30th CONGRESS OF THE INTERNATIONAL SOCIETY FOR FORENSIC GENETICS Universidade de Santiago de Compostela, 2025, pp. 209-215 DOI: https://dx.doi.org/10.15304/cc.2025.1869

# The Canine DNA Recovery Project: Current Findings and Next Steps

LOUISE DAWNAY<sup>1</sup>, PAUL RILEY<sup>1</sup>, MATT LEWIS<sup>1</sup>, SUZZANNE MCCOLL<sup>1</sup>, NICK DAWNAY<sup>1\*</sup>

<sup>1</sup>School of Pharmacy and Biomolecular Sciences, Liverpool John Moores University

\* Corresponding author at: email: n.m.dawnay@ljmu.ac.uk, tel: +44 151 2312485

### Abstract

The Canine DNA Recovery Project (CDnaRP) is a collaborative forensic project that aims to develop best practice methods for the collection and analysis of dog DNA recovered from attacked livestock and wildlife. The project works closely with police and forensic practitioners to optimise and standardise methods and also works with special interest groups including vets, rural insurers, farmers, and charities to ensure stakeholder awareness of the research and how it relates to livestock and wildlife offences involving canines. Our research to date has developed and validated a robust qPCR assay for canine DNA quantification. The method acts as an important quality control step in the forensic DNA analysis pipeline and has allowed us to assess the effectiveness of different DNA recovery techniques such as swabbing, taping, and cutting. Other areas of our research have revealed the extent to which PCR inhibitors and livestock DNA prevent PCR amplification and canine DNA profile interpretation and has identified mitigation steps leading to improved data quality. To build on these findings, our project has developed an Early Evidence Kit (EEK) for police and rural stakeholder groups to use in the event of a livestock attack. The distribution of these kits will allow a greater number of samples to be collected for the research and will begin to understand whether non-enforcement groups such as farmers themselves can collect admissible evidence in the future. Our presentation looks at the validation of this kit and considers their application in the UK Criminal Justice System.

### Keywords

Canine DNA, qPCR, Sample Recovery, Crime Scene.

### Introduction

In the United Kingdom, livestock attacks by dogs are a 'non-recordable offence', meaning police are not required to record their frequency. The most comprehensive

police data to date recorded 1705 incidents between 2013 and 2017, which resulted in 1928 livestock killed and 1614 livestock injured [1]. This is likely an underestimate as many incidents go unreported to police and data from insurance claims suggests thousands of attacks occur each year, costing £2.4 million in 2023 alone [2]. Reducing the frequency of dog attacks on livestock is a rural policing concern and is managed through initiatives including educational training programs [3], targeted police operations [4] and proposed changes to UK legislation [5]. Forensic support can aid police investigations by establishing a link between the suspect dog and attacked livestock through canine STR profiling, but this approach is not often used. Discussion with stakeholders identified issues within the sample collection and testing pipeline and led to the creation of the Canine DNA Recovery Project (CDnaRP), a multiphase, multi-stakeholder project led by Liverpool John Moores University, which aims to develop, promote, and apply best practice methods for the collection and analysis of canine DNA from attacked livestock and wildlife.

#### Methods

#### a. Community Building and CDnaRP Structure

The UK contains many stakeholders concerned with wildlife and rural crime including government, private companies, charities, professional societies, and academic researchers. The CDnaRP works with these groups to understand their needs, with stakeholders sitting on the project Scientific Advisory Board (Figure 1a).

#### b. Identifying Knowledge and Research Gaps

Discussion with police and forensic providers highlighted that the canine forensic DNA testing pipeline is underdeveloped and poorly characterised compared to that used in human forensic analysis (Figure 1b). Currently, there is no method to localize canine saliva on submitted swabs or samples, and only a single method (swabbing) recommended to recover DNA from attacked animals [6] despite human research suggesting other methods may offer advantages [7]. Issues with PCR inhibition during STR amplification have not been assessed despite evidence that many submitted samples fail to amplify during PCR. Finally, a standardized method for STR profiling for use in livestock attacks is needed, including robust STR chemistry that minimizes PCR inhibition and livestock amplification. Such laboratory improvements have to be balanced against the fact that police are often unable to collect a forensic sample due to limited resources and a statutory duty for the livestock keeper to dispose of the animal carcass [8].

# **Results and Discussion**

# a. Optimisation of recovery methods

A robust qPCR assay was developed to allow accurate quantification of recovered canine DNA [9]. The developed triplex assay amplifies MC1R for autosomal detection (Figure 1c), SRY for male canine detection (Figure 1d), and a synthetic IPC to detect the presence of inhibitors (Figure 1e). The assay was suitably sensitive and specific, and passed all common validation studies [10].



**Figure 1.** A) Structure of Canine DNA Recovery Project and its remit; B) Canine DNA Pipeline showing areas for development. Red = no current standardised method; Yellow = method needs assessment. C) qPCR amplification plot of MC1R; D) qPCR amplification plot of SRY; E) qPCR amplification plot of Internal positive control.

The qPCR assay was used to compare the amount of canine DNA recovered using three methods; a) swabbing, b) mini-taping, c) cutting wool. Recovery from

naturally shed wool spiked with canine saliva showed swabbing was the least effective method with significantly greater canine DNA recovery using scissors (Figure 2a). The same trend was observed when these methods were applied to real-world samples collected from livestock attacks in Spring 2023, although there was a ~20fold decrease in DNA yield (Figure 2b) suggesting mock samples were over-spiked. Results also revealed that average amounts of DNA recovered by swabbing (~20pg/ µl) was insufficient to generate a STR profile and provides one explanation for the observed analytical pipeline failures.



**Figure 2.** A) Amount of canine DNA (pg/µl) recovered from mock attack samples; B) Amount of canine DNA (pg/µl) recovered from real attack samples. Three different recovery methods and their associated materials used in collection; C) Scissor Kit, D) Mini-Tape Kit, E) Swab Kit.

Data also revealed instances where qPCR detected canine DNA but STR typing subsequently failed due to PCR inhibition. Assessment of methods to combat PCR inhibition, including different DNA extraction kits, sample dilution, and STR formulations and panels, is currently being written up as a technical note. Results of this research also found substantial amplification of livestock DNA using the Canine Genotype 2.1 Kit [11], which was much reduced when using CADNAP Panels 1 and 2 [10, 12], leading to an ongoing collaborative effort to develop a standardised approach for analysis of mixed canine:livestock samples. Guidelines are being developed for both Canine Genotype 2.1 Kit and CADNAP Panels 1 and 2 to allow analysis flexibility for forensic laboratories. Together these data support changes to the existing DNA recovery method and analytical pipeline used by UK police and forensic providers in response to livestock attacks.

# b. Widening participation through citizen science

An ambitious citizen science project has been launched to further assess and promote the developed sample collection methods by providing free training and collection kits to police, vets and livestock keepers. This will i) assess the use of methods across a wider group of individuals, ii) derive DNA data to augment existing data and assess reproducibility, and iii) serve to validate the methods and end-user groups.

The developed LAW (Livestock And Wildlife) DOG DNA Recovery Kits contain everything needed to successfully recover, preserve, and secure canine biological evidence at the scene of a livestock attack (Figure 2c-e). Kit release in Summer 2024 was well received by stakeholders and the rural community, resulting in positive press attention [25, 26]. KA future possibility is where the kits are used beyond the scope of the research project, with police, vets and livestock keepers undergoing certified training, to collect biological samples alongside digital data to submit for forensic analysis. This would require acceptance by the UK criminal justice community with the data collected being admissible in court on a case-bycase basis. While this may necessitate a change to current forensic regulations, it would solve issues associated with police response times, meaning evidence was collected early by the livestock keeper and/or vet before being transferred to the attending police officer when available.

Prior to wider adoption of new methods data needs to be reviewed, disseminated, and validated following community guidelines. To date, CDnaRP data has been distributed through a series of reports, scientific papers, conference presentations, professional working group meetings, and training events. Ultimately the CDnaRP will host these outputs through an online platform with links to online training materials for police, veterinary practitioners, and livestock keepers.

## Conclusion

The CDnaRP was established as a forum to develop, promote and apply best practice guidelines for the collection and analysis of Canine DNA from livestock and wildlife attacks. Results have shown the UK Canine DNA analysis pipeline can be further optimized with data being confirmed through the distribution of the LAW DOG kits. Data resulting from kit use in livestock attacks is pending and will be used to further assess different methods and use by end-user groups prior to making recommendations for wider adoption of sampling methods in the future.

## Acknowledgments

The authors would like to thank North Wales Police, National Police Chiefs Council (NPCC) Livestock Attack Working Group, Chartered Society of Forensic Science Animal Related Crime Working Group, National Wildlife and Rural Crime Units, Forensic Capability Network, National Sheep Association, Science and Advice for Scottish Agriculture (SASA) Wildlife Forensic Laboratory, Cellmark Forensic Services, NFU Mutual, Farmers Union Wales (FUW), Synergy Farm Health, IVC Evidencia.

### **Conflict of interest statement**

The work described has been funded by DEFRA (AW1414) and through LJMU Research and Innovation Services.

# References

[1] NPCC. Livestock Worrying Police Working Group Final report. Available at https://news.npcc.police.uk/resources/livestock-worrying-police-working-group-feb-2018. Accessed on 04.10.2024.

- [2] NFU Mutual. UK cost of livestock worrying rises by nearly 30%. Available at https://www.nfumutual.co.uk/media-centre/complacency-kills-as-uk-cost-oflivestock-worrying-rises-by-nearly-30/. Accessed on 04.10.2024.
- [3] Welsh Government Press Release. Cabinet Secretary welcomes new course addressing dog attacks on livestock in Wales. Available at https://www.gov.wales/ cabinet-secretary-welcomes-new-course-addressing-dog-attacks-livestockwales#:~:text=RDOC%20aims%20to%20support%20dog,roll%20out%20 of%20this%20course. Accessed on 04.10.2024.
- [4] Cheshire Constabulary. Operation Recall: new initiative launched to tackle livestock worrying. Available at https://www.cheshire.police.uk/news/cheshire/news/ articles/2023/10/operation-recall-new-initiative-launched-to-tackle-livestockworrying/. Accessed on 04.10.2024.
- [5] Animal Welfare (Kept Animals) Bill. Available at https://bills.parliament.uk/ bills/2880. Accessed on 04.10.2024.
- [6] PAW Forensic Working Group. Guidance document for the DNA swabbing of animal bite marks and sampling from suspected offending animals. Available at https://www.tracenetwork.org/wp-content/uploads/2018/05/Swabbing-of-bites-guidance-document-final-July-2017.pdf. Accessed on 04.10.2024.
- [7] Verdon TJ, Mitchell RJ, van Oorschot RA. Evaluation of tapelifting as a collection method for touch DNA. Forensic science international: Genetics. 2014 Jan 1;8(1):179-86.
- [8] DEFRA. Fallen stock and safe disposal of dead animals. Available at https://www.gov.uk/guidance/fallen-stock. Accessed on 04.10.2024.
- [9] Dawnay N. Optimising the collection of canine DNA from worried livestock for forensic identity testing. DEFRA Report AW1414. Available on request.
- [10] Dawnay et al 2024. Development of a qPCR assay for the quantification of canine autosomal DNA recovered from livestock attacks. Science and Justice, in press.
- [11] Dayton et al 2009. Developmental validation of short tandem repeat reagent kit for forensic DNA profiling of canine biological material. Croatian Medical Journal 50(3):268-85.
- [12] Berger et al 2014. Validation of two canine STR multiplex-assays following the ISFG recommendations for non-human DNA analysis. Forensic Science International: Genetics 8(1):90-100.