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PHARMACOEPIDEMIOLOGICAL STUDY OF FOREIGN SUBSTANCES IDENTIFIED IN POST RACE URINE SAMPLES ASSOCIATED A MORTALITY AT LA RINCONADA RACETRACK, CARACAS VENEZUELA, 2008-2012 A PRELIMINARY REPORT

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ABSTRACT

We report a pharmacoepidemiological study on foreign substance identifications at La Rinconada racetrack, Caracas Venezuela from 2008 to 2012. During this period 59904 horses started at La Rinconada in 4992 races of average field size 12 starters per race. At “La Rinconada” all winners and other randomly selected horses are tested yielding a total of 12.644 samples tested over the four year period of this review. Of these samples 126 were reported positive for at least one substance considered reportable under the rules of racing in Venezuela. No ARCI class 1 and two substances were identified in these samples. The most frequently identified substances were furosemide, 31 identifications, followers by no steroidal anti- inflammatory agents, 25 in total, and including phenylbutazone, dipyrone and Flunixin meglumine. Corticosteroids identified include dexamethasone and triamcinolone, for a total of 43 identifications. There were 5 bronchodilator identifications reported including clenbuterol and aminophylline. One identification of methocarbamol was reported, somewhat unusually, numerous identifications of pangamic acid, the supposed vitamin b-15. Review of these substances show that all of these substances are considered or have been classified as therapeutic medications and that no ARCI class 1 or 2 substances with a high potential to enhance performance have been reported during these 4 years of racing at “La Rinconada”.

KEY WORDS: Equine, Foreign Substances, Horses, Thoroughbreds.

INTRODUCTION:

Foreign substances in performance horses are defined as the "illegal application of any substance, except normal diet, that might modify the natural and present capacities of the horse at the time of the race" (Ungemach, 1985). Horse foreign substances cases worldwide have been reported since 1962 (Clarke, 1962), alkaloids (Debackere, et al., 1968), Trans-pai-oxocamphor (Fujii, et al., 1970), procaine (Kunde & Frey, 1971), sulpyrine, aminopiryne, antipirine (Momose & Tsuji, 1972), caffeine (Fujii, et al., 1972), chlorpromazine and phenobarbital (Fujii, et al., 1975), diazepam (Courtot, et al., 1975), corticosteroids (Schubert, 1977), local anesthetics (Delbeke, et al., 1981), psychotropic drugs (Jaeschke, 1983), etorphine (Woods, et al., 1986), morphine (McDonald, et al., 1988), betamethasone (Rodchenkov, et al., 1988), furosemide (theobromine (Delbeke & Debackere, 1991), methandienone (Hagedon, et al., 1992), non-steroidal anti-inflammatory drugs (Gonzalez, et al., 1996), dexamethasone (Ribeiro, et al., 1997), dipyrone (Klaus, et al., 1997), Cortisol (Brooks, 1999), clenbuterol (Harkins, et al., 2001), glycopyrrolate (Rumpler, et al., 2012), multiples foreign substances have been used to alter the performance purposes of the horse during the race, to the present 2013 with using anabolic steroids in Dubai and United Kingdom and USA.

Many of these foreign substances with multiple side effects, with direct consequences on the health of the horse and in many cases end up with irreversible damage and even the death, sudden dead or euthanasia of the horse. Also have been detected over the years illegal substances in horse racing have perfected the techniques of foreign substances detection in blood and urine. Test using gas liquid chromatography-mass spectrometry (1972), Radioimmunoassay (1986) enzyme-linked immunosorbent assay (ELISA) (1987), high-performance liquid chromatography (HPLC) (1990), particle concentration fluorescence assay (PCFIA) (1990), Ultra High Performance Liquid Chromatography (UHPLC) (2012).

The national and international regulations are rigorous in terms of therapeutic medications permitted for horse racing as well as sampling for post-race foreign substances control. But today are still no cases of illegal substances in horse racing severely affecting the equine industry worldwide.

The aim of this study was to describe a pharmacoepidemiological study foreign substances associated mortality in Thoroughbreds horses in the national racetrack “La Rinconada”, Caracas Venezuela.

MATERIAL AND METHODS:

Were studied 126 Thoroughbred horses (51 female and 75 male), between 2-5 years old, in the national race Track “La Rinconada” Caracas-Venezuela. During this period 59904 horses started at la Rinconada in 4992 races of average field size 12 starters per race. At “La Rinconada” all winners and other randomly selected horses are tested yielding a total of 12.644 samples tested over the four year period of this review. The Venezuela National Regulations of Horse Racing, in effect in 1995, states: Administration of any medication to horses registered for racing is prohibited, likewise any chemical, drug or substance of any nature which seeks to alter or modify in any way the horse’s normal or locomotors capacity (Tobin, et al. 2012).

No medication may be administered from five (5) days prior to participating in public competition until the horse ceases to be under the control of the horseracing authorities. Exceptions from compliance with the time provided herein, is the use of furosemide and phenylbutazone, which may be administered by veterinary prescription under the following scheme: The administration of furosemide may be up to four (4) hours before competition which will include a maximum dose of 250mg (Tobin, et al. 2012).

The administration of phenylbutazone may be up to twenty four (24) hours before the competition in which the animal will participate, at a dose not exceeding two (2) grams (in force, year 1995) (Tobin, et al. 2012). The equine registration card must record the administration of furosemide and/or phenylbutazone (Tobin, et al. 2012). Clinical signs are presented in the table number 1.

Samples of blood and urine for toxicology studies were taken immediately after death and analyzed using the competitive Enzyme-Linked Immunosorbent Assay (ELISA) specifically for Furosemide: Furosemide ELISA Kit (1042191 NEOGEN Corporation), Boldenone ELISA Kit (Cat.N.FA650 TECNA); Nandrolona:

Nortestosterone ELISA Kit (BIO K 208 BIO Diagnostic, Dexamethasone: Dexamethasone ELISA Kit (101519 BIOKITS). MaxSignal® Clenbuterol ELISA Test Kit (Bioo Scientific ISO CI#: SARA-2009-CA-0114-02-A); Caffeine/Pentoxifylline ELISA Kit (Neogen Corporation106419); Phenylbutazone ELISA Kit Neogen Corporation 104719-1; Nandrolone ELISA Kit Neogen Corporation 104619; Triamcinolone Acetonide ELISA Kit Neogen Corporation 105119; MaxSignal® Flunixin ELISA Test Kit BO_5050; MaxSignal® Acepromazine/Tricyclics ELISA Test Kit BO_5014 and Methocarbamol ELISA Kit from Neogen Corporation 108019. All equine were study by necropsy (Aluja and Constantino 2002). Samples of tissue were collected from the adrenal glands, gastric mucosa, pancreas, kidneys, liver, spleen, lungs, heart, skin and adenohypofisys (Aluja and Constantino 2002). Tissue sections were fixed in 10% buffered formalin, prepared and stained with Hematoxilin & Eosin (H&E) for light microscopy (Banks 1996).

RESULTS:

A total of 12.644 samples tested over the four year period of these review only 126 samples were positive approximately 0.99% and 4992 races 2.5% test positive.

Sudden Death Iatrogenic: This group of horses (26/126) was medicated injected into the jugular vein and developed a type I hypersensitivity glottis lung edema and sudden death. Were observed pulmonary edema, congestion and hemorrhage. Sub-endocardium petechial hemorrhage. Spleen with foci of acute coagulation necrosis. Hydronephrosis severed renal. Liver accentuated lobular pattern. The rest of the organs with edema, congestion and petechial hemorrhage. In all cases it was observed thrombophlebitis of the left jugular vein and hematoma. The histological sections showed severe pulmonary edema, congestion and hemorrhage. Diapedesis bleeding in heart tissue, liver and spleen. Severe acute renal tubular necrosis. Foreign substances detected in toxicological examination pangamic acid (21/26), boldenone (01/26), furosemide (02/26) and phenylbutazone (02/26).

Sudden death post race: This group of horses (32/126) presenting clinical characteristics, necropsy and histological compatible with pulmonary hemorrhage syndrome induced by exercise. These horses during the end of the race or after the race culminated cardio-respiratory collapse acute and sudden death. Epistaxis was observed only in 4 cases. Necropsy of these horses evidenced: massive hemothorax severe congestion and rupture marked bronchial and mediastinal arteries, subserosal petechial hemorrhage to caudo-dorsal lung lobes, severe edema in trachea, bronchus and lung parenchyma, severe bilateral pulmonary hemorrhage in lung parenchyma. Histopathological lesions showed severe congestion, marked edema, acute pulmonary hemorrhage due to rupture of focal bronchial arterioles, replete with red blood effusion. Foreign substances detected in toxicological examination furosemide (26/32), clenbuterol (03/32), aminophilines (02/32) and metocarbamol (01/32).

Metabolic syndrome: This category corresponded to horses (44/126) that developed side effects post-intoxication in the medium and long term. The clinical findings were consistent with polyuria, polydipsia, hyperglycemia, laminitis (tetra-laminitis) liver and kidney failure. The necropsy showed: weight loss, loss fatty subcutaneous, xantomathosis of subcutaneous tissue. Were observed multiple abscesses and cellulitis piodermas, Liver was swollen, friable and icteric, with focal telangiectasia, renal cortical and papillary necrosis, and equine gastric ulcer syndrome severed. Histopathology showed: liver with periacinar necrosis with a prominent acinar pattern and fatty degeneration severed necrosis and pancreas with vacuolar (glycogen) degeneration islets of langerhans, fibrosis and chronic, severe acute tubular degeneration, coagulation renal tubular necrosis, glomerulonephritis membranous. Hemorrhages in adrenal cortex, coagulation necrosis of the adrenal cortex and atrophy cortical severed. Foreign substances detected in toxicological examination Dexamethasone 20/44, Triancinolone 14/44, phenylbutazone (7/44) and simultaneous Furosemide (02/44), Boldenone (01/44) and Flunixin meglumine (1/44).

Secondary infectious and failure multiorgan: This category corresponds to the evolution of the

metabolic syndrome (22/126). Basically are secondary infections due to immunosuppression with bacteriemia and septicemia evolution. The commitment affects many organs with irreversible damage. clinical, necropsy and histological included pyothorax, septic peritonitis, bacteremia secondary to gastrointestinal tract disease, pneumonia, endocarditis, pyelonephritis, osteomyelitis, and bite wounds.Accompanied by failure liver with focal telangiectasia, pancreatic and renal. Foreign substances detected in toxicological examination Phenylbutazone (11/22), Dexamethasone 06/22, Dipyrone (04/22), Boldenone (02/22).

DISCUSION:

In the study period there were 126 cases of foreign substances with the death of the horses. The highest percentage of cases associated with metabolic syndrome was 35%. Secondly 25% corresponded to cases of sudden death associated with post-exercise induced pulmonary hemorrhage exercise. In third place with 21% sudden death after drug injection and development of type I hypersensitivity. The fourth and last place corresponded multiorgan failure and secondary bacterial infections with 19%. Relationship a detection of foreign substances by Group NSAIDs: Phenylbutazone 80%, 16% dypirona, flunixin meglumine 4%. The foreign substances detected were 82% anabolic and 18% triamcinolone. The bronchodilators were aminofilinas clenbuterol 60% and 40% respective; diuretics Furosemide only 100%; Methocarbamol 100%, 100% steroid dexamethasone. Other substance detected was Vitamins B15 (pangamic acid) 100%. These results agree with those reported in the literature worldwide where the prevalence of illegal substances cases associated with anabolic, steroids and clenbuterol predominate. To a lesser extent than furosemide and phenylbutazone is high the number of cases that are under therapeutic medications allowed by regulation Venezuela racing. Unlike other racing regulations in other countries that regulate the use of these two therapeutic medications (Tobin, et al., 2012), the foreign substances associated with NSAIDs, steroids anabolic and has as consequences the iatrogenic equine metabolic syndrome whose systemic complications affect the horse practically irreversible consequences and evolution to multiorgan failure and consequent secondary bacterial infections bacteremia and septicemia. Despite horses who died of pulmonary hemorrhage were medicated with furosemide was not possible to quantify the dose also were detected in 5 cases illegal bronchodilators. One possible reason that accounts for the development of induced pulmonary hemorrhage in horses exercising furosemide medicated can be associated with a pharmacological interaction of phenylbutazone with furosemide in these cases as well as a underdosing (concentration and volume) of furosemide and time application. The sudden death associated with the administration of therapeutic medications was associated mainly to the administration of a drug (trade B15 vitamin complex), which presents the main components being pangamic acid and sodium selenium has been reported to induce toxicity at high doses in the horse. The main limitation of this study was the quantification of foreign substances concentration which was observed only positivity but not the substances concentration. Although urine is the sample of choice for foreign substances medications tests in racehorses, it is rarely obtained following the sudden death of a racehorse on the track while racing (Uboh, et al., 1995). Thus, in the absence of urine and blood samples following sudden death, postmortem tissue samples are equally useful for forensic toxicological investigations of racehorses (Uboh, et al., 1995). Below is described the cases of illegal substances in horses most relevant and recent global. In Central and South America have reported cases of foreign substances in racehorses: 1991 Horse Racing Classic Caribbean (The Caribbean Horse Racing Confederation), Venezuela was disqualified for using banned therapeutic medications (phenylbutazone and lasix). Argentina was detected N-Butilescopolamina Bromuro in Thoroughbreds horses racing in 2012 and a case of acepromacine. Brazil cases of foreign substances Thoroughbreds horses racing was detected Flunixin meglumine in 2009. Chile in 2010 illegal substances for clenbuterol was detected in Thoroughbreds horses racing. Colombia: four Colombian Paso horses were positive a caffeine and theophylline in 2010, in Mexico two cases positive for clenbuterol at the Hippodrome of the Americas in 2011, in Panama 14 cases of horses was positive a cocaine in 2005, Puerto Rico nine cases positive a

Ethorphine in 2005, in Uruguay 12 cases was positive a caffeine in 2012, 17 foreign substances cases in 2011 and 29 illegal substances cases in 2010. Many of the causes of death have not been determined or related to foreign substances or poisoning. Studies are performed multidisciplinary to determine the etiology of sudden death in post-race horses. Reports in the literature suggest among its causes pulmonary hemorrhage induced by exercise (ruptured bronchial artery), aortic rupture, warfarin poisoning, heart failure and recently a case of acute renal failure. In conclusion, this study reported 126 cases of foreign substances in Thoroughbred horses with subsequent mortality. Multidisciplinary studies are needed to elucidate the foreign substances in horse racing worldwide, as well as immediate consequences in the short and medium term health of the horse.

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