results Summary

Results of Field Trial Year 1 are difficult to evaluate due to issues with experimental methodology. All methods have now been worked out and evaluated, but results from the initial trial were inconclusive. We did observe a general reduction in STEC placed in the soil, but we also observed persistence over the course of the 1 month study. The persistence observed was likely due at least in part to the cold weather conditions in November/December 2014. As such, the first trial is being considered a step in method development and validation.

In a lab-scale microcosm trial, we evaluated survival of *E. coli* O157 and O111 in soil with various amendments, including fresh dairy manure, sterile dairy manure, digestate, and no amendment. Results of this study are currently being statistically analyzed.

Submission number	**UofG2013-1735**		
Project Title	**Fate of invasive and herbicide resistant weed seeds after anaerobic digestion**		
Key words	**biogas, biomass, biosecurity, switchgrass, Phragmites**		
Lead Applicant	**Brandon Gilroyed**		
Organization	**University of Guelph**		
Start Date	**2014-06-02**	**End Date**	**2015-10-30**

Abstract

Anaerobic digestion (AD) is an important component of Ontario's developing bioeconomy. Feedstocks for AD systems include biomass crops and agricultural residues which contain seeds, some of which may be invasive weeds or carry herbicide resistant traits. Dissemination of invasive, weedy, or herbicide resistant plants in the agricultural ecosystem is undesirable for economic and environmental reasons. Due to the massive quantities of feedstock required for commercial AD operation, such systems concentrate material from a variety of sources which increases potential biosecurity risks. Seeds that enter the AD process and survive will subsequently be applied to agricultural land as part of the nutrient-rich digestate used as fertilizer. Although it has been previously established that seed survival is reduced during AD, most experiments to date have been conducted using lab scale equipment and have not focused on several species of interest in Ontario. We propose to investigate the survival of seeds from several perennial grass biomass species, common weed species, and weed species with demonstrated herbicide resistance, in an operating 250 kWe commercial AD system. The expected outcomes include an assessment of the risk posed by weed seeds entering AD systems, enabling prioritization of management strategies and development of BMP's for AD feedstocks.

Objectives

The overall objective of the project is to determine the risk that seeds from plants which are invasive, weeds, and/or herbicide resistant pose on agricultural land after AD in a commercial scale digester. Specific objectives include:

1. Determine seed survival of biomass crop seeds, including *P. virgatum*, *P. arundinacea*, and *P. australis*, in a commercial scale AD system
2. Determine seed survival of several weed species, including those with demonstrated herbicide resistant traits, such as giant ragweed, common ragweed, Canada fleabane, redroot pigweed, velvetleaf, green foxtail, lamb's quarters, and barnyard grass
3. Distinguish between the effects of anaerobic microbial degradation and time-temperature reduction in seed viability
4. Compare and contrast laboratory germination and tetrazolium staining to greenhouse germination and cold-moist stratification as tests for seed viability and dormancy

Results Summary

1. Anaerobic digestion reduced the viability of most plant seeds to zero after
2. Reduced seed viability can mainly be attributed to the effects of microbial activity in digestate, and not operating temperature of the digester.
3. Assessment of seed viability using either germination plus tetrazolium staining, or germination using cold/most stratification cycles in a greenhouse, provided similar results for all species tested.

We assessed two methods for determining seed viability after anaerobic digestion. In the first method, we germinated groups of 100 seeds aseptically in a growth chamber for 2 weeks. Seeds that did not germinate in 2 weeks were tested for dormancy using a tetrazolium staining technique.

Overall viability was determined by adding the number of germinated seeds with the number of positively stained seeds. In the second method, we surface planted groups of 500 seeds onto trays filled with potting mix and placed into a greenhouse to germinate. After 2 months in the greenhouse, trays were cold-moist stratified at 5˚C for 2 months, and this cycle was repeated three times to simulate 3 years of seasonal fluctuations. We found that both methods of assessing germination were very comparable for all species tested (phragmites, reed canary grass, switchgrass, and tomato).

We have discovered that there are differences in survival times based on the species of seed being tested. In our first set of experiments, we studied seeds from phragmites, reed canary grass, switchgrass, and tomato. Of these seeds, tomato was found to be the most durable. However, seed viability was reduced to 0 for all species within 7 days of exposure to anaerobic digestion. Our second set of experiments focused on additional plant species, including: *Echinochloa crusgalli, Conyza candensis, Ambrosia artemisiifolia, Amaranthus retroflexus, Chenopodium album, Sinapis arvensis, Abutilon theophrasti*, and *Hordeum jubatum*. Because of the large numbers of seeds and some species having seeds too small for effective tetrazolium staining, we were only able to monitor germinability and not viability of seeds. In this study, all seeds demonstrated total loss of germinability within 7 days of exposure to anaerobic digestion except for *A. retroflexus* (redroot pigweed) and *C. album* (lamb's quarters), which required up to 3 weeks of exposure to eliminate all germinability. The take home message from both studies is that plant seeds are rapidly made non-viable during anaerobic digestion. Biogas facilities typically operate at mesophilic temperatures in the 35-40˚C range. We wanted to determine if the observed reduction in seed viability over time was due to temperature alone, or whether the response was also due to biological factors. We performed an experiment where seeds were aseptically exposed to 38˚C temperatures in water, and compared those results to seeds placed in the biogas facility which was also operating at 38˚C. We found that the temperature-only seeds did not have reduced viability based on exposure time. This indicates that the biological (microbial) factors present in the biogas facility are necessary to explain the reduction in seed viability observed in our study.

We assessed viability of seeds using two different methods: germination plus tetrazolium staining, and germination using extended cold/moist stratification cycles in a greenhouse. Both methods gave similar results, though tetrazolium staining was labour intensive, unsuitable for small seeds, and susceptible to variance based on investigator skill and experience.

Submission number	**UofG2013-1622**		
Project Title	**Responsible antibiotic use on Ontario dairy farms – approaches, outcomes and attitudes**		
Key words	**dairy cattle antibiotics attitudes outcomes**		
Lead Applicant	**David Kelton**		
Organization	**University of Guelph**		
Start Date	**2014-05-01**	**End Date**	**2017-04-30**

Abstract

The recent Ontario Medical Association white paper entitled 'When antibiotics stop working' has brought the use of antibiotics in animal agriculture under scrutiny. Mastitis is the most costly disease of dairy cattle, and also the target for the greatest antibiotic use on dairy farms. The implementation of the Canadian Quality Milk (CQM) program has brought some standardization to recording of antibiotic use on dairy farms. While producers are subject to CQM audits as part of the provincial inspection program administered by Dairy Farmers of Ontario, there has not been a formal evaluation of the quality or quantity of the treatment records, the outcomes associated with mastitis treatment and antibiotic use, nor the attitudes of dairy farmers towards treatment recording or outcome assessment. The objectives of this study are to address these issues and to identify differences in approaches and attitudes among three distinct groups, tie-stall farms, free-stall parlour milked farms and farms utilizing robotic milking, to identify common and unique challenges. The outcomes of this work will contribute to the development of more effective strategies for treatment outcome assessment with the ultimate goal of decreasing the inappropriate use of antibiotics through the evaluation and elimination of ineffective treatment protocols.

Objectives

The goal is to increase understanding of the rationale, quantity and quality of antibiotic use for mastitis treatment and develop novel approaches to promote treatment and outcome recording in support of prudent antimicrobial use.

1. To use CQM treatment records to describe antibiotic use for the treatment of mastitis on Ontario dairy farms in terms of numbers and types of treatments, and compare use in tie-stall, free-stall parlour and free-stall robot milked herds.
2. To determine how often, and from what source, pathogen is used in selecting a protocol, and verify that treatment is appropriate for the pathogen.
3. To determine how often outcomes (success/failure) of treatment are recorded, and in their absence, use existing SCC and retreatment data to estimate success.
4. To investigate barriers to treatment and outcome recording to develop strategies that will support improved outcome assessment for more appropriate and prudent use of antibiotics.

Results Summary

Select Study 1 results:

First audit results were obtained for 3,981 herds. Producers who completed classroom training only were 1.5 times more likely to receive approval at first CQM validation than those with no training ($p=0.01$) and those producers who completed combination training were 2.0 times more likely to receive approval on first visit ($p<0.01$) compared to those producers who had not received training.

Increased participation in training programs offered prior to CQM validation is associated with an increase in the likelihood of a producer receiving approval at their first CQM audit. Completion of both on-farm and classroom training sessions provides the greatest odds of approval at first CQM visit.

Select Study 2 results:
Chi-square and one-way ANOVA were used to evaluate if DVM and non-DVM participants differed in their responses. Linear and logistic regression models were used to determine potential associations with advisor outcomes of interest. The advisor survey had a 51% response rate (n=99) and a mean survey completion rate of 79%. DVMs and non-DVMs responded differently when asked about impact on client relationship, if DVMs are the best choice as advisors, if CQM training was beneficial to DVMs, and the number of advisors in a practice. Providing combination training

Select Study 3 results:
Despite repeated requests and encouragement by the DFO board of directors, only 99 dairy producers voluntarily completed the survey, and only 91 of those provided their identification. 87 of these were matched to the original study population database. Of these 87 identified herds, 67 agreed to provide animal treatment records for review. Based on a detailed assessment of the 3,704 individual treatment events on these 67 farms, common missing information included milk and/or meat withdrawal times, drug dose, route of administration, and initialing treatment record by the individual delivering the treatment.

Submission number	**UofG2013-1548**		
Project Title	**Origin, biology and management of Hops Down Mildew: a threat to hops, cucumbers and other crops**		
Key words	**Hop, Pseudopernospora humuli, Downy mildew**		
Lead Applicant	**Mary Ruth McDonald**		
Organization	**University of Guelph**		
Start Date	**2014-05-20**	**End Date**	**2017-04-20**

Abstract

Hop is a re-emerging crop in Ontario with an increasing demand for high quality cones by brewers. Hop downy mildew is one of the most important diseases affecting hops in Canada. This project intends to study: a) The biology of *Pseudoperonospora humuli*, virulence and spread in Ontario. b) *Ps. humuli* systemic infection and overwintering in crowns and rhizomes in connection to yearly disease occurrence and effect on yield and hop yards' lifespan. c) Cross infection of *Ps. humuli* to cucurbits and Ps. cubensis to hops, due to close similarity between the two pathogens and overwintering of *Ps. humuli* in crowns and rhizomes. d) The effect of *Alternaria spp*. *and Botrytis spp*. on cone quality. e) Hop cultivar resistance to *Ps. humuli*, and the efficacy of fungicides and biofungicides in controlling hop downy mildew. f) Determine *if Ps. humuli* is present in hop plants and rhizomes arriving from the United States. Team members include OMAFRA specialists, scientists, researchers and a graduate student from the University of Guelph.

Objectives

Project's goal is to improve management of hop diseases by studying the biology of *Pseudoperonospora humuli* and characterizing cone pathogens through:

1. Identification and characterization of hop downy mildew, its effect on hop and cucumbers by:
    a) Surveying yards and wild hops for *Ps. humuli* isolates present in Ontario.
    b) Determining *Ps. humuli* impact in downy mildew management.
    c) Determining hop cultivar susceptibility to *Ps. humuli* and if races of the pathogen exist.
    d) Understanding *Ps. humuli* systemic infection and overwintering in hop crowns.
    e) Continuing initial work examining cross infectivity of *Pseudoperonospora humuli* and *Pseudoperonospora cubensis*) on cucurbits and hop.
2. Characterization of the cone pathogens *Botrytis spp*. and *Alternaria spp*. and their effect on cone quality.
3. Evaluation of reduced risk fungicides and biopesticides against the target pathogens.
4. Determining if *Ps. humuli* is present in hop plants and rhizomes arriving in Ontario from the United States.

Results Summary

The pathogen for cucurbit downy mildew (*Pseudoperonospora cubensis*) was collected and used in cross infection studies on greenhouse grown Hops. *P. cubensis* was able to cause disease on hop leaves though lesion size was smaller than those caused by *P. humuli*. Due to the low occurrence of hop downy mildew in 2015, cross infection studies on cucumber was not completed but is planned to be carried out in summer 2016.

Cone diseases were assessed at harvest at the Simcoe Research Station (SRS) and from samples collected from three commercial hop yards. *Alternaria* cone disorder was the most prevalent disease as it was found in seven of the nine cultivars grown at the SRS. The highest levels of Alternaria cone disorder was found in Sterling, Chinook and Centennial while Hallertauer, Cascade, Northern

Brewer and Galena had lower disease levels. *Alternaria* was also found in most of the diseased cone samples from the commercial hop yards. The most common cultivars showing Alternaria cone disorder symptoms in commercial yards were Nugget and Willamette. Other than *Alternaria*, *Botrytis sp* was also isolated from diseased cones. At the SRS, comparatively low levels of cone damage due to Botrytis and powdery mildew were found on some cultivars, however powdery mildew caused severe damage on Bertwell resulting in no marketable yield. Powdery mildew was not found in cones collected from commercial yards.

Three commercial hop yards were surveyed at different times during the 2015 season. Most of the plants in all three yards showed initial DM symptoms in the form of occasional spikes and dried DM lesions, however the disease did not progress further and impact on yield remained low. There was minor damage on cones at harvest, mostly caused by *Alternaria*.
In order to determine if *P. humuli* is present in hop plants and rhizomes, four hop rhizomes were collected from growers in 2015. The morphological analysis did not show any evidence of downy mildew in the collected rhizomes. However, the molecular analysis is yet to be carried out to further confirm this. In 2014, a molecular analysis protocol was developed and PCR analysis confirmed the validity of the protocol. In 2016 more rhizomes will be requested from growers and molecular analysis will be performed on samples collected in 2015 and 2016.

Submission number	**UofG2013-1731**		
Project Title	**Creation of an insect pest risk assessment tool for Ontario agriculture**		
Key words	**insect risk assessment; ontario agriculture**		
Lead Applicant	**Jonathan Newman**		
Organization	**University of Guelph**		
Start Date	**2014-06-02**	**End Date**	**2017-06-01**

Abstract

Invasive insect and mite pests cost the U.S. agriculture industry ~$14 billion/yr. In Canada, projected costs for five significant agricultural insect pests are $6.7 billion/yr. Although no estimates exist for Ontario, the province has more non-native species than any other province/territory, putting agricultural production at risk of serious losses due to damaging invasive insects. We propose to develop an insect pest risk assessment tool for Ontario that can be used to identify potential new invasive insects and to prioritize insect invaders for management. To do this, we will (1) identify life history traits linked to invasiveness in insects, (2) analyze pathways of insect invasion into Ontario and their associated probabilities, (3) determine concentrations of insect invaders in Ontario to identify hot-spots, and (4) develop a tool to estimate the potential for economic and environmental damage. Objectives 2–4 will also allow targeting of monitoring and other activities.

Objectives

We propose to develop a species-specific rapid risk assessment tool for invasive agricultural insects in Ontario. The tool will take into account the ability of invaders to establish and become abundant, pathways of introduction and spread, and the potential for invaders to cause economic and environmental damage. We will determine what insect traits are related to the potential for insects to invade and cause damage to the Ontario agriculture sector. We will also perform a combined analysis of pathways, invader concentrations, and economic distributions to identify regional risk levels. Our results will allow threat prioritization of high-risk potential and current invasive insects.

Results Summary

We have found that although there is some overlap in invasive traits between plants and insects, there are many unique traits related to invasion for both, especially related to insect behaviour. We have identified 12 of the 30 traits that were related to insect invasion do not have a clear analogue to plant traits, and therefore are unique to insects. This result suggests that a pest risk assessment developed for plant invasion may not be applicable for insects because traits that are important to insect invasion may be missing from the assessment. This may also be the case for other taxa. Our next step is to compile trait data for invasive and native insects to evaluate their potential to be used to predict future invasive insects.

Submission number	**UofG2013-1497**		
Project Title	**Characterizing Streptococcus suis from clinical cases and healthy-carrier pigs**		
Key words	***S.suis*, swine, serotype, virulence factors, antimicrobial resistance**		
Lead Applicant	**Robert Friendship**		
Organization	**University of Guelph**		
Start Date	**2014-06-16**	**End Date**	**2017-04-28**

Abstract

Losses due to diseases caused by *Streptococcus suis* are economically significant, and yet *S. suis* is commonly present on tonsils and in the respiratory tract of healthy pigs with no clinical effects. The serotypes and strains of *S. suis* vary in their ability to cause disease, and virulence factors (VFs) of *S. suis* are poorly understood. Likewise, outbreaks of *S. suis* disease tend to be triggered by complex environmental, management, and host factors which are not well understood.

The objectives of this study are: to investigate the distribution of *S. suis* serotypes, virulence factors, and antimicrobial resistance (AMR) profiles from clinical cases and healthy-carrier pigs; to determine the ability of a new multiplex PCR to identify *S. suis* serotypes; to investigate risk factors and treatment measures used on Ontario farms.

Nasal and tonsil swabs from healthy pigs and samples from clinical cases on 50 Ontario farms will be cultured for *S. suis.* Isolates from clinical cases across Ontario will also be included in the study. The isolates will be serotyped and tested for VFs and AMR. Furthermore, a survey will be conducted to examine on-farm risk factors, treatment-control strategies, and economic impact of *S. suis* infections on the study farms.

Objectives

The overall objective is to investigate characteristics of *Streptococcus suis* isolated from clinical and healthy-carrier pigs, as well as investigate risk factors associated with disease outbreaks and control measures commonly employed.

The specific objectives:

1. To determine the differences between *S. suis* isolates from clinical cases and isolates from healthy pigs including antimicrobial resistance patterns, virulence factors and serotypes (to improve our therapeutic approach and how we select strains for autogenous vaccines)
2. To investigate the agreement between coagglutination method and multiplex PCR for serotyping *S. suis* (to improve our diagnostic abilities in investigating outbreaks of disease)
3. To determine management and environmental conditions associated with outbreaks of streptococcal disease and to document the common approaches used to control an outbreak including therapeutics and management changes (in order find better methods of preventing and controlling *S. suis* diseases and reducing antibiotic use)

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Results Summary

Of 405 samples, 310 were collected from healthy and 95 from sick pigs. There was no significant difference between the recovery rate of *S. suis* from suckling versus nursery pigs (P =0.7), or from sows versus finishers (P =0.9). However, *S. suis* was more likely to be recovered from suckling and nursery piglets than from sows and finishers (P <0.001). *S. suis* was recovered more often from healthy pigs as opposed to sick pigs (P <0.001). However, it is possible that some the sick pigs tested might have been treated with antibiotics before sample collection. Seventeen serotypes were identified, with type 6 being the most common serotype. However, this type was only isolated from healthy pigs. Nineteen percent and 52% of isolates autoagglutinated or were untypable, respectively; untypable isolates were most likely to be recovered from healthy pigs (P <0.01). There was no agreement between the culture and PCR methods for *S. suis* detection.

Submission number	**UofG2013-1471**		
Project Title	**Complex mathematical and statistical modelling of between-farm disease transmission in the Ontario swine industry.**		
Key words	**swine; infectious diseases; agent-based modelling**		
Lead Applicant	**Rob Deardon**		
Organization	**University of Guelph**		
Start Date	**2014-07-01**	**End Date**	**2017-06-30**

Abstract

Farm-level disease transmission models, preferably based on high-quality data, can aid greatly in understanding how diseases spread. They can also facilitate the design of disease control/surveillance strategies for controlling/monitoring disease.

Highly complex models can be constructed that mimic many different possible mechanisms of disease transmission and population dynamics (e.g. spatial-, trade-, animal movement- and/or supplier-based networks). However, data we collect from such systems are usually incomplete; e.g. infection times and/or animal movement data may contain measurement error and/or be only partially observed. Approximate Bayesian computation (ABC) techniques can be used to fit such complex models to only partially observed data, without the substantial computational burden of a full data-augmented Bayesian approach.

Here we propose to develop realistic, complex regional-level animal-movement-based disease transmission models for Ontario swine, testing the plausibility of control and surveillance methods for diseases such as PRRS, PED and influenza. Simultaneously, we will use simulation studies to implement, develop and test ABC methods for fitting disease transmission models to data that might be collected in practice. Finally, the complex Ontario swine models above will be fitted to observed data (e.g. from Niagara region PRRS projects) using ABC methods, and then, risk-based surveillance & control strategies developed.

Objectives

1. Develop complex agent-based meta-population models (AMBs) of Ontario swine industry and disease propagation through such systems (Theme 2).
2. Test regional-level control/surveillance strategies for diseases such as PRRS and influenza in Ontario swine. (Theme 2).
3. Develop and implement sequential approximate Bayesian computational (S-ABC) methods that can be used to fit complex individual-level/agent-based models (ILM/ABM) to observed disease data. (Theme 1).
4. Validate S-ABC approach via spatial/network ILM-based simulation studies (Theme 1).
5. Apply S-ABC/ABM approaches to real data to gain insights into how to control PRRS (and other diseases such as PED, pending data availability) in Ontario swine industry. (Themes 1 & 2).
6. Apply S-ABC/ABM approaches to real data to gain insights into how to carry out risk-based surveillance for PRRS (and other diseases such as PED, pending data availability) in Ontario swine industry. (Themes 1 & 2).

Results Summary

PhD student Waleed Almutiry has successfully implemented code to simulate from ILMs that incorporate contact networks, and fit those models to epidemic data using MCMC, both when the contact networks are known, and when they are unknown to some degree. He has also successfully implemented S-ABC algorithms to do this model fitting, and explored which algorithm seemed to work best. He is currently working on analysing a real, complex data set (in this case the UK 2001 foot and mouth disease epidemic).

Ex-PhD student, and now postdoc, Gyanendra Pokharel, has carried out similar work using Gaussian process emulation techniques. Further, ex-PhD student, Rajat Malik has carried out similar work using data sampling based likelihood approximations.

PhD student Justin Angevaare has successfully implemented code to jointly simulate epidemic spread and pathogen sequence mutation, and fit those models to epidemic data using MCMC. When the pathogen sequence data has uncertainty associated with it, this is EXTREMELY computationally intensive and, thus, can only be used on small populations. He is thus, about to work on using S-ABC algorithms to do this.

PhD student Carolyn Augusta has been considering machine learning tools for carrying out statistical inference of such network-based models, and has recently successfully completed her qualifying exam.

Submission number	**UofG2013-1617**		
Project Title	**Microbiological Risks and Mitigation Strategies in the Application of Recycled Sand Bedding used in Dairy Operations**		
Key words	**Dairy, sand, pathogens, mistitis, decontamination,**		
Lead Applicant	**Keith Warriner**		
Organization	**University of Guelph**		
Start Date	**2014-09-01**	**End Date**	**2016-08-31**

Abstract

The Ontario dairy sector is critical to the economy of the Province with 4200 farms contributing to produce milk with a market value of $1.7bn each year. The industry is under constant pressure to increase productivity and decrease costs. One possible approach is to apply sand bedding for cattle which is acknowledged to increase milk yield by cows and enhance animal welfare. However, sand contributes significantly to waste management costs due to volume and requirement for ground injection. By recycling sand it is estimated that a small farm could save $7000 per year. However, by recycling sand it is possible to accumulate pathogens that could negatively affect herd health. The proposed project will assess the microbiology risk associated with recycled sand in dairy production and evaluate a selection of decontamination technologies to reduce pathogen carriage. The study will sample sand from three farms operating a sand bedding system. The samples will be screened for relevant pathogens and indicators along with the somatic cell count, in addition of milk yield of cows. Selection of intervention technologies will be based on efficacy and cost.

Objectives

The overall aim of the project will be to assess the microbiological risks on herd health of recycling sand bedding in dairy operations and identify decontamination interventions to reduce pathogen carriage. The specific objectives are:

1. Verify diagnostic methods to enumerate/detect pathogens and indicators.
2. Undertake sampling trials in three operations running standard sand bedding protocols and recycling system in parallel.
3. Validate sand decontamination methods based on UV, Advanced Oxidative Process, Steam and anti-microbial gasses.
4. Verify performance of selected sand decontamination methods in barn operations.
5. Final reporting and recommendations.

Results Summary

Sampling trials at commercial dairy operations

The first sampling visit was performed in May 2015 at which time fresh sand was placed in the stalls with additional sand being used to replenish sand lost during twice daily removal of manure. Pristine sand used to replenish that within the stalls harbored both coliforms and endospores but E. coli was rarely recovered. However, E. coli was recovered from sand taken from the stalls at approximately 3 log cfu with no significant difference between surface and at 15cm depth. The result would suggest that fecal contamination is introduced into the sand and persists or is replenished over time. Coliforms and endospore formers were also recovered with levels remaining stable over the 24 week sampling period. It was noted that the aerobic plate count was in the order of 7 log cfu and future 16S rRNA sequencing will be performed to probe the microbial populations within samples taken at different time points.

Sand Microcosm Studies

The highest persistence was observed for generic E. coli that did not significantly change in levels over the 35 day trial period. The inclusion of Bacillus probiotic did not significantly alter the

persistence of generic E. coli. A cocktail of STEC consisting on O157:H7 and O26 underwent a triphasic decline with an initial rapid decrease followed by a plateau region then further decrease.

No STEC survivors were recovered on samples taken at Day 35. Again, the probiotic preparation did not alter the decline although the Bacillus persisted throughout the trial period.

Although Klebsiella was introduced at 5 log cfu/g only 3.5 log cfu/g could be recovered at Day 0 suggesting a rapid decline in levels within an hour of inoculation. The remaining Klebsiella persisted in sand with no changes in levels for the initial 10 days then reduced by 1 log before again entering a persistent stage. Probiotic supplements did neither enhance nor decrease the persistence of the pathogen.

Similar to E. coli, there was no significant decrease in Staphylococcus levels over the course of the 35 day trial.

Submission number	**UofG2014-2194**		
Project Title	**Epidemiological investigations documenting early phase of the emergence of porcine epidemic diarrhea virus in Ontario swine herds**		
Key words	**Porcine Epidemic Diarrhea virus, emerging**		
Lead Applicant	**Terri O'Sullivan**		
Organization	**University of Guelph**		
Start Date	**2014-04-01**	**End Date**	**2017-04-30**

Abstract

Porcine epidemic diarrhea virus emerged in Ontario swine herds in late January of 2014. Initial outbreak investigation and subsequent bioassays identified porcine plasma as the most likely fomite by which the virus was introduced into Ontario swine populations. The role of feed, and other potential confounding factors has to be investigated using sound epidemiological design. Two complementary approaches will be used. First, matched case-control study will be conducted focusing on detailed management practices. Although the research question will be focused on feed, multiple other factors will be evaluated that could confound this association. All primary cases who agree would be included, and would be matched with a control herd of the same herd type, general herd size and time. Control status will be confirmed by parallel interpretation of serological and virological assays. Multivariable conditional logistic regression will be used to evaluate associations. Network data will be utilized using a variety of approaches.

The second approach will be the 2-cohort study. Exposed cohort will be a random sample of 20 herds that had the batch of feed with contaminated plasma. The non-exposed cohort will be a random sample of 20 herds which received only plant-based diet from the same feed supplier.

Objectives

The general objective of this proposal is to document all the circumstances surrounding the outbreak of PEDv in Ontario swine during the early phase. The More specifically, we aim to:

1. Evaluate association between the use of specific feed type and occurrence of herd outbreak of PEDv in swine herds between January 9th and March 1st, after adjusting for other confounding factors using case-control approach
2. Investigate other potential risk factors for infection with PEDv status in the early phase using case-control approach
3. Determine likelihood of developing PEDv infection after feeding a shipment of feed containing contaminated spray-dried plasma
4. Evaluating whether there is difference in risk of PEDv infection between herds which received the batch of contaminated plasma and the herds which received only a plant-based diet

Results Summary

The survival analysis on the customer-level data obtained from the involved feed company has been completed as a component of the cohort study. This customer-level data, AHL submission data, and OMAFRA public records allowed for statistical analysis in order to determine the likelihood of a PEDV outbreak between customers that did not receive the PCF (non-exposed) and customers that did receive the contaminated feed (exposed). Statistical analysis (consisting of Kaplan-Meier estimates of survival curves, log-rank tests, and Cox's proportional hazard models) demonstrated that exposed customers were 36.6 times more likely to have a PEDV outbreak compared to non-exposed customers ($P<0.012$). Additionally, the analysis demonstrated that customers that purchased more tonnage of feed or more Kg of SDPP were 7.5 times more likely to experience a PEDV outbreak

compared to those customers that purchased less or no KG of SDPP ($P<0.001$). Lastly, the analysis demonstrated that the number of feed deliveries did not have an effect on the likelihood of a PEDV outbreak ($P=0.02$) and hence the transport of feed (trucks) was not involved in the spread of the virus during the early phase of the outbreak in Ontario. These results show a very strong and statistically significant association between exposure to the contaminated feed and an outbreak of PED on an Ontario farm during the early phase of the PEDV outbreak in Ontario. Statistical analyses are now underway in even more detail to further evaluate the differences in the farms that experienced a PEDV outbreak vs. those that did not. This will further increase our confidence in the important findings highlighted in the cohort study. Basic descriptive statistics are completed on the case-control herd data.

Submission number	**UofG2013-1530**	**Funding Program**	**OMAFRA - U of G Research**
Project Title	**Assessment of the potential disease risks posed by wild turkeys (*Meleagris gallopavo*) to domestic poultry flocks in Ontario**		
Key words	**poultry, turkey, disease, transmission, risk**		
Lead Applicant	**Nicole Nemeth**		
Organization	**University of Guelph**		
Start Date	**2015-01-01**	**End Date**	**2017-12-31**

Abstract

Free-ranging wild turkeys (*Meleagris gallopavo*) have been increasing in number and expanding in geographic range since their reintroduction to Ontario in 1984. The interface between wild turkeys and commercial turkey flocks provides opportunities for pathogen spread, and information is needed on the potential disease risk posed by wild turkeys to Ontario's domestic poultry. A retrospective analysis of wild turkey diseases in the Canadian Cooperative Wildlife Health Centre database will provide a long-term perspective of detected diseases. Additionally, priority pathogens, including those that cause disease outbreaks or decrease fitness in commercial turkeys will be targeted for testing in hunter-killed wild turkeys. Knowledge of the prevalence and distribution of pathogens circulating among free-living wild turkeys combined with known transmission routes and pathogenesis will be used to assess the relative risk of pathogen transmission and subsequent disease among commercial turkey flocks, a valuable and growing component of the Ontario agricultural sector. Data generated from the proposed research will support emergency management preparedness and the formation of disease prevention strategies for Ontario poultry producers, as well as provide baseline disease surveillance records for Ontario wild turkeys.

Objectives

The objectives are four-fold: 1) Determine the presence and prevalence of important poultry-associated infectious agents in free-ranging wild turkeys in Ontario both retrospectively, through the Canadian Cooperative Wildlife Health Centre database, and prospectively, via sampling of hunter-killed wild turkeys; 2) Determine the distribution of these pathogens and identify disease "hotspots" through mapping and spatial analysis of the distribution of diseased or pathogen-carrying wild turkeys relative to disease-free turkeys; 3) Assess the potential risk of disease transmission from wild turkeys to commercial turkeys using a qualitative risk assessment framework that includes data gathered during this study as well as existing information from the scientific literature; 4) Provide baseline information about the health of wild turkeys in Ontario for future evaluation of disease emergence (e.g., expansion or increases in prevalence or distribution) as well as possible associations between disease prevalence and distribution relative to changes in land and water use and climate.

Results Summary

A long-term (22-year) retrospective study was completed in collaboration with the Canadian Wildlife Health Cooperative (CWHC) on diseases diagnosed in wild turkeys in Ontario. These results revealed that while the majority of turkeys (n=56) in the study were diagnosed as emaciation and trauma as the primary cause of death, small outbreaks of disease in Ontario wild turkeys occurred due to *Pasteurella multocida* and zinc phosphide toxicosis. A manuscript was submitted to the journal Avian Diseases and was favourably received (with acceptance anticipated shortly). The prospective portion of the study is on schedule, as 152 wild turkey carcasses were collected from hunters across southern Ontario, and all were necropsied and samples collected for testing of select pathogens that can infect both wild and domestic turkeys. Testing that has thus far taken place has included avian influenza viruses (0/150 wild turkey oropharyngeal swabs positive), avian poxvirus

(2/152; 1.3% with gross skin lesions that were PCR-positive for avian poxvirus), and Mycoplasma spp., including (150/152; 98.7% positive for ≥1 Mycoplasma spp. culture including primarily *M. gallopavonis* [96.7%], as well as *M. gallinaceum* [23.7%],*M. pullorum* [18.4%], and rarely *M. meleagridis* [2.0%], *M. iowae* [0.7%], and *M. synoviae* [0.7%]). Additional bacteria culture results include 105/152 (69.1%) cloacal swabs positive for E. coli, 2 (1.3%) positive for Campylobacter spp., and 0/152 for Salmonella. The E. coli isolates are currently undergoing antimicrobial resistance and susceptibility testing in collaboration with the Centre for Food-borne, Environmental and Zoonotic Infectious Diseases/Public Health Agency of Canada and Scott Weese Lab (UoG). Collaborations with the John Barta Lab (UoG) led to preliminary results: 79/104 (76.0%) fecal positives *for Eimeria spp*. We attempted to perform bacterial culture for *Pasteurella multocida, Erysipelothrix rhusiopathiae,* and *Ornithobacterium rhinotracheale*, and due to laboratory logistical issues, we will proceed to PCR to test for these agents and positives will be sequenced to compare with results from domestic turkeys. DNA extraction has just been completed from tissues for subsequent PCR for lymphoproliferative disease virus (LPDV) and reticuloendotheliosis virus (REV); depending upon the number of positive samples, all or a subset will be sequenced to compare with results from neighboring regions (e.g., the northeastern US). Testing for LPDV and REV in domestic turkeys and upland wild game birds has been expanded by collaborations with another UoG-Animal Health Laboratory collaboration and the CWHC. In addition, wild turkeys of potential diagnostic value (based on gross findings) have been selected and annotated in the database and histopathology will be pursued for these, to assess for any additional diseases. The development of our wild turkey disease database is ongoing, and when complete, we will seek the expertise of veterinary epidemiologist David Pearl (grant team member) to help with spatial and epidemiological analyses.

Submission number	**UofG2014-1999**		
Project Title	**Development and implementation of highly effective, high-throughput, and affordable diagnostic technologies to combat the emerging virus diseases faced by Ontario grape/wine industry**		
Key words	**Grapvevine, viruses, diagnostics, Reverse transcription-PCR, ELISA, grape decline, grapevine leafroll disease**.		
Lead Applicant	**Baozhong Meng**		
Organization	**University of Guelph**		
Start Date	**2015-05-01**	**End Date**	**2017-04-30**

Abstract

As the second largest fruit crop in Ontario, grape production reached 88,000 tonnes with farm-gate value of $100 million in 2013. The Ontario grape/wine industry has seen rapid growth and expansion. Ontario wines have garnered prestigious recognition in international market, and generate economic impact of $3.3 billion. Viruses are highly detrimental pathogens, responsible for substantial losses to production of premium grape and wines. Losses due to Fanleaf degeneration alone are estimated at $1 billion annually in France. Other diseases (Leafroll, Rugose wood, Quick decline and Red blotch) can result in total crop loss several years post-infection. This situation is further exacerbated by mixed infections with multiple viruses and winter damage, causing even greater destruction. Since 2013, there has been a sudden outbreak of viral diseases throughout Niagara region, raising widespread and serious concerns among growers, nurseries, and wineries. In response to this urgent need from the grape/wine industry, this project aims to: (i) develop highly effective and high-throughput diagnostic technologies targeting nine major grapevine viruses; and (ii) attain a comprehensive and accurate assessment on the prevalence and severity of major viruses in Ontario. Outcomes of this project will be pivotal to disease management and sustained profitability of Ontario grape/wine industry.

Objectives

This project serves as a starting point for a bigger and long-term goal, which is to make available highly effective and affordable technologies in response to the emerging viruses and viral disease outbreaks faced by Ontario grape/wine industry through development and implementation of sensitive, reliable, high-throughput and affordable diagnostic technologies. Ultimately, these technologies will play a pivotal role in the creation of robust, productive, sustainable and internationally competitive grape/wine industry in Ontario.

1. To produce quality antibodies for use in serological detection of six of the most important viruses (GLRaV-1, GLRaV-2, GLRaV-3, GVA, GVB, GRBaV).
2. To develop and validate ELISA and dot-ELISA for detection of these target viruses in using these “in-house” antibodies.
3. To assess the prevalence and distribution of nine major viruses in Ontario vineyards through a large-scale survey to attain an accurate assessment on the scope, severity and magnitude of the viral issue.

Results Summary

1. The capsid protein (CP) genes of all four viruses successfully amplified, cloned and sequence confirmed. The CP genes of all four viruses (GLRaV-1, GLRaV-2, GLRaV-3 and GRBaV) were amplified using PCR or RT-PCR and cloned into the intermediate vector pGEM-T Easy, and their sequences confirmed by DNA sequencing. Blast search revealed that our GLRaV-1 CP sequence is 92% to the Australian isolate, the GLRaV-2 sequence is

99% identical to isolate 93-955, the GLRaV-3 sequence is 99% identical to isolate 623 and the GRBaV sequence is 99% identical to isolate NY135.

2. The recombinant constructs in the shuttle vector with CP genes of four viruses were successfully generated. The cloned CP genes of all four viruses in pGEM-T Easy were subcloned into shuttle vector pFastBac HT-A, followed by sequencing confirmation.
3. The recombinant Bacmids containing CP genes of all four viruses have been successfully generated. After transformation of the shuttle vectors described above into DH10Bac, each expression construct contained in pFastBac HT-A was successfully mobilized into Bacmid through recombination.
4. The recombinant baculoviruses containing CP of all four viruses have been successfully produced. The recombinant Bacmid DNA with CP genes of these viruses were successfully purified and transfected into insect cells. We confirmed successful expression of recombinant CP for all four viruses through SDS-PAGE and Western blotting using His-specific antibody.
5. Conditions for expression of recombinant CP have been optimized. Pilot experiments revealed: (1) the titers of the baculoviral stocks being used for infection were between 2 to 5 x 108 pfu/ml; (2) monolayer culture produced considerably more proteins than suspension culture; (3) the best time window for isolating these recombinant proteins were 2-3 days post-infection.
6. The recombinant CPs of three viruses were successfully purified. French Press is better than the freeze-and-thaw method in releasing the recombinant proteins from insect cells. Buffer TKMN was best as judged by the amount of proteins produced. We successfully purified recombinant CP for all viruses except GRBaV. To purify enough proteins for immunization, 30 large flasks of culture infected with each recombinant baculovirus were required. The amount of recombinant CP produced from one flask of culture varies depending on the virus: 12.5 μg for GLRaV-1, 37.5 μg for GLRaV-2 and 90 μg for GLRaV-3.
7. Polyclonal antibodies against the recombinant CP of three of the four viruses are being produced. As stated earlier, antibodies produced from rabbits exhibit the propensity to give high background in a test due to non-specific reactivity to host proteins. To circumvent this potential issue, we have chosen rats for the purpose of antibody production in this project. We have completed the primary injection and the first booster injection. The final bleed will be collected in May.

Submission number	**UofG2014-1921**		
Project Title	**A risk assessment for tick-borne bovine anaplasmosis and its vector *Dermacentor variabilis* (American dog tick) in Ontario**		
Key words	**bovine anaplasmosis, *Dermacentor variabilis* (American dog tick), distribution modelling, risk assessment, climate change**		
Lead Applicant	**Jonathan Newman**		
Organization	**University of Guelph**		
Start Date	**2015-05-01**	**End Date**	**2017-04-30**

Abstract

Economic losses from tick-borne diseases are potentially huge for livestock producers, as are the costs of prevention and treatment of infection when it does occur. The aim of this project is to refine an existing species distribution model for *Dermacentor variabilis* (American dog tick), the primary vector for *Anaplasma marginale*, a rickettsial pathogen responsible for bovine anaplasmosis, and apply the model to Ontario. Distributions under both current and future climate change will be mapped. We will use map layers showing the locations of beef and dairy herds in Ontario to refine the risk maps. The maps will then be used by OMAFRA animal health specialists to prioritize surveillance, and plan for control should an outbreak occur.

Objectives

The main goals of this research are: (1) to refine the James et al. Dog Tick Distribution Model* (see example: http://www2.ca.uky.edu/gluck/q_jul11.asp) for use in Ontario; (2) use the refined model to map current suitable distribution, and hence risk, in Ontario; (3) apply data layers for Ontario Beef and Dairy operations to refine the risk map in #2; (4) project future risk using Ontario-specific climate change projections.

*James, A., Burdett, C., McCool, M.J., Fox, A., Riggs, P. In press. The geographic distribution and ecological preferences of the American dog tick in the United States. Medical and Veterinary Entomology.

Results Summary

Compared with the literature, the model we produced accurately represented the current distribution of *D. variabilis* based on climate suitability (temperature and precipitation conditions). The most influential variables included the maximum annual temperature, minimum annual temperature, and minimum annual precipitation. These variables represent the importance of annual temperature range and moisture availability to *D. variabilis*, consistent with literature.
The potential distribution of *D. variabilis* was projected to change by all climate change scenarios. All climate change scenarios projected a northward and westward expansion of the distribution of *D. variabilis*, encompassing much of Ontario where the tick currently does not occur or is uncommon. Climate change scenarios that project a greater amount of warming (i.e. RCP 6.0 and RCP 8.5) estimated a decrease in climate suitability in the southern USA, including parts of Texas where occurrence data indicate that *D. variabilis* is currently common. Overall, the area of suitable climate for *D. variabilis* in North America could increase up to 11.5% from present by 2050, and up to 12.2% from present by 2070. The maximum projected warming resulted in the least change in climate suitability. With all climate change scenarios, the overall increase in suitable climate comprised an increase in moderately suitable climate but a decrease in highly suitable climate, but still projected a net increase in suitability.

Preliminary results for bovine anaplasmosis risk assessment suggest that cattle in south-central Alberta, southwestern Saskatchewan, and southwestern Ontario are most at risk for potential increases in *A. marginale* transmission due to increased *D. variabilis* presence. In these regions, both

cattle numbers per county and probability of climate suitability are moderate-high. However, in Ontario, it is possible that risk will initially increase with climate change, and decrease over time. We are still working out how best to 'calculate' risk using available data and results, and considering other data, such as land use, to consider more variables that influence tickborne disease risk.

Submission number	**UofG2014-2095**		
Project Title	**Evaluation of Methods for On-Farm Euthanasia and Humane Depopulation of Commercial Meat Rabbits**		
Key words	**emergency management, production systems, animals, nonpenetrating captive bolt, carbon dioxide**		
Lead Applicant	**Patricia V. Turner**		
Organization	**University of Guelph**		
Start Date	**2015-05-04**	**End Date**	**2017-10-31**

Abstract

The development and validation of efficient, humane, and safe methods for on-farm euthanasia and depopulation of all livestock species, including meat rabbits, is a high priority for both provincial and federal governments and is important for meeting Canada's international animal welfare commitments to the World Organization for Animal Health (OIE). Currently, the only AVMA-approved methods for euthanasia of rabbits include cervical dislocation, which can't be used on animals exceeding 1kg, penetrating captive bolt, which is esthetically unpleasant and potentially dangerous for the operator when killing smaller animals, carbon dioxide inhalation after sedation, and barbiturate overdose, the last two methods of which require the presence of a veterinarian. Similarly, no humane and practical methods have been developed or approved for mass depopulation of commercial rabbits. This has created a significant animal welfare gap in that Ontario rabbit producers and regulatory authorities have limited acceptable methods available for culling rabbits post-weaning. We intend to address this deficiency by evaluating and comparing the efficacy, safety, efficiency, welfare impact, and operator impact of three physical methods (blunt force trauma, non-penetrating captive bolt, and cervical dislocation [only in animals <1kg]) and two gas methods (CO and CO2 inhalation) of euthanasia in cull meat rabbits.

Objectives

1. To evaluate the efficacy, safety, ease of use, esthetic acceptability, and welfare impact of three physical methods of euthanasia (cervical dislocation [2 methods - manual and mechanical, and only in animals <1 kg], blunt force trauma, and non-penetrating captive bolt) in preweaned, fryer (6-12 week-old), young adult (up to 8 mos), and adult cull meat rabbits (>8 mos).
2. To evaluate the efficacy, safety, ease of use, esthetic acceptability, and welfare impact of CO2 and CO inhalation for euthanasia of preweaned, fryer (6-12 week-old), youg adult (up to 8 mos), and adult cull meat rabbits (>8 mos).
3. To survey rabbit producers concerning their attitudes and preferences for euthanasia techniques in different age groups of rabbits as well as their understanding of when to cull.

Results Summary

Producer survey: producers have no reputable resources readily available for on-farm rabbit euthanasia training. They have largely taught themselves various techniques and they generally don't like performing it. Many will leave animals to die on their own because they aren't sure if they are doing procedures correctly. Almost no one indicated that they checked the animals after performing their euthanasia method to see if the animal was actually dead. This emphasized the important need for research into this area (on-farm euthanasia of meat rabbits) and development of training materials.

On-farm euthanasia - physical methods study: We evaluated the use of: nonpenetrating captive bolt (NPCB), assisted manual cervical dislocation (AMCD), cervical dislocation (CD), and blunt force trauma (BFT) in preweaned, weaned, and adult cull rabbits. For the NPCB work, we initially

validated the air compressor pressures using cadavers. CD was not an appropriate technique for larger rabbits, because of the strong muscle mass, which makes it difficult to achieve insensibility rapidly. Thus, this technique was only recommended for preweaned rabbits. AMCD was a good technique. There were 3 cases in which the technique had to be repeated in rabbits, largely related to insufficient training. This is an inexpensive device and one we are recommending. NPCB resulted in immediate insensibility in 100% of the rabbits. This is our preferred technique; however, we recognize that because of the expense, many producers will not be able to afford to purchase one. Our analysis included rabbit behaviour and reflex monitoring at the time of euthanasia, time to insensibility and death, gross damage to brain and skull, survey radiographs of the head and neck, and histopathology of the brain.

Submission number	**UofG2014-1885**		
Project Title	**Sustainable Management and Survey for Brown Marmorated Stink Bug in Ontario**		
Key words	**brown marmorated stink bug, invasive insect, sustainable management, survey, diagnostics, insecticide efficacy, biological control, overwintering aggregation management, farmer participatory training**		
Lead Applicant	**Cynthia Scott-Dupree**		
Organization	**University of Guelph**		
Start Date	**2015-05-04**	**End Date**	**2018-04-30**

Abstract

Brown marmorated stink bug (BMSB), *Halyomorpha halys Stål*, is an invasive pest native to East Asia. First identified in Pennsylvania in 2001, it has now been detected in 42 states and 2 provinces (Ontario and Quebec), and is of economic importance throughout the Mid-Atlantic US. There are more than 170 North American plant hosts, including broad spectrum of important fruit, vegetable and field crops, as well as landscape trees and shrubs. All are important agricultural commodities in Ontario. As of October, 2014 we know BMSB is established (i.e. breeding populations, adults and nymphs have been found) in Hamilton, London, Newboro, St. Catharines and Windsor. In addition we've had over 250 confirmed homeowner finds (with approximately 900 unconfirmed reports) and have caught BMSB during our survey efforts in 2014 using pheromone traps in agricultural areas on or near farms (i.e., tree fruits and grapes) at several locations. In 2014, many homeowners reported significant injury in ornamental plants, garden fruits and vegetables. The presence of high populations of BMSB in urban areas poses a risk of spillover into nearby agricultural areas and this substantially heightens grower concerns.

Objectives

This 3-year study will focus on the following objectives:

1. Phenology of BMSB in Ontario (T1);
2. Sustainable management strategies including assessment of action thresholds – biological control, chemical (contact and oral toxicity; more efficient delivering - timing and potential insecticides – singly and in mixes) and mitigation of overwintering aggregations (T1);
3. Development of diagnostic methods in tree fruits (apples and peach) - improve our ability to identify BMSB damage and differentiate damage caused by indigenous stink bug species (T1);
4. Continuation of the survey in urban, natural and agricultural areas and transportation corridors to determine spread and establishment of the BMSB in Ontario in 2015 and 2016(T2);
5. Develop a farmer participatory training program to facilitate pest management decision-making (detection / control) by growers and consultants (T1&T2; and
6. Develop an interactive online GIS resource to track BMSB in southern Ontario (T2).

Results Summary

In 2015, 191 sites were surveyed and BMSB adults were trapped at 9 agricultural, 5 rural, and 2 urban sites. BMSB has been detected in 17 counties, with established breeding populations in Hamilton (2012), London (2014), Newboro (2012), St. Catharines (2014), and Windsor (2014). The 2013-2015 surveys of BMSB have documented the spread of this pest across Ontario, with new locations found each season. The presence of high populations of BMSB in urban areas, where homeowners are reporting damage to landscape plants, and garden fruits and vegetables, poses a risk of spillover into nearby agricultural areas (i.e., apples, peaches, and grapes). As a result, research focused on developing sustainable pest management strategies for growers is imperative. Most chemical control strategies for BMSB have been developed in the US. We are presently undertaking direct and residual toxicity testing on 11 insecticides alone or in combinations and 2 biopesticides suited for control of BMSB nymphs in Canada - this is a new area of research since all the work in the US is on the adult stage. We believe that nymphs may be the weak link in the BMSB life cycle. Once the nymph toxicity test are complete we will move onto adults. In terms of conservation biological control strategies - a parasitoid belonging to the genus Anastatus has been reared from field collected BMSB eggs. Although this species appears to be rare in our field collections, it does appear to develop on and kill BMSB. We are presently attempting to establish a rearing colony of Anastatus so we can conduct further studies. Hopefully we have found an indigenous natural enemy that can be used in a sustainable pest management program. Indoor overwintering aggregations of BMSB were identified in the Hamilton area and will be monitor during Spring 2016 to determine when they start dispersing from overwintering sites, and when mating and then oviposition begins. This information will be of critical importance in establishing the timing/initiation of identified management tactics throughout the season. We are also working closely with the growers to enable them to begin monitoring for BMSB on their farms using pheromone traps. We are presently creating a YouTube video that will help the growers initiate pheromone monitoring on their farms and therefore provide us with essential survey in for this insect pest in Ontario.

Submission number	**UofG2014-1932**		
Project Title	**Novel PRRSV genotypes: tracking the emergence, transmission pathways, clinical impact, and pathogencity**		
Key words	**PRRS virus, outbreak investigation, transmission chain, pathogenecity, transportation, agent-based model, regional disease control program, surveillance**		
Lead Applicant	**Zvonimir Poljak**		
Organization	**University of Guelph**		
Start Date	**2015-06-01**	**End Date**	**2018-05-30**

Abstract

Several new PRRSV genotypes emerged in one of the regional disease control programs (DCP) which are known as PRRS area regional control and elimination (ARC&E) programs. Practitioners are reporting severe impact in several herds, and our ongoing research suggests that transportation network plays an important role. A need for further evaluation of PRRSV transmission pathways in this specific region, and detailed assessment of their clinical impact and pathogenicity has been identified.

Objectives

The overarching goal of this project is to investigate emergence, spread, and control of PRRSV in a specific Ontario area and to investigate pathogenicity, virulence and overall clinical impact of important PRRSV genotypes.

The proposed study aims to:

1. Determine the most likely transmission chain for specific PRRSV genotypes using molecular and classical epidemiological tools,
2. Characterize the transportation network from the largest transportation company in the area for the purposes of modelling infection control,
3. Determine clinical impact, and pathogenicity of one recently emerged PRRSV genotype in the area, and
4. Evaluate heterogeneity of PRRSV genotypes in one assembly yard over time.

The objectives will be accomplished by:

1. Estimating transmission chains using molecular and classical epidemiological approaches,
2. Performing network analysis of truck movements and agent-based modelling of infection control measures,
3. Performing pathogenicity study and assessing clinical impact through records, and
4. Conducting a longitudinal study of PRRSV circulation in assembly yard over one year and by sequence analysis of ORF5 of detected PRRS viruses.

Submission number	**UofG2014-2179**		
Project Title	**Development of avian reovirus as a biotherapeutic against poultry pathogens**		
Key words	**poultry, avian reovirus, biotherapeutics, induction of innate immune response, reduce the risk of infectious disease**		
Lead Applicant	**Eva Nagy**		
Organization	**University of Guelph**		
Start Date	**2015-07-01**	**End Date**	**2018-06-30**

Abstract

Infectious diseases represent a significant threat to the poultry industry, in terms not only of animal mortality and economic loss, but also the possibility of zoonotic spread to animals or agricultural workers. During an outbreak scenario, vaccines may not be available, or may have little post-exposure effect. Therefore biotherapeutics that can induce an antiviral response offers promise as a way of limiting disease and transmission. Often, attenuated microbes can serve as immunostimulatory agents. Specifically, microbes that possess double-stranded RNA (dsRNA) are known to stimulate antiviral cytokines. We isolated and partially characterized a novel avian reovirus, a class of viruses whose genome is composed of dsRNA, and whose mammalian counterparts have been shown to induce robust cytokine production. Preliminary work done in our lab shows that this virus is safe, as it does not result in embryo death when it is inoculated into embryonated eggs. We propose to test the efficacy of this virus as a post-exposure biotherapeutic that can limit the replication of other pathogens (eg. influenza virus) in both cell cultures and chickens. Furthermore, we will characterize the immune response induced by this virus to further our understanding of protection against viral diseases of poultry, in particular influenza virus.

Objectives

The overall objective of this study is to evaluate the potential of our novel avian reovirus (both live and inactivated forms) as a biotherapeutic for use against a range of poultry pathogens.

Specific objectives are as follows:

1. Study the innate immune response induced in avian cell lines by our novel reovirus, with focus on measuring production of interferons, as these are key mediators of antiviral immunity.
2. Compare the immunostimulatory efficacy (of this virus) in cell lines between live and inactivated viruses.
3. Determine the roles of individual reovirus genes in stimulating host-innate immunity.
4. Test the antiviral response induced by our reovirus against avian influenza virus. Specifically, investigate if treatment reduces virus titers.
5. Perform dose response experiments in chickens infected with avian influenza or other viruses, to evaluate the ability of reovirus to induce effective antiviral innate immunity.

Results Summary

1) Characterization of the intracellular innate immunity of cell lines

Cells were tested for VSV infectivity using various MOIs and incubation times. A virally encoded fluorescent protein (GFP) allowed monitoring VSV infection. Productive infection was observed in CH-SAH and QT-35 cells.

Polyinosinic-polycytidylic acid (poly I:C), a synthetic dsRNA, a potent immunostimulant, was used to induce the expression of endogenous IFNs. Cells were treated with extracellular or intracellular poly I:C. Poly I:C had toxic effects on CH-SAH cells above 3 ng/ml extracellular poly I:C, or beyond 0.5 ng intracellular poly I:C. DF1 and QT-35, tolerated higher concentrations of poly I:C.

Poly I:C treatment induced the expression of endogenous IFNs sufficient to establish the antiviral state in DF1 and QT-35 cells. CH-SAH cells failed to establish the antiviral state. Our studies showed that CH-SAH cells express endogenous IFNs, it is unknown whether their levels are insufficient to establish the antiviral state or their bioactivity has been compromised. Next, cells were treated with various concentrations of exogenous IFN-α for 6 h and then infected with VSV. All cells established a robust antiviral state suggesting that the Jak/STAT signaling is functional. GFP expression was not observed in all cells pre-treated with exogenous IFN-α, while its expression was observed in untreated cells. CPE was absent in infected CH-SAH and QT-35 when pre-treated with IFN-α.

2) Intracellular innate immunity to ARV-PB

Cells were infected with ARV-PB with various MOIs. As shown before, CH-SAH was highly sensitive to virus-induced cell killing and underwent rapid CPE. DF1 was highly resistant. Relative to CH-SAH, QT-35 underwent slower CPE.

Reoviruses are known to counteract IFN-stimulated antiviral state, we examined this. We first treated the cells with either poly I:C or exogenous IFN-α. Unlike VSV, ARV-PB1 killed CH-SAH and QT-35 in the presence of 10 ng/ml IFN-α. Concentrations above 30 ng/ml induced the antiviral state to ARV-PB1, though CPE was still observed. These observations suggest the ability of ARV-PB1 to counteract IFN response.

3) Induction of intracellular innate immunity by ARV-PB

QT-35 cells were the best system and selected for further studies because of their sensitivity to both VSV and ARV-PB1 and functional pathways for IFN expression and induction of antiviral state. Cells were infected and incubated at different time points, culture supernatants were harvested. Virus was inactivated by UV for 45 min. Supernatants were used as conditioned media for protection assays using VSV as indicator. Cells were incubated overnight with conditioned media and infected with VSV. If ARV-PB1 induces the expression of type I IFNs, incubation of fresh cells with conditioned media, which contain secreted IFNs, would be expected to induce the antiviral state. However, cells failed to induce the antiviral state suggesting that ARV-PB1 may not induce enough IFNs.

Submission number	**UofG2014-1925**		
Project Title	**Using network analysis and dynamic models to develop an understanding of the opportunities and challenges for disease control in equine populations.**		
Key words	**infectious disease, epidemiology, equine, disease transmission, simulation modeling, network analysis, risk assessment, intervention, contact patterns**		
Lead Applicant	**Amy Greer**		
Organization	**University of Guelph**		
Start Date	**2015-06-01**	**End Date**	**2018-06-01**

Abstract

Equine populations present a challenge for emergency management because of the diverse nature of their uses and the associated variability in movement patterns. Animal identification for all equines in Canada is a part of a strategy to ensure traceability and is an important step forward for the prevention and control of infectious diseases in horses. Traceability is a high priority for the Ontario Ministry of Agriculture and Food. The mobile nature of horses as they engage in events creates opportunities for the introduction and spread of infectious diseases within Ontario populations. However, little information exists that would allow for a thorough risk assessment of the epidemic potential that might exist for Ontario populations. We propose to integrate social network analysis and infectious disease transmission modeling in order to address this important knowledge gap. Understanding the network characteristics of equine populations will improve our ability to detect and control outbreaks before the disease becomes widespread. Our results will enable the creation of risk-based surveillance programs and control measures to prevent the spread of equine diseases in Ontario and also help to minimize the economic and emotional impact these diseases can have on the industry and farm families.

Objectives

Understanding infectious disease ecology is at the frontier of biological research, and a major long-term research question is: Under what circumstances can a pathogen invade and spread within a host population? The goal of this research project is to test the mechanisms leading to pathogen invasion and spread. We will use the network structure of Ontario horses and three pathogens (equine herpes virus (EHV), equine influenza (EI), and Streptococcus equi (strangles)) as a model system for advancing our understanding of the network factors controlling disease invasion and transmission, and how these factors interact creating epidemic potential within the equine population.

This research will address the following objectives:

1. Describe the network structure of the Ontario equine industry.
2. Determine how network structure influences the potential for disease introduction and spread within different equine populations in Ontario.

Results Summary

The first phase of this research aims to describe how contacts form between horses as they travel, and how this contact structure influences the risk of disease spread. Horse owners were asked to document their travel patterns over a competition season. These travel patterns will be analyzed using mathematical and graphical techniques to explore the qualities of horses and premises that may put them at higher risk of acquiring disease. As the potential for outbreaks increases, it is important to understand how contacts between horses can facilitate disease spread so the industry can prepare disease prevention strategies. The limited ability to describe this contact structure prevents an accurate estimate of epidemic potential. Data analysis is currently underway. This is the

first project in Ontario to use these unique methods to understand infectious disease spread. The outcomes will inform targeted disease surveillance programs to reduce the emotional and economic consequences of disease in the equine industry. Our results can assist knowledge-users in the industry to determine which disease control strategies would be effective in an outbreak. Exploring the relationship between horses as they travel is a fundamental step to inform strategies for disease management.

Submission number	**UofG2014-1868**		
Project Title	**Developing An Economic Model of Ontario's Pork Sector with Application to Disease Outbreak and Policy Response Analysis**		
Key words	**disease outbreak, economics, policy response,**		
Lead Applicant	**Alan Ker**		
Organization	**University of Guelph**		
Start Date	**2015-06-01**	**End Date**	**2017-04-30**

Abstract

The purpose of this project is to develop an econometric model of Ontario's swine sector: producer (farrow to finish, farrow to wean, finishing), processor, retailer. This model will be used to examine the consequences -- particularly the costs -- of various disease scenarios and subsequent intervention responses from trade partners, with particular emphasis on identifying the consequences for producers and consumers at each level of the value chain. More specifically, the model will permit users to synthetically apply shocks to a series of supply and demand equations to generate counterfactual outputs and prices at various stages of production for a given year. When compared with the baseline scenario, the counterfactual outputs can be used to calculate estimates of the net gain or loss at each stage along the value chain and across market participants. The model would provide the Ministry, the Policy Institute, and industry groups a tool to facilitate informed policy discussions in response to disease outbreaks. The main advantage of the proposed approach is its flexibility: the model can be used to assess any number of disease outbreak scenarios as they arise as well as a number of other potential market scenarios.

Objectives

The first objective of this project is to build an econometric model of Ontario's swine industry. The model would be grounded in historical data and use robust empirical methods. Most importantly, the model will capture the nuances of the industry. By developing a credible model, we can examine any number of "what-if" questions, especially changes to the industry resulting from exogenous shocks due to disease outbreak and potential retaliatory actions from trade partners.

The second objective of this project is to significantly increase the stock of human capital, which currently may be lacking, in the Ministry as well as the Department of Food, Agricultural, and Resource Economics (FARE) to estimate the economic impacts of disease outbreaks for not only the Ontario swine industry but other industries as well.

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