Original Article

Distribution and Risk Factors of Clinical Caseous Lymphadenitis in Small-Holder Goat Herds in Northeastern Thailand

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Abstract

Clinical caseous lymphadenitis was observed in 60 of 1,186 goats. A total of 34 small holder goat farms were investigated in 11 provinces in the northeastern part of Thailand. Approximately 66.67% of clinical caseous lymphadenitis goats were infected with *Corynebacterium pseudotuberculosis*, as confirmed by an ELISA test or bacterial culture. The average prevalence of clinical caseous lymphadenitis in herds was 6.36±4.30% (4.86-7.86; 95% CI). The abscessation of a superficial lymph node was commonly found in proximal (46.67%), middle (38.33%), and distal (15%) parts of the body. The location of the lesion was significantly associated (p<0.05) with positivity either from ELISA or bacterial culture, as goats with lesions in the distal part of the body showed a higher positivity (90%) than at the proximal (75%) and middle parts (45%) (p=0.02), respectively. No significant difference was observed for goat gender, age, or breed in terms of infection prevalence or displays of clinical signs of caseous lymphadenitis (p>0.05). Control and prevention measures should incorporate client education on a number of factors, including disease transmission to humans, clinical signs, impact on animal health and production loss and treatment.

Keywords: caprine, *Corynebacterium pseudotuberculosis* infection, factor, spatial distribution

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Introduction

Caseous lymphadenitis (CLA), a chronic infectious disease of small ruminants, is caused by the bacterium *Corynebacterium pseudotuberculosis*. It is a gram-positive pleomorphic rod-shaped bacterium that forms small, white, dry colonies surrounded by a narrow zone of β-hemolysis on blood agar after 48-72 hours of incubation at 37°C (Markey *et al*., 2013). *C. pseudotuberculosis* causes CLA worldwide in small ruminant herds; i.e., from 1996-2004, CLA was reported in 201 countries by the World Animal Health Organization (OIE) (Guimarães *et al*., 2011). CLA is a potential zoonotic disease, with the first case identified in humans in 1966 (Lopez *et al*., 1966). Occupational disease caused by CLA is found in shepherds, shearers, abattoir workers, butchers, veterinarians, neighbors, and farm visitors exposed to infected animals (Baird and Fontaine, 2007).

The mode of transmission from animal to animal is by direct contact with pus from abscesses or by ingestion, inhalation or indirect contact with contaminated materials, such as ear tags, shearing machines, castration tools, and feeders (Osman *et al*., 2018). In humans, the route of infection is by direct contact with CLA pus or by consuming contaminated products, such as unpasteurized milk or inappropriately cooked meat (Peel *et al*., 1997). The bacteria can also become airborne in testing laboratories, causing pneumonia in laboratory workers (Heggelung *et al*., 2015).

In goats, CLA displays two characterized forms of granulomatous lymphadenitis: superficial and visceral (Dorella *et al*., 2006). The superficial form can be observed as swellings or abscesses of superficial lymph nodes or subcutaneous tissues. The visceral form is recognized by pyogranulomatous lesions in the visceral lymph nodes and internal organs; this form cannot be detected by general appearance unless the animals show signs of chronic emaciation while the disease is progressing (Dorella *et al*., 2006). Cases of granulomatous nephritis can be detected by urine cultures (Ferrer *et al*., 2009).

CLA is a significant cause of economic loss that extends beyond the costs of long-term ill thrift of the animals, as the abscesses can downgrade the carcass price and increase the cost of carcass inspection and trimming, as well as lead to the condemnation of carcasses at the slaughterhouse (Paton *et al*., 1996; Windsor, 2011). In Thailand, information about CLA in goats is extremely limited. The aim of this investigation was to detect the prevalence of clinical CLA in small-holder goat herds being raised in the northeastern part of Thailand and to identify risk factors related to the disease to facilitate prevention and control.

Materials and Methods

Study design and Study area: This cross-sectional study was conducted from March 2016 to September 2018 to determine the prevalence of clinical CLA in goats in 11 provinces that had more than 25 small ruminant herds. In total, 1,186 goats in 34 small-holder goat herds from the provinces of Burirum, Chaiyaphum, Khon Kaen, Kalasin, Mahasarakham, Nong Bua Lamphu, Nakhon Ratchasima, Roi Et, Sakon Nakhon, Sisaket and Udon Thani (ICT, 2015) were included in this investigation. The study procedures were approved by the author's Institutional Animal Care and Use Committee (Record no. IACUC-KKU-73/61, date of approval 25 October 2018).

Sampling: A total of 60 clinical CLA cases were included. Sixty serum samples were collected for serology tests. Only three samples could be collected for bacterial identification; these were samples from two cases that had died from pneumonia due to gram negative bacterial infection. The owner allowed us to necropsy and biopsy the abscesses of mediastinal and right submandibular lymph nodes from the deceased goats for bacteriological identification. The procedures of Vandepitte *et al*. (2003) were followed for collection and transportation of specimens. In brief, the carcasses were necropsied in a closed necropsy room. The lymph node autopsy was done using an aseptic technique and the samples were transferred to sterile containers and shipped immediately at 2-8°C to the laboratory in the Faculty of Veterinary Medicine, Khon Kaen University. In the laboratory, the samples were processed using aseptic techniques by searing the sample surface with burning steel and opening the sample with sterile scissors and forceps. Pus was then collected with a sterile loop.

Questionnaires: All owners were interviewed for their management of risk factors associated with CLA. The studied factors included herd structure, herd and health management and knowledge about CLA. In addition, the animal gender, breed and age (as identified by the number of permanent incisors) were recorded. A physical examination for superficial lymph node enlargement or scars or abscesses induced by *C. pseudotuberculosis* was also performed on individual animals.

Serological tests: Blood samples were collected from the jugular vein into red 5 ml Vacutainer® tubes. All samples were transported on ice to the laboratory at the Faculty of Veterinary Medicine, Khon Kaen University, within six hours. All samples were centrifuged at 2,500 rpm for 10 minutes for serum collection and then stored at -20°C until analysis.

Antibody against CLA was detected using a CLA Indirect ELISA kit (IDvet, 310 rue Louis Pasteur-Grabels-FRANCE) according to the manufacturer's instructions. The optical densities (OD) of the samples and controls were measured at 450 nm using a spectrophotometer. The S/P % was determined based on the OD using the following equation:

$$S/P\% = \frac{(OD_{sample} - meanOD_{negativecontrol})100}{(meanOD_{positivecontrol} - meanOD_{negativecontrol})}$$

The results were classified according to the manufacturer's instructions, as follows: $S/P\%$ > 50, <40

to ≤ 50 and ≤ 40 were strongly positive, doubtful and negative samples, respectively.

Bacteriological identification: Pus specimens from three samples were processed by bacterial culture based on the method of Markey *et al.* (2013).

Epidemiological analysis: The spatial distribution was analyzed using Quantum GIS. The geographic coordinate system was used to produce the map for epidemiologic analysis.

Statistical analysis: Data were analyzed by EPI info 6 (v.6.0.4d, Centers for Disease Control and Prevention (CDC), USA, 2001). The associated risk factors were analyzed with the chi-square test, and statistical significance was determined as *p*-values less than 0.05.

Results

Prevalence, spatial distribution of CLA positive herds and epidemiological analysis: A positive ELISA test was registered in approximately 61.67% of the animals (37/60). Three serological-negative samples were detected by bacteriological culture from pus. The seroprevalence of clinical CLA in the tested herds was estimated at 6.36% ± 4.30% (95% CI 4.86-7.86). The average prevalence of clinical CLA and CLA-infected herds in each province are depicted in Fig. 1.

Clinical abscesses associated with serological analysis: A total of 51 cases showed clinical signs of lymphadenitis or multiple abscesses on superficial lymph nodes or reproductive organs (Fig. 2-1). Of these, 9 cases showed wound healing scars on superficial lymph nodes (Fig. 2-2) and one case had an open wound due to the abscess eruption through the skin at the popliteal lymph node. The CLA lesions were classified by location on the body as the proximal/front (parotid LN, submandibular LN and cervical LN), middle (prescapular LN, mediastinal LN, prefemoral LN, and lower abdomen), and distal/hind (gluteal LN, popliteal LN, mastitis, and orchitis) parts; their proportions per location are shown in Fig. 3.

The levels of antibody against CLA infection are shown in Fig. 4. Goats with clinical signs of CLA, including wound scars and superficial lymphadenitis (abscess), showed seropositivity at approximately 61.67%. The probability of seropositivity was 66.67% in goats with scars and 60.78% in goats with abscessed lymph nodes. None of the studied goats possessed both scars and abscessed lymph nodes.

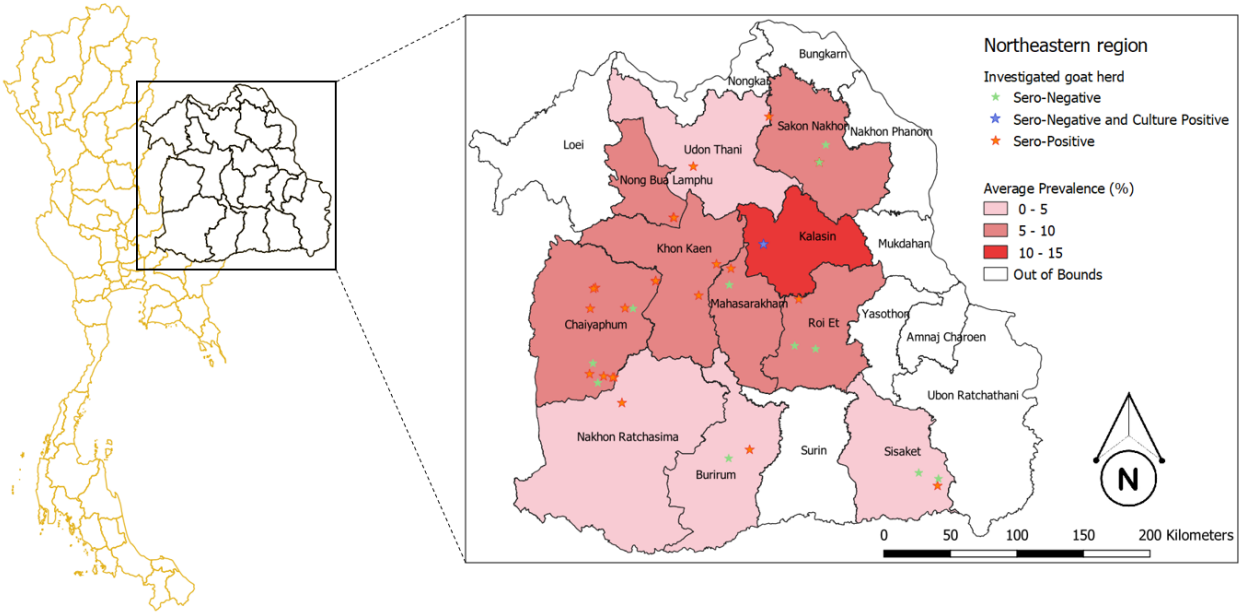

Figure 1 Spatial distribution of *Corynebacterium pseudotuberculosis*-positive herds and the average prevalence of CLA in Northeastern of Thailand.

Figure 2-1 Locations of enlarged lymph nodes or abscesses in caseous lymphadenitis cases. The enlargement of parotid (a), prescapular (b), and popliteal (c) lymph nodes is observed. Infections of the reproductive organs: mastitis (d) and orchitis (e).

Figure 2-2 Scars of CLA healing located on superficial lymph nodes in parotid (a) and prefemoral (b) lymph nodes.

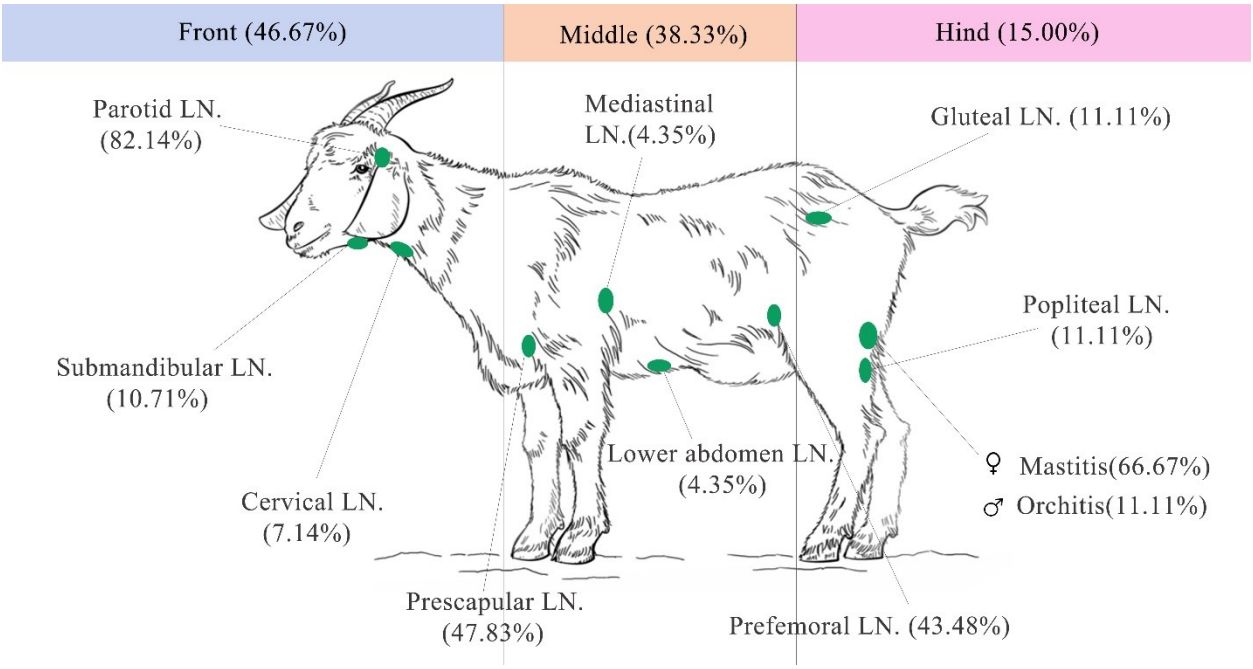

Figure 3 The proportions of clinical CLA lesions found in the proximal (front), middle, and distal (hind) parts of the body.

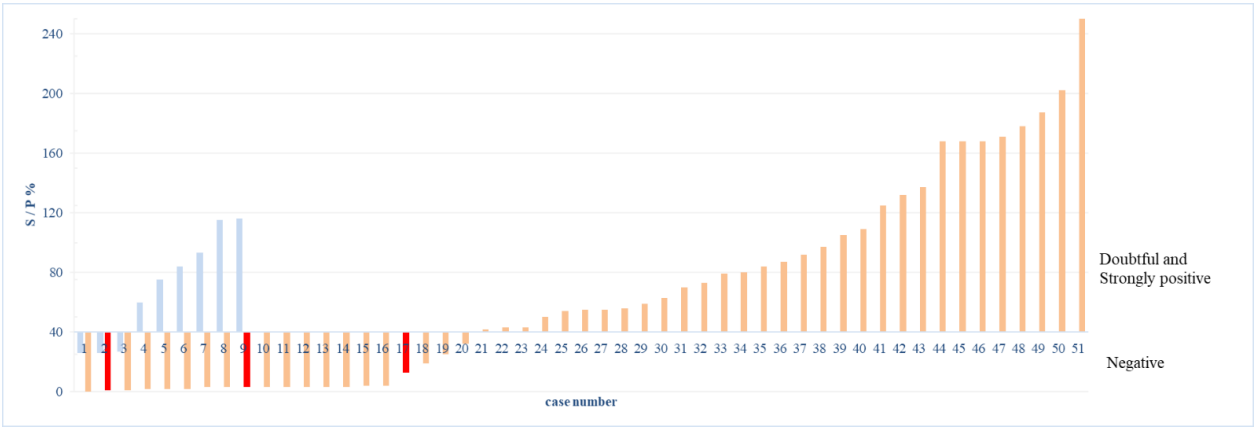

Figure 4 Antibody titer of CLA in clinical caseous lymphadenitis cases. The cut-off value is 40 (≤ 40 is negative, > 40 to 50 is doubtful, ≥ 50 is strongly positive). The blue bars showed the *S / P* % of serum from scar cases, the orange bars are from abscess cases, and the red bars are from seronegative cases that were positive by bacterial culture.

Table 1 Herd and health management factors related to caseous lymphadenitis (CLA) in small-holder goat herds in Northeastern Thailand

Factor	Category	Total number (%)
Herd size (number of goats)	< 30	18 (52.94)
	≥ 30	16 (47.06)
Time of herd establishment (years)	< 2	15 (44.12)
	≥ 2	19 (55.88)
Introduction of new goats to the herd	Yes	33 (97.06)
	No	1 (2.94)
Grouping by age	Mixed age	33 (97.06)
	Separated by age	1 (2.94)
Colostrum feeding	Mother reared	34 (100.00)
	Hand fed	0 (0.00)
Sexual reproduction	Natural mating	34 (100.00)
	Artificial insemination	0 (0.00)
Knowledge about CLA in goats	None	34 (100.00)
	Satisfied	0 (0.00)

Table 2 Individual animal factors associated with clinical caseous lymphadenitis (CLA) in small-holder goat herds in Northeastern Thailand

Factor	Category	No. of Positive (%)	Significance
Age (years)	< 2	23 (60.87)	p=0.68
	3	18 (66.67)	
	≥ 4	19 (73.68)	
Gender	Female	50 (66.00)	p=0.56*
	Male	10 (70.00)	
Breed	Anglo Nubian mixed	25 (68.00)	p=0.85
	Others	35 (65.71)	
Location of the lesion	Proximal	28 (75.00)	p=0.02
	Middle	22 (45.00)	
	Distal	10 (90.00)	

*Fisher's exact test

Risk factors

Herd and health management factors: The herd and health management factors for CLA, including herd size, the time of herd establishment, the introduction of new goats to the herd, grouping by age, colostrum feeding method, sexual reproduction method and the farmer's knowledge about CLA, are shown in Table 1.

Individual animal factors: Age, gender and breed were not significantly related to CLA infection (p>0. 05) (Table 2). However, CLA lesions in individual animals were commonly found in the proximal (46. 67%), middle (38.33%), and distal (15%) parts of the body, as shown in Fig. 3. The association between clinical CLA and seropositivity for caseous lymphadenitis was significantly different between the distal (90%), proximal (75%), and middle (45%) parts of the body (p=0.02) (Table 2).

Discussion

This study confirmed clinical cases of *C. pseudotuberculosis* infection in 11 provinces in Northeastern Thailand. Even though the seroprevalence of clinical CLA was small, this was only a preliminary study of CLA in this area.

Clinical CLA cases were mostly found in the proximal part of the goat body (46.67%) and were most frequently located on the parotid, submandibular, and cervical lymph nodes. This finding agreed with a previous study that reported the appearance of most of the clinical CLA on superficial lymph nodes of the head and neck (Oreiby *et al.*, 2014). Clinical CLA lesions are most commonly found on the superficial cervical lymph node in goats, unlike the case in sheep, where the lesions most frequently occur in the parotid lymph node (Al-Gaabary *et al.*, 2009). CLA was identified in the middle part of the body in 38.33% of our cases and mostly in the prescapular, prefemoral, and mediastinal lymph nodes, and in the lower abdomen. Fifteen percent of the CLA abscesses were found in the distal part of the body, primarily in the reproductive organs and gluteal and popliteal lymph nodes. Animals with CLA lesions in the distal part of the body showed 90% seropositivity, which was significantly higher than in goats with lesions on other parts of the body (p<0.05).

The location of abscesses in lymphadenitis depends on the location of the wound where *C. pseudotuberculosis* gained entry into the body. Most infected dairy goats have lesions on the head and neck, in the parotid, mandibular and superficial cervical (prescapular) lymph nodes, presumably due to injuries from thorns, splinters from wooden feeders or fences, combat wounds and scratching at lice (Smith and Sherman, 2009). Contact of these skin lesions with milk stands, feeders, fences or scratching posts can contaminate them with draining pus, leading to infection of other goats in the nodes about the head and neck (Smith and Sherman, 2009).

The analysis of individual factors showed that age, gender and breed were not associated with seropositivity or bacterial identification in the clinical CLA cases. The age factor from this study was consistent with that reported by Kaba *et al.* (2011), who showed no statistical difference in CLA infection in goat herds. However, another study found a significantly higher prevalence of CLA prevalence in female than in male animals and a higher prevalence in animals aged from 1 to 2 years and over 2 years (Al-Gaabary *et al.*, 2008).

In the present investigation, almost 100% of the farmers had the same herd and health management. They tended not to take precautions when introducing a new animal into the herd for expanding their herds, not to conduct CLA testing and not to group animals by age, while they managed colostrum by a mother-rearing system, used a natural mating system, and had only a limited knowledge of CLA as a disease. These factors might be associated with the incidence of clinical CLA in their herds, as the farmers may not be aware of the route of transmission of CLA. Therefore, differences in herd and health management factors might be associated with CLA infection. Oreiby *et al.* (2014) reported that the choice of breeding system has effects on the occurrence of CLA in small ruminant herds because CLA-infected animals can spread *C. pseudotuberculosis* to other animals in a short period due to the close contact and reduced air flow in stalls. In our study area, the breeding system involved mixing animals in the same barn, regardless of age and gender, and relying on a natural mating system. This management choice might cause CLA to spread easily

in the herd from CLA-infected animals to healthy ones, from adults to kids, and from bucks to does and is likely the reason why the factors of age and gender were not significantly associated with the disease in the present study. This finding is consistent with that of Kaba *et al.* (2011), who reported that adult goats infected with CLA could share the infectious agent with younger animals within the group. Moreover, the presence of seropositive males, superficial abscesses, respiratory signs of disease, poor condition of the animals, and the occurrence of reproductive failure in herd were identified as risk factors influencing the spread of CLA in goat herds (Kaba *et al.*, 2011).

Trading animals from herd to herd and sharing CLA-infected male breeders between herds are further risk factors. Although the sexual transmission of CLA has never been reported, males with CLA infection have decreased fertility performance, including decreased testosterone concentration and poor semen quality (Mahmood *et al.*, 2016). In our study, all the investigated farms used a mother-rearing system for colostrum feeding; therefore, CLA mastitis could have been transmitted to the kids during nursing.

The serological test used in this study is a useful tool for diagnosing the disease in the herd. However, the test could not identify all infected animals in the herd due to some false-negative results. The sensitivity of the ELISA test used in our study was 91.4% (95% CI, 81.36–96.26%) and the specificity was 100% (95% CI, 97.21–100%) (ID vet® validation data report, 2017). This could explain why some animals in our study showed CLA abscesses but were seronegative. Previous findings have demonstrated that the intradermal inoculation of 10^4 or 10^5 colony-forming units (cfu) of *C. pseudotuberculosis* could induce an abscess within 8–9 days and that the antibody titer increases within 15 days after the inoculation (Kuria *et al.*, 2001). Similarly, another study showed that subcutaneous inoculation with *C. pseudotuberculosis* at the dose of 10^5–10^8 cfu led to detection of antibodies against the exotoxin by ELISA approximately two weeks later (Chirino-Zárraga *et al.*, 2009).

In the present study, 20 clinical CLA cases that showed signs of lymphadenitis had seronegative results on the ELISA test. Since approximately two weeks must elapse post-infection for a test to be positive, pus collection for bacterial cultures is still necessary in clinical CLA cases that show negativity (Scott, 2011). Frequently, the organism is confirmed as *C. Pseudotuberculosis*; however, other bacteria, such as *Staphylococcus aureus*, *Arcanobacterium pyogenes*, and *Enterococcus faecalis*, might also be the cause (Smith and Sherman, 2009; Wichaiwong *et al.*, 2019). Cases with scar tissue may also show seronegative results when the agent is removed from scarred lesions (Kuria *et al.*, 2001). Goat kids younger than six months that are infected with CLA are also likely to show seronegative test results due to their underdeveloped immune systems (Chirino-Zárraga *et al.*, 2009), as was observed for the three-month-old goat kids in the present study.

Control, eradication and prevention measures are necessary due to the impact of CLA on animal production, reproduction and health and because of the danger of transmission to humans (Paton *et al.*, 1996; Williamson, 2001; Baird and Fontaine, 2007; Heggelung *et al.*, 2015; Mahmood *et al.*, 2016). When the disease is present in herds and the control measures fail, the seroprevalence of CLA can increase from 28.8% to 59.1% within two years (Baird and Malone, 2010). In endemic areas, the organisms can survive in environments such as soil, barns, equipment, and within infected tissues for periods ranging from three weeks to eight months (Garry, 2008; Guimarães *et al.*, 2011). Once the disease occurs in an animal, other animals living on the same farm that are at risk include not only sheep and goats but also cattle, since *C. pseudotuberculosis* can cause ulcerative necrotic skin lesions in cattle as well (Garry, 2008).

CLA-infected animals are treated by opening the abscess to drain abscessed nodes, cleansing the wound with a diluted disinfectant, (e.g., povidone iodine, or chlorhexidine) once a day, and isolating the treated animals for 20–30 days until the wound is healed (Williamson, 2001; Al-Gaabary *et al.*, 2009). Due to the potential of for zoonotic transmission, the surgeon who performs these surgical treatments needs to wear gloves during the surgery and any infected materials, such as gauze, needles, syringes, and blades, must be burned post-surgery (Smith and Sherman, 2009). The organism can persist in farm environments such as barns and nursing areas; therefore, the surgical area should be covered with plastic and paper prior to surgery. After surgery or during the wound dressing, the area should be cleaned with disinfectants (e.g., hypochlorite, formalin, cresol, or iodine) to kill *C. pseudotuberculosis* in the environment (Guimarães *et al.*, 2011; Osman *et al.*, 2018).

Antibiotic therapy, such as penicillin or tetracycline, should be administered for a few days after the spontaneous rupture or lancing of an abscess to prevent dissemination of the organism to other lymph nodes (Smith and Sherman, 2009). The use of off-label procaine penicillin G by a subcutaneous route for open draining and flushing of the abscess is acceptable for CLA treatment because the effectiveness is not significantly different from that achieved by the use of closed-system lavage combined with tulathromycin administration by subcutaneous or intralesional injection (Washburn *et al.*, 2009). A single dose of 2.5 mg/kg body weight of tulathromycin, injected subcutaneously or intralesionally, can maintain drug concentrations above the minimum inhibitory concentration (MIC) to kill *C. pseudotuberculosis* for at least 15 days, indicating that a single dose of tulathromycin will have long-term therapeutic effects for *CLA treatment* (Washburn *et al.*, 2013). Recently, penicillin showed good sensitivity for *C. pseudotuberculosis*, but the organism in Italy was resistant to ampicillin and gentamicin (Domenis *et al.*, 2018). In the present study area, information is needed on drug sensitivity.

Eradication and control measures, including the culling of CLA affected animals, combined with the clinical examination and serological detection of

infected animals, are recommended for the eradication of CLA in herds (Baird and Malone, 2010; Pépin and Paton, 2010). Recommended hygienic measures include isolating infected animals, feeding kids with non-mother rearing colostrum in cases of mastitis from CLA, and avoiding nails, wires and other sharp materials in housing facilities, since they can induce abscesses of the skin (Smith and Sherman, 2009; Windsor, 2011). Surgical instruments, needles, tattooers, milking machines, and shearing equipment should be sterilized between animals (Smith and Sherman, 2009). Insects can be CLA vectors; therefore, controlling external parasites is also necessary (Guimarães *et al.*, 2011). In cases of chronic respiratory disease, the animals should be culled or isolated from the other animals because the disease in its visceral form can be airborne; thereby encouraging CLA spread among the herd animals (Smith and Sherman, 2009). Animals with recurrent CLA abscesses after surgical treatment should be culled (Windsor, 2011). Contamination by CLA in the environment may be the source of new infections; therefore, housing facilities should be cleaned with disinfectant. For example, the bedding should be burned and newborns' umbilical cords should be dipped in iodine at birth (Smith and Sherman, 2009).

Vaccination is the one of the measures for controlling CLA. Windsor (2011) summarized the available vaccines for CLA; these included bacterin, toxoid, combined, live, and DNA vaccines. However, CLA vaccines have never been used in Thailand, especially in the studied area. This might require further research.

The prevention of CLA transmission into herds includes the introduction only of new animals that are from CLA-free herds (Smith and Sherman, 2009) and observation of new animals for clinical signs of CLA during a quarantine period, which should be at least 20 days before introducing the animal into a CLA-free herd (Kuria *et al.*, 2001). Screening for CLA by a serological test is also recommended during the quarantine period to identify CLA-free goats, as is screening adult goats in herds once a year for monitoring. In addition, animals with clinical signs of CLA or animals from CLA-infected herds should never be purchased and brought into the herd. Similarly, visitors, meat distributors or merchants, or anyone from outside the farm should be aware of CLA, as they can introduce the disease into the farm via contaminated equipment, clothing, and boots. Prevention control also entails the introduction of personal and vehicle biosecurity measures, such as cleaning contaminated vehicles and equipment with disinfectant, wearing fresh clothing before starting work on farms, and wearing clean and disinfected boots on farms. Because the CLA organism can cause human lymphadenitis, occupational exposure to CLA infected animals should be a concern for farm workers, veterinarians, meat inspectors, butchers, merchants, abattoir workers, and laboratory staff in *C. pseudotuberculosis* laboratory units (Peel *et al.*, 1997; Heggelund *et al.*, 2015). Basic personal hygiene, such as wearing gloves and masks and hand-washing with soap or disinfectant must be practiced when a risk of exposure to CLA animals exists.

The present investigation provides the first report of clinical CLA in the northeastern provinces of Thailand. The goat population in Thailand increased by 16% per year from 2013 to 2018, and by 81.58% during that period in northeastern Thailand (ICT DLD, 2018). However, the understanding of, and attention paid to, CLA remains limited in this region. Extension of knowledge in these areas would be useful for goat herders and public audiences to control and prevent CLA.

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Conflict of interest: The authors declare that they have no conflict of interest.

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