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The Utilization of the SDS-PAGE Salivary Protein Profiles for Clustering Analysis of Bali Cattle (*Bos sondaicus/javanicus*) and the *Taurine* or *Zebu* Cross Breeds in North Lombok District, Indonesia: A Preliminary Study

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Abstract

The main aim of this study is to investigate the salivary protein profile of Bali cattle (*Bos sondaicus/javanicus*) and their crossbred descendents with *Bos taurus* or *Bos indicus* in the country side farm of North Lombok District, Indonesia. A total of 52 samples of saliva collected from Bali cattle, a mix of Bali cattle and taurine or zebu cattle, and a pure taurine dairy cattle, were investigated through SDS-PAGE. Intra and inter specific relationships were estimated using Jaccard's similarity and Euclidean distance index. Dendograms based on cluster analysis, and hierarchical analyses using Ward's method were developed. It was revealed that cluster analyses of salivary proteins in the range of 17-45 kDa were able to be classified and disengaged from the breed or species in the population in this study. It can thus be concluded that saliva has a good prospect as an alternative biological material for phylogenetic studies in the population. It is explicable that further verification using molecular genetics technology, should be carried out before the concept is decided to be put into daily practice.

Keywords: *Bos sondaicus/javanicus*; *Bos indicus*; *Bos taurus*; Saliva; Dendogram

Introduction

Bali cattle (*Bos sondaicus/javanicus*) are an indigenous cattle breed of Indonesia, domesticated form of wild banteng [1,2]. These cattle are of beef cattle type, with relatively small body size, weighing about 250-350 kg, although there are cattle that also weigh up to 600-800 kg [3]. Farmers in Indonesia like to keep these cattle because they are easy to handle, have high adaptability, and are resistant to adverse conditions [4].

In the late 1970s or early 1980s, efforts to improve the quality of Bali cattle have been carried out through artificial insemination programs, or by the introgression of superior bulls of taurine and zebu breeds such as Simmentals, Herefords, Limousines and Brahman Angus or Brangus [5].

In further developments, many farmers are interested in the descendants of crossbred cattle, due to the increase in size. After more than three decades, certain regions in Indonesia seek to refine the local cattle, including Bali cattle to preserve them as the native cattle breed [6]. For these reasons, a screening method in the field or on farm is needed. There are some relationship analysis studies based on morphological phenotypes of body size or physical characteristics, which have been carried out to screen the local cattle breed from the cross cattle breed in a population. In addition, genetic information through DNA blood analysis has also been used as a gold standard. However, the latter, is admittedly still too expensive for farmers.

In the last decade, saliva has been used as an alternative biological material for studying the physiological status of the animal [7], as well as to compare one animal species to another [8]. An advantage of using saliva is that saliva is taken by a non-invasive procedure compared to blood which is taken invasively. This enables farmers, especially in the countryside, to agree for their cattle to be used as research subjects.

Based on these considerations, we have conducted a preliminary study on comparing the salivary protein profiles to analyze whether the protein profiles can be used as indicators of genetic relatedness between Bali cattle and the taurine or zebu crossbreed descendants in a community farm in North Lombok District, Indonesia.

Materials and Methods

Determination of subjects

The main material of this study is saliva, which is isolated from healthy, non-pregnant adult cattle, maintained by farmers in North Lombok District (~1200 m above sea level, masl) in Lombok Island, Indonesia (Figure 1). The cattle were fed King grass and the local roughages available around the farm mixed with rice bran twice a day, while the drinking water supplied *ad libitum*. A total of 23 cattle, selected by purposive sampling were used, consisting of Bali cattle, and cattle of taurine or zebu cross breeds. The type of cattle was determined based on physical characteristics from each type of cattle breed. Bali cattle are easily distinguished from taurine (*Bos taurus*) or zebu cattle (*Bos indicus*). Their coat color is very distinctive, usually reddish-brown, except for a clearly defined white area on the hindquarters that extends along the belly, and also white socks reaching from the hooves to just above the hocks compiled by Lindell [9].

Saliva was collected and treated as described previously [10] from oral cavity of the cattle, using disposable plastic pipettes. About 5-10 ml of saliva collected in disposable glass tubes containing preservative/bactericidal (0.2% v/v NaN_3) were placed in a cool box containing ice and immediately taken to the laboratory for further treatments.

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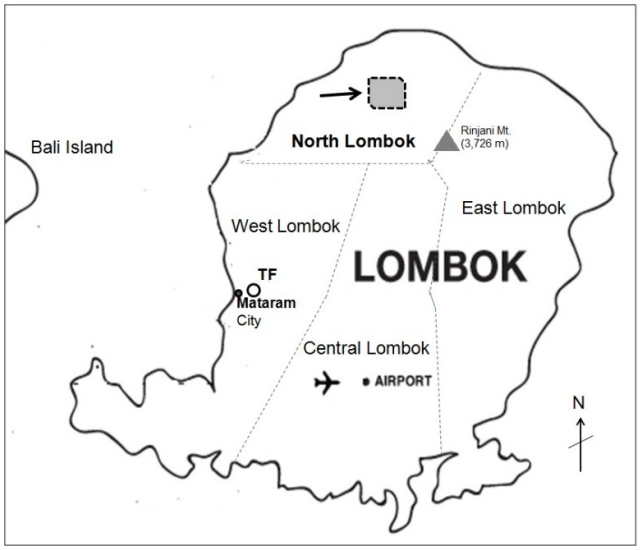

Figure 1: Map of Lombok, Indonesia, showing the location of the farm (arrow) in North Lombok District, and the Teaching Farm of Mataram University located in West Lombok District (TF, open circle) where the saliva samples were collected.

As a comparison, salivary profiles of 28 Bali cattle reared in the Teaching Faculty of Animal Science, Mataram University, West Lombok District were used as representation of the native local Bali cattle. These cattle do not have blood relations with those cattle in the study site in North Lombok District, and have never been crossed with other breeds. In addition, salivary profiles of Friesen Holstein cattle, as a representation of *Bos taurus* was also included (Courtesy: Dr. Wheeler TT, AgResearch Centre, Hamilton, NZ).

Sample preparation, protein estimation and SDS-PAGE profiling

In the laboratory, the saliva was centrifuged at 3000 rpm, 4°C, for 5 minutes, and the supernatant was aliquoated, and frozen at -80°C. Salivary protein concentration was determined using a NanoDrop 2000cSpectrophotometer (Thermo Fisher Scientific, USA) at a wavelength of 280 nm. All samples were further diluted with phosphate buffered saline at pH 7.4 and brought to a 1 mg/ml concentration. Electrophoresis was carried out under denaturing conditions in a discontinuous 1-D SDS-PAGE system using 12.5% acrylamide according to Laemmli [11], performed at a constant voltage of 100 V. Each sample was mixed with loading buffer and loaded at 10 µg per well after boiling for 4 minutes at 100°C. As a molecular weight standard, a protein molecular marker also ran along with the saliva samples according to the manufacture's instruction (Intron, Biotechnology). Gels were stained using Coomassie brilliant blue dye R-250 (Sigma, Aldrich), scanned and documented for analyzing purposes.

Band scoring and analysis

The banding patterns of the gel were analyzed manually and molecular weight values for various bands were calculated based on its relative migration distance or retardation factor (Rf), plotted into a graph as suggested by [12]. Interpolating the value from this graph then gave the molecular weight of the unknown protein band. The process was carried out with the assistance of Microsoft Office Excel 2007.

In this study salivary protein between the species in the region of 17-45 kDa molecular weights were preferred for analyzing due to some specific markers for bovine saliva that have been found in that range [10,13-15]. Only the unambiguous bands were scored or coded for the presence (scored 1) or absence (scored 0) of each saliva sample. Furthermore, similarity matrixes based on Jaccard's coefficient [16] were created for each group breed of cattle. Cattle of each group with Jaccard's similarity coefficient value of ≥ 0.75 were merged. With these values, dendograms were generated based on cluster analysis with Jaccard's and Euclidean similarity distance. In addition, a cluster tree was also elaborated using Ward's method. Tabulation and analyzing data were carried out using Excel Microsoft Office for Windows in combination with PAST (Paleontological Statistics) software package [17].

Results and Discussion

Morphological determination of the cattle used in this study

This study used local Bali cattle (*Bos sondaicus/javanicus*) and taurine or zebu crossbred cattle. The taurine or zebu crossbred cattle were introduced at the location of this study about 20-30 years ago. Some representations of photographs of the cattle, is presented in Figure 2. It is clear that, there are morphological differences between the local Bali cattle (Figure 2a) and the crossbreed cattle. Unfortunately, there is no good recording at the farm level, but from the farmers' testimony, it was informed that the cattle that they raise are the results of crossbreeding process. Furthermore, they named the cattle as Simbal after the Simmental and Bali cross (Figure 2b), Brangbal after the crossing of Brangus × Bali cattle (Figure 2c), Limbal for descendants crossing of Limousine and Bali cattle (Figure 2d), while Herbal is the nickname for the results of cross-breeding between Hereford and Bali cattle (Figure 2e).

SDS-PAGE salivary protein profiles

SDS-PAGE analysis had resulted about 7-12 protein bands for the salivary proteins of cattle in this study as presented in Figures 3a and 3b. Of the various bands obtained, in general, it appears that there are inter and intra-specific relationships between individual cattle. Of those bands generated, the bands with estimated molecular weight (Mr) between 17 and 45 kDa were selected. This is because that molecular weight range meets the requirements of manual calculation procedure based on Rf, as suggested by Hames [12]. Furthermore, as stated in the methods, from the 52 samples used, the individual cattle of the same breed that had homolog salivary protein profile were aligned and merged according to Jaccard's similarity value of ≥ 0.75. As a result, a total of 29 out of the 52 samples were developed (Table 1).

It can be seen in Table 1 that there are some predominant markers found in nearly all subjects (i.e., assigned as 1), namely proteins of 17 kDa, 18 kDa and 21 kDa. These protein markers are possibly in line with the peptides reported by Depamede [10] in Bali cattle saliva known as predicted zymogen granule protein 16 homologue (G3MZ19) with a nominal mass (Mr) 17.708 kDa, and Pancreatic adenocarcinoma upregulated factor-like (FINZ8) with a relative molecular weight (Mr) of 22.091 kDa. Furthermore a phylogenetic tree based on Bali cattle G3MZ19 gene has also been reported [15].

Phylogenetic analysis

In connection with the possibility of utilizing the salivary protein profiles to differentiate and determine relationships between local Bali cattle (*Bos sondaicus/javanicus*) and the descendant crossbreed between Bali cattle (*Bos sondaicus/javanicus*) and *Bos taurus* (Simbal,

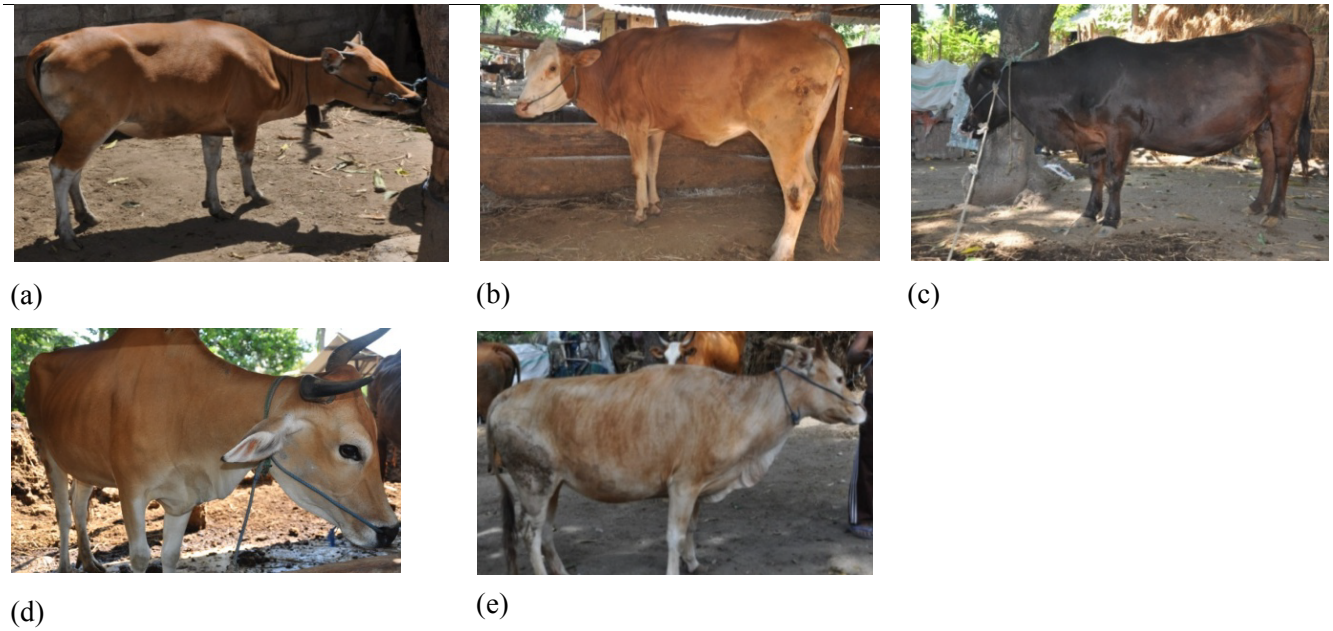

Figure 2: Representation of cattle used as source of saliva in this study reared by the farmers in Lombok North District (LND). Name of the type of species or breeds was based on the name given by the farmer. (a) Local Bali cattle; (b) Simbal: Simmental × Bali cattle crossbreed; (c) Brangbal: Brangus × Bali cattle crossbreed; (d) Limbal: Limousine × Bali cattle crossbreed; (e) Herbal: Hereford × Bali cattle crossbreeds.

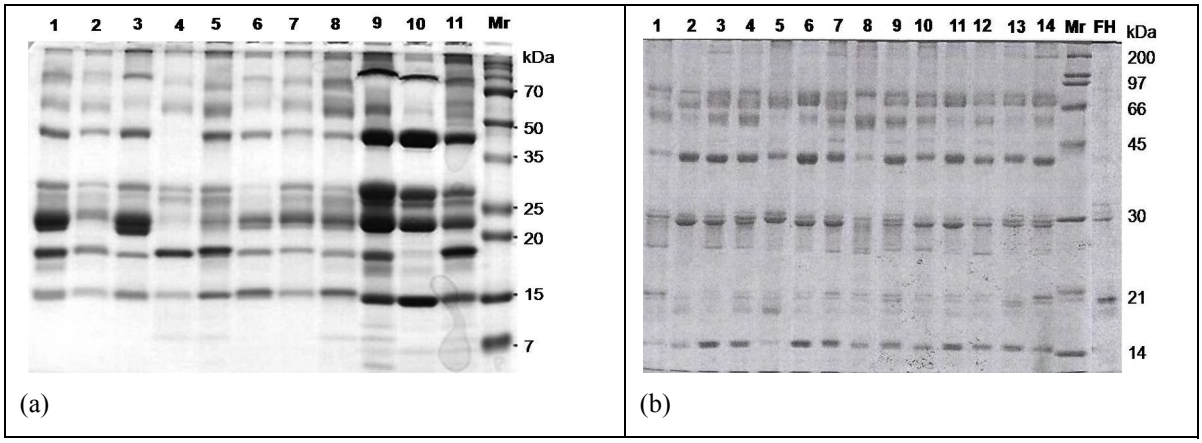

Figure 3: (a) Representation of protein profiles of Bali cattle saliva collected from Local Farm at North Lombok District (1-11). Simbal (1-2), Brangbal (3-5), Brahbal (6-8), Bali cattle (9-11). Mr: molecular weight marker. The protein profiles showing inter and intra specific relationships. (b) Representation of protein profiles of Bali cattle saliva collected from Teaching Farm (1-14) and a New Zealand dairy cattle saliva (FH). Mr: molecular weight marker.

Table 1: Molecular weight values for various bands of cattle salivary proteins based on SDSPAGE analysis after synchronization according to Jaccard's similarity distance within each cattle breed group.

No.	MW (kDa) / Sample Code	45	39	37	36	30	29	28	25	21	20	18	17
1.	Bal-KLU.01	1	0	0	1	0	0	0	0	1	0	1	1
2.	Bal-KLU.02	0	0	0	1	0	0	0	1	1	1	1	1
3.	Brahbal.01	0	0	0	1	0	0	0	1	1	0	1	0
4.	Brahbal.02	0	0	0	1	0	0	0	1	1	1	0	1
5.	Brahbal.03	0	0	1	0	0	0	0	1	1	0	1	0
6.	Brahbal.04	1	0	0	1	0	0	0	0	1	0	1	1
7.	Brangbal.01	0	0	0	1	0	0	0	1	0	1	1	0
8.	Brangbal.02	1	0	0	0	0	0	0	1	0	0	1	0
9.	Brangbal.03	1	0	1	0	0	0	0	0	1	1	0	1

10.	Herbal.01	1	0	0	1	0	0	0	1	1	0	1	1
11.	Herbal.02	0	1	0	0	0	0	0	0	1	1	0	1
12.	Herbal.03	1	0	1	0	0	0	0	1	1	0	0	1
13.	Herbal.04	0	0	1	0	0	0	0	0	1	0	1	1
14.	Limbal.01	1	0	0	1	0	0	0	0	1	1	0	1
15.	Simbal.01	0	0	0	1	0	0	0	1	0	1	1	0
16.	Simbal.02	1	0	0	1	0	0	0	1	1	1	1	0
17.	Simbal.03	0	0	0	1	0	0	0	0	1	1	0	1
18.	Simbal.04	0	0	1	0	0	0	0	0	1	1	0	1
19.	Bal. 1	0	1	0	0	0	0	1	1	1	0	1	0
20.	Bal. 7	0	1	0	0	0	0	0	1	0	0	0	0
21.	Bal. 10	0	1	0	0	0	0	1	0	1	0	0	0
22.	Bal. 11	0	1	0	0	0	0	1	0	0	0	1	0
23.	Bal. 14	0	1	0	0	0	0	0	1	1	0	1	0
24.	Bal. 15	0	1	0	0	0	0	1	0	0	0	1	0
25.	Bal. 17	0	1	0	0	0	0	1	1	1	0	0	0
26.	Bal. 19	0	1	0	0	0	0	1	1	0	0	1	0
27.	Bal. 21	1	1	0	0	0	0	0	0	0	0	1	1
28.	Bal. 24	0	1	0	0	0	0	1	0	0	0	1	0
29.	NZ. FH	0	0	0	0	1	1	1	0	1	1	0	0

No. 1-18: Cattle reared in NLD farm; **No. 19-28:** Cattle reared on Teaching Farm, Faculty of Animal Science, Mataram University. Bal-KLU: NLD-Bali cattle; Brahbal: Brahman × Bali cattle crossbreed; Brangbal: Brangus × Bali cattle crossbreed; Herbal: Hereford × Bali cattle crossbreed; Limbal: Limousine × Bali cattle crossbreed; Simbal: Simmental × Bali cattle crossbreed; Bal.: Bali cattle and **NZ. FH:** New Zealand Frisian Holstein cattle.

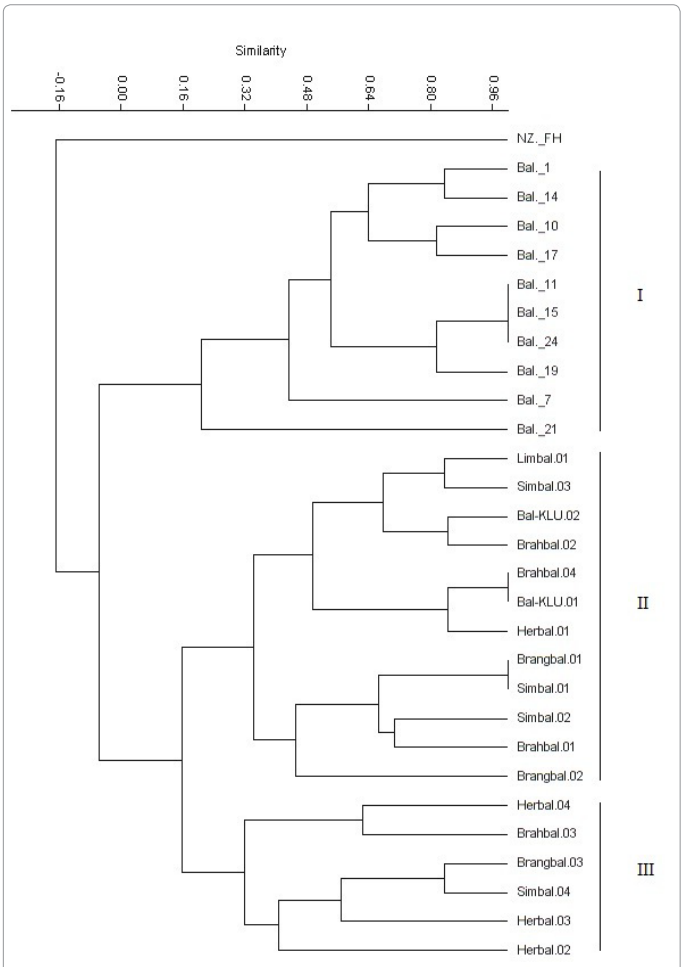

Figure 4a: A cluster analysis based on Jaccard similarity.

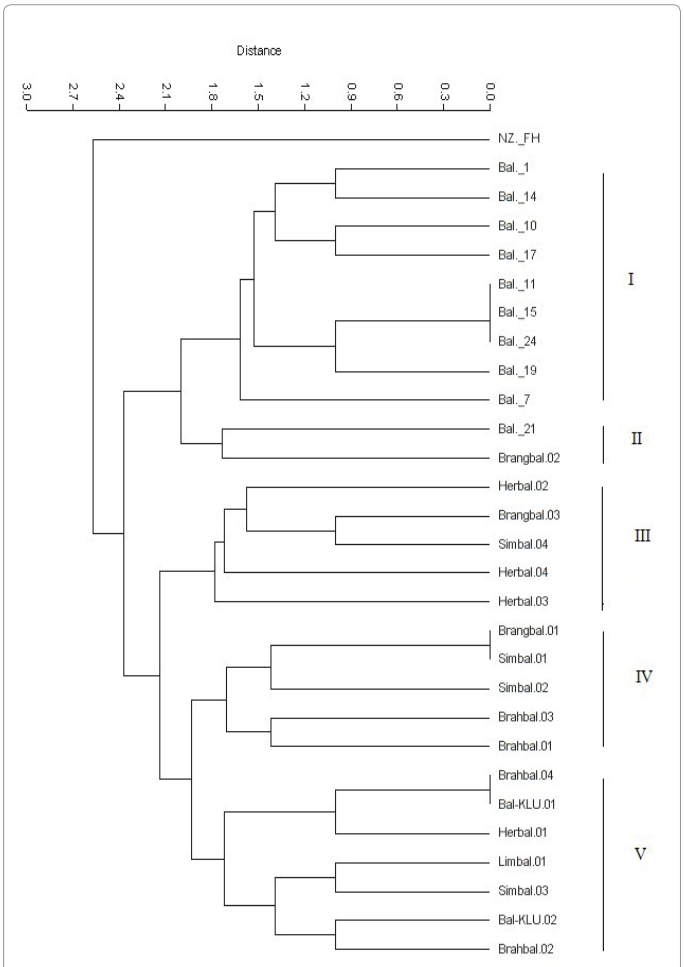

Figure 4b: A cluster analysis based on the Euclidean distance.

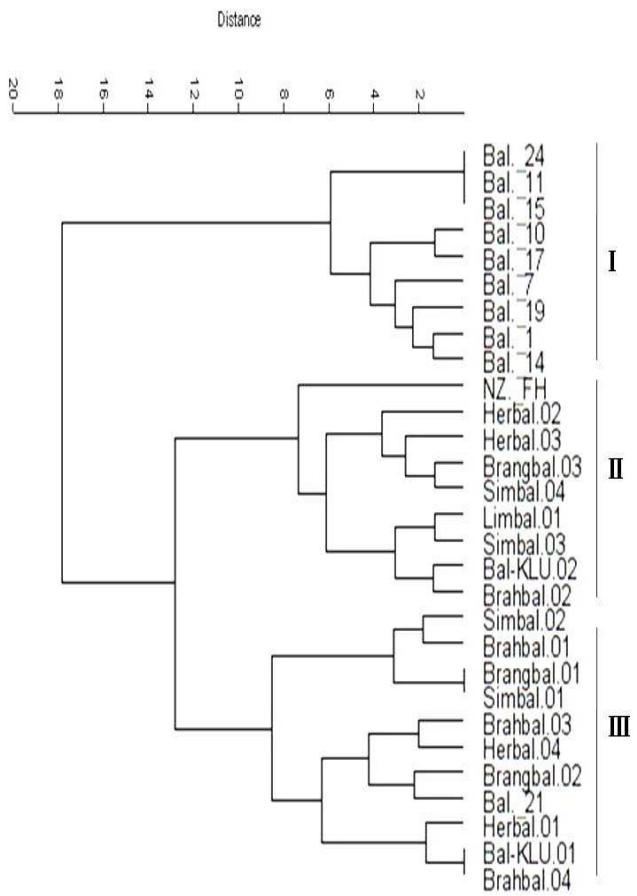

Figure 4c: Dendogram created by hierarchical cluster analysis of the data using Ward's method and Euclidean distance.

Herbal, and Limbal) or Zebu cattle/*Bos indicus* (Brangbal and Brahbal), the phylogenetic tree was constructed. Dendograms were constructed based on Cluster analysis with Jaccard's similarity and Euclidean distance matrix (Figures 4a and 4b) and hierarchical cluster based on Ward's method (Figure 4c).

The dendograms with Jaccard's similarity or Euclidean distance give only one major cluster i.e., the local breed (Bali cattle) and their crossbred descendants, with the New Zealand Friesian Holstein (NZFH) cattle in the out group position (Figures 4a and 4b). This is quite reasonable since NZFH is known as taurine dairy cattle, different from the others, which are of the beef type cattle. However, the dendogram that was generated based on hierarchical cluster analysis using Ward's method and Euclidean distance, gives two main clusters. Interestingly in this dendogram, NZFH dairy cattle is not in the out group position but fall into the group that consists of local crossbred cattle i.e., descendants of Brahman × Bali cattle crossbreeds (Brangbal) and Simmental × Bali cattle crossbreeds (Simbal).

If we observe closer, from all dendograms it can be clearly seen that the cattle in this study are grouped into orderly and organized clusters or sub clusters. It can be seen that all Bali cattle which were reared in the Teaching farm of Faculty of Animal Science, Mataram University fall into one sub cluster (I). The other sub clusters (II and III or II-V) consisted of the local cattle reared by the farmers at North Lombok District, the site of this study. The distribution pattern of the dendogram was in line with the nature of the samples. Bali cattle at the Teaching Farm were selected for research purposes based on the morphological characteristics of pure Bali cattle as compiled by [9]. On the other hand, the cattle reared by the farmers at the site of this study were of the progeny of crossbred between Bali cattle and *Bos taurus* or *Bos indicus* descendants. And this crossbreeding has been going on for a long time. It began with an artificial insemination program (late 1970s), and followed by the natural mating among descendent crosses without proper selection process conducted by farmers, which is quite a common practice in small holder farms [18,19].

Farmers carried out selection or cross mating their cattle, possibly without good management control. The crossbred cattle present until today, seem to be the cattle that have been able to survive better than others. The survival and distribution of the mixing crossbreds between imported breeds and the local breeds in North Lombok District is therefore quite reasonable since the district is a highland area (~1200 masl), which is suitable for the development of the offspring of crossbred cattle between native cattle (*Bos sondaicus/javanicus*) and the taurine or zebu descendant cattle [1,5,20].

In hierarchical analysis (Figure 4c) it can be clearly seen that New Zealand dairy cattle (NZFH) exist in cluster II, even though its position is separated from the local cross breed cattle. It is quite hard to explain about this, however we may speculate that the position of NZFH as representation of taurine cattle in this dendogram, strengthen the facts that cattle reared by the farmers at the site of this study were mixed crossbred cattle.

Overall, it can be seen that in general the lineage of the cattle in the site of the present study can still be traced based on their salivary protein profiles. In other words, saliva has potential prospect to be used as an alternative biological source to help the farmers examine their cattle descent. Especially for screening purposes on farms in which, taking blood samples is a serious concern. However obviously, it must be further followed by verification using molecular genetics technology, before it is decided to put it into daily practice.

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References

1. Mohamad K, Olsson M, van Tol HT, Mikko S, Vlamings BH, et al. (2009) On the origin of Indonesian cattle. PLoS One 4: e5490.
2. Nijman IJ, Otsen M, Verkaar EL, de Ruijter C, Hanekamp E, et al. (2003) Hybridization of banteng (*Bos javanicus*) and zebu (*Bos indicus*) revealed by mitochondrial DNA, satellite DNA, AFLP and microsatellites. Heredity 90: 10-16.
3. Martojo H (2003) Indigenous Bali Cattle: The Best Suited Cattle Breed for Sustainable Small Farming in Indonesia. In: The Relationship between Indigenous Animals and Humans in APEC Region. Chang HL, Huang YC (Eds.). Chinese Society of Animal Science, Taiwan, pp: 21-36.
4. Martojo H (2012) Indigenous Bali cattle is most suitable for sustainable small farming in Indonesia. Reprod Domest Anim 47: 10-14.
5. Setyawan AD (2015) Review: genetic diversity of local and exotic cattle and their crossbreeding impact on the quality of Indonesian cattle. Biodiversitas 16: 327-354.
6. Purwantara B, Noor RR, Andersson G, Rodriguez-Martinez H (2012) Banteng and Bali cattle in Indonesia: status and forecasts. Reprod Domest Anim 47: 2-6.

7. Lamy E, Mau M (2012) Saliva proteomics as an emerging, non-invasive tool to study livestock physiology, nutrition and diseases. J Proteom 75: 4251-4258.

8. Lamy E, Da Costa G, Silva FC, Potes J, Coelho AV, et al. (2008) Comparison of electrophoretic protein profiles from sheep and goat parotid saliva. J Chem Ecol 34: 388-397.

9. Lindell IC (2013) Phenotyping of Bali cattle and interviewing farmers in Indonesia-a minor field study. Department of Animal Breeding and Genetics, Swedish University of Agricultural Sciences.

10. Depamede SN (2013) Proteomic Analysis of a 14.2 kDa Protein Isolated from Bali Cattle (Bos Sondaicus/Javanicus) Saliva Using Single Dimension Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis and Matrix-Assisted Laser Desorption Ionization Time-of-Flight Tandem Mass Spectrometer. Ital J Anim Sci 12: e59.

11. Laemmli UK (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227: 680-685.

12. Hames BD (1998) Gel electrophoresis of proteins: A practical approach. 3rd edn. Oxford University Press, Oxford, New York, USA.

13. Wheeler TT, Haigh BJ, McCracken JY, Wilkins RJ, Morris CA, et al. (2002) The BSP30 salivary proteins from cattle, LUNX/PLUNC and von Ebner's minor salivary gland protein are members of the PSP/LBP superfamily of proteins. Biochim Biophys Acta 1579: 92-100.

14. Wheeler TT, Haigh BJ, Broadhurst MK, Hood KA, Maqbool NJ (2011) The BPI-like/PLUNC family proteins in cattle. Biochem Soc Trans 39: 1006-1011.

15. Depamede SN (2015) Expression of the Fragment Gene of Predicted Zymogen Granule Protein (G3MZ19) Homologue in the Bali Cattle (Bos Javanicus) Based on MALDITOF/TOF.MS Analysis of Bali Cattle Saliva. J Vet Sci Technol 6: 260.

16. Real R, Vargas JM (1996) The probabilistic basis of jaccard's index of similarity. Systematic Biology 45: 380-386.

17. Hammer O, Harper DAT, Ryan PD (2001) PAST: Paleontological Statistic software package for education and data analysis. Paleontologica Electronica 4: 1-9.

18. Talib C, Siregar AR, Budiarti-Turner S, Diwyanto K (2003) Implementation of a breeding program for Bali cattle. Technical issues at national and regional levels. Strategies to improve Bali cattle in Eastern Indonesia. ACIAR proceedings 110: 82-85.

19. Talib C, Siregar AR, Diwyanto K (2003) Options for genetic improvement of Bali cattle-assessing the strengths and weaknesses of alternative strategies. Option 3. Expensive technologies and AI deleted. Strategies to improve Bali cattle in Eastern Indonesia. ACIAR proceedings 110: 76-78.

20. Mohamad K, Olsson M, Andersson G, Purwantara B, van Tol HTA, et al. (2012) The Origin of Indonesian Cattle and Conservation Genetics of the Bali Cattle Breed. Reprod Domest Anim 47: 18-20.