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**Equine doping: perceptions, adverse effects and Cyprus hippodrome
case review for the years 2001-2010**

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**Φαρμακοδιέγερση σε ίππους: αντιλήψεις, ανεπιθύμητες ενέργειες
και αναδρομική παρουσίαση κρουσμάτων στην περίοδο 2001-2010
στον ιππόδρομο στην Κύπρο**

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ABSTRACT. Doping in race horses affects thousands of equine athletes worldwide. Illicit administration of medical compounds aims to improve the horse's performance either through altering the athletic capacity, or through covering the symptoms of pathologic conditions. Nicosia race track has a significant number of confirmed doping cases when considering the small equine population of the island, reflecting the lack of credibility of racing in Cyprus. The most frequently administered medications are anabolic steroids, human recombinant erythropoietin, stimulants, diuretics, non-steroidal anti-inflammatory drugs, local anesthetics, bronchodilators and coagulants. Interestingly, published literature on the effectiveness of these substances is inconclusive or contradictory. This article constitutes a cumulative presentation of confirmed doping cases in Nicosia race track and a brief review on the published literature regarding the effectiveness of the most commonly administered compounds.

Keywords: anabolic steroids; Cyprus racing; equine doping; equine performance manipulation

ΠΕΡΙΛΗΨΗ. Η φαρμακοδιέγερση αθλητικών ίπων αποτελεί διεθνή πραγματικότητα. Η παράνομη χορήγηση φαρμακευτικών ουσιών στοχεύει στη βελτίωση της επίδοσης του ίππου είτε μέσω της αύξησης της αθλητικής ικανότητας, είτε μέσω της κάλυψης συμπτωμάτων που προκαλούν παθολογικές καταστάσεις. Η λέσχη Ιπποδρομιών Λευκωσίας εμφανίζει σημαντικό αριθμό επιβεβαιωμένων θετικών δειγμάτων, λαμβανομένου υπόψη του μικρού αριθμού ιπποειδών. Οι συχνότερα χορηγούμενες ουσίες

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είναι τα αναβολικά στεροειδή, η ανθρώπινη ανασυνδυασμένη ερυθροποιητίνη, τα διεγερτικά, διουρητικά, τα μη στεροειδή αντιφλεγμονώδη, τα τοπικά αναισθητικά, τα βρογχοδιασταλτικά και τα πηκτικά του αίματος. Σύμφωνα με τη δημοσιευμένη βιβλιογραφία, η αποτελεσματικότητα αυτών των ουσιών στην αθλητική επίδοση των ίππων είναι ασαφής ή αμφιλεγόμενη. Το άρθρο αυτό αποτελεί συγκεντρωτική παρουσίαση των επιβεβαιωμένων θετικών κρουσμάτων φαρμακοδιέγερσης στον κυπριακό υπόδρομο, και μία συνοπτική μελέτη της δημοσιευμένης βιβλιογραφίας σχετικά με την αποτελεσματικότητα των συχνότερα χορηγούμενων ουσιών.

Λέξεις ευρετηρίασης: αναβολικά στεροειδή, Κυπριακός υπόδρομος, παραποίηση αθλητικής απόδοσης, φαρμακοδιέγερση ίππων

INTRODUCTION

Doping in sport horses is a global reality. It is defined as presence of a prohibited substance or its metabolites or markers in an athlete's bodily specimen. It is believed that thousands of horses suffer illicit use of medical substances and methods in order to achieve better performance in racing, show jumping, dressage, eventing, and endurance race. From these cases only a small percentage is being detected with obvious consequences against the responsible person(s).

It has been acknowledged that to offer a clean game to the horse racing audience, it is imperative need to ensure that the best care is provided to the equine athlete. International Federation for Equestrian Sports which is the international governing body of most equestrian sports (excluding horse racing) states on its official webpage (<http://www.feicleansport.org>): "Equestrian sport derives its credibility and public acceptance from the concept of fair play, the idea that the best athlete or team should win fairly and squarely, having competed under equitable conditions and under rules that are fair and applied evenly with true competence. To be valid and meaningful, competition results must be achieved on a level playing field. Doping and the inappropriate use of normal medications present a serious threat to the integrity and reputation of our sport, because they give athletes an unfair advantage and threaten the welfare of horses. Therefore, it is the responsibility of the entire equine community: athletes, veterinarians, grooms, managers, coaches, owners, officials and our National Federations, to join together to help combat doping and the inappropriate use of medications through better education and increased vigilance."

The most commonly used substances for horse doping can be divided in the following categories: (i) substances capable of acting on one of the body systems (the nervous s., the cardiovascular s., the respiratory s., the digestive s., the urinary s., the reproductive s., the musculoskeletal s., the blood s., the immune s. except for licensed vaccines against infectious agents, the endocrine s.; endocrine secretions and their synthetic counterparts) and (ii) substances capable of covering symptoms of diseases or syndromes, such as: (ii.i) anti-pyretics, (ii.ii) local anaesthetics and non-steroidal anti-inflammatory drugs (NSAIDS) to reduce inflammation and alter pain perception, (ii.iii) Analgesics and anti-inflammatory substances, (ii.iv) substances affecting blood coagulation and diuretics to cover the symptoms of exercise induced pulmonary haemorrhage, (ii.v) cytotoxic substances, antihistamines, local anaesthetics, muscle relaxants, respiratory stimulants, sex hormones, anabolic agents and corticosteroids.

CURRENT SITUATION IN CYPRUS HIPPODROME

Nicosia Race club was founded in the 19th century by English Army Officers and since 1960 it gained its nowadays structure. Anti-doping control is a responsibility of the Cyprus Turf Club. A urine sample is obtained from the winner of each race, and from horses with contradictory performance. The lab tests are performed in an independent laboratory in the United Kingdom. Detection of substances which are included in the prohibited list is convictable.

In the 2001-2010 decade, 1157 meetings with 9833 races took place in Nicosia race club. In these

Table 1. Analysis of the races, runners, starts and confirmed cases of doping in the Nicosia race club during the period 2001-2010.

Year	Races	Runners	Starts	Confirmed cases
2001	821	1091	7471	22
2002	913	1161	8515	13
2003	930	1223	8986	17
2004	946	1356	9349	14
2005	1020	1418	9741	14
2006	1002	1444	9832	20
2007	1069	1539	10724	5
2008	1055	1492	10478	13
2009	1028	1419	9402	13
2010	1049	1452	10302	30
Total	9833	13595	94800	161

races 13595 runners were recorded with 94800 starts (Cyprus Turf Club, 2001-2010). During this period 161 samples were found positive to substances included in the prohibited list (table 1). From these samples, 130 were detected with a single substance and 31 were detected with multiple substances; 44 horses were found positive to anabolic steroids (boldenone, testosterone, stanozolol), 30 horses were found positive to NSAIDS (ketoprofen, flunixin meglumine and phenylbutazone); 21 horses were found positive to alkaloids (caffeine, theophylline, theobromine, quinine), 5 horses were found positive to β -2 adrenergic agonists (clenbuterol, albuterol, formoterol), 5 Horses were positive to carbazochrome and 3 horses were positive to human recombinant erythropoietin. Finally, furosemide was traced in 6 horses. Drugs affecting the respiratory, nervous, haemopoetic, alimentary, urinary systems, but also anti-hypertensives, muscle relaxants and local anaesthetics were also detected. The list also includes butanoic acid, atropine, heptaminol, tranexamic acid and verapamil.

DISCUSSION

The number of positive samples during the years 2001-2010 is considerably high especially if the small number of races and starters is taken into con-

sideration. For instance, from a pool of 7346 runners in the 2006-2010 quinquennial, 81 cases were confirmed positives (ratio 1:90) while in the British race clubs the incidence for the same period was 93 confirmed cases (British Horseracing, 2006-2010); within 474364 runners (ratio 1:5100). Reasonably, it is suspected that these numbers are just the tip of the iceberg, and a number of cases is not detected as samples are rarely obtained from horses with poor performance.

The reasons of failure in the anti-doping rule in Nicosia race track in the authors' opinion can be summarised as: (i) lack of education of the people in the horse racing industry in Cyprus (trainers, Jockeys, Owners), (ii) lack of credibility of horse racing within Cypriot society, (iii) the penalties for anti-doping rule violations are not deterrent and (iv) the weaknesses of the anti-doping strategy of the authorities. Moreover, the fact that samples are obtained only from race winners makes it impossible to correlate drug administration and enhanced performance, as some non-winning horses may have been treated with illegal drugs. It is a fact that most of the decision making in horse doping is based on assumptions, perception and false expectations. The international literature on how the most commonly used substances are affecting performance is scarce and

only few studies have been published on this subject. Unsurprisingly, for the majority of the substances used in Cyprus it is true that no scientific evidence exists as to their pharmacokinetic abilities on the horse and their actual potential to enhance the performance of the equine athlete. Dissemination of these results would discourage people involved in equine sports industry from using such substances.

Anabolic steroids

Anabolic steroids are synthetic testosterone analogues. In this study, 44 horses in Nicosia race club were found positive to anabolic steroid compounds. They are amongst the most frequently used compounds as performance enhancers in horse racing. However the benefits of anabolic steroids use in sport horses are based on anecdotal documentation. Scientific evidence related with the expected positive consequences on athletic ability of anabolic steroids is rare and the result inconsistent. The physiological parameters response to the anabolic steroids administration vary, and in most cases not significant alterations are achieved on the parameters which directly affect sport horse performance.

A number of studies have investigated the result of anabolic steroids on performance. These studies investigated potential improvement in terms of direct performance improvement and indirect improvement through anabolic steroids effect on nitrogen balance, weight gain, red blood cell volume, muscle growth, and behavior (Pitts and Davis, 2007).

Researchers (Snow et al., 1982a; 1982b) have investigated a potential positive effect of anabolic steroids on nitrogen balance. One study has shown that nitrogen retention was significantly increased after anabolic steroid injection. It is speculated that anabolic steroids prevent training-related tissue breakdown, by promoting positive nitrogen balance. In the same studies there was no proven data to link anabolic steroids use with weight gain or avoid training-related weight loss. Body measurements had shown no significant changes between treated horses and control group.

Another research group, (Hyppa et al., 1995) has investigated the correlation between anabolic steroids and red cell volume. Red cell volume was increased by 20%. The same study investigated the

overall effect of anabolic steroids on performance ability through red cell volume. Surprisingly, according to the results of the study, the increase in red cell volume did not lead to increased performance or aerobic ability. Aerobic capacity was measured with the velocity achieved while heart rate is 200/min (V200); control horses showed positive correlation between red blood cell volume and performance ability while treated horses did not.

The anabolic steroids effect on muscle cells was investigated on several studies based on the effects they showed on several muscle markers. Muscle water content, muscle glycogen content, muscle enzyme activity, total mean protein concentration and the number of capillaries per unit fiber area were not proven to be significantly affected after administration of anabolic steroids (Snow et al., 1982a; Nimmo et al., 1982).

Other measurements with no consistent findings regarding anabolic steroids includes blood creatinine levels, total protein sodium, chloride, magnesium, phosphorus, white blood cell count serum cortisol levels, growth hormone levels and peak heart rate (Pitts and Davis, 2007).

The adverse effects of long term anabolic steroid use on horses should not be underestimated. Studies in humans and rats suggest that chronic use of anabolic steroids leads to a stiffer tendons with lower energy absorption potential, increasing the risk of tendon injury (Seynnes et al., 2013; Inhofe et al., 1995). Additionally, it has been associated with adrenal insufficiency (Dowling et al. 1993), aggressive behaviour, infertility and severe laminitis (Dowling 2002).

Non-steroidal anti-inflammatory drugs

NSAIDs, as antipyretics, analgesics and anti-inflammatory agents, are among the most commonly used medications in equine practice. Musculoskeletal injuries are usually treated with regimens from this category such as phenylbutazone, carprofen, meloxicam, and flunixin meglumine. The blocking action of NSAIDs on prostaglandin synthesis and therefore inflammation is achieved through cyclooxygenase inhibition in the arachidonic acid cascade. Even though musculoskeletal injuries have a complex background (age, racetrack surface, gender, training regime), it is speculated that NSAIDs are

associated with increased risk of injury. In a two year field study in a Kentucky race track (Dirikolu et al., 2008), injured horses were found with higher plasma concentrations of flunixin and phenylbutazone than non-injured horses. Finally, NSAIDs use does not come without consequences as they are among the etiologies of gastric ulceration (Murray, 1999) and renal papillary necrosis (Dowling, 2002).

Erythropoietin

Erythropoietin (EPO) is a peptide hormone. It is physiologically produced by kidney interstitial fibroblasts as a response to hypoxia if cardiopulmonary adjustments are not sufficient to prevent hypoxemia. The hormone stimulates erythropoiesis increasing red blood cell volume, and consequently increasing arterial partial pressure of oxygen (PaO₂). Interestingly, training horses in altitude or in very intensive regimes would not significantly increase EPO production and red blood cell volume. Negative feedback mechanisms prevent overproduction of EPO.

Altitude exercise has only a short term effect on EPO production. It is also suggested that EPO production is only enhanced during the first day of altitude exercise. Cardiorespiratory mechanism is capable of responding to the high altitude hypoxia after this short period. (Hinchcliff et al., 2008).

In an experiment where human recombinant erythropoietin (rhuEPO) was administered three times per week for three weeks to four mares (McKeever et al., 2006) an increase in red cell volume and aerobic capacity of the treaded horses is reported. In contrast, according to other studies (Jaussaud et al., 1994; Souillard et al., 1996), where rhuEPO was administered once, no positive alteration in the haemogram was noted.

It is therefore understood that when human recombinant erythropoietin is injected to horses, resting hematocrit is significantly elevated increasing blood viscosity. Trigger factors such a) splenic reserve mobilization and b) diuretic-induced fluid losses may lead to sudden death during exercise (Hinchcliff et al., 2008). RhuEPO administration to horses can potentially induce life-threatening anemia due to immune system reaction (Piercy et al. 1998). Three horses running in the Nicosia race track have

been found positive to EPO in the decade 2001-2010.

Caffeine

Caffeine (a xanthine alkaloid to which 7 horses were found positive) is also one of the drugs administered to stimulate horses and achieve better performance. In a study (Ferraz et al., 2008), the treatment group (n= 6, Arabian horses) received caffeine IV at 5 mg/kg 30 minutes prior exercise, while the control group of 6 Arabian horses received saline IV. The athletic potential of equine athlete is evaluated with the relation between velocity and heart rate. All horses followed the same exercise protocol on treadmill. The results of the study revealed that the caffeine-treated horses reached significantly increased velocities at the same levels of heart rate (10% at 180 beats/min and 11.7% at 200 beats/min) compared to the control group. It was also observed that caffeine-treated horses had significantly higher blood glucose and lower cortisol concentrations; they also had lower plasma insulin concentrations. Both control horses and caffeine-treated horses groups showed increased haematocrit (Hct) during exercise. However, exercise alone can lead to Hct alteration due to catecholamine induced splenic contraction as it can release up to 12 L of blood in the circulation (Hodgson et al., 2014). Caffeine induces adrenaline and noradrenaline release which is a factor that increases Hct. However, when administered at a dose of 2.5mg/kg (Savage et al., 2005), caffeine failed to enhance the performance potential during intense exercise.

Furosemide

Furosemide is very often used in race horses. During the time period of the study, 8 Nicosia race horses were found positive in that substance. The expectation from this loop diuretic is to manage Exercise Induced pulmonary hemorrhage (EIPH) symptoms. The efficacy of this widely abused treatment was investigated in early studies (Pascoe et al., 1985). In a field trial which included 167 Thoroughbred horses treated intravenously with either furosemide or placebo (saline) it was shown that furosemide significantly lowers the risk of EIPH incident (Hinchcliff et al., 2009).

Six Standardbred horses with known EIPH his-

tory were included in a clinical trial (Perez-Moreno et al., 2009), which demonstrated that administration of furosemide or furosemide – carbazochrome (antihemorrhagic agent administered additionally to furosemide treatment) combination to horses fails to reduce the severity of EIPH symptoms.

Etamsylate

Regardless the lack of scientific evidence, etamsylate (a non-thrombogenic anti hemorrhagic drug) is administered to racehorses competing in Cyprus as a masking agent to cover the epistaxis/bleeding caused by EIPH. Etamsylate is also frequently used after upper respiratory surgery to control bleeding in respiratory tract. *In vitro* and *in vivo* effects of etamsylate were investigated in a study that recruited seven and eight horses respectively (Segura et al., 2007). Etamsylate in different concentrations was incubated with citrated blood and using flow cytometry, P-selectin expression and annexin-V binding were measured. In the *in vivo* leg of this experiment, P-selectin expression was measured in blood samples obtained prior etamsylate administration and 1 and 2 hours post IV administration of 12.5 mg/kg etamsylate. P-selectin expression, leukocyte-platelet aggregate formation and annexin-V binding showed a significant increase in the *in vitro* study, but not in the *in vivo* experimentation.

Clenbuterol

During the time period of the study, 3 horses were found positive to clenbuterol. Clenbuterol as a β -2 sympathomimetic agent is administered as bronchodilator in clinical practice. Based on perceptions, it is administered to healthy performance horses, in order to increase oxygen intake—and therefore to increase aerobic performance. The aerobic capacity of the athletic horse is determined by the velocity corresponding at 4 mmol/L blood lactate concentration.

Long term effects of the administration of clenbuterol on performance have been investigated, with twenty-three unfit mares taking part in the study (Kearns and McKeever, 2002). In this study, the horses were divided in four groups: (i) exercise only, (ii) clenbuterol-treated only, (iii) exercise and clen-

buterol-treated and (iv) control. The study of the effects of clenbuterol on aerobic performance was based on performing tests like graded exercise test, aerobic capacity and exercise capacity test (ECT). Interestingly, the outcome of this study was that clenbuterol adversely affects aerobic performance, high-intensity exercise and the ability of the sport horse to recover from exercise, increasing the potential for cardiac and thermal injury. Moreover, excessive sweating was observed in treated horses while in the untreated ones sweating was in normal levels.

Findings of another study based on echocardiography (Sleepers et al., 2002) suggest that clenbuterol has detrimental effects on equine cardiac function causing changes in the structural dimensions increasing risk for aortic rupture.

CONCLUDING REMARKS

Undoubtedly, the doping issue in Cyprus is not negligible. The number of positive samples is significantly high considering the small size horse population. The large number of the trafficking substances, the lack of any scientific background of the people involved in equine doping and the potential side effects from this practice, reveal the inconvenient truth: the equine athlete is exposed to every individual's will to administer potentially harmful substances in order to gain advantage against its competition. It is well understood that it is a responsibility of the authorities to improve testing, in order to protect the equine athlete, but also the integrity and credibility of the horse racing product.

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CONFLICT OF INTEREST STATEMENT

The authors report no conflicts of interest.

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