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Detection of MMP-2 and MMP-9 in native goat breed of Tamil Nadu

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Abstract
The proposed study was carried out to detect the presence of MMP-2 and MMP-9 in serum of native goat breed of Tamil Nadu 'Kodi adu'. Four adult male and female Kodi breed of goats were selected. On gelatin zymogram reveals that the presence of three prominent bands at 92 kDa of MMP-9 and 72 and 62 kDa of MMP-2 were clearly observed in both the sexes. 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 62 kDa of MMP-2 was 2-4 times higher than 82 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 and 220 kDa of MMP-9 were also observed. Both latent form of 72 kDa MMP-2 and active form of 62 kDa MMP-2 observed as thicker bands and they showed maximum gelatinolytic activity as compared to marker. In addition, the 82 kDa of MMP-9 was also observed in female animals. It was concluded that there was no significant difference between the expression of MMP-9 and MMP-2 in both the sexes of Kodi goats. But the existence of active of 82 kDa of MMP-9 was observed in female animals. It was obvious that 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 widespread role in tissue remodeling and extra cellular degradation.

Keywords: Gelatin zymography, serum, MMP, gelatinase

Introduction
Chevon (goat meat) is most preferred and widely consumed meat in the country. Goat milk is known for easy digestibility and its health promoting traits. Goat is known for its utility like meat, milk, skin and manure, forms an important integral part in rural economy. Hence, goat rearing is otherwise called as Poor man's Cow'. The Goat population in the country in 2019 is 148.88 Million showing an increase of 10.1% over the previous census. As per the 20th Livestock census, the cattle population in Tamil Nadu rose to 95.19 lakh from 88.14 lakh registered in the 19th Livestock census. The population of goats increased to 98.89 lakh from 81.43 lakh during this period [1].

Matrix metalloproteinases are a family of zinc- and calcium-dependent proteolytic enzymes that possess the ability to degrade extracellular matrix and basement membrane components (Hulboy *et al*., 1997) [2]. MMP-9 is the largest member of the MMP family and is able to degrade a wide range of substrates, including type IV collagen, the principal type of collagen in the extracellular matrix of basement membranes [3]. MMP-2 is also capable of degrading type IV collagen, but has a different spectrum of activity (Xia *et al*., 1996) [4]. MMPs are expressed by a wide variety of reproductive tissues [2]. The MMP system has been strongly suggested to play a critical role in reproductive functions in humans and diverse animal species (Bai *et al*. 2005) [5]. Mostly, the MMPs have the capacity to degrade all the components of the extracellular matrix. '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 [6]. Thus, MMPs play a major role in cell behaviors such as cell proliferation, migration (adhesion/dispersion), differentiation, angiogenesis, apoptosis, and host defense.

The level of gelatinases in surrounding body fluids of actively remodeling tissue is indicative of basement membrane and extracellular matrix degradation under various physiological and pathological circumstances [7]. Gelatinases play an essential role in a number of normal physiologic processes as well as pathological events. Hence, this study was carried out to find out the existence of gelatinases in Kodi adu, a native goat breed 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 male and female animals of Kodi breed of goats were selected. In early morning before feeding the animals, blood samples were collected from all the eight animals in heparinised vacutainer. After collection, immediately the samples were transported to the laboratory and further evaluated for its protein content using standard procedure of Lowry's method (Lowry *et al*. 1951) [8]. 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

Serum samples were analysed by the method of modified SDS-PAGE (modification of Laemmli's method, 1970) [9] carried out by Heussen and Dowdle (1980) [10] 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) [11]. 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 both male and female Kodi breed goats, and the results were depicted in Fig -1. Gelatin zymogram suggests, three prominent bands at 92 kDa of MMP-9 and 72 and 62 kDa of MMP-2 were evidently present in both the sexes of Kodi adu. In addition, proforms of MMP-9, 220 and 135 kDa of MMP-9 are also present. All the latent and active forms of MMP-9 and MMP-2 are proteolytically active, and fully degraded the gelatin. The latent form of MMP-9 (92 kDa) was thicker than the active form. But in MMP-2, active form (62 kDa) is thicker than the latent form (72 kDa). The existence of gelatinases MMP-9 and MMP-2 was confirmed in serum of various domestic animals by various authors [12, 13, 14, 15].

In our earlier study, Krupakaran *et al* (2016) observed 220, 92, and 135 kDa of MMP-9 and 72 kDa of MMP-2 was observed in sheep serum and compared with the other species of domestic animals. Both the latent forms of MMP-2 and MMP-9 were exhibited. In another study of using lamb model, elevated level of MMP- 9 (220 kDa; Dimer), pro-MMP- 9 (92 kDa; Monomer) and pro MMP- 2 (72 kDa) were detected after the implantation of tissue engineered vascular graft. These MMP proteins help in the remodeling of tissues [16].

In the present study, MMP-2 (62 kDa) was very prominent in all the animals as compared to human markers (lane 5). In female animals, the active form of 82 kDa of MMP-9 was also present. The intensity of 62 kDa of MMP-2 was 3-5 times higher than 82 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 animals, 135 homodimer, 220 kDa of MMP-9 was also observed. The elevated level of MMP-2 and MMP-9 was observed due to remodeling of tissues during implantation.

Gelatinases have a prominent role in various reproductive functions. In female animals, the active form of MMP-9 was noticed. This might be due to individual animal physiological and reproductive status [14]. 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 [16].

Similarly, serum gelatinase (MMP-9) activity was increased in acute septicemic and chronically ill animals as compared normal animals noticed by Bannikov *et al*. (2011) [16]. They further concluded that the level of MMP-9 in acute and chronic metabolic disease was identical, and normal in healthy animals. Hence, the MMP's which were present in normal level and increased during external pressure or during internal physiological changes. Whenever extracellular degradation and tissue regeneration is there, the levels of MMP-2 and MMP-9 were elevated.

In our earlier study, goat breeds like Kodi and Tellicherry groups showed maximum gelatinolytic activity as compared to marker and other breeds. They showed 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 different reproductive status of the individual animals [14].

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 [17]. In addition, the development of very small blood vessels and process of intravasation require the presence of MMP-9 (Husslein *et al*., 2009) [18]. 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 remodeling and extra cellular degradation.

It was inferred that the expression of MMP-2 and MMP-9 were to be correlated with the reproductive status of individual animal as MMP played extensive role in tissue remodeling and extra cellular degradation. 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.

Gelatin zymographic analysis of MMPs in serum of Kodi breed goats

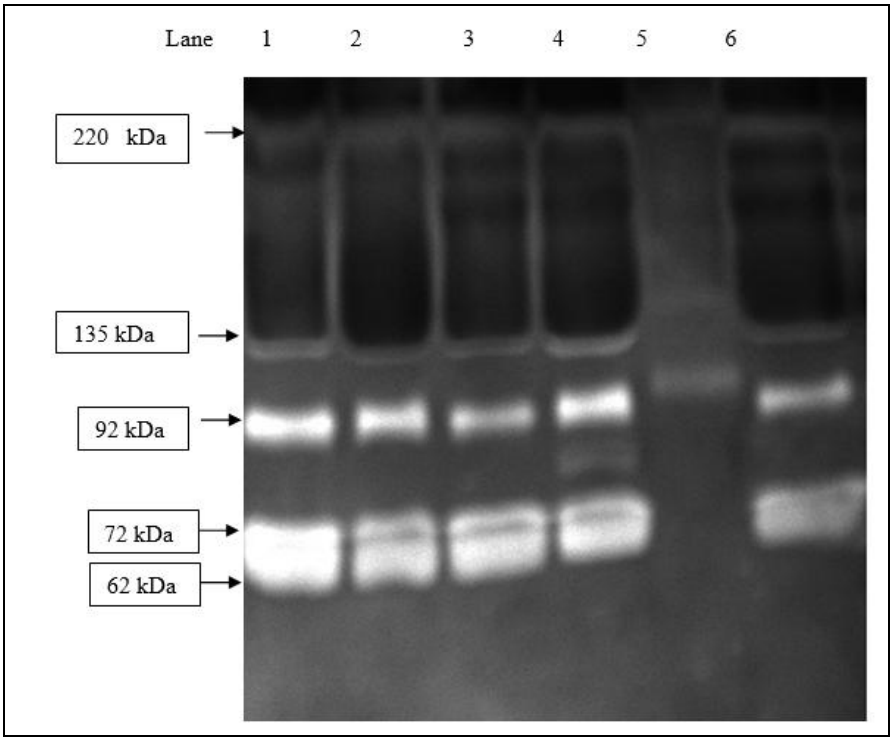

Fig 1: Gelatin zymographic analysis of MMPs in serum of Kodi breed goats
Lane 1, 2 & 3 - Kodi Male serum (10 microliters)
Lane 4 & 6 - Kodi Female serum (10 microliters) Lane 5- Human capillary blood Gelatinases (10 microliters)

Conclusion

It was concluded that there was no significant difference between the expression of MMP-9 and MMP-2 in both the sexes of Kodi adu. But in female animals, existence of active form of 82 kDa MMP-9 was also observed. 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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