Drug testing by horse hair analysis

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Using hair as an analytical sample to identify drug administration is not a new idea.

In the 19th century, hair analysis was used to detect the presence of arsenic in the bodies of suspected murder victims. Although these individuals had died up to 11 years previously, this retrospective forensic evidence ultimately led to the convictions of the perpetrators. Notably, in the 1960s, posthumous analysis identified arsenic in the hair of Napoleon. After defeat at Waterloo, the French Emperor was imprisoned on the island of St Helena up until his death in 1821. A rumour evolved and persisted that his British jailers had poisoned him with arsenic. This substance, however, was a common constituent of several domestic products at this time - including paints and dyes used in wallpaper printing - and subsequent hair analysis could not identify the actual source of the arsenic exposure.

Although hair analysis for the identification of drug abuse in people began more than 25 years ago, development of the technique for horses started only in the late 1990s, with the first research publications appearing in 2000.

Hair analysis benefits and applications

Although hair is a living tissue, it is a physically robust, water-free and extremely stable biological medium. These characteristics ensure that drug residues are protected against chemical and physical changes caused by metabolism, the environment or electromagnetic radiation, and thus degrade slowly over prolonged periods of time.

Analysis of horse hair can provide a range of information related to past drug use or misuse including:

- identity of the drug(s);
- time elapsed since drug administration;
- frequency of drug administration;
• estimate of dose;

• route of administration; and

• inference of partial clinical history.

The major attraction of hair analysis is that, unlike blood and urine testing, it can provide a detailed historical record of drug use in a horse, with retrospective detection possible for weeks, months or even years after administration. In this, it is complementary to blood and urine analyses, which can only identify "immediate" or very recent drug use - a time window of a few days for most drugs.

A further advantage of hair analysis is its ability to profile patterns of past drug use in horses. As mane growth is regular and continuous, consecutive sections of the hair from the follicle to the tip can be analysed individually. Therefore, a single hair sample, unlike a one-off blood or urine sample, if subjected to segmental analysis, can provide an estimate of not only the time elapsed since a drug was used, but also discriminate between single, repeated or continuous use of a drug (and).

Measurement of the relative concentrations of a parent drug and any resultant metabolites may enable the route of administration to be interpolated - whether oral, intravenous or topical.

Repeatability of testing is a further advantage, in that a second hair sample could be collected and tested weeks or months after the first, and yet yield the same drug identification result. This is not possible with blood or urine. Other beneficial characteristics of hair for drug testing include ease of collection, transportation and storage (no need for refrigeration).

Hair analysis cannot be used to replace the post-race urine or blood testing currently employed by horse racing’s regulatory authorities. However, hair testing may be beneficial to the racing and breeding industry in a number of other areas. The application of such testing as part of pre-purchase veterinary examination could bolster confidence in the physical wellbeing of a prospective purchase by identifying:

• recent use of anti-inflammatory drugs, such as the nonsteroidals phenylbutazone, flunixin and meloxicam, and the glucocorticoids, including dexamethasone and methylprednisolone, to mask lameness or other inflammatory conditions, and sedatives including detomidine and romifidine to hide behavioural problems; and

• past use of growth-promoting anabolic steroids, including nandrolone and stanozolol, and repartitioning agents, such as clenbuterol, to artificially enhance muscle development, aggression and stamina in young horses during breeding and training.

Hair analysis might provide evidence that is complementary to a positive post-race urine test if the finding is contested, such as when the trainer believes feed contamination to be the cause rather
than drug administration.

Prohibited substances can occur in feed or forage as natural constituents or contaminants. This was illustrated when a spate of post-race morphine positives arose from feed contaminated with poppy seeds. Application of sectional hair analysis in such a circumstance could discriminate between a single drug administration and an ongoing feed problem.

Alternatively, hair analysis potentially offers the regulatory authorities the opportunity to detect and deter the inappropriate in-training use of certain drugs to artificially enhance training capacity and performance.

This includes the use of:

- synthetic female hormones such as altrenogest to attempt to calm and render more manageable aggressive colts and entire horses; and

- stimulants and anabolic steroids to increase performance delay the onset of fatigue and boost recovery.

**How is hair analysis carried out?**

Hair analysis is a complex process involving several key stages, including:

- hair collection;

- sample preparation and decontamination;

- drug residue extraction and purification;

- instrumental analysis; and

- data analysis and interpretation.

The coat, mane or tail can all be used as sample matrices but, generally, hair from the mid region of the mane is preferred - owing to reduced variability in growth, less damage from tack or rugs and minimal contamination from urine and faeces. Sampled hair must be sufficiently long to cover the period of interest. Mane hair grows between 17mm and 25mm per month (three quarters to one inch per month) dependent upon breed. Duplicate samples (A and B) are secured with thread prior to collection by plucking or cutting at the skin surface () . Each sample is generally 4-5mm in diameter (about half the thickness of a pencil) and contains approximately 200 hairs. The B sample is stored in case a query necessitates repetition of the analysis.
Actively growing (anagen) hairs are used exclusively for the analysis and these are easily distinguished by microscopy. A rigorously tailored multi-stage wash procedure is employed to remove as much surface contamination as possible on the hair - such as dust, faeces and urine - while simultaneously maximising the retention of drug residues absorbed within the hair structure.

Decontaminated hair is cut into sequential sections, from the follicle outwards, of appropriate length (normally to render each section equivalent to one month of hair growth). The more distal hair sections represent older growth and, thus, periods further back in time. These sections are further chopped into fragments to increase the effectiveness of the subsequent drug extraction.

Drugs residues are extracted by incubating hair samples in organic solvents, or acid or alkaline solutions, and at elevated temperatures - typically 40-60°C. Under these conditions it takes several hours to fully extract drug residues. Therefore, a compromise is inevitably reached between time and efficiency, and extractions are typically performed in batches and conducted overnight. Extracts inevitably contain interfering substances, mainly of dietary origin, in addition to drug residues and these are removed by solidphase extraction prior to analysis. As levels of drug residues in hairs are extremely low, highly sensitive and selective analytical techniques, such as liquid chromatography-mass spectrometry (LC-MS), are required.

**Caveats in hair analysis**

A number of factors need to be taken into consideration when interpreting hair analysis data, including the colour and growth rate of the hair. Hair colour can have a profound effect on the level of drug residue present. This is because many, but not all, drugs bind extensively to the melanin pigments in the hair. Black hair contains greater quantities of eumelanin and, to a lesser extent, pheomelanin and, therefore, accumulates significantly higher levels of many drugs.

At the opposite end of the spectrum, grey or white hair contains virtually no pigment, hence drug levels are generally much lower. This melanin-to-drug binding effect is more pronounced for drugs that are weak bases, such as opioid analgesics, phenothiazine sedatives and cainic local anaesthetics. This hair colour effect has particular consequence when attempting to estimate the dose of drug that was originally used. Research has shown that higher hair drug concentrations result from higher doses and vice versa, but considerable variation exists between horses largely deriving from differences in hair colour, even when these differences appear subtle to the naked eye.

Consequently, the exact dose given to any individual horse cannot be quantified, but it is possible to infer whether the dose was high, moderate or low. If comparative quantitative values are desired, a correction factor needs to be applied that takes account of the variance in melanin content between samples. This can be achieved simply by co-analysis for melanin content and subsequently expressing drugs concentrations as nanograms per milligram melanin.
Although sequential hair analysis facilitates the estimation of the time that has elapsed between administration of a drug and collection of the hair sample, and the frequency of drug use to be determined, there are limitations to the precision of these two inferred pieces of information. Time of administration, in most instances, can only be established with a margin of error of plus or minus one month. This arises from the fact that although we know the mean and range in, for example, the growth rate of the thoroughbred mane, we do not know the exact growth rate in any individual horse.

Furthermore, individual hairs within a sample grow at slightly different rates and this can cause a broadening of the distribution profile (band spreading) that will reduce resolution and may mask repeated administrations that have occurred close together in time.

**Case study**

A two-year-old iron-grey colt presented with bilateral laminitis in the fore and hindlimbs four weeks after transportation. No obvious cause for the onset of the condition was immediately identifiable and the horse responded poorly to treatment. Common causative factors, such as trauma, excessive intake of grain or lush pasture and Cushing’s disease, were discounted. Injection of anti-inflammatory corticosteroids can precipitate laminitis in some horses, but there was no record of such drugs being administered in this case.

Nevertheless, sectional hair analysis was utilised to scrutinise the horse’s medication history and to specifically seek unrecorded drug treatments. As the horse was an iron grey, black-and-white mane hairs were analysed separately. Significantly, the analysis identified the presence of the corticosteroid dexamethasone phenylpropionate in the horse’s mane hair - an unrecorded administration. As expected, higher levels of the drug were detected in the black hairs ( ).

As this case study illustrates, the horse is an excellent species in which hair analysis can be applied effectively. Hair, however, is a complex tissue that can vary significantly between individuals and, for this reason, its analysis is not a routine procedure.

There is no generalised method for hair analysis, so specialist knowledge and a realistic view of its limitations is required to perform the technique and interpret the data successfully. Despite this, hair is a unique tissue for the retrospective detection of drug use in horses and its analysis is a valuable complement to other testing approaches.

Research on hair analysis for the detection of drug use in horses is comparatively recent and the efficacy and breadth of this technique continues to be actively studied.
Figure 1. A single hair sample, subjected to segmental analysis, can provide an estimate of the time elapsed since a drug was used, and also discriminate between single, repeated or continuous use of a drug.
Figure 2. Duplicate (A and B) hair samples taken from the mid-region of the mane of the horse are preferred.
Mane hair sample:
grey two-year-old TB colt

Corticosteroid

Dexafort preparation (Intervet UK)

Dexamethasone
phenylpropionate

Dexamethasone
phosphate
Figure 3. This mane hair analysis identified the presence of the cortkosteroid dexamethasone phenylpropionate.

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<th>Enrofloxacine</th>
<th>Ciprofloxacine</th>
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<th>Trimethoprim</th>
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TABLE 1. Analysis of hair follicles and drug detected (relating to Figure 1 below)