



## Case report

## Antimicrobial photodynamic therapy for caseous lymphadenitis abscesses in sheep: Report of ten cases



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### 1. Introduction

Caseous lymphadenitis abscesses (CLA) is a chronic infectious disease, reported as one of the major diseases that affects small ruminants herds around the world. The main cause for the cosmopolitan character of the disease is the global commercialization of infected animals associated with a faulty sanitation program [1].

*Corynebacterium pseudotuberculosis* is considered the main causative agent of CLA pathogenesis, nevertheless, other bacteria were also correlated as aetiological agents. The high prevalence of CLA represents some risks for the herd, such as the reduction of fertility, delayed development, gradual weight loss, occasional death of animals and condemnation of carcasses at the slaughterhouse [1].

The worldwide accepted diagnosis is the clinical identification of the abscesses in superficial lymph nodes that can be found in

several animals of a same herd. The most affected lymph nodes mandibular, parotid, popliteal, subiliac, superficial cervical, mammary and retropharyngeal. More rarely, internal lymph nodes from visceral organs can also be affected, leading to systemic changes and progressive weight loss [1].

The usual therapeutics consists in surgical drainage followed by systemic antibiotics, associated or not with local antiseptics solutions over the opened lesions [2,3]. However, the treatment period is long and, until now, there is no proven successful treatment available.

In this context, antimicrobial photodynamic therapy (APDT) could be an alternative approach for CLA. In fact, due to antimicrobial resistance worldwide, APDT has been used in the treatment of topical infections. Its action results from the interaction of a photosensitizer (PS), visible light and molecular oxygen. In the presence of oxygen, the activated PS can react with neighboring molecules by electron transfer, leading to the production of reactive oxygen species (type I reaction) or by energy transfer to oxygen, resulting in the production of singlet oxygen (type II reaction). Because PS accumulates in cell targets, only irradi-

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ated cells are impaired. APDT also takes advantage of its action on multiple targets not promoting microorganism resistance. Several strains of gram-positive and negative bacteria, fungi, protozoa, viruses and pathogenic algae were shown to be susceptible to the APDT [4,5].

Methylene blue (MB) is a PS commonly used in APDT associated to a red light to treat topical infections of different etiologies [5]. Thus, in this study, we investigated the feasibility of MB-mediated APDT to treat CLA in sheep after surgical drainage.

## 2. Case report

For this study, we used ten crossbreed sheep, adults, male and female, from the same herd, presenting lymph nodes enlargement characteristic of CLA. Inclusion criteria were the presence of a subcutaneous swelling in the lymph nodes, followed by external palpation to identify the consistency of the subcutis and presence of purulent exudate.

After the clinical diagnosis, we performed the surgical drainage. The sheep had their abscess fur removed by a shaver, and the skin was cleaned with alcoholic chlorhexidine solution. Then, a single surgical incision was made with a scalpel blade in order to open and drain the abscesses (Fig. 1A). Followed the surgical procedure, APDT was performed by applying 5 mL of MB (aqueous solution at a concentration of 60  $\mu$ M) (Fig. 1B) through syringe on the lesions. After 5 min of incubation in the dark, in order to allow MB uptake by the cells, the lesions were irradiated using a diode laser (wavelength, 660 nm; energy/point, 4 J; power, 100 mW; irradiance/point, 3.3 W/cm<sup>2</sup>; exposure time/point, 40 s; fluence/point, 133.3 J/cm<sup>2</sup>) (Fig. 1C). After treatment with APDT, the lesions were cleaned with 0.9% NaCl solution and then covered by a closure bandage to protect the site and avoid contamination. The treatment was repeated once a week until complete recovery (Fig. 1D). The evaluation of the lesions occurred by clinical analysis during thirty days and the recurrence rate was evaluated along six months. The

**Table 1**

Animals treated with antimicrobial photodynamic therapy, presenting the affected lymph node, time for complete healing and number of treatments.

Sheep ID	Affected lymph node	Healing time (days)	Number of treatments
1	Mandibular	12	1
2	Retropharyngeal	17	2
3	Mandibular	19	2
4	Parotid	17	2
5	Parotid	12	1
6	Retropharyngeal	16	2
7	Mandibular	12	1
8	Mandibular	13	1
9	Retropharyngeal	17	2
10	Parotid	18	2
Mean $\pm$ SD		15.3 $\pm$ 2.7	1.6 $\pm$ 0.5

SD: standard deviation.

clinical analysis was made by inspection of the lesion through the swelling reduction until complete recovery and closure of the skin, and with palpation to evaluate the presence of inflammatory process and purulent exudate.

All treated animals responded to the proposed protocol. After one week, no inflammatory signs and purulent exudate were observed. There was size reduction of all treated lymph nodes, returning to their normal sizes after two weeks. The highest prevalence of abscesses occurred in the mandibular (40%), followed by retropharyngeal (30%) and parotid (30%) lymph nodes. Submitted to 1 or 2 treatments, the time to full recovery was 19 days for the maximum and 12 days for the minimum. The average time of healing was 15.3 days (1.6 treatments) (Table 1).

Furthermore, no recurrences were observed in the treated lymph nodes during six months of follow-up and no adverse effects were observed in the treated animals during the experimental period.



**Fig. 1.** Abscess after surgical drainage (A); application of the photosensitizer methylene blue (B); irradiation with diode laser (C); complete wound healing after 2 weeks (D).

### 3. Discussion

Diseases caused by multidrug-resistant microorganisms are emerging in veterinary medicine due to the overuse of antibiotics, particularly in food animals. Thus, alternatives to conventional treatments must be developed.

In our study, APDT showed to be a suitable alternative approach for CLA as it presented a faster time of healing as much as no recurrence during six months of follow-up. This finding is awfully attractive since different results were observed by other authors, who reported a higher healing time (around 50 days) and adverse effects like pain [3]. Our study also shows a resolution rate of 100%, overcoming antibiotic therapies [2]. In addition, the absence of toxic residues commonly found in treatments with antibiotics is of pivotal importance to meat and milk industries and, consequently, to the human health.

A noteworthy remark is that MB showed be cost-effective and safe for the host cells. MB is a cationic PS of the phenothiazine family that retains characteristics that makes it particularly efficacious against infectious diseases [5]. Following photoactivation, MB induces both mechanisms type I and type II, with high quantum yield of singlet oxygen. Further, it has affinity with target microorganisms rather than host cells, causing efficient microbial reduction with short exposure times of red light [4].

Currently, microbial-drug resistance causes pronounced impact on morbidity and costs related to infections. Thus, the lack of selection of microbial strains that are resistant to photodynamic treatment ensures the repeatability of this therapeutic approach,

and MB could replace conventional antimicrobials allowing drug conservation and slowing resistance rates.

The application of APDT in veterinary medicine is still under investigation with suitable results in dermatological diseases [6]. We hope that this clinical trial will encourage colleagues and researchers in the development of subsequent protocols to improve APDT in clinical practice.

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