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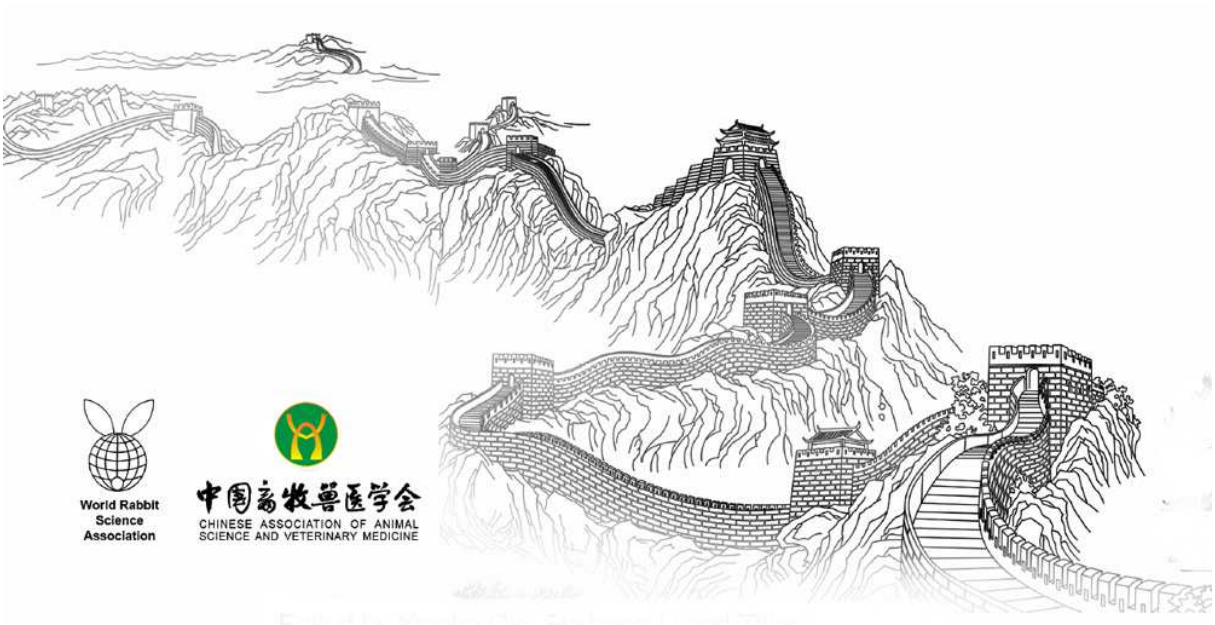

EVALUATION OF ANTIMICROBIAL RESISTANCE IN ONTARIO COMMERCIAL MEAT RABBITS

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ABSTRACT

Enteritis is common in commercial rabbit operations and is often treated empirically with antimicrobial agents. One area of significant concern is the off-label use of antimicrobials in rabbits and the potential for this to lead to the development of antimicrobial resistance, which may be transmitted to humans or other animals. This study examined feces from commercial rabbit populations for two potentially zoonotic bacterial agents of diarrhea, *Escherichia coli* and *Salmonella* spp., and also examined whether these bacteria demonstrated antimicrobial resistance to commonly used antimicrobials using standard microbiology culture and sequencing techniques. Fecal samples were collected from both does and fryers from 27 Ontario commercial rabbitries during winter and summer months (n=108 pooled samples from a total of 324 rabbits). *E. coli* was found to be 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 enterica* ser. 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.

Key words: Biosecurity, Zoonoses, Food safety

INTRODUCTION

Antimicrobials are commonly used in veterinary medicine to treat infectious disease, but also to prevent development of disease and for growth promotion in some food animals. While the development of antimicrobials has resulted in major declines in the prevalence of infectious diseases and improving animal health overall, the development of resistance to these antimicrobial agents has become a significant concern in human and veterinary medicine. Antimicrobial resistance (AMR) is defined as "the ability of microbes to grow in the presence of a chemical (drug) that would normally kill them or limit their growth" (NIH, 2009). As a result of developing resistance, bacteria that were once successfully eliminated by these drugs are now less responsive and can proliferate despite their presence.

Because of the potential significance of AMR for human health, the World Health Organization (WHO) has established a global AMR surveillance system, with the goal of "assessing the burden of AMR and [...] providing the necessary information for action in support of local, national, and global strategies" (WHO, 2011). It aims to aid in making appropriate evidence-based decisions with respect to antimicrobial use worldwide. In Canada, the Canadian Integrated Program for Antimicrobial Resistance Surveillance (CIPARS) was established in 2002 to monitor antimicrobial use and AMR in bacteria obtained from humans, animals, and animal-derived food products across Canada, with the goal of identifying

"appropriate measures to contain the emergence and spread of resistant bacteria among animals, food, and people". In animals and meat, the routinely examined bacteria are *Escherichia coli*, *Salmonella* spp., and *Campylobacter* spp. (CIPARS, 2013). These are of particular concern because of their zoonotic potential and likelihood to be transmitted through the food chain, as well as their ability to transmit resistance genes (Salyers, 1995). In Canada, surveillance of bacteria in animals and their meat products is done exclusively in cattle, chickens, turkeys, pigs, and horses (CIPARS, 2013). Smaller food-producing animal commodities, such as rabbits, sheep, and goats, are not included in this analysis. In addition, animals that are often in close contact with humans and therefore have potential to spread disease and AMR, such as pets or laboratory animal species, are also not routinely assessed. Recent studies have demonstrated, however, that despite their lesser presence, these species are still potential vectors for AMR bacteria. For example, in a study published in 2012 by Scott *et al* examining the presence of AMR in *E. coli* and *Salmonella* spp. in sheep flocks in Ontario, it was demonstrated that 13.1% of the total 849 *E. coli* isolates identified were resistant to at least one antimicrobial.

Because of the close working relationship between humans and meat rabbits, as well as between rabbits and other farm animal species, there is concern regarding the possibility of spread of *E. coli* and *Salmonella* spp. between human and rabbits, and, in particular, transmission of antimicrobial resistant strains of *E. coli* and *Salmonella* spp. between animal species. AMR has not been reported for any bacteria in rabbits in Canada; however, resistance has been reported for several bacterial species, including *E. coli* and *Salmonella* spp., in several other countries, suggesting that this is a distinct possibility for rabbits in Canada (for example, Badagliacca *et al*, 2012; Roug *et al*, 2013). Thus, the aim of our study was to identify the prevalence of *E. coli* and *Salmonella* spp, as well as levels of antimicrobial resistance within these bacteria, in Ontario commercial meat rabbits.

MATERIALS AND METHODS

Animals and experimental design

Separate fecal samples were collected from under the cages of does and fryers from 27 Ontario commercial rabbitries during both winter and summer months (n = 100 pooled samples from a total of 324 rabbits). Samples were aliquoted and frozen at -80C for further testing. Information about on-farm antimicrobial use was obtained at the time of fecal collection.

Bacterial isolation and susceptibility testing

Fecal samples were homogenized, inoculated into buffered peptone water (Becton Dickinson, ON, Canada), and then streaked onto MacConkey's agar and Xylitol Lysine Tergitol-4 (for *Salmonella* spp.) plates (Becton Dickinson) and incubated at 37^{o}C overnight. Three isolated presumptive colonies from plates with growth were positively identified for *E. coli* and/or *Salmonella* spp. and sent to the Laboratory for Foodborne Zoonoses in Guelph, Canada for susceptibility testing following the protocols of CIPARS, and using the broth microdilution method (Sensititre System; Trek Diagnostics, OH). *E. coli* and *Salmonella* spp. isolates from each sample were tested for susceptibility to the following antimicrobial agents (the respective breakpoints are included within parentheses): amoxicillin/clavulanic acid ($\geq$32ug/mL), ampicillin ($\geq$32ug/mL), azithromycin (no break-point available), cefoxitin ($\geq$32ug/mL), ceftiofur ($\geq$8ug/mL), ceftriaxone ($\geq$4ug/mL), chloramphenicol ($\geq$32ug/mL), ciprofloxacin ($\geq$1ug/mL), gentamicin ($\geq$16 ug/mL), kanamycin ($\geq$64ug/mL), nalidixic acid ($\geq$32ug/mL), streptomycin ($\geq$64ug/mL), sulfisoxazole ($\geq$512ug/mL), tetracycline ($\geq$16ug/mL), and trimethoprim/sulphamethoxizole ($\geq$4ug/mL).

Antimicrobial resistance gene detection for *Salmonella* Kentucky

Salmonella enterica ser. Kentucky lysates were prepared as described by Kozak et al, 2009. Using a set of novel multiplex PCR protocols performed using a Qiagen multiplex PCR kit (Qiagen, Mississauga, ON, Canada), testing for the β-lactamase gene $bla_{\text{CMY-2}}$ was conducted with 1x Qiagen multiplex PCR mixture,

1x Q-solution, and 1x primer mixture and 25uL test mixtures. Primers used were for CMYF and CMYR with the sequences GACAGCCTCTTTCTCCACA and TGGACACGAAGGCTACGTA, respectively.

Statistical analyses

The prevalence of *E. coli* and *Salmonella* spp. as well as the prevalence of AMR were calculated at a facility level using prevalence estimates with both upper (UL) and lower (LL) Sterne limits. Where the prevalence estimate was zero or one, the mean unbiased estimate (MUE) was calculated. Chi-squared tests were calculated on prevalence estimates, and when significant, a Fishers Exact post-hoc test was conducted. A p-value of ≤ 0.05 was considered to be significant for all statistics calculated.

RESULTS AND DISCUSSION

At least one *E. coli* isolate was obtained from 86% of samples and 3 *S. enterica* isolates (serovars London [2] and Kentucky [1]) were obtained (2 from the same farm) (Figure 1). Where at least one *E. coli* isolate demonstrated AMR, the prevalence of AMR was significantly higher in commercial rabbit samples than in pet rabbit samples (OR=infinite, MUE=83.85, LL=16.578, UL=infinity, $p<0.0001$) (comparisons with other rabbit samples were part of a second, parallel study not reported in this paper). There was no resistance identified in the *Salmonella* London isolates but resistance was present in the *Salmonella* Kentucky isolate (Figure 2). Resistance gene testing for the bla_{CMY-2} gene in the *Salmonella* Kentucky isolate confirmed it to be bla_{CMY-2} positive. Specific antimicrobial resistance is depicted in Figure 3.

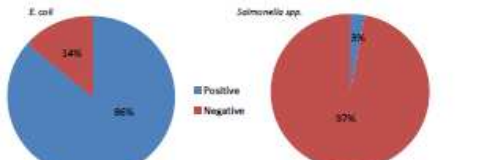

Figure 1. Proportion of commercial rabbit fecal samples positive for *E. coli* and *Salmonella spp.*

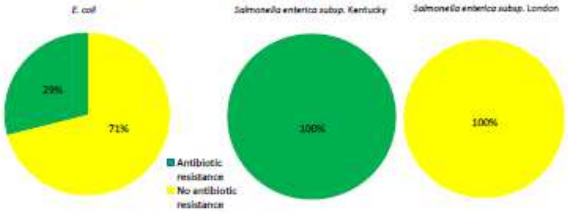

Figure 2. Proportion of *E. coli* and *Salmonella spp.* demonstrating AMR

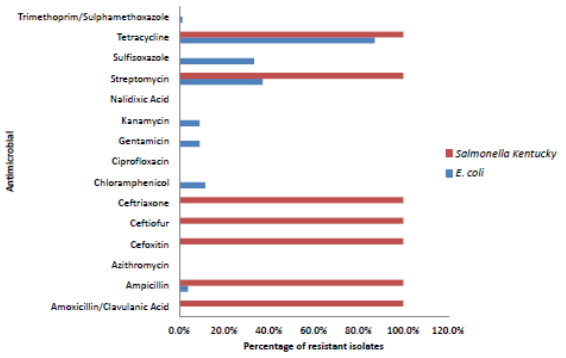

Figure 3. Proportion of *E. coli* (n=79) and *Salmonella* Kentucky (n=1) AMR isolates demonstrating intermediate or complete resistant to specific antimicrobials

A total of 18 of the farms reported routinely using at least one antimicrobial agent in food or water, one of which routinely used them in water and not feed, and the remaining used them exclusively in feed. Antimicrobial use was reported to occur in preweaned kits/nursing does, growing rabbits, and breeding animals on all farms that reported used antimicrobials. Eleven of the 18 farms also routinely administered antimicrobials to new animals/replacement stock. Salinomycin was the most commonly used antimicrobial agent. Table 1 summarizes all routinely reported antimicrobials used. Antimicrobials reported to be used infrequently for specific illnesses included tetracycline, trimethoprim-sulfadoxine, enrofloxacin, and penicillin, and these were predominantly administered by intramuscular injection.

Table 1. Antimicrobials agents used routinely on commercial meat rabbit farms in Ontario

Antimicrobial agent	Number of farms reporting routine use (n=18*)
Bacitracin methylene disalicylate	2
Chlortetracycline	3
Decoquinate	1
Diclazuril	1
Salinomycin sodium 6%	14
Sulfadimethoxine	1
Sulfamethazine	2
Tylan	3
Virginiamycin	7

*note: many farms reported use of >1 antimicrobial at the same time

No resistance to the antimicrobials listed as high priority within the WHO's "critically important category of antimicrobials important for human use" was identified in any of the AMR positive *E. coli* isolates; however, the *Salmonella* Kentucky isolate demonstrated resistance to two 3rd generation cephalosporins. These antimicrobials have been categorized as being of highest priority for risk management and their use is recommended to be limited exclusively to humans (WHO, 2011). While found in only one isolate, the appearance of resistance to this agent in meats rabbits is concerning and merits further investigation.

CONCLUSIONS

This study demonstrates that AMR strains of *E. coli* and *Salmonella* spp. are present on commercial meat rabbit farms, but at low levels, similar to other less intensively farmed animals in Canada, such as small ruminants. This population still bears monitoring because of the potential for transmission of bacteria and AMR to humans as well as other livestock species. Continued monitoring of this population may help to identify potential routes of antimicrobial transmission, as well as aid in the control of zoonotic diseases.

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