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Total RNA isolation from cell-free saliva of surti Buffalo: A non-invasive approach for biomarker detection

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Abstract
Saliva emerges as a particularly promising fluid resource for biomarker detection. Unlike milk and urine samples, saliva collection imposes no time restrictions, making it a convenient option for researchers and farmers alike. Non-invasive methods for collecting biomarkers in water buffaloes are indeed advantageous due to their ease, simplicity, and minimal stress on the animals. Saliva contains a diverse array of biomarkers that can provide valuable insights into the health, well-being, and physiological status of water buffaloes. These biomarkers can range from proteins and enzymes to hormones and genetic material, offering a comprehensive profile for monitoring various aspects of animal health and performance. Furthermore, saliva collection is relatively non-invasive, causing minimal discomfort or stress to the animals. This aspect is crucial for maintaining the welfare of the buffaloes while ensuring accurate and reliable biomarker analysis. By leveraging saliva as a rich source of biomarkers, researchers and livestock professionals can implement non-invasive monitoring programs to track the health, reproductive status, and disease susceptibility of water buffaloes effectively. This approach not only enhances animal welfare but also facilitates early detection of health issues and enables timely intervention strategies, ultimately contributing to improved management practices and productivity in buffalo farming operations.

Keywords: Cell free saliva, Total RNA isolation, Estrous cycle, Surti Buffalo

Introduction
Water buffalo saliva indeed possesses unique characteristics that make it a valuable biological fluid for biomarker detection (Maddu, 2019) [4]. Its clear appearance and basic pH of more than 9.0 distinguish it from other bodily fluids, such as blood or urine (Pandit *et* al., 2013, Sánchez *et al*., 2021) [9, 12]. The composition of water buffalo saliva is complex, reflecting contributions from various salivary glands, including the parotid, sublingual, and submandibular glands (Pasha *et al.*, 2018) [10]. Saliva is a mirror of the body because it is a plasma ultrafiltrate, this means most biomarkers found in blood are also present in saliva (Li *et al*., 2004; Onteru *et al*., 2016) [3, 8]. Saliva contains specific biomolecules like mRNA, miRNA, DNA, or protein that reflect the individual's physiology or disease condition. Biomolecules including hormones, metabolites, DNAs and RNAs from the blood can enter saliva through transcellular and paracellular routes (Ang *et al.*, 2011) [1]. The proteome of the whole saliva will be relevant to oral health and be crucial for the identification of meaningful biomarkers for oral disease (Muthukumar *et al.*, 2014; Shashikumar *et al*., 2018; Singha *et al*., 2022) [6, 13, 14]. Salivary levels of estrogen and progesterone vary at different phases of the estrous cycle in buffaloes (Ravinder *et al*., 2016) [11].

Materials and Methods
Collection of the saliva
Twelve Surti buffaloes (six dam-daughter pairs) maintained at Livestock Research Station, Navsari were selected for the study Saliva was collected during estrus and diestrus phases of

estrous cycle from all the twelve animals. The sample collection was generally done during morning hours on the day of estrus and diestrus. The animals were kept off feed and no water intake for at least 2 to 3 hours before saliva collection. About 10 ml saliva samples from each animal were collected in 15 ml sterile centrifuge tube using IV (Intra Venous) set tube. One cut end of the tube was intubated in mouth (lower jaw) of the animal and 20 ml syringe was attached with another end of the tube to aspirate the saliva. The aspirated saliva samples were transferred to sterile 15 ml centrifuge tube. The samples were immediately kept on ice and transferred to the laboratory.

Total RNA extraction

About 250 µL of supernatant or cell free saliva samples were transferred to microcentrifuge tubes for RNA extraction. Total RNA was extracted using TRIzol comprising of phenol and guanidinium isothiocyanate as follows. Microcentrifuge tube containing 250 µL of sample with 750 µL of TRIzol reagent were added. The mixture was vortexed and allowed to stand at room temperature for 10 min. After incubation, 200 µL of chloroform was added and the mixture was again vortexed vigorously for 15 sec. After 10 min incubation at room temperature, the mixture was centrifuged at 13000g for 10 min at 4°C. The aqueous supernatant containing total RNA was recovered and mixed with 600 µL isopropyl alcohol. The mixture was kept at -40 °C overnight. Next day the mixture was centrifuged at 13000g for 10 min at 4 °C to obtain the pellet. Supernatant was discarded and the pellet was washed with 1 ml of 75% ethanol followed by vortexing of 15 sec. The mixture was centrifuged at 13000g for 10 min at 4 °C. The Supernatant was discarded and the pellet was air dried. Properly air-dried pellet was dissolved in 20 µL of RNase free water. Isolated RNA was stored at - 80 °C till further use. Purity and quantification of RNA was done using Nanodrop spectrophotometric (Thermo Scientific ND 2000_C) analysis with the convention that one absorbance unit at 260 nm wavelength equals 40 µg RNA per ml. The samples with a 260/280 ratio between 1.8-2.0 were used for further processing. Moreover, quality and integrity of the total RNA was checked by performing denaturing formaldehyde agarose gel electrophoresis in 1 percent agarose gel and visualizing under UV light Gel documentation system. The RNA samples with two distinct bands of 28S and 18S were quite clear were considered as acceptable for further processing.

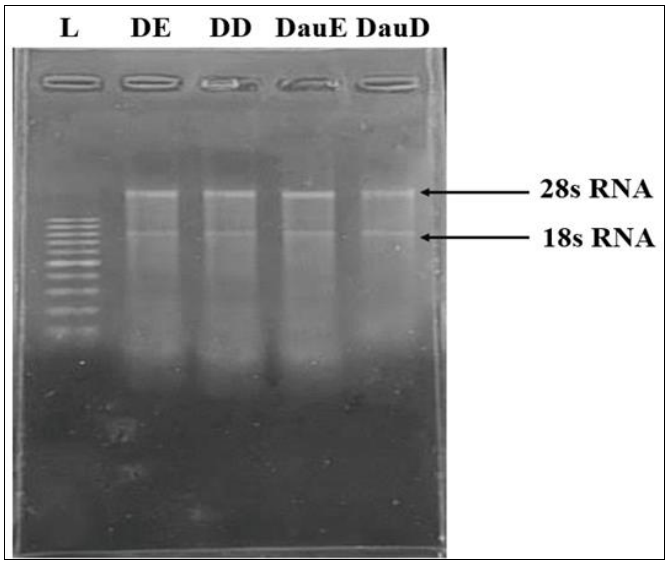
Results and Discussion

RNA isolation

About 15 - 150 ng/µL concentration of total RNA in 20 µL volume was obtained from 250 µL of saliva samples. Spectrophotometric reading of isolated RNA (after DNase treatment) at OD_{260} and OD_{280} was taken, which ranged between 2.0 to 2.2 which indicated that total RNA was without DNA and protein contamination. Average total RNA yield in all the 24 samples was 45.3±8.51 ng/µL. On an average total RNA yield was 905±170 ng from 250 µL of cell free saliva. Integrity of RNA was verified by 1 percent agarose gel electrophoresis where in single distinct bands of 28s RNA with almost twice the intensity compared to single compact band of 18s RNA were observed on visualization under gel documentation system.

The reports of RNA extraction from buffalo saliva were found at smaller extent. However, the report of RNA extraction from buffalo saliva was by Lal Krishna *et al.* (2021) [2] in which they could obtain 25.69±7.49 ng/µL (n = 8) total RNA from cell-free saliva. In another report from Surla *et al.* (2022) [15], average total RNA yield from six buffalo salivary samples was 385.35 ng.

Fig 1: Collection of saliva from lower jaw of mouth by IV set tube in Surti buffalo



L = ladder (100 bp), DE = Dam estrus, DD = Dam diestrus, DauE = Daughter estrus, DauD = Daughter diestrus

Fig. 2: One percent formaldehyde agarose gel electrophoresis of RNA samples

However, the RNA obtained in the present study was higher than reported by Lal Krishna *et al.* (2021) [2] and Surla *et al.* (2022) [15]. Saliva has been more widely used as source of biomarkers for diagnostic purpose in humans. Li *et al.* (2004) [3] reported that in human on an average 60.5±13.1 ng (n = 10) of total RNA was obtained from 560 µL cell-free saliva using RNA extraction kit. By comparing to human studies, Pandit *et al.* (2013) [9] reported highest RNA yield from human saliva as 987 ng/µL which is much higher than that obtained in the present study. Majem *et al.* (2017) [5] found 50-80 ng/ml RNA from human saliva. Oh *et al.* (2020) [7] reported on average 75.2±20.3 ng (n = 57) of total RNA from 500 µL of human saliva supernatant. Higher amount of total RNA from the human saliva samples as compared to buffalo saliva samples may be attributed to ease and convenience of sample collection and characteristic of the sample.

Conclusion

Saliva could be used as a non-invasive biological source for total RNA extraction in buffaloes. Despite appearing to be higher, the RNA quantity and quality found in buffalo saliva may be overestimated by the existence of plant phenolic chemicals, which are often found in high concentrations in the saliva of ruminants as a result of their diets. However, total RNA extracted from saliva could be further used for cDNA synthesis and relative gene expression studies.

References

1. Ang CS, Binos S, Knight MI, Moate PJ, Cocks BG, McDonagh MB. Global Survey of the Bovine Salivary Proteome: Integrating Multidimensional Prefractionation, Targeted, and Glycocapture Strategies. J Proteome Res. 2011;10(11):5059-5069. doi:10.1021/pr200516d
2. Lal Krishna GNS, Kumar OS. RNA Isolation from Cell Free-Saliva of Water Buffalo. Int. J Curr Microbiol Appl. Sci. 2021;10(7):752-764. doi:10.20546/ijcmas.2021.1007.082
3. Li Y, St. John MAR, Zhou X, Kim Y, Sinha U, Jordan RCK, Eisele D, Abemayor E, Elashoff D, Park N-H, Wong DT. Salivary Transcriptome Diagnostics for Oral Cancer Detection. Clin Cancer Res. 2004;10(24):8442-8450. doi:10.1158/1078-0432.CCR-04-1167
4. Maddu N. Functions of Saliva. In: Gokul S, editor. Saliva and Salivary Diagnostics. Intech Open; 2019. doi:10.5772/intechopen.84709
5. Majem B, Li F, Sun J, Wong DTW. RNA Sequencing Analysis of Salivary Extracellular RNA. In: Seymour GJ, Cullinan MP, Heng NCK, editors. Oral Biology. Springer New York; c2017. p. 17-36. doi:10.1007/978-1-4939-6685-1_2
6. Muthukumar S, Rajkumar R, Rajesh D, Saibaba G, Liao C, Archunan G, et al. Exploration of Salivary Proteins in Buffalo: An Approach to Find Marker Proteins for Estrus. FASEB J. 2014;28(11):4700-4709. doi:10.1096/fj.14-252288
7. Oh SY, Kang SM, Kang SH, Lee HJ, Kwon TG, Kim JW, Lee ST, Choi SY, Hong SH. Potential Salivary mRNA Biomarkers for Early Detection of Oral Cancer. J Clin Med. 2020;9(1):243. doi:10.3390/jcm9010243
8. Onteru SK, Baddela VS, Ravinder R, Kaipa O, Nayan V, Singh P, et al. Direct Saliva Transcript Analysis as a Novel Non-Invasive Method for Oestrus Marker Detection in Buffaloes. Biomarkers. 2016;21(2):99-101. doi:10.3109/1354750X.2015.1118549
9. Pandit P, Cooper-White J, Punyadeera C. High-Yield RNA-Extraction Method for Saliva. Clin Chem. 2013;59(7):1118-1122. doi:10.1373/clinchem.2012.197863
10. Pasha S, Inui T, Chapple I, Harris S, Holcombe L, Grant MM. The Saliva Proteome of Dogs: Variations Within and Between Breeds and Between Species. Proteomics. 2018;18(3-4):1-7. doi:10.1002/pmic.201700293
11. Ravinder R, Kaipa O, Baddela VS, Singhal Sinha E, Singh P, Nayan V, et al. Saliva Ferning, an Unorthodox Estrus Detection Method in Water Buffaloes (*Bubalus bubalis*). Theriogenology. 2016;86(5):1147-1155. doi:10.1016/j.theriogenology.2016.04.004
12. Sánchez J, Fuentes N, Ibañez-López FJ, López-García I, Gutiérrez AM. A Multi-Herd Study Shows That Saliva Is More Than a Reflection of Serum Biomarkers in Pigs. Animal. 2021;15(12):1-12. doi:10.1016/j.animal.2021.100413
13. Shashikumar NG, Baithalu RK, Bathla S, Ali SA, Rawat P, Kumaresan A, et al. Global Proteomic Analysis of Water Buffalo (*Bubalus bubalis*) Saliva at Different Stages of Estrus Cycle Using High Throughput Mass Spectrometry. Theriogenology. 2022;110:52-60. doi:10.1016/j.theriogenology.2017.12.046
14. Singha S, Pandey M, Jaiswal L, Dash S, Fernandes A, Kumaresan A, et al. Salivary Cell-Free HSD17B1 and HSPA1A Transcripts as Potential Biomarkers for Estrus Identification in Buffaloes (*Bubalus bubalis*). Animal Biotechnology. 2022;1-11. doi:10.1080/10495398.2022.2105228
15. Surla GN, Kumar LK, Vedamurthy VG, Singh D, Onteru SK. Salivary TIMP1 and Predicted miR-141, Possible Transcript Biomarkers for Estrus in the Buffalo (*Bubalus bubalis*). Reprod Biol. 2022;22(2):100641. doi:10.1016/j.repbio.2022.100641