

## Short Communication

# AGEING AND THE AMOUNT OF DNA EXTRACTED FROM BAWEAN DEER (*Axis kuhlii*) FECES

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### ABSTRACT

Noninvasive genetic sampling is the most reliable approach to collect endangered species which are typically rare and elusive. However, the approach is hampered by poor quality and quantity of DNA amount, and therefore, a pilot study is required. Our current short communication article reports the first noninvasive genetic sampling approach using feces of critically endangered Bawean deer (*Axis kuhlii*) to test the effect of aging on the amount of DNA extraction. Fecal samples of the Bawean deer were collected from the Bawean Deer Captive Unit in Sangkapura Village, Bawean Island, East Java. The samples were grouped into two different categories, namely: 1) New samples: for feces that had just been expelled for less than a day and 2) Old samples: for feces that were estimated to have been expelled for more than a day. There were 11 New samples and 16 Old samples. The samples were extracted using the QIAGEN Mini Stool Kit. The DNA quantification was carried out by using a fluorometer. The results of the extraction between the two categories were analyzed by the Kruskal Wallis test. The results showed that the mean rank value of the New samples was better (15.27) than the Old samples (13.13). The P value of the Kruskal Wallis test, however, was higher than the Asymp significant value, indicating that there was no meaningful differences ( $P = 0.49$ ) between the two samples categories. The work described in this short communication article is a preliminary result and an important step in the study of Bawean deer population genetic.

**Keywords:** Bawean deer, biodiversity, critically endangered, DNA, noninvasive sampling

### INTRODUCTION

Conservation strategies for endangered species require genetic information (Whiteley *et al.* 2015) that can be difficult to obtain using a traditional method such as invasive sampling and destructive sampling. In small and elusive wildlife populations, noninvasive DNA sampling was successfully employed for obtaining genetic data (Kohn & Wayne 1997). However, the effective use of noninvasive samples for genetic analyses of wildlife populations is hampered by typically poor quality and low quantity of the DNA obtained (Taberlet & Waits 1999).

In large animals, DNA samples can be obtained from fluids, hair and feces gathered in

the field. However, these types of samples usually provide DNA of low quantity, quality, and integrity, causing the difficulties in analyzing some molecular markers (Venegas *et al.* 2020). DNA of the depositor is present in sloughed intestinal epithelial cells (Waits & Paetkau 2005). DNA degradation in fecal DNA is enhanced by ageing of the samples and by the action of variable environmental conditions (e.g., temperature, humidity, exposure to sun or rain) (Nsubuga *et al.* 2004). The effectiveness of genetic analysis using fecal samples would be improved if some of the factors affecting DNA yield could be identified and used as guidelines in sample collection.

Recent study has analyzed the effect of ageing and environmental condition on the amount of DNA extraction from deer feces

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(Brinkman *et al.* 2010; Ebert *et al.* 2021; Gupta *et al.* 2018). However, these studies were conducted on temperate climate conditions. To our knowledge, such study has not been conducted in tropical environments which have different UV radiation from sunlight and humidity.

Our current short communication article reports the first noninvasive genetic approach using feces of critically endangered Bawean deer (*Axis kublii*) to test ageing on the amount of DNA extraction.

## MATERIALS AND METHODS

### Sample Collection

The Bawean Deer feces were collected from the Bawean Deer Captive Unit at Bawean Island, East Java. The fecal samples were categorized as New for feces that has probably been deposited for less than one day and Old for feces that has been deposited for more than 1 day. The two categories of feces were differentiated by evaluating its appearance and by monitoring the depositor on the captivity.

The feces were collected using a feces container added with silica granules and labeled.

The fecal samples were collected on August 2021. The fecal DNA were immediately extracted at the Laboratory of Bioresources and Veterinary Technology, UGM Vocational College.

### DNA Extraction

The DNA extraction was carried out by using QiAmp DNA Stool Mini Kit (QiaGEN) following the manufacturer's protocol. The results of the extraction (double stranded DNA only) were measured by a fluorometer (Quantus Fluorometer, Promega).

## RESULTS AND DISCUSSION

The 27 collected Bawean deer feces were categorized as the New and Old feces. The new category is characterized by the moist and glittering appearance, while the old category is relatively dry and harsh in appearance (Figs. 1 & 2).



Figure 1 Bawean Deer feces categorized as New with the moist and glittering appearance



Figure 2 Bawean Deer feces categorized as Old with the dry and harsh appearance

The DNA extraction showed different quantities of DNA at different sample conditions as shown in Table 1.

Table 1 Quantification of DNA Extraction from Bawean Deer Feces

New samples (ng/μL)	Old samples (ng/μL)
0.615	0.73
0.993	1.245
2.061	0.805
1.512	0.73
0.079	1.106
2.857	1.655
12.224	6.352
2.613	8.82
5.845	0.066
7.123	0.207
3.179	7.727
	1.189
	3.057
	1.972
	2.743
	3.379

Kruskall Wallis test showed that the New feces samples resulted to better DNA quantification compared to the Old feces samples (Mean rank = 15.27). However, there is no significant difference between the two categories (P = 0.477).

Table 2 Kruskall Wallis Analysis

Quantity	Sample	N	Mean Rank
	New	11	15.27
	Old	16	13.13
	Total	27	

Table 3 Kruskal Wallis Test (P > 0.05)

	Quantity
Kruskal-Wallis H	0.477
df	1
Asymp. Sig.	0.490
a. Kruskal Wallis Test	
b. Grouping Variable: Sample	

This finding is in line with the work of Agetsuyama-Hanigara *et al.* (2017) showing that it is recommended to use fecal samples not older than 3 days old for genetic analysis of the Sika Deer (*Cervus nippon yakushimae*).

This study shed the light on the importance of collecting the New fecal samples for studying Bawean deer genetics in a natural population with a noninvasive sampling approach. However, the finding is only limited to DNA quantity which can come from bacteria, fungi, or any other genetic sources in the feces. Further research is required to explore the optimum condition for successful species-specific Bawean Deer DNA amplification.

## CONCLUSION

The data showed that the New fecal samples provided better results of DNA extraction than the Old fecal sample. Nevertheless, the differences are not significant.

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