



Aureomycin® and Aureo S700®: Mechanism of Action Against *Mannheimia haemolytica* Growth and Virulence

Denny Hausmann, DVM

Bovine Respiratory Disease (BRD) represents a major economic hurdle all cattle feeding operations must overcome. Many pathogens, both viral and bacterial, are involved in the BRD complex with stress playing a large role in initiating the disease. *Mannheimia haemolytica* is a primary pathogen involved in the BRD complex. It plays an extensive role in BRD in all cattle, being the major bacterial pathogen involved in death due to pneumonia in feedlot cattle.

There are several virulence factors associated with the disease process through which *M. haemolytica* affects its host. Those factors include:

- A bacterial capsule which promotes bacterial invasion and adherence to host tissue
- Proteins in the outer bacterial membrane that elicit the immune response
- Adhesions which enhance colonization of the bacteria
- Neuraminidase, an antigenic, glycoprotein enzyme which reduces viscosity of the respiratory mucous, allowing closer bacterial apposition to the cell surface
- Lipopolysaccharide, a major surface structure of gram negative bacteria that acts as an endotoxin
- Leukotoxin, which is a exotoxin secreted by the bacterial cell

These factors make it possible for *M. haemolytica* to avoid clearance and host defenses, multiply in lung tissue, and lyse macrophages and neutrophils also found in the lungs. These are all factors which enhance lung injury.⁽¹⁾ Of all these factors, leukotoxin is considered to be the primary virulence factor.^(1,2,3,4)

Leukotoxin is produced by *M. haemolytica* during log-phase growth,⁽³⁾ and attracts macrophages and modulates them. Leukotoxin produced by *M. haemolytica* is cytotoxic only for ruminant leukocytes,⁽¹⁾ which may in part be due to a specific binding site found on bovine leukocytes.⁽⁵⁾ It is leukotoxin that is

responsible for the characteristic pathology of pneumonic pasteurellosis,⁽⁴⁾ which is demonstrated by the observation that when leukotoxin is inactivated, pulmonary lesions decrease in spite of bacterial colonization.⁽¹⁾

At low levels, leukotoxin stimulates the production of inflammatory mediators by neutrophils and mononuclear phagocytes. These cells then undergo apoptosis (or programmed cell death). At higher doses, leukotoxin causes the cells to swell and lose their viability.⁽⁶⁾ Since the ability of leukotoxin to cause apoptosis is concentration dependent, it is conceivable that the process of cell death contributes to an ineffective host-defense response, dependent on leukotoxin concentration in pneumonic lesions.⁽³⁾

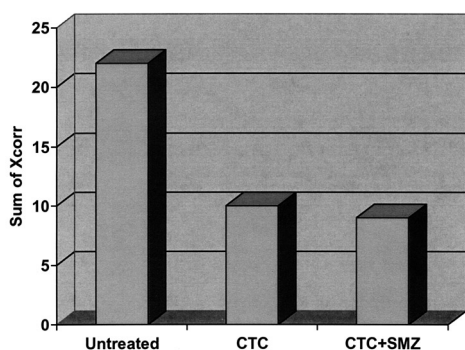
When antimicrobials are administered to cattle to prevent, control or treat disease, they do not kill all the target pathogens. Rather, the success of an antimicrobial against pathogens is dependent upon a number of factors beyond the calf itself; which include pharmacokinetics of the particular drug, and the ability of the drug to inhibit or kill the bacteria. However, the animal itself must eliminate the bacteria on its own through utilization of a healthy immune system. Thus, any interference with growth or pathogenicity of the bacteria within the calf is beneficial. Typically, the projected success when treating clinical bacterial disease with antimicrobials is determined by establishing a MIC (lowest antimicrobial concentration at which there is no growth after incubation) for the antimicrobial with respect to a specific pathogen.

Previous studies have shown that sub-MIC (less than MIC) levels of antimicrobials can have a positive impact on pathogens.⁽⁷⁾ Most recently, Reeks and others observed that sub-MIC levels of Aureomycin (chlortetracycline) and Aureo S700 (chlortetracycline and

sulfamethazine) as low as one-sixteenth MIC can inhibit growth of *M. haemolytica* and *H. somnus*.⁽⁸⁾

One of the methods of identifying and quantifying virulence factors utilizes proteomics, which is the large-scale study of proteins, particularly their expression under different environmental conditions. A recent proteomics study by Nanduri et al.⁽⁶⁾ examined the impact of sub-MIC levels of both Aureomycin and Aureo S700 on protein expression of *M. haemolytica*. Expression of proteins involved in energy production, nucleotide metabolism, translation, and the bacterial stress response were affected when *M. haemolytica* was cultivated in the presence of one-fourth the MIC level of Aureomycin and Aureo S700. More importantly, it was found that one-fourth MIC level of Aureomycin significantly inhibited the expression of *M. haemolytica* leukotoxin A, which is the secreted exotoxin. One-fourth MIC level of Aureo S700 significantly inhibited expression of both leukotoxin A and its activator, leukotoxin C. (Graph 1.) This study, for the first time, demonstrated a plausible explanation at the molecular level for the impact of sub-MIC levels of Aureomycin and Aureo S700 on pasteurellosis and its corresponding lung damage.

Expression of leukotoxin A in untreated *M. haemolytica* and in *M. haemolytica* exposed to 1/4 MIC of Aureomycin (CTC) and Aureo S700 (CTC+SMZ). Adapted from Nanduri et al.



Untreated vs. CTC: P=0.02
 Untreated vs. CTC+SMZ: P=0.002

Xcorr is a quantitative score on how well the actual mass spectrum from a peptide matches its theoretical mass spectrum; the sum of Xcorrs is calculated from all the individual Xcorr values derived from all the peptides identified from the protein.

When Aureomycin or Aureo S700 is administered, a reduction in morbidity is seen, with an improvement in response of sick cattle to BRD treatment with injectable antibiotics being frequently encountered.⁽⁹⁾ As stated previously, factors that improve the ability of the calf to control the disease process and allow the immune system to function maximally is beneficial. Inhibition of growth of *M. haemolytica* and *H. somnus* and reduction in *M. haemolytica* leukotoxin expression may be some of the mechanisms responsible for the efficacy of Aureomycin and Aureo S700 in lowering BRD morbidity, and improving response to BRD therapy.

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