Survival Analysis of Clinical Cases of Caseous Lymphadenitis of Goats in North Shoa, Ethiopia

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1.Introduction

Abscess disease, commonly known as Morel’s disease and caseous lymphadenitis (CLA), is a bacterial disease of sheep and goat causing abscesses in lymph nodes of the body and internal organs, especially the lung. It is a contagious disease distributed worldwide and lacks effective control measures. Once introduced into a sheep or goat flock, it is very difficult to control because of its poor response to treatment, its ability to persist in the environment, and the limitations in detecting subclinically infected animals [1, 2]. CLA is a disease caused by Corynebacterium pseudotuberculosis bacteria. It is commonly found in animals, such as sheep, goat, and cattle, from where the infection is transmitted to humans. These bacteria are Gram-positive, nonmotile pleomorphic rods (about 0.5 μm in width) that often present a characteristic Chinese letter or palisade arrangement in the smear. C. pseudotuberculosis is a mycolic acid-containing facultative intracellular parasite that multiplies inside the macrophage. Its natural habitat is skin, mucus membranes, and gastrointestinal tract of normal sheep and soil of sheep pens [3, 4]. Accurate CLA diagnosis is based primarily on clinical observations (external abscesses) [5] and the identification of C. pseudotuberculosis by phenotypic and biochemical tests; this is important to differentiate this bacterium from other abscess inducing pathogenic agents, such as Arcanobacterium pyogenes or Pasteurella multocida [2, 6, 7].
It causes considerable economic losses to sheep and goat producers as it reduces milk yield and meat and wool production. Also, it decreases reproductive efficiencies. It is the main cause of condemnation of sheep carcasses in slaughterhouses in Australia, one of the world’s largest producers of meat and wool [8, 9]. Unfortunately, it is the internal abscesses that are fatal, whereas external abscesses are generally responsible for disease transmission [1]. In Ethiopia, recent findings indicate that 15% prevalence of caseous lymphadenitis was reported from goats slaughtered in Luna Export Abattoir originated from district area of Borena Range Land [10] and 10% prevalence of local abscess was also reported in Boer goats of Adami Tulu Agricultural Research Center Nucleus site being the major disease next to GIT parasites, keratoconjunctivitis, ticks, and respiratory problems [11]. 4.7% prevalence of caseous lymphadenitis was also reported from the Boer goat breeding station of Southern Ethiopia [12]. In Ethiopia, the disease was not yet characterized at farm level. Therefore, the objective of this study is to estimate the prevalence, incidence, and risk factors of caseous lymphadenitis and to identify the causative bacteria.

2. Materials and Methods

2.1. Study Area and Animals. The study was conducted at the on-station Boer × Central Highland Goat cross-breeding program carried out at Ataye Research site, Debre Birhan Agricultural Research Center, Ethiopia. The site is located in central Ethiopia and the climate is characterized by bimodal rainfall consisting of the long rainy season (June-September), short rainy season (February-May), and dry season (October-January) [13]. The site’s geographic coordinate reference is 10°35’ N latitude and 39°93’ E longitude and is located at 1491 m above sea level altitude (Figure 1).

The study animals were a mix of different goat breed groups including Boer, Boer cross with Central Highland Goat, and Central Highland Goats. The flock was managed semi-intensively with grazing and supplement. The supplement includes ad libitum grass hay, chopped pasture (Napier grass, Desmodium spp., and vetch), and concentrated supplement based on their body weight. Detail summary of breeding, feeding, management system, and data recording of the flock are presented in [14, 15].

2.2. Clinical Examination and Sample Collection. The study was carried out on a goat farm maintained under a semi-intensive system in the lowland of North Shoa, Ataye District, Ethiopia. The study was conducted over a period of 8 years (June 2011 to January 2019) using a longitudinal (both retrospective and prospective) follow-up study design. A total of 1025 goats of breeds, 287 Boer goat, 515 Boer × CHG, and 223 CHG), were followed up and clinically examined for the presence of enlarged and abscessed superficial lymph nodes and internal caseous abscess during postmortem examination. Tissue samples from 32 goats with lesions suspected of caseous lymphadenitis were taken after shaved and disinfected with 70% alcohol or savlon, and then an incision was made with a sterile blade and the samples were transported in icebox to the microbiology laboratory of Debre Birhan Agricultural Research Center.

2.3. Descriptive Statistics and Survival Analysis. Data collected from clinical cases and laboratory tests were entered into an Excel spreadsheet. Clinical records were rearranged as time-to-event data form in the Excel spreadsheet. The starting point of follow-up was the date (day/month/year format) that the goat joined the farm through birth, purchase, or transfer from other research centers. The event/failure time was the date (day/month/year format) a goat shows CLA clinical sign. Since the disease is characterized by a recurrent relapse of abscess due to poor response to antibiotic treatment, the data were rearranged according to the counting process data layout with multiple observations per subject [16]. Goats that fail in the first time will be again recorded using the first failure time as the initial time. Censored observations are goats that leave out the farm by death (the most common one), by transfer, or end of the study period.

The estimation of the survivor function was computed as follows [17]:

\[ \hat{S}(t) = \prod_{j: t_j < t} \left(1 - \frac{d_j}{n_j}\right), \]

(1)

where \( \hat{S}(t) \) is the value of survival function at a time \( t_j \), \( n_j \) is the number of goats without caseous lymphadenitis cases at time \( t_j \), and \( d_j \) is the number of goats with caseous lymphadenitis cases at time \( t_j \). Survival curves were constructed with the Kaplan–Meier method, and we used the survival curve plotting macro %NEWSURV [18].

The risk factors associated with caseous lymphadenitis were assessed using multivariate Cox regression analysis adjusted for sex, age, breed, season, and year. Statistical analyses were performed using SAS statistical software version 9.4 [19]. We used the PROC PHREG option called COVSANDWICH that corrects for the dependence of repeated events on the same goat over time. This option invokes a method variously known as the robust variance estimator or the modified sandwich estimator, developed for Cox regression by Allison [16] and Lin and Wei [20]. The proportional hazard assumption of covariates was checked using PH ASSES option, and for the violated variables, we add time interaction in the model. Year when the goat joined the farm was used as a stratifying variable. \( p \) values <0.05 were considered as statistically significant. Then, Weibull proportional hazard model for the caseous lymphadenitis case of a particular goat at a time \( t \) was designed as follows:

\[ \lambda(t) = \lambda_0(t) \times \exp(Age_x + Sex_x + Season_x), \]

(2)

where \( \lambda(t) \) is the risk of caseous lymphadenitis or probability of goats being caseous lymphadenitis positive at time \( t \), \( \lambda_0(t) \) is the baseline hazard function with shape parameter \( p \) and scale parameter \( \lambda \) of the Weibull distribution or \( \lambda_0(t) = \lambda \rho (\lambda t)^{\rho - 1} \), \( Age_x \) is fixed effect of the \( x^{th} \) age of goat when joined the farm, \( Sex_x \) is fixed effect of the \( x^{th} \) sex of goat, and \( Season_x \)
is fixed effect of the \( k \)th season of the year when the goat joined the farm.

2.4. Bacteriological and Biolog MicroStation with GEN III Microplate System Bacteria Rapid Identification. Thirty-two pus samples taken from suspected caseous lymphadenitis abscess were cultured on 7% sheep blood agar for 48–72 h at 37°C, and bacterial colonies were identified on the basis of morphological characteristics, biochemical tests, i.e., catalase, urease, trehalose, xylose, maltose, and glucose fermentation tests, and reverse CAMP test (antagonistic haemolysis between \( \text{Corynebacterium pseudotuberculosis} \) and \( \text{Staphylococcus aureus} \) for phospholipase D (PLD) production [4]. Catalase, PLD, and urease-positive and nitrate-negative cultures were considered positive for \( \text{C. pseudotuberculosis} \).

Out of 32 bacterial cultures, 12 samples of well-grown bacterial colonies on blood agar were transported to the National Animal Health Diagnostic Center, Sebeta, in icebox for further bacterial species identification. OmniLog (fully automated coated microplate-based bacterial identification system), that is, GEN III microplate with protocol A method, was used to test suspected colonies. A single colony grown on Biolog Universal Growth (BUG) agar medium was selected and emulsified into “inoculating fluid A” (IF A). According to the manufacturer’s instructions, cell density of the bacterial inoculum was measured for a specified transmittance (90 to 98%) using a turbid meter, as specified in the user guide. For each isolate, 100 \( \mu l \) of the cell suspension was inoculated into each of the 96-well coated microplate, using an automatic multichannel pipette, and incubated aerobically at 33°C for 22 h. The OmniLog identification system automatically reads each microplate and provides identification called species/subspecies ID, and then, the results were printed (GEN III database, version 5.2.01). The results were also read in the Biolog MicroStation reader after 22 h incubation outside GEN III incubation.

3. Results

3.1. Descriptive Statistics and Survival Analysis. During the overall eight-year clinical follow-up study period, out of 1,025 goats examined, we found 214 abscess cases clinically diagnosed as caseous lymphadenitis (Figures 2(a) and 2(b)). In the recurrent survival data analysis, there were 1,138 observations and 214 cases (81.2% of goats with a prevalence of 18.8% were censored due to mortality, transfer to other places, or end of the study period) and the incidence rate was 0.14 cases per animal year. The prevalence was highest in Boer \( \times \) CHG goats (21.03%). From a total of 214 cases, 130 have occurred once and they did not relapse, and 84 cases are
twice or more relapse cases (Table 1). The incidence rate was 0.14 cases per animal year.

There was a significant difference between breed, season, and age (Figure 3). Newborn kid starts clinical cases by around 6

months of age and the failure rate is steady reaching their median survival time at 36.8 months (nearly 3 years old) (Figure 3(b)). At the end of the study, the cumulative failure rates were 68% for newborn and 64% for yearlings. The hazard is higher for newborn goats starting from first year interval up to the end while the hazard is relatively lower in the first three years for yearling goats and higher after three years of age (Table 3). The survival rate is poor for Boer cross with local Central Highland Goat and goats joined the farm during the dry season (Figures 3(a) and 3(c)).

Proportional hazard regression analysis of risk factors using the robust variance regression of recurrent events and year as a stratifying variable showed that age, sex, and breed were associated with \( p \text{ value} < 0.05 \) caseous lymphadenitis occurrence (survival) and season of the year when the goat joined the farm was not associated with caseous lymphadenitis survival. Newborn goats compared with yearling age goats have 4.801 times higher probability of being infected with caseous lymphadenitis \( p \text{ value} < 0.0001 \). Female goats

### Table 1: Summary of caseous lymphadenitis cases with order of recurrence.

| Breed         | Positive (%) | 1st case | 2nd case | 3rd case | 4th case | 5th case | 6th case |
|---------------|--------------|----------|----------|----------|----------|----------|----------|
| Boer goat     | 314          | 53 (16.88)| 34       | 12       | 4        | 2        | 1        |
| Boer × CHG    | 585          | 123 (21.03)| 72       | 28       | 11       | 7        | 4        | 1        |
| CHG           | 239          | 38 (15.9) | 24       | 9        | 3        | 2        | 0        | 0        |
| Total         | 1138         | 214 (18.80)| 130      | 49       | 18       | 11       | 5        | 1        |

Note: CHG: Central Highland Goat.
have 1.856 times higher rate of being infected compared with male goats (\(p\ value = 0.0023\)). Pure Boer goat breeds have 4.217 times higher rate of being infected (\(p\ value < 0.0001\)), and Boer cross with Central Highland Goat breeds have 4.562 times higher rate of being infected (\(p\ value < 0.0001\)) compared with local Central Highland Goat Breed goats (Table 4).

### 3.2. Bacteriological and Biolog MicroStation with GEN III Microplate System Results

From 32 pus samples of CLA suspected goats, we were able to recover pure colonies of bacteria (Figure 2(c)). All of the samples were found to be positive for *C. pseudotuberculosis* based on cultural examination. All isolates of *C. pseudotuberculosis* were Gram-positive pleomorphic rod (Figure 2(d)), catalase-positive, urease-positive, antagonistic haemolysis with *Staphylococcus aureus* for phospholipase D (PLD) production, and nitrate-negative [4]. Out of 12 bacterial cultures tested using Biolog MicroStation with GEN III microplate system results, 10 were *C. pseudotuberculosis* and 2 of them were *C. ulcerans*.

### 4. Discussions

#### 4.1. Descriptive Statistics and Survival Analysis

The present study shows the prevalence of CLA as 18.8% and the incidence rate as 0.14 cases per animal year. The cumulative failure rates were 68% for newborn aged goats and 64% for yearling aged goats. This result indicates a high rate of CLA infection rate at Ataye goat farm. The cases were characterized by poor response to local and systemic antibiotic treatments. The farm veterinarians cost a considerable time to treat the frequent cases including the recurrent abscess following incision, local treatment by iodine and salvon, and systemic antibiotic treatments using long-acting oxytetracycline.

The current prevalence is in line with reports abroad, 19.23% prevalence by Al-Gaabary et al. [21], 26% prevalence by Paton [22], and in Ethiopia 15% by Fikre and Abraha [10]. The current port is relatively higher than most reports in Ethiopia 4.7% by Molla [12], 11.7 by Abebe and Sisay [23], and 10% by Hunduma et al. [11]. The variations in the disease frequency between different studies may be attributed to the differences in the management systems and climatic conditions in each study where the viability of the causative organism in the contaminated environment is greatly affected by ambient temperature, and it may also be attributed to the endemic nature of the disease which leads to a variation in animal immunity and the degree of animal susceptibility [21]. In Australia, the prevalence of CLA had decreased from over 50% in the 1770s to approximately 20% in the late 1990s through the use of recommended CLA vaccine [22, 24]. The higher rate of infection in our study was due to semi-intensive management of goats, a higher density of flocks on the smaller grazing land, higher density at the barn, and the lower quarantine practice of clinical cases in the farm where all of them aggravates the transmission rare of the disease. There is also no vaccine available in the country.

The most affected superficial lymph node was the parotid lymph node. Similar results were reported in [2, 10]. These results may be attributed to the habit of goats that tend to scratch their shoulders and heads against walls and fences or any hard objects, resulting in a high percentage of superficial parotid, mandibular, cervical, and prefemoral lymph node infection because it drains the shoulder region [10].

The hazard of CLA was higher (\(p < 0.001\)) in the newborn than in the yearling age group. This result contradicts from other findings [10, 21], where age groups below 1 year are at a lower risk. This may be due to variation in the type of study and age classification method. In this result, new cases are started at around 6 months of age. The higher incidence rate in the newborn is due to the poor immunity of kids.

Female goats were at higher (\(p < 0.0023\)) risk of infection than male goats. Similar reports indicated a higher prevalence of CLA in female groups [21, 25]. These results may be attributed to the fact that does usually reared for older ages than bucks, as well as a relatively higher number of does usually reared in one group resulting in a high rate of contact. However, a few number of males are kept together mainly for the mating purpose of the farm [14, 15].

Breed variation was the other important risk factor in the proportional hazard analysis. Boer and Boer cross with local goats have 1.856 times higher rate of being infected compared with male goats (\(p\ value = 0.0023\)). Pure Boer goat breeds have 4.217 times higher rate of being infected (\(p\ value < 0.0001\)), and Boer cross with Central Highland Goat breeds have 4.562 times higher rate of being infected (\(p\ value < 0.0001\)) compared with local Central Highland Goat Breed goats (Table 4).

### Table 2: Summary of caseous lymphadenitis cases with lymph node location.

| Goat breed   | Parotid LN | Prescapular LN |Undefined LN | Prefemoral LN | Mandibular LN | Pharyngeal LN | Multiple LNs | Skin area | Supramammary LN | Cervical LN | Visceral organs LN | Overall cases, N (%) |
|--------------|------------|---------------|-------------|---------------|--------------|---------------|--------------|-----------|------------------|-------------|------------------|-------------------------|
| Boer goat    | 21         | 10            | 2           | 2             | 3            | 4             | 2            | 1         | 2                | 3           | 3                | 53/314 (16.88)         |
| Boer × CHG   | 55         | 21            | 12          | 13            | 4            | 7             | 7            | 2         | 1                | 1           | 0                | 123/585 (21.03)        |
| CHG          | 16         | 5             | 0           | 8             | 4            | 0             | 0            | 2         | 2                | 0           | 1                | 38/239 (15.90)         |
| Total (relative %) | 92 (42.99) | 36 (16.82) | 14 (6.54) | 36 (10.75) | 11 (5.14) | 11 (5.14) | 5 (2.34) | 4 (1.87) | 5 (2.34) | 4 (1.87) | 214/1138 (18.80) |

Note. CHG: Central Highland Goat; CLA: caseous lymphadenitis; LN: lymph node.
Central Highland Goats were at higher risk \((p < 0.0001)\) compared with the local Central Highland Goat breeds. Generally, the imported Boer goats are at higher risk to various disease conditions [26]. This may be due to the higher resistance of local breeds due to adaptation [12].

4.2. Bacteriological and Biolog MicroStation with GEN III Microplate System Results. We recovered 32 \(C.\) pseudotuberculosis bacteria from all 32 cultured abscess samples. From 12 pure culture samples tested using the Biolog-based test, 10 of them were \(C.\) pseudotuberculosis and 2 of them were \(C.\) ulcerans positive. However, all of the 12 samples were \(C.\) pseudotuberculosis positive based on the biochemical test method. The Biolog-based test screened the 2 samples as \(C.\) ulcerans. The current result is in line with most previous findings. \(C.\) pseudotuberculosis were the most frequent isolated bacteria of clinical CLA abscess [10, 21, 23, 25, 27]. The present result of 2 \(C.\) ulcerans based on the Biolog test is the first report in Ethiopia. Although the differentiation between \(C.\) ulcerans and \(C.\) pseudotuberculosis was based on a single biochemical reaction, the trehalose test, the present results are in agreement with previous studies [28] and indicate that the microorganism isolated in the present study was \(C.\) ulcerans. There are also reports indicating that \(C.\) ulcerans was isolated from clinical cases of goat [29]. \(C.\) ulcerans is an emerging zoonotic disease that causes diphtheria like illness in humans [30, 31].

5. Conclusion and Recommendations
Clinical and bacteriological study of the caseous lymphadenitis indicates that the high prevalence of caseous

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**Figure 3:** Kaplan–Meier survival function curve of caseous lymphadenitis cases from birth or entry to farm to 60 months of the follow-up period.
lymphadenitis in this goat farm. Moreover, repeated abscess occurs after treatment. Laboratory work reveals that *C. pseudotuberculosis* and *C. ulcerans* were isolated bacteria that cause abscess disease. Due to the regular abscess after treatment, it indicates poor response of the disease to different antibiotics and antiseptic chemicals. Control and prevention in sheep and goat farms should focus on quarantine and culling of positive animals and vaccination with the CLA vaccine.

**Data Availability**

The datasets used to support the findings of this study are available from the corresponding author upon request.

**Conflicts of Interest**

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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### Table 3: Life table of CLA cases stratified based on the age of goats.

| Age group | Interval (months) | Total | Cases | Lost | Cumulative failures | Hazard | 95% CI hazard |
|-----------|------------------|-------|-------|------|---------------------|--------|-------------|
|           |                  |       |       |      |                     |        | Lower       | Upper       |
| Newborn   | 0-12             | 764   | 61    | 439  | 0.11                | 0.010  | 0.007       | 0.012       |
|           | 12-24            | 264   | 48    | 80   | 0.30                | 0.020  | 0.014       | 0.026       |
|           | 24-36            | 136   | 21    | 53   | 0.44                | 0.018  | 0.010       | 0.025       |
|           | 36-48            | 62    | 9     | 17   | 0.53                | 0.015  | 0.005       | 0.025       |
|           | 48-60            | 36    | 5     | 22   | 0.62                | 0.019  | 0.002       | 0.035       |
|           | 60-72            | 9     | 1     | 4    | 0.68                | 0.013  | 0.000       | 0.038       |
|           | 72-84            | 8     | 0     | 4    | 0.68                | 0.000  |             |             |
| Yearling  | 0-12             | 374   | 16    | 103  | 0.05                | 0.004  | 0.002       | 0.006       |
|           | 12-24            | 255   | 16    | 82   | 0.12                | 0.007  | 0.003       | 0.010       |
|           | 24-36            | 157   | 4     | 33   | 0.15                | 0.002  | 0.000       | 0.005       |
|           | 36-48            | 120   | 15    | 33   | 0.27                | 0.013  | 0.007       | 0.020       |
|           | 48-60            | 72    | 11    | 22   | 0.40                | 0.017  | 0.007       | 0.026       |
|           | 60-72            | 39    | 3     | 13   | 0.46                | 0.008  | 0.000       | 0.017       |
|           | 72-84            | 23    | 1     | 6    | 0.48                | 0.004  | 0.000       | 0.013       |
|           | 84-96            | 16    | 3     | 12   | 0.64                | 0.029  | 0.000       | 0.062       |
|           | 96-108           | 1     | 0     | 1    | 0.64                | 0.000  |             |             |

**Note.** CI: confidence interval; St. err: standard error; HR: hazard ratio; CHG: Central Highland Goat.

### Table 4: Proportional hazard regression analysis results of explanatory variables effect on caseous lymphadenitis with robust standard errors.

| Variables                  | Parameter estimate | Standard error | St. err ratio | HR     | 95% CI hazard |
|----------------------------|--------------------|----------------|---------------|--------|---------------|
| Age Newborn vs. yearling   | 1.56880            | 0.29319        | 0.827         | 4.801  | [2.702–8.529] |
| Sex Female vs. male        | 0.61824            | 0.20285        | 1.159         | 1.856  | [1.247–2.762] |
| Breed Boer vs. CHG         | 1.43907            | 0.22716        | 0.657         | 4.217  | [2.702–6.582] |
| Breed Boer × CHG vs. CHG   | 1.51778            | 0.37440        | 0.912         | 4.562  | [2.190–9.503] |
| Season Summer vs. short rain| 0.19403           | 0.29058        | 1.208         | 1.214  | [0.687–2.146] |
| Season Dry vs. short rain  | 0.04796            | 0.17486        | 0.948         | 1.049  | [0.745–1.478] |

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