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This review accompanies the relevant episode of the Cutting Edge veterinary podcast. In each episode of this podcast, 3rd year students in the University of Calgary's veterinary medicine program fill you in on the most up-to-date literature and evidence-based practices on topics that matter to you, the practising veterinarian.

A Comparison of Caseous Lymphadenitis (*Corynebacterium pseudotuberculosis*) Treatments In Small Ruminants

STUDENTS: Emma Mandolesi-Kahanyshyn, Sarah Kulle and Allison Kwantes
FACULTY MENTORS: Drs. Brielle Rosa and Karen Liljebjelke

Background

Caseous lymphadenitis (CLA) is a chronic, highly infectious disease of economic importance found worldwide in small ruminants, costing 15-17 million dollars annually[1,2]. Economic consequences include carcass condemnation, decreased production, reproductive losses, and death[1,3]. Historically, CLA was implicated as the third leading cause of economic loss to the sheep industry of the western United States[4]. CLA impacts both sheep and goats, however, despite goats having more CLA lesions at slaughter, research has indicated that there is higher prevalence and morbidity in sheep[5,6]. A 2009 study surveying over one thousand small ruminants determined prevalence of CLA to be 50.47%[5]. In some countries, CLA is not a notifiable disease, therefore, it has been suggested that the true prevalence is currently underestimated[7]. This emphasizes the importance of CLA, and why it is essential to have effective treatments to enhance animal welfare and economics[5]. Canadian goat and sheep production has gradually increased in the past decade[8,9]. Alberta is the second leading province in goat meat production and is ranked among the top three sheep producing provinces[8,10]. The economic implications associated with CLA are even more relevant for Canada's small and growing sector, warranting a review of CLA management options to protect Canadian flocks.

The pathological agent, *Corynebacterium pseudotuberculosis,* is a gram-positive aerobe which causes specific disease syndromes in sheep, goats and horses[1,11]. *C. pseudotuberculosis* targets the lymphatic system and can cause superficial and/or visceral lymphadenopathy, and in the skin and viscera it is commonly characterized by caseous abscessation. Pathogen spread can occur due to various management practices on farms (ie. shearing, tail docking), environmental contamination from lesions rupturing, or by direct contact between animals via skin penetration or respiratory/oral routes[1,4,12]. Once an individual is infected, the disease is difficult to eliminate due to poor responses to therapeutics, pathogen virulence factors, and diagnostic limitations[1,11,12].

The most widely accepted control measures for CLA includes the treatment of valuable individuals, aggressive culling, implementation of strong biosecurity protocols, and/or vaccination[1,4]. In this review, we focus on individual animal treatments, specifically comparing open drainage with lavage and closed system lesion management with intralesional or systemic antimicrobial therapy. The proposed benefit of closed-system management is the reduced risk of environmental contamination. Surgical removal also reduces this risk; however, it has higher costs and increased risks associated with general anesthesia[1,4]. Antimicrobials that have shown some efficacy against CLA include penicillin, oxytetracycline, and tulathromycin[1,4,13]. We reviewed the literature to determine the susceptibility of *C. pseudotuberculosis* to the stated antimicrobials, as well as their efficacy in treating CLA. Additionally, we examine the efficacy of different vaccines in treating and/or preventing CLA in small ruminants.

<u>Closed versus open lavage</u>

As mentioned, one option for the individual CLA treatment is lavage, which can be done either via closed or open methods. The research in this area is limited not only by the number of publications but also the number of individuals included in each study. There is no clear evidence to say which method is most effective. A 2009 study is the most current research that comes closest to comparing these methods; however, their use of different antimicrobial drugs confounds the comparison of such lavage methods[4]. This study included three treatment groups: Group 1 was treated via open drainage, saline lavage and subcutaneous (SC) procaine penicillin G (PPG) injection, Group 2 was treated with closed lavage of lesions and an intralesional injection of tulathromycin, and Group 3 was treated with closed lavage and a SC injection of tulathromycin. From this study, the authors concluded that needle distension lavage combined with tulathromycin could be an acceptable alternative to open drainage and flushing. This was based on the similar resolution proportions of 92.9%, 83.3% and 82.4% for groups 1, 2 and 3, respectively. A suggested benefit is a decrease in environmental contamination when using closed system drainage. Note that the study lacked a control group comparing whether closed system lavage without an antibiotic was comparable.

Another study compared two intralesional injection groups and a control group that used the conventional treatment method of open drainage and iodine lavage[11]. Iodine tincture and sodium hypochlorite were used as intralesional treatments and although using intralesional injections is not the focus of this review, the results could be used to consider the implications of closed drainage and intralesional injection of antimicrobials. In both intralesional groups, natural rupture occurred in all animals' post-treatment. The material from five out of the six ruptured lesions tested positive for *C. pseudotuberculosis,* therefore failing to reduce the risk of environmental contamination. Edema was also found in the regions of the lymph nodes belonging to the intralesional treatment groups[11]. This was proposed to be due to irritation of the products used at high concentrations. One advantage suggested from using intralesional treatments was a shortened clinical stage of disease (which in this study was one month shorter than the conventional treatment). Like the study described above, due to the small sample size, incompletely described methods and an unclear control group, there is weak evidence to make conclusive statements regarding closed versus open drainage.

<u>Antimicrobials</u>

Antimicrobials are commonly used to treat infections in small ruminants but are often used in an extra-label manner since there are few drugs approved for small ruminants in

Canada. With regards to CLA, there are data available on outcomes after treatment with oxytetracycline, penicillin and tulathromycin, with penicillin and oxytetracycline as the most used treatments in practice[14]. The use of tulathromycin has been explored due to its ability to penetrate tissue chambers in bovine and swine, suggesting a potential new solution to CLA management[15].

Oxytetracycline:

Oxytetracycline (OTC) is a broad-spectrum antimicrobial drug which distributes to most tissues; It has historically been used in the treatment of gastrointestinal and respiratory disease[13,16]. It is effective against aerobic gram-positive and gram-negative bacteria and has been suggested to be effective against *C. pseudotuberculosis*[13,17]*.* Reported doses of injectable OTC range from 2-20 mg/kg SID or q3-5d depending on the formulation used[13,17,18]*.* OTC may be effective at penetrating encapsulated lesions due to its moderate lipid solubility[13]. Specifically, in comparison to tulathromycin, suggested benefits of OTC include lower cost, higher availability, and a shorter withdrawal time. Currently in the literature there is only one study with weak to moderate evidence supporting the use of OTC as a treatment for CLA[13]. Treatment groups consisted of sheep that received an OTC at a dose of 20 mg/kg administered by percutaneous injection into a chamber inoculated with *C. pseudotuberculosis*, percutaneous injection into an uninoculated control chamber, or IM injection in the cervical region. Results indicated sheep that received OTC via IM injection achieved drug concentrations >1 ug/mL (MIC for isolate, 0.5 ug/mL) in all *C. pseudotuberculosis* inoculated tissue chambers but these all remained culture positive for *C. pseudotuberculosis* throughout the entire study. The authors suggested that MIC levels were not maintained long enough to eliminate *C. pseudotuberculosis*. In comparison, intra-chamber administration of OTC effectively eliminated the *C. pseudotuberculosis* isolate <48 hours after treatment. *In situ* efficacy would require further studies to determine.

Penicillin:

There are limited data on the pharmacokinetics and pharmacodynamics of penicillin in goats. Doses for goats are often extrapolated from sheep or cattle doses, despite the differences in metabolism that exist between these species[19,20]. For small ruminants, penicillin has historically been used for the treatment of clinical mastitis, diarrhea and pneumonia[14]. It has also become an option for CLA treatment in part since it has a broad spectrum of action and covers multiple gram-positive organisms, therefore covering co-infections that may arise[4]. PPG has been approved for used in sheep in USA, (with the dose of 660U/kg of body weight for 4-7days) but still is not approved for goats[19]. Penicillin is the most common drug residue detected in tissue and milk, and although rare, anaphylactic reactions resulting from meat residues have been noted[19,20]. Due to this, and the fact that penicillin is off label for goats, there can be no detectable levels of penicillin in tissues which complicates penicillin at a CLA treatment choice. Derbyshire's study of mastitis in goats indicated that goats with mild to moderate mastitis infections responded well to penicillin, but those with severe infections were unable to recover, which begs the question how effective penicillin may be for severe CLA cases, especially with extensive visceral involvement[21]. On the other hand, in the 2009 study by Washburn, intralesional PPG injection (20,000U/kg) yielded better results than intralesional tulathromycin injection[4]. Additionally, Al-Gaabary et al's study examining sheep treated with PPG on shearing days showed that with sufficient hygienic measures, prophylactic PPG injections were effective at minimizing CLA prevalence in affected flocks[5]. Although there is little evidence supporting its use as a CLA treatment, penicillin may have some efficacy for CLA treatment and prevention.

Tulathromycin:

The pharmacological properties of tulathromycin may justify its use in the treatment of CLA in sheep and goats. Tulathromycin has a high volume of distribution and is eliminated slowly; providing a prolonged period of tissue concentration above the MIC for common bacterial pathogens of the bovine and swine respiratory tract[15,22]. Tulathromycin also has gram positive activity; therefore, it should be effective against *C. pseudotuberculosis*[15,22]. The safety of tulathromycin use in goats has been demonstrated in small-scale studies involving young juveniles and mature adults[23,24]. Washburn et al. demonstrated that 25 mg/kg SC injections (ten times the recommended dose in cattle and swine) were well tolerated in yearling goats, aside from a transiently increased creatine kinase and some genotoxic effects[23]. In a different study, 2.5, 7.5, and 12.5 mg/kg SC injections given weekly for three weeks were all well tolerated in juvenile goats, with no significant differences in physical or histological parameters[24]. The ability to safely give multiple injections is considerable due to the antimicrobial's primarily bacteriostatic mechanism of action[15,22]. The known safety of repeat injections would allow practitioners to treat persistent abscesses with more confidence. As for efficacy of tulathromycin in the closed-system treatment of CLA, the evidence is not strong. Neither the use of single dose (2.5 mg/kg) intralesional or SC tulathromycin combined with closed system lavage was significantly better in resolving superficial CLA compared to open-system lavage and SC PPG[4]. Intralesional injection of 2.5 mg/kg tulathromycin into implanted tissue chambers did maintain a concentration above the pathogen's MIC (2 ug/mL) for 15 days; however, some chambers showed no decline in pathogen growth[28]. Additionally, intralesional injections had the same success rate as SC injections for inhibiting pathogen growth; suggesting that both routes of administration could be acceptable[28]. Overall, tulathromycin has been shown to be safe to use extra-label in goats, however it's efficacy in treating CLA is mixed. Further studies are required to determine true efficacy, as well as studies from the field to determine efficacy *in situ*.

Within the considerations for any antibiotic used, the importance of antimicrobial resistance (AMR) cannot be overstated. Since there is little on-label data for small ruminants, if not given proper protocols, producers may overestimate body weight and often give dosages higher than the ones prescribed[14]. In a 2017 study, milk from 100% of the goat bulk tanks (n = 26) and 87% of the sheep bulk tanks (n =47) sampled contained bacteria with resistance to penicillin, emphasizing the potential for the development of AMR in these species[30]. Additionally, we must consider the importance of these antimicrobials to human medicine. Oxytetracycline and penicillin are highly important antimicrobial drugs, whereas tulathromycin is a critically important antimicrobial according to the World Health Organization[31]. These classifications further emphasize the importance of antimicrobial stewardship if practitioners do choose to use these antimicrobials.

<u>Vaccines</u>

In addition to using lavage or antimicrobials, vaccine treatments have also been attempted to treat or prevent CLA. Vaccines have the potential to be an effective preventative measure or treatment during a CLA outbreak, especially due to their ease of delivery and safety[32]. Currently, the most accepted vaccine for treating CLA is Glanvac-6, but Case-Bac is also available in Canada[6,33]. Both are toxoid vaccines labelled only for sheep; there are no vaccines labelled for use in goats in Canada for the management of CLA[12,33,34]. Attempts have been made to use other formulations (live, DNA); however, these had limited ability to treat CLA. Glanvac-6 has been shown to limit the spread of infection beyond the initial infection site but provides varied protection, ranging from 25-90% reduction in new CLA lesions following vaccination of a previously unvaccinated herd[6,32].

In terms of using vaccines as a protective measure, there have been limited studies indicating their functionality. In a Sutherland et al study 81 sheep were challenged with *C. pseudotuberculosis* three months after completing a two-vaccination series against CLA, with 27 sheep still developing CLA lesions[2]. This could indicate that vaccines may not effectively prevent CLA, but more research is required to deepen understanding. In addition to the Sutherland et al study, Windsor suggested that vaccines may not be the most effective method of CLA prevention since *C. pseudotuberculosis* shedding may still occur years after vaccination[32]. Although data are limited on the benefits of prophylactic preventative vaccines, multiple sources have determined that a protocol of at least two doses administered one month apart, in addition to annual boosters, (and according to Windsor, potentially more vaccinations around lambing and weaning season), are needed to have any successful prophylactic protection[32,35]. Ultimately, vaccinations can decrease microbial contamination, therefore reducing spread of disease and associated economic and animal losses; but completely eliminating *C. pseudotuberculosis* infections is unlikely.

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