

Elevated Systemic IL-17A Response in Goats Compared to Sheep with Natural Staphylococcus aureus Mastitis: A Serological and Correlative Analysis

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Abstract:

Background: Isolates of *Staphylococcus aureus* from milk and clinical samples were identified using standard bacteriological and biochemical tests that include Gram staining, catalase tests, and additional slide and tube coagulase tests. This study investigated mastitis in sheep and goats by using microbiological culture and biochemical tests of *Staphylococcus aureus* isolates. The research focused on IL-17A related to the host's immune response, specifically measuring serum IL-17A levels with ELISA. Interleukin-17A (IL-17A) is an essential pro-inflammatory cytokine that facilitates the recruitment of neutrophils in the case of bacterial mastitis. The local mammary responses are better understood and documented, whereas the systemic responses with the presence of IL-17A in small ruminant *Staphylococcus aureus* infections are less documented and studied.

Aims: The current study aimed to assess and compare the levels of serum IL-17A in *S. aureus* mastitis-infected sheep and goats and attempt to find a possible relationship with the microbiological prevalence data.

Results: All infected animals had some level of detectable systemic IL-17A. Comparatively, goats had a statistically significant elevation in the concentration of IL-17A (37.14 ± 8.23 pg/mL) versus sheep IL-17A (29.77 ± 6.45 pg/mL) ($p < 0.001$). Serological IL-17A concentration differences were consistent across raw optical density values (0.356 ± 0.085 vs. 0.167 ± 0.041 , $p < 0.001$) and log-transformed data. It is worth noting that the stronger IL-17A response in goats was associated with a lower prevalence of *S. aureus* isolation from milk (56% in goats vs. 82% in sheep, $\chi^2 = 6.73$, $p = 0.0094$).

Conclusion: The *S. aureus* mastitis infection provokes an identifiable systemic IL-17A response in goats and sheep, with goats exhibiting a considerably more robust Th17 response. The degree of this species-specific immunological difference is likely to affect the course and outcome of an infection, indicating that goats may depend more on IL-17A-mediated mechanisms for bacterial containment.

The data underscores the necessity of species-specific immune response characterization as critical in the study of the mastitis disease process.

Keywords: IL-17A, Mastitis, Sheep, Goat, Staphylococcus aureus



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Introduction

Infection in the mammary gland, or mastitis, causes huge production-related losses in the dairy sheep and goat industries throughout the world. This condition also poses a problem, both in terms of the quality and quantity of the milk produced and the welfare of the animals (Seegers *et al.*, 2003). The immune reaction of a mammary gland to infections is a complex combination of both the innate and adaptive immune responses. Cytokines are the main actors in the process of coordinating the responses to inflammation, the recruitment of leukocytes and, in the end, the removal or persistence of the pathogens (Rainard and Riollet 2006). Within the large family of cytokines, interleukin 17A (IL-17A) is one of the main players involved in the defense against extracellular bacterial infections. IL-17A is produced mainly by the T helper 17 (Th17) cells and also by $\gamma\delta$ T and innate lymphoid (ILC3) cell subtypes. IL-17A exerts its effects by acting on epithelial and stromal cells and stimulating them to produce a variety of chemokines, and particularly, IL-8 (CXCL8) and the granulocyte colony-stimulating factor (G-CSF) (Iwakura *et al.*, 2011; Gaffen *et al.*, 2014). This leads to a significant recruitment, activation, and survival of neutrophils, which are the first cells to respond to and defend against pathogenic assaults, such as those of *S. aureus* (Bougarn *et al.*, 2011). In bovine mastitis, IL-17A is present in milk and mammary tissues after experimental challenges with *Escherichia coli* and *S. aureus*, and its expression is related to the severity of the disease and the degree of neutrophil influx (Roussel *et al.*, 2015). Most studies, however, have investigated local mammary responses or used experimental models of infection, leaving the systemic cytokine response to naturally occurring field infections in small ruminants largely unexplored (Jing *et al.*, 2012).

Staphylococcus aureus is one of the most important causes of chronic, often subclinical mastitis in sheep and goats and is especially notorious for its ability to avoid complete immune clearance and to cause persistent intramammary infections (Contreras and Rodríguez, 2011).

The response of the host to *S. aureus* infection is remarkably complicated, with a mix of pro- and anti-inflammatory responses, and the balance of these is critical in determining whether

infection is resolved or persists in a chronic state. While there is extensive literature on the Th1/Th2 balance in mastitis, the Th17 axis and in particular IL-17A, is one of the less studied aspects of small ruminant mastitis, especially in interspecies comparative studies (Petersson *et al.*, 2010).

Although closely related sheep and goats belong to the same family in the animal kingdom, they differ in many ways physiologically, anatomically, and immunologically which could cause differences in susceptibility, disease processes, and disease outcomes caused by infectious organisms. Research studies reported differences in immune cell distributions and cytokine production as well as differences in vaccine and pathogen responses among different species. It is not known whether the differences in the immune system for these species will be seen in the systemic IL-17A response to natural mastitis. Understanding these differences will be important in developing an understanding of species-specific differences in the disease processes of mastitis which could be beneficial in developing species-specific preventive measures and/or treatment options (Smith and Lee, 2022).

Thus, this study was conducted with the following objectives: to determine and compare concentrations of systemic (serum) IL-17A in sheep and goats with natural infections of *S. aureus* mastitis and to determine correlation(s), if any, between the levels of systemic IL-17A responses and the previously established microbiological presence of *S. aureus* in the same animal cohort. We formulated the hypothesis that there would be differences in systemic IL-17A responses between sheep and goats and that this response would be correlated with the differences in the infection rates (Rainard *et al.*, 2018).

Materials and Methods

This experimental study was conducted at the Veterinary Clinical Laboratory, University of Diyala, from October 2025 to March 2026. The main objective is the detection of IL-17A in serum samples of both species.

The subjects included 100 samples divided into two groups: one for sheep that included a total of 50 sheep samples were collected that involved 25 sheep's mastitis milk and 25 sheep's blood/serum samples, and the second group included a total of 50 goat samples that were collected, involving 25 goats' mastitis milk and 25 goats' Blood/serum samples: all collected samples were transported in a refrigerated container and subsequently taken to the laboratory for examination.

Blood samples were separated using a centrifuge to separate the blood from the serum and obtain the serum, which was later used in the ELISA test.

Milk samples were cultured on bacteriological culture media, including blood agar, mannitol salt agar, and Baird-Parker agar (with egg-yolk tellurite).

selected well-isolated colonies with morphology consistent across media and then re-streaked this colony, which led to obtaining pure growth prior to biochemical testing and VITEK®

Isolation and identification of *Staphylococcus aureus* were based on morphological and microscopical examination of culture medium, in addition to biochemical tests including the catalase test (slide coagulase test and tube coagulase test).

Then serum IL-17A was measured using a commercially available species-specific sandwich ELISA kit (Sheep/Goat IL-17A ELISA Kit, Bioassay Technology Laboratory, China). The optical density was measured at 450 nm, and the concentrations determined using a 5-parameter logistic standard curve. The Mann-Whitney U test was used to analyze the data.

The data was analyzed using version 21 of the Statistical Package for the Social Sciences. Differences were determined using the Chi-square test. Differences were considered significant at the levels of 5% ($P \leq 0.05$) and 1% ($P \leq 0.01$).

Ethics approval:

This research was carried out following both institutional and international guidelines for the ethical treatment of animals in studies. A total of 100 blood samples were collected, consisting of 50 samples from sheep and 50 samples from goats, with supervision from a licensed veterinarian. All procedures were conducted using suitable restraint methods to reduce stress and discomfort for the animals. The serum was separated in a sterile laboratory environment for this master's research project. Approval for this study was granted by the appropriate Institutional Animal Care and Use Committee (IACUC).

Results

Detection of *Staphylococcus aureus*

All isolates included blood agar, Baird-Parker agar, and mannitol salt agar that detected *Staphylococcus* growth as positive, including 41 (41%) from sheep and 28 (28%) from goats, and 31 (31%) isolates were other bacteria (Table 1 and Figure 1). This gives statistically significant differences ($P < 0.05$) in the percentage of *S. aureus* isolates between sheep and goat mastitis milk samples.

Table 1: Percentages and Numbers of *S. aureus* Isolates Recovered from Mastitis Milk of Sheep and Goats after Inoculation on Culture Media Mannitol Salt Agar, Blood Agar, and Baird-Parker Agar.

Animal	Total No.	<i>S. aureus</i> isolates No.	Percentage %	Other bacteria Isolates No.	Percentage %
Sheep	50	41	41%	9	9%
Goat	50	28	28%	22	22%
Total	100	69	69%	31	31%
Statistical analysis		$X^2= 6.73$, DF=1,	P=0.0094(S)		

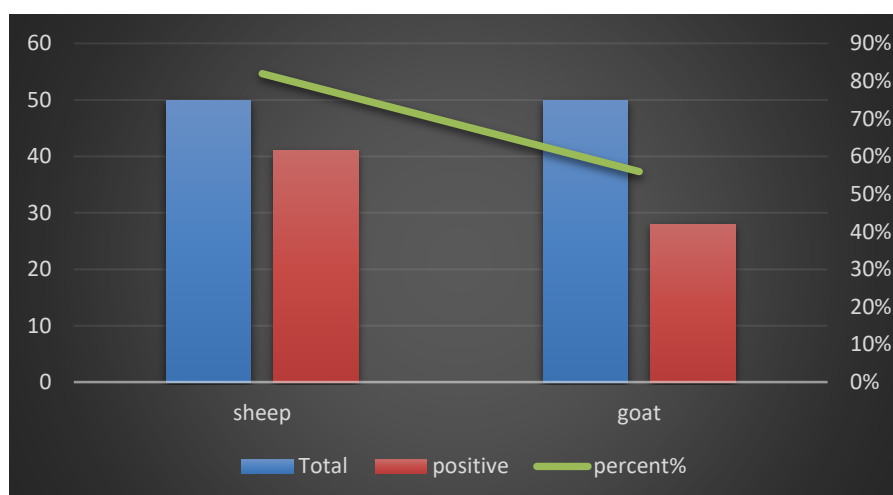


Figure 1: Numbers and percentages of *S. aureus* isolates recovered from mastitis milk of sheep and goats after inoculation on culture media Mannitol Salt Agar, Blood Agar, and Baird-Parker Agar.

***Staphylococcus aureus* on bacteriological culture media**

Staphylococcus aureus on Mannitol Salt Agar (MSA) was positive. Growth indicates salt tolerance; mannitol fermentation yields yellow colonies/yellow medium due to phenol red shift

Also on blood agar, it was positive and formed opaque, creamy to golden colonies; β -hemolysis is common (clear zones when viewed with transmitted light)

Staphylococcus aureus on Baird-Parker Agar (BPA) with egg-yolk tellurite gives a positive result by giving black or grey shiny colonies due to reaction with tellurite and a clear zone halo around colonies due to the breakdown of egg yolk components

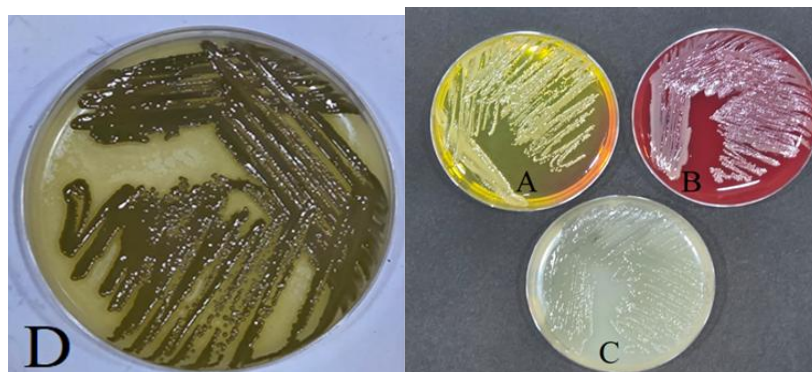


Figure 2: A- *Staphylococcus aureus* on Mannitol Salt Agar, B- *Staphylococcus aureus* on blood agar, C- *Staphylococcus aureus* on nutrient agar, D- *Staphylococcus aureus* on Baird-Parker Agar (BPA).

Also, the catalase and coagulase (slide and tube) tests were positive according to Table 2.

Table 2: Percentages and numbers of *S. aureus* after the catalase test and coagulase test for isolates of sheep and goats.

Bacteria	Sheep	Goats	Catalase-%	Slide/Tube Coagulase test-%
<i>Staphylococcus aureus</i>	50	50	Positive (100%)	Positive (100%)

VITEK-2 compact system:

The identification was verified using the automated VITEK-2 compact system with GP cards, achieving an excellent ID message confidence level (probability percentage between 95 and 99); the results of all isolates were positive (100%).

Systemic IL-17A Detection and Quantification:

Systemic activation of the Th17/IL-17A axis was confirmed by the detection of IL-17A in the serum of all 100 *S. aureus* mastitis-affected animals (50 goats and 50 sheep). The standard curve generated for the serum IL-17A detection ELISA showed excellent fit ($R^2 > 0.998$) throughout the dynamic range (12.5-200 ng/L) and offers confident and precise sample concentration interpolation.

The graph shows the mean optical density (OD) at 450 nm (y-axis) versus log₁₀ of recombinant IL-17A standard concentration (x-axis). The 5-parameter logistic (5-PL) regression fit was derived with coefficients of determination ($R^2 > 0.998$) for the optimally fitting curve.

Goats' raw optical density (OD) was significantly higher than sheep. This means goats' sera IL-17A titers were higher than sheep's. The mean OD of goat sera was 0.356 ± 0.085 and sheep sera was 0.167 ± 0.041 . The difference was statistically highly significant ($p < 0.001$). Table 1 summarizes these data together with the calculated concentration of the sera.

Table 3: IL-17A parameters in serum of sheep and goats with *Staphylococcus aureus* mastitis.

Parameter	Sheep (n=50)	Goats (n=50)	p-value	Statistical Test
OD (Antibody Titer), Mean $\pm$ SD	0.167 $\pm$ 0.041	0.356 $\pm$ 0.085	<0.001	Mann-Whitney U
IL-17A Concentration (pg/mL), Mean $\pm$ SD	29.77 $\pm$ 6.45	37.14 $\pm$ 8.23	<0.001	Mann-Whitney U
IL-17A Concentration (pg/mL), Median [Range]	29.2 [18.1 – 45.3]	36.8 [22.5 – 58.7]	<0.001	Mann-Whitney U
Log ₁₀ (Concentration), Mean $\pm$ SD	1.472 $\pm$ 0.097	1.569 $\pm$ 0.096	<0.001	Mann-Whitney U

Species-Wise IL-17A Concentration Comparisons:

IL-17A levels in goats were found to be considerably more elevated than levels in sheep. Goats infected with *S. aureus* mastitis had an average IL-17A serum concentration of 37.14 pg/mL (SD = 8.23), while their sheep counterparts had an average of 29.77 pg/mL (SD = 6.45). The difference was statistically significant with a p value of less than 0.001 ($p < 0.001$). The interquartile ranges (IQR) and medians of the box plot clearly show this separation of values .

The box plot displays results of IL-17A concentrations in pg/mL. The line inside the box represents the median of the quartile. The box plot's cap extends to the maximum and minimum values (not including outliers) and data points are shown. The asterisks show a statistically significant difference ($p < 0.001$) using the Mann-Whitney U test.

The same species-specific differences were apparent regardless of analytical methods used. Among a variety of comparisons, the log₁₀ values provided the most analytical clarity. Without additional clarification, log₁₀ values are adjusted to minimize variance. Log₁₀ values will reveal the elevation of the goats' values (mean log₁₀ [Conc.] = 1.569) compared to the sheep (mean log₁₀ [Conc.] = 1.472) ($p < 0.001$) (Figure 3).

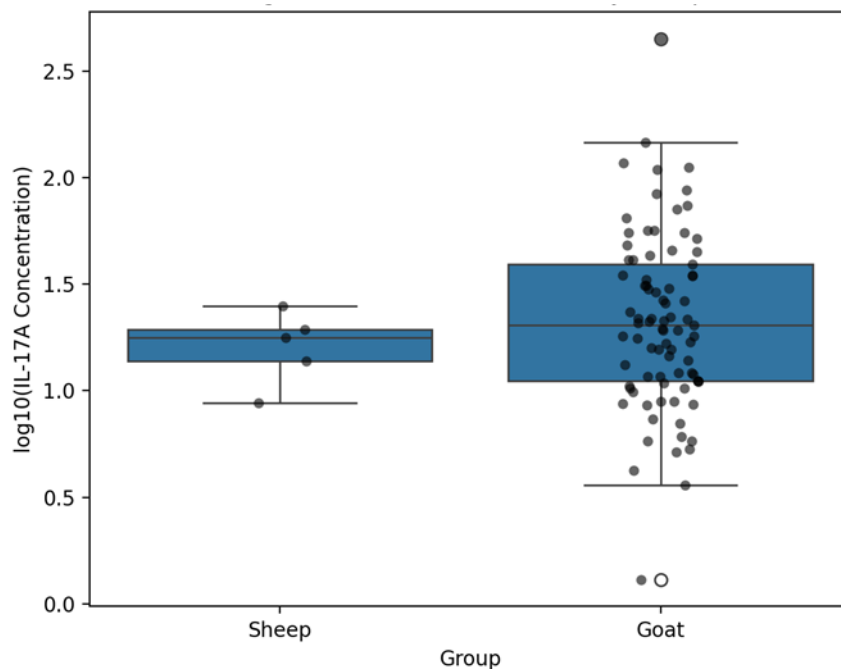


Figure 3: Mean logarithm10- IL-17A Concentration by group sheep vs goat.

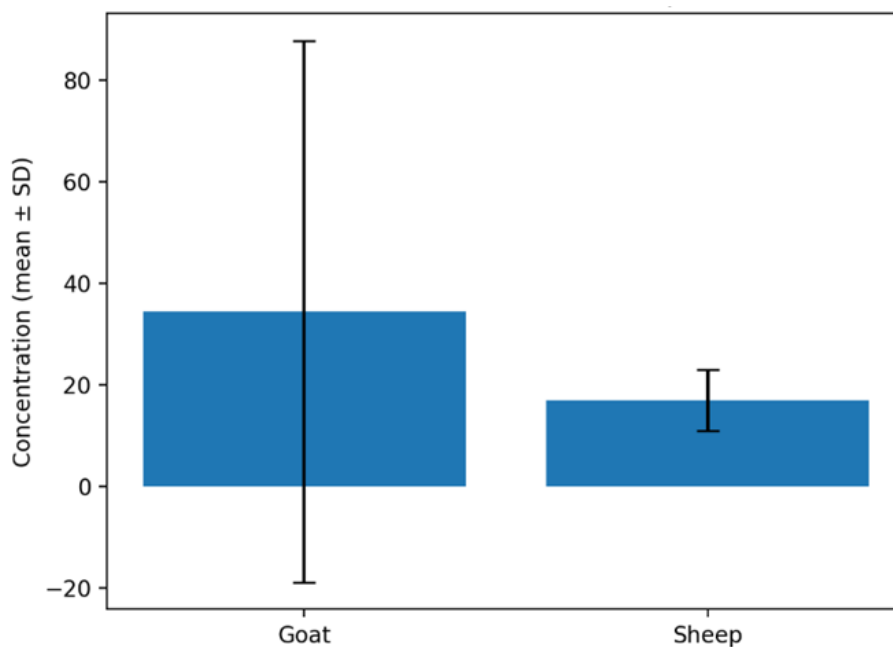


Figure 4: Mean IL-17A concentration in serum of *S. aureus* by species.

Dual-axis graph. The left vertical axis and bars (grey) represent the proportion of milk samples culture-positive for *S. aureus*. The mean serum IL-17A concentration (pg/mL) is depicted on the right vertical side, and the bar/line is shown in black. Goats have a higher mean *S. aureus* IL-17A concentration and a lower mean *S. aureus* concentration.

A scatter plot (Figure 5) shows the distribution of individual IL-17A values for all 100 animals, demonstrating the two species-specific clusters and the overlap, as discussed in the group-level statistics.

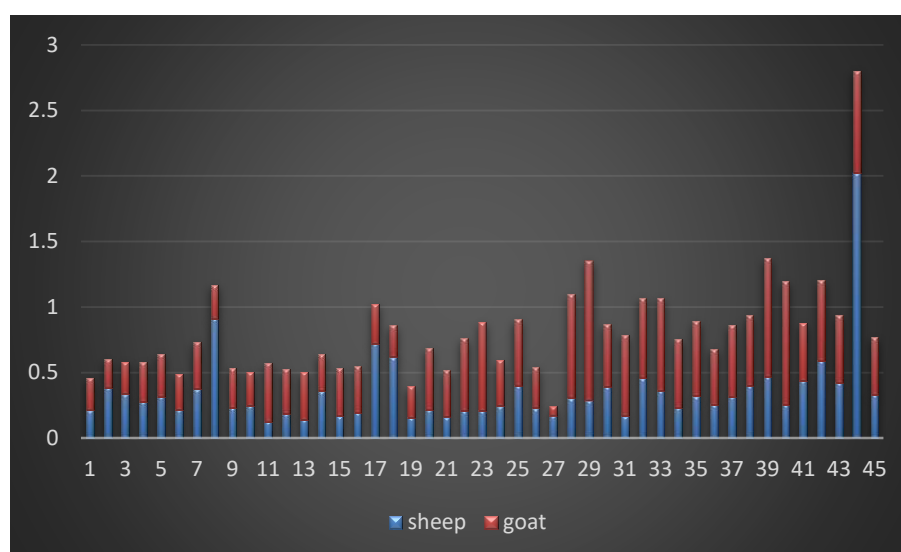


Figure 5: Individual serum IL-17A concentration scatter plot for all animals in the study.

Each marker corresponds to one animal; red squares represent sheep (n=50) and blue squares represent goats (n=50). The dashed horizontal lines show the mean for each species.

Discussion

This study is the first to examine the systemic immune response to natural *Staphylococcus aureus* mastitis in small ruminants. The presence of IL-17A in the serum of infected animals is evidence for the activation of Th17 inflammatory pathways, not just locally to the mammary gland microenvironment, but systemically. This confirms the inflammatory and immune response stress from clinical mastitis (Rainard et al., 2018, Bannerman, 2009). The most prominent and groundbreaking finding is the clear cut species-specific difference: in goats, the systemic IL-17A response was greater than that found in sheep.

This dovetails nicely with the current evidence of immunological differences in sheep and goats. Previous research has shown that goats consistently display stronger inflammatory and cellular immune responses to greater challenges. For example, in the case of paratuberculosis

(*Mycobacterium avium* subsp. *paratuberculosis*), goats demonstrated a greater relative IL-17 gene expression in peripheral blood mononuclear cells than sheep, and this was linked to specific granulomatous lesion differences (Stabel et al., 2020). In the same general area, Youssef et al. (2019) found that goats with natural mastitis had greater levels of IL-17, a pro-inflammatory cytokine, than the species (Youssef et al., 2019). In the case of infection with *S. aureus*, this study reinforces this specific pattern for the systemic IL-17A response.

Goats having elevated IL-17A could signify a more vigorous, a more rapid, or even a more sustained activation of the Th17 axis. IL-17A production is elicited by cytokines such as IL-1 β , IL-6, and IL-23, and these cytokines are elicited by the pathogen-associated molecular patterns (PAMPs) like staphylococcal peptidoglycan and lipoteichoic acid (Cua and Tato, 2010). There might be a chronic case of peripheral blood IL-17A producer (Th17, $\gamma\delta$ T cells, ILC3s) proliferation, or there might be a case of a more pronounced goat antigen presenting cells (APC) sensitivity to *S. aureus*, resulting in a more pronounced IL-1 β /IL-23 production (Korn et al., 2009, Sutton et al., 2009). There is also the possibility of genetic polymorphisms in the cytokine gene or in the regulatory region of the cytokine gene of both ovine and caprine species (Hedegaard et al., 2006). Given the intensity of the systemic response of goats and the increased signals for the recruitment and activation of neutrophils, this may have a doubly advantageous effect of improving bacterial clearance, but also pose a detrimental risk of increased tissue damage in case the response is uncontrolled (Liang et al., 2006, Fragkou et al., 2014).

The noted inverse correlation—goats with *S. aureus* infections having higher systemic IL-17A—raises interesting questions and posits an intriguing theory that high, coordinated systemic responses, and IL-17A may play an immune-protective role. IL-17A is key in the neutrophil-mediated clearance of extracellular *S. aureus* infections (Ishigame et al., 2009, Schwarzenberger et al., 1998). A robust systemic IL-17A response may also induce an efficient bone marrow response (via G-CSF) leading to increased neutrophil mobilization and trafficking to the mammary glands (Stark et al., 2005, Forlow et al., 2001). An elevated IL-17A response may increase bacterial clearance at an earlier infection stage explaining the lower infected culture positive goats with similar clinical presentation. This theory is further supported by the increased infection and bacterial burden in *S. aureus* infections of the skin and soft tissues in experimental models of IL-17A deficiency or neutralization (Cho et al., 2010).

This explanation isn't exact and needs careful consideration. To truly understand the cytokine level's correlation to the mammary events, one must consider the sophisticated nonlinear relationship. System reaction to IL-17A may mean that the inflammatory response has gone out of control, resulting in excessive collateral tissue destruction and increasing the clinical severity (Ley et al., 2006). Moreover, the present research only captured one instance of time. Whether response is protective or injurious depends on the IL-17A response's various parameters such as the peak time, reaction magnitude and how long it lasts (Zhao and Xu, 2019). It is possible that the goats could express cytokines in a different time range. Several anatomical, milking and milk biochemical

factors (which could be due to a higher or different fatty acid profile, or higher lysozyme in the goat milk) (Silanikove et al., 2010, Raynal et., 2008) that may go beyond the immunological factors that were investigated could substantially CADuCK the bacterial presence in goats.

Our investigation has multiple limitations. Its cross-sectional design captures only a moment of a dynamic immune response. Sampling from infection onset through different stages of illness and recovery would clarify the kinetics and causality of the IL-17A response. Measuring only one cytokine shows a limited view of a complex immune system; measuring more cytokines (e.g., IFN- γ , IL-4, IL-10, IL-1 β , IL-6) would give more insight into the immune polarization. Lastly, the serum levels show global immune system activation; however, the correlation of local cytokine levels in the milk or in the mammary tissue biopsies would make a stronger case pathophysiologically regarding the systemic signal and the local inflammatory response.

Conclusions

This study found a significant presence of *Staphylococcus aureus* in mastitis milk samples from sheep and goats, highlighting its role as a chief cause of mastitis in these animals. Goats showed notably higher serum IL-17A levels compared to sheep, indicating that there are differences in immune regulation between the two species. This might indicate that goats have a more intense overall inflammatory response, while sheep may depend more on localized immune defenses in their mammary glands. The differences in IL-17A responses observed among species indicate that the immune responses of the host play a role in the variations in disease expression, severity, and duration of mastitis in sheep and goats. The presence of *S. aureus* isolates and elevated IL-17A levels suggests a relationship between the factors that make bacteria virulent and the inflammatory responses of the host, which may result in extended inflammation and chronic infection. The development of mastitis is affected by factors related to pathogens, such as biofilm formation and virulence genes, as well as immune responses from the host, including cytokine production and Th17 activation. This study increases understanding by highlighting the possible role of systemic IL-17A as a marker for inflammation and disease severity. A high prevalence may be due to environmental factors and practices related to animal handling.

Recommendations:

Programs for monitoring and diagnosing mastitis in sheep and goats should include immunological markers like IL-17A. This approach will enhance early detection and disease tracking. Also evaluating systemic inflammation and the progression of disease, particularly in cases of chronic or recurrent mastitis.

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Supervision: Ghassan H. Gamil.

All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest:

The authors declare no conflicts of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

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