EQUINE DRUG TESTING IN THE 21ST CENTURY
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INTRODUCTION

The Penn Vet Equine Pharmacology Laboratory’s mission is To Promote the Welfare of the Equine Athlete while maintaining the Integrity of Equine Sport through Pharmacological and Forensic Research (1). The Pennsylvania Equine Toxicology and Research Laboratory’s mission is To Perform Equine Drug Testing for the Commonwealth of Pennsylvania’s Six Racetracks. I consider it a privilege to be the Director and Acting Director, respectively, of these laboratories, and I’d like to start with acknowledging all of the wonderful people who work in the laboratories, my current and past mentors, the members of the Pennsylvania Racing Commissions, and many of the racing industry stakeholders who share my passion for ensuring the welfare of the horse while maintaining the integrity of equine sport.

Funding for these laboratories is provided primarily by the Pennsylvania Department of Agriculture, State Horse Racing Commission (2). Grants have been provided by the Racing Medication and Testing Consortium. Additional monies and/or equipment have been donated to the Penn Vet Equine Pharmacology Laboratory by the Pennsylvania Harness Horsemen Association at Pocono Downs and Chester Downs, the Meadows Standardbred Owners Association, and the Horsemen Benevolent and Protective Association at Penn National and Presque Isles Downs. I have no commercial financial disclosures to declare.

The practice of racing horses has existed for centuries as the “sport of kings”. Race training requires an intense exercise program and an exceptional nutritional program to optimize the development and performance of the horse’s biological machinery. Especially in the last 100 years, the serendipitous discovery and the targeted development of pharmaceutical agents that can clearly enhance and/or modify the development and performance of the body has created an ethical dilemma for competitors, regulatory bodies, and fans of all sports. The intended purpose of such agents is to relieve suffering, to aid the healing process, and/or to maintain the welfare of the patient. However, the use of these agents in equine athletes training for competition, and/or during competition – termed “doping” – can give the user an unfair advantage over other competitors who have trained without the use of performance altering or enhancing drugs, and some drugs can compromise the safety and welfare of the competitors. In an effort to maintain the integrity of the sport and to ensure the welfare of the horse, the use of all drugs was banned by regulatory agencies, and legislatively by many states within the USA, and these states and some countries around the world, still operate legally under a “zero tolerance” policy when it comes to drug use in equine athletes.

DRUG TESTING PROCEDURES

To enforce the ban on pharmaceutical assistance during competition, drug testing procedures and laboratories were established. In PA, testing began at each individual race track in 1964, and was centralized at the Pennsylvania Equine Toxicology and Research Laboratory (PETRL) in 1986 in West Chester PA. Modern day procedures require blood and urine samples to be collected from the winner and one or two other competitors (i.e. “specials”). Horses must report directly to the test barn from the race track at the conclusion of the race for sample collection, and only authorized personnel are allowed to enter this area. In addition, horses are selected randomly for pre-race testing to prevent the use of alkalizing agents (i.e. “milkshaking” with sodium bicarbonate).

Samples are collected and packaged by the Commission’s veterinarian and/or their designee, and all samples are assigned a barcode and entered into a Laboratory Information Management System (LIMS). The samples are barcoded so that the laboratory personnel cannot identify the horse from which the sample came or the owner and trainer of the horse, and this sample ID number is also used to track the tests performed on each sample at the laboratory. The appropriate packaging of the samples with the corresponding paperwork is very important because a continuous “chain of custody” must be documented for each sample from the time the sample is collected to the time the sample is delivered to the testing laboratory and throughout the testing process at the laboratory.

Following sample collection at the racetrack, part of the sample is sent to PETRL for testing and the other part of the sample is kept in a double-locked freezer at the race track (i.e. two locks). One lock can only be opened by the Commission staff and the other can only be opened by a representative from the horseman’s association, ensuring both representatives are present for sample addition/removal. If a drug is reported by PETRL, the trainer can request the “split sample” (i.e. the sample maintained at the race track) to be sent to another comparable drug testing laboratory for testing; a list of acceptable laboratories is provided by the Commissions. The second laboratory must confirm PETRL’s reported finding in order for penalties to be assigned for the finding.

The testing procedures are similar for the Fédération Equestre Internationale (FEI), which is the regulatory body that oversees Olympic Equestrian Sports (3). The USA National Federation is the United States Equestrian Federation (www.usef.org)(4). Samples are collected by FEI Official Veterinarians; veterinarians interested in helping to collect samples for testing can apply to become first a Permitted Treating Veterinarian and then an Official Veterinarian at FEI level events. See the FEI website’s Anti-Doping and Veterinary Matters Tab for more information on the FEI Anti-Doping effort and on becoming an FEI-approved veterinarian (5).
ANALYTICAL METHODOLOGIES

PETRL receives approximately 35,000 samples per year for drug testing from the six racetracks in PA. During the summer racing season, PETRL receives as many as 1000 samples per week. Blood and urine samples are routinely used to test for approximately 600 drugs using a multitude of technologies. The samples are first analyzed with several screening methods (i.e. methods which look for a large number of drugs but do not provide quantitative information). If a drug is suspected in a sample, the sample is then subjected to a specific confirmatory test. The confirmatory test confirms the presence of the suspected drug by comparison of the sample’s results to the result obtained for a certified reference standard (positive control); this test also provides quantitative information. The tests performed for each sample are recorded on paper and tracked electronically using LIMS.

Drug testing was originally performed using thin layer chromatography, however this technology is now obsolete in most drug testing laboratories due to the development of faster, more specific, and more sensitive methods: enzyme linked immuno-sorbent assay (ELISA) and mass spectrometry (MS). Mass spectrometry is coupled with a variety of separation and/or ionization techniques including liquid chromatography (LC), gas chromatography (GC), and inductively coupled plasma (ICP). In addition, DNA testing can now be performed to confirm the identity of the horse from which the sample was collected if the chain of custody is questioned. Hair testing is also a new method being implemented by some laboratories.

ELISA is a relatively cheap and sensitive method that uses antibodies to detect the presence of drugs. A good explanation of how the ELISA works can be found on Neogen’s website, a manufacturer of many drug testing ELISA kits for horses (6). Because antibodies often will bind to multiple members of the same drug family, ELISA can only be used as a screening method to suggest the presence of a prohibited drug. A confirmatory method must then be performed to identify which drug is present and to accurately quantify the amount present.

Mass spectrometry is the preferred method for confirming the presence of a drug, and this technology can also be used for screening samples for multiple drugs (7). It is more specific than ELISA because the drug is processed by several steps that provide identifying information. First, an extraction step uses the chemical nature of the drug to group drugs with similar properties together (e.g. basic drugs and acidic drugs are grouped together). Second, chromatography physically separates the drugs from each other by passing them through a solid column filled with a material that will bind to the drugs. The length of time a drug remains inside the column (i.e. retention time) will depend on the size of the drug, how well it binds to the substance in the column, and on the solvents flowing through the column. By using different types of columns and solvents, a particular drug can be isolated from the other components of the sample. The eluent from the column flows directly into the mass spectrometer. There are different types of mass spectrometers, but all of them measure the mass and charge of the drug which is the m/z ratio. Sometimes a drug is also subjected to a process inside the mass spectrometer to create fragments of the drug. The fragmentation pattern (i.e. m/z ratio of the fragments produced) and the ion ratio (i.e. the proportion of those fragments to each other) provide additional identifying information. If the retention time, m/z ratio, fragmentation pattern, and ion ratio match between the sample and the reference standard, according to the criteria set forth by the Association of Official Racing Chemists (9), then the drug has been confirmed in the sample.

Ongoing technological developments in mass spectrometry and data acquisition software have significantly increased the ability of drug testing laboratories to identify and quantify drugs. Within the last 5 years, the sensitivity of our analytical methods have improved in some cases by 1000 fold e.g. drugs that were accurately quantified at plasma concentrations ranging between 1 µg/mL (0.001 mg/mL) and 1 ng/mL (0.000001 mg/mL) can now be accurately quantified at 1 pg/mL (0.00000001 mg/mL). This is the equivalent of being able to accurately measure the concentration of dissolving 4 grains of salt (assuming 0.7 mg/grain) in an Olympic size swimming pool (2,500,000 L)! This dramatic increase in sensitivity is an advantage for detecting substances of abuse that should never be found in the horse e.g. dermorphin – a synthetic opioid agonist (8), but it has created a nightmare with regard to therapeutic drugs used by practicing veterinarians, which in some cases (e.g. sotolol) can now be detected days to weeks after there is no longer a measureable pharmacological effect.

MEDICATION RULES

Who makes the rules for Olympic equestrian sports?

Organized anti-doping efforts for human and equine sport have been in existence since the beginning of the 20th century. However, human deaths and scandals due to doping prompted the formation of the World Anti-Doping Agency in 1999, which now creates the rules and regulations for human Olympic sports (10). Dr. Arne Ljungqvist, who was vice-president of WADA from 2008 to 2013, served as a consultant to the FEI from 2008 to 2010 in order to develop a similar approach for Olympic equestrian sports (11). These rules include a list of prohibited drugs (i.e. The Prohibited List) and this list is updated annually by the FEI Prohibited Substances List Expert Group. This group is composed of sport veterinarians, pharmacology and toxicology specialists, and researchers (3). Anyone can submit suggestions for consideration by using a form found on the FEI website under the Anti-Doping and Controlled Medication tab.

Who makes the rules for racing?


In the United States, each state is responsible for regulating racing. The members of the Racing Commission (or in some states the Gaming Commission), which are appointed by the governor, are responsible for developing and enforcing the rules and regulations in their state, including those pertaining to drug testing. They receive guidance from a number of sources. The Association of Racing Commissioners International (ARCI) was established in 1934 to try to unify rules and regulations across state lines, and many Commissioners are members of this organization (12). In 2003, racing industry representatives supported the formation of the Racing Medication and Testing Consortium (RMTC) so that it could provide guidance to ARCI and others specifically regarding drug testing (13). The RMTC Scientific Advisory Committee, of which I am a member, meets approximately every 6 months to discuss and formulate recommendations pertaining to medications and best laboratory practices. The horsemen and women associations (e.g. The Horsemen’s Benevolent and Protective Association) also provide input to the Racing Commissions regarding their interests with respect to drug policies and testing. Some states have an equine medical director, typically a veterinarian, employed by the Racing Commission to provide them guidance. And some states (e.g. PA) support a research laboratory so that drug questions specific to their jurisdiction can be immediately and confidentially addressed with targeted research in pharmacology and analytical chemistry.

Which drugs are prohibited for racing?

The first list of prohibited drugs for racing was generated by ARCI in 1991, and includes a classification scheme with 5 categories (12). Class 1 drugs are those with the highest potential to compromise the integrity of the sport or the welfare of the horse and include DEA scheduled drugs (e.g. morphine). Class 2 includes drugs that could be used therapeutically, but also have a high potential to compromise the integrity of the sport and/or welfare of the horse (e.g. anesthetics including local blocking agents). Class 3 drugs are those with moderate potential (e.g. bronchodilators and anabolic steroids). Class 4 drugs are those with some potential but less potential than Class 3 drugs (e.g. nonsteroidal anti-inflammatories and corticosteroids), and Class 5 are those with essentially no potential (e.g. anti-ulcer medications) but are prohibited to be given in many jurisdictions within 24 hours (e.g. PA) or 48 hours (e.g. CA) of the race. ARCI also recommends penalties appropriate for the classified drugs. With regard to “zero tolerance” ARCI states: "Found substances or drugs not included in these guidelines should be treated as Class 1 violations warranting a Class A penalty unless otherwise advised by consultation with Racing Commissioners International (RCI) and/or the Racing Medication and Testing Consortium (RMTC).” (12) In addition, as of 2012, the Thoroughbred Owners and Breeders Association annually issues a list of drugs that must be included in the drug testing program for Graded and Listed Stakes races.

Every year, more drugs are added to the lists of prohibited drugs. However, the speed at which approved medications and illegal substances have been discovered, developed, and made available for purchase has consistently surpassed the ability of forensic chemists to develop analytical methods for identifying individuals who have administered these substances to their horse. It was estimated in 2011 that 8,969 unique compounds are approved for therapeutic or experimental use in humans and animals, and synthetic chemists have created libraries of over 100,000 compounds (14). Many of these substances do have the potential to compromise the integrity of the sport and/or the welfare of the animal, however, many do not. It is up to the researchers and research sponsors, in collaboration with private investigators, to prioritize which drugs are studied first. Historically, much of the focus of this work has been only on the identification of these drugs with little regard to the concentration measured – zero tolerance means that legally any measureable amount is a violation. The dramatic increase in sensitivity that has been achieved over the last 5 to 10 years, however, has forced a re-evaluation of this philosophy for drugs routinely used by practicing veterinarians to treat disease.

THRESHOLDS AND WITHDRAWAL TIMES

A threshold is the concentration below which a drug will be ignored in a sample (i.e. thresholds allow exceptions to the zero tolerance rule). The first drug to be assigned a threshold for racing horses was phenylbutazone (threshold = 2 µg/mL), the most commonly used non-steroidal anti-inflammatory. A threshold can be set in plasma or urine. A threshold in plasma is recommended if the drug can be confirmed and quantified in plasma after it is no longer having a measureable pharmacological effect (e.g. flunixin meglumine). A threshold in urine is recommended if the pharmacological effect is measurable after the drug can no longer be confirmed in plasma (e.g. clenbuterol). For short acting drugs, the threshold is set to correlate with 24 or 48 hours to ensure no drugs have been administered during this time frame – this assures compliance with the “no drug administration” rule stated earlier.

A withdrawal time is the recommended length of time needed for the concentration to fall below the threshold (or limit of confirmation if there is no threshold). A withdrawal time can be estimated for a particular drug only if the threshold/limit of confirmation is known and pharmacokinetic data are available, however it can never be guaranteed. Pharmacokinetic studies are typically performed in a relatively small number of healthy animals (6 to 20) and the data are extrapolated to the entire racing population. Ideally population pharmacokinetic studies would be performed to ensure the pharmacokinetic model generated with the small number of horses applies to the population, however the resources to do these intensive studies are limited. Thus, a safety factor is applied to try to account for the limitations of the data at hand. The RMTC calculates the upper 95/95 tolerance limit to provide withdrawal guidance, and more information describing this calculation can be found on their website (13).

Thresholds and associated withdrawal times have now been proposed for 30 medications by the RMTC, and the RMTC scientific advisory committee is working with the American Association of Equine Practitioners (AAEP) Racing Committee (15) to
identify which drugs should be prioritized for research efforts going forward. Because the Center for Veterinary Medicine (16, 17), the branch of the Food and Drug Administration responsible for the approval of veterinary drugs, does not require pharmacokinetic data for the approval of equine drugs, and because of extra-label drug use, these data have primarily been generated in academic research laboratories.

WHAT IS APPROPRIATE DRUG USE?
This question has yet to be answered and is the subject of much debate in the United States and globally. Many feel that no drugs should be used. Many others consider it cruel to prohibit the use of drugs that have a medical benefit, even if they compromise integrity. Currently, it is up to racing commissioners to decide the answer to this question for race horses.

RESOURCES AND REFERENCES