Overview of Dietary, Environmental, and Endogenous Substances of Regulatory Concern in Racing Horses

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There are many everyday substances, part of the normal and ordinary diet, brought into the environment by humans or specifically synthesized within the horses that are possible drug-test contaminants. Authors' addresses: The Maxwell H. Gluck Equine Research Center, Dept. of Veterinary Science, College of Agriculture, University of Kentucky, Lexington, KY 40506 (Tobin, Harkins, and Woods); Departamento de Morfologia e Fisiologia, Animal Faculdade de Ciencias Agrariais e Veterinarian de Jaboticabal, Unesp. Sao Paulo, Brazil (Queiroz-Neto); Truesdail Laboratories, Inc., Tustin, CA 92680 (Stanley); and Kentucky Racing Commission, 4063 Iron Works Pike, Lexington, KY 40511.

1. Introduction

With the increasing sensitivity of equine drug testing, the incidence of detection of dietary, environmental, and endogenous substances in postrace blood and urine samples has increased. The purpose of this review is to bring together information on this subject for equine practitioners, commission veterinarians–equine medical directors, and association veterinarians. We have categorized these substances under three sections: dietary substances, environmental substances, and endogenous substances.

2. Dietary Substances

A. Definition

A dietary substance is a substance that is part of the normal and ordinary feeding of horses. Here we are concerned with substances that yield materials in postrace samples that trigger administrative actions. With dietary substances, there are generally clear-cut geographic, seasonal, and food-source influences on the appearance of these materials in postrace samples.

B. Salicylate

Salicylic acid (salicylate), the prototype nonsteroidal anti-inflammatory drug (NSAID), is found in the postrace urine of all horses and has long been recognized as normal in horse urine. The problem for the regulator comes in distinguishing between salicylate from natural sources, such as alfalfa hay, and salicylate administered to horses as topical applications or rubs such as methylsalicylate.

Approaching this problem, the authorities analyzed large numbers of samples from horses racing in England and Canada and also samples from experimental horses. From these studies, thresholds of

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6.0 μg/ml and 750 μg/ml of salicylate in plasma and urine, respectively, were established. These thresholds are internationally recognized, and the 750 μg/ml threshold in urine is being reviewed for adoption as the urinary threshold for this agent in California. A critical factor in the development of this threshold was the identification of alfalfa hay as a cause of high urinary concentrations of salicylate. We are not aware of any recent administrative actions based on the identification of salicylate. Salicylate is an Association of Racing Commissioners International Class 4 agent.[1]

C. Hordenine

Hordenine is a plant alkaloid that is closely related both structurally and pharmacologically to epinephrine. It gets its name from *Hordeum vulgare* or barley, a common source of hordenine. Other common sources include Reed Canary grass, brewers grains, and sprouting barley. When administered intravenously, hordenine produces clear-cut but very transient pharmacological effects. There is no evidence to suggest that the oral administration of hordenine is associated with any pharmacological effects.

Like salicylate, hordenine is likely to be found in a large number of postrace urine samples if they are examined at a high enough sensitivity. There are, however, regional reports of unusually high concentrations of hordenine being found in the postrace urines of horses racing in Minnesota and also in Queensland. It seems likely that these geographically related high concentrations of hordenine are also seasonally related.[2]

Despite that fact that hordenine is routinely detectable in a small percentage of the horses racing in any given jurisdiction, there is at this time no formal threshold for hordenine. Hordenine is not classified by ARCI. The last administrative actions taken on hordenine in U.S. racing took place in West Virginia in 1987. Some European laboratories, however, report the presence of hordenine to their authorities.

D. Dimethyl Sulfoxide

Dimethyl sulfoxide (DMSO) is found in all horse urines and also, in small amounts, in rainwater. It is found in many feed ingredients and in relatively large amounts in Lucerne hay. DMSO in the urine of untreated horses is thought to be entirely of dietary origin. DMSO and its metabolite, dimethylsulfone (MSM), therefore occur normally in horse urine. In horses on a diet of Lucerne hay, urinary concentrations as high as 5 μg/ml of DMSO in urine have been reported.

From these findings, DMSO should not be reported for administrative action on the basis of a qualitative finding only, but it should be quantified and be present in excess of a defined threshold. DMSO is a Class 5 substance in the ARCI classification system. As such, DMSO is considered an agent with essentially no ability to affect the outcome of a race, and it is generally considered to be of little regulatory interest. DMSO is often readily identifiable in postrace urine samples, and some U.S. Laboratories report DMSO to their authorities. The international thresholds for DMSO are 1 μg/ml in plasma and 15 μg/ml in urine.[3]

E. Morphine

Morphine is found in significant quantities in hay grown in certain parts of Australia and worldwide in poppy seed used in baked products such as bagels and muffins. Occasional low concentrations of morphine or its metabolizes in postrace horse urine in eastern Australia have been traced to horses eating feed contaminated with poppy capsules. Another possible source of morphine is codeine, which is metabolized to morphine in man and presumably also in the horse; the pharmacological activity of codeine in man may be due to its metabolism to morphine. Findings of morphine in postrace urine samples may therefore be associated with contaminated hay in certain geographic areas, inadvertent feeding of poppy seed bagels, accidental contamination from prescription codeine, or morphine from other sources.

Because poppies grow wild in Australia, there are clear seasonal and geographic influences on the incidence of morphine identifications in this country. No published or unpublished thresholds for morphine have been reported; however, Australian researchers use chemical-ionization GC/MS procedures to identify equine urine samples in which morphine is derived from *Papaver setigerum* contamination of cereal crops.[4] Morphine is an ARCI Class 1 agent.

F. Scopolamine

Scopolamine is an alkaloid that is closely related to atropine that is available as a pharmaceutical agent and also from various plant sources. Pharmaceutical scopolamine has clear-cut if limited applications in equine medicine. The most common plant source of scopolamine in the U.S. is Jamestown or jimson weed, which grows wild across much of the southern United States. Jimson weed is occasionally abused by teenagers seeking a pharmaceutical high.

Scopolamine identifications are rarely reported in racing horses, and unequivocally distinguishing between pharmaceutical scopolamine and scopolamine from plant sources is far from trivial. Experiments in which pharmaceutical scopolamine was administered have shown that, within the limitations of postrace testing, it was not possible to distinguish clearly between jimson weed scopolamine and pharmaceutical scopolamine. Although jimson weed also contains atropine, limited experimental studies suggest that atropine from jimson weed is much less likely to be detected in horse urine than scopolamine. In contrast, a seasonal finding of jimson weed in close association with horses and an associated finding of scopolamine in postrace urines clearly raises the possibility of environmental contamination.[5]

Within the past 2 years, a number of scopolamine

identifications have been made in the U.S. and elsewhere. Scopolamine is an ARCI Class 3 agent. To our knowledge, no published or unpublished thresholds for scopolamine have been reported.

G. Bufotenine

Bufotenine or *NN*-dimethylserotonin is an indole alkaloid found in the leaves and seeds of *Piptadenia* and also from *Amanita*. Bufotenine is also secreted by the skin glands of toads, where it may have deterrent, antibacterial and antifungal properties. Bufotenine is also hallucinogenic, and materials from frog and toad skin are sometimes ingested for their hallucinogenic effects.

At least one identification of bufotenine in a postrace urine sample has been reported in the U. S., and a number of identifications have been made outside the U.S. Although no penalty was assessed, no formal threshold for this agent in postrace urine exists. Bufotenine is not classified by ARCI. As yet, no seasonal or geographic associations for bufotenine identifications have been reported.

H. Arsenic

Arsenic is ubiquitous in nature and is found in all horse urines. However, it can be used as a tonic in small amounts and as a stopper in large amounts. A threshold was therefore needed to distinguish between normal and unusually high concentrations of arsenic in postrace urines. Crone and his coworkers analyzed 4000 postrace samples in Hong Kong between 1983 and 1988. These data yielded a skewed distribution that was normalized by performance of a cube-root transformation. From this distribution, the threshold was set at 4 plus standard deviations from the mean. The international threshold for arsenic is now 0.3 μg/ml of arsenic in urine.[3] Although it is highly likely that there are geographic influences on arsenic concentrations in postrace urine, these are not described. Arsenic is not classified by ARCI.

3. ENVIRONMENTAL CONTAMINANTS

A. Definition

Environmental contaminants are substances brought into the environment of the horse by man and that are unlikely to be found in horses not closely associated with man. Horses may be exposed to these materials prerace, in which case metabolizes of the materials will be found in the postrace samples. Identification of the parent contaminant in the absence of metabolizes is presumptive evidence of postrace contamination.

B. Caffeine

Caffeine is the most widely used psychoactive agent in the world. It is consumed daily by humans in considerable (125 mg) amounts. In the horse, caffeine's long plasma half-life and relative ease of detection make it surprising that caffeine is relatively rarely reported in postrace samples from racing horses.

Caffeine is extensively metabolized in the horse, as only approximately 2–3% of a dose is excreted in the urine as unchanged caffeine. In contrast, ~30% of the dose is excreted as theophylline, theobromine, and paraxanthine.

A finding of caffeine in a urine sample with associated metabolizes generally means that caffeine went through the horse. The finding of caffeine without any associated metabolizes means that the caffeine did not go through the horse, with the implication that the caffeine resulted from postrace contamination. A less obvious source of contamination is human sweat. All coffee drinkers excrete caffeine in their sweat; as the sweat evaporates, the cutaneous concentration of caffeine increases. Skin and sweat can therefore contain significant amounts of caffeine. If caffeine is detected in a postrace sample, the sample should be analyzed for metabolites. In the absence of metabolizes the likelihood is that the caffeine present is due to postrace contamination.

Because of the widespread environmental presence of caffeine, Hong Kong has an unpublished threshold of 0.01 μg/ml in plasma and 0.03 μg/ml in urine. Malaysia also has an in-house threshold of 0.01 μg/ml in plasma.[6] Caffeine is an ARCI Class 2 agent.

C. Theobromine

Theobromine is 3,7-dimethylxanthine, and along with theophylline and paraxanthine it is a major metabolite of caffeine in the horse. For two decades theobromine was the most commonly identified material in horse urine in England. This theobromine originated from caffeine in cocoa beans and cocoa husk. When cocoa husk is administered, theobromine predominates in the urine; in the early 1980's, large numbers of calls were being made on these agents.

It proved very difficult to remove cocoa husk from the feed, so the approach was taken of developing a threshold. Studies were carried out at the Horse Racing Forensic Laboratory in England, and 2 μg/ml in urine was established as the regulatory threshold.[7] Theobromine is an ARCI Class 4 agent.

D. Nicotine

Nicotine is ubiquitous in the human environment and is occasionally identified in postrace urine samples from horses. As far as we know, the metabolism of nicotine in the horse has yet to be described; however, in man cotinine and *trans*-3-hydroxycotinine are its major urinary metabolites. From what is known of the metabolism of nicotine in humans, the likelihood of free nicotine entering horse urine by any route other than contamination is very small. In the absence of cotinine or other nicotine metabolites, a nicotine identification is presumptive evidence of postrace contamination.[8] Nicotine is not currently classified by ARCI.

E. Cotinine–*Trans*-3 -Hydroxycotinine

Cotinine–*trans*-3-hydroxycotinine are the major urinary metabolizes of nicotine in man. Their identification in horse urine in significant concentrations is presumptive evidence that the horse was exposed to nicotine, such as bedding or tobacco stalks. These agents are not classified by ARCI.[8]

F. Cocaine

Cocaine is ubiquitous in certain human environments, and cocaine and or its metabolizes have been found in tongue ties, in saliva samples from horses entering races, and in postrace urine samples from horses. Most of these identifications have been at relatively low concentrations, and their pharmacological and forensic significance is often unclear.

In Illinois, control of the use of cocaine on tongue ties has been implemented by use of a prerace cocaine enzyme-linked immunosorbent assay. Application of this test allows prerace detection of cocaine contamination; the trainer is then invited to withdraw his or her horse, and most trainers elect to do so. This approach avoids the problem of determining the source, pharmacological effect, and forensic significance of trace detections of cocaine or its metabolizes in postrace urine. Determining the significance of traces of cocaine or its metabolizes in postrace samples is made more difficult by the fact that cocaine spontaneously hydrolyzes to breakdown products difficult to distinguish from authentic metabolites.[9] Cocaine is classified as an ARCI Class 1 agent.

4. Endogenous Substances

A. Definition

Endogenous substances are substances that are specifically synthesized within the horse and are independent of dietary or other sources.

B. Hydrocortisone

Hydrocortisone is an endogenous corticosteroid hormone produced by the adrenal gland that is essential to normal life. It is also available as an injectable pharmaceutical and its release in the horse can be specifically stimulated by administration of adrenocorticotropic hormone. As a way to control its use in racing horses, a urinary threshold has been established.

Research by Stenhouse and Ralston in Western Australia and at the Horse Racing Forensic Laboratory in England suggests that 1 μg/ml in urine is an acceptable threshold, with only 1/10,000 probability of a normal horse exceeding this concentration.[10,11]

C. Testosterone

Testosterone is normal in the plasma and urine of geldings and fillies, but at very low concentrations. Testosterone can also be used for its anabolic actions in fillies and geldings. For this use of testosterone to be controlled, a threshold for this agent is required. The Australian authorities use a threshold of 100 rig/ml of testosterone, although by what method this threshold was devised is not quite clear.[4]

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