Elucidating the immune suppressive effects of antibiotics in cattle

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Introduction

The impact of antibiotic therapy on antimicrobial drug-resistance is well known in the cattle industry yet the potential effects of antibiotic administration on immune modulation is unknown. Data from various animal models indicate that some antibiotics can suppress adaptive immune responses. Azithromycin for example, can suppress inflammation and innate immune defenses inferring that indiscriminate use of antibiotics may paradoxically increase susceptibility to infection and interfere with vaccine immunity. For cattle, we aim to identify classes of immune modulatory antibiotics to determine whether treatment interferes with vaccine responses. We hypothesize that macrolide and tetracycline antibiotics will significantly suppress immune responses in cattle which will be tested using assays that evaluate immunity in vitro.

Materials and Methods

Adaptive immune responses to antibiotics is assessed by analysis of bovine peripheral blood monocytic cells (PBMC) from either naïve or antibiotic-treated animals. PBMCs from naïve animals are treated with various concentrations of different classes of antibiotics in vitro and proliferative responses of T cells are determined by DNA synthesis assays. T cell activation assays are determined by Con-A mediated upregulation of MHCII and IL-2R (CD25) by flow cytometry. PBMCs will also be treated with LPS and IgG secretion levels determined by ELISA. Comparisons between untreated and antibiotic-treated PBMC will identify potential effects on vaccine IgG titers. Similar analysis will be performed on antibiotic-treated animals. Thus far, we have initially analyzed immune cells from PBMC in 5 treatment groups (n=5 each). Both technical and biological replicate groups consisting of control vs. experimental (antibiotic treatment groups) as nonparametric continuous or grouped variables are compared using either a one-tailed or two-tailed Mann-Whitney U test. P values for statistical variance of biological replicates are determined using Repeated Measures ANOVA with Tukey 's multiple comparison tests. Statistical analyses will be performed using GraphPad Prism 8.

Results

In preliminary tests, four different antibiotics (azithromycin, tulathromycin, tilmicosin, and enrofloxacin) were tested at various concentrations on immune stimulated naïve bovine PBMC. Results show that treatment of PBMC with clinically relevant plasma concentrations (~ 0.1 μg/ml) of each antibiotic resulted in significant reduction in T cell proliferation. Additionally, treatment of PBMC with the azithromycin resulted in significant reduction in T cell activation as determined by reduced CD25 expression. Finally, treatment of PBMC with tilmicosin resulted in significant inhibition of MHCII upregulation, consistent with reduced T cell activation. Similar results were noted in tests evaluating tulathromycin, gamithromycin, and oxytetracycline. Therapeutic administration of these antibiotics thwart optimal T cell activation in PBMC in vitro and show reduced ability of T cells to upregulate MHCII and CD25. Since preliminary data suggest that immune modulation occurs when T cells are treated with pharmacologically relevant doses of antibiotics in vitro. Further testing will determine the extent of immune modulation by six classes of antibiotics. These data will help determine the immune modulating potential and long-term effectiveness of various antibiotics and overall effectiveness in face of emerging resistant pathogens.

Significance

Lower proliferative responses and decreased CD25 expression on antibiotic-treated T cells in vitro or in vivo suggest a direct negative impact of antibiotics on host immunity. The continued use of antibiotics in the industry may in fact be, in addition to imposing selection for resistant pathogens, diminishing host immunity. The assessment of host immune modulation imposed by routinely-used antibiotics will expedite creative approaches to agricultural animal health issues.