ORIGINAL ARTICLE

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Occurrence of caseous lymphadenitis in goats from a slaughterhouse in the Brazilian Northeast

Ocorrência de linfadenite caseosa em caprinos provenientes de um abatedouro no Nordeste brasileiro

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ABSTRACT: Caseous lymphadenitis (CLA) is a chronic zoonosis that affects small ruminants globally. CLA is sometimes characterized by its visceral form, which may be associated with late diagnosis during *postmortem* inspection only. Alternatively, serological diagnosis could be used in the subclinical CLA cases detection due to its high sensitivity. Here, the occurrence of CLA in goats slaughtered in Mossoró, Rio Grande do Norte (RN) state, Brazil, was determined and indirect Enzyme Linked Immunosorbent Assay (ELISA) and microbiological culture methods were compared. Serum samples were collected from 150 goats and used to perform indirect ELISA using a supernatant of *Corynebacterium pseudotuberculosis* as an antigen. Goats were inspected after slaughter and fragments from internal organs suspicious of CLA were collected for microbiological culture. Indirect ELISA detected anti-*C. pseudotuberculosis* antibodies in 11.33% (17/150) of goats, while *C. pseudotuberculosis* was detected by microbiological culture in only 2% (3/150). The kappa index (κ = 0. 275) presented a weak agreement between ELISA and microbiological culture. In conclusion, despite the considerable proportion of CLA in goats from a slaughterhouse in Mossoró-RN, there was low detection of *C. pseudotuberculosis* in microbiological culture, denoting the higher sensitivity of ELISA. These facts could be associated with the long incubation period of *C. pseudotuberculosis* and the early seroconversion of goats infected.

KEYWORDS: *Capra hircus*; *corynebacterium pseudotuberculosis*; seroprevalence.

RESUMO: A linfadenite caseosa (LC) é uma zoonose crônica que afeta pequenos ruminantes em todo o mundo. A LC é por vezes caracterizada pela sua forma visceral, que pode ser diagnosticada tardiamente apenas durante a inspeção *post mortem*. Alternativamente, o diagnóstico sorológico pode ser utilizado na detecção de casos subclínicos de LC devido à sua alta sensibilidade. Neste trabalho, foi avaliada a ocorrência de LC em caprinos abatidos em Mossoró, Rio Grande do Norte (RN), Brasil e comparados os métodos de imunoabsorção enzimática indireta (ELISA) e cultura microbiológica. Amostras de soro foram coletadas de 150 caprinos e utilizadas para realização de ELISA indireto utilizando sobrenadante de *Corynebacterium pseudotuberculosis* como antígeno. Os caprinos foram inspecionados após o abate e fragmentos de órgãos internos suspeitos de LC foram coletados para cultura microbiológica. O ELISA indireto detectou anticorpos contra *C. pseudotuberculosis* em 11,33% (17/150) dos caprinos, enquanto *C. pseudotuberculosis* foi detectado por cultura microbiológica em apenas 2% (3/150). O índice kappa (κ = 0,275) apresentou fraca concordância entre ELISA e cultura microbiológica. Conclui-se que apesar da considerável proporção considerável de LC detectado por ELISA em caprinos provenientes de um abatedouro de Mossoró-RN, houve baixa detecção de *C. pseudotuberculosis* em cultura microbiológica, denotando a maior sensibilidade do ELISA. Esses fatos podem estar associados ao longo período de incubação de *C. pseudotuberculosis* e à soroconversão mais precoce dos caprinos infectados em relação à formação de granulomas.

PALAVRAS-CHAVE: *Capra hircus*; *corynebacterium pseudotuberculosis*; soroprevalência.

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INTRODUCTION

Ninety-four percent of Brazilian goats are in the Northeast region, where the economic activity is characterized as subsistence farming and serves local trade (Carvalho *et al.*, 2020). In 2018, Rio Grande do Norte (RN) state had the sixth largest goat herd in the Northeast with 439,429 animals. Potiguar West, the region of RN where Mossoró city is located, held 218,830 goats (approximately 50% of the RN herd) (IBGE, 2018).

In this context, sanitary management tends to be poor and some diseases such as caseous lymphadenitis (CLA) are highly prevalent (Farias *et al.*, 2018). CLA is a chronic granulomatous infectious that mainly affects sheep and goats, characterized by two clinical forms that can coexist: the superficial form of CLA with infection of external lymph nodes (parotid, submandibular, pre-scapular, and sub iliac); and the visceral form with abscessing of different internal organs, for examples liver, lungs, uterus, kidneys, spleen, encephalon, and also internal lymph nodes (Aftabuzzaman; Cho, 2021; Santarosa *et al.*, 2014). In a study conducted in Spain, from 147 culled sheep with compatible CLA lesions, 107 presented the visceral clinical form of the disease (72.79%), while only 32 animals were affected by the superficial form (21.77%). In addition, eight animals were found to be affected by both the visceral and the superficial presentations (5.44%) (Ruiz *et al.*, 2020).

As some infected animals do not develop external lesions, the disease sometimes is only diagnosed on *post-mortem* examination (Abebe; Sisay Tessema, 2015). Abscesses in goats are suggestive of CLA and microbiological analysis followed by biochemical assays is considered the gold standard for diagnosis of CLA (Alves *et al.*, 2021). On the other hand, serological tests, such as ELISA, are an alternative for the detection of asymptomatic cases of CLA (Rebouças *et al.*, 2013), and thus, can act as important instruments for ensuring CLA diagnosis and control (Oreiby, 2015).

CLA is distributed globally and causes important economic losses for ovine and caprine breeders due to body wasting, subsequently reduced meat, wool, and milk yields and segregation of affected animals, and condemnation of affected carcasses and skins in abattoirs (Osman *et al.*, 2018).

Farias *et al.* (2018) found seropositive goats in 94.5% (55/52) of examined farms in RN state, showing remarkable dissemination of CLA. Furthermore, 33% (221/663) of tested goats in the Brazilian Northeast were seropositive for *C. pseudotuberculosis*. However, the prevalence of CLA in animals slaughtered in RN is not known. This study aims to determine the prevalence of CLA in goats from a slaughterhouse in Mossoró, RN, and to analyze the agreement between indirect ELISA and microbiological culture in the identification of *C. pseudotuberculosis*.

MATERIAL AND METHODS

Study Region

The study was conducted in a slaughterhouse of the Municipal Inspection Service, located in Mossoró city, Rio Grande do Norte state, Brazil (lat 5° 11' 17" S, long 37° 20' 39" W). This slaughterhouse receives bovine, ovine, and caprine species from Mossoró and surrounding municipalities, including some in the Ceará state.

Sample Animals, Blood Collections, and Serum Characterization

All procedures involving animals were approved by the Ethics Committee on the Use of Animals (CEUA) of the Federal Rural University of the Semi-Arid under approval number 18/2018. The procedures were performed following those established by the National Council for the Control of Animal Experimentation (CONCEA).

To obtain the minimum number of animals to use in this prevalence study, the formula for simple random sampling described by Thrusfield (2007) was used. We considered the weekly media of 60 slaughtered goats, and the following parameters: maximum sampling error of 7.5% and confidence level of 95%. Thus, 150 goat serum samples were used as samples here and were collected between August and September 2018.

Before blood collection, the goats were randomly selected, and an ante-mortem examination was performed through inspection and palpation of the main lymph nodes searching for some skin scars or external lesions suggestive of CLA. Additionally, data from goats regarding breed, age, gender, and weight were taken. It was not possible to administer questionnaires to the goat's owners, since the animals were left in the slaughterhouse throughout the week without any programmed schedule.

Blood collection was performed through jugular venipuncture and previous asepsis using 70 percent ethanol. The blood samples were placed in identified sterile tubes without anticoagulants and put into isothermal boxes, and subsequently transported to the Laboratory of Vaccinology and Applied Immunology (LAVIA) for serum collection and storage at –20 °C. After slaughter, the carcass was examined macroscopically aiming to identify the presence and position of internal granulomas. Samples of purulent material from suspected CLA lesions were collected for microbiological identification (Rebouças *et al.*, 2013).

Microbiological Analysis

Purulent samples were taken from abscesses suggestive of CLA and cultured on Brain Heart Infusion (BHI) agar, at 37 °C

for 48 h, for later confirmation through biochemical tests. Colonies with a morphology similar to *C. pseudotuberculosis* were grown in BHI broth under stirring at 140 rpm at 37 °C for 48 h, and then a smear of the colonies with morphological similarity to *C. pseudotuberculosis* was performed and stained using Gram's method. Cell morphology was evaluated using an optical microscope (Tripathi; Sapra, 2022). Gram-positive colonies were subjected to biochemical tests for maltose, glucose, sucrose, urea, esculin, and nitrate according to the methodology described by MacFaddin (2000).

Indirect Elisa

To determine the seroprevalence, an indirect ELISA using proteins secreted from the 1002 strain of *C. pseudotuberculosis* as an antigen was performed (Seyffert *et al.*, 2010). The antigen was collected from the *C. pseudotuberculosis* culture supernatant after 48 hours in BHI broth. The antigen was diluted in 1:100 proportion using 0.5 M carbonate-bicarbonate buffer (pH 9.6). Ninety-six-well plates (Nunc-Immuno Plate Maxisorp) were adsorbed with 100 µL of the coating solution, following incubation for 18 h at 4 °C. Plates were washed three times with 0.05% PBS-Tween (pH 7.6) (PBS-T) and incubated for two hours at 37 °C with 200 µL per well of blocking solution (PBS-T added to 5% skimmed milk powder). A new three-step washing was performed and 50 µL of the serum diluted in PBS-T (1:100) was added to each well, followed by incubation for 1 hour at 37 °C. The plates were washed and incubated with a peroxidase-labeled anti-goat IgG (diluted in 1: 10,000 with PBS-T). After a five-step washing, 50 µL/well of a chromogenic substrate (0.4 mg/mL ortho-phenylenediamine dihydrochloride in citrate-phosphate buffer containing 0.04% hydrogen peroxide at 30%, pH = 5.0) was added to each well, and the plate was incubated for 15 min in the dark at room temperature. The reactions were stopped using a 5% sulfuric acid solution (25 µL/well) and absorbance was taken at 492 nm using an ELISA spectrophotometer. Each sample was evaluated in duplicate, using appropriate positive and negative controls. Duplicates with differences greater than 20% were repeated to confirm the results. The cut-off point used was 0.35, with a specificity of 98.5% and a sensitivity of 93.5%.

Statistical Analysis

Real and apparent prevalence, confidence limits, and positive and negative predictive values were obtained using the EpiTools epidemiological calculator (https://epitools.ausvet.com.au/), which considers assay sensitivity and specificity, total sampling, and the number of positive animals.

Due to the non-normality of the data, Fisher's exact test was performed to assess the correlation between the variables sex, age, and seropositivity. To verify the influence of the sex variable on seropositivity, odds ratio (probability ratio) and Baptista–Pike were used. Statistical analyses were performed using GraphPad Prism 8.0.1 software.

The assessment of agreement between microbiological and serological tests was performed using the kappa index (κ), also obtained using the GraphPad Prism 8.0.1 software. The kappa index (κ) follows the classification described by Landis and Koch (1977): where 0 indicates no agreement; 0.1–0.39 = poor agreement; 0.4–0.59 = moderate agreement; 0.6–0.79 = substantial agreement; and 0.8–1.0 = almost perfect agreement.

RESULTS AND DISCUSSION

Sample Characterization

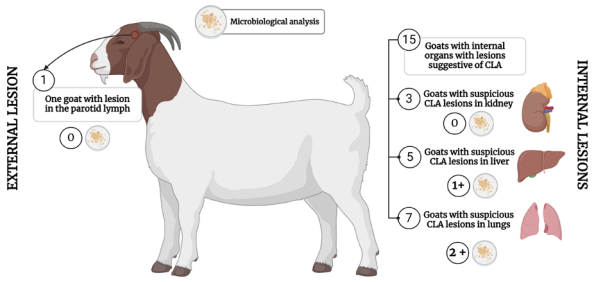
All goats assessed here had no defined racial pattern (SRD), with an average age of 34.6 months, equivalent to 2.8 years. From the 150 goats studied, 54.6% (82/150) of the sampled animals were males, while 45.3% (68/150) were females.

Regarding *ante-mortem* and *post-mortem* examinations, only a six-year-old female weighing 20 kg had an external lesion in the parotid lymph node compatible with CLA, on the other hand, 15 animals presented internal organs with lesions suggestive of CLA. The goat with the highest number of lesions was a six-year-old female weighing 38 kg with granulomas in the liver, kidneys, and lungs. Purulent exudate and the fragments of organs from suspected goats were collected for bacterial isolation.

Microbiological Characterization

Fifteen animals (10% of the sample) presented lesions suggestive of CLA in their organs, and these were located mainly in the lungs, liver, and kidneys. Cultivation in the BHI medium provided bacterial growth in approximately 48 hours. In total, 48 colonies were stained with Gram, with only one Gram-negative, and the remaining Gram-positive. The colonies on a cream-to-orange coloration, dry, opaque, and concentrically ringed, were submitted to biochemical tests (from six animals). After biochemical analysis, only three positive samples for *C. pseudotuberculosis* coming from different animals were identified. The colonies were derived from abscesses located in the liver (for two goats) and in the lung (for one goat), as shown in Figure 1.

Although considered the gold standard in the diagnosis of CLA, the microbiological culture of the etiologic agent is not practical, is time-consuming, and not always possible, as the animal may be asymptomatic, without granulomas (Farias *et al.*, 2018). In addition, bacterial isolation and biochemical characterization are laborious, and the presence of subclinical cases can provide an opportunity for the spread of the disease within and between herds (Yitagesu *et al.*, 2020).



Source: own authorship with Biorender.com.

Figure 1. The number of lesions and location of internal and external lesions compatible with caseous lymphadenitis in slaughtered goats. One goat had an external lesion compatible with caseous lymphadenitis but was not positive for caseous lymphadenitis in the microbiological analysis. Fifteen goats had internal lesions compatible with caseous lymphadenitis, and three of them had a positive result in the microbiological analysis. One of these lesions was in the liver and two in the lungs.

Serological Tests

Indirect ELISA was capable of detecting anti-*C. pseudotuberculosis* antibodies in 11.33% of goats (17/150). Table 1 shows the serological prevalence according to sex. A positive correlation between female goats and seropositivity was observed using Fisher's exact test (p = 0.0002). Similar data were found previously by Yitagesu *et al.* (2020), where females showed a greater risk of contamination than male goats. Additionally, a previous study of slaughtered sheep showed a higher prevalence of CLA in females than in males (Issa *et al.*, 2021).

Despite the different distribution of goats between sex and the higher sample number of males, the occurrence of CLA was significantly higher in females. The odds ratio indicated that females are 11.32 times more affected than males, which can be explained by the longer permanence of females in the herd than males and by the chronic features of CLA (Al-Gaabary; Osman; Oreiby, 2009).

Moreover, most seropositive goats here had an age higher than two years old (Table 2). Serological prevalence was numerically higher in animals aged two years or over, nevertheless, Fisher's Exact Test showed no correlation between seropositivity and age (p = 0.0709).

Silva *et al.* (2020) described, in Pernambuco, that two-year-old goats had a higher CLA prevalence. Farias *et al.* (2018) demonstrated a positive correlation between age and seropositivity in sheep and goats, with older animals being more stricken by CLA. Here, a low number of young animals was seropositive, which may be related to the short time for CLA exposure factors and long incubation period. CLA has an incubation period of between two and four months, before it may be detected clinically (Baird, 2003). Figure 2 shows the distribution of seropositive goats concerning sex and age.

Microbiological prevalence (2%) was lower than serological prevalence (11.33%), indicating the higher sensitivity levels of ELISA. All goats positive in the microbiological culture were also positive in ELISA, however, 14 animals were detected only in the ELISA test, which generated a kappa index (κ = 0.275) with a weak agreement between serological and microbiological tests (CI 95% = 0.026, 0.524). A previous study performed by Nassar *et al.* (2016) corroborates the low sensitivity of the microbiological test. From 202 sheep with lymph node enlargement slaughtered, Nassar *et al.* (2016) identified 38 (34%) as *C. pseudotuberculosis* positive by microbiological culture, and 110 (54%) by PCR, which also generated a weak agreement (κ = 0.193). The authors concluded that molecular diagnosis is more efficient, reproducible, and faster than microbiological culture.

The weak agreement between indirect ELISA and microbiological tests assessed in the present study may be attributed to two main factors. First of all, in CLA, as previously mentioned, granulomas may take up to four months to appear (Baird, 2003), on the other hand, goats infected with *C. pseudotuberculosis* have early detectable IgG levels from days 6 to 11 post-infection (Paule *et al.*, 2003). Therefore, goats detected by indirect ELISA but not by microbiological assays could be in the early stages of infection. Another situation that should be considered is the differential diagnosis. Nassar *et al.* (2016) isolated from lesions similar to CLA granulomas many other agents different from *C. pseudotuberculosis*, such

Table 1. Serological prevalence of slaughtered goats in Mossoró-RN according to sex. Asterisks (*) in the same column indicate statistical significance.

Sex	Seronegative	Seropositive	Total	Serological prevalence
Female	53	15*	68	22.06% *
Male	80	2*	82	2.43% *

Table 2. Serological prevalence of slaughtered goats in Mossoró-RN according to age. Asterisks (*) in the same column indicate statistical significance.

Age	Seronegative	Seropositive	Total	Serological prevalence
< 2 years	64	4	68	5.88%
≥ 2 years	69	13	82	15.85%

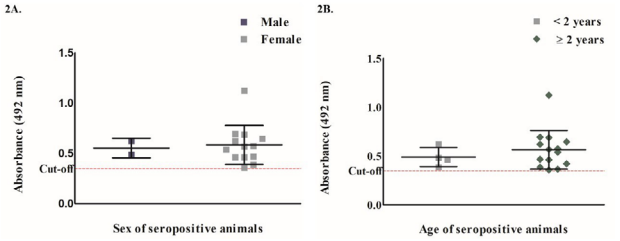


Figure 2. A. Distribution of seropositive goats by sex. B. Distribution of seropositive goats by age. Error bars consider mean and standard deviation.

as *Staphylococcus* sp., *Bacillus* sp., *Enterobacteria*, *Pseudomonas* sp., *Escherichia coli*, *Acinetobacter* sp., *Serratia marcescens*, *Streptococcus* sp., *Citrobacter freundii*, *Klebsiella ozaeneae*, *Actinomyces* sp., *Klebsiella pneumoniae*, *Enterobacter gergoviae*, and non-fermenting Gram-negative bacilli. Additionally, in our study, we did not perform microbiological tests aiming to identify other microorganisms.

Here, the apparent prevalence was 11% (CI 95%: 7–17%) while the real prevalence considering sensitivity and specificity parameters of indirect ELISA was 10% (CI 95%: 6–17%). The predictive positive value was 88%, while the predictive negative value was 99%, indicating that a negative result is more reliable than a positive one (Akobeng, 2007). It is important to highlight that ELISA is unable to distinguish positive results between truly infected animals and previously exposed animals like recovered or vaccinated animals (Pacheco *et al.*, 2007; Williamson, 2001).

A previous study performed in the Brazilian Northeast region found higher prevalence levels (approximately 30%) in the field than we showed here for slaughtered goats (Farias *et al.*, 2018). In a study conducted in a slaughterhouse with the Federal Inspection Service in Paraíba state, Brazil, 15.9% (236/1,466) of slaughtered sheep presented macroscopic lesions similar to CLA. Bacteriological culture was performed and in 74.5% (43/51) of samples, *C. pseudotuberculosis* was isolated (Souza *et al.*, 2011). In another sheep slaughterhouse with the Federal Inspection Service in Minas Gerais state, Brazil, 43.7% of 805 slaughtered sheep were CLA-seropositive (Guimarães *et al.*, 2011).

However, it is important to mention that most sheep and goat slaughters in the Brazilian Northeast are clandestine, occurring without inspection of health standards in inappropriate places (Nogueira Filho; Figueiredo Júnior; Yamamoto, 2010). Therefore, we hypothesized that to avoid carcass condemnation, merchants choose only animals from better sanitary conditions to be slaughtered under legal conditions with the Municipal Meat Inspection Service, while those in poor health condition could be illegally slaughtered. Additional studies in this area might be conducted to assess the impact of clandestine slaughter on food security.

CONCLUSION

Despite the seroprevalence of CLA for goats slaughtered in Mossoró, Rio Grande do Norte (11.33%) being below that previously found in the Brazilian Northeast, clandestine slaughter should be considered in sampling for future studies. Additionally, indirect ELISA proved to be a faster and more sensitive technique than microbiological culture for CLA diagnosis and should be considered especially in the detection of subclinical cases, which represents a challenge to disease control.

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REFERENCES

ABEBE, D.; SISAY TESSEMA, T. Determination of *Corynebacterium pseudotuberculosis* prevalence and antimicrobial susceptibility pattern of isolates from lymph nodes of sheep and goats at an organic export abattoir, Modjo, Ethiopia. **Letters in Applied Microbiology**, v. 61, n. 5, p. 469–476, 2015.

AFTABUZZAMAN, M.; CHO, Y. Recent perspectives on caseous lymphadenitis caused by *Corynebacterium pseudotuberculosis* in goats – A review. **Korean Journal of Veterinary Service**, v. 44, n. 2, p. 61-71, 2021.

AKOBENG, A. K. Understanding diagnostic tests 1: Sensitivity, specificity and predictive values. **Acta Paediatrica**, v. 96, n. 3, p. 338–341, 2007.

AL-GAABARY, M. H.; OSMAN, S. A.; OREIBY, A. F. Caseous lymphadenitis in sheep and goats: Clinical, epidemiological and preventive studies. **Small Ruminant Research**, v. 87, n. 1–3, p. 116–121, 2009.

ALVES, L. L. *et al*. Multiplex PCR for molecular characterization of isolated strains of granuloma in sheep from state of Sergipe and Antimicrobial Susceptibility Test (AST). **Brazilian Journal of Development**, v. 7, n. 5, p. 49131–49147, 2021.

BAIRD, G. Current perspectives on caseous lymphadenitis. **In Practice**, v. 25, n. 2, p. 62–68, 2003.

CARVALHO, J. S. *et al*. Characterization of goat and sheep production in the state of Sergipe, Northeast of Brazil. **Acta Veterinaria Brasilica**, v. 14, n. 2, p. 121–131, 2020.

FARIAS, A. M. *et al*. Soroprevalência da infecção por *Corynebacterium pseudotuberulosis* em caprinos no Nordeste brasileiro utilizando técnica de imunoabsorção enzimática (ELISA-indireto). **Pesquisa Veterinária Brasileira**, v. 38, n. 7, p. 1344–1350, 2018.

GUIMARÃES, A. S. *et al*. High sero-prevalence of caseous lymphadenitis identified in slaughterhouse samples as a consequence of deficiencies in sheep farm management in the state of Minas Gerais, Brazil. **BMC Veterinary Research**, v. 7, n. 1, p. 1-5, 2011.

IBGE. **PPM – Pesquisa da Pecuária Municipal**. 2018. Disponível em: <https:/www.ibge.gov.br/estatisticas/economicas/agricultura-e-pecuaria/9107-producao-da-pecuaria-municipal.html?edicao=25474&t=resultados>. Acesso em: 22 fev. 2023.

ISSA, N. A. *et al*. Prevalence and molecular investigation of caseous lymphadenitis among the slaughtered sheep at Duhok Abattoirs; experimental infection with *Corynebacterium pseudotuberculosis* in rabbits. **Iraqi Journal of Veterinary Sciences**, v. 35, n. 2, p. 263–270, 2021.

LANDIS, J. R; KOCH, G. G. The measurement of observer agreement for categorical data. **Biometrics**, v. 33, n.1, p. 159-174, 1977.

MACFADDIN, J. **Biochemical tests for identification of medical bacteria**. Philadelphia: Williams and Wilkins, v. 113, n. 7, 2000.

NASSAR, A. F. DE C. *et al*. Diagnostic comparison of *Corynebacterium pseudotuberculosis* through microbiological culture and PCR in sheep samples. **Arquivos do Instituto Biológico**, v. 82, p. 01–06, 2016.

NOGUEIRA FILHO, A.; FIGUEIREDO JÚNIOR, C. A.; YAMAMOTO, A. **Mercado de carne, leite e pele de caprinos e ovinos no Nordeste**. Fortaleza: Banco do Nordeste do Brasil, 2010.

OREIBY, A. F. Diagnosis of caseous lymphadenitis in sheep and goat. **Small Ruminant Research**, v. 123, n. 1, p. 160–166, 2015.

OSMAN, A. Y. *et al*. The epidemiology and pathophysiology of caseous lymphadenitis: A review. **Journal of Veterinary Medicine and Research**, v. 5, n. 3, p. 1–7, 2018.

PACHECO, L. G. C. *et al*. Multiplex PCR assay for identification of *Corynebacterium pseudotuberculosis* from pure cultures and for rapid detection of this pathogen in clinical samples. **Journal of Medical Microbiology**, v. 56, n. 4, p. 480–486, 2007.

PAULE, B. J. A. *et al*. Experimental *Corynebacterium pseudotuberculosis* primary infection in goats: Kinetics of IgG and interferon-γ production, IgG avidity and antigen recognition by Western blotting. **Veterinary Immunology and Immunopathology**, v. 96, n. 3–4, p. 129–139, 2003.

REBOUÇAS, M. F. *et al*. Development of an indirect ELISA to detect *Corynebacterium pseudotuberculosis* specific antibodies in sheep employing T1 strain culture supernatant as antigen. **Pesquisa Veterinária Brasileira**, v. 33, n. 11, p. 1296–1302, 2013.

RUIZ, H. *et al*. The relevance of caseous lymphadenitis as a cause of culling in adult sheep. **Animals**, v. 10, n. 11, p. 1962, 2020.

SANTAROSA, B. P. *et al*. Meningoencefalite supurativa por *Corynebacterium pseudotuberculosis* em cabra com linfadenite caseosa: Relato de caso. **Veterinária e Zootecnia**, v. 21, n. 4, p. 537-542, 2014.

SEYFFERT, N. *et al*. High seroprevalence of caseous lymphadenitis in Brazilian goat herds revealed by *Corynebacterium pseudotuberculosis* secreted proteins-based ELISA. **Research in Veterinary Science**, v. 88, n. 1, p. 50–55, 2010.

SILVA, T. I. B. DA *et al*. Clinical-epidemiological study of caseous lymphadenitis in goats in the state of Pernambuco. **Revista de Ciências Agroveterinárias**, v. 19, n. 3, p. 329–336, 2020.

SOUZA, M. DE F. *et al*. Linfadenite caseosa em ovinos deslanados abatidos em um frigorífico da Paraíba. **Pesquisa Veterinaria Brasileira**, v. 31, n. 3, p. 224–230, 2011.

THRUSFIELD, M. **Veterinary epidemiology**. Third edition ed. Oxford: Blackwell Science Ltd., 2007.

TRIPATHI, N.; SAPRA, A. **Gram staining**. Treasure Island: StatPearls Publishing, 2022.

WILLIAMSON, L. Caseous lymphadenitis in small ruminants. **Veterinary Clinics of North America: Food Animal Practice**, v. 17, n. 2, p. 359-371, 2001.

YITAGESU, E. *et al*. Survival analysis of clinical cases of caseous lymphadenitis of goats in North Shoa, Ethiopia. **Veterinary Medicine International**, v. 2020, p. 8, 2020.