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Research Article

Genetic Diversity of Gorontalo Local Cattle Based on Microsatellite DNA

Safriyanto Dako[1], Nibras Karnain Laya[1], Syukri I Gubali[1], Ari Ardiantoro[2], V.M Ani Nurgiartiningsih[3], Gatot Ciptadi[3], Desinta Wulandari[3], Suyadi Suyadi[3]*

[1]Department of Animal Husbandry, Faculty of Agriculture, Gorontalo University, Gorontalo 96128, Indonesia; [2]Biotechnology Laboratory, Faculty of Animal Science, Universitas Brawijaya Malang, East Java 65145, Indonesia; [3]Faculty of Animal Science, Universitas Brawijaya Malang, East Java 65145, Indonesia.

Abstract | Microsatellites are one option for characterizing cattle populations; that information can be used as a basis for the development and maintenance of local cattle. This study aims to evaluate genetic diversity and relationships using microsatellite markers between the populations of Gorontalo cattle in Gorontalo Province, Indonesia, using 3 microsatellite markers. A total of 126 samples of local bovine blood were collected, which consisted of North Gorontalo (n=28), Gorontalo (n=52), Bonebolango (n=37), PO (n=3), and Bali (n=6), were used in this study. The Genomic DNA Mini Kits were used for DNA extraction for an analysis fragment in the microsatellite DNA region using ILSTS017, HEL13, and BM1818 primers. A total of 74 alleles were identified across entire populations. The expected heterozygosity ranged from 0.407±0.216 (Bali) to 0.716±0.050 (Gorontalo), and the observed heterozygosity ranged from 0.471±0.084 (Bonebolango) to 0.778±0.222 (PO). F statistical analysis includes F_{IS} 0.038, F_{IT} 0.248, and F_{ST} 0.231. The three microsatellite markers were moderate (0.25-0.5) to highly informative (PIC>0.5). The research showed that Bali cattle were distinct from all other cattle populations, while Gorontalo-Bonebolango admixture and North Gorontalo cattle were mixed with the PO cattle population. In conclusion, markers used were highly informative and polymorphic in investigating genetic diversity in Bonebolango, Gorontalo, and North Gorontalo populations, while the genetic relationship among cattle populations was divided into two main clusters, i.e., the Bali and PO populations, which closely reflect the breeding process in the research area. This information will be useful for future development and maintenance of local cattle in Gorontalo Province, Indonesia.

Keywords | Microsatellite, Genetic diversity, Genetic relationships, Local cattle, Gorontalo

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***Correspondence** | Suyadi Suyadi, Faculty of Animal Science, Universitas Brawijaya Malang, East Java 65145, Indonesia; **Email:** suyadi@ub.ac.id
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INTRODUCTION

The increased demand for livestock products as human food illustrates an increase in human quality of life but challenges the genetic availability of livestock. The development of local livestock as a provider of genetic resources needs to be preserved because local livestock have the ability to adapt to their own environment. This genetic ability is expected to produce more productive livestock (Gobel et al., 2021; Pribadi et al., 2015; Ilham et al., 2016).

Gorontalo province is located in Indonesia, specifically between Central Sulawesi and North Sulawesi. The potential for cattle in Gorontalo Province is 192,229 heads (BPS, 2020). There are several types of cattle that are well developed in the Gorontalo region, such as Bali cattle, PO cattle,

Table 1: Bovine microsatellite markers information used in study.

Marker	Chromosome	Primer sequences (5'>3')	Primer attachment temperatures (°C)	Length of DNA base (Bp)	Genebank access
ILSTS017				105-125	
HEL13 (D11S15)	11	F: TAAGGACTTGAGATAAGGAG R: CCATCTACCTCCATCTTAAC	52-57	178-200	X65207
BM1818 (D23S21)	23	F: AGCTGGGAATATAACCAAAGG R: AGTGCTTTCAAGGTCCATGC	56-60	248-278	G18391

Brahman cattle, and other local cattle that have not been identified. Local cattle that have not been identified are often called Diiti/ordinary/Local cattle. The existence of local cattle has a different phenotypic appearance compared to Bali and PO cattle. Naturally, livestock that adapts to the environment will bring up different phenotype variations in the population. Muladno (1994) state that genetic change can be used for the process of evolution of livestock populations/breeds by looking at the genetic distance of a population. Adaptability of local livestock describes a relative ability of an individual to survive and reproduce next generation to ensure continued survival of the population. Adaptations are mutations or genetic changes that help an organism or animal survive in its environment. Genetic adaptation is a biological characteristic with a heritable basis that improves reproduction and/or survival and results from evolution by natural selection. Increased animal performance based on genetic improvement results in more product produced per animal. Genetic improvements made in one generation are passed on to the next (Naskar et al., 2012; Mueller and Eenennaam, 2022). Efforts to maintain the genetic abilities that are formed in local cattle require adequate information, including qualitative characteristics, morphometrics, productivity, and genetics information, so that these local livestock can be developed in the future.

Microsatellites are an option for characterizing cattle breeds or populations (Metta et al., 2004; Rincon et al., 2007); they are also used to answer questions related to genetic diversity and genetic relationships among cattle populations (Rincon et al., 2007; Sun et al., 2008; Chaudhari et al., 2009). Microsatellite loci are widely used because of their high polymorphism, co-dominance, and relative abundance in the genome (Rincon et al., 2007; Karthickeyan et al., 2009). The study of microsatellite DNA diversity in local Indonesian cattle has been reported previously (Satriani et al., 2002; Sarbaini, 2004; Winaya et al., 2007; Abdullah, 2008; Sutarno et al., 2015; Septian and Sumantri, 2015; Agung et al., 2015, 2019), but has never been reported in local cattle in Gorontalo. It is necessary to have an overview of the genetic diversity analysis of local Gorontalo cattle based on microsatellite DNA analysis. The importance of this information can be used as a basis for the development and maintenance of local cattle. This study aims to evaluate genetic diversity and relationships using microsatellite markers between the populations of Gorontalo cattle in Gorontalo Province, Indonesia.

MATERIALS AND METHODS

Blood samples and DNA isolation

All procedures related to animal use in this study were approved by the Animal Care and Use Committee of Brawijaya University under regulation number 145-KEP-UB-2022 (Ethical Clearance). A total of 126 samples of local bovine blood were collected from 3 different regions: Gorontalo (n = 52), Bonebolango (n = 37), and North Gorontalo (n= 28), plus the PO (n= 3) and Bali (n= 6) population samples in the Gorontalo province, were used in this study. Meanwhile, blood samples were analyzed at the Biotechnology Laboratory, Faculty of Animal Science, Universitas Brawijaya, Malang. Blood was collected from the vena coxygealis of the cattle. The DNA was isolated using Genomic DNA Mini Kits. The DNA isolation procedures followed the protocol instructions.

Primer and DNA amplification

A total of 3 bovine microsatellite markers were used in the PCR process. The markers consisted of ILSTS017, HEL13, and BM1818 (Table 1). The PCR reaction contained a mixture of 1 µL DNA template (50–100 ng/µL) and 30 µL PCR premix. The PCR premix consisted of 0.4 µL primer (10 pmol/ µL), 15 µL Go Taq Green Master Mix (Promega, USA), and 14.2 µL Nucleus Free Water (NFW). The PCR thermal cycler conditions are shown in Table 2. The PCR product visualization methods were based on Susilorini et al. (2022). Microsatellite fragment analysis was conducted at First Base Laboratory, Selangor, Malaysia.

Table 2: The PCR thermal cycler condition.

PCR Step	Temperature (°C)	Time	Number of cycle
Pre-denaturation	95	5 minutes	Once
Denaturation	95	30 s	35 times
Annealing	58	45 s	
Extension	72	1 minute	
Final extension	72	5 minutes	Once

Microsatellite data analysis

The fragment analysis result was processed to *get al*lele size data. Allele data was then converted using Convert version 1.31 for further analysis. The converted data were analyzed using Cervus version 3.0.7 to generate observed heterozygosity (HO), expected heterozygosity (HE), frequency/number of alleles, polymorphism information content (PIC) values, and Hardy–Weinberg (HW) equilibrium. GenAlEx 6.51 b2 was used to generate genetic differentiation (FST), the rate of inbreeding between populations (FIS), the rate of inbreeding in populations (FIT), and principal coordinate analysis (PCoA) to determine the genetic relationship between livestock breeds. Genetic structures were analyzed using POPTREEW (POPTREEW website version) (Takezaki et al., 2014) to generate the reconstruction of phylogeny trees between populations and genetic distance.

RESULT AND DISCUSSION

Microsatellite polymorphisms and genetic diversity

Studies on the determination of genetic diversity in Gorontalo cattle based on microsatellite markers aren't available, so this study was conducted to find information about its diversity. The polymorphism of molecular genetics and the F-statistic belonging to each locus are summarized in Tables 3 and 4. A total of 74 alleles were detected through the 3 markers analyzed in entire populations: 8 alleles were detected in Bali cattle, 9 alleles in PO cattle, 20 alleles in Bonebolango cattle, 21 alleles in Gorontalo cattle, and 16 alleles in North Gorontalo cattle. The observed heterozygosity (OH) was 0.474, 0.407, 0.737, and the expected heterozygosity (EH) was 0.712, 0.658, and 0.829 for ILSTS017, HEL013, and BM1818, respectively.

Table 3: F Statistics (F_{IS}, F_{IT}, F_{ST}) between five cattle population.

Lokus	N	Ho	He	PIC	HW	F_{IS}	F_{IT}	F_{ST}	Nm
ILSTS017	95	0.474	0.712	0.699	***	0.412	0.530	0.200	0.999
HEL013	86	0.407	0.658	0.601	**	0.067	0.214	0.157	1.342
BM1818	95	0.737	0.803	0.803	***	-0.017	0.157	0.068	3.442
Mean						0.154	0.265	0.142	1.928
SD						0.131	0.140	0.039	0.764

Note: N=number of sample; Ho=observed heterozygosity; He=expected heterozygosity; PIC=polymorphism information content; HW= Hardy-Weinberg Equilibrium; FIS= inter-population inbreeding rate; FIT= inbreeding rate in population; FST= genetic differentiation; Nm= gene flow.

Table 4: Number of alleles (Na), observed heterozygosity (Ho), and expected (He), and Polymorphism Information Content in five cattle population in each loci.

Pop	Lokus	N	Na	Ne	PIC	Ho	He	HW
Bali	ILSTS017	6	3	1.946	0.424	0.167	0.530	ND
	HEL013	6	5	3.600	0.680	0.833	0.788	ND
	BM1818	6	6	5.143	0.777	0.833	0.879	ND
PO	ILSTS017	3	2	1.800	0.346	0.000	0.533	ND
	HEL013	3	3	2.571	0.535	1.000	0.733	ND
	BM1818	3	4	3.600	0.671	1.000	0.867	ND
Bolongo	ILSTS017	22	8	2.907	0.627	0.364	0.671	ND
	HEL013	17	4	2.429	0.513	0.412	0.606	ND
	BM1818	22	8	4.523	0.754	0.636	0.797	ND
Gorontalo	ILSTS017	41	9	3.543	0.682	0.488	0.727	*
	HEL013	37	5	2.700	0.564	0.351	0.638	*
	BM1818	41	7	5.033	0.777	0.732	0.811	ND
Gorontalo Utara	ILSTS017	23	4	2.574	0.553	0.696	0.625	ND
	HEL013	23	3	2.266	0.489	0.304	0.571	ND
	BM1818	23	9	5.290	0.785	0.783	0.829	ND

Note: N=number of sample; Na= Number of alleles; Ne= Mean number of effective alleles; Ho= observed heterozygosity; He= expected heterozygosity; PIC= polymorphism information content; HW= Hardy-Weinberg Equilibrium.

Table 5: Number of alleles (Na), observed heterozygosity (Ho), and expected (He), and polymorphism information content in five cattle breeds observed.

Breed	N	Na±SD	Ne±SD	Ho±SD	He±SD	PIC
A	6	4.67±0.88	3.563±0.923	0.611±0.222	0.671±0.096	0.627
B	3	3.00±0.58	2.657±0.521	0.667±0.333	0.593±0.081	0.518
C	22	6.68±1.33	3.286±0.634	0.471±0.084	0.674±0.056	0.631
D	41	7.00±1.16	3.759±0.682	0.524±0.111	0.716±0.050	0.674
E	23	5.33±1.86	3.377±0.961	0.594±0.147	0.660±0.077	0.609

Note: A= Bali cattle; B= PO cattle; C= Bolongo; D= Gorontalo; E= North Gorontalo; N= number of sample; Na= Number of alleles; Ne= Mean number of effective alleles; Ho= observed heterozygosity; He= expected heterozygosity; PIC= polymorphism information content; SD= standart deviation.

The genetic diversity belonging to each population was shown in Table 5. EH ranged from 0.407±0.216 (Bali) to 0.716±0.050 (Gorontalo), whereas OH ranged from 0.471±0.084 (Bonebolango) to 0.778±0.222 (PO). The EH value was higher than OH in all populations except in the PO and Bali populations.

Expected heterozygosity in all loci showed a higher value than observed heterozygosity, indicating that all loci deviated from HWE. The deviation of loci from HWE can be caused by several factors, such as selection, genetic drift, inbreeding, the presence of null alleles, selection in favor of homozygotes, and the Wahlund effect (Rahal et al., 2021). The EH value was lower compared to Algerian cattle (Rahal et al., 2021), but higher than the previous study in Indonesian cattle (Sutarno et al., 2015). The highest level of heterozygosity was in locus BM1818, which was higher than previous research in Red Steppe cattle using BM1818 loci, which obtained heterozygosity values of 0.692 (OH) and 0.701 (EH) (Kramarenko et al., 2018). The highest heterozygosity in the Gorontalo population (0.716±0.050) could be explained by the lack of a breeding program. The EH > OH value indicates low heterozygosity. Smith and Wang (2014) states that heterozygosity influenced by the variety of alleles and the frequency of each allele at each locus. Heterozygosity values range from 0 to 1. If heterozygosity equals 0 (zero), then the population being measured has a very close genetic relationship, and if the value is equal to 1 (one), then the population being measured has no genetic relationship.

The F-statistics consisting of F_{IS}, F_{IT}, and F_{ST} were 0,038, 0248, and 0.231, respectively. All microsatellite loci were deviated from the Hardy-Weinberg Equilibrium (HWE), meanwhile for all loci in each population, only locus ILSTS and HEL013 in Gorontalo cattle showed the deviation from HWE. The presence of null alleles in the study was between 6.15% (BM1818) and 24.01% (HEL013). The F_{ST} value of 23.1% indicated that 23.1% of the total genetic variation was due to allele differentiation between breeds, while the remaining 76.9% was due to the difference among individuals within the breed across the 3 markers. The average F_{ST} value of 0.231 indicated that the genetic difference in the population was high (0.15-0.25). Hartl and Clark (2007) state that the F_{ST} value of 0-0.05 (small), 0.05-0.15 (medium), 0.15-0.25 (high), and > 0.25 (very high). Obtained F_{ST} values were higher than Egyptian cattle (El-Sayeed et al., 2016), Algerian cattle (Rahal et al., 2021), South African Nguni cattle (Sanarana et al., 2016), and cattle raised in Turkey (Demir and Balcioğlu, 2019), but these values were lower compared to previous research in Indonesian cattle (Agung et al., 2019). The F_{IS} values were 0.412, 0.067, and -0.017 for ILSTS017, HEL013, and BM1818, respectively. The obtained F_{IS} value was used to gain a better understanding of the degree of inbreeding and endangered potentiality. A F_{IS} value less than 0.05 means the breeds are not in danger, 0.05-0.15 means the breeds are potentially endangered, 0.15-0.25 means the breeds are minimally endangered, 0.25-0.40 means the breeds are endangered, and >0.40 means the breeds are critically endangered (Simon and Bchenauer, 1993; El-Sayeed et al., 2016).

PIC values were found to be polymorphic between 0.601 (HEL013) and 0.803 (BM1818). The PIC value based on each population ranged from 0.353 to 0.674, which indicated a moderately to highly informative value. In the present study, the PIC values of three markers were highly informative (> 0.5), whereas the PIC values for ILSTS017 loci in the Bali and PO populations had low informative values (0.25–0.5). The PIC values in previous research on Egyptian cattle were 0.45 and 0.64 (El-Sayeed et al., 2016); those of Taro White cattle were 0.536 (Heryani et al., 2019); those of Zimbabwean Sanga cattle were 0.666–0.682 (Gororo et al., 2018); and those of Eastern European cattle were 0.752 (Illie et al., 2015). The PIC result showed that the markers are highly informative for the characterization of the three populations used in the study, i.e., the Bonebolango, Gorontalo, and North Gorontalo populations. The PIC value for identifying genetic diversity should be greater than 0.5.

Genetic structure and relationships

The result of genetic distance was used as basic data to create the visualization of a phylogeny tree (Table 6), which revealed the closest genetic distance between

Gorontalo-Bonebolango (0.037), followed by Gorontalo – North Gorontalo (0.043) and Bonebolango-North Gorontalo (0.078), respectively, while the farthest genetic distance was between Bali – PO (2.109). Estimation of the pair-wise F_{ST} value revealed the highest genetic differentiation between PO and Bali (0.206) and the lowest genetic differentiation between Bonebolango-North Gorontalo (0.011), Gorontalo-North Gorontalo (0.012), Bonebolango-Gorontalo (0.013), and PO-North Gorontalo (0.029). Low genetic differentiation indicated high genetic similarity between populations.

Table 6: Population pair-wise Fst (bottom diagonal) and Nei's standard genetic distance (top diagonal).

Populasi	Bali	PO	Bolon-go	Goronta-lo	Gorontalo Utara
Bali	***	0.053	0.959	0.714	0.807
PO	0.015	***	1.233	0.931	1.030
Bolongo	0.134	0.174	***	0.037	0.078
Gorontalo	0.104	0.142	0.008	***	0.043
Gorontalo Utara	0.128	0.166	0.017	0.010	***

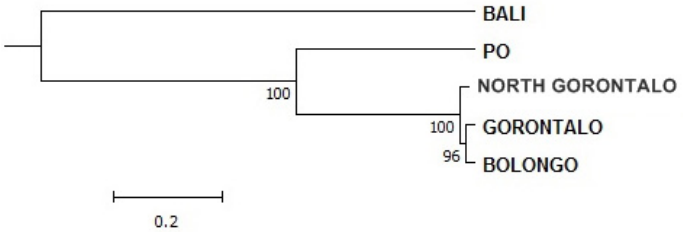

Figure 1: The reconstruction of the UPGMA phylogeny tree of Gorontalo cattle populations with Nei genetic distance.

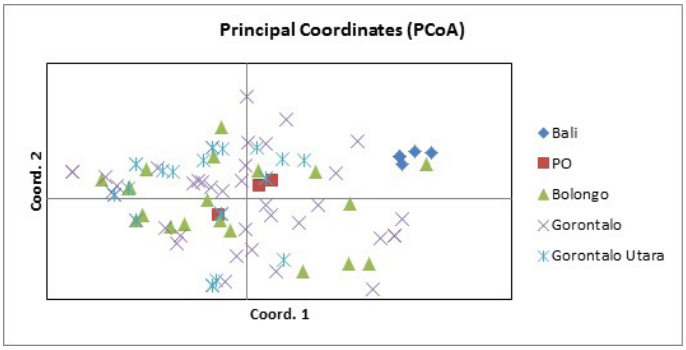

Figure 2: Principal coordinate analysis (PCoA) based on 3 microsatellite loci from 95 individual cattle in Indonesia. Bali= ◆; PO= ■; Bolongo= ▲; Gorontalo = ×; Gorontalo Utara= ✳.

Genetic distance analysis shows that the genetic distance between populations ranged from 0.037 to 2.109, while the pair-wise F_{ST} value ranged from 0.011 to 0.206. The reconstruction of the phylogeny tree based on Nei's genetic distance is shown in Figure 1. The figure revealed two main clusters of cattle populations. The first cluster consisted of Bali cattle, and the second cluster consisted of PO cattle. Clusters of PO population were divided into two subpopulations, i.e., the North Gorontalo population and the Bonebolango–Gorontalo population. The principal coordinate analysis (PCoA) result (Figure 2) supported the visualization of the phylogeny tree. It is shown that Bali cattle clearly separate from other cattle, while the population of PO cattle forms a cluster that contains local cattle in three different populations as subpopulations.

The reconstruction of a phylogeny tree using five cattle populations revealed a high gene share between Gorontalo and Bonebolango. The PCoA analysis confirmed (Figure 3) that Bali populations are distinct from other populations studied, whereas Gorontalo-Bonebolango admixture and North Gorontalo were mixed with the PO population. This is due to the distribution pattern of the arrival of cattle to Gorontalo province, which starts from North Gorontalo, and then the incoming cattle will be selected. After that, the cattle will be distributed to other areas, such as Gorontalo and Bonebolango. Cattle that entered North Sulawesi province (before Gorontalo province separated from North Sulawesi province) were dominated by PO cattle. Then, in 2000 (UU No. 38 of 2000), when Gorontalo officially became a separate province from North Sulawesi, the province of Gorontalo was dominated by Bali cattle. This distribution pattern allows for differences between the cattle populations in North Gorontalo and other areas. The similarity in cattle between the populations of Gorontalo and Bonebolango can be due to the fact that the two regions are close together, which allows for inbreeding between livestock and causes the appearance of the same genetics.

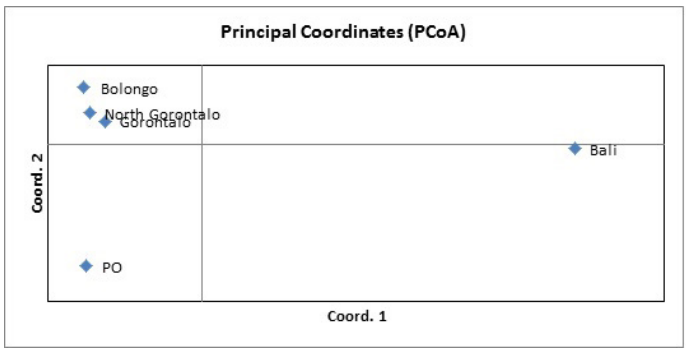

Figure 3: Principal coordinate analysis (PCoA) based on each population.

The populations of Bali and PO cattle form a different cluster. This is because Bali and PO cattle are different species; Bali cattle are included in *Bos sondaicus*, while PO cattle are in *Bos indicus*. The existence of PO cattle for more than two decades can cause a high share of genetic pool between PO cattle and local cattle in Gorontalo province. Samples of cattle in the populations of North Gorontalo, Gorontalo, and Bonebolango are local cattle in each of these

regions. The phenotypic appearance of the three cattle in the area has a dominant mixture of PO phenotype, a little Bali phenotype, and both cattle's phenotypes (Suyadi et al., 2014; Laya et al., 2020; Domili et al., 2021; Gobel et al., 2021). However, they are not said to be PO and Bali cattle (Figures 4, 5, and 6), but local cattle for each region. The study could serve as guidelines for future genetic studies on the development and maintenance of local cattle in Gorontalo Province, Indonesia.

Figure 4: Local cattle population of Gorontalo Regency.

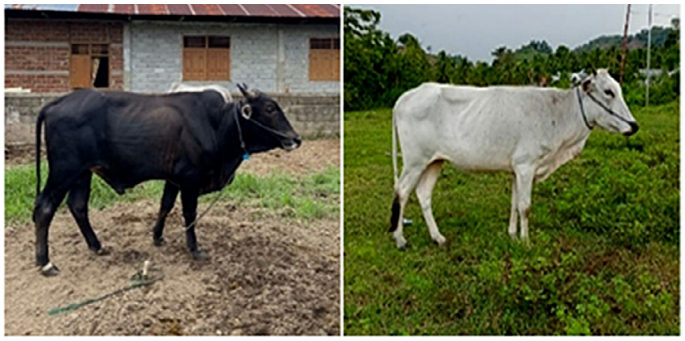

Figure 5: Local cattle population of North Gorontalo.

Figure 6: Local cattle of the Bonebolango population.

CONCLUSIONS AND RECOMMENDATIONS

Three markers used were highly informative and polymorphic in investigating genetic diversity in three cattle populations in Gorontalo Province, i.e., Bonebolango, Gorontalo, and North Gorontalo. There are two main clusters: (1) Bali cattle, which are distinct from all other cattle populations; and (2) the PO population, which includes the North Gorontalo, Bonebolango, and Gorontalo subpopulations and closely reflects the breeding process state in the research area. This information will be useful for the future development and maintenance of local cattle in Gorontalo Province, Indonesia, and it is recommended to standardize local Gorontalo cattle as typical local Gorontalo cattle.

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NOVELTY STATEMENT

1. Gorontalo cattle come from PO and Bali cattle which have been raised by the community for a long time.
2. Gorontalo local cattle are a new cluster resulting from a decrease in the genetic quality of PO and Bali cattle.

AUTHOR'S CONTRIBUTION

SD, SS, VMAN and GC idea and design. SD, NKL, SIG, AA and DW material sample collection and lab analysis. DW, SD and NKL write the manuscript. SD and SS: Revision.

Conflict of interest

The authors have declared no conflict of interest.

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