OAHN FINAL REPORT

Project #: OAHN-10

Project Title: Developing a Health and Disease Surveillance Network for Ontario Mink Farms

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Start date: Feb 1, 2016 **End date:** Dec 31, 2017

Executive Summary

This project evaluated the occurrence of infectious diseases on a limited number of Ontario mink farms over a two-year period. It proved difficult to recruit producers to participate in this program. Fecal PCR shedding patterns were evaluated on 100% of Ontario mink farms for both 2016 and 2017. Mink astrovirus, hepatitis E virus, ad porcine rotavirus were detected in some samples from some farms in both 2016 and 2017, generally at modest levels. Antimicrobial resistance was also detected on 3 of 44 and 5 of 42 farms for 2016 and 2017, respectively, including on one farm in 2017 with high levels of resistance to multiple agents. Because of the known poor biosecurity standards on mink farms, this suggests that antimicrobial use should be monitored and controlled for these animals in the future.

Objectives:

The original objectives of this project were three-fold:

a) to provide partial support for semi-annual on-farm veterinary herd health visits to Ontario mink farms in 2016 and 2017 and support for diagnostic pathology work-up (including ancillary testing) of a subset of animals found dead on participating farms;

b) using validated assays, to test tissue samples from mink pathology submissions for emerging or known disease-inducing viruses, including AD (with sequencing of positive samples), mink astroviruses, mink hepatitis E virus, and mink rotavirus; and

c) to conduct mink welfare audits with producers to ensure that their management practices support optimal mink health.

Because no farms participated in herd health visits for either 2016 or 2017, this objective was removed and replaced with evaluating fecal samples collected from females from all Ontario mink farms for the presence of the same emerging or known disease-inducing viruses.

Similarly, because the Canadian Mink Breeder Association decided to launch their own animal welfare assessment scheme, this objective was replaced with an assessment of antimicrobial resistance of

relevant enteric bacteria (i.e., *Salmonella* spp. and *E. coli*) from fecal samples collected from females from all Ontario mink farms in 2016 and 2017. This work was conducted in collaboration with the Public Health Agency of Canada.

Materials and methods

For each diagnostic submission, gross and microscopic evaluations were conducted on tissues submitted from a maximum of 5 animals per producer. Culture and susceptibility typing was done on all relevant samples as well as testing for distemper, avian influenza, Aleutian disease, and mink enteritis virus. CIE testing was also available to producers for up to 200 blood samples.

For viral PCR assays, specific assays had been developed and validated in the Turner lab. Pooled fecal samples from under the cages of up to 3 adult females were collected, mixed in the bag, then frozen at -70C until processed.

Results

a) **2016 disease surveillance summary**

- For 2016, in entirety, we had 5 farms submit samples in total, and 1 farm was able to submit twice. There were no herd health visits conducted under this project funding. The results suggest a high value for farms when submitting mink cadavers to the AHL for processing. There is a high level of infectious disease on farm – both viral and bacterial – and regular submissions would assist the herd veterinarians in making appropriate recommendations to better manage the health of animals on-farm.

Testing provided valuable information on animal mortality, identifying:

Farm A: canine distemper infections, mixed bacterial pneumonia and cystitis, intestinal hemorrhage
Farm B: Aleutian Disease, severe mixed bacterial rhinitis (necrotizing dermatitis)
Farm C: canine distemper infections + mixed bacterial rhinitis
Farm D: Aleutian Disease
Farm E: mixed bacterial infections underlying death in 3 of 5 animals (cystitis, meningoencephalitis, bronchitis, and peritonitis

CIE Testing for AD

- Three producers submitted samples for CIE testing in the fall of 2016. There are no results on the final Sapphire reports for 2 of the farms (16-068365, 16-068393), and Virology has been contacted to clarify the interpretation.
- Farm D (16-090111) 20 of 24 samples tested positive for AD

2017 disease surveillance summary

- For 2017, we had 5 farms submit in total, and 1 farm submitted twice while another submitted three times. There were no herd health visits conducted under this project funding.
  Testing provided valuable information on animal mortality, identifying:

  Farm F: disseminated bacterial gastroenteritis (*Neisseria* sp.), pyothorax (*Pasteurella* spp.), unspecified hemorrhagic pneumonia, and hepatic lipidosis.
  Farm G: Stress hemorrhage due to cold ambient temperatures and failure to transition to solid diet.
  Farm H: Septicemia
  Farm J: Hemorrhagic pneumonia and coliform septicemia
  Farm K: Bacterial pneumonia (*S. delphi, E. coli*), hemorrhagic pneumonia, bacterial cystitis (*S. delphi*), vertebral osteomyelitis, chronic diaphragmatic rent, coliform enteritis, and distemper.

  b) As part of this study, we analyzed fecal samples from mink kits and adult females for the presence of mink astrovirus, porcine rotavirus and mink hepatitis E virus (summer 2014 and summer 2015 – mink kits and adult females, winter 2016 and winter 2017 – adult females only). Previous studies from Europe have shown these viruses to cause disease in farmed minks, as well as being a risk factor for human transmission within the farm and to outside populations. These viruses have never been evaluated before in Canadian mink and no vaccines exist to prevent these infections from occurring on mink farms.

  Gastrointestinal astrovirus can cause diarrhea and possible embryonic or kit mortality, while in humans it causes symptoms similar to stomach flu. It primarily affects young, old or immunocompromised animals and is normally self-resolving with mild diarrhea and abdominal pain. The virus can also be found in healthy mink and may require other co-factors to cause disease. It is currently unknown whether mink astrovirus can be transmitted to humans or other animals. Good handwashing and hygiene practices on-farm will eliminate the risk of virus transmission.

  Porcine rotavirus or similar strains are normally found in pigs, and are transmitted through ingestion of undercooked or raw meat. Porcine rotavirus in mink can cause outbreaks of diarrhea, and the virusoften exists in conjunction with other pathogens. In humans, porcine rotavirus can cause diarrhea, more often in young children. Again, good handwashing and hygiene practices on-farm eliminate the risk of virus transmission.

  Hepatitis E virus (HEV) has a primary effect on the liver, causing liver damage and sometimes, diarrhea. In mink, the virus has been linked to outbreaks of diarrhea and increased on-farm mortality of younger animals. Whether mink hepatitis E virus can infect humans is unknown; however, pig and rabbit virus variants can cause significant disease in humans.

	2014					
	Astrovirus		Rotavirus		Mink Hepatitis E	
	Adult	Kits	Adult	Kits	Adult	Kits
Positives - individual						
Positives - overall	16	27	0	0	2	11
Total # tested	87	86	87	86	87	86

	2015					
	Astrovirus		Rotavirus		Mink Hepatitis E	
	Adult	Kits	Adult	Kits	Adult	Kits
Positives - individual						
Positives - overall	9	17	3	4	6	15
Total # tested	96	98	96	94	96	98

(Note: the above tables have an open line to allow us to customize the results in reports sent to individual farms – only the total positives are included for the OAHN report). Information is also presented graphically below.

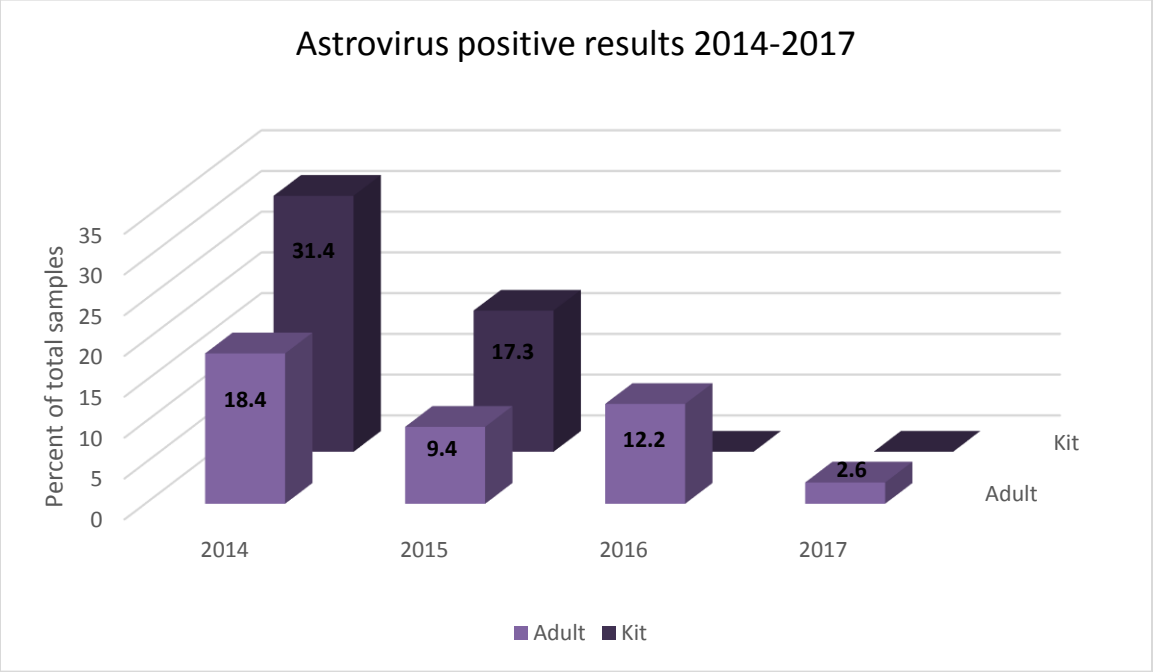
	2016		
	Astrovirus	Rotavirus	Mink Hepatitis E
	All Adult Females		
Positives - individual			
Positives - overall	5	1	4
Total # tested	41	41	41

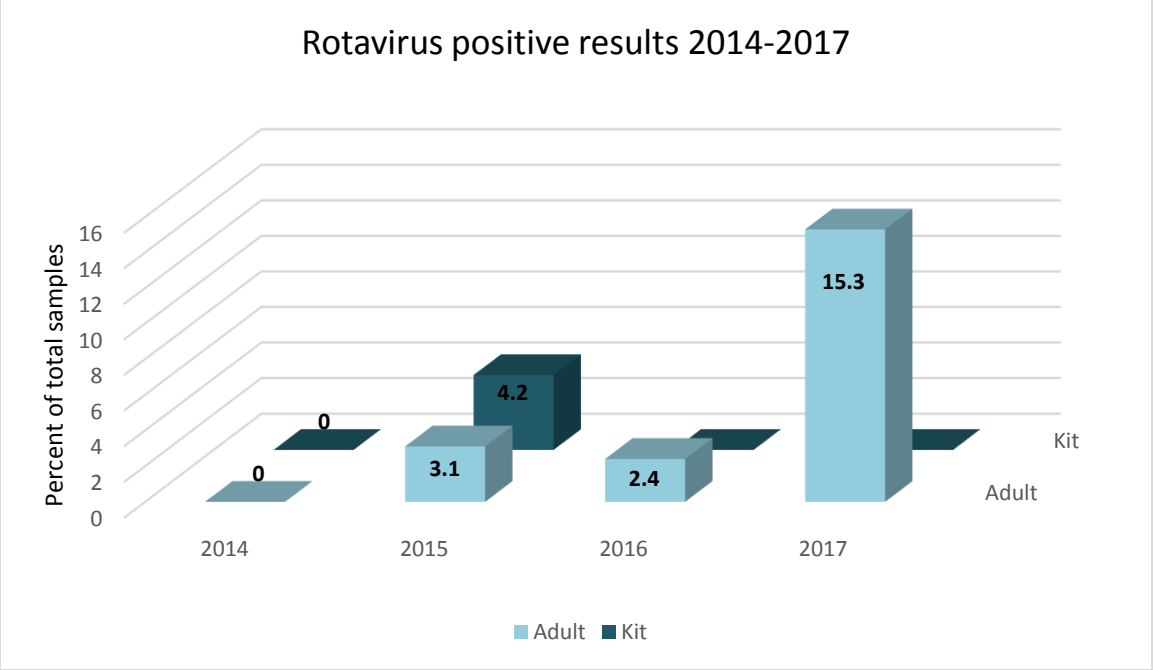
	2017		
	Astrovirus	Rotavirus	Mink Hepatitis E
	All Adult Females		
Positives - individual			
Positives - overall	1	6	4
Total # tested	39	39	39

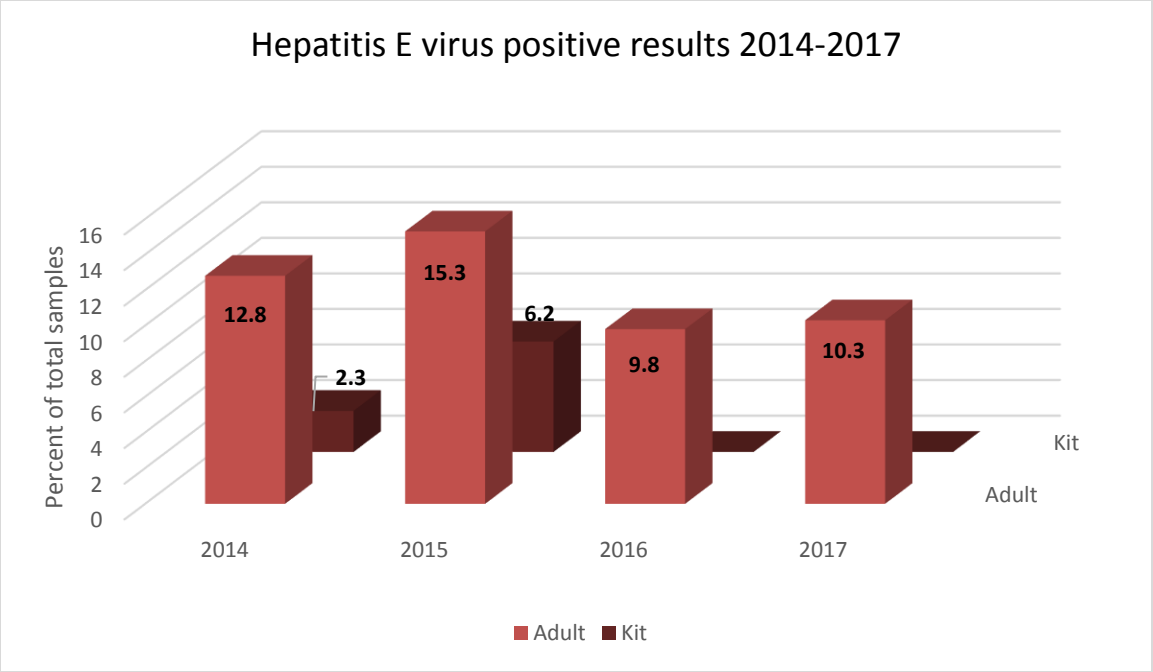
Co-infections or infection with more than one virus type, were also found in several samples as seen below. Co-infection can result in a higher risk of active disease. (AV = astrovirus, HEV = mink hepatitis E virus, RV = rotavirus). The following table summarizes the number of samples per year that demonstrated virus co-infections.

	Adults	Kits
2014	AV & HEV – 2 samples	AV & HEV – 3 samples
2015	None	AV & HEV – 1 sample RV & HEV – 1 sample
2016	None	N/A
2017	AV & RV – 1 sample	N/A

Virus PCR assay results:







c) AMR evaluation of fecal bacteria

In addition to specific viral testing, samples were also submitted to the Public Health Agency of Canada as part of an ongoing project for antimicrobial resistance (AMR) testing. This test isolates and identifies specific bacterial strains present in feces (such as *E. coli* and *Salmonella* species) and determines bacterial sensitivity or resistance to common antibiotics. As most gut bacteria are readily transmissible to humans, if these bacteria are resistant to antibiotics, this can transfer the resistance to infected human hosts. This becomes very important in antibiotic therapy for many infections, and in preventing antimicrobial resistance in other organisms. Appropriate biosecurity on the farm, proper use of antibiotics (drug type, dose, and duration of treatment) under the recommendation and supervision of a veterinarian, and good hygiene practices are essential in reducing the spread of antibiotic-resistant bacteria. Canada is monitoring AMR in all farmed animal species as part of a global project overseen by the World Health Organization. Because the genetic information for antimicrobial resistance can be passed between different bacteria in animals and humans there is significant interest in monitoring resistance even if farmed animals do not enter the food chain.

In 2016, 12 different strains of *E. coli* were isolated from adult female mink fecal samples from eight of 38 farms. Of these, three farms were positive for resistance to one or more of five antimicrobials.

2016	Ciprofloxacin	Streptomycin	Sulfisoxazole	Tetracycline	Trimethoprim-Sulphamethoxazole
Farm 1			Resistant	Resistant	Resistant
Farm 2	Resistant	Resistant			
Farm 3				Resistant	

In 2017, 10 different strains of *E. coli* were isolated from adult female mink fecal samples from seven of 37 farms. Of these, five farms were positive for resistance to one or more of eight microbials.

2017	Amoxicillin /Clavulanic Acid	Ampicillin	Cefoxitin	Ceftriaxone	Gentamicin	Streptomycin
Farm 1	--	--	--	--	--	--
Farm 2	--	Resistant	--	--	Resistant	Resistant
Farm 3	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant
Farm 4	--	--	--	--	--	--
Farm 5	--	--	--	--	--	--

2017 cont'd	Sulfisoxazole	Tetracycline	Trimethoprim/Sulphamethoxazole
Farm 1	--	Resistant	--
Farm 2	--	Resistant	--
Farm 3	Resistant	Resistant	--
Farm 4	--	Resistant	--
Farm 5	Resistant	Resistant	Resistant

Applications

The results of this study have confirmed the utility of diagnostic pathology services for monitoring health conditions on mink farms. Infectious diseases – both bacterial and viral – are highly common on mink farms and can have devastating effects. No true incidence of prevalence estimates can be produced because of the low participation rate. It was unfortunate that despite being offered repeated opportunities to participate in more in-depth on-farm herd health assessments, the vast majority of Ontario mink producers elected not to participate. In informal polling of producers at regional meetings there was no consistent reason offered for this. In a few cases, concern was expressed about the lack of expertise of veterinarians visiting farms; however, the producers were being offered this service free. In general, Ontario mink producers are used to working relatively independently.

The results of the viral PCR assays do not conclusively demonstrate active viral infections, since the PCR assay can detect virus passing through the gastrointestinal tract. However, they strongly suggest that mink-specific astrovirus and hepatitis E virus are present within Ontario mink herd populations. Both kits

and adults are infected, although increased numbers of adults were demonstrated to be shedding hepatitis E compared with kits in all years. Porcine rotavirus was detected in low levels in the feces of adult mink in 2015-2017, and may be related to feeding undercooked or contaminated pork products. All 3 viruses are thought to have zoonotic potential, which raises some concerns, given the known low level of personal hygiene when working in mink farms as well as the variable biosecurity practices.

Levels of antimicrobial resistance were generally low and consistent with levels seen in other minor use species, such as sheep. Slightly more resistance was noted in 2017 compared with 2016 (5 of 42 vs 3 of 44 in 2016) and all 5 farms with AMR in 2017 demonstrated resistance against tetracycline in 2017. One farm demonstrated resistance in 2017 to all antimicrobials tested, except trimethoprim/sulpha. Enhanced veterinary oversight and tracking of antimicrobial use on mink farms will be beneficial in monitoring this trend.

Discussion / Suggestions for next steps

It is clear that the Ontario mink industry would benefit from closer relationships with veterinarians. Producers submitted specimens in this study when large outbreaks were occurring. The diagnostics conducted were beneficial in helping to plan a course of action on the affected farms. We anticipate that relationships with veterinarians will increase with increased regulation and oversight of antimicrobial drug prescription and use in animal species in Canada.

The importance of PCR detection of mink hepatitis E and astrovirus and porcine rotavirus are difficult to determine without further study of viral pathogenicity, time course of infection and shedding, and the impact of co-infections on the health and production of mink.

Communications

Scientific papers:

Xie XT, Macdonald RE, Tapscott B, Nagy E, Turner PV. Fecal shedding patterns of astrovirus, rotavirus, and hepatitis E virus in adult female and weaned farmed mink (*Neovison vison*). (submitted)

Xie XT, Kropinski A, Tapscott B, Weese JS, Turner PV. 2018. Prevalence of fecal viruses and bacteriophage in Canadian farmed mink (*Neovison vison*). Microbiol Open (accepted).

Compo NR, Gomez DE, Tapscott B, Weese JS, Turner PV. Fecal bacterial microbiota of Canadian commercial mink (*Neovison vison*): Yearly, life stage, and seasonal comparisons. (submitted)

Posters: (note: this does not include posters presented at the OVC Grad Research Day)

Xie XT*, Nieto-Gomez D, Nagy E, Weese JS, Turner PV. Eukaryotic and phage viruses present in the commercial mink fecal virome: a metagenomic study. CPHAZ Symposium, University of Guelph, Guelph, ON, May 23, 2017.

Other:

N. Compo. DVSc thesis, August, 2017.