CSI Rhino - putting forensics into practice

This is the second part of an article by Rhino expert **Felix Patton** about how CSI - Crime Scene Investigation - techniques beloved of TV drama series are being used in the fight against poaching and wildlife trafficking.

A carcass has been found, cut into four pieces so it could be hidden in a gully covered in bush. It is old and there is little flesh left. The size and shape of the skull indicate it is of a sub-adult White rhino. The horns have been carefully removed representing 'poached by humans' rather than 'naturally taken', often by hyena. The skin of the head is remarkably well preserved and many of the wrinkle patterns on the side of the face are intact. These act like fingerprints as every rhino has a unique profile pattern so a photograph is taken to compare with the reserve database.

Specialists take forensic samples of skin, nail and hair for later DNA analysis.

Back at the reserve's rhino office, the photograph of the right profile wrinkle patterns is compared with the database and clearly identified as sub-adult male 172.

Two weeks later, in a village not far from the reserve, two men are arrested attempting to find a buyer for two rhino horns. The origin of these horns is not known. A photo of the horns appears in the Kenyan Daily Star newspaper and is compared with the reserve database. Bingo! Another match with sub-adult male white rhino 172.

Problem – this information, while useful in getting a quick link between the poached rhino and the criminals, is based on expert interpretation of the photographs and so open to conflicting opinions. It is not robust enough evidence to enable a judicial conviction. The solution is DNA analysis. If the DNA profile from the carcass matches the DNA profile from the horns then the evidence will be robust enough for a conviction, but only if it has been forensically collected and tested in a suitably licenced laboratory.

This case, a real situation that occurred in 2014 in Kenya, was made easier by the short time and geographical distance between the carcass being discovered and the horns being found. In many more cases the time gap may be months or years and the geographical distance can cross continents with most African poached rhino horn ending up in the Far East. There have been two main developments to overcome these obstacles – microchips and a rhino DNA profile database.

Microchips are passive transponders, no bigger than a grain of rice, which transmit a unique serial number when scanned by a reader. The life span of a microchip is indefinite as energy is obtained from the reader. Microchipping a rhino involves placing one (each with its unique code)

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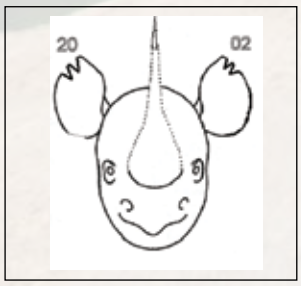

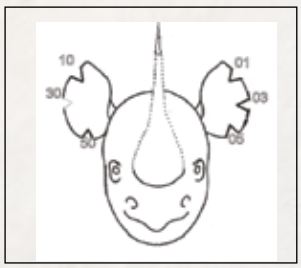

in each horn of the rhino and under its skin at the shoulder and the rump. After this, the rhino can be identified by the number of the microchip. Once applied, microchips become the primary source of individual identification confirmed by DNA analysis.

For microchips to be useful in providing judicially acceptable evidence of illegal activity, the processes involved in inserting the chips must be carried out forensically. Since the rhino has to be anaesthetised in order to plant the chips, other useful operations are often carried out in tandem – most usually sample collection for DNA profiling and ear notching.

To protect the integrity of the samples for DNA analysis and ensure the chain of custody from sample collection to evidentiary report, DNA sample collection kits have been produced by the Veterinary Genetics Laboratory of the University of Pretoria in South Africa. One component of the kit is a drill bit for making the holes in the horns to put in the microchip. While drilling the holes, horn waste is saved for DNA extraction. The holes are then filled with dental acrylic. A special applicator is used to inject the two chips under the skin.

A blood sample is taken from a readily accessible vein in the foot of the rhino where a small clipping is cut from the nail. Hair samples are collected from the tail. Each of the samples are kept in special containers which are referenced accordingly.

Skin samples are taken from the ear either as a result of notching or, where notches are already present, as a small sliver from the top of the ear. Ear notching is a method of making an individual rhino more effectively and efficiently identified. It entails cutting V-shaped areas at specific points on the ears of the rhino, which represent numbers (see illustration NOTCH 99 + 22). This is especially useful in making a preliminary identification of a rhino that has been poached and therefore does not have its main identification feature – the horns. The pieces of

skin removed from cutting the notches are ideal for DNA analysis.

All the samples are sealed in a special collection bag and sent to a recognised laboratory for analysis. The first lab approved for this work was the Veterinary Genetics Laboratory (VGL) of the University of Pretoria. A successful project in 2009 resulted in the VGL developing a method to extract DNA from rhinoceros horn and produce an individual DNA profile from the horn. VGL went on to develop a central standard secure database of rhinoceros DNA profiles named RhODIS (Rhino DNA Index System) based on CODIS, the human database of the FBI. Two datasets are combined – a forensic dataset derived from poaching cases and a routine dataset derived from live rhinos when ear notched, treated for injury, dehorned and so on plus stockpiles of horns collected from natural deaths. Since its launch in June 2010, there are now over 13,500 individuals on the database not only from South Africa but also from Namibia, Zimbabwe, Botswana, Malawi and Kenya.

A new $1.5 million wildlife forensic and genetic laboratory has been established by the Kenya Wildlife Service at their headquarters in Nairobi such that DNA profiling can be carried out for animals from East and Central Africa. The facility complies with standards established by the Kenya Accreditation Service as well as special requirements established by the Judiciary, the Ministry of Justice, National Cohesion and Constitutional Affairs and by the International Laboratory Accredit Cooperation (ILAC) Guidance for Forensic Science Laboratories.

KWS started thinking about a forensic laboratory some 15 years ago when differentiating between common meat and bush meat was proving a challenge. Wild animals were being slaughtered for their meat and seizures done but there was no concrete evidence to prove it was really meat harvested from wildlife. The only means of separating the two was by the anatomical features of the animals. Poachers

started deboning the bush meat before sending it to the markets making it very difficult to conclude whether the meat was from wildlife.

KWS started thinking of science and tried protein tests but these proved ineffective for wildlife. Elimination evidence was then used with samples taken to the government chemists. The scientists could tell if the samples were from domestic animals or not but not what type of animal or which animal the product was from. The way ahead was DNA analysis.

The new lab has several roles:

1. Boosting prosecution in the illegal trade of bush meat overcoming new techniques employed by bush meat dealers, which have made it difficult to produce court-admissible evidence.
2. Advancing the prosecution of poachers and smugglers by providing traceability of trophies (especially elephant ivory and rhino horn) linking them with specific poaching incidences.
3. Population genetics and molecular diagnostics for tracking the genetic status of declining wildlife populations and determining isolated and specific wildlife gene pools that require specific protection.
4. The diagnosis of wildlife diseases.
5. Conservation research with anyone studying ecology and requiring the use of molecular tools as part of their work being supported by the laboratory.

Central to obtaining accurate DNA profiles are two pieces of equipment – the QIAcube and the Rotor-Gene Q. A QIAcube was donated to KWS by German based QIAGEN and its partner Bio-Zeq to enable the lab to fully automate the preparation of the sample taken from confiscated ivory, horn or meat. The Rotor-Gene Q provides genetic fingerprinting of the sample through streamlined, easy-to-use real-time PCR analysis. This specialist equipment needs careful calibration and regular servicing ,which will require ongoing funding.

In order to make the laboratory fully operational, a multi-national delegation conducted a two-day training workshop and site inspection. The workshop included a mock trial designed to expose KWS investigators and laboratory staff to the rigors of testifying at trial including potential pitfalls and defense challenges.

Since forensic work is highly specialized, seven staff were sent to various institutions in Israel, United States, Canada and South Africa for training.

With the application of forensic evidence, it is hoped that there will be more convictions of poachers and wildlife traffickers that, in turn, will boost conservation efforts. KWS could not confirm the results of the DNA tests on the carcass of rhino 172 and the recovered horns but the other evidence mentioned previously suggests they will show them to be from the same animal so a conviction should result. The first of many positives for 'CSI Rhino Kenya' in the future.