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Exploring Matrix Metalloproteinases as Biomarkers in Male and Female Tellicherry Goats using Gelatin Zymography

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ABSTRACT: The presence of Matrix Metallo Proteinases (MMPs) in the serum of the Tamil Nadu goat breed Tellichery was determined by a relative investigation. These experimental goats were split into two groups based on their sex and age. Each group had 12 animals: Group I had 12 male goats and Group II had 12 female goats. Before concentrate feeding, blood samples were taken in the morning. Gelatin zymography was applied to these serum samples. In both sexes showed major bands at 72 kDa of MMP-2 and at 220, 135 and 92 kDa of MMP-9. All latent forms of MMP-9 (220, 135, and 92 kDa) were detected as weak bands in female serum samples (Lanes 1, 6, 7). Since the MMP-2 (72 kDa) band was so noticeable, it was clear that MMP-2 activity was far greater than MMP-9 activity. In female serum samples, MMP-2 was roughly two to three times more intense than MMP-9. The 92 kDa and 135 kDa forms of MMP-9 were more noticeable in male serum samples (Lanes 2, 4, 5), while the 220 kDa form was less noticeable. Males exhibited higher levels of MMP-2 activity than females, which may indicate higher levels of gelatinase expression overall. Male samples had active forms of MMP-9 (210, 125, and 82 kDa), while female samples only contained latent forms. According to the study's findings, both sexes exhibited MMPs. In a similar vein, male goats expressed more MMP-2 than female goats, but female goats expressed more MMP-9. Because MMPs are involved in the buildup of extracellular matrix and are linked to the concentric remodeling of tissues, the results showed that sex influences the expression of MMP profiles in caprine species.

Keywords: Tellichery goats, MMP-2, MMP-9, Gelatin Zymography.

INTRODUCTION

Goats are highly valued for their diverse utility, as they provide meat, milk, skin, and manure, contributing significantly to the rural economy. Due to their economic importance, goats are often referred to as the "poor man's cow." According to the 20th livestock census, the goat population in the country reached 148.88 million in 2019, reflecting a 10.14% increase compared to the 19th livestock census (Livestock Census, 2019). Matrix Metalloproteinases (MMPs) are zinc-dependent enzymes that operate within the extracellular environment and have the ability to degrade extracellular matrix (ECM) proteins (Nagase *et al*., 2006). These enzymes play a crucial role in various physiological and pathological functions, including cell proliferation, migration, angiogenesis, differentiation, apoptosis, and immune responses. The MMP system has been strongly linked to reproductive processes such as folliculogenesis, pregnancy, and parturition in both humans and various animal species (Bai *et al*., 2005). While extensive research has been conducted on MMPs in domestic animals like cattle, buffaloes, and sheep, studies focusing on goat breeds remain limited.

The Tellicherry goat is particularly valued for its high fertility rate, early maturity, high prolificacy, strong maternal instincts, and adaptability to diverse management systems and environmental conditions, making it an important breed for reproduction. Therefore, this study aimed to investigate the presence of Matrix Metalloproteinase activity in Tellicherry goat breeds.

MATERIAL AND METHODS

This study was conducted at the Department of Veterinary Physiology and Biochemistry, TANUVAS—Veterinary College and Research Institute, Salem, Tamil Nadu, India.

A. Collection of evaluation of serum

A total of twenty four healthy Tellicherry goats were chosen for the study, consisting of equal numbers of males from Group I (n=12) and females from Group II (n=12), all with comparable body weight and age. Blood samples were taken from all six animals early in the morning prior to feeding, utilizing vacutainers. The gathered samples were promptly taken to the lab for assessing protein content using the standard Lowry's technique (Lowry *et al*., 1951). The blood samples were centrifuged at 3000 rpm for 15 minutes to isolate the serum, which was then examined for protein levels with a spectrophotometer. A reference standard curve was created utilizing varying concentrations of bovine serum albumin (BSA). The serum samples were kept at -20°C for additional analysis.

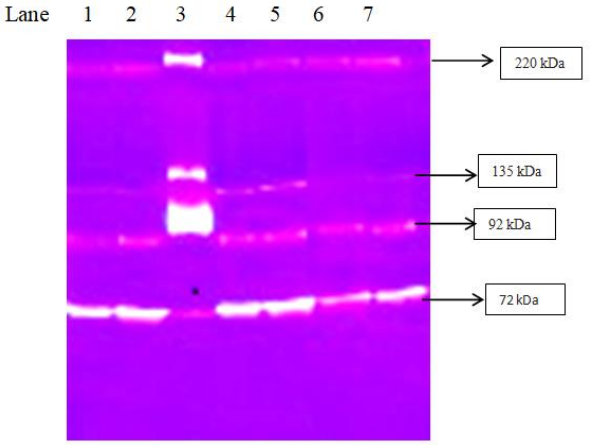
B. Gelatin zymography

A revised SDS-PAGE technique, grounded in Laemmli's method (Laemmli, 1970) and subsequently adjusted by Heussen and Dowdle (1980), was utilized to analyze serum samples. Additional procedures were conducted following the method outlined by our laboratory (Prakash Krupakaran *et al.,* 2016). The gelatin zymogram was standardized with human capillary blood gelatinases, following the technique of Makowski and Ramsby (1996). The reference marker for assessing the zymogram bands was gelatinase from human capillary blood, in accordance with the procedure outlined by Makowski and Ramsby (1996), and additional processing was executed as described by Prakash Krupakaran *et al*. (2016).

RESULTS AND DISCUSSION

A. Expression of Gelatinases (MMP-2 and MMP-9) in Serum Samples

The gelatin zymogram for Tellicherry goats is shown in Fig. 1. All serum samples underwent gelatin zymography. Each serum sample displayed proteolytic activity, as indicated by the complete degradation of gelatin. Major bands were identified at 220, 135, and 92 kDa corresponding to MMP-9, and at 72 kDa for MMP-2 across all groups. In the Tellicherry breed, notable bands were detected at 92 kDa and 72 kDa, representing the latent forms of MMP-9 and MMP-2, respectively. Additionally, in each group, two lytic bands were found at 220 and 135 kDa, denoting the proforms of MMP-9. However, in all serum samples from the Tellicherry breed, the active forms of MMP-9 and MMP-2 were absent. MMP-2 (72 kDa) was notably more prominent in all species compared to the human marker (lane 5). Our findings align with those reported by Bannikov *et al.* (2011); Newby *et al.* (2014) for bovine species, as well as Prakash Krupakaran *et al.* (2015) for buffaloes and again in 2016 for Jersey crossbred. Consistent with these findings, Prakash Krupakaran *et al.* (2016) noted that in Jersey bull serum, the latent form of MMP-2 (72 kDa) was more prominent than that of the MMP-9 monomer (92 kDa) in cattle, across various sex and age groups.



Lane 1, 6 and 7 – Female Tellicherry breed goats serum samples

Lane 2, 4 and 5 – Male Tellicherry breed goats serum samples

Lane 3 - Human capillary blood gelatinases as marker

Fig. 1. Gelatin zymography of Matrix MetalloProteinases in serum samples of male and female Tellicherry breed goats (13 microliters of sample Loaded in each well).

B. Expression of MMP-2 in Tellichery Goats

In male groups (G I: lane 2, 4, 5), major bands observed at 220, 135, 92 of MMP-9 and 72 kDa MMP-2. In male goats, the intensity of the latent MMP-2 form (72 kDa) was 2-3 times raised as compared to the female groups (G II: lane 1, 6, 7). Similarly, in G II (lanes 1, 6, 7), latent form of MMP-2 was observed at 72 kDa. The intensity of MMP-2 in female was higher than MMP-9 but lower than male groups. Similar results were observed by Prakash Krupakaran *et al*. (2015); Balamurugan *et al*. (2024). The intensity of the latent MMP-2 form (72 kDa) in this study was consistent with findings from regular cyclic buffaloes, as reported by Prakash Krupakaran *et al*. (2015). Overall, a comparison of MMP-2 and MMP-9 revealed that MMP-9 was predominantly present in its active form, whereas MMP-2 was primarily found in its latent form observed in the recent report of Balamurugan *et al.* (2024). MMP-2 activity was more intense in males compared to females, suggesting a greater overall expression of gelatinases.

C. Expression of MMP-9 in Tellichery Goats

In female Serum Samples (Lanes 1, 6, 7), all latent forms of MMP-9 (220, 135, and 92 kDa) were present as faint bands. The MMP-2 (72 kDa) band was highly prominent, indicating that MMP-2 activity was significantly higher than MMP-9 activity. The intensity of MMP-2 in female serum samples was approximately 2–3 times stronger than that of MMP-9. Variations in band formation could be attributed to several factors, including the animal's health, reproductive status, fertility, productivity, and nutritional intake. Among the

female lanes, Lanes 6 and 7 showed more intense bands compared to Lane 1. In females, MMP-2 and MMP-9 play a key role in follicular development, ovulation, and corpus luteum remodeling. The predominance of MMP-2 in female samples suggests its active involvement in ovarian tissue remodeling, extracellular matrix degradation, and folliculogenesis. The presence of only latent MMP-9 in female serum samples indicates that its activation may be highly regulated during different reproductive phases. Further, estrogen and progesterone in females regulate MMP-2 and MMP-9 expression, influencing reproductive cycles and pregnancy maintenance.

In Male Serum Samples (Lanes 2, 4, 5): The 135 kDa and 92 kDa forms of MMP-9 appeared prominently, whereas the 220 kDa form was less distinct. MMP-2 activity was more intense in males compared to females, suggesting a greater overall expression of gelatinases. Active forms of MMP-9 (210, 125, and 82 kDa) were detected in male samples, whereas only latent forms were observed in female samples. Our results were in agreement with the results of earlier reports from the same laboratory. The presence of gelatinases MMP-9 and MMP-2 in the serum of various domestic animals has been previously documented by several researchers (Balamurugan *et al*., 2017; Balamurugan *et al*., 2020; Prakash Krupakaran *et al*., 2015; Prakash Krupakaran *et al*., 2016). In Males, the stronger expression of active MMP-9 in male serum samples suggests its involvement in testicular remodeling, spermatogenesis, and sperm motility regulation. Active MMP-9 has been linked to extracellular matrix degradation in testicular tissue, which facilitates sperm release and maturation. Further, testosterone in males stimulates extracellular matrix remodeling, contributing to higher MMP-9 activity. Apart from that, differences in gelatinase expression may also be influenced by nutritional status, metabolic activity, and overall productivity of the animals. Higher MMP-2 levels in females may indicate greater metabolic turnover in reproductive tissues, whereas higher MMP-9 activity in males suggests its role in muscle and connective tissue remodeling.

B. Impact of sex on gelatinase activity expression

To examine sex-based differences in gelatinase activity, gelatin zymography was performed on samples from both male and female subjects. Results revealed that the intensity of the inactive form of matrix metalloproteinase-2 (MMP-2, 72 kDa) was approximately 1.5 times greater in males than in females. In contrast, the inactive form of MMP-9 was more intense in females compared to males. Additionally, males showed two prominent bands at 220 kDa and 135 kDa—representing proenzymatic forms of MMP-9—while these bands were even thicker in females, indicating a stronger expression of pro-MMP-9. These findings are in agreement with previous research conducted by Bonnema *et al*. (2007); Belo *et al*. (2009); Kusnierova *et al*. (2015); Cancemi *et al*. (2020), which consistently demonstrated that MMP-2 levels are higher in males and MMP-9 levels are more pronounced in females. Cancemi *et al*. (2020) further observed in human studies that pro-MMP-2 activity significantly increases with age in men, but not in women, suggesting that sex hormones may regulate MMP expression. This idea is supported by Berg *et al*. (2014), who highlighted the hormonal influence on MMP levels.

Kusnierova *et al*. (2015) also observed that plasma MMP-2 levels positively correlate with age, especially in individuals over 49 years. They found that MMP-3 levels were influenced by both age and sex, with reduced levels in younger individuals and especially in females under 47. Although plasma MMP-9 levels were unaffected by age, they were found to be sex-dependent, being lower in females. Their overall conclusion was that while MMP-2 and MMP-3 levels are age-dependent, MMP-3 and MMP-9 are primarily influenced by sex. In contrast to these results, Belo *et al*. (2009) studied obese versus healthy children and found no significant sex-based differences in levels of MMP-2, MMP-8, MMP-9, pro-MMP-9, TIMP-1, or TIMP-2. Specifically, pro-MMP-9 activity was 0.99 in girls compared to 0.76 in boys, and MMP-2 values were 1.59 in both groups.

However, supporting our current observations, Sathyamoorthy *et al*. (2015) investigated MMP levels in tuberculosis-infected patients and reported higher MMP-1, MMP-3, MMP-8, and MMP-9 in males than in females. Notably, MMP-8 levels in plasma were 1.51 times greater in males, and this was not linked to disease severity. This aligns with the broader view that males often mount stronger inflammatory responses than females, as discussed by Guerra-Silveira & Abad-Franch (2013). Further evidence suggests that estrogen may play a protective role in females. This might be due to female sex hormones help regulate immune responses. Smith *et al*. (2007) reported that female neutrophils exhibit reduced MMP-9 expression during menstrual phases with high estrogen levels. These hormonal effects may explain why females tend to show higher MMP-9 levels in some contexts, despite their regulatory influence. Overall, these findings suggest that gelatinase expression patterns—particularly for MMP-2 and MMP-9—are influenced by both sex and age. Hormonal and physiological conditions, unique to each sex and life stage, likely contribute to these observed differences in enzyme expression.

CONCLUSIONS

The gelatin zymography results confirm that sex significantly influences the expression of gelatinases (MMP-2 and MMP-9) in Tellicherry goats. Females predominantly exhibit latent MMP-9 with stronger MMP-2 activity, likely associated with reproductive tissue remodeling. In contrast, males show higher active MMP-9 expression, suggesting its involvement in testicular function and extracellular matrix remodeling. These findings highlight the importance of gelatinase activity in reproductive physiology and provide insights

into potential biomarkers for fertility and reproductive health in goats.

FUTURE SCOPE

The role of MMP2 and MMP9 levels can serve as biomarkers for infectious and inflammatory diseases in Tellicherry goats, such as mastitis, pneumonia, and arthritis. These MMPs play a role in ovarian follicle development, corpus luteum function, and uterine remodeling. Investigating MMP expression patterns during different reproductive stages can improve breeding efficiency and fertility management. MMP2 and MMP9 are involved in extracellular matrix degradation and muscle remodeling. Studying their activity could help in improving meat tenderness and quality, providing valuable insights for the meat industry. Comparative analysis of MMP activity in Tellicherry goats and other breeds can help understand breed-specific physiological adaptations.

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Conflict of lnterest. None.

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