Admixture study of Ongole grade cattle based on genome-wide SNP data

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Abstract. We present population structure analysis of two differed Ongole grade cattle strains in Indonesia i.e., common Ongole grade (PO) and Kebumen Ongole grade (PO Kebumen). The aim of this study was to identify the genomic characteristic of both PO and PO Kebumen cattle strains. In this study, genotyping method of 15 PO and 25 PO Kebumen was carried out using an Illumina BovineSNP50 Bead Chip. In order to deeper analyses, we used additional 50K SNP data of 20 PO, 20 Nellore, 20 Brahman, 20 Simmental, and 20 Limousin that freely available in the data repositories. An estimation of the genetic population study was carried out using following approaches: pairwise fixation indices, heterozygosity and Nei’s standard genetic distance, and ancestral based admixture. These analyses revealed a B. taurus influence in PO but none in PO Kebumen. Furthermore, we also identified the close admixture of PO Kebumen to Nellore. Since Nellore was originated from Ongole cattle, we presume the PO Kebumen is the maintained Pure Ongole Breed in Indonesia.

1. Introduction

Ongole grade (PO) cattle are an important breed on the livestock development in Indonesia. It is because PO cattle have the potential on beef production, adaptable on tropical and under conditions of low external input in Indonesia [1]. Mature weight of bull (1.5-2 years old) reached 250-330 kg while cow was 280–300 kg (2-3 years old) in East Java [2]. Percentage of carcass of Ongole grade was around 49%
of carcass [3]. This cattle breed is believed to be the result of grading up system for local cattle in Java with Sumba Ongole cattle in 1905 to 1929 [4,5]. In 1991, the PO population was the highest one, reaching 46% of the total cattle population in Indonesia [4]. However, the current conditions are different, there has been a dominance artificial insemination (AI) in local cattle using straw from the sub-tropical cattle breeds [6].

Since 2010, a research team from the Assessment Institute for Agricultural Technology of Central Java carried out research and development of PO cattle, which are widely maintained throughout the Urut Sewu area, Kebumen District, Central Java. Based on the results of the study, the performance of PO cattle population in the area is superior than the common PO in other regions or those compared to Indonesian National Standard for Ongole grade [7,8]. Ongole grade cows in Kebumen regency (Karangreja, Tanggulangin and Brecong sub district) on 25-72 months have wither height at 135-136 cm, while the chest circumference and body length were 59-161 cm and 135-138 cm, respectively [8]. Ongole in the other sub district at Kebumen i.e Klirong, Buluspesantran, Mirit, Puring, Ambal and Petanahan have almost the same of body size and weight, in mature PO cows (more than 24 months) have 371.98 kg for body weight, 130.97 cm for wither height, 143.07 cm for body length and 165.53 cm for chest circumference while mature PO bulls have body weight, wither height, body length, and chest circumference were 457.39 kg, 137.45 cm, 138.04 cm, and 175.19 cm, respectively [9]. Therefore, the PO in Kebumen was determined to be the different PO strain by the Ministry of Agriculture (Decree No. 358/Kpts/PK.040/6/2015), i.e. Kebumen Ongole grade (PO Kebumen). Even so, many people still have question, what the difference is between common PO and PO Kebumen cattle.

Molecular techniques approach is one of tools to distinguish ancestral of cattle and also to investigate the potential gene that associated with phenotype traits. Recently, we are in genome sequence era and Illumina company released many types of microarray for bovine i.e BovineSNP50 BeadChip (53,218 SNPs), BovineHD Genotyping BeadChip (777,000 SNPs), and GeneSeek GGP Bovine 150K that used to genome-wide analysis. Based on history, the microarray SNP Chips was created from long studies and was begun from Quantitative Trait Loci Study. Quantitative Trait Loci (QTL) is a technique for mapping in genome-wide inference of the relationship between genotype at various genomic location and the phenotype for a set of quantitative traits in terms of the number, genomic positions, effects, interaction and pleiotropy of QTL. The primary of QTL mapping is to localize chromosome regions that significantly affected quantitative trait in population [10]. In April 2001, SNP Research Center (SRC) in Tokyo Japan collaborated with Human Genome Center (HGC), Institute of Medical Science (IMS), University Tokyo, and the Japan Science and Technologies (JST) begun to develop technologies in whole genome sequencing in human with identify 150,000 SNPs. The first draft of sequence was published in 2001 and then 2002, Tanaka’s group research in SRC successfully identified a susceptibility gene for myocardinal infraction (the first GWAS (Genome Wide Association Study) in the world) [11]. And now, many studies used this technique to identify candidate gene and SNP for create a marker tool included in livestock. Several economic traits in beef cattle was explored as like growth traits [12], carcass traits [13] and reproductive traits [14]. Furthermore, GWAS studies were applied in dairy cattle [15] and goat [16].

In another way, the data of genotyping from microarray analysis can be used to know the ancestral through Admixture analysis. For examples, utilization of BovineHD Genotyping BeadChip (777K) in research was the study of adaptive introgression from indicine cattle into white cattle breeds from Central Italy [17]. Another study in 2020 has published which concern to know the pattern of admixture, divergence, and ancestry of African cattle populations determined from genome-wide SNP data [18].

This study is intended to provide answers to these questions by exploring the genetic structure of the population and identifying the ancestral admixture of both PO and PO Kebumen. In order to get more accurate results, we used the Bovine 50K SNP data which were collected from various sources. The results of this study are expected to be useful as a material that can be considered in the future policy development of Ongole grade in Indonesia.
2. Materials and Methods

2.1. Cattle Genome Data Sources
Cattle genome data used in this study amounted to 140 samples, all of them were the result of standard genotyping process using Bovine 50K SNP chip (Illumina). The Ongole grade (PO1) and Kebumen Ongole grade (PO Kebumen/KEB) were collected from Sudrajad [19], with amount 15 and 25 samples, respectively. The additional 20 samples of Ongole grade (PO2) were accessed in the publication appendix [20], while the other cattle breeds, i.e. Nellore (NEL), Brahman (BRH), Simmental (SIM), and Limousin (LIM) samples, 20 samples of each were downloaded from the public data repository (http://datadryad.org/; [21]).

2.2. Genome Data Quality Control
The low quality of genome data was eliminated using the PLINK v1.07 program [22] based on the following quality control criteria: 1) SNP variations will be maintained if the Hardy-Weinberg Equilibrium value is not less than 1 x 10^{-4}; 2) SNP call rate is greater than 90%; 3) Minor allele frequency less than 1%; 4) Empty SNP variants in each individual and empty genotypes in each variant must not exceed 10% [19]. We used the autosome in analyses.

2.3. Genomic Population Structure Analysis
A series genomic population study was carried out by the following approaches: 1) Pairwise fixation indices using 2 R packages i.e., hierfstat [23] and StAMPP [24]; 2) Heterozygosity and Nei’s standard genetic distance [25]; 3) Ancestral based admixture using fastStructure [26] and visualized using R v.3.2.2 [27].

3. Results and Discussion
The summary of the genome structure analyses results are written in Table 1. Data were distinguished based on the cattle population, hence, there were two Ongole grades (i.e. PO1 and PO2). Based on the table, it can be seen that in general the heterozygosity of taurine cattle (SIM and LIM) was higher compared to those in indicine (PO1, PO2, KEB, NEL, and BRH). The same results were found in previous studies. Iranian cattle, Jersey and Holstein cattle (taurine) have heterozygosity value 0.32 and 0.36, respectively while heterozygosity of Brahman, Sistani, Kermani, Pars, and Nadji cattle (indicine) were 0.23, 0.23, 0.27, 0.26 and 0.31, respectively [28]. The other study reported that African taurine (N’Dama and Sheko) have heterozygosity value 0.27 and 0.26, respectively and then European taurine (Brown Swiss, Hereford, Holstein, Jersey and Limousin) have 0.33-0.38 of heterozygosity value. Indicine cattle like Brahman, Nelore and Gir have 0.22, 0.20, and 0.20, respectively [29].

Meanwhile, the inbreeding coefficient (F_{IS}) was opposite, indicine has higher value than taurine. The same result and pattern of F_{IS} value was found in several previous studies [28,29]. Furthermore, Kebumen Ongole Grade (KEB) has lower heterozygosity and moderate inbreeding coefficients compared to those in Ongole Grade (PO1 and PO2). This gives an indication that the variant in the PO1 and PO2 cattle population has a higher level of diversity. This is possible because there were intensive Artificial Insemination (AI) applied using both indicine and taurine straws. Since 1990s, many PO cattle were mated with taurine cattle (Simmental and Limousin) and resulted in Simpo or Limpo crossbred. The farmer or breeder supporting the program crossbreeding because the calves have a good performance and faster growth compared with PO cattle. At the age of the three years, the hybrid cattle (Simpo and Limpo) are able to reach 800 kg but in PO cattle only has a half of it [30]. Meanwhile, stock of frozen semen Limousin and Simmental cattle at BBIB Singosari were higher than the other taurine and indicine cattle. In 2020, Artificial Insemination Institute Singosari (Balai Besar Inseminasi Buatan) has 557,762 frozen semen doses of Limousin and 285,657 doses of Simmental (https://bbibsingsosari.ditjenphk.pertanian.go.id). It showed that taurine cattle (Simmental and Limousin) were favourable cattle breed for breeding mating.
However, the application of AI is very limited in Kebumen Ongole Grade (KEB) population due to the breeding system and farmer preference. Farmers prefer to use natural mating system for their cows with the superior bull in their area. They have their own qualifications regarding the superior KEB bull. Previous study reported that 91.44% of farmer in Kebumen region used natural mating for their cattle [31]. The use of limited bull in breeding system will reduce the genetic variation [32].

### Table 1. Summary of the cattle population structure

| Population Structure | Cattle Population\(^4\) | PO1 | PO2 | KEB | NEL | BRH | SIM | LIM |
|----------------------|--------------------------|-----|-----|-----|-----|-----|-----|-----|
| No. Samples          |                          | 15  | 20  | 25  | 20  | 20  | 20  | 20  |
| \(H_0\)\(^1\)        |                          | 0.24| 0.27| 0.21| 0.21| 0.24| 0.48| 0.48|
| \(H_e\)\(^2\)        |                          | 0.19| 0.21| 0.17| 0.16| 0.19| 0.34| 0.35|
| F\(IS\)\(^3\)        |                          | -0.15| -0.17| -0.16| -0.18| -0.15| -0.31| -0.30|

\(^1\)Observed Heterozygosity; \(^2\) Expected Heterozygosity; \(^3\) Inbreeding coefficients; \(^4\) PO1 and PO2: Ongole grade, KEB: Kebumen Ongole grade, NEL: Nellore, BRH: Brahman, SIM: Simmental, LIM: Limousin

We have conducted population differentiation analyses by the \(F_{ST}\) and Nei’s genetic distance (Table 2). Both \(F_{ST}\) and Nei's analyses showed the relatedness levels of populations. Based on Table 2, we got several evidence:

1. There were different conditions and relatedness levels of PO population in Indonesia. It might be caused by the different management and breeding system on each PO population. PO1 was originated from Yogyakarta and Boyolali while PO2 was originated from East Java. Among Indonesian Ongole grade, KEB is closer to PO1 than PO2.
2. KEB is closer to NEL than BRH. This is an affirmation that KEB is closely related to NEL. This also dismissed the notion that KEB are descendant of BRH.

### Table 2. Pairwise \(F_{ST}\) (lower diagonal) and Nei's genetic distances between populations (upper diagonal)

| Cattle Population\(^1\) | PO1     | PO2     | KEB     | NEL     | BRH     | SIM     | LIM     |
|--------------------------|---------|---------|---------|---------|---------|---------|---------|
| PO1                      | 0       | 0.0031  | 0.0023  | 0.0036  | 0.0040  | 0.0400  | 0.0379  |
| PO2                      | 0.0009  | 0       | 0.0032  | 0.0044  | 0.0047  | 0.0380  | 0.0358  |
| KEB                      | 0.0004  | 0.0015  | 0       | 0.0030  | 0.0038  | 0.0415  | 0.0394  |
| NEL                      | 0.0016  | 0.0026  | 0.0015  | 0       | 0.0048  | 0.0421  | 0.0400  |
| BRH                      | 0.0018  | 0.0027  | 0.0021  | 0.0030  | 0       | 0.0398  | 0.0377  |
| SIM                      | 0.0383  | 0.0368  | 0.0408  | 0.0411  | 0.0387  | 0       | 0.0118  |
| LIM                      | 0.0359  | 0.0344  | 0.0384  | 0.0388  | 0.0364  | 0.0087  | 0       |

\(^1\) PO1 and PO2: Ongole grade, KEB: Kebumen Ongole grade, NEL: Nellore, BRH: Brahman, SIM: Simmental, LIM: Limousin

We performed admixture analysis with \(K=2\) to \(K=3\) in order to get detail of genetic composition of the cattle populations. The result is depicted in Figure 1 and showed clear discrepancy between indicines and taurines breed i.e., colored as brown and blue, respectively. There were hybridization cases on both Ongole grade cattle populations (PO1 and PO2) with taurines. PO 2 from East Java has higher taurine composition than PO1 from Yogyakarta and Boyolali (Figure 1) and it caused genetic distance of both Ongole grade was a little bit far to Kebumen Ongole grade (KEB) (0.31 to 0.15). In this study it was found that Kebumen Ongole grade (KEB) has no taurine composition or pure indicine while in other ongole grade such hybridization exists. The other study, based on partial cytochrome b gene in mitochondrial DNA, showed that Kebumen Ongole grade have the same sequence and indicated have the same origin from maternal inheritance with Brahman cattle [33]. Brahman cattle is produced by
cross-breeding Kankrej, Gujarat, Nellore (or Ongole) and the Gir (or Gyr) strains (https://bee2live.com/story-brahman-cattle-breed-106-104641 ). There are two types of Brahman cattle American, Red Brahman and Gray Brahman. The American Gray Brahman is primarily a mixture of Guzerat and Nellore with some influence from other Zebu breeds while The Red Brahman is a mixture of Gir and Indu-Brazil with some Guzerat influence. In both of the Red and Gray Brahman cattle have a small influence of European breeds that were used in the grading up process [34].

Kebumen Ongole grade (KEB) mixture is similar to NEL (Nellore cattle), pure of indicine. This result supporting the hypothesis that KEB is close to NEL. The Ongole cattle breed, also known as Nellore, is the native cattle from the coastal districts Guntur, Prakasham and Nellore of Andhra Pradesh [35] and classified of Zebu breed cattle. Based on history, Nellore cattle in Brazil was originated from India. The first Ongole cattle arrived in Brazil at the end of the 18th century [36] or 150 year Before Present (yBP) [37] and rapidly developed in Brazil. Nellore breed cattle is exclusively explored for meat production.

**Figure 1.** Ancestral based admixture for Ongole grade cattle in Indonesia (PO1, PO2, PO Kebumen/KEB) compared to other indicine (Nellore/NEL, Brahman/BRH) and taurine (Simmental/SIM, Limousin/LIM)

Genetic characterization through admixture study is important to investigate the composition breed in the cattle and for conservation-making decisions in livestock or the breeding program. In this study, we have found interesting information about Kebumen Ongole Grade. Based on admixture study using Bovine DNA SNP Chip 50K, Kebumen Ongole Grade tend to close mixture with Nellore or Ongole breed cattle (Zebu cattle) rather than Brahman cattle. So, we presume the Kebumen Ongole Grade (KEB) is the maintained Pure Ongole breed in Indonesia.

### 4. Conclusion
There was genetic evidence to distinguish common PO and PO Kebumen. The hybridization cases might occur in common PO but has not been proven in PO Kebumen. PO Kebumen can be presumed as the maintained Pure Ongole breed in Indonesia.

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