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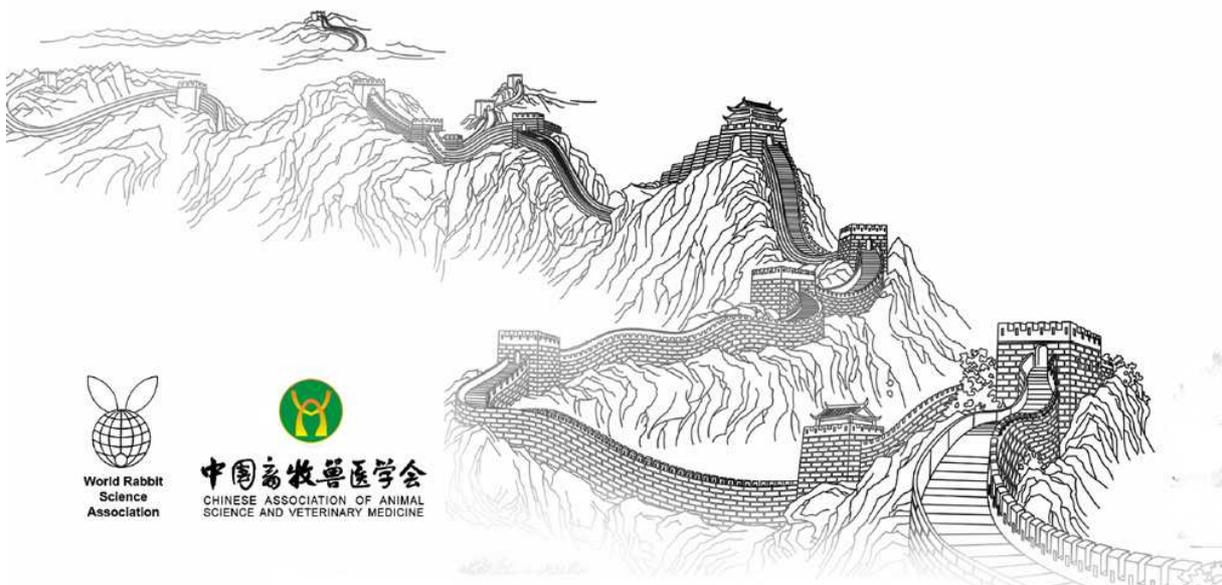
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## PREVALENCE OF PATHOGENIC VIRUSES WITHIN ONTARIO COMMERCIAL MEAT RABBITS

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### ABSTRACT

The Ontario commercial rabbit industry produces an important alternative source of meat, and Ontario farms represent approximately 40% of Canadian operations. Production losses from birth to weaning range up to 36%, largely from infectious enteric and respiratory diseases. The purpose of this study was to assess the prevalence of rabbit-specific rotavirus, astrovirus, and hepatitis E virus (HEV) in Ontario commercial meat rabbits and compare to other rabbit populations, including research, animal shelters, and pets. Pooled rabbit fecal samples (n=108) from healthy animals were collected from 27 commercial farms (separate samples from fryers and does) in both summer and winter to determine whether there was a seasonal difference in virus shedding. Fecal samples were extracted and PCR assays conducted to look for the presence of rabbit-specific rotavirus, astrovirus, and HEV. Our results show that of the tested Ontario commercial rabbit farms, 48% were positive for astrovirus and 2 were positive for rotavirus, with no detection of rabbit. One sample from a group of commercial fryers, submitted for post mortem analysis from 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.

**Keywords:** zoonoses, rabbit rotavirus, rabbit astrovirus, rabbit hepatitis E virus, pathology

### INTRODUCTION

The Ontario commercial rabbit industry forms a small but important alternative source of meat for Ontario consumers and typical operations range from 50 to 1200 does and are often family run operations. Processors estimate that domestic market demands are 200% of current supply; however, producers are hampered from increasing productivity by the high levels of enteric and respiratory disease. Recent surveys have indicated that losses from birth to market range from 24% to 36%. In 2006, an Ontario-wide outbreak of infectious idiopathic enteritis resulted in high herd mortality exceeding 40% (Kylie, et al, 2016, unpublished). Because of the narrow profit margin, producers are less likely to submit moribund animals for full diagnostic work-up and it has been difficult to gain an adequate epidemiologic picture to assist the industry with herd management, efficiency, and productivity.

The European scientific community considers lapine rotavirus to be a significant co-factor in rabbit enteritis complex. Rabbit astrovirus has been reported in association with enteritis in commercial meat rabbits in Europe and China (Martella et al, 2011; Stenglen et al, 2012) but is not routinely tested for in Canadian rabbit fecal samples. Further, in 2011, a newly emerging hepatitis E virus (HEV) was discovered in commercial US and Chinese rabbitries with prevalence up to 36% (Cossaboom et al, 2011; Zhao et al, 2009). Whether the virus is zoonotic is unknown; however, viral sequence analyses demonstrated homology to pig and human virus variants. The prevalence of these agents in Ontario and

Canadian commercial rabbits is unknown. Significant mixing of rabbits occurs during lairage as well as during transportation of live rabbits for slaughter in the U.S. The combination of industry-wide enteric disease with unregulated antimicrobial use and newly identified rabbit pathogens suggested the need for an in-depth study.

## MATERIALS AND METHODS

### Animals and experimental design

108 pooled fresh fecal samples (both healthy fryers and does, representing at least 324 rabbits) were collected from 27 commercial farms in summer and winter months, to evaluate whether virus shedding was occurring and if so, whether there were temporal or seasonal differences in virus shedding patterns. In addition, 14 individual or pooled fecal samples were evaluated from cases of clinically sick rabbits presenting for post mortem through the Animal Health Laboratory, that is the provincial diagnostic laboratory associated with the University of Guelph. Samples were returned to Guelph where they were crushed, mixed, and frozen in aliquots at -80C for further analysis. All animal procedures and use were approved by the University of Guelph Animal Care Committee (AUP10R087).

### RNA Extraction and PCR Assays for Rabbit Viruses

Fecal samples (0.2g) were thawed, RNA was extracted and denatured, and cDNA was synthesized using the QuantiTect Reverse-Transcriptase cDNA synthesis kit (Qiagen, Mississauga, ON, Canada). Lapine rotavirus, astrovirus, and HEV specific RT-PCR assays were conducted (Table 1).

Table 1: PCR Conditions for Rabbit-Specific Virus Assays

	Rotavirus <sup>a</sup>	Astrovirus <sup>b</sup>	HEV <sup>c</sup>
<b>Forward Primer</b>	RRV-VP7-F1	MDS-119fwd	RabdegF2
<b>Reverse Primer</b>	RRV-VP7-1062	MDS-120rev	Rabdeg22
<b>Annealing Temp</b>	49°C	57°C	45°C
<b>Extension*</b>	72°C, 90s	72°C, 30s	72°C, 60s 1 <sup>st</sup> round, 30s 2 <sup>nd</sup> round
<b>Positive Control</b>	BRV8-De	Bov13-14b	+GSP (HEV cDNA gene specific primers)

\*29 rounds of extension for all. <sup>a</sup>Matthijnsens et al (2006), <sup>b</sup>Stenglein MD et al (2012), <sup>c</sup>Cossaboom et al (2011)

PCR products were subsequently evaluated by gel electrophoresis (see astrovirus example, Figure 1). Positive samples were sequenced and compared using BLAST and CLUSTALW for known viruses.

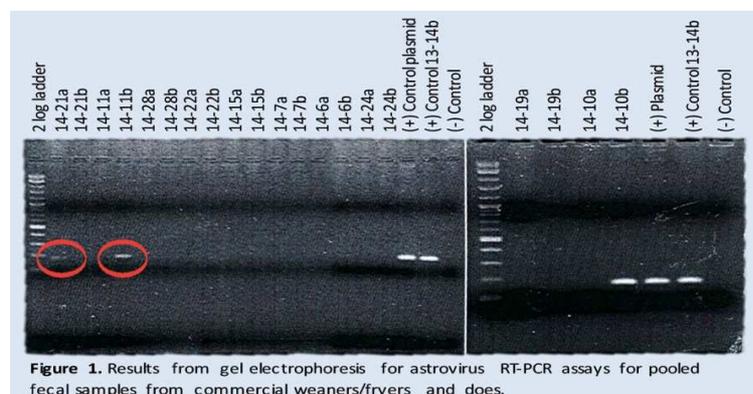


Figure 1 : Results from gel electrophoresis for astrovirus RT-PCR assays for pooled fecal samples from commercial weaners/fryers and does

## RESULTS AND DISCUSSION

The overall results of the prevalence of virus shedding, by rabbit age and season are shown in Table 2. Fecal virus shedding was more common in younger animals and rabbit astrovirus shedding was found in pooled fecal samples on 48% of farms evaluated. Samples that were positive on one sampling were not necessarily positive on the second sampling and vice versa. Does were more likely to be shedding virus in the summer months, although there was no difference in astrovirus shedding by season in younger growing rabbits. Shedding of rotavirus and HEV was not detected in does while rotavirus shedding was only noted in growing rabbits on one farm that was experiencing an outbreak of diarrhea at the time of sampling. On that farm, both rotavirus and astrovirus were detected in feces from healthy and sick growing rabbits, but neither virus was detected in does. Both rotavirus and HEV were detected in the feces of a sick rabbit from a farm not included in the survey, which was presented for pneumonia.

**Table 2:** Prevalence of Fecal Virus Shedding on Ontario Commercial Meat Rabbit Farms by Rabbit Age and Season

Virus	Does		Growers/Fryers	
	Winter	Summer	Winter	Summer
Astrovirus	2/27 (7%)	5/27 (19%)	7/27 (26%)	6/27 (22%)
Rotavirus	0	0	1/27 (4%) <sup>a</sup>	0/27 +1 <sup>b</sup>
Hepatitis E virus	0	0	0	0/27 +1 <sup>b</sup>

<sup>a</sup>2 positive pooled samples were detected on the same farm, 1 from healthy growers and 1 from growers with diarrhea

<sup>b</sup>1 positive individual sample from a post mortem specimen submitted for an outbreak of pneumonia. The same animal was positive for both rotavirus and hepatitis E

Rabbit enteritis complex (REC) is a common disease condition of commercial rabbits characterized by diarrhea and related pathological effects. REC results in serious economic losses in the commercial rabbit industry as it is a major cause of morbidity from birth to weaning (Peeters et al, 1984). The development of REC is multifactorial and may involve bacterial agents as well as viral agents, including rotavirus (Percy et al, 1993) and other viruses. Rabbit rotavirus is considered an important co-factor in the pathogenesis of REC in rabbits from European farms. The results of this study suggest that rotavirus is less common in both healthy and sick commercial rabbits in Canada. We did not assay for the presence of rotavirus antibodies, so cannot determine whether animals had been infected at younger ages and had cleared the virus or had never been infected.

Rabbit astrovirus is a novel astrovirus that was recently identified with over 40% prevalence in young rabbits with enteritis in Italy (Martella et al, 2011). Astrovirus infection is a very common cause of diarrhea in children and it is thought to be important in the development of enteritis complex of turkeys, but its role in the pathogenesis of REC is still unknown. This study suggests that rabbit astrovirus is highly prevalent in growing and breeding rabbits in Canada, although it is found in both healthy and sick rabbits. More work needs to be done to evaluate its role in rabbit enteritis.

In recent years, HEV has been detected in rabbits in three geographically separate regions. In Beijing, China, 57% of farmed Rex rabbits were found to have anti-HEV antibodies, and 7.5% were positive for the presence of HEV RNA (Zhao et al, 2009). In Virginia, US, 49% of commercial rabbits of various breeds were infected with HEV, as detected through fecal shedding, viremia or seropositivity (Cossaboom et al, 2011). Prevalence of HEV RNA in France was determined to be 7% and 23% in farmed and wild rabbits, respectively (Izopet et al, 2012). Genome analyses and comparisons of the various rabbit strains revealed similarities to established HEV genotypes 3 and 4, but sequence divergence suggests that these strains represent their own distinct genotype (Izopet et al, 2011, Zhao et al, 2009). As genotypes 3 and 4 are transmissible between humans and other animal species, this novel strain may also hold zoonotic

potential. Our study suggests that rabbit HEV is uncommon but not completely absent from Ontario commercial meat rabbit populations. Stock from commercial rabbit farms is occasionally sold to pet stores and directly to people interested in purchasing rabbits as pets. We have also detected rabbit-specific HEV in the feces of clinically healthy companion pet rabbits in Ontario (results not shown). Fecal shedding of rabbit HEV is a potential public health concern as rabbit-specific virus has been shown to infect cynomolgus macaques with resultant development of hepatitis (Liu et al, 2013).

## CONCLUSIONS

This study determined that rabbit astrovirus is shed in the feces of clinically healthy growing and breeding Ontario rabbits at high prevalence throughout the year, although the significance of this is unknown. Rotavirus and HEV are both shed with very low prevalence, primarily in the feces of clinically diarrheic young, growing rabbits.

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## REFERENCES

- Cossaboom CM, Cordoba L, Dryman BA, Meng X. 2011. Hepatitis E Virus in Rabbits, Virginia, USA. *Emerg Infect Dis.* 17(11): 2047-2049.
- Izopet J, Dubois M, Bertagnoli S, Lhomme S, Marchandeanu S, Boucher S, Kamar N, Abravanel F, Guerin J-L. 2012. Hepatitis E virus strains in rabbits and evidence of a closely related strain in humans, France. *Emerg Infect Dis.* 18(8):1274-1281.
- Kylie J, Brash M, Whiteman A, Sears W, Slavic D, Weese JS, Turner PV. 2016. Causes of on-farm mortality and biosecurity practices on Ontario commercial meat rabbit farms. *Can Vet J (in preparation)*.
- Liu P, Bu QN, Wang L, Han J, Du RJ, Lei YX, Ouyang YQ, Li J, Zhu YH, Lu FM, Zhuang H. 2013. Transmission of hepatitis E virus from rabbits to cynomolgus macaques. *Emerg Infect Dis.* 19(4):559-65.
- Matthijnsens J, et al. 2006. Full genomic analysis of human rotavirus strain B4106 and lapine rotavirus strain 30/96 provides evidence for interspecies transmission. *J Virol.* 80(8):3801-10.
- Martella V, Moschidou P, Pinto P, Catella C, Desario C, Larocca V, Circella E, Banyai K, Lavazza A, Magistrali C, Decaro N, Buonavoglia C., 2011. Astroviruses in Rabbits. *Emerg Infect Dis.* 17:2287-2293.
- Peeters JE, Pohl, Charlier G. 1984. Infectious agents associated with diarrhoea in commercial rabbits: a field study. *Ann Vet Res.* 15(3):335-340.
- Percy DH, Muckle CA, Hampson RJ, Brash M. 1993. The enteritis complex in domestic rabbits: A field study. *Can Vet J.* 34:95-102.
- Stenglein MD, Velazquez E, Greenacre C, Wilkes RP, Ruby JG, Lankton JS, Ganem D, Kennedy MA, DeRisi JL. 2012. Complete genome sequence of an astrovirus identified in a domestic rabbit (*Oryctolagus cuniculus*) with gastroenteritis. *Virology* 9:216:216-226.
- Zhao C, Zhongren M, Harrison TJ, Feng R, Zhang C, Qiao Z, Fan J, Hongxia M, Li M, Song A, Wang Y. 2009. A novel genotype of hepatitis E virus prevalent among farmed rabbits in China. *J Med Virol.* 81:1371-1379.

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