

Effects of production practices on *Mannheimia haemolytica*

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Abstract

Mannheimia haemolytica is a leading contributor to bovine respiratory disease (BRD). Various vaccines are available for prevention or control of BRD due to *M. haemolytica*; published research indicates that vaccines can decrease BRD morbidity. Several antimicrobials are labeled for treatment or control of BRD due to *M. haemolytica*. Historically, metaphylaxis (mass antimicrobial medication at arrival) has been shown to reliably decrease BRD morbidity in high-risk cattle. Until recently, antimicrobial resistance (AMR) in *M. haemolytica* has been rare, even in cattle given antimicrobials. However, in the past 5 years multiple reports described *M. haemolytica* isolates from fatal BRD cases that are resistant to many or most antimicrobials. Additionally, nasopharyngeal shedding of AMR *M. haemolytica* by large proportions of live high-risk cattle following metaphylaxis has been reported. This information suggests that response rates following antimicrobial administration for BRD treatment or control may begin to decline. However, to date, reported death rates have not been high in cattle described to be shedding AMR *M. haemolytica*, and retrospective studies have not revealed a direct relationship between prevalence of AMR *M. haemolytica* and treatment failure. Properly designed prospective studies are needed to more clearly define the impact of AMR *M. haemolytica* in cattle populations.

Key words: *Mannheimia haemolytica*, serotypes MDR, antimicrobial resistance

Résumé

La bactérie *Mannheimia haemolytica* est l'une des causes majeures du complexe respiratoire bovin (CRB). Plusieurs vaccins sont disponibles afin de prévenir ou contrôler le CRB impliquant *M. haemolytica*. Les données publiées indiquent que les vaccins peuvent réduire la morbidité reliée au CRB. Plusieurs antimicrobiens sont approuvés pour le traitement ou le contrôle du CRB impliquant *M. haemolytica*. Historiquement, il a été démontré que l'utilisation d'antimicrobiens en métaphylaxie (médication massive à l'arrivée) réduisait de façon fiable la morbidité reliée au CRB chez les bovins à haut risque. Jusqu'à tout récemment, la résistance antimicrobienne chez les bactéries *M. haemolytica* était assez rare même chez les bovins recevant des antimicrobiens. Toutefois, lors des cinq dernières années, il y a eu plusieurs rapports d'isolats de *M. haemolytica* impliqués dans des cas

fatals du CRB qui résistaient à plusieurs ou à la plupart des antimicrobiens. De plus, l'excrétion nasopharyngée de bactéries *M. haemolytica* résistantes a été rapportée dans une grande proportion de bovins en vie à haut risque suivant l'utilisation d'antimicrobiens en métaphylaxie. Cette information suggère que le taux de réponse suivant l'administration d'antimicrobiens pour le traitement du CRB ou son contrôle commence peut-être à diminuer. Toutefois, le taux de mortalité rapporté à ce jour chez les bovins excréteurs de bactéries *M. haemolytica* résistantes n'est pas très élevé et des études rétrospectives n'ont pas démontré un lien direct entre la prévalence de bactéries *M. haemolytica* résistantes et l'échec du traitement. Des études prospectives bien conçues sont nécessaires afin de mieux cerner l'impact des bactéries *M. haemolytica* résistantes sur les populations de bovins.

Mannheimia haemolytica: the agent

Although *M. haemolytica* can be found innocently living on mucosal surfaces in normal cattle, it can also cause infection leading to a variety of diseases, including bronchopneumonia, pleuropneumonia, otitis, sinusitis, and mastitis. Different serotypes of the bacteria have been recognized based on reactivity with antiserum raised in laboratory animals; serotype A2 is most often a commensal in cattle, while serotypes A1 and A6 are most often isolated from cases of bovine respiratory disease (BRD). In contrast, serotype A2 is the most common cause of disease in sheep (ref). Recently a genotyping approach has been developed which can distinguish *M. haemolytica* isolates causing BRD from those isolated from healthy cattle,¹ and MALDI can be used to rapidly identify disease-associated genotypes² can be rapidly identified by Matrix-assisted Laser Desorption/Ionization (MALDI).

Like any microbe that can cause disease, this pathogen has multiple virulence factors that enable pathogenicity. As the bacteria is gram negative, the cell wall contains lipopolysaccharide (endotoxin), meaning that infection due to *M. haemolytica* may be accompanied by signs of endotoxemia. A polysaccharide capsule helps the bacteria evade the host immune response, and iron binding proteins help the bacteria to trap needed iron in spite of host mechanisms aimed at iron sequestration. While these factors all contribute to pathogenicity, the bacteria's leukotoxin is the most characteristic virulence factor. Leukotoxin, which is secreted by the bacteria, specifically kills ruminant leukocytes, including neutrophils and macrophages. This means that host immune cells sent

to fight infection are specifically killed by a toxin the bacteria produces. Moreover, the leukotoxin binds specifically to a leukocyte surface molecule, CD18, which is a component of the cell surface complex LFA-1 which is expressed in higher numbers when leukocytes come into contact with the bacteria. This essentially means that the natural response of leukocytes fighting off *M. haemolytica* make them more vulnerable to killing by leukotoxin produced by the bacteria.

Disease due to *M. haemolytica*

Mannheimia haemolytica is best known for causing fibrinous, sometimes necrotizing bronchopneumonia or pleuropneumonia; stocker and feedlot cattle are particularly susceptible, with the agent being the most common bacteria isolated in acute cases of stocker and feedlot BRD. The agent has also been reported to cause BRD which can be fatal in preweaning beef calves, and a few descriptions of severe fatal BRD in lactating dairy cattle exist. In contrast, *M. haemolytica* is less commonly reported to be associated with BRD in dairy calves, where *Pasteurella multocida*, *Mycoplasma bovis*, and other mycoplasmas are the more prevalent bacteria. While less common, *M. haemolytica* can also be isolated in pure culture from cases of sinusitis, otitis, and mastitis in cattle, and the agent can also cause these diseases, as well as bronchopneumonia or pleuropneumonia in sheep and goats.

Vaccination to Control Bovine Respiratory Disease (BRD) due to *M. haemolytica*

Vaccines to control and prevent BRD due to *M. haemolytica* are available, both containing the bacteria alone or in combination with other agents. Available vaccines contain either modified live *M. haemolytica*, bacterins containing inactivated bacteria, toxoids containing leukotoxin, or bacterial extracts containing leukotoxin and other bacterial products. All licensed vaccines have shown protection in experimental challenge studies; more compelling evidence comes from field trials, where vaccination is tested in cattle managed conventionally. Vaccines for *M. haemolytica* are among the best-supported vaccines used for control of BRD agents, as multiple clinical trials have been published which test the benefit of *M. haemolytica* vaccines, with many showing benefit. A recent meta-analysis evaluating the available published research concluded that *M. haemolytica* vaccines significantly decreased BRD morbidity but not crude mortality in feedlot cattle and beef and dairy calves.⁷

Antimicrobial (AM) use and Antimicrobial Resistance

The premier role of *M. haemolytica* as a cause of BRD prompted the development and marketing of several antimicrobials designed to control and treat disease due to the agent. While AM use in high-risk cattle for metaphylaxis or BRD treatment has been common for decades, until recently

resistance to AM used for BRD, particularly to newer drugs, did not seem to be a concern. A survey of 461 Mh isolates from fatal BRD cases between 1988 and 1992 indicated that the majority were susceptible to all AM tested for which the Clinical and Laboratory Standards Institute (CLSI) has established breakpoints determining resistance for BRD treatment.¹² The highest proportion of resistance by Mh was seen for tetracycline (43% resistant). Similarly, researchers evaluating Mh isolates collected from cattle dying of BRD between 1994 and 2002 found them to have stable and high rates of susceptibility to ceftiofur and enrofloxacin, while resistance to tetracycline was more prevalent.¹³ Canadian researchers evaluating cattle sampled by nasopharyngeal swabbing at feedlot entry and within 30 days of feedlot exit found relatively low rates of AM resistance, in spite of treatment for BRD with tulathromycin or ceftiofur, as well as in-feed delivery of tylosin and chlortetracycline for liver abscess control.³ Of 409 Mh isolates, these researchers found 100% to be susceptible to ceftiofur, enrofloxacin, and florfenicol. Sixteen of 409 isolates were resistant to oxytetracycline, and 1 was resistant to tilmicosin. They found no trend suggesting a relationship between AM therapy and the development of resistance. In a similar but larger study,¹¹ researchers sampled over 5,000 cattle at 4 feedlots at entry and again at 1 later time point (between 33 and 202 days-on-feed). All cattle were on feed containing tetracycline, 31% received injectable tetracycline for BRD treatment, and 23% received injectable macrolide for BRD. Twenty percent of the cattle were culture-positive for Mh on second sampling. In spite of relatively high rates of AM exposure, only 6% of Mh isolates were resistant to more than 1 AM. However, Noyes et al did find that cattle exposed to a penmate given injectable AM were at 24 times greater odds of having a multiple drug-resistant (MDR) Mh isolated from them, suggesting that MDR Mh may be transmitted from cattle treated with AM to cattle that are not treated with AM. A small experimental trial showed no macrolide resistance in Mh isolated from cattle over a 28-day period when cattle were treated with tilmicosin, tulathromycin, or tylosin,¹⁴ although resistance did increase in *Enterococcus* isolates collected from feces of the cattle during the trial.

An important question for which there is currently no clear answer is: if MDR Mh are present in a group of cattle, does infection with these MDR Mh make the cattle less likely to respond to AM therapy for BRD? It seems obvious that Mh resistant to a given AM would cause infection that is poorly responsive to that AM, but the data available to date do not clearly confirm this. McClary et al¹⁰ reported that, in cattle in 16 different clinical trials where tilmicosin was used for BRD treatment, the rate of treatment success was not significantly different for cattle that had a tilmicosin-resistant Mh isolated from a nasopharyngeal swab collected before treatment, as compared to cattle that had a sensitive Mh isolated. However, the number of cattle that harbored a resistant Mh before treatment was small (n = 6), and there appeared to be a trend toward a difference in response, with

a treatment success rate of 62% for cattle with a susceptible isolate, vs 38% for cattle with a resistant isolate ($P = 0.08$). Similarly, in a 2012 paper, researchers in Oklahoma reported that AM resistance patterns from Mh isolated from the lungs of cattle that died of BRD did not seem related to the drugs administered before death.⁶ Although these studies did not identify evidence for a clear link between AM use in cattle and AMR harmful to cattle health, a 2013 report by Lubbers and Hanzlicek⁹ gained widespread attention among cattle veterinarians. This report described resistance patterns from 389 Mh isolates from lung tissue collected at necropsy of cattle on 266 unique premises and submitted to the Kansas Veterinary Diagnostic Laboratory between 2009 and 2011. Of 55 Mh isolates submitted in 2009, nearly 35% were *pan susceptible* (susceptible to all AM tested), while only 5% were *resistant* to 5 AM. In contrast, of 179 isolates submitted in 2011, only 17% were *pan susceptible*, while 35% were *resistant* to 5 AM. The authors acknowledged that the samples represented a biased population, given that they were from cattle that most likely failed to respond to AM treatment for BRD, and they originated from a relatively small geographical region within the US. However, the increase in proportion of highly resistant Mh over a relatively short period of time suggested that in at least some regions of the country, MDR Mh resistant to most classes of AM used for treating BRD were becoming easy to find.

Soon after this report, Klima et al⁵ described AM susceptibility of 55 Mh isolates from feedlot cattle in Nebraska, Texas, and Alberta (Canada). All isolates were grown from lung tissue or postmortem nasopharyngeal swabs of cattle that died due to BRD. Of the 55 Mh isolates, 72% were resistant to at least 1 AM, and 30% were resistant to drugs in more than 7 AM classes. The MDR Mh were all isolated from cattle in Nebraska or Texas; pulsed field gel electrophoresis (PFGE) indicated that 8 isolates from Nebraska were a clonal subpopulation. Relevant to this work, in 2012 Michael et al reported finding an integrative conjugative element (ICE $Pmu1$) in *Pasteurella multocida* that included 12 AM resistance genes. Using primers based on the sequence of genes included in ICE $Pmu1$, Klima et al found that MDR Mh from Nebraska and Texas all contained a similar sequence. Since then, an ICE encoding resistance to 3 classes of AM was identified in Mh, ICE $Mh1$;¹³ and 3 more have been identified in Mh from US sites.⁴ Because ICE contain features that allow them to be transmitted from 1 bacterium to another through conjugation, they can easily move horizontally between bacteria within a genus, or across genera. Consistent with this, ICE $Mh1$ recently described in Mh was transferred to *P. multocida* by conjugation.²

Taken together, the recent literature indicates that 1) AM treatment of cattle populations does not always lead to an obviously high prevalence of MDR Mh in the weeks following treatment, although cattle in contact with treated cattle may be at increased risk of acquiring MDR Mh; 2) MDR Mh are becoming easier to find in certain cattle populations,

with some isolates demonstrating resistance to as many as 7 classes of AM; and 3) a direct relationship between prevalence of MDR Mh in a cattle population and rates of BRD treatment failure has not been clearly defined, though the research to date has largely addressed this question through retrospective studies, which may be flawed by bias or confounding.

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