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Existence of gelatinases in goat breeds of Tamil Nadu

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Abstract
A study was undertaken to analyse the gelatinase activity through gelatin zymography in serum of various native goat breeds of Tamil Nadu. Four adult male and female native breeds goat viz. Jamunapari, Kanni, Kodi and Tellicherry were selected. On gelatin zymogram reveals that the presence of three prominent bands at 220, 92 kDa of MMP-9 and 72 kDa of MMP-2 were clearly observed for all goat breeds. All the three forms of MMP are proteolytically active, completely degraded the gelatin. Both latent forms of MMP-2 and MMP-9 were exhibited and the latent bands were observed as thicker bands than the active form. The intensity of 72 kDa of MMP-2 was 2-4 times higher than 92 kDa of MMP-9. The level of expression of 72 kDa of MMP-2 was constant as compared to 92 kDa of MMP-9. In all the groups, 135 homodimer or MMP-9 was also observed. Further, in Kodi and Tellicherry groups showed maximum gelatinolytic activity as compared to marker by showing more intensity in 72 kDa of MMP-2. In addition, the 62 kDa of MMP-2 was also observed. The level of expression of 72 kDa band was constant compared to that 92 KDa. It was concluded that there was no significant difference between the expression of MMP-9 and MMP-2 in both the sexes of each breed. Further, there is more up regulation of MMP-2 mediated through MMP-9 activity observed in goat serum. It was inferred that the expression of MMP-2 and MMP-9 were to be correlated with reproductive status of individual animal since MMP played extensive role in tissue remodelling and extra cellular degradation.

Keywords: Gelatin zymography, serum, MMP, gelatinase

Introduction
India occupies first position in terms of goat population and milk production. Chevon (goat meat) is most preferred and widely consumed meat in the country. Since ancient times goat milk has traditionally been known for its medicinal properties and has recently gained importance in human health due to its proximity to human milk for easy digestibility and its all round health promoting traits. Goat is a multi facet animal known for its utility like meat, milk, skin and manure, forms an important component of rural economy. The country has 135 million goat and 33.01 million farmers holders contributes to economically weaker section of society [1].

Enzymes responsible for the collagen and other protein degradation in extracellular matrix (ECM) are matrix metalloproteinases (MMPs). Collagen is the main structural component of connective tissue and its degradation is a very important process in the development, morphogenesis, tissue remodelling, and repair. Mostly, the MMPs have the capacity to degrade all the components of the extracellular matrix. ‘’Matrix metallo peptidases (MMPs), also known as matrix metallo proteinases or matrixins, are metalloproteinases are that are calcium-dependent zinc-containing endopeptidases [2]. These enzymes are known to be involved in the cleavage of cell surface receptors, the release of apoptotic ligands (such as the FAS ligand), and chemokine /cytokine inactivation [3]. Thus MMPs play a major role in cell behaviors such as cell proliferation, migration (adhesion/dispersion), differentiation, angiogenesis, apoptosis, and host defense.

This family of endoproteases has been considered essential in a number of normal physiologic processes as well as pathological events. Nowadays, specific MMPs are targeted to induce locally for cardiac abnormalities; thereby MMPs may represent a promising and novel therapeutic target. Hence, this study was carried out to find out the existence of gelatinases in certain goat breeds of Tamil Nadu.

Materials and Methods

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

Collection and evaluation of serum

Four healthy animals of Jamunapari, Kanni, Kodi and Tellicherry breed of goats were selected in every breed. Before feeding the animals, blood samples were collected from all the four groups in a heparinised vacutainer in early morning hours. After collection, immediately the samples were transported to the laboratory and further evaluated for its protein content using standard procedure of Lowry's method [4]. The blood samples were centrifuged at 3000 rpm for 15 min. serum was separated and further analyzed for its protein content by using 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.

Gelatin zymography

The serum samples were analysed by the method of modified SDS-PAGE (modification of Laemmli's method, 1970) [5] carried out by Heussen and Dowdle (1980) [6] by the addition of co-polymerizing substrate of gelatin (0.3%) (final concentration was 0.15% to the resolving gel (8%). The samples were electrophoresed at 100V for 20 min. Renaturation was carried out with 2.5% Triton X-100 for 3 hrs on a mechanical shaker with a mild agitation. Then developing was done by incubating the gel in 10 mM $CaCl_2$, 0.15 M NaCl and 50 mM Tris pH 7.5.for 18 hrs at 37 °C. The gel was stained with 0.25% coomassie brilliant blue for 2 hrs, followed by destaining with destaining solution for 1 hr and finally the gel was washed with distilled water.

Analyzing the results of gelatin zymogram

Human capillary blood gelatinase was used as the standard marker for comparing the zymogram bands based on the procedure of Makowski and Ramsby (1996) [7]. The blood was collected from a capillary and weighed in a tarred polypropylene tube using analytical balance by using a fingerstick puncture. Samples were added with 20X volume of Laemmli buffer and thoroughly mixed. Then the aliquots were stable for 3 months at -20 °C.

Results and Discussion

On gelatin zymography, it was confirmed that MMP-2, MMP-9 were present in the serum samples of all goat breeds, and the results were depicted in Fig No-1. Gelatin zymogram suggests, three prominent bands at 220, 92 kDa of MMP-9 and 72 kDa of MMP-2 were evidently present in both the sexes of all goat breeds. All the three forms MMP-9 and MMP-2 are proteolytically active, and fully degraded the gelatin. The latent forms existed in both MMP-2 and MMP-9 but the latent bands were observed as thicker bands than the active form. The results were agreed by various authors [8, 9, 10]. The existence of gelatinase was confirmed in serum of various domestic animals by various authors [8, 9, 10]. In our earlier study, we observed 220, 92, and 135 kDa of MMP-9 and 72 kDa of MMP-2 was observed in goat serum. Our results are in accordance with the results of Cummings *et al.* (2012) [11]. He observed that higher level of MMP- 9 (220 kDa; Dimer), pro-MMP-9 (92 kDa; Monomer) and pro MMP-2 (72 kDa) found in lamb model, after the implantation of tissue engineered vascular graft.

Gelatinases plays a significant role in tissue remodelling and implantation. Whenever extracellular degradation and tissue regeneration is there, the levels of MMP-2 and MMP-9 were elevated. MMP-9 expression was increased in goat during *Listeria meningoencephalitis* infection as compared to normal goat [12].

Further, MMP-2 (72 kDa) was very prominent in all the breeds as compared to human markers (lane 7 and 8). The intensity of 72 kDa of MMP-2 was 2-4 times higher than 92 kDa of MMP-9. The level of expression of 72 kDa of MMP-2 was constant as compared to 92 kDa of MMP-9. In all the groups, 135 homodimer or MMP-9 was also observed. Hence, the MMP's which were present in normal level is increased during external pressure or during internal physiological changes.

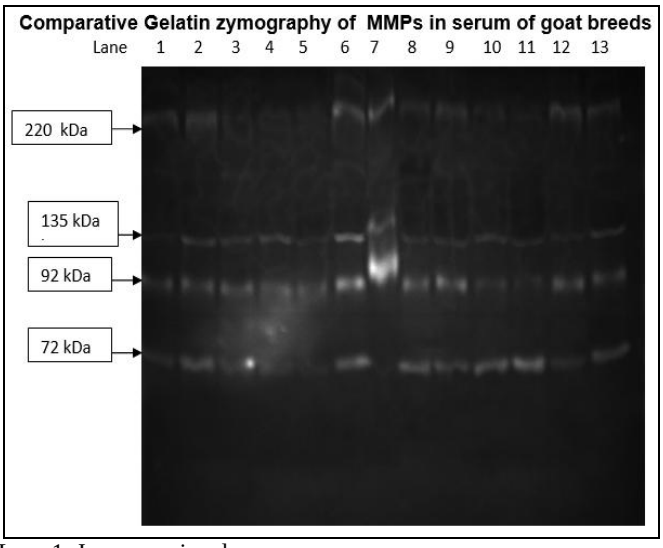
In another study, serum MMP 9 concentrations in healthy cows increased significantly when compared with sera of acutely septic and chronically ill animals. In contrast, serum concentrations of Hp–MMP 9 complexes found almost exclusively in sera from acutely septic animals but not in chronically ill and normal cattle. Hence, Serum haptoglobin–matrix metalloproteinase 9 (Hp–MMP 9) complex could be used as a biomarker of systemic inflammation in cattle [13].

Further, in Kodi and Tellicherry groups showed maximum gelatinolytic activity as compared to marker and other breeds by showing more intensity of 72 kDa of MMP-2. In addition, the active form of MMP-2 62 kDa was also observed. The level of expression of 72 kDa band was constant compared to that 92 kDa MMP-9. This might be due to the different reproductive status of the individual animals. Several documents revealed that the myocardial and blood level of MMP-9 increase in animal model HF. The importance of MMP-9 is more than MMP-2 but in animal models such as pig and dog, the duration of MMP-2 elevation is longer [14].

Gelatinases have a prominent role in various reproductive functions. Higher gelatinase activity was found in atretic follicles than in normal follicles. The active form MMP-2 and proMMP-9 come into existence in follicular fluid may be a key indicator of atresia [15]. The level of gelatinases in surrounding body fluids of actively remodelling tissue is indicative of basement membrane and extracellular matrix degradation under various physiological and pathological circumstances [16].

Circulating MMP-2 and MMP-9 are inversely associated with large artery stiffness but not with wave reflections in healthy persons. This finding implies that these gelatinases may have a possible role in the determination of arterial function and has potential implications for their involvement in the pathophysiology of cardiovascular diseases [17]. Similarly, detection of the active form of MMP-9 is more important than active MMP-2 because the active form of MMP-9 was only seen in patients with DCM [18].

MMP-9 is known to participate in trophoblast cells invasion and to be involved in the formation of new blood vessels and thus it is called a trigger of angiogenesis [19]. In addition, the development of very small blood vessels and process of intravasation require the presence of MMP-9 [20]. Altered maternal serum matrix metalloproteinases MMP-2, MMP-3, MMP-9, and MMP-13 observed in severe early- and late-onset preeclampsia. It was inferred that the expression of MMP-2 and MMP-9 were to be correlated with reproductive status of individual animal as MMP played extensive role in tissue remodelling and extra cellular degradation.



Lane 1. Jamunapari male
Lane 2. Jamunapari male
Lane 3. Jamunapari female
Lane 4. Kanni male
Lane 5. Kanni female
Lane 6. Kodi female
Lane 7. Human capillary blood gelatinases as marker
Lane 8. Kodi male
Lane 9. Tellicherry female
Lane 10. Tellicherry female
Lane 11. Tellicherry male
Lane 12. Jamunapari male
Lane 13. Jamunapari male

Fig 1: Comparative Gelatin zymogram of MMPs in serum of goat breeds (15 microliters of serum in each well)

Conclusion

It was concluded that there was no significant difference between the expression of MMP-9 and MMP-2 in both the sexes of each breed. Further, there is more up regulation of MMP-2 mediated through MMP-9 activity observed in goat serum. Since, gelatinases might have important functions in various reproductive activities, they have to be targeted as a therapeutic tool to augment animal production.

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