FREQUENCY AND AMR OF SALMONELLA AND CAMPYLOBACTER ISOLATED FROM SMALL-RUMINANT FECAL SAMPLES FROM THE U.S., AUSTRALIA AND BAHAMAS

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Abstract- The study objectives were to determine presence and antimicrobial resistance (AMR) of *Salmonella* (n=522) and *Campylobacter* (n=233) from small-ruminant fecal samples (from viscera within abattoirs and farm pens) in the U.S. (n=400; n=146), Australia (n=4) and Bahamas (n=118; n=83) over a three-year period. Pathogens were isolated using traditional microbiological methods and the identities determined through biochemical confirmation. AMR testing was done following the National Antimicrobial Resistance Monitoring Systems (NARMS) guidelines. *Salmonella* presence was 5.3% in the U.S., 41.5% in the Bahamas, and absent from Australia. *Campylobacter* detection was 76.0% in the U.S., 91.6% in the Bahamas, and 100% in Australia. *Salmonella* resistance to Tetracycline was more frequent in isolates from the U.S. than the Bahamas (28.6% and 9.5% respectively). *Campylobacter* multi-drug resistance (MDR) was detected in isolates from the U.S. (100%) and Bahamas (20%).

Key words- antimicrobial resistance, lamb, goat

I. INTRODUCTION

Pathogens, such as *Salmonella* and *Campylobacter*, are often studied in cattle, pork and poultry species, however, less research has been done to determine their presence in small-ruminants. Globally, each year, 8.3 million and 95.6 million illness are attributed to foodborne acquired *Salmonella* and *Campylobacter* respectively [1, 2]. With the increase in consumption of small ruminants, especially in developing countries, more information is needed on the pathogen presence in these species. Antimicrobial use in human and animal medicine over time has created selection and opportunity for the emergence of AMR bacteria [3] The role of resistant microorganisms in the environment, humans and animals has increased cognizance of the relationship between human and animal health, and the universal responsibility to address global health. However there are gaps current within the current AMR surveillance systems of food-producing livestock, food products, humans and the environment. Small-ruminants, such as lambs and goats, serve as a meat source within the U.S., Australia and Caribbean. A better understanding of the presence and AMR patterns of these pathogens in lambs and goats is needed. This objectives of this study were to 1) determine the presence of *Salmonella* and *Campylobacter* and 2) determine AMR from isolated pathogens in small-ruminant feces from the U.S., Bahamas and Australia.

II. MATERIALS AND METHODS

For *Salmonella* detection, a total of 522 small-ruminant fecal samples were collected for analysis from the U.S (n=400), Bahamas (n=118) and Australia (n=4). A total of 233 fecal samples for *Campylobacter* detection were collected in the U.S. (n=146), Bahamas (n=83), and Australia (n=4). Small-ruminant fecal samples from each country were either collected from pen floors on farms or from the descending colon immediately after evisceration during the harvest process. For *Salmonella* and *Campylobacter*, feces were subjected to selective enrichment, cultured for phenotypical colonies and confirmed using latex agglutination. Isolates were frozen with 20% glycerol at -80°C. AMR was determined by using the micro-broth dilution (Sensititre®) and the National Antimicrobial Resistance Monitoring Systems (NARMS) panel with resistance determined according to the Clinical and Laboratory Standards Institute (CLSI) recommended breakpoints. A U.S. veterinary permit for importation and transportation of controlled materials and organisms and vectors (USDA-APHIS research permit #114031) was obtained, for the transportation of fecal samples to the U.S. for analysis.

III. RESULTS AND DISCUSSION

From 522 fecal samples analyzed for *Salmonella*, pathogen presence was 5.3% in the U.S., 41.5% in the Bahamas, and no *Salmonella* was detected in samples from Australia; isolates were collected from the U.S. (n=21) and Bahamas (n=49). From 233 fecal samples analyzed for *Campylobacter*, frequency of detection was 76.0% in the U.S., 91.6% in the Bahamas, and 100% in Australia; isolates were obtained from the U.S. (n=111), Bahamas (n=76), and Australia (n=4). Of the frozen isolates reserved, 35 *Salmonella* isolates and 33 *Campylobacter* isolates were selected for recovery and AMR testing.

Two *Salmonella* isolates were MDR, one from the U.S. and one from the Bahamas U.S. and Bahamas *Salmonella* isolate resistance differed by antibiotic type. U.S. isolates were resistant to Chloramphenicol (14.3%) and Streptomycin (7.1%), while isolates from the Bahamas expressed resistance to Clavulanic acid (4.8%), Ampicillin (4.8%) and Cefoxitin (4.8%). The frequency of resistance to Tetracycline was higher in isolates from the U.S. than the Bahamas (28.6% and 9.5% respectively). *Campylobacter* isolates had resistance to Clindamycin (90.9%), Florfenicol (75.8%), Erythromycin (72.7%), Tetracycline (69.7%), Azithromycin (42.4%), Nalidixic Acid (33.3%), Ciprofloxacin (30.3%) and Gentamicin (9.1%). Of the 33 isolates tested, 24 were found to be multi-drug resistant (MDR). The most MDR was detected in isolates from the U.S. (100%), followed by the Bahamas (20%), with no resistance detected in isolates from Australia. Fluoroquinolone resistant *Campylobacter* spp. have been identified as a high priority pathogen for new research and development of antibiotics as a result of global antibiotic resistance concerns [4]. The resistance pattern found in *Campylobacter* isolates from small-ruminants supports this need.

IV. CONCLUSION

While small-ruminants, such as lambs and goats, are important species raised in the Caribbean for food and supplemental income, data regarding pathogen presence in these species from the Caribbean is very limited [5]. The frequency of *Salmonella* and *Campylobacter* detected in this study provides data about the presence of these two pathogens from small-ruminants in three countries. Furthermore, the AMR results from this study confirm the need to address AMR in pathogens from livestock that will enter the food supply system. Within the U.S., concerns about AMR in livestock entering the food supply have motivated regulatory changes regarding sub-therapeutic use in livestock. While the U.S. already has established compliance guidelines in place for antibiotic use in lambs and goats, recent changes in the marketing of antimicrobial products to prescription or veterinary feed directive have the potential to change access and usage of these products in small-ruminants [6]. In the Bahamas, however, antibiotic usage in livestock is not regulated. With *Salmonella* spp. and *Campylobacter* spp. considered two of the leading bacteria causing foodborne illness in humans from the Caribbean, antibiotic resistance of these pathogens has the potential to impact the burden of human illness [5]. With the abundance of small-ruminants distributed globally, and their ubiquitous presence in developing countries, an increased global understanding and surveillance of the presence of these products is crucial to reducing the global burden of AMR.

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REFERENCES

- 1. Majowicz, S.E., Musto, J, Scallan, E., Angeulo, F.J., Kirk, M., O'Brien, S.J., Jones, T.F., Fazil, A., Hoekstra, R.M. (2010). The global burden of nontyphoidal *Salmonella* gastroenteritis. Clinical Infectious Disease. 50(6):882 889.
- Kirk, M.D., Pires, S.M., Black, R.E., Caipo, M., Crump JA, Devleesschauwer B, et al. (2015) World Health Organization Estimates of the Global and Regional Disease Burden of 22 Foodborne Bacterial, Protozoal, and Viral Diseases, 2010: A Data Synthesis. PLoS Med 12(12): e1001921. doi:10.1371/journal.pmed.1001921
- Laxminarayan, R., T. Van Boeckel and A. Teillant. (2015), "The Economic Costs of Withdrawing Antimicrobial Growth Promoters from the Livestock Sector", OECD Food, Agriculture and Fisheries Papers, No. 78, OECD Publishing. http://dx.doi.org/10.1787/5js64kst5wvl-en
- 4. WHO.WHO publishes list of bacteria for which new antibiotics are urgently needed. 27 Feb 2017. Geneva. Accessed 1 April 2017. Available at: http://www.who.int/mediacentre/news/releases/2017/bacteria-antibiotics-needed/en/
- 5. Guerra, M.M.M., de Almeida, A.M. & Willingham, A.L. Trop Anim Health Prod (2016) 48: 1095. doi:10.1007/s11250-016-1082-x
- 6. FDA. 2016. Veterinary Feed Directive Update. U.S. Food and Drug Administration. Accessed 2 April 2017. Available at: https://www.fda.gov/AnimalVeterinary/NewsEvents/CVMUpdates/ucm507355.htm