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Distribution of prolific Garole sheep in West Bengal, India

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Summary
The Garole is a prolific breed of sheep. High prolificacy in sheep carrying the Booroola gene (*FecB*) is the result of a mutation in bone morphogenetic protein receptor-IB (BMPR-IB) (Wilson *et al.*, 2001a,b) which had previously been identified in Garole sheep from the Sunderban region of West Bengal (Davis *et al.*, 2002) . There is evidence that the breed has originated from the sheep brought by the Tibetan traders and traded in the plains of Bengal during the seventeenth till the nineteenth century. The present study was carried out to remap the distribution of the Garole sheep within the state of West Bengal (India) using the presence of the BMPR-IB mutation in the sheep flocks reared at different locations within the state of West Bengal. The breeding tract of Garole sheep was initially thought to be in the districts of 24 Parganas, South and North alone. However, the results from the present study indicate that the sheep is also reared in the district of Midnapur (East), besides in Jalpaiguri and Cooch Behar districts situated in northern parts of the state. The results of the present study indicate that the breeding tract of Garole sheep extends up to Jalpaiguri and CoochBehar districts of West Bengal at 26°16′ and 27°0′ North latitude and 88°4′ and 89°53′ East longitude. This study also indicates that the ancestors of the Garole sheep have migrated from China/Tibet, during the trading between West Bengal and Bangladesh during the seventeenth century till the early-twentieth century.

Keywords: *Garole sheep, FecB mutation, distribution, West Bengal, India*

Résumé
Le Garole est une espèce prolifique de mouton. Haut prolificacy dans le mouton qui porte le gène de Booroola (*FecB*) est le résultat d'une mutation dans BMPR-IB (Wilson *et al.*, 2001a, b) qui avait été précédemment identifié dans le mouton de Garole de la région de Sunderban de Bengale d'ouest (Davis *et al.*, 2002). Il y a de la preuve que l'espèce a provenu du mouton amené par les commerçants tibétains et échangé dans les plaines de Bengale pendant le dix-septième jusqu'à le dix-neuvième siècle. L'étude présente a été exécuté à remap la distribution du mouton de Garole dans l'état de Bengale d'ouest (l'Inde) utilisant la présence de la mutation de BMPR-IB dans les troupeaux de mouton a élevé aux emplacements différents dans l'état de Bengale d'ouest. L'étendue élevant de mouton de Garole a été pensée au début pour être dans les quartiers de 24 Parganas, le Sud et Nord seul. Cependant, les résultats de l'étude présente indiquent que le mouton est aussi élevé dans le quartier de Midnapur (l'Est), en plus dans Jalpaiguri et les quartiers de Behar de Cooch ont situé dans les parties du nord de l'état. Les résultats de l'étude présente indiquent que l'étendue élevant de mouton de Garole s'étend en haut aux quartiers de Jalpaiguri et CoochBehar de Bengale d'ouest à 26°16′ et 27°0′ la latitude du nord et 88°4′ et 89°53′ la longitude de l'Est. L'étude présente indique aussi que les ancêtres du mouton de Garole ont migré de Chine/Tibet, pendant le commerce entre Bengale et Bangladesh d'ouest pendant le dix-septième siècle jusqu'à le premier vingtième siècle.

Mots-clés: *mouton Garole, mutation du gène* FecB*, distribution, Bengal occidental, Inde*

Resumen
La Garole es una prolífica raza de ovejas. La alta prolificidad en las ovejas portadoras del gen Booroola (*FecB*) es el resultado de una mutación en BMPR-IB (Wilson *et al.*, 2001a, b), que había sido previamente identificado en el ganado ovino Garole de la región de Sunderban de Bengala Occidental (Davis *et al.* 2002). Existen pruebas de que la raza tiene su origen en las ovejas traídas por los comerciantes tibetanos y comerciaban en las llanuras de Bengala desde el siglo XVII hasta el siglo XIX.

El presente trabajo se realizó para reasignar la distribución geográfica de las ovejas Garole dentro del estado de Bengala Occidental (India), utilizando la presencia de la mutación BMPR-IB en los rebaños de ovejas criadas en distintos lugares dentro del estado de Bengala Occidental. Inicialmente se pensó que el área de cría de las ovejas Garole fuera en distritos de 24 Parganas, Sur y Norte solo.

Sin embargo, los resultados del presente estudio indican que las ovejas también se crían en el distrito de Midnapur (este), además de en los distritos de Jalpaiguri y Cooch Behar, situado en el norte del estado. Dichos resultados indican que la zona de cría de ovejas Garole se extiende hasta Jalpaiguri y CoochBehar, distritos de Bengala Occidental a 26°16′ y 27°0′ latitud Norte y 88°4′ y 89°53′ longitud

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Este. Asimismo, el estudio señala que los ancestros de las ovejas Garole fueron traídos desde China / Tíbet, a consecuencia del comercio entre el Oeste de Bengala y Bangladesh desde el siglo XVII hasta comienzos del siglo XX.

Palabras clave: *oveja Garole, mutación Fec B, distribución, Bengala Occidental, India*

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Introduction

The highly prolific Garole breed of sheep is prevalent in the state of West Bengal and Bangladesh (Ghalsasi and Nimbkar, 1993; Bose and Moitra, 1995; Sandip Banerjee and Soma Banerjee, 2000). Various authors (Ghalsasi and Nimbkar, 1993; Bose and Moitra 1995; Singh and Bohra 1996; Sharma *et al.*, 1999; Sahana *et al.*, 2001) have reported that the breeding of Garole sheep is localized in the Sunderban region of West Bengal and Bangladesh. Bose and Moitra (1995) reported that the habitat of the breed spans between 21° and 23° North latitude and 87° and 89° East longitude, spanning an area of 4 226 km^2. Sharma *et al.*, (1999) observed that the highest elevation inhabited by the Garole sheep is 200 m above the sea level. The present study was carried out to study the distribution of the Garole sheep within the state of West Bengal in India and also to study the reasons for prolificacy in sheep flocks reared in the districts of Jalpaiguri and Cooch Behar in India.

This project was done to identify the presence of the bone morphogenetic protein receptor IB (*BMPR-IB*) gene, which affects the fecundity of sheep in different sheep flocks reared in West Bengal. The presence of BMPR-IB mutation was initially identified in Garole sheep, reared in the Sunderban region of West Bengal and Bangladesh. The historical findings indicate that wool was traded from Tibet. Sheep were also used to carry merchandise by the Tibetan traders to Jalpaiguri and Cooch Behar districts from the seventeenth till the early-twentieth century. The *FecB* gene as observed in the flocks in the above-mentioned districts have been introduced to the local ovine population by the crossings with the sheep of Tibetan origin that occurred during the past, as has been observed in the present study (Binny, 2005). Davis (2004) reported that one copy of the *FecB* gene increases ovulation rate in Booroola Merino by about 1.5 and two copies by 3.0. These extra ovulations in turn increase litter size by 1.0 and 1.5, respectively. High prolificacy in Booroola sheep is due to a non-conservative mutation (q249r) in a highly conserved intracellular kinase signalling domain of the BMPR-1B expressed in the ovary and granulosa cells (Mulsant *et al.*, 2001; Wilson *et al.*, 2001a, b). The BMPR-1B, also known as ALK-6, is a member of the transforming growth factor-β (TGF-β) superfamily. These are multifunctional proteins that regulate growth and differentiation in many cell types. Members of this family play essential roles during embryogenesis in mammals, amphibians and insects as well as in bone development, wound healing, haematopoiesis and immune and inflammatory responses (Massague, 1998; Letterio and Roberts, 1998).

There are historical records indicating large scale trading of livestock in the districts of Jalpaiguri and Coochbehar, during the seventeenth till early decades of the twentieth century. The great silk route from China via Tibet terminated at the districts of Coocb Behar and Jalpaiguri (Figures 8 and 9). The exchange of goods from the hills took place in the regions and many fairs that sprang up at different places in this region. The fairs were generally held in winter and continued roughly for 4 weeks. The largest of such fairs one took place at Darwani (presently in Rangpur district of Bangladesh; latitude 25° 45′00″ and longitude 89°15′ 00″) where, according to a contemporary source, around 50 000 visitors participated. Here along with the traditional products, various live animals such as elephants, camel, sheep were sold here from the neighbouring states such as Bihar (Ratna Sarkar and Indrajit Ray, 2006). Similar fairs were also held at Panga Barabhita, Badarganj, Birat in different parts of Rangpur district and in Rangpur town itself (Ratna Sarkar and Indrajit Ray, 2006).

As sheep from different parts of eastern India and Tibet were traded in these fairs at Rangpur and adjoining area, there were chances of crossing among different breeds in the region as indicated in present results from Jalpaiguri and Cooch Behar region (Zone-3) of West Bengal. The aim of the present study was to study the prevalence of FecB mutation in sheep reared at different locations of West Bengal and to correlate the historical findings regarding the migration of sheep from Tibet to plains of Bengal during the past centuries.

Materials and methods

The state of West Bengal is situated between 22°34′10.92″ N and 88°22′10.92″E latitude and longitude. The state is situated in the eastern part of India, to the south is situated the Bay of Bengal, to the East is Bangladesh, the North is guarded by the Himalayas and in the West is situated the state of Jharkhand. The study was carried out using multistage sampling technique. For the study the state of West

Bengal was divided into six zones, where there could have been possibilities of the existence of sheep with high incidences of prolificacy.

The Zone-1, situated in the Midnapur (East) district 22°30′ 00″N and 87°30′00″E, located away from the Sunderbans and is divided by the river Hoogly, near to the Bay of Bengal. Zone-2, the district 24 Parganas (North) is situated between latitude: 23°15′ North–22°11′ North and longitude: 89°5′ East–88°20′ East, a part of the district comes under the Sunderban region. Zone-3, the districts of Jalpaiguri and Cooch Behar, is situated between 26°16′ and 27°0′ North latitude and 88°4′ and 89°53′ East longitude is in the Northern part of the state at the foothills of the Himalayas, the place is situated nearly 800 km away from the currently known Garole sheep breeding tract. The Cooch Behar and Jalpaiguri districts boundary Bangladesh in the south, state of Assam in the east, Bhutan in the north and the district of Darjeeling in the west. Zones 4, 5 and 6 district 24 Parganas (South) located between latitude North 20′20″ South 22′06 and longitude East 88′20″ West 88′60″ and is the district where maximum Garole population is found and is taken as a control district. The locations of the various districts are presented in Figure 1.

The present literature reviewed on the breeding tract of Garole breed indicates that the same is limited only to 24 Parganas (North and South) in West Bengal.

A total of 114 of sheep were included in the present study. The locations/areas/regions were the blood samples were collected from the sheep have been presented in Table 1.

The samples were collected through multistage sampling process and the villages within the zones were selected randomly and purposive sampling (for their prolific status) was done within the village. Sheep with prior history of multiple births and lambs and rams born from multiple births were considered for the present study. The history of multiple births in the sheep was obtained from the rearers by formulating informal questioners. There were also instances where the blood was collected from the ewes with multiple lambs at foot. The blood was collected by puncturing the veins of the animal's ear. The samples were collected in duplicate on easi Trace™ DNA labels (Hally Labels, Christchurch, New Zealand) and were analysed for the presence of the BMPR-IB mutation.

Preparation of blood samples

DNA was extracted from each 2.0 mm punch using alkaline lysis at 75 °C as described by Rudbeck and Dissing (1998). The punches were incubated for 5 min in 20 µl 0.2 M NaOH and then 180 µl 0.04 M Tris-HCl, pH 7.5 was added. An aliquot of 1–5 µl of extract was used per polymerase chain reaction (PCR).

Forced restriction fragment length polymorphism PCR for *FecB*

The process was carried out as per the methodology suggested by Davis *et al.* (2002). However, in the present process easi Trace™ labels were used. PCR was carried out using a modification of the forced Restriction fragment length polymorphism (RFLP) method described by Wilson *et al.* (2001a, b). The primer TestR15 has been engineered to introduce a point mutation such that PCR products from the *BMPR-1B* gene with the Booroola mutation contain an *Ava*II (New England Biolabs, Beverly, MA, USA) restriction site (G|GACC),whereas products from non-carriers of the mutation lack this site. Genomic DNA (~100 ng) was amplified as described by Wilson *et al.* (2001a, b). The samples on easi Trace™ DNA labels were punched (single 1.2 mm punch) and were amplified in a 25 µl reaction volume using an alternative primer set CCAG AGGACAATAGCAAAGCAAA, Test F2, and CAAG ATGTTTTCATGCCTCATCAACACGGTC (Test R15).

The amplification was carried out using 35 cycles at 94 °C for 15 s, 60 °C for 30 s and 70 °C for 30 s, followed by 72 °C for 5 min and 99 °C for 15 min. The 190 bp product was then digested using *Ava*II. The resulting products were separated by electrophoresis on a 5 percent agarose gel and visualized with ethidium bromide. Products containing the FecB mutation yield 160 and 30 bp fragments, while non-carrier products remain uncut at 190 bp.

Results

The results obtained from the present study are presented in Table 2. The results indicate that, FecB was observed in most of the samples analysed from all the zones. The results also show that the samples from zones, 1, 2, 4, 5 and 6 are having either BB or B+ mutation, as evident from Figure 2.

The results further indicate that the sheep reared at Jalpaiguri and Cooch Behar districts of West Bengal at 26°16′ and 27°0′ North latitude and 88°4′ and 89°53′ East longitude too carry the BMP IB mutation. However, results from this zone indicates the presence of all the three genotypes i.e. BB, B+ and ++ . Suggesting that, in the past some out crossing has taken place among the sheep breed reared in the districts of North Bengal (Figures 3–5).

Historical findings

The erstwhile Government of Bengal records indicate that sheep were brought to Bengal by the traders from Tibet (Rennie, 1866; Firminger, 1920; Hunter, 1879). Therefore, there are possibilities that the *FecB* gene may also be present in the Sipsu sheep breed of Bhutan. The breeding tract of the Sipsu sheep is South Bhutan, adjacent to Cooch Behar and Jalpaiguri districts of India. Dorji

Figure 1. Location of all the zones from where the blood samples were collected.

Table 1. Zones from where the samples were collected

Zones	Neighbouring villages	District
1	Mahisadal, Geokhali	Midnapur (East)
2	Tona, Machibhanga, Rajarhat	24 Parganas (North)
3	Mynaguri, Malbazar, Dhupguri, Changrabadha, Pundibari*	Jalpaiguri, Coochbehar*
4	Joynagar Mazilpur (Hari Narayanpur, Rajapur Korabeg, Sripur and Uttar Durgapur)	24 Parganas (South)
5	Mandir Bazar (Laksmi-Narayanpur Dakshin, Laksmi-NarayanpurUttar, Mathurapur Paschim and Mathurapur Purba	24 Parganas (South)
6	Villages adjoining Basanti, Canning	24 Parganas (South)

*signifies that the villages around Punibari (from where the samples were collected) are situated in the district of Cooch Behar.

et al. (2003) reported that the Sipsu sheep is also a prolific sheep breed, in the region. Grenard (1974) reported that the sheep were used as a beast of burden by the transhumance community in Tibet. Pemberton (1839) in his observations also mentioned that the caravans from Tibet usually comprised mules, ponies, horses, yaks, sheep, etc. who could negotiate the narrow rugged paths in the mountains. Sheep was reared in Tibet for wool and most of the wool was traded to eastern India from the Jelep la pass, via Chumbi valley to Kalimpong, presently in Darjeeling district of West Bengal. Many traders from Bhutan terminated their journey at Cooch Behar, disposing their wares there. It was a commercial centre that developed from the sixteenth century onwards as a centre of exchange among various traders from Tibet, Bhutan, Sikkim and India, The last leg of the journey for the traders was from Cooch Behar to Rangpur. Hunter (1876) reported that the price of a Tibetan sheep was lower than that of a Nepali sheep (Bonpala). As the former had a lower body weight the Tibetan sheep could carry a load of 15–20 kg each as against the carrying capacity of 6–12 kg for the Bhutanese sheep and goat (Ratna Sarkar and Indrajit Ray, 2005).

Discussion

The presence of the *BMPR IB* gene both in Small Tail Han and Hu sheep of China (Yan Ya-Dong *et al.*, 2005; Chu *et al.*, 2007), support the present results. The Hu sheep originated from Mongolian sheep as early as in the Song Dynasty (AD 420–479). Mongolian sheep were introduced from the pastoral region of North China to the Taihu lake basin which borders the present provinces of Zhejiang and Jiangsu (Feng *et al.*, 1996). The migration of the ancestors of the sheep to India during the colonial era via the silk route may have led to the transfer of the gene to the sheep reared in West Bengal. Bell (1928) reported that merchants from Bhutan traded with China through Tibet during the nineteenth century. The major items of trade between China and Bhutan were sheep and woolen products besides other items such as rock salt and silk. Moreover, as the traders travelled long, it is logical to conclude that they used the rams as beast of burden. The rams being slightly heavier could take more loads. Moreover, ewes could delay movements of caravans due to lambing en-route. Therefore, suggesting that the *FecB* gene in sheep reared in the plains of Bengal and Bangladesh could have been transferred from the paternal linage only, similar inference regarding the transfer of *FecB* gene through paternal lines has been suggested by Davis (2008). This inference is in accordance with the mitochondrial (mt) DNA results, which suggests that the Hu sheep has haplotypes A, B and C (Chen *et al.*, 2006), while Garole has only haplotype A, (Meadows *et al.*, 2005). The mt DNA results on both the breeds suggest that the two breeds have distinctly different maternal lines while may be have similar paternal lines.

The currently available literatures on ovine breeds of India (Acharya, 1982; ICAR, 2002) indicate that the sheep reared in the Jalpaiguri and the Cooch Behar districts of

Figure 2. Garole ewe carrying *FecB* gene with her twins, Zone-4.

Table 2. Presence of *Fec B* gene in sheep at different locations studied.

Zones[1]	Number of observations	Fec BB/Fec BB	Fec BB/Fec B+	FecB+/FecB+	% homozygous
1	16	15	1	–	93.8
2	21	17	4	–	81
3	36	25	9	2	69.4
4	20	20	–	–	100
5	8	8	–	–	100
6	13	13	–	–	100

[1]Refer to Figures 1 for different zones.

Figure 3. Sheep carrying *FecB* gene, with her triplets, Moynaguri, district: Jalpaiguri, West Bengal, Zone-3.

Figure 4. Garole ewe with FecB mutation and her quadruplets, a case of super foetation, Zone-3.

Figure 5. Owner of sheep reared at Jalpaiguri, North Bengal, Zone-3.

India are of Bonpala breed. The Bonpala sheep (Figure 6) is a large breed of sheep and is reared in the district of Darjeeeling and some parts of the neighbouring state of Sikkim (Vij *et al.*, 1997). The Bonpala is not a prolific breed and does not harbour the BMPR-IB mutation.

At the termination of the journey at Cooch Behar and furthermore at Rangpur the sheep and ponies were traded off as it was not worthwhile to take them back all the way to Tibet. These animals were purchased by the local traders who then transported the sheep by boats to the southern parts of Bengal (now Bangladesh and West Bengal).

Figure 6. Bonpala sheep from Sikkim.

Hunter (1875) in his report on the Sunderban region of erstwhile Bengal reported that sheep were not traditionally reared by the farmers of the region. William Milburn (1813) reported trading of sheep at Fulta port which is situated on the bank of the river Hoogly, opposite to Geokhali in Zone-1.

The traded sheep at Fulta were the ones that were transported from Rangpur to the Sunderbans and were allowed to regain their condition at the local villages.

The sheep traded from Fulta were slaughtered for consumption by the sailors en route to different ports. It is possible that some of these sheep might have escaped or may have been dropped off or exchanged by the sailors in Java, the hypothesis is in accordance with the report by Bradford and Inounu (1996) regarding the progenitors of the Javanese thin-tailed sheep. They were also in opinion that of the Javanese thin-tailed sheep probably originated from the sheep brought from Bengal and Bangladesh. The presence of *FecB* gene in Garole sheep strongly supports the earlier claim by Austin (1943) and Guthrie (1957) regarding the transportation of Bengal sheep to Australia in the eighteenth century.

The present findings conclude and support the historical findings that the breeding tract of Garole sheep extends up to the Northern districts of West Bengal and is not limited to Sunderbans alone. Binny (2005) was in the opinion that the sheep transported to New South Wales, Australia during the nineteenth century by the ship Shar Hormuzear (from Bengal) were in fact the long haired Tibetan sheep. It can therefore be concluded that the *FecB* gene of the Garole sheep may have its origin from the ovine breeds of Tibet which were traded in the Northern parts of Bengal during the colonial era.

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