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Non-Invasive Method for Recovery of Nucleic Acids in Farm Animals Using Nylon Flocked Swabs

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Abstract: Sample collection in livestock animals is difficult because choosing proper device for collecting samples is uncertain. Flocked swabs are the most recent advancement in single-use specimen collecting equipment. Flocking is the application of multi-length fibres to an adhesive-coated surface to improve sample collecting method and process. Since the onset of the animal collection swabs, nylon flocked technology has become a new tool in the field of healthcare especially diagnostic. Advantages of non-invasive methods include painless collection ensuring the best possible animal welfare. Nylon flocked swabs can be utilized for a host cell sample collection providing high quality and enough amount of DNA. In the present study, through nasal swab high amount of DNA was identified for goat (124.03 ng/µl) as compared to bovine (96.734 ng/µl) and pig (87.638 ng/µl). Thus nylon flocked nasal swabs showed a good performance for Veterinary diagnosis, although this would be an alternative promising specimen collecting tool.

Keywords: *Non-invasive*, DNA, *Bos taurus indicus*, *Sus scrofa domesticus*, *Capra hircus*

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Introduction

In veterinary clinical practice molecular marker play an important role in sex identification, marker assisted selection, percentage testing and genomic disease diagnostics. For decades, genetics has been used to improve animal breeding and genetic research and DNA testing are accelerating globally. Parentage verification, phenotypic features, and other types of genetic testing are commonly used in the sector (e.g., coat colour determination of red vs. black). Specimens are used to give supporting evidence for determining the cause of disease or death in animals, as well as for disease monitoring or surveillance (Adamska *et al.,* 2012).

Live animal samples may include blood, hair, feathers, excrement, or ectoparasites, as well as samples collected by swabbing lesions or orifices. Only fully trained persons who are skilled in the

necessary techniques should attempt to collect blood samples from living animals. Blood collection necessitates suitable animal restraint to avoid traumatic injury to both the animal and the investigator taking the samples. When the sampling procedure will cause more than little or transient discomfort or anxiety, anesthetics may be required. Blood collection and an aesthetic protocol may also need to be reviewed by an Institutional Animal Care and Use Committees (IACUC) before being used in the field (McClure *et al.,* 2009).

Because of the simplicity of the materials used and the availability of blood, blood sampling has been the "go to" method for DNA extraction. Blood sampling required the assistance of a skilled professional, typically a veterinarian. After collection, blood coagulates unless Ethylenediaminetetraacetic acid (EDTA) is added which act as an anticoagulant agent. This allowed for more storage time before processing. A chemically treated paper card is also used on which the blood could be applied. Because of the chemical-based preservation and drying, the DNA would not degrade. Any bacteria that had the potential to degrade the sample would be destroyed by paper. When dried, the Flinders Technology Associates (FTA) card can be stored at room temperature without deterioration. The sampling of DNA from this card required only a 3mm punch, which was then re-suspended in solution for genomic analysis of the DNA (FAO, 2006; McClure *et al.,* 2009).

Swabs are exceptionally useful for testing the presence of infections in large numbers of dead or living animals. To detect mucosal shedding of a pathogen, tracheal (usually used on deceased birds), oral pharyngeal, cloacal, and nose swabs are routinely utilized (Bersev and Liebscher, 1983).

Nasal swabs may be useful for detecting viruses transmitted by respiratory secretions, but they may not be useful for detecting bacteria involved in a mouth infection since random swabbing of the oral cavity would likely return a mixture of common oral and environmental bacteria. Although swabs are convenient, they frequently give the worst circumstances for germ survival while in route to a diagnostic laboratory. To guarantee effective microorganism recovery, appropriate collecting devices, must be used (Koneman *et al.,* 1997). The objective of this study was to demonstrate the quality and quantity of host DNA collected using in nasal swab.

Materials and Methods

Study Animals:

Nasal samples were collected from Bovine (*Bos taurus indicus*, n=15), Pig*(Sus scrofa domesticus*, n=15) and Goat (*Capra hircus*, n=15) without harming the animals. The time required for collection and DNA extraction was recorded.

Nasal Swabs design:

Nylon-flocked-swab- a sterile dry swab was aseptically taken from its packing and swabs inserted into the zig whole evenly and then swabs flock to already prepared 9:2 ratio adhesive, and then carry on the electrostatic machine for flock. The flock machine has nylon fibres on the bottom side and upper side with adhesive (Polyurethane Adhesives) to pass negative charges by an electric gun and spread out in rod plate. Under the airflow of the substrate adsorbed, the fibre anchored at perpendicular alignment into the substrate surface of Swab.

Collection of the Sample:

The Samples were collected at University Research Farm (URF) -TANUVAS, Chennai, Tamil Nadu (Fig. 1). 15 samples were collected from each animal-- bovine, goat and pig. The samples were collected using sterile flocked nylon Swabs (Manufacturer: TranScience Innovative Technologies, Veterinary Incubation Foundation (VIF), Tamil Nadu Veterinary and Animal Sciences University (TANUVAS), Chennai, Tamil Nadu). Swabbing was performed by holding the swab at a 30° angle to the surface to be sampled. While

Fig. 1: Collection of the sample for bovine, pig and goat using Nasal Swab.

moving the swab in one direction, the swab's head was rotated gently and thoroughly over the surface. The swabbing motion's linear direction was altered by 90°, and the surface was thoroughly swabbed once more. By altering the direction of the swabbing action by 135°, a third covering of the surface was completed. The sample collected swab was introduced into TE buffer (5 ml).

DNA Collection and Extraction from Nasal Swabs:

100 µl of collected samples were taken for DNA extraction. 20 µl of Magnetic beads and 20 µl of Proteinase K were added in 2.0 ml centrifuge tube, then vortexed for 3 min at maximum speed. 560 µl of lysis buffer was added and vortexed and the tube was placed in a magnetic stand for 2 min, then the supernatant was discarded. 900 µl of Wash-I solution was added and vortexed, the tube was placed in a magnetic stand for 2 min and supernatant was discarded, then 900 µl of Wash-II solution was added (repeat it for 2 times) and vortexed. The tube was placed in a magnetic stand for 2 min, the supernatant was discarded and the sample was dried for 10 min, finally elution buffer of 40 µl was added, vortexed and the tube was placed in a magnetic stand for 2 min , the magnetic particles were discarded then carefully DNA solution was collected (TrueScreen DNA Extraction Kit).

Agarose Gel Electrophoresis for the Separation of DNA:

The DNA samples were loaded in 0.8% agarose gel prepared using 0.5X TBE buffer containing 0.5 µg/ml ethidium bromide. In brief, 1 µl of 5X loading dye was mixed with 2 µl samples and were loaded and electrophoresis was performed at 75V power supply and with 0.5X TBE as electrophoresis buffer for about 35 min, until the bromophenol blue front migrating to almost the bottom of the gel. The molecular standard used was a 2-log DNA ladder (NEB). The gel was visualized in a UV transilluminator and the image was captured under UV light using Gel documentation system (Bio-Rad) (Diaz *et al.,* 2010; Nazemalhosseini-Mojarad *et al.,* 2011).

Nucleic acid (DNA) Purity 260/280 using multimode reader:

Extracted DNA samples were checked for the purity using Multimode reader. SparkControl software enables easy select of the “NanoQuant Nucleic Acid Quantitation” control bar. For individual blanking, blank values for each sample

position are subtracted from the sample values measured in the same position. 2 µl of samples was loaded in the multi-reader plate after the quantification of nucleic acid DNA each selected sample and then result is expressed in ng/µl quantification of DNA after run out in the multimode reader. The SparkControl software automatically measure all wavelengths for nucleic acid quantification, using 310 nm as a reference wavelength for internal correction. The measurements yielded a full spectrum from 200 to 1,000 nm, as well as the 260/280 and 260/230 ratios. In a microplate format, absorbance i.e., quantification of DNA works on samples ranging from around 0.25 ng/µL to about 125 ng/ µL by using Tecan Infinite M200 Pro plate reader.

Data Analysis:

The data were evaluated to see how the quality and quantity of DNA differed between samples. To compare the concentrations of DNA to the concentrations of protein, the A260/A280 ratio was utilized. The predicted quality of the samples gathered was determined by ratios. In order to observe DNA quantity and collection time for each animal, data were retrieved from excel spread sheets. All experimental data involved analysis of variance using one way ANOVA test for significance range $p<0.001$.

Results and Discussion

The Gel electrophoresis results showed the presence of DNA tested samples. Figure 2 shows the quantity of DNA in bovine, goat and pig samples. Gel comparisons were performed in this investigation to determine the detection limits for the DNA and Marker (controls). Geldoc system, staining was found to be superior to ethidium bromide staining for detecting the presence of DNA and its fragments because this stain is comparatively more sensitive. There have been few studies that illustrate the benefits of Gel doc system over conventional staining procedures (Huang and Fu, 2010).

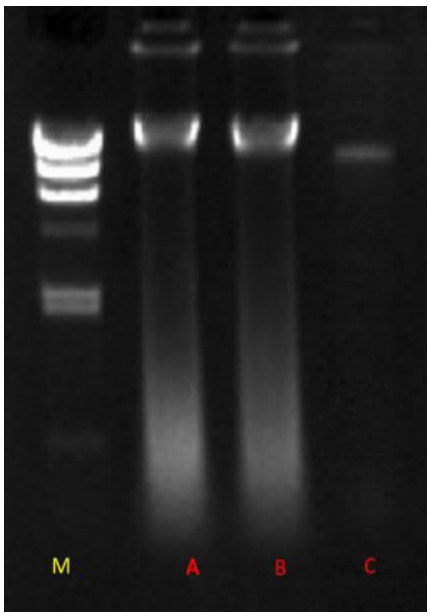

Fig. 2: DNA in Gel electrophoresis analyzed in the sample such as M- Marker, A-Goat, B-Pig and C- Bovine.

The A260/A280 ratios of extracted DNA of Bovine, Pig, and Goat were analyzed. The absorbance ratios (A260/A280) of the goat average 1.708 and their DNA recovered range 124.63 ng/µl (Table 1). The absorbance ratios (A260/A280) of the pig average 0.908 and their DNA recovery range 87.638 ng/µl (Table 2).The Bovine ratio average 1.61 and Bovine DNA sample showed presence in 96.734 ng/µl (Table 3). Goat was found in the highest DNA recovery range in 124.63 ng/µl compared to other animal samples. Experimental sample like Bovine, Pig and Goat and their standard deviation of DNA have been illustrated in Tables 1-3. Selected animals samples of DNA (Bovine, Pig and Goat) recovery was significant ($p <0.001$) (Fig. 3). Impurities must be kept to a minimum in order to avoid inhibiting enzymatic activities or interference with gel migration patterns (Adamska *et al.,* 2012). Genomic DNA is a necessary component for performing molecular applications including genomic studies (Lundblom *et al.,* 2011; Chacon-Cortes *et al.,* 2012). LeBlanc *et al.* (2020) compared combined oropharyngeal and nasal swabs. Other investigations (Wehrhahn *et al.,*

Table 1: DNA quantification of the sample for goat

	A260	A280	Ratio (A260/A280)	DNA (ng/ µl)
Mean	27.84	17.2	1.618	1240.33
Average	2.784	1.72	1.61	124.03*
Standard Deviation	0.118	0.201	0.588	1.389

* p <0.001

Table 2: DNA quantification of the sample for Pig

	A260	A280	Ratio (A260/A280)	DNA (ng/ µl)
Mean	21.66	12.77	1.69	876.38
Average	2.16	1.27	1.696	87.638*
Standard Deviation	0.063	0.08	0.79	0.908

* p <0.001

Table 3: DNA quantification of the sample for Bovine

	A260	A280	Ratio (A260/A280)	DNA (ng/µl)
Mean	22.24	13.01	1.708	967.34
Average	2.224	1.31	1.708	96.734*
Standard Deviation	0.109	0.097	0.174	1.495

* p <0.001

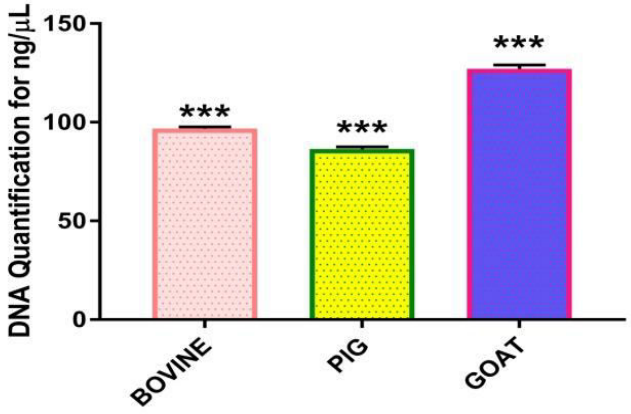

Fig. 3: Quantification of DNA analysis of Bovine, Pig and Goat of ANOVA is significant (p <0.001).

2020; Apecchi *et al.,* 2020) discovered that the combined swab samples collection of RNA contains a higher percentage of positive detection than the reference nasal swabs.

Conclusion

In conclusion nasal swab sampling method is the alternative collecting host DNA samples in livestock animals for various veterinary applications.

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