Biological Forum
an International Journal 17(2): 67-70(2025)
ISSN No. (Print): 0975-1130
ISSN No. (Online): 2249-3239

Gelatin Zymography of Matrix Metalloproteinases in Serum Samples of Kangayam Breed Cattle

T.C. Balamurugan[1], V. Haripriya[2], R. Prakash Krupakaran[3], G. Anandhi[4], E.L. Aadhie Shrie[2], M. Vishal[2] and M. Swathi[2]*

[1]*Assistant Professor, Department of Veterinary Physiology and Biochemistry, TANUVAS-VCRI, Salem (Tamil Nadu), India.*
[2]*UG Scholar, TANUVAS-VCRI, Namakkal, (Tamil Nadu), India.*
[3]*Professor, Department of Veterinary Biochemistry, TANUVAS-VCRI, Namakkal (Tamil Nadu), India.*
[4]*PG Scholar, Department of Veterinary Biochemistry, TANUVAS-VCRI, Namakkal (Tamil Nadu), India.*
Tamil Nadu Veterinary and Animal Sciences University, Chennai (Tamil Nadu), India.

(Corresponding author: T. C. Balamurugan)*
(Received: 02 May 2024; Revised: 20 May 2024; Accepted: 10 June 2024; Published online: 17 February 2025)
(Published by Research Trend)

ABSTRACT: The current study was carried out to ascertain the demonstration of gelatinases *viz.*, MMP-2 and MMP-9 in serum of Kangayam cattle, an indigenous cattle breed of Tamil Nadu. Experimental animals were divided into two groups *viz.*, Group I Male (2-4 yr), Group II Female (2-4 yr). Gelatin zymography was done on all serum samples, and it was established that the chief bands were found at 220, 125, and 92 kDa of MMP-9 and 72 kDa of MMP-2 in both groups. Two noticeable bands were observed at 92 kDa and 72 kDa, which represent the pro-forms of MMP-9 and MMP-2, respectively. Additionally, two lytic bands were found in each group at 220, 125 kDa, which represent the proforms of MMP-9; similarly, the older groups displayed thicker bands at 135 and 220 kDa, which indicate the expression of proenzymatic forms of MMP-9. In order to determine the sex difference, the latent form of MMP-2 (72 kDa) was 1.5 times more intense in male groups than in female groups. Male groups had a lower level of latent form MMP-9 intensity compared to female groups. Comparably, two more noticeable bands at 220 and 125 kDa were observed in the male groups, showing the proenzymatic forms of MMP-9 and confirming that they are the lytic bands of MMP-9. It was determined that both groups had proven expression of gelatinase activity. However, male groups expressed more MMP-2 than female groups did, whereas female groups expressed more MMP-9 than male groups. Therefore, because MMPs are involved in the buildup of extracellular matrix and are linked to concentric remodeling of tissues, age-dependent changes in MMP profiles occurred as a function of age.

Keywords: Gelatin zymography, MMP-2, MMP-9 Kangayam cattle, Bands.

INTRODUCTION

Kangayam cattle are an excellent draught breed and are predominantly found in the Kangayam, Dharapuram, Perundurai, Karur, and Palani taluks of Tamil Nadu, southern India (Akila *et al.*, 2012). According to the 19th Livestock Census, India has a cattle population of 190.9 million, making it a major livestock species. Cattle account for approximately 37.3% of India's total livestock population and 14.7% of the global cattle population. India has 43 registered native cattle breeds, broadly classified into dairy, draught, and dual-purpose breeds based on their primary utility in dairying or agricultural work. Indigenous farm animal breeds have adapted to harsh climatic conditions with minimal management inputs, including limited feed, fodder, and healthcare. Additionally, they efficiently convert low-quality feed into nutrient-rich outputs, making them well-suited to tropical environments and playing a crucial role in agriculture (Vinothraj *et al.*, 2024).

Matrix metalloproteinases (MMPs) are a family of zinc-dependent enzymes involved in the degradation of extracellular matrix components. Among them, MMP-2 (gelatinase A) and MMP-9 (gelatinase B) specifically target gelatin and type IV collagen, playing significant roles in various physiological processes in cattle. MMP-2 and MMP-9 are involved in the remodeling of the endometrial extracellular matrix during the estrous cycle and pregnancy. Their activity is regulated by various factors, including cytokines and hormones, ensuring proper tissue remodeling for successful implantation and maintenance of pregnancy. The presence of MMP-2 and MMP-9 in bovine semen has been detected through gelatin zymography, suggesting their role in semen quality. These enzymes may influence sperm motility and overall fertility.

Elevated levels of MMP-9 have been associated with inflammatory conditions such as mastitis in cattle. Monitoring MMP-9 activity can serve as a biomarker for early detection and management of such diseases.

Assessing the activity of MMP-2 and MMP-9 in reproductive tissues and semen can provide insights into fertility status, assisting in the selection and breeding of high-quality livestock. Hence, the present study was conducted to find out the presence of gelatinases in the Kangayam cattle breed of Tamil Nadu.

MATERIALS AND METHODS

The current study was carried out at the Department of Veterinary Physiology and Biochemistry, TANUVAS-Veterinary College and Research Institute, Salem, Tamil Nadu, India.

A. Collection and evaluation of serum

Twelve healthy male (Group I; n-12) and female animals (Group II; n-12) of the Kangayam breed of cattle were selected. Blood samples from each animal were collected in a heparinized vacutainer during the early morning before feeding the animals. The samples were transported to the laboratory immediately and evaluated for protein content using the standard procedure of Lowry's method (Makowski and Ramsby 1996). The blood samples were centrifuged at 3000 rpm for 15 min, and the separated serum was analyzed for protein content by photometric estimation of blue color by using a spectrophotometer. The standard curve was built by using various concentrations of bovine serum albumin (BSA) as standard. The serum samples were stored at -20°C for further analysis.

B. Gelatin zymography

The serum samples were subjected to modified SDS-PAGE (modification of Laemmli's method carried out by Heussen and Dowdle (1980) with the addition of a co-polymerizing substrate of gelatin (0.3%), and further procedures were carried out by the method described from our laboratory (Prakash Krupakaran *et al*., 2016).

C. Analyzing the results of gelatin zymography

Human capillary blood gelatinase was used as the standard marker for comparing the zymogram bands as per the procedure carried out by Makowski and Ramsby (1996), and further methods were described by Prakash, krupakaran *et al*. (2016).

RESULTS AND DISCUSSION

Gelatin zymography was performed on all serum samples from the two groups, and the results are depicted in Fig. 1. Since every serum sample totally broke down the gelatin, they were all proteolytically active. Human capillary blood gelatinases were used as molecular weight markers, helping in the identification of MMP bands in the serum samples (Lane 1, 7, 13). Lane 2, 3, 10, 11, 12 (male cattle serum) and Lane 4, 5, 6, 8, 9 (female cattle serum). It was verified that the main bands were seen in both groups at 220, 135, and 92 kDa of MMP-9 and 72 kDa of MMP-2. The latent forms of MMP-9 and MMP-2 are represented by the two significant bands that were found in the Kangeyam breed at 92 kDa and 72 kDa, respectively. Additionally, two lytic bands representing the performs of MMP-9 were detected in each group at 220 and 135 kDa. However, the active forms of MMP-9 and MMP-2 are not found in any of the Kangeyam breed's blood samples. Prakash Krupakaran *et al*. (2016) and Balamurugan *et al*. (2023) in bovine, Prakash Krupakaran *et al*. (2015) in bovine, and Balamurugan *et al*. (2017) in ovine species were all in agreement with the findings of the current study.

Effect of sex on the expression of gelatinase activity. To find out the relationship between the sexes, the gelatin zymograms of male and female animals were compared. In the Kangeyam breed, in male groups (G I: lane 2, 3, 10, 11, and 12), the intensity of the latent form of MMP-2 (72 kDa) was 1.5 times higher than that the female groups (G II: lanes: 4, 5, 6, 8, 9). In male groups (G I: lane 2, 3, 10, 11, and 12), two more prominent bands were noticed at 220, 125 kDa revealed that they are the lytic bands of MMP-9, indicating the pro-enzymatic forms of MMP-9. As compared to male groups, the female groups (G II: Lanes: 4, 5, 6, 8, 9) showed thicker bands at 125 and 220 kDa, indicating the expression of pro-enzymatic forms of MMP-9 was very clear. At 220 kDa, the samples from female animals showed more prominent bands than those of male animals. At 135 kDa of MMP-9 (pro MMP-9), no bands were observed in any of the samples, but at 125 kDa of MMP-9, all the samples showed prominent bands. In line with the present results, Prakash Krupakaran *et al*. (2016); Balamurugan *et al*. (2023) in bovine and Balamurugan *et al.* (2017) in ovine species were observed. But one difference observed in this study was that the preform of MMP-9 was observed at 125 kDa instead of 135 kDa. This might be due to breed variation and the individual physiological status of the animal.

The latent form of MMP-9 (92 kDa) was 1.5 times more intense in the female groups (G II: Lanes: 4, 5, 6, 8, 9) than in the male groups (G I lane 6, 8 and G II lane 7, 9). Similar observations were noticed in the earlier reports of Balamurugan *et al.* (2023) in bovine and Balamurugan *et al.* (2024) in buffaloes. In both male and female animals, the latent form of MMP-2 (72 kDa) was visible in all the animals. The intensity of MMP-2 (72 kDa) was 2-4 times higher than the latent form of MMP-9 (92 kDa). As compared to female group, the male group showed more intensity of MMP-2. This is corroborated with earlier reports of Balamurugan *et al*. (2023) in bovine, Balamurugan *et al*. (2024) in buffaloes, and Cancemi *et al*. 2020 in humans. According to a study by Cancemi *et al*. (2020), in humans, there was a discernible rise in Pro-MMP-2 activity with age in men but not in women. This is because, particularly in women, the expression of MMPs is also influenced by hormonal status (Berg *et al*., 2014).

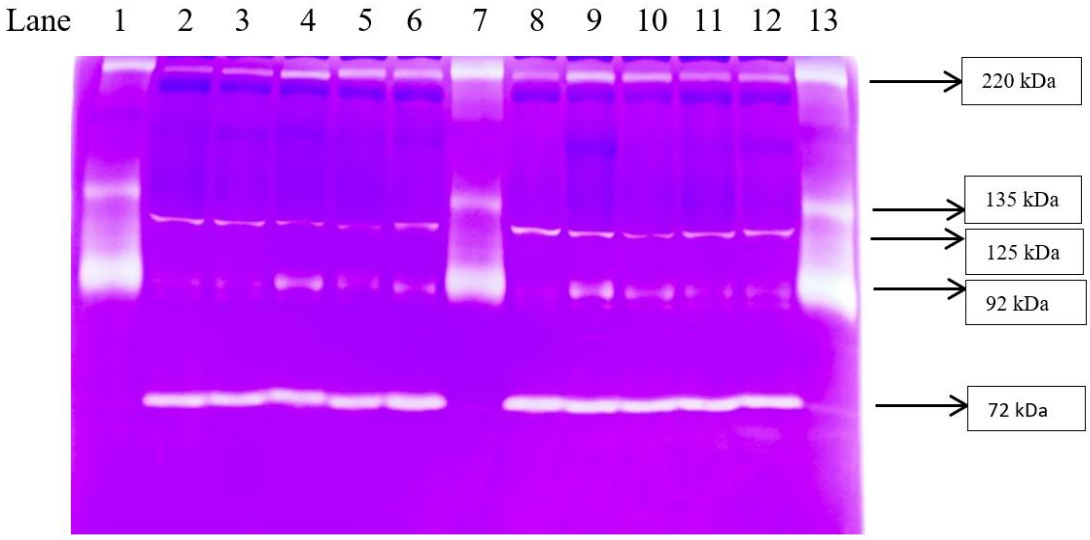

Fig. 1. Gelatin zymography of MMPs in serum of Kangayam cattle (13 microlitres of serum in each well).

This demonstrates unequivocally that female animals express more MMP-9 than male animals do. Female neutrophils have been reported to exhibit lower MMP-9 during the menstrual cycle when estrogen levels are greater (Smith *et al.*, 2007), and female sex hormones may be protective. Furthermore, within the age and sex categories, there may be a correlation between the expression levels of gelatinases and each individual animal's physiological state.

CONCLUSIONS

It was determined that both sexes had proven expression of gelatinase activity. The expression of MMP-9 was higher in female groups than in male groups, while MMP-2 was higher in male groups than in female groups. Further, there is more up regulation of MMP-2 mediated through MMP-9 activity observed in Kangayam cattle serum. Because MMPs are involved in the formation of extracellular matrix and are linked to the concentric remodeling of tissues, age-dependent changes in MMP profiles were observed as a function of age.

FUTURE SCOPE

The role of MMP-2 and MMP-9 could be further explored for their active role in various physiological mechanisms and how it augments the reproduction potential of the Kangeyam cattle.

Acknowledgements. The authors are grateful to the Dean, VCRI, Namakkal and Dean, VCRI, Salem and TANUVAS for providing the necessa1y facilities.
Conflict of Interest. None.

REFERENCES

Akila, N., Senthilvel, K. & Velusamy, K. (2012). Productivity performance of Kangayam cattle. *Indian Journal of Animal Sciences 82*(11), 1440-1441.

Balamurugan, T. C. & Prakash Krupakaran, R. (2020). Detection of MMP-2 and MMP-9 in native goat breed of Tamil Nadu. *Journal of Entomology and Zoology Studies, 8*(5), 1136-1139.

Balamurugan, T. C., Prakash Krupakaran R., Kibson C., Rajendiran S., Iswarya, R. & Perumal, P. (2017). A comparative gelatin zymography of matrix metalloproteinases in serum of native sheep breeds of Tamil Nadu. *Journal of cell and tissue research, 17* (3), 6239-6242.

Balamurugan, T. C., Prakash Krupakaran, R., Anandhi, G., Senthamil Pandian, C. & Perumal, P. (2024). A comparative gelatinase expression study between Indian hilly (Toda and Bargur) and plain (Murrah) bubaline species. *Journal of the Andaman Science Association, 29*(1), 38-44.

Balamurugan, T. C., Prakash Krupakaran, R., Anandhi, G., Senthamil Pandian, C. & Perumal, P. (2023). Influence of sex and age on serum matrix metalloproteinase expression in bovine species. *Journal of the Andaman Science Association*, *28*(2), 218-224.

Balamurugan, T. C., Prakash Krupakaran, R., Visha, P., Murugavel, S. & Pradheep, N (2020). Existence of gelatinases in goat breeds of Tamil Nadu. *Journal of Entomology and Zoology Studies*, *8*(1), 1019-1021.

Berg, G., Schreier, L. & Miksztowicz, V. (2014). Circulating and adipose tissue matrix metalloproteinases in cardiometabolic risk environments: pathophysiological aspects. *Hormone Molecular Biology and Clinical Investigation*, *17*, 79–87.

Cancemi, P., Aiello, A., Accardi, G., Caldarella, R., Candore, G., Caruso, C., Ciaccio, M., Cristaldi, L., Di Gaudio, F., Siino, V. & Vasto, S. (2020). The Role of Matrix Metalloproteinases (MMP-2 and MMP-9) in Ageing and Longevity: Focus on Sicilian Long-Living Individuals (LLIs). *Mediators of Inflammation*, *5*, 8635158.

Heussen, C. & Dowdle, E. B. (1980). Electrophoretic analysis of plasminogen activators in polyacrylamide gels containing sodium dodecyl sulfate and copolymerized substrates. *Analytical Biochemistry, 102*, 196–202.

Makowski, G. S. & Ramsby, M. L. (1996). Calibrating gelatin zymograms with human gelatinase standards. *Analytical Biochemistry, 236*, 353–356.

Prakash Krupakaran, R, Balamurugan, T. C., Arunkumar, S., Guru D. V. Pandiyan & Perumal, P. (2015). Alterations in gelatinase activity during different reproductive stages of reproduction in Murrah

buffaloes. *Indian Journal of Animal Science*s, *85*(5), 458-461.

Prakash Krupakaran, R., Balamurugan, T. C., Lakshmi, R. D., Sheeba, A. & Perumal, P. (2016). Comparative study on serum matrix metalloproteinases in various species of domestic animals. *Indian Journal of Animal Sciences, 86*, 545–549.

Smith, J. M., Shen, Z., Wira, C. R., Fanger, M. W. & Shen, L (2007). Effects of menstrual cycle status and gender on human neutrophil phenotype. *American Journal of Reproduction and Immunology*, *58*, 111–119.

Vinothraj, S., Kumar, S. R., Inbaraj, C., Saravanan, R. & Kumaravel, P. (2024). Study on morphological parameters of Rekla Race Breed- Kangayam in Tamil Nadu. *International Journal of Veterinary Sciences and Animal Husbandry*, *SP-9*(4), 17-19.

How to cite this article: T.C. Balamurugan, V. Haripriya, R. Prakash Krupakaran, G. Anandhi, E.L. Aadhie Shrie, M. Vishal and M. Swathi (2025). Gelatin Zymography of Matrix Metalloproteinases in Serum Samples of Kangayam Breed Cattle. *Biological Forum, 17*(2): 67-70.