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A Welfare Review of Different Doping Agents in Equine Sports

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Abstract

Doping has always been a part of equine sports, which presents a number of challenges regarding the protection of the horse from an animal welfare point of view. This issue goes beyond the health effects of doping agents on horses, as the consequences of such practices can be of importance regarding the image of the sport, the financial investments made, the protection of the rider's health and the reputation of the horse track. That is why governments are trying to put in place laws to prevent doping from occurring, and developing more and more efficient methods of analysis to control for doping. The main current issues regarding the battle against doping is the non-uniformity of laws across the world, which creates a lot of confusion. Veterinarians have an important role regarding the implementation of a system which can ensure the safety of both horses and riders.

Contents

1 List of abbreviations

PBZ (phenylbutazone)

NSAID (nonsteroidal anti-inflammatory drug)

CPK (creatine phosphokinase)

MSTN (myostatin)

IFHA (International Federation of Horseracing Authorities)

FEI (International equestrian Federation)

LC-MS (liquid chromatography-mass spectrometry)

SPE (solid-phase extraction)

GC-MS (gas chromatography-mass spectrometry)

HPLC-UV (high performance liquid chromatography–diode array detection)

NACE-MS (nonaqueous capillary electrophoresis–mass spectrometry)

MDPV (3,4-methylenedioxypyrovalerone)

EIPH (exercise-induced pulmonary hemorrhage)

FNCF (National Federation of French races)

EADCM (Anti-Doping and Controlled Medication Regulations)

2 Introduction

Doping is the act of using enhancing drugs to increase performance. Any drug usage non intended for therapeutic treatment is considered doping. In case of horses doping practices are mostly prevalent in endurance or speed racehorses, such as Arabians or thoroughbreds, and high-level competing show horses. Doping agents are used to increase the gains acquired if a horse wins a race and there was a lot of betting on this horse for example. This topic is relevant from a veterinary point of view because such practices can be dangerous for animals, for riders, and make it impossible for a fair assessment of horse performance during a race or competition. The term « doping » was first introduced from racing in the US, as it seemed to be more common practice in this part of the world.

Doping of horses starts alongside doping of human athletes, with remedies which were available at the time, usually more natural than the chemicals which are available today. During the Roman Empire, horses taking part in races with chariots were given hydromel. It is an alcoholic beverage made from the fermentation of water and honey, which has anti-inflammatory and antibiotic properties. Other substances, acting as stimulants, were given to horses such as coffee, tea, white wine etc. Veterinarians also recommended anise seeds and sandarac; a resin obtained from the tree *Tetraclinis articulata*. Anise seeds are anti-fungal, antibacterial, anti-inflammatory and helps reduce stomach ulcers. Sandarac reduces muscle cramps, promotes relaxation, and has anti-parasitic effects. However, used in high dosage, doping substances can be lethal, and more and more breaks out to the public eye. In the 19th century, the Unites States of America (I guess you meant the country, not the continent in general known for their famous horse races, started giving alkaloids such as morphine, cocaine, and heroin to their horses to enhance their performance. It has then spread out to European countries. In 1903, there was even a case of Belgian smugglers at the border of the country giving their horses stimulants to escape the authorities. Later on, at the end of the 2nd World War, amphetamines were also used, and progressively corticosteroids, diuretics and hormones (De Mondenard J, 2000). The most frequently reported drugs between 1949 and 1974 are listed in Table 1.

Regulations about doping were slowly held in place to try and solve the issue. It started in the 17th century, in which exciting substances were banned in races. But doping wasn't really much of an issue until the 20th century, where they started testing for substances using the horse's body fluids, such as urine, blood and saliva (Clarke et al., 1976).

During the 20th century, people really started writing articles in veterinarian journals about cases of thoroughbreds dying during races because of doping, which raised awareness. To this day there are regulations held in place to prevent doping, but they are constantly evolving, as there are new drugs available on the market, new application methods, new methods for the detection of doping, and more and more studies on the effect of the drugs on horses.

Table 1: Twelve most frequently reported drugs internationally between 1949 and 1974. The number of cases involving the drug are shown as a percentage of all positive cases for the three different periods (Clarke et al., 1976).

	1949-64 (per cent)	1965-69 (per cent)	1970-1974 (per cent)	Number of times reported, 1949-1974
Procaine	20	20	17	721
Caffeine	17	11	6	496
Amphetamine	15	10	6	471
Phenylbutazone	4	9	16	326
Methylphenidate	2	14	7	212
Methylamphetamine	2	5	3	135
Theobromine	3	5	2	131
Dipyrone	3	4	4	129
Oxyphenbutazone	1	3	5	92
Barbiturates	6	0	0	86
Pentazocine	0	2	3	69
Morphine	2	1	0	62

3 Literature review

This literature review's main focus will be on the type of sports in which doping is most widely practiced, the health effects of doping agents on horses to see whether or not it is a real issue, and the role of the veterinarian in the battle against doping.

3.1 Sports most prevalent for doping

In the equine sports industry, there are two distinct fields: one is racing, either gallop, trot, or jump. The other are horse shows, with a variety of disciplines such as dressage, jumping, cross-country, hunter etc.

In galloping races, thoroughbreds are overrepresented for their speed and agility. In trotting races, the *« trotteur français »* is mostly used, as it is known to possess speed, strength, agility and a combative personality.

In horse shows, there are more diverse breeds used, however in France the main one is *« Selle Français »*, as it is quite polyvalent and a relatively stable temperament, which is necessary for a good performance under stress.

Doping occurs mostly in the racing industry as there are more financial gains for winning than in horse shows, and the training of racehorses is quite different. Their training is more strenuous and intense, meaning the risk for injuries and any pathology developing is higher than in other disciplines. Moreover, usually they start training at an earlier age, as young as 2 years old, and a thoroughbred fully matures up to 8 years old, meaning their musculoskeletal system is not fully developed yet to sustain such physical demands. This can cause the appearance of tendinitis, joint issues, back problems, therefore lameness. To counter this, some trainers are then willing to use medication, feed or other methods to mask health issues and increase performance. The career of a racehorse is much shorter than that of a sports horse, usually stopping on average around 4 or 5 years old and not 12 years old.

In horse shows, medication misuse is not as much of a problem, as horses have more time to rest and do not need to be at peak performance during each training. However, other methods during training such as putting spikes on the jumps to incent the horse to jump higher or performing surgery on the flexor tendons to reduce the sensation of pain during training can be problematic. It is another debate which I will not cover in the next parts.

This thesis will cover mainly the horse racing industry, with medication and other procedures used as doping agents, and the regulations put in place by governments to counteract this practice.

3.2 Mechanism of action of doping substances and negative effects on horses

Although horses have been known to already having impressive physical abilities, they have frequently been given doping agents to increase training performance, the number of races, and ultimately racing performance. The main health issues horses encounter while training are joint and tendon inflammation, respiratory diseases and colic.

There are different pharmacological classes of drugs used to enhance athletic performance. We will detail for each class, the compounds commonly used, their mechanism of action and the potential clinical effects of these drugs on horses.

Since the early 1950's, veterinarians started treating horses with phenylbutazone (PBZ) which is a nonsteroidal anti-inflammatory drug (NSAID). Phenylbutazone inactivates prostaglandin H and prostacyclin synthase which reduces the inflammation of tissues. This drug is usually used for the short-term treatment of pain and fever in animals. Phenylbutazone was not too expensive, easy to administer, and had minimal side effects, so, due to the intensive training regimen of horses, with a mix of long daily gallops and shorter sessions to develop stamina and speed, it became a drug of choice to relieve symptoms of lameness and keep the horse in training or racing. The usage of certain drugs were not as regulated in veterinary medicine as it was in human medicine, especially in stables which required a larger quantity. The latter would rely on veterinary supply companies and not the prescription of a veterinarian who would have been on-site. Moreover, no clinical trials had really been done to ensure the safety of this drug on horses. Although there had been research about the drug's toxicity in humans, research in veterinary medicine was not as developed, because most veterinarians worked in a more isolated setting, so the transfer of knowledge between the veterinary and human medical community was nonexistent. PBZ was nonetheless put on the restricted list of drugs, and could only be prescribed by a veterinarian (Worboys and Toon, 2018).

The first public concerns of PBZ occurred during the Kentucky Derby in 1968 (Worboys and Toon, 2018), when the substance was found in urine samples of the winner. The horse was then revoked to last place and it is the horse in second place who became the winner. This decision was up for debate in court for several years and is still an ongoing debate to this day.

Certain owners and trainers claimed the usage of such substances should be permitted as it is equivalent to other means of boosting performance like training and breeding, unlike

giving depressants which would be unfair as it is stopping one own's or others horses from performing to their full capacity (Worboys and Toon, 2018).

As PBZ was still allowed for use during training, a study in 1960 was conducted to examine phenylbutazone's pharmacokinetics in different animals, and its half-life in order to compare it to horses (Worboys and Toon, 2018). The aim of the study was to inform trainers how long before the race they had to withdraw the drug, and authorities on how it could be detected through blood and urine. The results stated that the half-life of PBZ was relatively short in horses, as it is around twelve times higher in humans. Moreover, the effects of PBZ can still occur at very low levels in the plasma and the tissues, which means that the tests used to screen for the drug my not be sensitive enough to detect its presence. Table 2 is used to present PBZ's pharmacokinetics.

Table 2 : Species differences in the metabolism of phenylbutazone (Burns et al. 1960)

Species	Biological half life in hours
Man	72
Dog	6
Rabbit	3
Rat	6
Guinea pig	5
Horse	6

The animal welfare concerns back then were not so much about the toxicity of the drug itself, although there has been some speculation about the toxic effect of metabolites due to the administration of larger quantities of the drug to horses, but the relief of pain perceived by horses allowed them to work at full capacity. This meant the increase of injuries, as the horses were physically strained. As people became more aware of the toxicity of PBZ for humans, veterinarians started questioning its safety for horses as well (Worboys and Toon, 2018).

In 1983, a report (Worboys and Toon, 2018) stated that in high dosage, in ponies, PBZ could cause serious side effects like swelling, ulceration of the mouth and gastrointestinal tract, and even death. Thoroughbreds seemed to tolerate it more, with only hypoproteinemia reported as a side effect. Also in 1983, a study from Peter Lees and his colleagues at the Royal Veterinary College (Lees et al., 1983a, b; Lees and Higgins, 1985) confirmed that

PBZ could induce kidney and liver damage if given at high dosage. Other reported side-effects could be depression, inappetence, melena, diarrhea and abdominal edema. The veterinarians, conducing such clinical experiment, affirmed that PBZ could be used safely by reducing the dosage, keeping the horse hydrated, and stopping its administration at any signs of side-effects. It was thought out that PBZ had a lower toxicity in horses than humans, however a growing number of research pointed that horses had similar side-effects to humans. Avoiding long-term use and using alternatives, like other NSAIDs were advised. This raises the question of its usage in racing, as lameness in horses can be chronic and the tolerance to PBZ building up as it is used repeatedly, so an increase in dosage might be required to obtain the same effects. In the early 1980's, PBZ was banned from use in human medicine, however to this day it is still widely used in horses, as the side-effects reported are lower in horses than humans (Worboys and Toon, 2018).

Another class of drugs used are anabolic steroid hormones such as testosterone, nandrolone, boldenone and stanozolol. Physiologically, those hormones increase the growth of muscles and tissues by activating androgen receptors in skeletal muscle fibers, which stimulates proteogenesis. It is most commonly used by veterinarians to increase appetite, weight gain, tissue repair and accelerate recovery. However, certain trainers can misuse the drug, in order to maximize muscle growth in foals. When given in a high dosage, repeatedly, steroid hormones can create an early ossification of cartilages. It can also lead to a faster rate of growth of muscles compared to tendons, which can result in chronic lameness and even the rupture of the tendon while exercising. Furthermore, the usage of such hormones can result in an electrolyte imbalance, thus an increase in the retention of sodium, blood pressure and oedema occurrence. Lastly, later on, reproduction issues can occur such as a decrease of fertility in stallions and mares (Grandjean, 2010).

Another class of drugs are stimulants and tranquilizers. Stimulants usually act on the neuroendocrinian pathway to increase muscle movement, thus speed, or as a bronchodilator, therapeutically used for chronic obstructive pulmonary disease. Whereas tranquilizers, through the neuroendocrinian pathway as well, will reduce sensitivity to stress and pain. Tranquilizers are commonly used by veterinarians if a horse seems highly nervous or during a more painful procedure.

Side effects of tranquilizers can result in difficulties with perception, balance and incoordination. The consequences of these can be reduced performance and increase of trauma injuries, due to a lack of adaptability to sudden potential dangerous situations on the track. Certain tranquilizers, like diazepam and acepromazine, can increase the level of

creatine phosphokinase (CPK) in the blood plasma, which means that muscle cells are being destroyed, sometimes induce a respiratory depression.

Side effects of stimulants are mostly a reduced capacity of the body to perceive when it is reaching its physiological limits, thus running the risk of exceeding them and degrading its motor movements, as well as leading to a potential dysfunction of the cardio-respiratory system (Grandjean, 2010). Other side effects can be anxiety and muscle tremors. The most commonly used stimulants are catecholamines, amphetamines and caffeine.

Beta-blockers, such as timolol, atenolol and propranolol are commonly used as the treatment of arrythmias and decreasing heart rate. Through the neuroendocrine pathway they can also reduce the impact of stress hormones, thus improving performance on the short-term, which makes it appealing to racing trainers. However, these drugs can negatively impact the cardiac, respiratory and metabolic function. In the muscle cell, the blockage of the beta 2 receptor stops glycogenolysis, and the blockage of the beta 1 receptor stop lipolysis, which depletes the cell from intracellular energy substrates. The blockage of the beta 2 receptor decreases the elimination of lactates, which decreases osmotic water retention. It also decreases the level of fatty acids in the blood, generated from adipose tissue. The blockage of beta 1 decreases the rate of blood perfusion and decreases the level of blood glucose, generated from the liver. All of these can lead to a dysfunction of the cardio-vascular system.

The last class of drugs to mention are diuretics, particularly furosemide, used in the US. This drug is commonly used therapeutically as a treatment to exercise-induced pulmonary hemorrhage (Moreira et al., 2021). This condition causes the horse to bleed from the lungs, so this drug will help in clearing the lungs from an excess in fluid. As this diuretic also increases the quantity of urine excreted, trainers use this drug to reduce the horse's weight during the race, thus increasing its speed. However, this can cause dehydration and kidney damage.

In sum, most of these drugs are initially used by veterinarians as part of a therapeutic treatment plan, however they can be misused in order to enhance or decrease their horses or other's horses' performance, which is a concern from an animal welfare point of view.

3.3 Gene therapy as an enhancement of performance

In the sports and racing horse industry, breeding has been used for a long time in order to select for specific breeds with the attributes required for the demands of the discipline. It is highly regulated, for example, the thoroughbreds cannot be artificially inseminated to ensure there is no cross-breeding and preserve other breeds. Lineage must also be traceable and written in the horse's passport.

Stated in this article (Tozaki et al., 2020), gene therapy can be used as treatment for injury or chronic diseases in horses, as some diseases can be the consequence of certain gene expression, like polysaccharide storage myopathy 1, which is caused by the mutation of glycogen synthase 1. The insertion of exogenous genes called « transgenes » using plasmid or viral vectors, in living cells, in order to produce the target protein for months, is a process described as gene transfer. On the other hand, gene silencing suppresses translation of the protein in the cells by hybridizing the mRNA with artificially synthesized antisense oligonucleotides. Gene silencing can treat certain neurological diseases (Tozaki and Hamilton, 2021).

On healthy horses though, gene therapy can be used to enhance performance during training and racing. Gene transfer can improve certain metabolic processes which can modify the horse's nutritional needs or physical performance. Gene silencing can improve racing performance by inhibiting the expression of myostatin (MSTN), which reduces the growth of skeletal muscle (Tozaki and Hamilton, 2021). However, completely suppressing this gene can have a negative effect on the horse's health, which would hinder its performance as well. Another method used is gene editing, which consists of genetically modifying the embryo using CRISPR-Cas9, all of which is prohibited by the International Federation of Horseracing Authorities (IFHA) and the Fédération Equestre Internationale (FEI) organizations (Tozaki and Hamilton, 2021). The genetic modification of the egg poses a serious threat to the thoroughbred breed as the modified genes can be inherited. A thoroughbred can be taken out of the Stud Book if it does not meet the requirements of live breeding by a pure-bred stallion. Gene editing can become a problem if the genes are edited in an animal once it was born, as not all of the cells will express the edited gene, resulting in the horse being « mosaic » (Tozaki and Hamilton, 2021). In order to avoid such phenomenon, it has been suggested to edit gametes instead of embryos (Tozaki and Hamilton, 2021).

Gene therapy has not been a widely spread doping practice so far, so authorities are not really looking into implementing strict screening, however, as prevention, laboratories are developing anti-gene doping tests.

4 Aims

First, I will explain how I have gotten the idea of writing a thesis on this topic. During some of my equine internships in France, I was accompanying a vet to his regular visits, in an area where there are many race horses, trotting and galloping. During one of the visits, there was a horse with symptoms of lameness and inflammation to one of his carpal joints. This horse had to participate in a race a couple of weeks after the visit. The veterinarian gave this horse anti-inflammatories and calculated the days until the race to know if there would still be traces of the medication in the horse's blood during the race. It is after this internship that I decided it was an interesting topic, as it is relevant in today's medical practice, and was actually pretty common in the industry, but no one really talked about it.

The aim of this study is to analyze the potential effects of doping agents on the horse's health and welfare, which would justify the establishment of regulations against the use of such methods. The horse sports and racing industry is known to be of great financial value, whether it is through people biding during a race, buying/selling horses, attending shows, freezing semen for reproduction around the world, or even watching on television. The financial and popularity rewards of winning are enough for certain owners and trainers to risk their horse's health using sometimes questionable methods to enhance their performance and training abilities.

Doping agents could be a real threat to the sport, as not only does it put the horses in danger, but also the riders as well. Moreover, the question of « fair-play » arises, as horses should be judged based on their performance in equal circumstances for it to be valid. Of course, there is always a bit of luck at play, as horses are live beings, which means they could not be at their best during the competition or getting injured, but any horse can encounter these issues, so it does not present any unfair advantage. Some trainers have disputed, as seen earlier, that doping agents were just another means of enhancing performance like training or breeding, so if such practices are allowed, why would using certain substances or therapies not allowed?

In the current situation, some procedures are banned, like gene therapy, but others like the use of different therapeutic drugs are solely regulated, with many grey areas concerning the withdrawal period, the threshold above which the concentration is too high, the sampling methods used, the difference in regulations across countries and the role of the veterinarian. Testing for doping is usually done randomly on site during a race or show, by a habilitated agent of the racing track or a veterinarian.

The analysis of the effects of doping agents will help shed more light on the current debates regarding doping in equine sports, why it is an important matter for authorities, trainers and veterinarians, as well as ways that the governments are ensuring the safety of horses and their riders by enforcing new laws and developing new anti-doping tests.

5 Materials and method

I looked mostly on the internet for articles, on websites like science direct. As my thesis is a review on the welfare of horses, I looked at any article which would present different doping agents. I used mostly the key words doping in race horses, or doping in equine sports. There was not a lot of literature available in English, so I found as well for French articles. As the racing industry in France is well known throughout the world, mostly in Normandie, I was able to find information regarding the different substances used, the sampling methods, regulations in place and the role of the veterinarian. Most articles are stating the situation in the US, the UK and France, as those countries host or trade the most race horses throughout the world.

First, the history of doping introduces the meaning of doping, different ways of doping and the evolution of such practices. Then, some articles stated the different drugs which could be misused, with their mechanism of action and side effects. There were also several articles on gene therapy, testing for it, so I put it in a separate chapter. The articles used for the literature review explained the different effects of doping agents on the horse's health, which could serve as building blocks for the next part of my thesis which would focus more on the regulations established. Then some of the articles highlighted the debate on whether such actions were justified, how could we improve the system, how do the rules differ from country to country and is it effective or people will just find new ways of going around it. The role of the veterinarians is also mentioned in those articles, because they have access to all of the therapies, injections and anti-doping tests. Some of the articles were presenting

doping, the effects on horses of doping substances, the methods of analysis of such substances and then regulations put in place. Certain articles also compared horse doping to human doping which I developed a bit in the chapter concerning the effects of PBZ in horses and humans, and again in the results concerning regulations, as certain substances are banned in human sports but not in equine sports. It is relevant because certain research concerning doping in human sports could have been useful to veterinarians, and it also highlights the isolation many veterinarians face during their clinical experiments, not being able to build as efficiently on previous knowledge, as well as a lack of regulations concerning the use of certain restricted substances, which could in turn be misused for doping. Almost all of the articles described well the debates around doping which I put in the results and discussion section, as it is always evolving and never static. Rules are also quite contested by trainers because their horse can be disqualified if traces of doping is found, thus it has an impact on the outcome of the race. The articles gave an overview of the current situation concerning doping, but there is no straight answer, as this issue is very complex and integrates many different aspects, from ethical to financial and legal. Moreover, every country has its own set of rules, which confirms there is no black and white as not every country has been able to agree on a single way of proceeding.

6 Results

From the previous findings, if doping agents indeed have a negative effect on horses, it is then necessary to explain how to detect those substances in order to prevent people from misusing them in the first place.

6.1 Health effects

As seen in the literature review, we can draw as a conclusion that certain medications used for therapeutic reasons, can have negative side effects on horses. The main class of drugs concerned on this issue are anti-inflammatories, stimulants, tranquilizers, anabolic steroid hormones, beta-blockers and diuretics. When the horse is sick, usually those side effects are minimal compared to the damaging effects of the disease, so the benefice-cost ratio is still favorable. However, when such drugs are used on healthy horses to improve their performance and not for healing purposes, the side effects can have much more negative effects on the horse's health. The side effects can either have a direct impact on the horse's

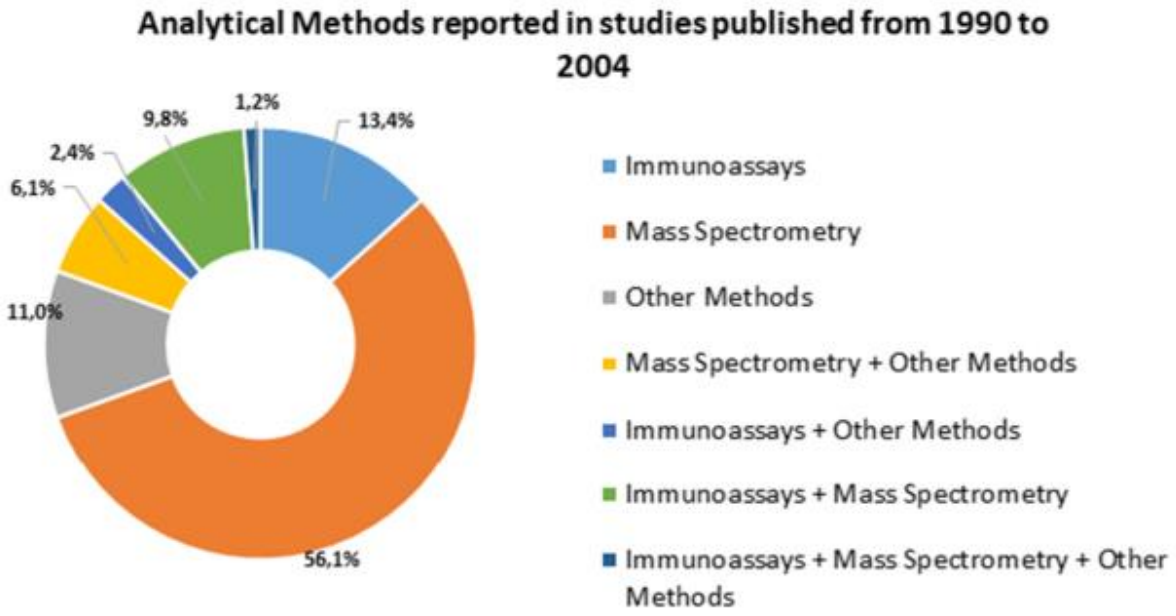
body, or sometimes it is not so much side effects which are problematic, but relieving the brain's perception of pain and tiredness can increase the risk of the horse pushing above its physiological limits, leading to injury or even death.

Another therapy which can be used as doping is gene therapy, which can also harm the horse's health by transferring, silencing or editing gene expression. The risk of gene expression not being uniformed is higher, which can create other health issues. It also presents an issue regarding the genetic lineage of racing and sports horses, as usually there is a couple of donors from breeding stallions throughout the world and if one of them is genetically modified, all of its offsprings can inherit this genetic modification. The stallion and offsprings can be disqualified from getting the appropriate certification which makes them of much less financial value.

6.2 Analytical methodologies

As stated in a review (Moreira et al., 2021) about the history of development of analytical methodologies from 1990 to 2019, the 2018 Anti-Doping and Drug Testing Program conducted by US horse racing regulatory bodies found substantial compliance with medication rules. After testing a total of 258,920 samples, 1561 violations were uncovered, meaning that 99.4 % of all tests found the horse to be compliant with the rules. During the 1990's with increasing support for research on anti-doping analytical methods, there was an increase of the availability of liquid chromatography-mass spectrometry (LC-MS) for detecting illicit substances, which was much more accurate and sensitive than previous tests. The used methods can be seen on Figure 1 and 2.

For screening purposes, a multi-drug analysis approach is much more efficient than a single-drug analysis, as the list of prohibited substances is quite large and there can even be drugs used simultaneously. The parents drug may not be present in the blood, but the presence of metabolites can indicate that the drug was indeed consumed prior to testing. The development of a generic mixed-mode solid-phase extraction (SPE) procedure, involving ion-exchange chromatography has enabled the indication of multiple exogenous peptides with sequence diversity from equine plasma and urine in a single procedure. Figure 3 presents the most commonly used matrices to detect doping agents.



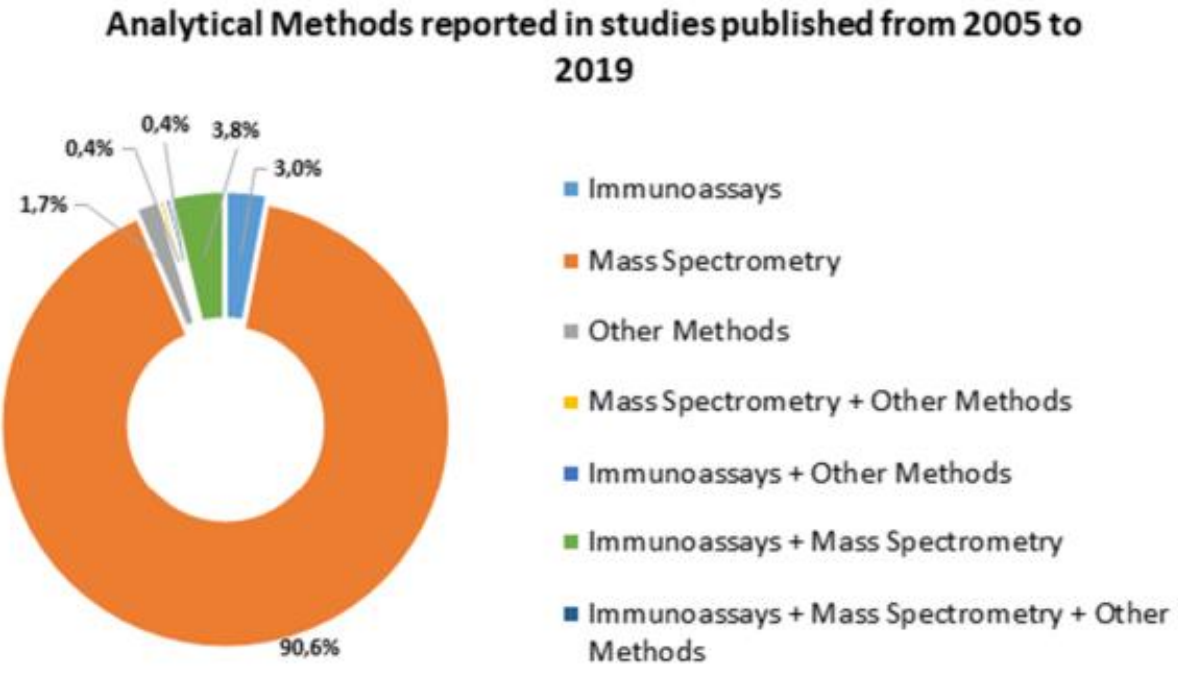
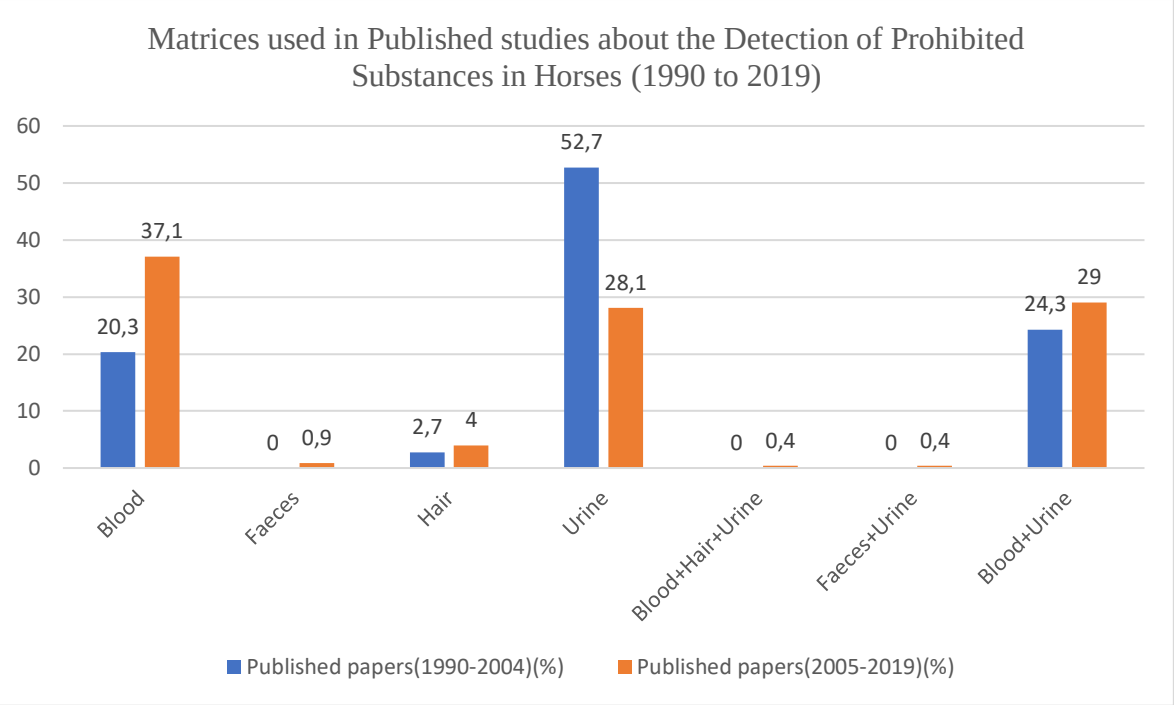


Figure 1 and 2 : Analytical detection methods for the analysis of biological matrices for doping control purposes (%) from 1990 to 2004 (Figure 1) and from 2005 to 2019 (Figure 2), (Moreira et al., 2021).

Figure 3: Percentage of published papers per analytical matrix, reporting the collection and analysis of biological matrices for doping control purposes in horses from 1990 to 2004 and from 2005 to 2019 (Moreira et al., 2021).



Every procedure has its advantage and disadvantage regarding screening for doping agents, however equine plasma and serum has been a preferred method for developing tests, as blood can be collected anytime compared to urine. Plasma contains a lot of proteins which need to be filtered in order to extract the analytes targeted, however it can be an easier matrix than urine to work with, in order to detect parent compounds. It is also easier to link pharmacological effects of the drugs to plasma than urinary concentrations, making it a point of reference for the tolerated threshold. As the therapeutic dose is usually smaller than the doping dose, this matrix makes it possible to differentiate both practices.

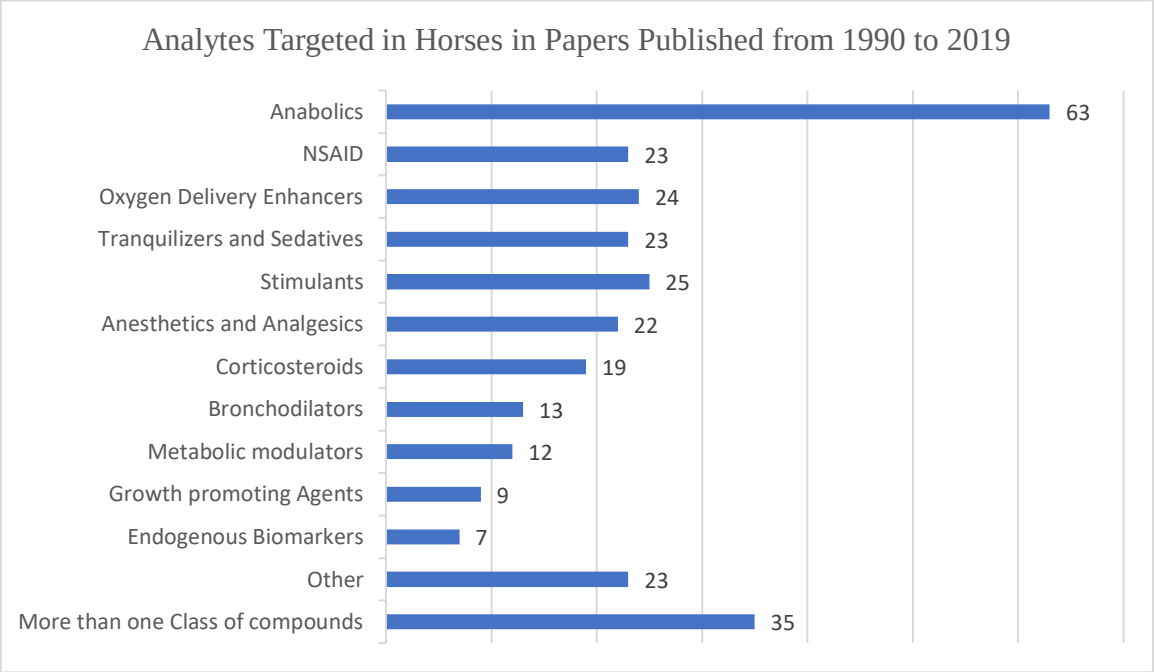
However, some authors have argued that urine can be collected in larger amounts, using a non-invasive procedure. Blood requires more expertise for sample collection and increased sensitivity of tests with the restricted amount of blood available. Urine is also an easier matrix to work with, for the detection of metabolites from the parent compound, thus making it still the most common matrix used by laboratories.

The age of the horse can also be a factor in which matrix to use. Urine is hard to collect in younger horses, so faeces can be used, especially for the detection of growth hormones, however it requires a laborious pretreatment and is quite a slow procedure. A solvent extraction combined with SPE might be a solution, in order to accelerate the procedure.

Horse hair has also been commonly used, as it can detect compounds in a larger window of several weeks to months, it is easy to transport, preserve, and is a non invasive procedure. Hair from the mane and tail is more suitable than hair from the rest of the body as it grows at a constant rate, about 2 cm per month, and not seasonally. The hair strands can be segmented in order to match the time of exposure to the drug. Basic drugs can reach concentrations 10 times higher in darker than lighter hair. It could be related to pH of melanocytes and keratinocytes which is between 3 and 5, and the affinity of basic drugs to melanin. Excretion of the compounds in hair also depends on its lipophilic properties as hair is a lipophilic material and repels water, so more sensitive methods are required for hydrophilic compounds. Cross-contamination from sweat or the environment can also occur in hair samples, which is why it needs to be thoroughly washed and decontaminated before analysis. Impurities can therefore lead to false positives. Moreover, due to light exposure or damage of hair strands, the concentration of drugs in hair can diminish, leading to false negatives. That is why hair can be a costly and lengthy procedure, without always being accurate (Moreira et al., 2021).

I will now go into detail as to which matrix is preferably used for each specific prohibited substances and how they are detected.

Figure 4: Number of published papers, by targeted drugs from 1990 to 2019, regarding the control of doping in horses (Moreira et al., 2021).



The first class of drugs are anabolic steroid hormones. Most of the commercial available ones are in the esterified form and often used as intramuscular administration. When the esters reach the bloodstream, they are hydrolyzed by esterase enzymes into free steroids. Due to this intense hydrolyzation, it is very difficult to detect esters from steroid hormones. Some methods have been able to detect nandrolone laurate up to 14 days' post-administration in plasma, nandrolone dodecanoate up to 120 days in hair and up to 23 days in plasma, testosterone decanoate up to 133 days and of testosterone propionate up to 120 days in hair and of a mixture of esters of testosterone up to 8 days in plasma. Gas chromatography-mass spectrometry (GC-MS) and LC-MS have been able to detect multiple anabolic steroids in equine plasma. Nonetheless, anabolic steroids were not banned in racing horses in the US until 2008 (Moreira et al., 2021).

There are some forms of anabolic steroids though, like fluoxymesterone, mesterolone, 17alpha-methyltestosterone, mestanolone, methandienone, methandriol and oxymetholone, which are administered orally. Those hormones are excreted in a shorter time compared to the ones for intramuscular administration. The parent compound does not stay in the horse for long as it is extensively metabolized, so mostly its metabolites are detected. Initially some metabolites could be detected from 12h to 24h after ingestion, in the horse's urine, but

as research progressed, they found other metabolites which could be detected 48h after ingestion (Moreira et al., 2021).

Another class of drugs that are frequently used are NSAID's. The main methods used to detect metabolites of NSAID are GC-MS, high performance liquid chromatography–diode array detection (HPLC-UV) and LC-MS in either urine or plasma. Another type of important NSAID are COX-2 inhibitors such as firocoxib and celecoxib which have been detected using LC-MS (Moreira et al., 2021).

As another class of drugs, tranquilizers and sedatives most commonly used are clonidine, guanabenz, and xylazine. Clonidine is administered intravenously and can be detected in equine plasma with the GC-MS method up to 2h after administration for a dosage of 0.025 mg/Kg. Guanabenz, on the other hand, can be detected up to 8h after an administration of 0.04 mg/Kg intravenously. Xylazine, which is normally used to sedate horses prior to surgical procedures, used to be detected using its metabolites in equine urine, some of which could be identified up to 2h after administration and others up to 25h post-administration. However, recent research has demonstrated that if given 200 mg of xylazine to thoroughbreds, it was possible to detect the parent compound in plasma for more than 70h, using LC-MS (Moreira et al., 2021).

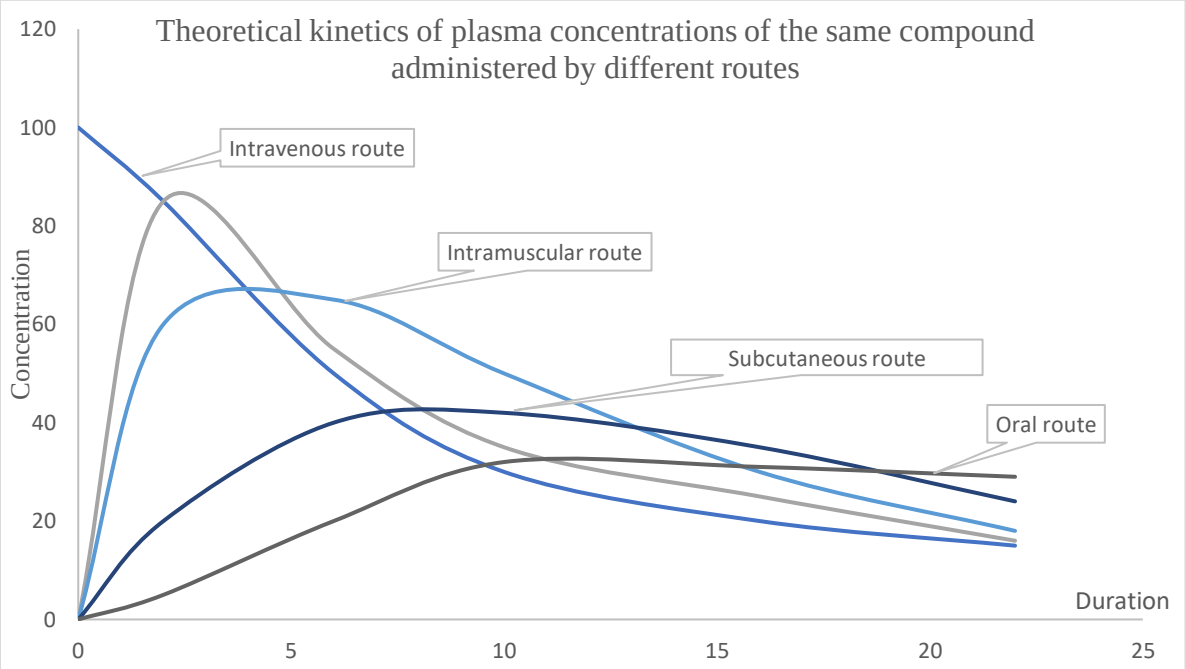
Stimulants, on the other hand, such as amphetamines, can be detected using nonaqueous capillary electrophoresis–mass spectrometry (NACE-MS) in equine plasma (Moreira et al., 2021). Another popular stimulant is 3,4-methylenedioxypyrovalerone (MDPV), which is a recreational drug belonging to the class of cathione. It has been detected in equine plasma using LC-MS. Its limit of detection ranges from 0.02 ng/ml to 0.5 ng/ml. Methods of analysis used for methylxanthine can also detect caffeine and theophylline in urine and blood, as well as theobromine in urine. The latter, which is a metabolite of caffeine, can be present in several horse feeds, so it is important for trainers to be vigilant on which feed they use during racing season, not feeding their horses chocolate and for the analysts to consider the ratio of theobromine to caffeine in order to make sure it is actually doping. The caffeine threshold limit to control for doping is 250 ng/ml in serum and 1000 ng/ml in urine for 72h before the race. It has been stated though, that the acidification of urine, through the administration of ascorbic acid, can increase the excretion of such compounds. Urine is a widely used matrix to detect caffeine, as most of the compounds concentrate in the urinary bladder (Moreira et al., 2021).

Diuretics, which are also therapeutically used for exercise-induced pulmonary hemorrhage (EIPH), can mask the usage of other doping agents as it increases the quantity of urine, thus

decreases the concentration of other doping agents, or altering the urine's pH, making it difficult to detect other substances. The most commonly detected diuretic in equine plasma or urine is furosemide, usually administered 2h prior to racing. The use of furosemide for EIPH is routinely used in the US, however it is banned in most racing countries (Moreira et al., 2021).

Figure 5 is used to show that screening must take into account the type of administration of the drug as well, as if it is administered intravenously, it will reach the blood much quicker than orally, however its concentration will drop at a faster pace. Moreover, certain medication is administered as long-acting and slow-release tablets, which could be detected in tissues for a longer period of time.

Figure 5 : Theoretical kinetics of plasma concentrations of the same compound administered by different routes (Grandjean, 2010).



6.3 Regulations and laws

Regulations and laws concerning doping in races are in place for different reasons. First, it was to protect the interests of people biding during horse races and the reputation of horse tracks. Then, it was a means to ensure the safety of riders and horses (Gadot, 2005).

There are 3 main principles forging the anti-doping battle:

- Good health and absence of medication: the horse should be in good health during a competition. It is hard to define the health status of a horse, so the criteria which has been used is the total of active substance and medication in the horse's organism.
- Transparency during training and throughout the horse's career. Any treatment administered during training should be reported. It should also not enable the horse to keep training if it is presenting any pathologies incompatible with the physical demands of racing.
- The non-utilization of prohibited practices such as physical or chemical procedures which could enhance performance.

The difference between the anti-doping policies for horses and humans is that in humans, the absence of any trace of medication or active substance is not realistic. It is not possible

to prohibit the human athlete from drinking coffee, tea, wine or taking any kind of contraceptive. Therefore, it is still possible for people with any pathology which directly does not interfere with the practice of their sport to participate in competitions (Gadot, 2005).
In France, the anti-doping regulations of races is written in the code of law of racing. The code of law is specific to each racing discipline, galloping and trotting. These codes are enacted by a strict procedure: study of the proposition by the commission of the code, then approved by the governing body and voted by the committee. The supervisory ministry has to approve as well. They ensure that the anti-doping policies are similar in both disciplines (Gadot, 2005).
Currently, the regulations state that there is a list of permanent interdictions, which means the utilization of such substances is prohibited under any circumstances. This includes: anabolic steroids, growth factors, substance acting on the erythropoiesis, synthetic oxygen transporters, any substance having properties similar to those mentioned previously and blood transfusion (Gadot, 2005).
Then, there is a list of substances permitted during training but prohibited of use during racing. The usage of such products must be justified and prescribed by a veterinarian. It includes substances acting on the nervous, digestive, cardiovascular, respiratory, urinary, reproductive, musculoskeletal, endocrine and immune system. Antipyretics, analgesics, anti-inflammatories and cytotoxic substances are also included in this category (Gadot, 2005).
The threshold above which the substance's concentration detected is considered doping are internationally defined by official analysts and veterinarians and inserted in the code of racing. These substances can be present in the everyday feed of the animal or as endogenous compounds to horses, thus there is a little margin (Gadot, 2005).
The substances which can be present in the feed of horses :

- Arsenic : 0.3 µg/ml in urine.
- Diméthyl sulfoxide : 15 µg/ml in urine or 1 µg/ml in plasma.
- Salicyilic acid : 750 µg/ml in urine or 6,5 µg/ml in plasma.
- Theobromine : 2 µg/ml in urine.

The endogenous substances of horses include :

- Hydrocortisone : 1 µg/ml in urine.
- Estranediol in intact males : the ratio of the mass concentration of the free and conjugated forms is equal to 1, in urine.

- Testosterone in castrated males : 0.02 µg/ml in urine.
- Testosterone in females (except pregnant mares) : 0.055 µg/ml in urine
- Free carbon dioxide : 37 µmol/l in plasma.
- Methoxytyramine : 4 µg/ml in urine.

Certain procedures are also regulated. Any horse which has undergone a procedure which modifies the air route or its composition in the trachea or nasal cavity cannot participate in a race. In galloping races, covered mares cannot participate after 150 days following the previous coupling. In trotting races, no female can participate if she has been bred at 2 or 3 years old, after the 15th of September of the year in which she has been bred, and in the 150 days following the birth of her progeny. It is not possible in France to participate in a race without having obtained the appropriate accreditation, which includes in the contract, respecting the code of law. This contract applies to owners, trainers and riders (Gadot, 2005). During racing, screening is carried out by veterinarians, either employed or officially certified by the « Fédération Nationale des Courses Françaises » (FNCF). Two samples are taken and remain anonymous. The first sample is analyzed in France in the lab of the FNCF, and the other one is kept for further investigation in a lab from another country, designated by « l'Association des entraineurs », in case the first sample comes back positive. The report is sent in a sealed envelope back to the FNCF and the trainer gets a copy of the report. An appeal to the commission can be made if the sanctions are heavy (Gadot, 2005).
In the code of law, the trainer, which is primarily in charge of the horse's training and wellbeing, is considered sole responsible in the case of reported doping. The owner of the horse can also face some of the consequences and charges (Gadot, 2005).
The sanctions in the case of positive samples are :

- Automatically disqualified.
- Fine for the trainer, up to 15 000 euros
- Suspension or withdrawal of the trainer's license
- Suspension of the horse (up to 6 months in case of anabolic steroids), or withdrawal for the rest of its life
- Publication of the incident on the official racing newsletter.

In the trotting industry, the horse is suspended immediately after being notified, while waiting for a decision.

Galloping race competitions occur worldwide, whereas trotting races occur more often within European limits, thus there is more uniformity concerning laws (Gadot, 2005).

The international federation of horse racing authorities is mainly trying to harmonize the laws and procedures of the 55 countries which host gallop races. The main regulations and laws are gathered in the « Accord international pour les courses et l'élevage » (Gadot, 2005). In the trotting industry, the European Union of the « Trot » has the same function.

In the battle against doping, it is hard to align different philosophies and regulations, with on the one hand, the USA, Canada, Australia, New-Zealand, and on the other hand, the rest of the world (Gadot, 2005).

In France, during the year 2003, more than 1.5 billion euros has been spent on screening during races, sampling has been done in more than 96% of races on all of the winners, the first 5 horses ranked, the first 3 horses in group I races and the 2 first horse in group II races, as well as random sampling. The number of samplings has doubled in the past 15 years. Sampling can also be done on horses during training or horse temporarily taken out of training, which has been put in place since 1995. For the gallop races of 2003, random sampling by drawing of lot has been carried out on 3400 horses, and only 3 positive cases have been detected and sanctioned. Around 0.30 to 0.45 % positive cases have been reported per year, attesting for the efficiency of the anti-doping strategy. The sensitivity of the tests used for screening is always improving, thus it is possible to detect an active substance in the organism way before the effects of the drug start appearing. However, European countries are trying to limit the sensitivity of these tests, as these drugs can be used as part of a therapeutic plan by the veterinarian which is not necessarily considered doping, depending on its concentrations (Gadot, 2005).

In the US, the Horseracing Integrity and Safety Act was signed into law in 2020, in an effort to unify laws throughout the country. Some delays in implementation were due to some states and industry organizations, such as the National Horsemen's Benevolent and Protective association, resisting those changes. After the act was fully implemented, the number of deaths decreased by 38% (Block and Amundson, 2024).

6.4 Position and role of the veterinarian

In equine sports, the veterinarian should not be perceived as the enemy which is trying to reinforce a coercive policy. Concerning doping, its role is prevention and information. He should be helping in providing the technical and scientific data used in the writing of laws against doping, and in charge of sampling during controls at horse tracks. The role of the veterinarian is to discuss with trainers, riders and owners the best way to prepare their horses for races (Grandjean, 2010). It is not in anyone's interest for a breach of trust to occur between trainers and veterinarians as they are both working hand in hand during the whole life of the horse.

7 Discussion

Horseracing and horse shows are both considered equine sports. It is therefore essential to keep « the spirit of sport ». As stated in the FEI's Equine Anti-Doping and Controlled Medication Regulations (EADCM), the values we find in sport are ethics, fair play and honesty, health, athlete's rights, excellence in performance, character and education, fun and joy, teamwork, dedication and commitment, respect for rules and laws, respect for self and other participants, courage, community and solidarity. Doping goes against all of these values, as it is usually done in secrecy and does not reflect integrity of practice.

Doping is a delicate subject which will only grow bigger concerns as time moves on. The use of medication or other procedures in itself, as part of a justified treatment plan by a veterinarian, is not a problem, however it can be misused on healthy horses and horses which cannot physically compete. Such practices can endanger the horse's health and their rider's during training and competitions.

Anti-doping rules in horses and humans are quite different, they do not have the same set of rules. Some substances like PBZ are not as dangerous for animals as humans, so the rules are not as strict, but other substances such as stimulants like caffeine may be more permitted in humans than horses, as people drink more tea and coffee as part of their daily routine.

To ensure the safety of horses, governments are putting in place laws across the country and worldwide, controls against doping during training and competitions, and sanctions for anyone who does not comply with the rules. I think there is still some improvement to be made concerning the harmonization of laws in between countries, as the disparity decreases the efficiency of such laws. If one country or state is not as strict, people will just go around the system and race their horses where it is most beneficial for them. However, there is a

fine line between the laws being fair and them being too strict. It is very unlikely for a horse to be perfectly healthy during its entire career and not needing anything to boost its performance or prevent any further injuries. The screening should take into account any beneficial treatment the horse has taken, as sometimes it is better for the horse to be pain-free than not getting any treatment at all. The veterinarians have an important role in ensuring the safety of horses as they mostly are aware of the horse's condition when asked for regular check-ups. They should be up to date in the regulations to inform the trainers, as well as authorities if needed, and to prescribe an appropriate treatment. They must be careful in the types and quantity of drugs they use, especially if any is left to the trainer. They should also be transparent about the treatments given which are documented and accessible to authorities. They must also be vigilant if they suspect any traces of doping in the horses they examine and notify authorities for reinforced surveillance. It would also be beneficial for veterinarians to share their knowledge with their colleagues about the effects of different drugs they use, as well as with the human medical field, which has more means to doing research on similar doping agents. Lastly, they should help in writing anti-doping laws, as they work closely with trainers and horse tracks.

I think it is important for veterinarians to be tactful with trainers, as without trust, it is difficult to work towards a common goal, and any remark of the veterinarian to the trainer could not be well received. If the trainers think the veterinarian is not taking them into account, they might be even more willing to finding loopholes and cheating the system, instead of confiding in their veterinarian. This could hinder the whole process of the anti-doping battle which is to value teamwork, integrity of the sport and protect the health of horses and riders.

8 Summary

In sum, doping has always existed throughout history under different forms. With the development of technology, as well as the emerging pharmaceutical industry, it has permitted for people to find new ways of cheating the system, but also more effective screening measures. This thesis mainly presents the issues on doping in thoroughbreds in the racing industry, but doping agents can also be used in other sports horses, for which there are similar regulations. It has been proven by research that if misused, several medications and procedures can bear negative consequences on the horse's health. There also has been some cases of death reported during racing. Moreover, a lot of debates around fair play in racing and the reputation of horse tracks are still ongoing. Laws in place against doping are not black and white, as different factors need to be weighed in, and not every veterinarian agrees on which substance is really harmful for horses, as seen with PBZ. Most of the laws revolve around a list of prohibited substances during training and during competitions. There are also indications on the minimum threshold acceptable for each drug detected, and the preferred methods of analysis. The FEI is trying to set the same rules for each racing country, however, there is some resistance from important organizations which makes it difficult to implement the same rules everywhere. This can reduce the efficiency of the system and create a lot of confusion, as horses are traveling everywhere and might not comply with the rules of the hosting country. It is important for veterinarians, trainers, riders and authorities from different countries to work together, exchange information, and work on building a system which is fair and financially viable for all parties. The current situation is that all of the racing countries have laws against doping, some more strict than others. Pharmaceutical companies and laboratories must also be held accountable in this fight against doping, as their research and products can easily be misused, and sanctions given to trainers are based on the lab results. Doping in equine sports is a very important issue, with a lot a stake, and yet it is still a very controversial topic and not spoken often about. That is why, for the safety of horses and people participating in the sport, as well as the image of the sport, it is essential to keep on working on the system to improve it and make it as efficient as possible.

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10 Figures and tables

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