DETERMINATION OF THE BOARD OF DIRECTORS OF THE HORSERACING INTEGRITY AND SAFETY AUTHORITY, INC. RELATING TO FUROSEMIDE

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BOARD RESOLUTIONS

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HORSERACING INTEGRITY AND SAFETY AUTHORITY, INC.

April 24, 2026

The following resolutions were unanimously approved by the Board of Directors of the *Horseracing Integrity and Safety Authority, Inc. (the "Authority") on April 24, 2026:*

WHEREAS, the Horseracing Integrity and Safety Act of 2020 (the "Act") directs the Board of the Authority (the "Board") to consider whether a modification to the prohibition on the use of furosemide (commonly referred to as "Lasix") in Covered Horses in the 48-hours prior to a race (the "Furosemide Prohibition") is warranted;

WHEREAS, under the Act, the Board must vote to unanimously adopt each of the following findings to effectuate a modification of the Furosemide Prohibition:

(i) That the modification is warranted.
(ii) That the modification is in the best interest of horse racing.
(iii) That furosemide has no performance enhancing effect on individual horses.
(iv) That public confidence in the integrity and safety of racing would not be adversely affected by the modification.

WHEREAS, the Furosemide Advisory Committee submitted a 158-page report to the Board (attached hereto as Exhibit A to Tab 1) summarizing the independent research studies, discussing considerations relevant to the control of furosemide, and offering conclusions and recommendations regarding future policy for the regulation of furosemide;

WHEREAS, the Board has determined that a modification (the "Modification") to the Furosemide Prohibition is warranted and that the Furosemide Prohibition shall be modified to permit the use of furosemide in the 48-hours prior to a race for all Covered Horses except for (a) two-year-old Covered Horses and (b) Covered Horses competing in stakes races (as defined in 15 U.S.C. § 3051(17));

WHEREAS, until such time as the Federal Trade Commission approves the updated regulations submitted by the Authority with respect to the administration of furosemide, the administration of furosemide pursuant to the Modification shall be governed by the administration rules of the applicable State Racing Commission that were in place on May 21, 2023;

WHEREAS, the Modification will not result in any change to the regulatory treatment of furosemide for Covered Horses that has been in effect since the inception of the Authority's Anti-Doping and Medication Control Program on May 22, 2023 because the Modification simply allows continuation of the exemptions (which every state racing commission applied for and received) set forth in the Act;

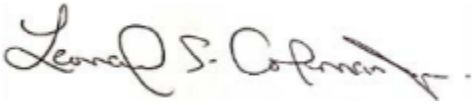
WHEREAS, the Board adopts the following findings: (i) that the Modification is warranted; (ii) that the Modification is in the best interest of horse racing; (iii) that furosemide has no performance enhancing effect on individual horses; and (iv) that public confidence in the integrity and safety of racing would not be adversely affected by the modification; and

WHEREAS, the adoption of these findings in and of themselves satisfies the Act, the Board has determined to also adopt the findings attached at Tab 1.

NOW, THEREFORE, BE IT RESOLVED, that the Modification to the Furosemide Prohibition is warranted and that the Furosemide Prohibition shall be modified to permit the use of furosemide in the 48-hours prior to a race for all Covered Horses except for (a) two-year-old Covered Horses and (b) Covered Horses competing in stakes races (as defined in 15 U.S.C. § 3051(17));

FURTHER RESOLVED, that the Board adopts the following findings: (i) that the Modification is warranted; (ii) that the Modification is in the best interest of horse racing; (iii) that furosemide has no performance enhancing effect on individual horses; and (iv) that public confidence in the integrity and safety of racing would not be adversely affected by the modification; and

FURTHER RESOLVED, that the officers of the Authority be, and each of them hereby is, authorized, empowered and directed, in the name and on behalf of the Authority, and under its corporate seal or otherwise, to execute and deliver such other instruments and documents, and to take such further action, as may be deemed necessary, appropriate or convenient, in order to implement the provisions of the foregoing resolutions, the authority for the execution and delivery of such other instruments and documents, and the taking of such further action by such officers, to be conclusively evidenced hereby.



Leonard S. Coleman, Jr., Secretary

Charles P. Scheeler, Chair

TAB 1: BOARD FINDINGS

FINDINGS OF THE BOARD OF DIRECTORS OF THE HORSERACING INTEGRITY AND SAFETY AUTHORITY, INC. RELATING TO FUROSEMIDE

I. INTRODUCTION

The Horseracing Integrity and Safety Act of 2020 (the "Act") directs the Board (the "Board") of the Horseracing Integrity and Safety Authority, Inc. (the "Authority") to consider whether a modification to the prohibition on the use of furosemide (commonly referred to as "Lasix") in Covered Horses[1] in the 48 hours prior to a race (the "Furosemide Prohibition") is warranted. *See* 15 U.S.C. § 3055(e)(3)(A)-(B). Under the Act, the Board must vote to unanimously adopt each of the following findings to effectuate a modification of the Furosemide Prohibition:

(i) That the modification is warranted.
(ii) That the modification is in the best interest of horse racing.
(iii) That furosemide has no performance-enhancing effect on individual horses.
(iv) That public confidence in the integrity and safety of racing would not be adversely affected by the modification.

Id.

For the reasons set forth herein, and as established in the Board Resolutions dated April 24, 2026, the Board unanimously adopts each of the above findings and determines that the Furosemide Prohibition shall be modified to permit the use of furosemide in the 48 hours prior to a race for all Covered Horses except for (a) 2-year-old Covered Horses and (b) Covered Horses competing in stakes races (as defined in 15 U.S.C. § 3051(17)).[2] Although the modification will take effect on May 23, 2026, it will not result in any change to the regulatory treatment of furosemide for Covered Horses that has been in effect since the inception of the Authority's Anti-Doping and Medication Control Program (the "ADMC Program") on May 22, 2023.[3]

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[1] A "Covered Horse" is defined as "any Thoroughbred horse, or any other horse made subject to this chapter by election of the applicable State racing commission or the breed governing organization for such horse under section 3054(k) of this title, during the period (A) beginning on the date of the horse's first timed and reported workout at a racetrack that participates in covered horseraces or at a training facility; and (B) ending on the date on which the Authority receives written notice that the horse has been retired." 15 U.S.C. § 3051(4).

[2] As defined in the Act, "[t]he term 'stakes race' means any race so designated by the racetrack at which such race is run, including, without limitation, the races comprising the Breeders' Cup World Championships and the races designated as graded stakes by the American Graded Stakes Committee of the Thoroughbred Owners and Breeders Association." 15 U.S.C. § 3051(17). For the avoidance of doubt, furosemide shall not be administered in the 48 hours prior to a race to any 2-year-old Covered Horse or any Covered Horse competing in a stakes race.

[3] Every state racing commission applied for and received a three-year exemption to the Furosemide Prohibition, which began on May 22, 2023 and allowed for race day furosemide administration to all Covered Horses except for (a) 2-year-old Covered Horses and (b) Covered Horses competing in stakes races.

II. STATUTORY BACKGROUND

In 2020, Congress enacted the Act to establish a comprehensive, national regulatory framework for Thoroughbred racing in the United States. Central to the Act is the recognition of the Authority, a private, independent, self-regulatory, nonprofit corporation, for purposes of developing and implementing a horse racing anti-doping and medication control program and a racetrack safety program for Covered Horses, Covered Persons, and Covered Horseraces. 15 U.S.C. § 3052(a).

The Authority's ADMC Program establishes, among other things, a centralized testing and results management process, a uniform list of prohibited substances, and standardized penalties for violations that are efficiently and consistently applied across the United States.[4] As required by the Act, a cornerstone of the ADMC Program is the prohibition on "the administration of any prohibited or otherwise permitted substance to a covered horse within 48 hours of its next racing start." 15 U.S.C. § 3055(d). This 48-hour prohibition took effect on the commencement of the ADMC Program (May 22, 2023). The Act, however, permitted state racing commissions to apply to the Authority for a three-year exemption from the 48-hour prohibition with respect to the use of furosemide[5] for all Covered Horses except for (a) 2-year-old Covered Horses; or (b) Covered Horses competing in stakes races. 15 U.S.C. § 3055(f). All state racing commissions overseeing covered horseraces applied for and received the three-year exemption. The exemption expires on May 22, 2026. *Id.*

The three-year exemption period ran concurrently with a three-year study period mandated by the Act, which directed the Authority to convene a Furosemide Advisory Committee (the "Committee" or "FAC")—comprised of horse racing anti-doping and medication control industry experts–to commission and monitor independent scientific research on the administration of furosemide to horses during the 48-hour period before the start of a race as well as the effect of furosemide on equine health and the integrity of competition.[6] 15 U.S.C. § 3055(e). Over the last three years, the FAC monitored five scientific studies funded by the Authority and conducted by independent investigators across the country, including research teams at the University of Florida, Washington State University, Texas A&M University, the Virginia Polytechnic Institute and State University, and the Center for Clinical and Translational Research.

Under the Act, the FAC is to submit to the Board–on or before May 22, 2026—"a written report on the [furosemide] study. . . that includes recommended changes, if any, to the [48-hour] prohibition" on the administration of furosemide. 15 U.S.C. § 3055(e)(2).

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[4] The Authority's ADMC Program is administered by the Horseracing Integrity & Welfare Unit ("HIWU"), an independent anti-doping and medication control enforcement organization.

[5] Furosemide is classified as an S7 Controlled Medication Substance on the ADMC Program's Prohibited List.

[6] A full list of Furosemide Advisory Committee members is available here: About Us - Horseracing Integrity and Safety Authority.

III. THE FUROSEMIDE ADVISORY COMMITTEE REPORT AND RECOMMENDATIONS

The FAC submitted a written report to the Board summarizing the independent research studies, discussing considerations relevant to the control of furosemide, and offering unanimous conclusions and recommendations regarding future policy for the regulation of furosemide. *See* HISA Furosemide Advisory Committee, Official Report, *Results of Furosemide Study and Recommended Changes to the Current Prohibitions for Consideration by the HISA Board of Directors* (Mar. 31, 2026) (the "FAC Report").[7] The FAC's recommendations to the Board provide in pertinent part as follows:

> *The FAC recommends modification of the current furosemide policy. Specifically, the FAC makes the following recommendation based upon a review of the scientific evidence — including the existing body of peer-reviewed literature, the results of research funded by HISA, FAC internal research, and the FAC's observations and analysis of the Thoroughbred racing industry in the United States during the past three years of regulatory oversight by HISA.*
>
> *These recommendations are made without bias or conflict of interest and are offered from the perspective of unconditional advocates for equine welfare and the future success of horse racing worldwide. These recommendations are intended to provide an ethically-balanced approach to address equine welfare, integrity, public confidence and industry sustainability issues.*
>
> *Given the primary importance of equine welfare, and in recognition of the scientific information that:*
>
> - *EIPH occurs in two-year-old racehorses; severity and incidence increase with age.*
> - *If a horse experiences EIPH, it is at increased risk for repeated episodes of EIPH.*
> - *Repeated episodes of EIPH can cause irreversible pathologic changes in the lung.*
> - *Furosemide is the only FDA-approved medication that has been shown to decrease the incidence and severity of EIPH in horses.*
> - *Recent scientific evidence has determined that regular administration of furosemide to horses in appropriate doses and on a weekly schedule does not cause harm to the horse.*
> - *There is no scientific evidence that supports the current ban of furosemide administration to two-year-old horses on race day.*

[7] A copy of the FAC Report is attached hereto as **Exhibit A**. Enclosed with the FAC Report are the final reports from each of the five studies monitored by the FAC, along with other supporting materials.

- *When confounding variables are controlled, the preponderance of scientific research over the past 40 years does not support the conclusion that furosemide is performance enhancing.*

the FAC recommends:

The current regulatory policy regarding furosemide, prohibiting its use in two-year-olds and in horses racing in Named or Graded Stakes races in the forty-eight (48) hours prior to a race, be modified to permit the current strictly regulated administration of furosemide to horses competing in all races, except for Graded Stakes races that implicate commercial breeding potential.

In so doing, we recognize that there is no scientific justification for prohibiting the administration of furosemide in horses competing in Named and Graded stakes races and conclude that such prohibition is grounded in historic concerns about the marketability of elite North American horses in the international markets. This recommendation will require refining the definition of "stakes races' to limit the prohibition of the administration of furosemide in the forty-eight (48) hours prior to a race only to Listed and Graded stakes races. This recommendation is specifically intended to eliminate the practice of converting Named Stakes races to handicap or overnight races in order to avoid the prohibition on furosemide use in those events.

Additional Recommendations:

1. *Continued research be conducted to better understand the long-term effects of the race day administration of furosemide in 2-year-old horses.*
2. *Continued research be conducted to better understand and further characterize the implications of limited furosemide use, as noted above, upon the issue of integrity and competitive fairness in racing.*
3. *Continued research be conducted to investigate the physiologic effects of furosemide on racehorses other than reduction of differential pressure in terminal airways of racehorses.*
4. *The U.S. Thoroughbred racing industry conduct a thorough evaluation of current stable management practices at racetracks and training centers, with the goal of identifying best practices to improve air quality in stabling areas, reduce exposure to small particulate matter and other airborne irritants, and to mitigate risk factors associated with EIPH in Thoroughbred racehorses.*
5. *The U.S. Thoroughbred racing industry prioritize animal welfare over political or commercial interests in imposing regulatory policy governing race day use of furosemide. The FAC believes that placing equine welfare as the foremost priority will, in the long term, best serve the integrity, sustainability, and public trust of the Thoroughbred racing industry.*

As stewards of the horse, and to the degree that we employ horses for sport and/or entertainment, we are morally obligated to do everything humanly possible to reduce the risk for compromise of equine welfare related to that endeavor.

Finally, while the committee understands that there are limitations to the ability of science to inform policy decisions, these recommendations are supported by strong scientific evidence with a focus on equine welfare, while taking into consideration the social, economic and political realities of our time.

FAC Report, pp. 46-47 (emphasis added).

IV. DISCUSSION

Pursuant to the Act, after receipt of the FAC Report, the Board may modify the Furosemide Prohibition upon the unanimous adoption of each of the following findings:

(i) That the modification is warranted.
(ii) That the modification is in the best interest of horse racing.
(iii) That furosemide has no performance-enhancing effect on individual horses.
(iv) That public confidence in the integrity and safety of racing would not be adversely affected by the modification.

15 U.S.C. § 3055(e)(3)(B). As set forth herein and as established in the Board Resolutions dated April 24, 2026, the Board unanimously determines that the Furosemide Prohibition shall be modified to permit the use of furosemide in the 48 hours prior to a race for all Covered Horses except for (a) 2-year-old Covered Horses and (b) Covered Horses competing in stakes races (as defined in 15 U.S.C. § 3051(17)) (the "Modification"). Each of the findings are discussed in turn below.

(i) A Modification of the Furosemide Prohibition is Warranted.

The Board has determined that the Modification of the Furosemide Prohibition is warranted because (1) it is in the best interest of horse racing; (2) there is no scientific evidence that furosemide has a performance enhancing effect on individual horses; and (3) public confidence in the integrity and safety of racing would not be adversely affected.

(ii) A Modification of the Furosemide Prohibition is in the Best Interests of Horse Racing.

The Board has determined that the Modification of the Furosemide Prohibition is in the best interests of horse racing.

Furosemide is the only U.S. Food and Drug Administration-approved medication that has been shown to decrease the incidence and severity of exercise-induced pulmonary hemorrhage (EIPH), a condition affecting a significant percentage of Thoroughbreds. *See* FAC Report, pg. 38 ("EIPH prevalence may exceed 60-70% in racing populations."). "The use of furosemide to control the

incidence of EIPH in Thoroughbreds on race day was gradually adopted by 38 racing State regulators between 1976 and 1995. Since then, furosemide has been legalized in all racing States for use on race day under tightly controlled rules and is the only medication permitted to be administered to a horse on race day." *Id.* at 3.

Although furosemide "is the only consistently demonstrated pharmacologic intervention that reduces EIPH severity," race-day administration of furosemide to Thoroughbreds remains a "controversial and polarizing issue" in the sport.[8] *Id. at* 3, 38. The FAC summarized the ongoing furosemide debate as follows:

> *Proponents for the use of furosemide to control EIPH in racehorses on race day have justified its use on the basis of the best interests of the health and welfare of the horse. Research has shown that once a horse experiences an episode of EIPH, it is at increased risk for repeated episodes over time. For that reason, proponents have argued that furosemide is a benign medication that has prophylactic value to prevent or minimize EIPH. They further maintain that if the medication is banned, horsemen will be forced to use inhumane methods to treat EIPH such as withdrawing food and water from the horse for substantial periods of time prior to racing or withdraw the horse from racing altogether. Critics have opposed the treatment of racehorses with furosemide on race day because they contend research has shown it can enhance performance and provide an unfair advantage for horses that are treated. For years, critics also have maintained that treatment with furosemide on race day can dilute the urine of horses and mask or compromise post-race testing for prohibited substances. Other critics have argued that repeated treatment with furosemide is detrimental to the health and welfare of the horse because chronic use of the medication disrupts the normal electrolyte and mineral balance in the horse that can predispose it to fatal musculoskeletal injury. These statements have not been scientifically proven.*

Id. at 3 (emphasis added).

The Board concludes that adopting the Modification is in the best interests of horse racing. A few of the relevant considerations are set forth in the following paragraphs.

First, the use of "furosemide has been shown to mitigate the severity of [EIPH] in racehorses. EIPH occurs during high-speed exercise as the result of small capillary vessel rupture within the lung." *Id.* This condition affects a significant percentage of Thoroughbreds to varying degrees, "from very minor amounts of blood only seen endoscopically after a race to severe bleeding from the nostrils, termed epistaxis." *Id.*

[8] Presumably, the importance of this issue gave rise to the Congressional mandate to conduct a three-year study period on the use of furosemide.

Without access to furosemide on race day, horses may be retired or withdrawn from racing competition due to health and safety concerns. The potential impact of this could be significant as approximately 76% of all Covered Horses (excluding 2-year-old Covered Horses since they were not exempt from the Furosemide Prohibition) that competed in a race in 2025 were administered furosemide on race day.[9] A prohibition on race-day furosemide could result in more horses being retired over a relatively short period, placing additional pressure on an aftercare industry that is already operating with limited resources and funding.[10] Further, this could also lead to fewer horses in racing, smaller field sizes, and cancellation or consolidation of races due to insufficient entries.[11] Potential downstream consequences of this include reduced number of mares bred, reduced wagering handle, diminished fan engagement, and increased economic pressure on racetracks and other industry participants. *See* Comment from Patricia Wonderley[12] (A prohibition on furosemide "will decimate the industry and stop horse handicapping."); *see* Comment from Thoroughbred Owners of California, California Thoroughbred Trainers, and California Thoroughbred Breeders Association[13] ("Implementing any changes to the current policy would prove highly disruptive to horsemen in all regions at a time when key economic indicators such as field size are closely monitored."); *see* Comment from HISA's Horsemen's Advisory Group[14] ("Out of a deep concern for the health of the horses; doing all we can to continue to support the industry and all of the people it employs both directly and indirectly; and thus for the good of racing in general, the members of the HISA Horsemen's Advisory Group unanimously recommends that the HISA Board of Directors votes to continue the use of race day Lasix along the exact guidelines that currently exist. Failure to do so will cause an immediate severe and future shrinkage of the industry across the board and likely threaten its overall viability. Conversely, continuation of this policy will go a long way towards improving consistency, planning, and overall cooperation among all horsemen's groups as we move forward.").

Second, and as described in further detail below in subsection (iii), the FAC concluded that the available scientific evidence does not support the concern that furosemide is a performance-enhancing substance. *See* FAC Report, p. 44 ("A performance enhancing drug is defined as one

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[9] As reported in the HISA Portal, in 2025, there were 25,578 unique Covered Horses administered furosemide on the day of a race. This accounts for approximately 76% of all Covered Horses (excluding 2-year-olds) that competed in at least one race in 2025. Additionally, more than 200 Covered Horses were placed on the Veterinarians' List for epistaxis in 2025.

[10] As noted, more than 25,000 Covered Horses were administered furosemide on the day of a race in 2025. If the Furosemide Prohibition were to take effect, it is possible that a significant percentage of these Covered Horses could be retired or withdrawn from racing, which would place additional demands on aftercare organizations.

[11] A prohibition on race-day furosemide could have unintended consequences for the horses that remain active in racing. If a meaningful number of horses are retired or withdrawn from racing due to a furosemide prohibition, the remaining population of horses could face elevated health risks due to increased demands placed upon a smaller, more limited population.

[12] A copy of this comment is attached hereto as **Exhibit B**.

[13] A copy of this letter is attached hereto as **Exhibit C.**

[14] This letter was submitted on behalf of the 17 members of HISA's Horsemen's Advisory Group, which consists of trainers, veterinarians, breeders, and owners with more than 400 years of combined experience in the racing industry. A copy of this letter is attached hereto as **Exhibit D**.

that builds muscle, increases strength, enhances endurance, acts as a stimulant or speeds recovery. Furosemide has not been shown to do any of these.").

Similarly, the FAC concluded that there is little risk that race-day furosemide administration masks the detection of other controlled or prohibited substances:

> *Research has demonstrated that furosemide-associated diuresis ceases approximately 3 hours post-administration. As such, the potential for a dilute urine sample after 3-4 hours post-administration is relatively small. Because furosemide is a potent diuretic that causes dilution of urine, lowering urine solute concentration, which can mask the presence of prohibited substances, urine specific gravity (USG) is used in equine drug testing to help ensure urine samples are not too dilute for the detection of prohibited substances, thus invalidating post-race drug testing. The measurement of USG alongside regulating the control of a maximum furosemide dose, route of administration, and time (in proximity of post-time) in which furosemide can be administered on race day largely eliminate the "masking" concerns associated with race day furosemide use and the ability of laboratories to detect prohibited substances. Most racing regulatory authorities have employed a USG threshold to determine the reliability and appropriateness of a sample for detection of prohibited substances.*
>
> *Furthermore, because US labs largely test in blood (including paired blood and urine post-race samples), this minimizes sole reliance on potentially diluted urine samples. To that end, the target substance, concentration of that substance, and interaction of multiple drugs (furosemide and potential prohibited substance) in a horse at one time are also impact variables to consider regarding the detection of a prohibited substance.*
>
> *Overall, because of the above listed controls for race day administration, measurement of USG in urine samples of horses treated with furosemide, and routine screening in blood alongside urine, the resulting impact of race day furosemide administration on detection of prohibited substances in a post-race sample is minimal.*

Id. at 37 (emphasis added).

While the available body of scientific research supports the above findings, the Board recognizes that the existing research is not without limitations and does not necessarily resolve all questions and issues regarding the administration of furosemide, including the impact, if any, on 2-year-old horses. *Id.* at 47 (FAC recommending additional research on (1) the long-term effects of the race-day administration of furosemide in 2-year-old horses; (2) the implications of limited furosemide use on the integrity and competitive fairness in racing; and (3) the physiologic effects of furosemide on racehorses other than reduction of differential pressure in terminal airways of

racehorses.) In the absence of more definitive research and understanding, the Board finds that maintaining the regulatory framework in place since May 22, 2023—i.e., permitting race day furosemide for all horses except for 2-year-olds and horses competing in stakes races—is in the best interests of horse racing.

The Board agrees with the FAC that additional research is warranted on the long-term effects of race-day administration of furosemide in 2-year-old horses.[15] The FAC Report included several, some non-statistically significant, findings that warrant further investigation before permitting furosemide administration to 2-year-old horses. *See* Bayly W., Meyer J., Sanz M., Leguillette R., Cohen N. Does pre-race administration of furosemide to Thoroughbred racehorses prolong their racing careers? Appendix F, FAC Report, p. 73 (the "Bayly Study")("When trainer fixed effects were considered, there was a suggestion that the ban on furosemide may have actually increased career starts by +1.04. However, the 95% Confidence interval was -0.85 to +2.93 with p = 0.28, indicating that this was not a significant finding); *see* DeNotta S. Effects of repeated furosemide administration on electrolyte homeostasis and bone density in exercising Thoroughbreds, Appendix G, FAC Report, p. 87 (the "Denotta Study") ("Bone material strength index scores (BMSi) in both groups increased over the course of the study, the increase was less pronounced in treatment horses (mean change = 1.6) when compared to controls (mean change 4.0). Although this difference was not statistically significant (p = 0.381) in this study, further investigation to better understand how chronic furosemide affects bone strength is warranted."); *see* White-Springer S. Effects of repeated furosemide administration on basal mitochondrial function and energy production capacity of the gluteus medius muscle, FAC Report pp. 22-23 ("Repeated furosemide administration did not alter mitochondrial oxygen consumption capacity or the maximal ability to produce ATP, key determinants of a horse's aerobic performance capacity. However, furosemide did reduce mitochondrial efficiency of ATP production, meaning more cellular input was required to generate the same amount of energy."); *see* Boone A., White-Springer S. Risk Factors for Epistaxis Incidence in United States Thoroughbred Racehorses in 2024, FAC Report p. 149) ("No significant interaction or correlation was observed between previous epistaxis and finish position.").

The question, therefore, is whether a ban on the use of furosemide for 2-year-old horses should continue until such research is completed. Equine safety and welfare are of the utmost importance and must be the central focus in answering this question. Based on the FAC Report, there is no compelling evidence that indicates that the existing prohibition on furosemide for 2-year-old horses is detrimental to their career. A few of the relevant research findings are noted below:

[15] The Board requests that the members of the FAC submit a proposal to the Board containing a recommended plan for additional research on the long-term effects of race-day administration of furosemide in 2-year-old horses and any other issue(s) involving the race day administration of furosemide.

- "Prohibiting routine race day furosemide administration to 2-year-olds did not decrease their racing careers as it did not have a substantial effect on the number of career starts over a horse's first five years of racing." *See* Bayly Study, p. 73.
- "The gap between 2YO and 3YO starters narrowed by only 0.27 starts, a small, negative change that was not statistically significant. If banning furosemide for 2-year-olds materially harmed durability, the gap should have narrowed substantially as 2-year-old careers shortened relative to those of 3-year-olds. This did not occur ($p = 0.41$)." *Id.* at pp. 72-73.
- "When all 2-year-old starts in the U.S. that took place from 2017-2021 are compared, the risk of a horse not finishing a race as a 2-year-old was not significantly different for horses racing without race day furosemide ($p = 0.123$)." *See* Waller A. Examining associations between furosemide treatment and racehorse health and safety, Appendix J, FAC Report, p. 126 (the "Waller Study").
- "After controlling for age, sex, and racing class level, early career furosemide administration was not associated with outcomes relating to overall racing career performance and longevity. There were no differences in any of the examined metrics of racing career performance and longevity between horses that competed with versus without race-day furosemide treatment for all their 2-year-old races. Specifically, the presence/absence of early-career furosemide administration did not have subsequent long-term effects on lifetime earnings, racing career length, lifetime starts, number of times placed in the top 3, or average lifetime speed figures." *Id.* at p. 129.

Based on the FAC Report, the welfare impact on the 2-year-old ban appears to be neutral. Thus, other factors should be considered as part of this analysis, such as the perception by the public and industry stakeholders of moving from a ban to allowing furosemide on 2-year-olds—a move that could be perceived as a step backward on safety and welfare and further distancing U.S. racing from international counterparts. Given the absence of evidence indicating that the existing prohibition is harmful to a racing career, the need for further research, and the potential negative perception both domestically and internationally, the Board finds that maintaining the existing prohibition for 2-year-olds is in the best interests of horse racing.

With respect to stakes races, the FAC concluded that the prohibition on race-day furosemide is "grounded in historic concerns about the marketability of elite North American horses in the international markets" and recommended continuing the prohibition for listed and graded stakes races only. FAC Report, pp. 46-47. The Board agrees that aligning U.S. stakes races with international medication-free standards enhances the credibility and global standing of U.S. racing. However, the Board believes that maintaining a uniform prohibition across all stakes races is in the best interests of racing at this time. A uniform approach provides clear rules for horsemen, veterinarians, regulators, the betting public, and other industry stakeholders. A bifurcated approach for stakes races would increase the risk of confusion and could lead to inadvertent noncompliance.

Other broader considerations weigh in favor of maintaining the current prohibitions. Since the inception of the ADMC Program, equine racing-related fatality rates have declined and public confidence in the safety and integrity of the sport has improved. Moreover, it is noteworthy that industry stakeholders are advocating for the continuation of the status quo:

> *This letter from the Thoroughbred Owners of California (TOC), California Thoroughbred Trainers (CTT), and California Thoroughbred Breeders Association (CTBA) states our support for the continuation of the current policy, which prohibits the use of furosemide (Lasix) in all 2-year-old races and all stakes' races.*
>
> *This policy has served the California racing and breeding industry well, striking a balance that has worked effectively over the past three years. It has also contributed greatly to record results at horse sales across the nation. The increased participation from international buyers at horse sales has helped restore confidence in the quality of our bloodstock and the image of our racing. The current policy has delivered the desired results.*
>
> *Implementing any changes to the current policy would prove highly disruptive to horsemen in all regions at a time when key economic indicators such as field size are closely monitored. In the absence of any compelling scientific evidence to the contrary, our three organizations strongly recommend maintaining the status quo.*

Letter from Thoroughbred Owners of California, California Thoroughbred Trainers, and California Thoroughbred Breeders Association (emphasis added) (Exhibit C).[16]

> *We have discussed the topic of Lasix extensively during our monthly meetings and with our fellow horsemen at racetracks, sales, and breeding farms. We acknowledge that the use of race day Lasix may be the single most important topic that horse racing is currently facing. We would like you to know that, among horsemen, this is not a polarizing topic - the overwhelming majority of horsemen favor the continued use of race day Lasix. We have given this very careful consideration. Out of a deep concern for the health of the horses; doing all we can to continue to support the industry and all of the people it employs both directly and indirectly; and thus for the good of racing in general, the members of the HISA Horsemen's Advisory Group unanimously recommends that the HISA Board of Directors votes to continue the use of race day Lasix along the exact guidelines that currently exist. Failure to do so will cause an immediate severe and future shrinkage of the industry across the board and likely threaten its overall viability. Conversely, continuation*

[16] Collectively, these groups represent thousands of Thoroughbred owners, trainers, and breeders throughout the country.

of this policy will go a long way towards improving consistency, planning, and overall cooperation among all horsemen's groups as we move forward.

Letter from HISA Horsemen's Advisory Group (Exhibit D).

Multiple trainers commented on the use of furosemide as part of an article published in *Thoroughbred Daily News*:

> *"I think we're protecting the breed by not allowing 2-year-olds to race on it," trainer Graham Motion said. "We're protecting the stud book by not allowing the best fillies and colts in the country to run on Lasix in stakes races. So we're protecting the breeding program. As for the bread-and-butter daily racing, I think Lasix is something that only helps the horses. I think it's working, and I don't really see the reason to take it away. I think the present system is working and I think it's going to make life very difficult for the day-to-day racing and the guys that have the claimers to operate without it."*
>
> *"I am hoping that they will keep Lasix," [Linda Rice] said. "I think that it could be damaging to lose Lasix for the bulk of racing. It could be damaging to the field size and the number of horses that can continue to race. And we're already having a problem with field size. It could be difficult to maintain the numbers that we need to keep racetracks going. So I'm hoping that they will decide to keep Lasix. As far as I'm concerned, I'm okay with 2-year-olds not having Lasix. I think that's fine. I'm actually for that. I think that gives them a chance to grow to full maturity and race as a 3-year-old. But I do think that it will have a negative effect on racing in general if they remove Lasix."*
>
> . . .
>
> *Like Motion, Pletcher also said that the current rules are working and should not be changed.*
>
> *"What they're doing now seems to be working fine," he said. "I think everyone has kind of found a way to compromise, and what they've done is a good compromise. The platform that HISA ran on was one where they were going to allow some flexibility on the Lasix, and so I think they'd lose some goodwill turning back on that."*

Will HISA Ban Lasix in All Races? Its Board of Directors Will Soon Decide, Thoroughbred Daily News (Feb. 25, 2026).[17]

[17] The only other comment received by the Board that is not referenced in this document is a comment submitted on behalf of the American Association of Equine Practitioners (AAEP) requesting that the Board "continue to allow race-day Lasix with the current prohibitions of 2-year-olds and stakes races, until the findings are available for review by the broader industry." A copy of the AAEP letter is attached hereto as **Exhibit E**.

For these reasons and others, the Board finds that the Modification is in the best interests of horse racing.

(iii) Furosemide Has No Performance-Enhancing Effect on Individual Horses

The Board adopts the conclusion of the FAC that furosemide does not have a performance-enhancing effect on individual horses.

Notably, "performance enhancing" is not defined in the Act. Under the FAC's definition, "[a] performance enhancing drug is defined as one that builds muscle, increases strength, enhances endurance, acts as a stimulant, or speeds recovery." FAC Report, p. 43. Based on a review of earlier observational studies conducted prior to the Act, along with research commissioned and monitored by the FAC, the FAC concluded that "[t]here is no scientific evidence to support the statement that furosemide is a performance enhancing drug." *Id.*

Research commissioned as part of the FAC's charge includes Dr. Sally Denotta's controlled investigation that examined repeated furosemide administration in exercising Thoroughbreds using a prospective randomized placebo-controlled treadmill design over a 14-week period.[18] *Id. at* 20-21, 38-39. The study measured body weight and hydration status, packed cell volume, plasma electrolyte concentrations, urine specific gravity, cardiac function during exercise, and skeletal bone density. *Id.* at 39. The FAC summarized Dr. Denotta's findings as follows:

> *Body weight: There was no sustained difference between treated and control horses.*
>
> *Electrolytes: There were no clinically meaningful changes in serum sodium, potassium or chloride.*
>
> *Hydration markers: Urine specific gravity did not differ from pre-administration levels 48 hours post-administration.*
>
> *Cardiac performance: Echocardiographic measures during exercise were unchanged.*
>
> *Bone health: Bone density and skeletal strength were unaffected.*
>
> *Importantly, repeated administration did not produce cumulative physiologic effects during the study period. These findings indicate that repeated furosemide administration does not produce systemic physiologic changes consistent with direct performance enhancement. Any benefit may be consistent with a reduction of pulmonary bleeding during intense exercise.*

Id. at 39 (emphasis added).

[18] *See* Denotta Study.

While Dr. Denotta's study found that there was no *sustained* difference in body weight between treated and control horses, the FAC Report does cite research indicating that a horse may experience a temporary reduction of body mass following administration of furosemide. FAC Report, p. 122 ("For example, furosemide treatment results in an acute, transient uresis and accompanying body weight loss of 2%."). The FAC concluded, however, that "[r]educed body mass could theoretically lower the metabolic costs of locomotion, similar to the advantage associated with lighter carried weight. However, the magnitude and consistency of this effect appear small across studies." *Id.* at 38.

Dr. Amanda Waller conducted a "retrospective, observational, case-crossover study design . . . to assess the effect of prerace administration of furosemide on racing performance in individual Thoroughbred racehorses competing in North America from 2020-2024."[19] *Id*. at 30. Dr. Waller's research indicated that race-day furosemide "was not significantly associated with winning speed figures, finishing position, or percentage of the field beaten." *Id.* at 40.

The FAC also reviewed "earlier observational racing studies" from 1985, 1999, and 2005 that reported "modest performance improvements among horses receiving furosemide." *Id.* at 39-40. However, regulatory changes to race-day furosemide administration, coupled with the more recent findings from studies commissioned by the FAC, "suggest earlier reported performance differences may reflect confounding variables rather than pharmacological enhancement." *Id.* at 40. Based on their review of the available research, the FAC concluded:

> *The current body of scientific and racing evidence strongly supports the role of furosemide in reducing exercise-induced pulmonary hemorrhage in racehorses. There is no scientific evidence to support the statement that furosemide is a performance enhancing drug. A performance enhancing drug is defined as one that builds muscle, increases strength, enhances endurance, acts as a stimulant or speeds recovery. Furosemide has not been shown to do any of these.*

Id. at 43 (emphasis added).

(v) Public Confidence in the Integrity and Safety of Racing Would Not Be Adversely Affected by the Modification.

The Board has determined that the public confidence in the integrity and safety of racing would not be adversely affected by modifying the Furosemide Prohibition and allowing the current furosemide exemptions to continue.

Since May 22, 2023, race-day furosemide administration has been permitted in all Covered Horses except for (a) 2-year-old Covered Horses; and (b) Covered Horses competing in stakes races. There are various economic indicators that suggest public confidence has increased in the integrity and

[19] Waller AP. Examining associations between furosemide treatment and racehorse health and welfare (2025). This study was commissioned and monitored by the FAC.

safety of racing over the last three years. For example, since 2023, several racing organizations have announced significant construction and renovation projects combined totaling more than $1 billion, reflecting long-term confidence in the future of the industry. These projects include:

- In 2023, the New York Racing Association, Inc. (NYRA) announced plans for a $455 million project to build new thoroughbred racing facilities at Belmont Park.[20]
- In October 2023, Keeneland announced plans for major capital investment projects, which are expected to cost nearly $93 million.[21]
- In 2024, the Maryland Stadium Authority announced plans for a $400 million project to demolish, design, and reconstruct Pimlico Race Course in Baltimore and to build a new training facility.[22]
- In July 2024, Churchill Downs, Incorporated announced plans for an $80-$90 million renovation to the existing grandstand at Churchill Downs Racetrack.[23]

New construction projects are not the only indicator of economic growth and confidence in the industry. North American Thoroughbred sales have increased since the inception of the Authority's ADMC Program. In 2022—the last full year prior to inception of the ADMC Program—the average price for horses sold was $88,256.00.[24] By 2025, the average price increased to $107,649.00, a nearly 22% increase.[25] Moreover, Keeneland, Fasig-Tipton, and Ocala Breeders Sales—three of the largest national auction houses in the United States—have each announced record sales over the last three years. A few examples are cited below:

- September 2025: The 2025 Keeneland September Yearling Sale concludes with record $531,500,000 in sales, "the highest gross ever realized at a Thoroughbred auction worldwide."[26] Buyers represented 33 countries with participation from Asia, the Middle East and Europe.
- October 2025: Fasig-Tipton Kentucky October Yearlings Sale "smashes records" for gross sales, average price, and median price.[27]

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[20] *See* NYRA Secures New York State Approval to Construct a New Belmont Park, https://pastthewire.com/nyra-secures-new-york-state-approval-to-construct-a-new-belmont-park/

[21] *See* Keeneland Makes Historic Announcement: Construction of a New Paddock Building and Enhancements to the Saddling Paddock, https://www.keeneland.com/media/news/keeneland-makes-historic-announcement-construction-new-paddock-building-and-enhancements

[22] *See* Redevelopment of the Pimlico Racing Facility and New Training Facility, https://mdstad.com/redevelopment-pimlico-racing-and-laurel-park-racing-facilities

[23] *See* Churchill Downs Incorporated Reveals Grandstand Club and Pavilion Renovation Plan for Churchill Downs Racetrack, https://www.churchilldownsincorporated.com/churchill-downs-incorporated-reveals-grandstand-club-and-pavilion-renovation-plan-for-churchill-downs-racetrack/

[24] *See* Bloodhorse, State of the Market, https://www.bloodhorse.com/horse-racing/thoroughbred-sales/state-of-the-market

[25] *Id.*

[26] *See* American Horse Publications, Keeneland September Concludes with Record $531.5 Million in Sales, https://www.americanhorsepubs.org/newsgroup/keeneland-september-concludes-with-record-531-5-million-in-sales.

[27] *See* Bloodhorse, Fasig-Tipton KY October Yearling Sale Smashes Record, https://www.bloodhorse.com/horse-racing/articles/288158/fasig-tipton-ky-october-yearlings-sale-smashes-records.

- November 2025: Keeneland's November Breeding Stock Sale reported to be the highest-grossing November sale since 2007.[28]
- December 2025: Fasig-Tipton announces the "the highest grossing digital sale ever" with "421 horses sold for $10,607,900."[29]
- January 2026: Keeneland's two-day January Horses of All Ages Sale concluded with "total sales for 700 horses of $53,580,300 . . . to record the highest gross for the auction since 2008."[30]
- March 2026: Ocala Breeders' Sales Co. announces OBS March Two-Year-Olds in Training Sale concludes with record gross; ties mark for most seven-figure horses.[31]

The number of international horses competing in premier races in the United States has also increased since inception of the ADMC Program in 2023. For example, the Breeders' Cup World Championships, one of the largest events in global horse racing, has seen an increase of 12.6% in international based starters in Breeders' Cup races over the last three years compared to the three years preceding the ADMC Program. This uptick in international participation reflects, at least in part, a heightened level of confidence and trust in the safety and integrity standards in Thoroughbred racing in the United States.

For these reasons and others, the Board finds that public confidence in the integrity and safety of racing would not be adversely affected by the Modification.

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[28] *See* Keeneland Sales, Keeneland November Breeding Stock Sale Concludes with Nearly $246 Million in Total Sales, https://www.keeneland.com/media/news/keeneland-november-breeding-stock-sale-concludes-nearly-246-million-total-sales.

[29] *See* Thoroughbred Daily News, 'Highest Grossing Digital Sale Ever': Treadstone Tops Fasig-Tipton December Digital Sale https://www.thoroughbreddailynews.com/highest-grossing-digital-sale-ever-treadstone-tops-fasig-tipton-december-digital-sale/.

[30] *See* Keeneland Sales, Gross Sales Exceed $53 Million for Two-Day Keeneland January Horses of All Ages Sale, https://www.keeneland.com/sales/news/gross-sales-exceed-53-million-for-two-day-keeneland-january-horses-of-all-ages-sale/

[31] *See* Ocala Breeders' Sales Co., OBS March Sale Concludes with Record Gross; Ties Mark for Most Seven-Figure Horses, https://obssales.com/blog/2026/03/12/obs-march-sale-concludes-with-record-gross-ties-mark-for-most-seven-figure-horses/.

EXHIBIT A: FUROSEMIDE ADVISORY COMMITTEE REPORT

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Lexington, KY, 40507

Web: www.hisaus.org

HISA Furosemide Advisory Committee

Image: Dr S. Palmer

Official Report

Results of Furosemide Study and Recommended Changes to the Current Prohibitions for Consideration by the HISA Board of Directors

March 31, 2026

Table of Contents

INTRODUCTION

Furosemide is a diuretic medication first approved by the FDA in 1966 to treat edema (fluid retention) in people with a variety of serious medical conditions such as liver, kidney, and heart disease and high blood pressure. In veterinary medicine, furosemide has been shown to mitigate the severity of exercise-induced pulmonary hemorrhage (EIPH) in racehorses. EIPH occurs during high-speed exercise as the result of small capillary vessel rupture within the lung. EIPH can vary in severity from very minor amounts of blood only seen endoscopically after a race to severe bleeding from the nostrils, termed epistaxis. Efforts to reduce the incidence of EIPH include both good stable management practice and medication.

The use of furosemide to control the incidence of EIPH in Thoroughbreds on race day was gradually adopted by 38 racing State regulators between 1976 and 1995. Since then, furosemide has been legalized in all racing States for use on race day under tightly controlled rules and is the only medication permitted to be administered to a horse on race day.

To say that race day administration of furosemide to racehorses in the United States has been a controversial and polarizing issue is an understatement. Proponents for the use of furosemide to control EIPH in racehorses on race day have justified its use on the basis of the best interests of the health and welfare of the horse. Research has shown that once a horse experiences an episode of EIPH, it is at increased risk for repeated episodes over time. For that reason, proponents have argued that furosemide is a benign medication that has prophylactic value to prevent or minimize EIPH. They further maintain that if the medication is banned, horsemen will be forced to use inhumane methods to treat EIPH such as withdrawing food and water from the horse for substantial periods of time prior to racing or withdraw the horse from racing altogether. Critics have opposed the treatment of racehorses with furosemide on race day because they contend research has shown it can enhance performance and provide an unfair advantage for horses that are treated. For years, critics also have maintained that treatment with furosemide on race day can dilute the urine of horses and mask or compromise post-race testing for prohibited substances. Other critics have argued that repeated treatment with furosemide is detrimental to the health and welfare of the horse because chronic use of the medication disrupts the normal electrolyte and mineral balance in the horse that can predispose it to fatal musculoskeletal injury. These statements have not been scientifically proven.

From a global perspective, furosemide administration on race day is unique to the United States. As a result, there has been international pressure to eliminate the use of furosemide on race day in the United States in order to achieve global conformity for the Thoroughbred horse breeding industry. No matter the absence of scientific information to support it, there is a marked bias against US Thoroughbreds because of the permitted administration of furosemide on race day.

RELEVANT REGULATORY HISTORY

In 2001, in the first major united industry effort to achieve medication uniformity, the Racing Medication Testing Consortium (RMTC) was formed by industry stakeholders to develop and promote uniform medication rules, practices and laboratory testing by all racing jurisdictions. Given the controversy regarding possible performance-enhancing effects of furosemide, the RMTC recommended that furosemide administration be permitted to all racehorses on race day under certain regulatory controls in order to eliminate any potential competitive advantage and the possibility of masking in drug testing.

In 2011, as the internal debate about the race day use of furosemide continued unabated, an International Summit on Race day Medication, with particular focus on EIPH and furosemide, was held at Belmont Park Racetrack. This summit was jointly sponsored by the American Association of Equine Practitioners, the National Thoroughbred Racing Association (NTRA) and the RMTC. The leading scientific experts and researchers on EIPH, racing officials, veterinarians, horsemen and track executives from the United States, United Kingdom, Ireland, Hong Kong, South Africa, Australia and United Arab Emirates participated in this summit. The goal of the summit was to discuss the topic of race day medication from a global perspective in an effort to determine what is best for the horse and for the horse racing industry. Efforts to reach a consensus regarding the use of furosemide on race day were confounded by conflicting international views, and no changes to current regulatory policy were recommended.

In 2017, a group of industry stakeholders sought federal legislation to create federal oversight of the use of drugs and medications in Thoroughbred racing, including a ban on the race day use of furosemide. The legislation was the subject of a public hearing but was not passed out of the House Energy and Commerce Committee.

At the same time, the RMTC, working at the behest of the NTRA and the Association of Racing Commissioners International (“ARCI”), recommended a set of uniform rules and practices for the use of drugs and medications in horses, including new controls on the use of race day furosemide. The NTRA adopted these recommendations for its uniform Code of Standards for member organizations, and the ARCI did the same for its model recommended uniform national regulatory rules and protocols for racing jurisdictions. These rules and protocols: 1) allowed the trainer to determine whether the use of race day furosemide was in the best interests of the health and welfare of the horse; 2) required furosemide to be administered on race day only by a regulatory appointed and authorized veterinarian; 3) limited the time at which furosemide could be administered to not less than 4 hours prior to a race; and 4) provided that furosemide could be administered intravenously only at a dosage not less than 150 mg nor more than 500 mg. To ensure regulatory compliance, post-race testing required demonstration of a furosemide concentration in serum or urine consistent with appropriate administration. If the specific gravity of urine was below 1.010 or no urine sample was collected, the serum sample concentration of the drug could not exceed 100 ng/ml. These recommended practices and protocols, however, were voluntary and not mandatory. The contentious debate about the permitted use of furosemide on race day did not abate.

In 2020, Congress enacted the Horseracing Integrity and Safety Act (the “Act”) which recognized the Horseracing Integrity and Safety Authority, Inc. (the “Authority”), a private, independent, self-regulatory, nonprofit corporation, for purposes of developing and implementing a horseracing anti-doping and medication control program and a racetrack safety program for covered horses, covered persons, and covered horseraces. Among other things, the Authority was authorized to develop and propose to the Federal Trade Commission (the “FTC”) uniform national safety and anti-doping rules for Thoroughbred racing (the Act and the Authority are jointly and severally referred to hereinafter as “HISA”). The rules only take effect if approved by the FTC.

The Act imposed new uniform requirements for the use of furosemide on race day. Effective with the commencement of HISA’s anti-doping program, May 22, 2023, the use of furosemide in the 48 hours prior to a race was prohibited in all two-year-old horses and horses competing in stakes races named as such by racetracks (“Named stakes”), races comprising the Breeders Cup World Championships and races designated as graded stakes by the American Graded Stakes Committee (collectively referred to hereinafter as “Listed and Graded stakes”). For all other races, the use of furosemide on race day was permitted provided the state racing commission applied for and received a 3-year exemption from HISA. HISA’s regulatory controls for those races mirrored the ARCI recommended protocols. Subsequently, all racing jurisdictions applied for and received the exemption.

Additionally, HISA was required to convene an advisory committee comprised of horseracing anti-doping and medication control industry experts, to conduct a study during the 3-year exemption period on the administration of furosemide to racehorses during the 48-hour period before the start of a race, including the effect of furosemide on equine health, the integrity of competition and any other matter the authority considered appropriate. The committee was mandated to submit a report to the HISA Board on its study and any recommended changes to the current prohibitions prior to the expiration of the 3-year period.

This report summarizes the work of the Furosemide Advisory Committee (FAC) over the past 3 years and offers conclusions and recommendations to the HISA Board regarding future regulatory policy for the regulation of furosemide.

HISA Furosemide Advisory Committee

On June 21, 2023 HISA announced the formation of the FAC to oversee the 3-year study on the administration of furosemide within 48 hours of a covered horserace, including the effect on equine health and the integrity of competition, as mandated by the Act.

Over the past 3 years the following individuals have served as members of the FAC:

Emma Adam, DVM, PhD, DACVIM, DACVS, is a veterinarian with over 30 years of racing experience in the United States, United Kingdom, France and Australia. She is board-certified in equine medicine following a residency at Texas A&M University and in surgery following a residency at the New Bolton Center at the University of Pennsylvania. Dr. Adam also received her Ph.D. in musculoskeletal science from the University of Kentucky's Gluck Equine Research Center. Dr. Adam has served as a member of the Grayson-Jockey Club's Research Advisory Committee. She currently serves as Vice President of the American Association of Equine Practitioners (AAEP) and sits on the Veterinary Advisory Committee for the Horserace Betting Levy Board in the UK.

Alan M. Foreman, Esq. is Chairman of the Thoroughbred Horsemen's Association (THA) and Vice-Chairman of the Racing Medication and Testing Consortium (RMTC). Foreman is considered one of the racing industry's leading attorneys in the United States and an expert in the regulation of drugs and medications in the equine sport. He is the national Ombudsman for the Horseracing Integrity and Welfare Unit.

Scott Hay, DVM, is a veterinarian and partner at Teigland, Franklin and Brokken, where he focuses on lameness, performance evaluations and purchase examinations. He also serves on the RMTC's Scientific Advisory Committee. Dr. Hay previously served as President of the American Association of Equine Practitioners (AAEP) and as a member of the Grayson-Jockey Club's Research Advisory Committee.

Ted Hill, VMD, is a racing steward for the Jockey Club with more than 23 years of experience. He previously served as Chief Veterinarian for the New York Racing Association (NYRA). Dr. Hill received his VMD from the University of Pennsylvania. Dr. Hill was unable to sustain his role on the FAC and resigned his position in November 2023 for personal reasons.

Rob Holland, DVM, Ph.D., is a respiratory and infectious disease specialist in Lexington, Kentucky and partner at Holland Management Services, Inc., a consulting practice offering solutions in outcomes research and veterinary medicine. Dr. Holland has worked with the Kentucky Horse Racing Commission (KHRC) and has expertise in equine drug testing processes.

Heather Knych, DVM, Ph.D., DACVCP, is a Professor of Clinical Veterinary Pharmacology and Head of the Pharmacology Section at the K.L. Maddy Equine Analytical Chemistry Laboratory at the School of Veterinary Medicine at the University of California, Davis. Dr. Knych is board-

certified in Clinical Veterinary Pharmacology and has an extensive publication record in the areas of pharmacokinetics and pharmacodynamics. She received her DVM and Ph.D. in Pharmacology and Toxicology from the University of California, Davis, where she also conducted her residency in Veterinary Pharmacology. Dr. Knych is considered one of the foremost veterinarian pharmacologists and an expert in her field. She has also served on the Research Advisory Committee for the Grayson Jockey Club.

Ryan Murphy was the Executive Director for the Partnership for Clean Competition (PCC), the leading anti-doping research organization dedicated to the protection of clean athletes. Prior to joining the PCC, Murphy served as Program Officer with the Sports Diplomacy division at the U.S. Department of State and prior to that as Senior Manager for Sport & Competition for Special Olympics International. Murphy has also worked as an International Doping Control Officer for International Doping Tests & Management. In addition to his work at the PCC, Murphy serves as an Adjunct Professor for the Sports Industry Management Master's program at Georgetown University. Ryan moved positions in November 2024, and resigned his position on the FAC.

Foster Northrop, DVM, is a practicing racetrack veterinarian with more than 35 years of industry experience. He has served on the boards of the KHRC and the American Association of Equine Practitioners (AAEP), as well as the RMTC's Scientific Advisory Committee. Unfortunately, Dr. Northrop had to resign from the FAC in February 2025 for personal reasons.

Scott Palmer, VMD, DABVP, Equine Practice serves as the FAC Chair. Dr. Palmer is a former President of the AAEP and the American Board of Veterinary Practitioners (ABVP). He also chaired the New York Task Force for Racehorse Health and Safety from 2011 to 2013 and served as a clinician and referral surgeon at the New Jersey Equine Clinic for 38 years. He is currently a member of the RMTC's Scientific Advisory Committee, the Horseracing Testing Laboratory Committee, the Association of Racing Commissioners International's (ARCI) Drug Testing and Standards Committee and the ARCI's Equine Welfare and Veterinarians Committee. He is highly respected as the current Equine Medical Director for the New York State Gaming Commission.

N. Edward Robinson, BVetMed, Ph.D., is a nationally recognized veterinarian, academic and researcher who spent nearly 50 years at Michigan State University's College of Veterinary Medicine, during which he spent more than 20 years as the Matilda R. Wilson Professor of Large Animal Clinical Sciences. Dr. Robinson has spent his career researching lung disease in horses. He received his veterinary degree from the Royal Veterinary College in London and a Ph.D. from University of California, Davis. Unfortunately, Dr. Robinson resigned from the FAC in August 2023, for personal reasons.

Corinne Sweeney, DVM (HIWU Appointment), is an American College of Veterinary Internal Medicine (ACVIM) specialist and has spent the past 44 years at the University of Pennsylvania School of Veterinary Medicine. She has served as a member of the Pennsylvania State Horse Racing Commission since 2008 and previously served as Chair of the ARCI in 2019. She is a

certified Organizational Ombuds Practitioner and has served as the Penn Vet Ombuds since 2015. Throughout her career she performed clinical research and is well published in the area of EIPH.

Sarah White-Springer, Ph.D. holds the Link Endowed Chair in Equine Science and is an Associate Professor of Equine Physiology with a dual appointment in the Departments of Animal Science and Kinesiology & Sport Management at Texas A&M University. As an equine exercise physiologist, her research focuses on skeletal muscle health and adaptations to exercise and diet. Dr. White-Springer was the first to investigate skeletal muscle mitochondrial bioenergetics in horses in the United States and works closely with the racehorse industry to identify novel predictors of performance and fatigue. Dr White-Springer joined the FAC in January 2024.

HISA support for the FAC has been provided by Dr. Jennifer Durenberger, Dr. Shari Silverman, Samuel Reinhardt, Esq. and Niki Robb.

RELEVANT HISA STATUTE

Section 3055. Horseracing Anti-Doping and Medication Control Program

5(d) PROHIBITION. —Except as provided in subsections (e) and (f), the horseracing anti-doping and medication control program shall prohibit the administration of any prohibited or otherwise permitted substance to a covered horse within 48 hours of its next racing start, effective as of the program effective date.

(e) ADVISORY COMMITTEE STUDY AND REPORT. —

(1) IN GENERAL.—Not later than the program effective date, the Authority shall convene an advisory committee comprised of horseracing anti-doping and medication control industry experts, including a member designated by the anti-doping and medication control enforcement agency, to conduct a study on the use of furosemide on horses during the 48-hour period before the start of a race, including the effect of furosemide on equine health and the integrity of competition and any other matter the Authority considers appropriate.

(2) REPORT.—Not later than three years after the program effective date, the Authority shall direct the advisory committee convened under paragraph (1) to submit to the Authority a written report on the study conducted under that paragraph that includes recommended changes, if any, to the prohibition in subsection (d).

(3) MODIFICATION OF PROHIBITION.—

(A) IN GENERAL.—After receipt of the report required by paragraph (2), the Authority may, by unanimous vote of the Board of the Authority, modify the prohibition in subsection (d) and, notwithstanding subsection (f), any such modification shall apply to all States beginning on the date that is three years after the program effective date.

(B) CONDITION.—In order for a unanimous vote described in subparagraph (A) to effect a modification of the prohibition in subsection (d), the vote must include unanimous adoption of each of the following findings:

(i) That the modification is warranted.
(ii) That the modification is in the best interests of horse racing.
(iii) That furosemide has no performance enhancing effect on individual horses.
(iv) That public confidence in the integrity and safety of racing would not be adversely affected by the modification.

(f) EXEMPTION. —

(1) IN GENERAL. —Except as provided in paragraph (2), only during the three-year period beginning on the program effective date, a State racing commission may submit to the Authority, at such time and in such manner as the Authority may require, a request for an exemption from the prohibition in subsection (d) with respect to the use of furosemide on covered horses during such period.

(2) EXCEPTIONS. —An exemption under paragraph (1) may not be requested for—

(A) two-year-old covered horses; or

(B) covered horses competing in stakes races.

(3) CONTENTS OF REQUEST. —A request under paragraph (1) shall specify the applicable State racing commission's requested limitations on the use of furosemide that would apply to the State under the horseracing anti-doping and medication control program during such period. Such limitations shall be no less restrictive on the use and administration of furosemide than the restrictions set forth in State's laws and regulations in effect as of September 1, 2020.

(4) GRANT OF EXEMPTION. —Subject to subsection (e)(3), the Authority shall grant an exemption requested under paragraph (1) for the remainder of such period and shall allow the use of furosemide on covered horses in the applicable State, in accordance with the requested limitations.

WORK PRODUCT OF THE FUROSEMIDE ADVISORY COMMITTEE

The inaugural meeting of the FAC was held on July 12, 2023 via Zoom. The FAC met regularly over the past 3 years to execute its charge as defined in Section 6 of The Act. Organizational matters for the FAC included creation of a request for research proposals (RFP), the development of a rubric to evaluate and score those proposals for funding by HISA and a proposed timeline for FAC funding and deliverables:

First Disbursement:	January 31, 2024
Interim Report Due:	January 31, 2025
Second Disbursement:	January 31, 2025
Final Report due on or before:	January 31, 2026
Disbursement of final payment (last 20% of award):	February 28, 2026

On August 8, 2023, the FAC issued a request for proposals for targeted research on the administration of furosemide to racehorses during the 48-hour period before the start of a covered race (see **Appendix B**). In evaluating applications, the FAC paid particular attention to the impact of the proposed work as it applies to horseracing in North America. Up to $1,000,000 was proposed to be available each year for two years to fund projects. The deadline for research proposals was November 15, 2023.

To assist investigators in creating proposals that would specifically address the need of the HISA Board to make an informed decision regarding the potential modification of the furosemide ban currently in effect, the FAC provided the following examples of potential areas of investigation:

- What are the long-term economic implications for US Thoroughbred racing of prohibiting furosemide administration within 48 hours of a race (using metrics such as frequency of starts, career length, number of starts per year, age at retirement, and field size)?
- What are the effects of furosemide administration within 48 hours of racing on equine health and welfare?
- Does the use of furosemide within 48 hours of racing jeopardize racing's social license to operate?
- Would administration of furosemide within 48 hours of racing adversely affect public confidence in the integrity of horse racing?
- Does administration of furosemide within 48 hours of racing enhance racing performance?
- Is administration of furosemide alone or in combination with any other medication within 48 hours of racing associated with exercise-associated sudden death?
- Is the absence of furosemide administration within 48 hours of a race associated with exercise-related sudden death?
- What factors such as environmental conditions, stable management, pre-existing medical conditions, age, and race distance and/or frequency, and the use of furosemide and its repeated use within 48 hours of a race are associated with EIPH?

- What alternatives to administration of furosemide within 48 hours of racing effectively mitigate or reduce the prevalence of EIPH in Thoroughbred racehorses?
- What are the acute & chronic effects of EIPH on horse health and performance?
- What are the effects of chronic furosemide administration on the musculoskeletal system, nervous system, cardiovascular system, respiratory system, nervous system, and immune system?
- Does the administration of furosemide within 48 hours of racing mitigate the increase in severity of EIPH over time?

Eight research proposals were received by the FAC before the November 15, 2023 submission deadline. All proposals were reviewed by the FAC and scored according to the rubric described in **Appendix C**. On September 9, 2024 the FAC reported to the HISA Board and recommended funding of the following 3 research proposals (see **Appendix D**).

1) **Does Pre-Race Administration of Furosemide to Thoroughbred Racehorses Prolong Their Racing Careers?**
   Principal Investigator: Warwick Bayly, BVSc, PhD, DACVIM, Professor, Department of Veterinary Clinical Sciences, Washington State University.

2) **Effects of Repeated Furosemide Administration on Electrolyte Homeostasis and Bone Density in Healthy Adult Exercising Thoroughbreds.**
   Principal Investigator: SallyAnne L. DeNotta, DVM, PhD, DACVIM., Clinical Assistant Professor, Large Animal Medicine, University of Florida College of Veterinary Medicine.

3) **Examining Associations Between Furosemide Treatment & Racehorse Health and Welfare.**
   Principal Investigator: Amanda Waller, BSc, PhD, Research Scientist, Center for Clinical and Translational Research, Nationwide Children's Hospital, Ohio.

Subsequent to a review of the initial research proposals, it was apparent that none of the proposals would provide the scope of data required to meet all of the remit for the HISA Board. In particular, they did not specifically address (i) the purported performance enhancing effect of furosemide, nor (ii) the question of whether public confidence in the integrity and safety of racing would be adversely affected by the modification. In light of this analysis, the FAC recommended issuing another request for proposals to address this question and create a poll to answer the question of public confidence.

On July 30, 2024, a specific request for proposals to investigate the potential performance enhancing effect of furosemide was issued (see **Appendix E**).

Additionally, it was brought to the attention of the FAC that HISA staff was assembling a spreadsheet listing confirmed episodes of epistaxis (bleeding from one or both nostrils) as documented by regulatory veterinarians at HISA covered racetracks. As of September 2, 2024, 137 cases of epistaxis were reported. While no statistical analysis had been completed to date,

HISA staff noted that the percentage of racing-associated epistaxis in horses running in races in which furosemide was not permitted appeared to be disproportionate to the percentage of horses running in races where furosemide was permitted. That is, a higher number of horses exhibiting epistaxis were not administered pre-race furosemide. With this finding in mind, Dr. White-Springer agreed to work with HISA staff and one of her post-graduate students to statistically analyze these data to determine what risk factors might be identified as being associated with epistaxis in races under current furosemide regulation. An additional aim was to determine if there was a statistically significant difference in the incidence of epistaxis in horses in races in which furosemide was prohibited compared with those in which furosemide was permitted (see Boone study).

Six research proposals specifically aimed to answer the question "Is furosemide a performance enhancing drug" were received by the FAC before the October 15, 2024 submission deadline. All of these proposals were reviewed by the FAC and scored according to the rubric described in **Appendix C.** On February 25, 2024, the FAC reported to the HISA Board and recommended funding of the following research proposal:

Examining associations between furosemide treatment and racehorse performance.
Principal Investigator: Amanda Waller, BSc, PhD, Research Scientist, Center for Clinical and Translational Research, Nationwide Children's Hospital, Ohio.

HISA FUNDED RESEARCH STUDIES & CONCLUSIONS

Bayly: Does pre-race administration of furosemide to Thoroughbred racehorses prolong their racing careers?

Bayly W, Meyer J, Sanz M, Leguillette R., Cohen N., University of Washington College of Veterinary Medicine

Full final report in Appendix F.

***Objectives**:*

1. Do Thoroughbred racehorses in the lower quintile (the lowest of 5 equal groups of horses that received pre-race treatment with furosemide per lifetime start) have significantly fewer starts and shorter careers.

2. Do horses diagnosed with EIPH grades =3 or =4 have significantly fewer starts post-diagnosis than horses with EIPH grades < 3. [Higher EIPH scores indicate a greater degree of blood observed in the lower trachea on endoscopic exam]

This research evaluated the above-captioned objectives using 3 separate studies. This is a triangulation research approach wherein the findings of 3 studies with differing methods are used to test a single hypothesis. Common findings among 3 independent studies substantiate the veracity of the conclusions.

Study #1:

This study compared the effect of the proportion of a horse's starts in which it received pre-race furosemide on the longevity of its racing career defined as number of lifetime starts and career duration from 2011-2019 in the group of horses that had their first start in 2011.

Materials & Methods:

Jockey Club performance data on 17,451 Thoroughbreds from the 2008-2009 foaling cohorts that began racing in the United States in 2011 was examined to assess whether historical race day medication practices allowed inference regarding the impact of furosemide on career duration as the dataset contained baseline information on each individual horse and information on the individual races for each horse throughout their careers until the end of 2019, thus providing up to 9 years of follow-up data.

Statistical Analyses:

The evaluated outcome was career longevity measured as total career starts, with career termination as the event of interest. Horses still actively racing at the end of the observation period were right censored at their last observed start, as their ultimate career length could not be ascertained. Given their foaling years, horses were 10-11 years old by the end of 2019, and the number still racing was minimal.

The analyses used 3 models to assess how furosemide administration influenced career length: the Cox proportional hazard model, Kaplan-Meier survival curves, and a marginal structural model.

Results:

The models provided no meaningful reliable insight to achieving the aim of the study for the following reasons and are therefore not presented in detail.

Furosemide use in the 2011 cohort was effectively ubiquitous by the early stages of racing participation, leaving no valid untreated comparison group and no clinically meaningful variation in exposure. This limitation arose from historical medication practices themselves and could not be resolved through alternative modeling strategies due to fundamental limitations present in this dataset. 14,202 horses (81.4%) received furosemide in 100% of their starts and 2,767 horses (15.9%) received it in 1-99% of their starts. Only 2.8% of horses (482 of 17,451) never received furosemide during their careers.

The few horses that never received furosemide was not a random sample of untreated horses. They were predominantly horses that failed early in their careers, before trainers had the opportunity to place them on furosemide. 35.5% had only one career start. This created a severe selection bias. By the third career start, 97% of horses had received furosemide at least once. This near-universal adoption eliminated any meaningful comparison group.

In addition to the near universal adoption of furosemide early in horses' racing careers, the available data lacked critical clinical covariates that simultaneously influenced both furosemide use and career continuation. The most relevant unmeasured confounders were:

- Underlying musculoskeletal health: Horses with subclinical orthopedic issues may have been managed differently, affecting both medication decisions and career duration.
- Subclinical injury status: Early indicators of developing injuries were not captured in race records but likely heavily influenced trainer decisions about medication and racing continuation.
- Trainer-driven veterinary decision-making: Trainers' clinical judgment about when to use furosemide was informed by private information about a horse’s condition that was not available to investigators.
- Management intensity: The overall level of veterinary attention and preventive care a horse received likely affected both furosemide use patterns and career outcomes.

Conclusions:

1. Near universal furosemide use (97% by race 3) eliminated valid comparison groups. With only 2.8% of horses never receiving furosemide, there was insufficient variation to estimate treatment effects.
2. The 'Never Furosemide' group was systematically different from treated horses. With 35.5% one-and-done horses (vs. 4.3% in the Always Furosemide group), this

comparison group consisted largely of early failures who never had the opportunity to be treated with furosemide.

3. These limitations cannot be overcome with statistical adjustments. The problems were structural, not analytical as a valid “no furosemide” comparison group essentially did not exist.
4. Investigators were unable to determine whether regular pre-race or pre-breeze treatment with furosemide did or did not have a protective effect on horses’ health and promoted career longevity.

Study #2:

The second study compared the effect of pre-race furosemide administration on the number of race starts made in the first 4.5 years of racing careers of horses that had their first start in 2015 (before HISA regulation) and those that had their first start in 2021 (after HISA regulation).

Materials & Methods:

Two distinct but related causal questions were asked:

1. Did the 2021 furosemide ban harm the career longevity of 2-year-old horses exposed to it, based on total race starts up to August 31, 2025? This question related to the effect of the policy. The availability of cohorts of contemporaneous control groups (2-year-olds that received furosemide and 3-year-old first-time starters who were never subject to the ban in 2021) facilitated comparisons that provided the answer to this question.
2. Did denying furosemide to a horse causally affect its career length? This question pertained to the effect of the drug; ie, did furosemide use itself (rather than factors correlated with its use) extend racing careers. Although the Jockey Club data anonymized tracks, the tracks that banned furosemide were identified by determining 2-year-old furosemide usage at the anonymized track. If this usage was 0%, the track was identified as a “ban track”.

Statistical Analyses:

The question related to the effect of the policy banning furosemide administration to 2-year-olds (ie, did the policy banning furosemide administration to 2-year-olds do any harm?) was answered using a Difference-in-Differences (DiD) analysis by comparing treated horses (2-year-old starters) to contemporaneous controls (3-year-old first-time starters) who were never subjected to the ban. The 3-year-old first-time starters served as an internal control group. They raced under identical industry conditions, with the same trainers, for the same purses, with same pandemic-era disruptions, except for one key difference: they were never subject to the 2-year-old furosemide ban. This structure sharply limited statistical confounding and enabled the policy effect to be isolated.

To answer the question regarding the drug effect, ie, whether furosemide itself (rather than factors correlated with its use) extended racing careers, an Instrumental Variables (IV) used track ban status as an instrument that generated exogenous variations in furosemide exposure. This was possible because the partial ban on furosemide also created variation across tracks: at

ban tracks, furosemide was prohibited for 100% of 2-year-old races; at non-ban tracks, it was permitted and used in approximately 78% of races. This variation formed the basis of the IV analysis.

Additionally, comparable data was available for 2- and 3-year-olds that had their first race starts in 2015 when there was no ban of any type on the pre-race administration of furosemide. Horses in the 2015 cohort were followed until December 31, 2019. Those in the 2021 cohort were tracked through August 31, 2025, providing up to 4 years of post-exposure follow-up when the horses from each cohort that were still racing would have been 6 or 7 years old, depending on their ages in 2015 or 2021.

Results:

Across both cohorts, horses debuting at age 2 consistently accumulated more lifetime starts than those debuting at age 3. This gap likely reflected opportunity and, possibly, biology; i.e.. an earlier debut provided more time to race and biological factors that might have delayed the start of a horse's racing career until it was 3 years old may have also impacted the longevity of that career.

The gap between 2-year-old and 3-year-old starters narrowed by only 0.27 starts, a small, negative change that was not statistically significant. If banning furosemide for 2-year-olds materially harmed durability, the gap should have narrowed substantially as 2-year-old careers shortened relative to those of 3-year-olds. This did not occur ($p = 0.41$).

The first phase of the IV analysis examined whether the instrument (ban-track status) actually affected treatment (furosemide denial) and found that racing at a ban track increased the probability of furosemide denial by approximately 78 percentage points.

When trainer fixed effects were considered, there was a suggestion that the ban on furosemide may have actually increased career starts by +1.04. However, the 95% Confidence interval was -0.85 to +2.93 with $p = 0.28$, indicating that this was not a significant finding.

Conclusions:

Prohibiting routine race day furosemide administration to 2-year-olds did not decrease their racing careers as it did not have a substantial effect on the number of career starts over a horse's first five years of racing. There was no significant change in the difference in career starts of 2- and 3-year-olds first-time starts in the 2015 and 2021 cohorts. The persistent advantage of 2-year-old starters over 3-year-old first-time starters in terms of lifetime starts reflected debut timing and opportunity, not pharmacological protection.

Study #3

The third study assessed the impact of EIPH severity on the number of subsequent race starts and career duration through August 31, 2025 (a period of 12-18 months) of all horses that were endoscopically examined after racing in the period from February 15 – August 31, 2024.

Materials & Methods:
2,561 video recordings of post-race tracheoendoscopic veterinary examinations of Thoroughbred racehorses were evaluated by three veterinarians for evidence of EIPH, and its severity graded on a scale of 0 - 4 using whole numbers only, with Grade = 4 representing EIPH of greatest severity and a Grade = 0 indicating that there was no visual evidence of EIPH. Horses were classified by their maximum (worst) EIPH grade observed across all endoscopic examinations, not necessarily their first observation. This ensured classification by the true severity ceiling.

The relationship between maximum EIPH grade and a) periods between races and b) declines in class of races, was analyzed for the period between the date the maximum grade was assigned and Augst 31, 2025. Data from 22,873 races were evaluated. Absence durations were calculated from consecutive race dates within each horse's record.

Statistical Analysis:
To assess the relationship between the most severe EIPH grade and the number of subsequent race starts, the Poisson regression was used to model racing frequency with an offset for follow-up time (log-transformed years), yielding incidence rate ratios (IRR) comparing each EIPH group to the reference (Grade 0 - 1). Age at scoping was included as a covariate.

To examine whether EIPH severity, measured by consensus endoscopic grading, was associated with prolonged absence from racing, the outcome variables (>90 days or >120 days to next start) were depicted as binary indicators for whether the horse was absent >90 or >120 days before their next recorded start. Horses in the >120 days cohort were also included in the > 90 days group. Absence variables were calculated from consecutive race dates within each horse's record. Horses with no subsequent race in the dataset were coded as missing, leaving 20,863 race-level observations for prolonged absence analyses. The chi-square (χ^2) test or Fisher's exact test when expected counts < 5 were used with $p < 0.05$ considered significant. The association between EIPH severity grade and whether the class of race in which a horse competed was also assessed as a negative relationship between the 2 could be interpreted as a negative effect of severe EIPH. Sustained class decline was defined as a drop in race class level from which the horse never recovered and represented functional career deterioration while the horse was still actively racing. Race classes were assigned the following: stakes = 5, allowance/AOC = 4, maiden special weight = 3, claiming = 2, maiden claiming = 1. A class drop was considered "permanent" if the horse never subsequently raced at the prior class level or higher. Only the first permanent drop was counted as the event.

Results:
Fifty-three veterinarians provided 2,460 videos of sufficient quality from 2,010 horses for consensus EIPH Grades to be determined. Fifty-six horses (2.7%) were reclassified to higher severity groups compared to using their first recorded grade. The mean follow-up performance period was 468 days.

Career Starts

Higher EIPH severity was associated with reduced post-scoping racing frequency. After adjusting for age at scoping, Grade 2 horses raced 9% less frequently than Grade 0-1 horses and Grade 3-4 horses raced 15% less frequently. The most severe bleeders (Grade 4) averaged 6.5 starts per year compared to 8.0 for Grade 0 horses — a 19% reduction. Each additional year of age was associated with a 7% increase in racing frequency. Unlike analyses of single-race performance or binary retirement outcomes, which showed non-monotonic patterns (see below), racing frequency demonstrated a clear severity gradient, suggesting this metric may best capture the cumulative career impact of EIPH on lifetime starts by a horse and its career duration.

Career Duration

Absences of >90 and >120 days, respectively, from racing had no association with EIPH grade of severity. Despite excellent statistical power (>20,000 observations), absence rates for both periods were virtually identical across all EIPH severity levels (5.45 ± 0.2% for >90 days; $p = 0.91$; 3.63 ± 0.4% for >120 days; $p = 0.10$).

Class Drop

EIPH severity showed no association with a sustained drop in the class of races that horses competed in regardless of their EIPH grade where drop rates were virtually identical or their age.

Limitations of this study:

1. Single EIPH measurement: Horses were assigned a consensus grade from one scoping event. EIPH severity fluctuates between races, so this may not have captured a horse's true bleeding tendency.
2. Absence as a proxy: Prolonged absence may reflect many factors (training decisions, minor injuries, scheduling) beyond EIPH-related pathology.
3. Censoring: Horses with no subsequent race following endoscopy (n = 2,010) were excluded from absence analyses. If horses with EIPH grades ≥ 3 were more likely to be retired, this could bias the results.
4. Confounding: This analysis did not adjust for potential confounders such as age, trainer, track surface, or medication status.

Conclusion:

EIPH severity was clearly associated with racing frequency but not with prolonged (> 90 days or >120 days) absences from racing, or with sustained class decline which was used as an objective measure of competitive performance trajectory. These findings suggested that while EIPH did not appear to impair racing career outcomes when measured by some functional performance metrics, horses with severe EIPH raced less often than horses with either no or only mild endoscopic EIPH grades.

DeNotta: Effects of repeated furosemide administration on electrolyte homeostasis and bone density in healthy adult exercising Thoroughbreds.

Sally DeNotta, DVM, PhD, DACVIM; University of Florida College of Veterinary Medicine.

Full final report in **Appendix G**.

Objectives:

1. Examine how long-term furosemide treatment affects extracellular and intracellular electrolyte balance, parathyroid and calcium homeostasis, and urinary electrolyte excretion.
2. Determine if horses receiving serial doses of furosemide develop pharmacologic tolerance and reduced physiologic response to treatment.
3. Examine the effects of repeated administration of furosemide on bone density and strength using minimally invasive methods of measurement DEXA scan and Bonescore® OsteoProbe® microindentation.
4. Evaluate the effects of serial dosing with furosemide on cardiac function before, during, and after exercise.

Materials & Methods:

This was a prospective, blinded, placebo-controlled study in which 9 horses were administered 250 mg (5 mL) intravenous furosemide weekly for 14 weeks (treatment), while 6 horses were administered a similar volume of weekly saline placebo (control) (Figure 1). Horses were matched by bodyweight and randomly allocated to groups, and study personnel were blinded to group identification throughout the study. Study horses were trained to run on an EquiGym high-speed equine treadmill and maintained fitness throughout the study with 3x/week exercise sessions. All horses were group housed in outdoor paddocks at the University of Florida College of Veterinary Medicine throughout the study, and all were fed the same diet consisting of free choice grass hay, commercial pelleted equine feed, and maintenance dose oral vitamin E supplementation.

All horses were screened and confirmed to be negative for bisphosphonates by the UK Equine Analytical Chemistry

Blood and urine samples were serially collected throughout the study, and samples were processed and stored in a -80C laboratory freezer for batch analysis upon study completion. Electrolyte assays were performed in the UF College of Veterinary Medicine Clinical Pathology Lab, while serum PTH and iCa assays were performed at the Michigan State Veterinary Diagnostic Lab. Horse hydration status was assessed via serial body weight measurements as well as packed cell volumes (PCV) and urinary specific gravity (USG). At three timepoints (pre-study, mid-study, post-study), horses underwent telemetric ECG evaluation to assess for any underlying cardiac arrhythmias.

To evaluate bone density, DEXA scans were performed immediately before and immediately after study completion. To evaluate bone strength, BoneScore® Osteoprobe® micro-indentation

was performed immediately before and after study completion. This novel method for bone strength evaluation is new to the equine veterinary realm, and this study is the first to allow for comparison between traditional DEXA scan bone density and micro-indentation mechanical bone strength.

Following completion of the study outlined above, an additional study period added to further investigate Aim 2 and the common anecdotal observation that horses develop a tolerance for furosemide after repeated doses, i.e., the medication has less profound physiologic effects in horses that have received the medication previously. To test this hypothesis, body weight, urine and blood samples were collected for PCV, USG, electrolyte analysis, and urinary fractional excretions from all study horses on day 100 (9 horses that had received furosemide weekly for 14 weeks and 6 horses that had received only placebo), after which all 15 horses were administered 250 mg (5 mL) of intravenous furosemide. Blood was collected at 7 and 24 hours for electrolyte analysis. Body weight, blood, and urine samples were then obtained 48 hours after furosemide administration, and the response to treatment was compared between the two groups.

Results:

1. Weekly administration of 250 mg (5 mL Salix®) of intravenous furosemide for 14 weeks in treadmill exercised Thoroughbreds (3 – 5 years of age) in this study did not affect long term hydration status, electrolyte homeostasis, or bone strength using the above-described techniques during the study period.
2. No differences in heart rate before, during, or after exercise were observed between groups.
3. Cardiac arrythmias were not detected with greater frequency in treated horses when compared with controls under study conditions.
4. Horses administered weekly doses of furosemide at 250 mg intravenously did not demonstrate a reduced physiologic response when compared to horses that had received placebo over the 14-week study period.

Conclusions:

1. Overall, the results of this study suggest that weekly administration of 250 mg of intravenous furosemide to young exercising Thoroughbreds for 14 weeks does not affect long term hydration status, electrolyte homeostasis, or bone strength.
2. The physiologic response to repeated furosemide treatment did not decrease over time.

White-Springer: Effects of repeated furosemide administration on basal mitochondrial function and energy production capacity of the gluteus medius muscle.

Sarah White-Springer, Departments of Animal Science and Kinesiology and Sport Management, Texas A&M University, College Station, TX

Full final report in **Appendix H**.

Objectives:

This study was conducted as an add-on to Dr. DeNotta's study and aimed to determine if repeated furosemide administration impacted the horse's ability to generate energy in a highly propulsive hindlimb muscle, the gluteus medius.

Materials & Methods:

As detailed above, mature Thoroughbred horses were matched by bodyweight and randomly allocated to be administered either 250 mg (5 mL) intravenous furosemide (5 mares, 4 geldings) or a similar volume of saline (3 mares, 3 geldings) weekly for 90 days. Study personnel remained blinded to group identification throughout the study and data analysis. All horses were trained to run on an EquiGym high-speed equine treadmill and maintained fitness throughout the study with 3x/week exercise sessions. All horses were group housed in outdoor paddocks at the University of Florida College of Veterinary Medicine throughout the study, and all were fed the same diet consisting of free choice grass hay, commercial pelleted equine feed, and maintenance dose oral vitamin E supplementation.

After 90 days of treatment, muscle samples were collected from the gluteus medius muscle in the proximal portion of the left hindlimb. Tissue was aseptically collected using a minimally invasive 12-gauge tissue collection needle. Muscle samples were flash-frozen in liquid nitrogen and stored at -80°C or stored at 4°C until being analyzed for live-cell mitochondrial capacities within 24 hours following collection.

Frozen muscle samples were analyzed for activity of the mitochondrial enzyme, citrate synthase (CS) by kinetic colorimetry as a proxy for mitochondrial volume density. Additionally, permeabilized fibers were analyzed for mitochondrial oxidative phosphorylation and electron transfer capacities by high-resolution respirometry, and production of ATP was quantified in mitochondria isolated from the same sample by high-resolution fluororespirometry.

Results:

1. Ninety days of weekly furosemide administration did not alter mitochondrial respiratory or ATP production capacities, nor did the utilization of complexes of electron transfer differ between horses receiving furosemide and those that did not.
2. Repeated furosemide reduced mitochondrial efficiency of ATP production.

Conclusions:

Repeated furosemide administration did not alter mitochondrial oxygen consumption capacity or the maximal ability to produce ATP, key determinants of a horse's aerobic performance

capacity. However, furosemide did reduce mitochondrial efficiency of ATP production, meaning more cellular input was required to generate the same amount of energy. This decreased efficiency demonstrates that repeated furosemide does not enhance performance through improved basal cellular energy metabolism.

Johnson: Examination of the effect of chronic furosemide administration on gene transcription in the gluteus medius muscle of Thoroughbred Racehorses

Sally Johnson and Giovanna Lungu, School of Animal Sciences, Virginia Tech, Blacksburg VA

Full final report in **Appendix I**.

Objectives:

This study was an additional add-on to Dr. DeNotta's study and exploited the muscle samples collected by Dr. White-Springer. The objective was to determine if repealed furosemide affects gene transcription to enhance muscle recovery following exercise.

Materials & Methods:

Training causes muscle adaptions that include altered gene expression to meet the metabolic demands for increased shortening velocity. The ability of furosemide administration throughout the training program to affect gene transcription was examined by RNA sequencing. Gluteus medius biopsies (~100 mg) were harvested from racing age Thoroughbreds (4.0 ± 0.17 yr) administered saline (CON, n = 6; 3 females, 3 males) or furosemide (n = 9, 5 female, 4 male) as described above.

For details of experimental design, please refer to DeNotta et al, "Effects of repeated furosemide administration on electrolyte homeostasis and bone density in healthy adult exercising Thoroughbreds." Total RNA was isolated using TRIzol coupled with spin-column purification, as described previously. Total RNA (RIN > 8.0) was used for polyA mRNA isolation and cDNA library construction. Adapters and low-quality base reads were removed from the raw sequencing reads using fastp (v0.24.2). The trimmed reads were aligned to the reference genome (EquCab 3.0, GCA_002863925.1) using the STAR aligner (v2.7.11). Quantification was performed using Salmon (v1.10.2) at the transcript level. The Rtximport package (v1.30.0) was utilized to import the transcript-level quantification results and to summarize the reads at the gene level. Statistical computation was performed using the Omics Playground (v 3.5.0). Gene-level testing for identification of differences was performed with DESeq2 (Wald test), edge R (QLFtest), and limma-trend. The Benjamini–Hochberg corrected false discovery rate of $q < 0.05$ was used for all bioinformatic analyses.

Results:

The 15 samples were sequenced at an average depth of 20.8 ± 1.9 M reads with exon transcripts representing 79.5 ± 0.22% of the total. Intron reads comprised 14.1 ± 0.18% and intergenic reads represented 6.4 ± 0.10% of the total. Pearson correlation analysis of the sequencing reads (transcripts per million, TPM) ranged from 0.97-1.0 indicating strong similarity amongst the samples. Alignment to the Equus caballus genome demonstrated a total of 12,756 transcripts were expressed within the muscle that fell into 4 major categories with approximately 19% classified as cytochrome family genes. Using the conventional false discovery rate (FDR) of $q < 0.05$, no differences in expression were noted between saline and furosemide treated horses. Reducing the stringency of the test to $q < 0.10$ also produced no measurable differences between treated and control horse skeletal muscle transcriptomes.

Clustering the data into gene sets prior to analysis (GSEA, Fishers exact test) yielded no differences between control and furosemide treatment. Thus, cursory investigation of fit, unexercised Thoroughbred skeletal muscle demonstrates that furosemide has no effect on basal gene expression. The integrity of the dataset was assessed by examining sex-specific gene expression (DESeq2, edgeR) at $q < 0.05$. Results demonstrate that eukaryotic translation initiation factor 2 subunit 3, Y-linked-like (LOC100066299) was expressed exclusively in males by comparison to females, and LOC102150010, an uncharacterized gene carried on the X chromosome, was greater in females than males. Thus, the libraries can identify differentially expressed genes. In addition to these sex chromosome genes, modest differences ($\log2 FC > 0.5$) in FKBP prolyl isomerase 5 (FKBP5), malonlyl CoA decarboxylase (MLYCD) and glutamate decarboxylase like 1(GADL1) were noted.

Conclusions:

Using multiple statistical methods, no differences in gene expression in gluteus medius (rump) muscle as a function of repeated furosemide administration were detected. These results provide supportive evidence that repeated use of the drug did not offer an advantage regarding basal muscle function as measured by gene expression of racing-age Thoroughbreds.

Waller: Examining associations between furosemide treatment and racehorse health and safety.

Amanda Waller, PhD, The Research Institute at Nationwide Children's Hospital

Full final report in **Appendix J**.

Objectives:

Recent retrospective data from the U.S. Thoroughbred racehorse population was analyzed to gain insights towards answering the following questions:

1. What are the effects of race day furosemide administration (absence or presence) on the health and welfare of Thoroughbreds racing in the USA? (Aim #1)
2. Is the presence or absence of race day furosemide administration associated with exercise-related sudden death? (Aim #1).
3. Is the presence or absence of race day furosemide administration associated with an increased risk of race-related fatal musculoskeletal injury? (Aim #1)
4. What are the long-term social and economic impacts of prohibiting race day furosemide administration to the U.S. Thoroughbred racing industry (using metrics such as frequency of starts, career length, career earnings, average field size, etc)? (Aim #2)
5. Does the absence of early-career furosemide administration negatively affect overall career performance and longevity? (Aim #2)
6. What is the effect of race day furosemide administration on racing performance? (Aim #3)
7. Does race day furosemide treatment adversely affect the integrity of competition? (Aim #3)

Aim #1 – Racing Related Injuries & fatalities

Experimental Methods:

The present study used a retrospective observational cohort analysis to assess the association between prerace administration of furosemide and i) fatal musculoskeletal injury, or ii) exercise-associated sudden death, in two-year old Thoroughbreds that raced in the U.S. from 2020-2024. Importantly, by holding age constant between the two cohorts which raced with and without furosemide, and confining the analysis to the immediate time period before and after the widespread furosemide ban was enacted, the influence of confounding variables that plagued previous studies (ex: age and trainer) were vastly mitigated.

The study cohort consisted of all starts in 2-year-old Thoroughbred races in the United States from 2020 through 2024. Data on fatal injuries were taken from a publicly available database previously shown to have excellent accuracy with the Equine Injury Database (von Hippel, 2019). Once relevant fatalities were identified from the database and categorized according to type, further confirmation of the fatal injury and information on furosemide use during the fatality race was extracted from another publicly available database (Equibase Company LLC, 2025). The outcome variable of interest was racing-associated fatal injury due to musculoskeletal injury or sudden death. For musculoskeletal, the terms for inclusion were any racing associated

catastrophic injuries resulting from fractures or tendon/ligament injury or head/cervical trauma which resulted in death or euthanasia within three days of the race. For sudden death, racing-associated fatality descriptions included the terms sudden death, pulmonary hemorrhage, EIPH, post-exertional distress, collapsed and died, suspected cardiac, etc. Race-related sudden death is defined by cases of sudden death that occur during exercise or within one hour post-race (Lyle et al 2012, Diab et al 2019).

Statistical Analysis

Statistical analysis and graphing were performed using SigmaPlot 12.0 and GraphPad Prism 10 software packages. Chi-squared test was used to test associations between the incidence of racing-related fatality and race-day furosemide treatment, with statistical significance established as $p<0.05$ with a 95% confidence interval. A priori power analysis was conducted using SigmaPlot prior to study commencement to determine the required sample size to detect a projected odds ratio of 0.5 (without/with furosemide; reciprocal= 2.0) for the association between race day furosemide treatment and racing-related fatality, based on a presumed baseline musculoskeletal and sudden death fatality rate prevalence of 1.60 and 0.13, respectively (Hitchens et al 2019, Bennet & Parkin 2022), an alpha of 0.05, and a power of 80%, and using a two-sided chi-square test. A minimum sample size of 19,375 and 123,077 race starts per furosemide group was calculated to be necessary for the musculoskeletal and sudden death outcomes of interest, respectively.

Results:

This study cohort which spanned the years 2020 through 2024 consisted of N = 25,326 and 78,524 total race starts for horses treated with and without furosemide, respectively.

1. Pre-race administration of furosemide was not significantly associated with the risk of racing-related musculoskeletal fatality, sudden death, or all-cause racing-related fatalities per 1000 starts.

Given the lower overall incidence of sudden death compared to musculoskeletal fatalities, the retrospective study period was further extended to a seven-year time frame that encompassed multiple racing seasons both before and after the widespread furosemide ban (2018 through 2024). The final seven-year study cohort consisted of N= 65,770 and 82,889 total race starts for horses treated with and without furosemide, respectively.

2. Pre-race administration of furosemide was not significantly associated with the risk of racing-related musculoskeletal fatality, racing-related sudden death, or all-cause racing related fatality.

Since furosemide treatment also has the potential to affect non-fatal health and safety outcomes, statistical analysis of an additional dataset was performed. The inclusion criteria for this dataset was all starts in graded stakes races for Thoroughbreds racing in the U.S during the study time period. The predictor variable was presence/absence of race day furosemide treatment. The outcome variable of interest was horse related Did Not Finish (DNF) incidents. DNFs attributed to non-horse factors (jockey falls, equipment failures, gate issues, etc) were excluded.

3. The incidence of a horse not finishing in a Graded Stakes race was numerically higher for horses racing without race day furosemide, for both the Pre- and Post-ban time periods studied.
    a. There was a tendency towards this becoming significant for the time period after the furosemide ban however the analysis was underpowered (p = 0.073, power 67.9%).
4. When the data was combined into all U.S. Graded Stakes races taking place from 2017-2024, the number of horse-related DNF incidents per 1000 starts was 4.84 and 5.35 for horses competing with/without race day furosemide, respectively (p = 0.550; power 53.4%).
5. In 2-year-olds specifically, the incidence of a horse not finishing in a race was again numerically higher for horses racing without race day furosemide, for both the Pre- and Post-ban time periods studied.
    a. There was a tendency for this difference to become significant for the time period after the furosemide ban (p = 0.067).
6. When all 2-year-old starts in the U.S. that took place from 2017-2021 are compared, the risk of a horse not finishing a race as a 2-year-old was not significantly different for horses racing without race day furosemide (p = 0.123).

Aim #2 - Furosemide & Career Longevity

Experimental Methods:

Individual level comparison

A retrospective observational case control study was employed to compare key metrics of career performance in Thoroughbreds that raced exclusively on furosemide as 2-year-olds, versus a matched cohort of controls that did not receive furosemide for any races during their 2-year-old year. Specific dataset inclusion criteria encompassed Thoroughbreds racing in the USA with three or more starts during their 2-year-old season (2020), at least one of which was a Listed Stakes or Black-Type class of race. Horses in the “Yes” cohort received race day furosemide treatment for all their 2-year-old starts. Horses in the “No” cohort were matched controls such that they were direct competitors to the Yes group horses in at least one Listed Stakes/Black-Type race in their 2-year-old season, and all their 2-year-old starts were conducted without race day furosemide treatment. Thus, matched pairs were matches in racing age, sex, and racing class. Outcome variables of interest included lifetime earnings, career length, lifetime starts, top 3 placings and average lifetime speed figures. Information on furosemide use during each race and measures of racing career performance were extracted from a publicly available database (Equibase Company LLC, 2025).

Group level comparison

To further investigate whether race day furosemide treatment is associated with career longevity and race participation, an additional group-level analysis of was performed. The cohort consisted of 2-year-old Thoroughbred racehorses racing in the North America between 1992 and 2025. Outcome variables of interest were A) 2-year-old starts per total foal crop, B) 2-year-

old average starts per horse, and C) 2-year-old starters per total foal crop. A linear regression analysis for each outcome variable vs time was performed to identify the industry trends over time. The outcome values for the most recent years since the furosemide ban were compared to the overall trendline to assess the ban's overall effects to date. Annual racing data from the five years immediately preceding the widespread furosemide ban in 2-year-old races (2015 to 2019) was compared to post-ban seasons (2021 to 2024). The data for annual 2-year-old racing parameters were extracted from a publicly available database (The Jockey Club, 2025), and normalized to each year's respective annual foal crop.

Statistical Analysis

Statistical analysis and graphing were performed using SigmaPlot 12.0 and GraphPad Prism 10 software packages. Linear regression analysis was used to assess trends in two-year old races over time. Welch's t-test or Wilcoxon signed-rank test were used to probe for differences in career metrics between furosemide cohorts. Statistical significance was established as $p<0.05$ with a 95% CI. A priori power analysis estimated a minimum sample size of N=18 horses/matched cohort was required to achieve adequate statistical power (0.80 with alpha= 0.05).

Results:

1. After controlling for age, sex, and racing class level, early career furosemide administration was not associated with outcomes relating to overall racing career performance and longevity.

To study the effects of the furosemide ban in 2-year-old races at the group population level, the annual racing statistics for 2-year-old North American Thoroughbred racehorses racing from 1992 through 2024 were normalized to each year's respective foal crop data, and linear regression analysis was performed to show the industry trend over time.

2. Apart from the anomalous COVID-19 season of 2020, the overall state of 2-year-old racing have been at or above the trendline since the furosemide ban.
3. Excluding the outlier Covid-19 year of 2020, the five-year time periods immediately before (Pre) and after (Post) the furosemide ban were compared:
    a. Rates of 2-year-old starters were higher in the Post-ban group.
    b. Relative to annual foal crop, the number of 2-year-old starts and starters were higher after the furosemide ban ($p = 0.03$ and $P<0.001$, respectively).
    c. The average starts/horse was decreased post-ban ($p = 0.002$).

<u>Aim #3 – Furosemide & Racing Performance</u>

Experimental Methods:

Group level comparison

A retrospective observational cohort analysis was employed to assess the relationship between prerace administration of furosemide and racing performance in a large, controlled population of

U.S. Thoroughbred racehorses. The dataset consisted of all American Graded Stakes races which were run every year over a consecutive five-year period from 2019-2023. Graded Stakes that were not run every year or not run under an identical format year-over-year were excluded. Importantly, the influence of confounding variables was vastly mitigated by limiting the inclusion criteria for the study population of interest to graded stakes races that took place annually, both immediately before and after a widespread ban on furosemide was initiated. Information on furosemide use during the race and measures of racing performance were extracted from a publicly available database (Equibase Company LLC, 2025). The official winning Equibase® Speed Figure was chosen as the primary outcome variable

Individual level comparison

A retrospective, observational, case-crossover study design was used to assess the effect of prerace administration of furosemide on racing performance in individual Thoroughbred racehorses competing in North America from 2020-2024. Information on furosemide use during a race and measures of racing performance were extracted from a publicly available database (Equibase Company LLC, 2025). To reduce variation in performance outcomes due to extrinsic and intrinsic factors, statistical performance comparisons was limited to pairs of On/Off furosemide races run at identical distances, track surfaces, and racing class, under fast/good conditions, and occurring within the same racing season (same racing age /calendar year) and quarter. A random cross-section of individual horses was screened for matched pairs of completed races On/Off race day furosemide that meet the specific race-inclusion criteria. The outcome variables of interest for each race were the horse's official Speed Figure for that race, along with the placing/finishing position, and percentage of the field beaten.

Statistical Analysis:

Statistical analysis and graphing were performed using SigmaPlot 12.0 and GraphPad Prism 10 software packages. One-way analysis of variance (ANOVA) was used to compare year-over-year race performance outcomes and the treatment effect of furosemide administration. Paired t-test was used to compare individual race performance outcomes and furosemide treatment effect (presence or absence of race day administration). Shapiro- Wilk and Brown-Forsythe tests were used to confirm normality and equal variance, respectively. Statistical significance was established as $p<0.05$ with a 95% confidence interval (two-tailed).

At the group level comparison, a priori statistical power calculation using the 2017-2019 graded stakes race data (ie; prior to/absent from any year-over-year modifications in furosemide regulatory status) determined a minimum sample size of n= 20 races per group was needed in order to detect a 1% difference in the race winning Speed Figure with vs without permitted race day furosemide treatment, at a 5% level of significance (two sided) and with 80% power, and assuming an average standard deviation of 2 speed figure points. At the individual level comparison, a priori power calculation determined a minimum sample size of n= 30 races per group was needed to detect a 5% difference in the means of the race performance outcome variables of interest.

Results:

To control for racing age and the different time frames of enacting the furosemide ban, the extracted data was separated into four distinct cohorts for subsequent statistical analysis: A) Two-year-old Graded Stakes races, which enacted the furosemide ban starting in 2020. Three-year-olds and upwards Graded Stakes races were separated by year of eventual furosemide ban into B) races which enacted the ban starting in 2021, C) races which enacted the ban in 2023, and D) the races which had no ban and continued to permit race day furosemide through 2023.

1. There were no significant differences in racing performance in Graded Stakes races conducted before vs after permitted race day furosemide, in any cohort group, for any year-over-year period studied.

Finally, a retrospective, observational, case-crossover study design was employed to assess the effects of prerace administration of furosemide on racing performance at the individual horse level. A random cross-section of individual horses was screened for matched (race surface, distance, racing class, racing age and season) pairs of completed races On/Off furosemide. Importantly, the use of the case- crossover design and tightly controlled race matching strategy ensured that the influence of potential confounding variables was minimized.

2. There were no significant differences in racing performance in Graded Stakes races conducted before vs after permitted race day furosemide, in any cohort group, for any year-over-year period studied.
3. When the race performance of individual race horses was compared over matching pairs of races in which they competed with vs without race day furosemide treatment, there was no significant association between a horse receiving race day furosemide and the concomitant speed figure ($p = 0.670$), finishing position ($p = 0.130$), or percentage of the field beaten achieved in that race ($p = 0.340$).

Conclusions:

1. In a large, retrospective cohort of two-year-old Thoroughbreds racing in the U.S., neither the presence nor absence of race day furosemide treatment was associated with risk of racing-related sudden death or musculoskeletal fatality. However, there was a tendency for an increased relative risk of not finishing in a race for 2-year-olds that did not receive pre-race furosemide.
2. In a retrospective observational case control cohort comparing U.S. thoroughbreds that raced exclusively on furosemide as 2-year-olds versus a matched cohort of controls that did not receive furosemide for any races during their 2-year old year, neither the presence nor absence of race day furosemide showed an association with lifetime starts, racing career length, or additional key metrics of overall career performance and longevity.
3. Relative to annual foal crop, the number of 2-year-old starts and starters were higher after the furosemide ban was enacted.
4. In a retrospective cohort of racehorses competing in North American Thoroughbred Graded Stakes during the multi-year time period directly before and after the widespread

furosemide ban was enacted, race-day furosemide treatment did not affect racing performance as measured by year-to-year winning Speed Figures.

5. Using a tightly controlled race matching strategy, a retrospective observational case-crossover study of individual Thoroughbred racehorses competing in North America from 2020-2024 revealed no significant association of pre-race furosemide treatment with race performance outcomes (Speed Figure, finishing position, & percentage of the field beaten).

FAC INTERNAL RESEARCH STUDIES & CONCLUSIONS

Boone: Risk Factors for Epistaxis Incidence in United States Thoroughbred Racehorses in 2024

Alana Boone, Sarah White-Springer, PhD; Texas A&M University, Shari Silverman, HISA

Full final report in **Appendix K**.

Objective:

1. Apply statistical analyses and modeling techniques to evaluate the relationships between external variables and the likelihood of epistaxis occurrence with assistance from artificial intelligence in conjunction with machine learning.

Materials & Methods:

The dataset for this study included epistaxis cases documented at Horse Integrity and Safety Authority (HISA) regulated racetracks during the 2024 racing season. Horses presenting with epistaxis, unrelated to physical trauma, were identified via post-race examinations by regulatory veterinarians. Respective information was collected for each confirmed epistaxis case and corresponding data were collected for all other horses participating in the same race to be used as contemporary control horses. The complete dataset included 202 documented epistaxis cases and 1,286 non-epistaxis cases, yielding a total of 1,488 Thoroughbred horses.

A range of variables potentially associated with epistaxis were collected, including racetrack information, race history, race information, environmental information, and horse history. Racetrack variables included track, surface, distance, and condition. Race history variables included days since last race, previous epistaxis incidences, previous unsound incidences, lifetime starts, lifetime earnings, and furosemide (Lasix) use from the previous race. Race information variables include trainer, time of race, race number, off odds, type of race, finish position, purse, Lasix use, and blinkers. Environmental information included weather (cloudy, clear, rainy, showery), hourly temperature, moisture content, and barometric pressure. Horse history variables included horse, sex, and age in months. Finish position was defined as the horse's placing relative to the total number of horses competing in the race. Off odds represented the betting odds assigned to each horse at race start. Lasix use was categorized into four groups based on current and prior race administration: Yes, indicating horses that used Lasix in both the current race and the previous race; On, indicating horses that did not use Lasix in the previous race but did use it in the current race; No, indicating horses that did not use Lasix in either the previous or current race; and Off, indicating horses that used Lasix in the previous race but did not use it in the current race. Data were obtained from veterinary records reported to the Horseracing Integrity and Safety Authority (HISA) and from Equibase horse racing records.

Data were explored for missing values and standardized via their respective categorical and numerical values. The inherently categorical variables were converted into numerical codes to

be used as quantifiable variables for machine learning. Pairwise correlations among predictors were assessed using Pearson correlation coefficients (r) visualized in a correlation heat map. Variables with correlation coefficients ≥ 0.7 were excluded to mitigate multicollinearity, which can inflate variance in parameter estimates and reduce interpretability of individual predictor effects. Based on this criterion, Lasix use last race and lifetime starts were removed due to strong correlations to Lasix and age, respectively. Horse name was excluded as a non-informative identifier and trainer was removed due to excessive variability.

The dataset used in this study is notably imbalanced, with 202 epistaxis cases and 1,286 non-epistaxis cases. Class imbalance is a common challenge in machine learning classification tasks because models trained on skewed class distributions tend to be biased toward the majority class and can perform poorly on the minority class. To address this issue during training, techniques designed to mitigate class imbalance were used. Synthetic minority oversampling technique (SMOTE) was used to generate synthetic examples of the minority class to produce a more balanced training set. Additionally, 5-fold cross-validation on was employed as a resampling approach by using a holdout variation.

Conclusions:

Epistaxis occurrences were significantly related to previous epistaxis episodes and finish position. Horses with previous epistaxis episodes were more likely to have future episodes. Horses with epistaxis had decreased racing performance, predominantly finishing in the last quartile of their field. Although off odds was not a statistically significant predictor of epistaxis, its frequent selection by machine learning models and comparatively low p-value (0.2016) suggest a potential indirect or contextual role. In this cohort, 72% of horses that displayed epistaxis and 73% of horses that did not display epistaxis had received race-day furosemide. Twenty-eight percent of horses experiencing epistaxis and 27% of horses that did not exhibit epistaxis were running off furosemide. Furosemide use did not impact epistaxis occurrence (p = 0.72).

Non-HISA Funded Recent Research

One HISA FAC grant application was not funded due to concerns of the FAC with the quality of study design; however, the researchers still executed the project and shared their findings with the FAC via videoconference. This research was conducted by Michele Sezgin, Joel Greenhouse, Joseph Appelbaum, and was supervised by Ron Yurko at the Statistics and Data Science Department of the Carnegie Mellon University. The study was entitled “Does Lasix Make Horses Run Faster?”. Their modeling-based study examined ~100,000 race results involving approximately 28,000 horses between 1991 and 2024 garnered from a proprietary database that is not publicly available. The data related to 2-year-old racehorses that raced both with and without furosemide. The metric they compared was finishing in the money (1st, 2nd, or 3rd). The results of their conditional logistic regression and mixed effects logistic regression analyses indicated that there was a slight increase in the odds of a horse finishing in the money when receiving furosemide. The FAC were able to review and discuss this project with the authors and among themselves. Additionally, the authors presented an abstract of their results at the New England Symposium on Statistics in Sports in September 2025. The abstract and their poster are included as **Appendix L**.

The FAC found that the limitations of the study included the exclusive use of 2-year-olds where the variable of racing experience could not be accounted for in the model. There is a feeling among trainers and experienced track practitioners that initial starts are less likely to include the use of furosemide and that, as the number of races increases, furosemide would be administered to that horse. Another salient point is that no data were available to correlate race finish position with EIPH. Indeed, as with other studies, the question of whether furosemide administration in and of itself is a performance enhancing drug, or is it the mitigation of EIPH that leads to a better finishing position, remains unanswered.

ENVIRONMENTAL CONSIDERATIONS

It is well documented that small airway inflammation is near ubiquitous in stalled horses and that this condition is associated with an increased risk of EIPH [1-5]. It is the observation of the FAC members' experience that jurisdictions in other countries place a far greater emphasis on the reduction of environmental allergens in stabling facilities and through management practices to reduce small airway inflammation.

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DOES FUROSEMIDE INTERFERE WITH DRUG TESTING?

Furosemide is a diuretic medication that increases urine production, producing a dilute urine for a period of time post administration. Urine and blood are the two common biologic media that are used for equine drug testing. The dilution of urine can make detection of controlled or prohibited substances more difficult because drugs in urine are reported as a concentration of the drug in urine. If urine is diluted the concentration of all medications may be reduced depending on their specific disposition and pharmacodynamics. This is relevant in regard to the limit of detection of drug testing methodologies.

Expert professional opinion was sought to better understand if modern race day furosemide practices posed problems for the detection of controlled or prohibited substances.

The following commentary was obtained:
Regarding the potential impact of furosemide on regulatory biological samples and an analytical laboratory's ability to detect prohibited substances.

Each racing jurisdiction establishes the regulation for dose, route, and time of administration. Doses are between 150mg (minimum) to a maximum of 500mg intravenously administered intravenously during a 30-minute window of time that is 4 to 4.5 hours prior to post time for each eligible race.

Research has demonstrated that furosemide-associated diuresis ceases approximately 3 hours post-administration. As such, the potential for a dilute urine sample after 3-4 hours post-administration is relatively small. Because furosemide is a potent diuretic that causes dilution of urine, lowering urine solute concentration, which can mask the presence of prohibited substances, urine specific gravity (USG) is used in equine drug testing to help ensure urine samples are not too dilute for the detection of prohibited substances, thus invalidating post-race drug testing. The measurement of USG alongside regulating the control of a maximum furosemide dose, route of administration, and time (in proximity of post-time) in which furosemide can be administered on race day largely eliminate the "masking" concerns associated with race day furosemide use and the ability of laboratories to detect prohibited substances. Most racing regulatory authorities have employed a USG threshold to determine the reliability and appropriateness of a sample for detection of prohibited substances

Furthermore, because US labs largely test in blood (including paired blood and urine post-race samples), this minimizes sole reliance on potentially diluted urine samples. To that end, the target substance, concentration of that substance, and interaction of multiple drugs (furosemide and potential prohibited substance) in a horse at one time are also impact variables to consider regarding the detection of a prohibited substance.

*Overall, because of the above listed controls for race day administration, measurement of USG in urine samples of horses treated with furosemide, and routine screening in blood alongside urine, the **resulting impact** of race day furosemide administration on detection of prohibited substances in a post-race sample is minimal.*

Please see **Appendix M** for a reference that provides historical context for the control of race day furosemide use in consideration of equine drug testing and detection of prohibited substances.

SCIENTIFIC EVIDENCE ON PURPORTED PERFORMANCE ENHANCING EFFECTS OF FUROSEMIDE & EXERCISE-INDUCED PULMONARY HEMORRHAGE

The following review of scientific publications over the last 40 years provides context for the findings of the currently funded HISA research studies.

Introduction

Furosemide (Lasix™, Salix™) is widely administered to Thoroughbred racehorses to mitigate exercise-induced pulmonary hemorrhage (EIPH), a condition characterized by bleeding from pulmonary capillaries during high-intensity exercise. Numerous veterinary studies demonstrate that furosemide reduces the severity and frequency of pulmonary bleeding in athletic horses. Despite its therapeutic benefits, race day administration of furosemide remains controversial because of the perception that it may provide a direct performance advantage. Distinguishing between therapeutic mitigation of pulmonary bleeding and true pharmacologic performance enhancement is therefore central to evaluating the role of Lasix in racing. Understanding this issue requires evaluating both well-controlled physiologic experiments and observational racing studies.

Physiologic Basis of EIPH

Exercise-induced pulmonary hemorrhage is common in racing Thoroughbreds. During maximal exercise, pulmonary arterial pressures may exceed 90–100 mmHg, producing mechanical stress on pulmonary capillaries. This extreme vascular pressure can lead to capillary stress failure, allowing blood to enter the airways.

Research consensus indicates:

- EIPH prevalence may exceed 60–70% in racing populations
- repeated episodes may impair respiratory function
- furosemide is the only consistently demonstrated pharmacologic intervention that reduces EIPH severity

Potential Mechanisms of Action:

Weight-Loss Hypothesis: Furosemide produces rapid diuresis after intravenous administration. Racehorses may lose approximately 8–10 kg of fluid, roughly 2–3% of body mass, prior to racing depending on the dose administered. Reduced body mass could theoretically lower the metabolic cost of locomotion, similar to the advantage associated with lighter carried weight. However, the magnitude and consistency of this effect appear small across studies (Hinchcliff et al., 1993; Zawadzkas et al., 2006).

Pulmonary Pressure Reduction: A second mechanism involves pulmonary vascular physiology. Furosemide decreases plasma volume and venous return, lowering pulmonary vascular pressure during intense exercise. By reducing pulmonary capillary stress, the drug decreases the likelihood and severity of pulmonary hemorrhage. Under this framework, improved

performance could occur indirectly through preservation of respiratory function, rather than direct pharmacologic enhancement.

Controlled Physiologic Evidence: A controlled investigation conducted by DeNotta (2025) examined repeated furosemide administration in exercising Thoroughbreds using a prospective randomized placebo-controlled treadmill design over a 14-week period.
Investigators measured:

- body weight and hydration status
- packed cell volume
- plasma electrolyte concentrations
- urine specific gravity
- cardiac function during exercise
- skeletal bone density

Key Findings:

- Body Weight: There was no sustained difference between treated and control horses.
- Electrolytes: There were no clinically meaningful changes in serum sodium, potassium, or chloride.
- Hydration markers: Urine specific gravity did not differ from pre-administration levels 48 hours post-administration.
- Cardiac performance: Echocardiographic measures during exercise were unchanged
- Bone health: Bone density and skeletal strength were unaffected.

Importantly, repeated administration did not produce cumulative physiologic effects during the study period. These findings indicate that repeated furosemide administration does not produce systemic physiologic changes consistent with direct performance enhancement. Any benefit may be consistent with a reduction of pulmonary bleeding during intense exercise.

Observational Racing Evidence: Earlier observational racing studies reported modest performance improvements among horses receiving furosemide.
For example:

- Soma (1985) reported faster race times in EIPH-positive horses treated with furosemide.
- Gross et al. (1999) reported small improvements in finishing position in a large North American dataset.
- Hinchcliff et al. (2005) in a 744-horse study in Melbourne concluded that horses with EIPH severity grade ≥ 1 finished farther behind the winner of the race than horses that had no evidence of EIPH.

However, the US studies were conducted when medication use was voluntary, introducing trainer selection bias, since horses with known EIPH were more likely to receive treatment. Additionally, at that time the administration of furosemide was not strictly regulated in terms of

dose and time of administration. More recent analyses have taken advantage of regulatory changes restricting race day furosemide administration.

A comprehensive analysis by Waller (2025) evaluated North American racing data using:

- retrospective cohort analysis
- case-control analysis
- case-crossover analysis

After adjusting for confounders such as race class, trainer effects, and historical performance, race day furosemide was not significantly associated with:

- winning speed figures
- finishing position
- percentage of the field beaten

The study also reported no significant association with:

- racing-related sudden death
- catastrophic musculoskeletal injury
- career longevity

These findings suggest earlier reported performance differences may reflect confounding variables rather than pharmacologic enhancement.

Evaluating medication effects in racing populations presents several methodological challenges. Common confounders include:

- trainer selection bias
- survivorship bias
- regression to the mean
- underlying EIPH severity
- track surface and race conditions
- widespread use of furosemide limiting the number of age/race matched untreated controls for substantive statistical analyses.

Historically, voluntary medication use meant trainers effectively controlled treatment assignment. Modern regulatory restrictions create natural experimental conditions that reduce selection bias and improve causal interpretation. The current exemption has allowed monitoring of select groups of horses racing without furosemide treatment.

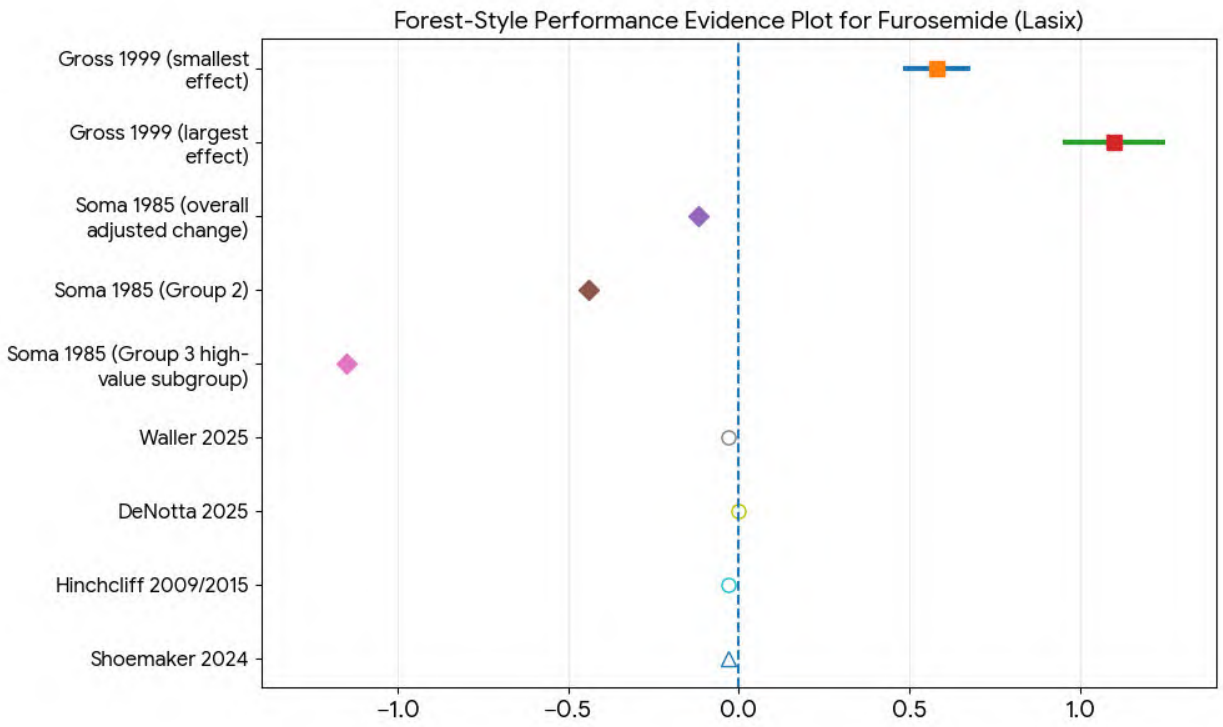

Figure 1. Forest plot summarizing estimated performance effects of prerace furosemide administration across representative racing and physiologic studies. Across the literature, most modern studies cluster around zero, indicating no statistically significant independent performance effect once confounding variables are addressed. Square (■) + horizontal line = verified extractable estimate with 95% confidence interval (CI); Diamond (◆) = extracted mean change, no CI reported; Open circle (○) = no demonstrated independent performance effect; Open triangle (Δ) = indirect/associative evidence, not a direct furosemide effect. Symbols to the left of 0 = no demonstrated or null effect/faster time if negative metric; symbols to the right of 0 = reported improvement. Note: Only Gross 1999 provides directly extractable performance estimates with variance. Soma 1985 provides extracted adjusted mean changes without pooled variance. Waller, DeNotta, Hinchcliff, and Shoemaker are placed on or slightly left of the no-effect line to reflect no demonstrated independent performance enhancement in the currently available commensurate data.

Table 1. Extractable Evidence on Furosemide (Lasix) and Racehorse Performance

Study	Outcome	Extracted Data	CI Available	Poolable	Performance Enhancement	Notes
Soma 1985	Adj time (s/km)	-0.13 overall; subgroup -0.44, -1.14	No	Limited	Mixed	Subgroup-dependent
Gross 1999	Race time (sec)	0.56 ± 0.04 to 1.09 ± 0.07 faster	Yes	Yes	Yes	Large Dataset
Hinchcliff 2015	Consensus	No numeric performance	No	No	Not demonstrated	Mechanistic only; Large Dataset
Shoemaker 2024	Association	Indirect; EIPH-performance	No	No	Not demonstrated	Not direct Lasix
DeNotta 2025	Physiology	No performance effect	No	No	No	Physiologic only
Waller 2025	Outcomes	No significant association No performance effect	No	No	No	Modern dataset

Conclusion:

The current body of scientific and racing evidence strongly supports the role of furosemide in reducing exercise-induced pulmonary hemorrhage in racehorses. There is no scientific evidence to support the statement that furosemide is a performance enhancing drug. A performance enhancing drug is defined as one that builds muscle, increases strength, enhances endurance, acts as a stimulant or speeds recovery. Furosemide has not been shown to do any of these.

References

1. DeNotta S. Effects of repeated furosemide administration on electrolyte homeostasis and bone density in exercising Thoroughbreds. HISA Furosemide Advisory Committee Report. 2025.
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3. Hinchcliff KW, McKeever KH, Muir WW 3rd, Sams R. Effect of furosemide and weight carriage on energetic responses of horses to incremental exertion. Am J Vet Res. 1993 Sep;54(9):1500-4. PMID: 8239140.
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6. Hinchcliff KW, Couetil LL, Knight PK, Morley PS, Robinson NE, Sweeney CR, van Erck E. Exercise induced pulmonary hemorrhage in horses: American College of Veterinary Internal Medicine consensus statement. J Vet Intern Med. 2015 May-Jun;29(3):743-58. doi: 10.1111/jvim.12593. PMID: 25996660; PMCID: PMC4895427.
7. Shoemaker S, Wang Y, Sellon D, Gold J, Fisher A, Bagshaw J, Leguillette R, Sanz M, Bayly W. Prevalence and severity of exercise-induced pulmonary hemorrhage in 2-year-old Thoroughbred racehorses and its relationship to performance. J Vet Intern Med. 2024 Mar-Apr;38(2):1167-1176. doi: 10.1111/jvim.17003. Epub 2024 Feb 16. PMID: 38363079; PMCID: PMC10937470.
8. Waller AP. Examining associations between furosemide treatment and racehorse health and welfare. HISA Final Report. 2025.
9. Zawadzkas XA, Sides RH, Bayly WM. Is improved high speed performance following frusemide administration due to diuresis-induced weight loss or reduced severity of exercise-induced pulmonary haemorrhage? Equine Vet J Suppl. 2006 Aug;(36):291-3. doi: 10.1111/j.2042-3306.2006.tb05555.x. PMID: 17402434.

CURRENT SCIENTIFIC EVIDENCE SUMMARY OF FUROSEMIDE RESEARCH

Research is a systematic and objective process of collecting information to gain a better understanding of a specific problem or answer a specific question. Research is typically conducted in 5 stages:

- Pose a question or evaluate a hypothesis
- Gather information and/or perform experiments to test that hypothesis
- Perform unbiased statistical analysis of the findings of the investigation
- Draw conclusions that either support or refute the hypothesis
- Communicate the results of the investigation

Publication of research requires peer review of the investigator's findings by qualified experts to ensure that the methods used in the study are appropriate, and that conclusions of the authors are valid and supported by the data presented in the study.

It is important to understand that there are limitations of any scientific study. These limitations provide context and often identify the need for further research to gain a better understanding of the problem or question. The most important limitation of scientific research is that some questions of policy simply cannot be reduced to a scientific hypothesis for which the evidence needed to prove it is available.

In a fully transparent effort to provide the HISA Board with the best possible information to help them make an informed decision regarding the furosemide policy for US horseracing, the FAC offers the best scientific information for which strong evidence is available (what we know) with the full knowledge that the Board must make decisions related to the furosemide policy based on both science and opinion.

There is strong scientific evidence to support the following statements:

- EIPH is a common condition in the Thoroughbred racehorse.
- EIPH of Grades 3 and 4 reduces the performance of racehorses.
- If a horse experiences EIPH, it is more likely to experience it again.
- Repeated episodes of EIPH compromise respiratory health.
- Furosemide treatment prior to high-speed exercise mitigates the degree of EIPH.
- Repeated furosemide treatment as it is currently used on race day has not been associated with electrolyte imbalance or reduced bone strength in exercising Thoroughbred racehorses.

There is no scientific evidence to support the following statement:

- Furosemide is a performance enhancing drug. A performance enhancing drug is defined as one that builds muscle, increases strength, enhances endurance, acts as a stimulant or speeds recovery. Furosemide has not been shown to do any of these.

- Furosemide administration under current strict regulation masks the detection of other controlled or prohibited substances

MODIFICATION RECOMMENDATIONS FROM THE FAC TO THE HISA BOARD

The FAC recommends modification of the current furosemide policy. Specifically, the FAC makes the following recommendation based upon a review of the scientific evidence — including the existing body of peer-reviewed literature, the results of research funded by HISA, FAC internal research, and the FAC's observations and analysis of the Thoroughbred racing industry in the United States during the past three years of regulatory oversight by HISA.

These recommendations are made without bias or conflict of interest and are offered from the perspective of unconditional advocates for equine welfare and the future success of horse racing worldwide. These recommendations are intended to provide an ethically-balanced approach to address equine welfare, integrity, public confidence and industry sustainability issues.

Given the primary importance of equine welfare, and in recognition of the scientific information that:

- EIPH occurs in two-year-old racehorses; severity and incidence increase with age.
- If a horse experiences EIPH, it is at increased risk for repeated episodes of EIPH.
- Repeated episodes of EIPH can cause irreversible pathologic changes in the lung.
- Furosemide is the only FDA-approved medication that has been shown to decrease the incidence and severity of EIPH in horses.
- Recent scientific evidence has determined that regular administration of furosemide to horses in appropriate doses and on a weekly schedule does not cause harm to the horse.
- There is no scientific evidence that supports the current ban of furosemide administration to two-year-old horses on race day.
- When confounding variables are controlled, the preponderance of scientific research over the past 40 years does not support the conclusion that furosemide is performance enhancing.

the FAC recommends:

The current regulatory policy regarding furosemide, prohibiting its use in two-year-olds and in horses racing in Named or Graded Stakes races in the forty-eight (48) hours prior to a race, be modified to permit the current strictly regulated administration of furosemide to horses competing in all races, except for Graded Stakes races that implicate commercial breeding potential.

In so doing, we recognize that there is no scientific justification for prohibiting the administration of furosemide in horses competing in Named and Graded stakes races and conclude that such prohibition is grounded in historic concerns about the marketability of elite North American horses in the international markets. This recommendation will require refining the definition of

"stakes races' to limit the prohibition of the administration of furosemide in the forty-eight (48) hours prior to a race only to Listed and Graded stakes races. This recommendation is specifically intended to eliminate the practice of converting Named Stakes races to handicap or overnight races in order to avoid the prohibition on furosemide use in those events.

Additional Recommendations:

1. Continued research be conducted to better understand the long-term effects of the race day administration of furosemide in 2-year-old horses.
2. Continued research be conducted to better understand and further characterize the implications of limited furosemide use, as noted above, upon the issue of integrity and competitive fairness in racing.
3. Continued research be conducted to investigate the physiologic effects of furosemide on racehorses other than reduction of differential pressure in terminal airways of racehorses.
4. The U.S. Thoroughbred racing industry conduct a thorough evaluation of current stable management practices at racetracks and training centers, with the goal of identifying best practices to improve air quality in stabling areas, reduce exposure to small particulate matter and other airborne irritants, and to mitigate risk factors associated with EIPH in Thoroughbred racehorses.
5. The U.S. Thoroughbred racing industry prioritize animal welfare over political or commercial interests in imposing regulatory policy governing race day use of furosemide. The FAC believes that placing equine welfare as the foremost priority will, in the long term, best serve the integrity, sustainability, and public trust of the Thoroughbred racing industry.

As stewards of the horse, and to the degree that we employ horses for sport and/or entertainment, we are morally obligated to do everything humanly possible to reduce the risk for compromise of equine welfare related to that endeavor.

Finally, while the committee understands that there are limitations to the ability of science to inform policy decisions, these recommendations are supported by strong scientific evidence with a focus on equine welfare, while taking into consideration the social, economic and political realities of our time.

APPENDIX A: Section 6 (e) of The Horseracing Integrity and Safety Act of 2020

HORSERACING ANTI-DOPING AND MEDICATION CONTROL PROGRAM.

(e) ADVISORY COMMITTEE STUDY AND REPORT.—

(1) IN GENERAL.—Not later than the program effective date, the Authority shall convene an advisory committee comprised of horseracing anti-doping and medication control industry experts, including a member designated by the anti-doping and medication control enforcement agency, to conduct a study on the use of furosemide on horses during the 48-hour period before the start of a race, including the effect of furosemide on equine health and the integrity of competition and any other matter the Authority considers appropriate.

(2) REPORT.—Not later than three years after the program effective date, the Authority shall direct the advisory committee convened under paragraph (1) to submit to the Authority a written report on the study conducted under that paragraph that includes recommended changes, if any, to the prohibition in subsection (d).

(3) MODIFICATION OF PROHIBITION.—

(A) IN GENERAL.—After receipt of the report required by paragraph (2), the Authority may, by unanimous vote of the Board of the Authority, modify the prohibition in subsection (d) and, notwithstanding subsection (f), any such modification shall apply to all States beginning on the date that is three years after the program effective date.

(B) CONDITION.—In order for a unanimous vote described in subparagraph (A) to effect a modification of the prohibition in subsection (d), the vote must include unanimous adoption of each of the following findings:

(i) That the modification is warranted.

(ii) That the modification is in the best interests of horse racing.

(iii) That furosemide has no performance enhancing effect on individual horses.

(iv) That public confidence in the integrity and safety of racing would not be adversely affected by the modification.

(f) EXEMPTION.—

(1) IN GENERAL.—Except as provided in paragraph (2), only during the three-year period beginning on the program effective date, a State racing commission may submit to the Authority, at such time and in such manner as the Authority may require, a request for an exemption from the prohibition in subsection (d) with respect to the use of furosemide on covered horses during such period.

(2) EXCEPTIONS.—An exemption under paragraph (1) may not be requested for—

(A) two-year-old covered horses; or

(B) covered horses competing in stakes races.

(3) CONTENTS OF REQUEST.—A request under paragraph (1) shall specify the applicable State racing commission's requested limitations on the use of furosemide that would apply to the State under the horseracing anti-doping and medication control program during such period. Such limitations shall be no less restrictive on the use and administration of furosemide than the restrictions set forth in State's laws and regulations in effect as of September 1, 2020.

(4) GRANT OF EXEMPTION.—Subject to subsection (e)(3), the Authority shall grant an exemption requested under paragraph (1) for the remainder of such period and shall allow the use of furosemide on covered horses in the applicable State, in accordance with the requested limitations.

APPENDIX B: Initial Request for Proposals

HISA Furosemide Advisory Committee: Request for Proposals (RFP) – *Scheduled to be issued September 1, 2023.*

Contents

a. Scope of the Request for Proposals

The Horseracing Integrity and Safety Act of 2020 (https://www.congress.gov/bill/116th-congress/house-bill/1754/text) (as amended) (the "Act"), recognizes the Horseracing Integrity and Safety Authority, Inc. ("HISA"), a private, nonprofit corporation, for the purposes of *inter alia*, developing and implementing uniform rules, regulations and protocols for a national Racetrack Safety Program and Anti-Doping and Medication Control ("ADMC") Program.

In this regard as it relates to the ADMC Program, Section 6, subsection (d) of the Act prohibits the administration of any prohibited or otherwise permitted substance to a Covered Horse within 48 hours of its next racing start, effective as of the ADMC's Program's effective date, which was May 22, 2023. The Act specifically prohibits the administration of furosemide within 48 hours of a Covered Race to 2-year-old Covered Horses and Covered Horses competing in stakes races, except that State Racing Commissions may apply to HISA for a 3-year exemption from the furosemide prohibition for all other Covered Races. As of May 22, 2023, all State Racing Commissions have applied for and received the 3-year-exemption.

The Act further specifies that HISA shall, no later than the ADMC Program's effective date, convene an advisory committee comprised of horseracing anti-doping and medication control industry experts, including a member designated by the anti-doping and medication control enforcement agency, to conduct a study on the use of furosemide on horses during the 48-hour period before the start of a race, including the effect of furosemide on equine health and the integrity of competition and any other matter the HISA considers appropriate. Pursuant to the Act, the HISA has convened a Furosemide Advisory Committee ("FAC").

No later than May 22, 2026, the FAC shall submit to HISA a written report on the study it has conducted that includes recommended changes, if any, to the prohibition in Section 6, subsection (d) of the Act

After receipt of the report, the HISA Board of Directors may, by unanimous vote, modify the prohibition of furosemide administration within 48-hours of a Covered Race, provided that the Board unanimously adopts each of the following findings:

(i) That the modification is warranted.
(ii) That the modification is in the best interests of horse racing.
(iii) That furosemide has no performance enhancing effect on individual horses.
(iv) That public confidence in the integrity and safety of racing would not be adversely affected by the modification.

To fulfill its congressional mandate, the FAC seeks proposals for research on the administration of furosemide to Covered Horses during the 48-hour period before the start of a Covered Race. In evaluating applications, the FAC will pay particular attention to the impact of the proposed work as it applies to horseracing in North America. Up to $1,000,000 will be available each year for two years to fund projects. The total grant funding available being $2,000,000 over two years.

Examples of research proposals may include but should not be limited to:

- What are the long-term economic implications for U.S. Thoroughbred racing of prohibiting furosemide administration within 48 hours of a race (using metrics such as frequency of starts, career length, number of starts per year, age at retirement, and field size)?
- What are the effects of furosemide administration within 48 hours of racing on equine health and welfare?
- Does the use of furosemide within 48 hours of racing jeopardize racing's social license to operate?
- Would administration of furosemide within 48 hours of racing adversely affect public confidence in the integrity of horse racing?
- Does administration of furosemide within 48 hours of racing enhance racing performance?
- Is administration of furosemide alone, or in combination with any other medication, within 48 hours of racing associated with exercise-associated sudden death?
- Is the absence of furosemide administration within 48 hours of a race associated with exercise-related sudden death?
- What factors such as environmental conditions, stable management, pre-existing medical conditions, age, and race distance and/or frequency, and the use of furosemide and its repeated use within 48 hours of a race are associated with Exercise-induced pulmonary hemorrhage (EIPH)?

- What alternatives to administration of furosemide within 48 hours of racing effectively mitigate or reduce the prevalence of EIPH in Thoroughbred racehorses?
- What are the acute & chronic effects of EIPH on horse health and performance?
- What are the effects of chronic furosemide administration on the musculoskeletal system, nervous system, cardiovascular system, respiratory system, nervous system, and/or immune system.
- Does the administration of furosemide within 48 hours of racing mitigate the increase in severity of EIPH over time?

The HISA FAC will compile and evaluate applications in order to make recommendations for disbursement of funds to successful applications.

Twenty percent (20%) of the total budget will be paid upon completion and submission of the Final Report on or before January 10, 2026. Project findings must not be published or shared publicly prior to January 10, 2026. However, project findings are expected to be published in peer-reviewed journals, after the Final Report has been approved.

Deadlines:

Application submission:	November 15, 2023
Decision for funding:	December 15, 2023

Schedule for Awarded Grants:

First Disbursement:	January 10, 2024
Interim Report:	January 10, 2025
Second Disbursement:	January 31, 2025
Final Report on or before:	January 10, 2026
Disbursement of final payment (last 20% of award):	January 31, 2026

b. Grant Guidelines

Principal Investigator(s): The principal investigator (PI) should have some professorial rank and salary provided, if employed by an academic institution, or industry/private entity equivalent. Salary and Fringe benefits should not be allocated for the Principal Investigator, Co-Investigators, and faculty of the applicant institution and/or any collaborating institution(s).

Financial Notes:

- Salary and Fringe Benefits cannot be allocated for Principal Investigator(s) or faculty of the applicant or collaborating institution(s).
- Stipend (+/- tuition support for students) for technical staff, graduate students, and/or post-doctoral scholars may be supported and assessed on a case-by-case basis.
- Up to 25% for indirect costs may be allocated to an institution. 25% is NOT computed on any salary allocations.

- Twenty percent (20%) of the total budget will be paid upon completion and submission of the Final Report on or before January 10, 2026.

c. Formatting Instructions

- Grant applications to be uploaded/sent as a single .pdf document prior to the submission deadline without exception. The total file must not exceed 3MB in size. Please follow the instructions for each portion of the application. Failure to do so may forfeit your consideration for funding.
- Arial font, 11-point type, regular (not bold) type, with a page size of 8.5 x 11 inches, single spaced is acceptable. Pages numbered continuously in the RESEARCH PLAN Items 1- 4 which must not to exceed 15 pages. Upper and lower case in the body of the plan.
- The PDF version of the Research Plan may include graphics, tables, and diagrams as needed.
- No URL links should appear anywhere in the application.

d. Application Information

The Grant Application serves as a contract. It is understood and agreed by all the undersigned if the application is approved, and a grant made, it will be according to the terms of the project application and to the stipulations set forth in the accompanying instructions, and conditions of grants that will be and are herewith made a part of the grant agreement. All applicants must comply with appropriate regulations pertaining to the use of any animals covered by the Animal Welfare Act in any proposed study. The policy on humane care and use of vertebrate animals shall require that grant applications for research employing such animals be accompanied by a letter stating that the Institutional Animal Care and Use Committee or equivalent has reviewed and approved the research project for funding as requested. Applications involving human subjects must be reviewed and approved by the applicants Institutional Review Board (IRB) or equivalent.

Grant proposals may be reviewed prior to receipt of such statements of approval by the HISA FAC, but funds will not be awarded without a letter signed by an individual qualified and authorized to represent the application institution.

All documents will be submitted in .pdf format. Incomplete applications, those not adhering to formatting guidelines, or exceeding page limits will not be reviewed. Applications shall serve as a contract.

e. Application Sections

RESEARCH PLAN

The research plan should have the following headings (sub-headings may be used at your discretion). **Do not exceed a total of 15 pages for RESEARCH PLAN items 1 through 4.**

1) The Research Problem and Background Information: The research problem and its impact on North American horseracing. Cite existing work concerning the problem and the relevance of that work to this grant proposal. (Max two (2) pages)

2) Hypothesis (HI, H2, etc.): Specific aims (Al, A2, etc.) and goals identified for each hypothesis. It should be clear how each aim relates to the hypothesis, how they will be performed and in what sequence, and how they will relate to the overall industry problem. (One (1) page)

3) Preliminary Studies: If the proposed research will build on recent previous studies or if pilot projects have been completed for this application, summarize the findings, and relate them to the proposed research. (Zero to three (0-3) pages)

4) Experimental Methods and Design: Planned Data acquisition and analysis to include statistical methods, expected results if applicable, potential pitfalls and plans for dealing with them.
Do not exceed a total of 15 pages for RESEARCH PLAN items 1 through 4.

5) References: Literature cited in proposal only. (Three (3) pages max)

6) Appendix: Include difficult to reproduce items such as color pictures if necessary or previously published articles by the investigators that have a direct relationship to this project. (Three (3) pages max)

7) Budget:

Detailed Line-Item Budget: Budgets include, but are not limited to, the following items:

- All essential costs for the success of the program and detailed justification of such must be included in the Budget Narrative.
- Administrative costs: Costs necessary for the effective administration of the project may include salaries for award recipient organization staff/students/post-doctoral scholars, fringe benefits, and other direct and indirect costs submission.
- Equipment: May be allowed but must be justified in the budget narrative.
- Clinic/Meeting/Publication costs
- Other justifiable expenses directly related to supporting project activities.

While there is no rigid ratio of administrative costs to program costs, proposals in which the administrative costs do not exceed 25% of the total requested HISA grant funds will be more competitive under the cost-effectiveness review criterion.

Budget Narrative: A budget narrative must be included and provide detailed explanation for each budget line-item where the activity purpose or means of calculation may not be clear or require further justification. It should include at minimum:

- (1) Formulae used to calculate salary costs, benefits, travel, and per diem costs for multiple people; training materials and expenses based on participant number, etc.
- (2) Supportive explanation and justification for activities and expenses determined essential, but that may not be understood from the narrative or may otherwise be unclear to reviewers.
- (3) Breakdown of costs associated with relatively high amount line-item requests that would reasonably be questionable by reviewers. (e.g., if requesting $50K for equipment).

8) Calendar of funding: In the budget please be sure to provide dollar amounts expected to be paid on the disbursement dates:

Schedule for Awarded Grants:

First Disbursement	January 10, 2024
Second Disbursement	January 31, 2025
Disbursement of final payment (last 20% of award)	January 31, 2026

9) Calendar of Activities: The anticipated duration of the agreement will be up to two years. Provide a monthly plan of action or timetable that demonstrates the timing and organization of your responsibilities as the recipient. A Gantt chart or equivalent may be useful here.

10) Financial Officer:
Provide the name, email address, phone number and mailing address of the institutional financial officer who will receive and disburse funds.

f. Required Information

BIOGRAPHICAL SKETCHES - limit two (2) pages per person.

Provide the following information for key professionals on the List of Personnel beginning with the Principal Investigator.

This information should include the following:

- NAME and TITLE

- EDUCATION: Begin with baccalaureate or other initial professional education and include postdoctoral training.
    - INSTITUTION AND LOCATION
    - DEGREE YEAR CONFERRED
    - FIELD OF STUDY
- RESEARCH AND/OR PROFESSIONAL EXPERIENCE: Concluding with present position, list in chronological order previous employment and experience.
- PUBLICATIONS: List the titles and complete references to publications during the past three years and representative earlier publications pertinent to the application.

OTHER SUPPORT current and pending

- Please include Investigator's Name, Entire Support Dates, Percent of Effort, Source of Support, Annual Cost, Title of Support, Overlap
- Include all federal, non-federal, and institutional grant and contract support. If none, state "none".
- If any proposals/grants overlap, duplicate, or are being replaced or supplemented by the present application, delineate, and justify the nature and extent of the scientific and budgetary overlaps or boundaries.
- Beginning with the Principal Investigator, complete the following for each of the professionals named in the biographical sketches.

RESOURCES AND ENVIRONMENT RELATIVE TO PROPOSAL FACILITIES (1 page)

LETTERS OF SUPPORT: Letters from collaborating stakeholders or institutions where support of sample acquisition, processing of samples or data is to be performed.

SIGNATURE PAGE: See page 9.

g. Submission Process

Submissions are **due November 15, 2023, 11.59pm ET.** Applications must be emailed to Dr. Jennifer Durenberger (jennifer.durenberger@hisaus.org) and Mr. Sam Reinhardt (samuel.reinhardt@hisaus.org) whereupon an email acknowledging receipt of the application correctly formatted will be returned to the applicant by email the following day confirming receipt. If you do not receive an email within two (2) business days, please contact Dr. Jennifer Durenberger and Mr. Sam Reinhardt.

h. Grant Evaluation Process

Review Criteria: Technically eligible applications will be competitively reviewed according to the criteria stated below. The criteria listed below are not rank ordered. In reviewing

applications, the FAC will pay particular attention to the impact of the proposed work as it applies to horseracing in North America.

(i) **Project Planning and Ability to Achieve Objectives in the Given Timeframe:** Project objectives should be stated clearly and should reflect the applicant's expertise in the subject area. Objectives should respond to the focus criteria in this announcement and should relate to the current conditions in North America. A detailed agenda and relevant work plan should explain how objectives will be achieved in the given time frame and should include a timetable for completion of major tasks.

(ii) **Merit of the Proposal:** Background, Hypothesis(es), Specific Aim(s), Expertise of Investigators and Organizational Support: Study design, justification of the proposal, and feasibility of addressing the objectives of the study will be important assessment criteria, as will impact on horseracing in North America. Collaborative strengths within applications will be assessed.

(iii) **Cost-effectiveness:** Overhead and administrative costs in the proposal budget, including salaries and subcontracts for services, should be kept to a minimum, and adequately explained in the budget.

(iv) **Project Monitoring and Evaluation:** Proposals should include a detailed plan to monitor and report progress of the project. They should also include (per Section e(4)) alternative strategies should problems be encountered during the execution of the research.

i. Non-Disclosure Agreement

Successful grant applicants will be asked to sign non-disclosure agreements related to work performed on this proposal.

j. HISA Contact Information

Please contact the following people with questions:

- Dr. Jennifer Durenberger: jennifer.durenberger@hisaus.org
- Mr. Sam Reinhardt: samuel.reinhardt@hisaus.org

Signature Page

Official Signing for Applicant Organization:
This form must be filled out by an official from the applicant's organization and submitted as an attachment in order for an application to be considered complete.
Name:
Title:
Institution:
Address Line 1:
Address Line 2:
City, State, Zip:
Work Phone:
Mobile Phone:
Fax:
E-Mail:

I certify that all expenditures made with grant funds awarded by the HISA FAC are for appropriate grant purposes in accordance with the provisions of the application, grant terms and conditions, and all other awarded documents.

Signature:

Printed Name:

Title:

Date

APPENDIX C: Scoring Rubric for Grant Proposals

Primary PI	
Grant Title	
Institution	
Amount Req	

	Project planning & feasibility of completion	**Scientific Merit of Proposal**	**Impact on the Issue**	**Budget & Cost-effectiveness**	**Project Monitoring & Alternative Strategies**	**Overall Impression (incl personnel, resources, writing, clarity)**	**Total**
	Max 100	Max 150	Max 100	Max 50	Max 50	Max 50	Max 500
Score Reviewer 1							0
Score Reviewer 2							0
Score Reviewer 3							0
Score Reviewer 4							0
Range Minimum	0	0	0	0	0	0	0
Range Maximum	0	0	0	0	0	0	0
Mean							0

APPENDIX D: Initial Research Proposals Recommended for Funding

Does Pre-Race Administration of Furosemide to Thoroughbred Racehorses Prolong Their Racing Careers?
Principal Investigator: Warwick Bayly, BVSc, PhD, DACVIM, Professor, Department of Veterinary Clinical Sciences, Washington State University.

The primary objective of this study is to determine the impact of severe EIPH on horses' careers and the overall economic health of the Thoroughbred racing industry. There are three specific aims of this study. The specific objective of Aim 1 is to assess whether regular treatment of horses during their careers was associated with more career starts and greater longevity than horses that raced without furosemide treatment. The specific objective of Aim 2 is to investigate the impact of banning the administration of furosemide to 2-year-olds on the duration of their careers and their number of lifetime race starts. The specific objective of Aim 3 is to determine the extent to which severe EIPH impacts the number of subsequent race starts, the period between them and, when applicable, the time between the diagnosis of severe EIPH and retirement.

Effects of Repeated Furosemide Administration on Electrolyte Homeostasis and Bone Density in Healthy Adult Exercising Thoroughbreds.
Principal Investigator: SallyAnne L. DeNotta, DVM, PhD, DACVIM., Clinical Assistant Professor, Large Animal Medicine, University of Florida College of Veterinary Medicine.

The primary objective of this study is to examine the effects of repeated administration of furosemide on electrolyte hemostasis, parathyroid response, and urinary electrolyte excretion in exercising healthy adult Thoroughbred racehorses. The secondary objective of this study is to examine the effects of repeated administration of furosemide on bone density and strength using minimally invasive methods of measurement DEXA scan and OsteoProbe. In addition, ECG monitoring will occur during exercise and at 24 hours post workout, in an attempt to better elucidate cardiac rhythm dynamics in actively exercising furosemide-treated horses.

Examining Associations Between Furosemide Treatment & Racehorse Health and Welfare.
Principal Investigator: Amanda Waller, BSc, PhD, Research Scientist, Center for Clinical and Translational Research, Nationwide Children's Hospital.

The objective of this study is to examine the effects of race day furosemide treatment on the health and welfare of Thoroughbred racehorses, and the integrity of the sport of horse racing. The central hypothesis of this study is that prohibition of furosemide within 48 hours of racing is likely to result in negative consequences to equine welfare, as indicated by increased racing fatalities and decreased career longevity, among other factors.

APPENDIX E: Request for Proposals – Performance Enhancement

HISA Furosemide Advisory Committee: Request for Proposals (RFP) – *Scheduled to be issued July 30, 2024.*

Contents

a. Scope of the Request for Proposals

The Horseracing Integrity and Safety Act of 2020 (https://www.congress.gov/bill/116th-congress/house-bill/1754/text) (the "Act"), preempts the state-by-state regulation of the Thoroughbred racing industry by requiring the enactment and implementation of uniform rules, regulations and protocols relating to equine health, safety, and welfare and anti-doping and drug testing. The Act creates an Authority ("HISA") to, inter alia, adopt the uniform rules, regulations and protocols for the national Safety and Anti-Doping and Medication Control Programs.

In this regard as it relates to the Anti-Doping program, Section 6, subsection (d) of the Act prohibits the administration of any prohibited or otherwise permitted substance to a covered horse within 48 hours of its next racing start, effective as of the Anti-Doping and Medication Program's effective date, which was May 22, 2023. The Act specifically prohibits the administration of furosemide within 48 hours of a covered race to 2-year-old horses and horses competing in stakes races, except that State Racing Commissions may apply to HISA for a 3-year exemption from the furosemide prohibition for all other covered races. As of May 22, 2023, all State Racing Commissions have applied for and received the 3-year-exemption.

The Act further specifies that HISA shall, as of the program effective date, convene an advisory committee comprised of horseracing anti-doping and medication control industry experts, including a member designated by the anti-doping and medication control enforcement agency, to conduct a study on the use of furosemide on horses during the 48-hour period before the start of a race, including the effect of furosemide on equine health and the integrity of competition and any other matter the Authority considers appropriate. As required, the HISA has constituted a Furosemide Advisory Committee ("FAC").

Not later than three years after the program effective date, the FAC shall submit to HISA a written report on the study it has conducted that includes recommended changes, if any, to the prohibition in Section 6, subsection (d) of the Act

After receipt of the report, the HISA Board of Directors may, by unanimous vote, modify the prohibition of furosemide administration within 48-hours of a covered race, provided that the Board unanimously adopts each of the following findings:

(i) That the modification is warranted.
(ii) That the modification is in the best interests of horse racing.
(iii) That furosemide has no performance enhancing effect on individual horses.
(iv) That public confidence in the integrity and safety of racing would not be adversely affected by the modification.

If the Board fails to modify the current policy, the use of furosemide within 48 hours of all covered races will be prohibited as of May 23, 2026.

REQUEST FOR PROPOSALS
The FAC seeks proposals or research to specifically address the question:

Is furosemide a performance enhancing drug in Thoroughbred racehorses?

In evaluating applications, the FAC wishes to point out that study design and data analysis must be sensitive to factors such as racing surface, age of horse, distance of race/exertion, environmental conditions such as humidity and altitude that may influence outcome and/or the texture of the track surface. They will also pay particular attention to the impact of the proposed work as it applies to horseracing in North America. To be considered eligible for funding, at least one outcome must be an objective, quantifiable measure of performance.

Up to $1,000,000 will be available to fund projects.

The HISA FAC will compile and evaluate applications in order to make recommendations for disbursement of funds to successful applications.

Twenty percent (20%) of the total budget will be paid upon completion and submission of the **Final Report on or before January 10, 2026**. Project findings must not be published or shared publicly prior to January 10, 2026. However, project findings are expected to be published in peer-reviewed journals, after the Final Report has been approved.

Deadlines:
Application submission: October 15, 2024

Decision for funding: ` November 15, 2024

Schedule for Awarded Grants:
First Disbursement: January 10, 2025
Final Report on or before: January 10, 2026
Disbursement of final
payment (last 20% of award): January 31, 2026

b. Grant Guidelines

Principal Investigator(s): The principal investigator (PI) should have some professorial rank and salary provided, if employed by an academic institution, or industry/private entity equivalent. Salary and Fringe benefits should not be allocated for the Principal Investigator, Co-Investigators, and faculty of the applicant institution and/or any collaborating institution(s).

Financial Notes:

- Salary and Fringe Benefits cannot be allocated for Principal Investigator(s) or faculty of the applicant or collaborating institution(s).
- Stipend (+/- tuition support for students) for technical staff, graduate students, and/or post-doctoral scholars may be supported and assessed on a case-by-case basis.
- Up to 25% for indirect costs may be allocated to an institution. 25% is NOT computed on any salary allocations.
- Twenty percent (20%) of the total budget will be paid upon completion and submission of the Final Report on or before January 10, 2026.

m. Formatting Instructions

- Grant applications to be uploaded/sent as a single .pdf document prior to the submission deadline without exception. The total file must not exceed 3MB in size. Please follow the instructions for each portion of the application. Failure to do so may forfeit your consideration for funding.
- Arial font, 11-point type, regular (not bold) type, with a page size of 8.5 x 11 inches, single spaced is acceptable. Pages numbered continuously in the RESEARCH PLAN items 1- 4 which must not to exceed 15 pages. Sentence case in the body of the plan.
- The PDF version of the Research Plan may include graphics, tables, and diagrams as needed.
- No URL links should appear anywhere in the application.

c. Application Information

The Grant Application serves as a contract. It is understood and agreed by all the undersigned if the application is approved, and a grant made, it will be according to the terms of the project application and to the stipulations set forth in the accompanying instructions, and conditions of

grants that will be and are herewith made a part of the grant agreement. All applicants must comply with appropriate regulations pertaining to the use of any animals covered by the Animal Welfare Act in any proposed study. The policy on humane care and use of vertebrate animals shall require that grant applications for research employing such animals be accompanied by a letter stating that the Institutional Animal Care and Use Committee or equivalent has reviewed and approved the research project for funding as requested. Applications involving human subjects must be reviewed and approved by the applicants Institutional Review Board (IRB) or equivalent.

Grant proposals may be reviewed prior to receipt of such statements of approval by the HISA FAC, but funds will not be awarded without a letter signed by an individual qualified and authorized to represent the application institution.

All documents will be submitted in .pdf format. Incomplete applications, those not adhering to formatting guidelines, or exceeding page limits will not be reviewed. Applications shall serve as a contract.

d. Application Sections

RESEARCH PLAN

The research plan should have the following headings (sub-headings may be used at your discretion). **Do not exceed a total of 15 pages for RESEARCH PLAN items 1 through 4.**

11) The Research Problem and Background Information: The research problem and its impact on North American horseracing. Cite existing work concerning the problem and the relevance of that work to this grant proposal. (Max two (2) pages)

12) Hypothesis (HI, H2, etc.): Specific aims (Al, A2, etc.) and goals identified for each hypothesis. It should be clear how each aim relates to the hypothesis, how they will be performed and in what sequence, and how they will relate to the overall industry problem. (One (1) page)

13) Preliminary Studies: If the proposed research will build on recent previous studies or if pilot projects have been completed for this application, summarize the findings, and relate them to the proposed research. (Zero to three (0-3) pages)

14) Experimental Methods and Design: Planned Data acquisition and analysis to include statistical methods, expected results if applicable, potential pitfalls and plans for dealing with them.
Do not exceed a total of 15 pages for RESEARCH PLAN items 1 through 4.

15) References: Literature cited in proposal only. (Three (3) pages max)

16) Appendix: Include difficult to reproduce items such as color pictures if necessary or previously published articles by the investigators that have a direct relationship to this project. (Three (3) pages max)

17) Budget:

Detailed Line-Item Budget: Budgets include, but are not limited to, the following items:

- All essential costs for the success of the program and detailed justification of such must be included in the Budget Narrative.
- Administrative costs: Costs necessary for the effective administration of the project may include salaries for award recipient organization staff/students/post-doctoral scholars, fringe benefits, and other direct and indirect costs submission.
    - Up to 25% for indirect costs may be allocated to an institution. 25% is NOT computed on any salary allocations
- Equipment: May be allowed but must be justified in the budget narrative.
- Clinic/Meeting/Publication costs
- Other justifiable expenses directly related to supporting project activities.

Budget Narrative: A budget narrative must be included and provide detailed explanation for each budget line-item where the activity purpose or means of calculation may not be clear or require further justification. It should include at minimum:

- (1) Formulae used to calculate salary costs, benefits, travel, and per diem costs for multiple people; training materials and expenses based on participant number, etc.
- (2) Supportive explanation and justification for activities and expenses determined essential, but that may not be understood from the narrative or may otherwise be unclear to reviewers.
- (3) Breakdown of costs associated with relatively high amount line-item requests that would reasonably be questionable by reviewers (e.g., if requesting $50K for equipment).

18) Calendar of funding: In the budget please be sure to provide dollar amounts expected to be paid on the disbursement dates:

<u>Schedule for Awarded Grants:</u>

First Disbursement	January 10, 2025
Disbursement of final payment (last 20% of award)	January 31, 2026

19) Calendar of Activities: Please provide a monthly plan of action or timetable that demonstrates the timing and organization of your responsibilities as the recipient. A Gantt chart or equivalent may be useful here.

20) Financial Officer:

Provide the name, email address, phone number and mailing address of the institutional financial officer who will receive and disburse funds.

e. Required Information

Beginning with the Principal Investigator, complete the following (BIOGRAPHICAL SKETCH and OTHER SUPPORT) for each key professional included on the proposal:

1) BIOGRAPHICAL SKETCHES - limit two (2) pages per person.

This information should include the following:

- NAME and TITLE
- EDUCATION: Begin with baccalaureate or other initial professional education and include postdoctoral training.
  - INSTITUTION AND LOCATION
  - DEGREE YEAR CONFERRED
  - FIELD OF STUDY
- RESEARCH AND/OR PROFESSIONAL EXPERIENCE: Concluding with present position, list in chronological order previous employment and experience.
- PUBLICATIONS: List the titles and complete references to publications during the past three years and representative earlier publications pertinent to the application.

2) OTHER SUPPORT current and pending

- Please include Investigator's Name, Entire Support Dates, Percent of Effort, Source of Support, Annual Cost, Title of Support, Overlap
- Include all federal, non-federal, and institutional grant and contract support. If none, state "none".
- If any proposals/grants overlap, duplicate, or are being replaced or supplemented by the present application, delineate, and justify the nature and extent of the scientific and budgetary overlaps or boundaries.

RESOURCES AND ENVIRONMENT RELATIVE TO PROPOSAL FACILITIES (1 page)

LETTERS OF SUPPORT: Letters from collaborating stakeholders or institutions where support of sample acquisition, processing of samples or data is to be performed.

SIGNATURE PAGE: See page 9.

f. Submission Process

Submissions are **due October 15, 2024, 11.59pm ET.** Applications must be emailed to Dr. Jennifer Durenberger (jennifer.durenberger@hisaus.org) and Mr. Sam Reinhardt (samuel.reinhardt@hisaus.org) whereupon an email acknowledging receipt of the application correctly formatted will be returned to the applicant by email the following day confirming receipt. If you do not receive an email within two (2) business days, please contact Dr. Jennifer Durenberger and Mr. Sam Reinhardt.

g. Grant Evaluation Process

Review Criteria: Technically eligible applications will be competitively reviewed according to the criteria stated below. The criteria listed below are not rank ordered. In reviewing applications, the FAC will pay particular attention to the impact of the proposed work as it applies to horseracing in North America.

(v) **Project Planning and Ability to Achieve Objectives in the Given Timeframe:** Project objectives should be stated clearly and should reflect the applicant's expertise in the subject area. Objectives should respond to the focus criteria in this announcement and should relate to the current conditions in North America. A detailed agenda and relevant work plan should explain how objectives will be achieved in the given time frame and should include a timetable for completion of major tasks.

(vi) **Merit of the Proposal:** Background, Hypothesis(es), Specific Aim(s), Expertise of Investigators and Organizational Support: Study design, justification of the proposal, and feasibility of addressing the objectives of the study will be important assessment criteria, as will impact on horseracing in North America. Collaborative strengths within applications will be assessed.

(vii) **Cost-effectiveness:** Overhead and administrative costs in the proposal budget, including salaries and subcontracts for services, should be kept to a minimum, and adequately explained in the budget.

(viii) **Project Monitoring and Evaluation:** Proposals should include a detailed plan to monitor and report progress of the project. They should also include (per Section e(4)) alternative strategies should problems be encountered during the execution of the research.

h. Non-Disclosure Agreement

Successful grant applicants will be asked to sign non-disclosure agreements related to work performed on this proposal.

i. HISA Contact Information

Please contact the following people with questions:

- Dr. Jennifer Durenberger: jennifer.durenberger@hisaus.org
- Mr. Sam Reinhardt: samuel.reinhardt@hisaus.org

APPENDIX F: Bayly Final Report

Final Report

DOES PRE-RACE ADMINISTRATION OF FUROSEMIDE TO THOROUGHBRED RACEHORSES PROLONG THEIR RACING CAREERS?

Submitted by Warwick Bayly, James Meyer, Macarena Sanz, Renaud Leguillette, Noah Cohen

Contributing Investigators: Debra Sellon, Sandra Yucupicio, Erin Pinnell, Alasdair Botting, Zuzanna Pietras

Executive Summary

Hypothesis 1: That thoroughbred racehorses in the lower quintile of pre-race treatment with furosemide per lifetime start have significantly fewer starts and shorter careers.

Despite large datasets and extensive application of multiple statistical analysis models and methods, we failed to prove the first hypothesis. Particularly germane to this hypothesis was the finding that there was no difference in the number of career starts over a 5-year period in 2-year-olds that had their first race starts in 2015 and 2021, respectively, despite the fact that pre-race administration of furosemide was close to 100% in the 2015 cohort and 0% in the majority of the equivalent 2021 group. Prohibiting routine race-day furosemide administration to 2-year-olds did not harm their racing careers.

Hypothesis 2: That horses diagnosed with EIPH grades =3 or =4 have significantly fewer starts post-diagnosis than horses with EIPH grades < 3.

With respect to the impact of EIPH severity as assessed tracheoendoscopically, no significant association was identified between the likelihood of a prolonged absence from racing, or a drop in the class of races and EIPH severity. Additionally, even though pre-race furosemide administration attenuates the severity of EIPH, we were unable to demonstrate that this had a positive effect on career longevity. However, severe EIPH severity had a negative effect on the number of subsequent race starts that horses had after undergoing post-race tracheoendoscopic examination in the spring and/or summer of 2024.

This report documents the findings from a series of observational studies, each of which was designed to evaluate **the association between furosemide use and thoroughbred racehorse career longevity.** Because a controlled experimental design was not feasible, we relied on observational studies which had limitations due to the inevitable existence of biases that were impossible to control. Because of these limitations, we constructed a 3-

pronged or triangulation approach in order to substantiate the veracity of our findings based on the principle of consistency; ie, despite differences in the nature of the studies, all 3 had findings in common with respect to determining whether furosemide use had an impact on the career longevity of American thoroughbred racehorses. Collectively, data from 45,001 horses that had 737,628 race starts were received and analyzed across three cohorts. This provided substantial statistical power. The findings and conclusions we have drawn are summarized in the following paragraphs. These are based on the results of multiple analyses of the collected data.

The study sought to test 2 hypotheses:

1. That thoroughbred racehorses in the lower quintile of pre-race treatment with furosemide per lifetime start have significantly fewer starts and shorter careers.

2. That horses diagnosed with EIPH grades =3 or =4 have significantly fewer starts post-diagnosis than horses with EIPH grades < 3.

Study #1

Aim: *To compare the effect of the proportion of a horse's starts in which it received pre-race furosemide on the longevity of its racing career in terms of lifetime race starts and career duration from 2011 – 2019 in the cohort of horses that had their first start in 2011*.

Methods

Jockey Club performance data on 17,451 Thoroughbreds from the 2008-2009 foaling cohorts that began racing in the United States in 2011was examined to assess whether historical race day medication practices allowed inference regarding the impact of furosemide on career duration as the dataset contained baseline information on each individual horse and information on the individual races for each horse throughout their careers until the end of 2019, thus providing up to 9 years of follow-up data.

Statistical Analyses

The evaluated outcome was career longevity measured as total career starts, with career termination as the event of interest. Horses still actively racing at the end of the observation period were right-censored at their last observed start, as their ultimate career length could not be ascertained. Given their foaling years, horses were 10-11 years old by the end of 2019, and the number still racing was minimal.

The analyses used 3 models to assess how furosemide administration influenced career length: the Cox proportional hazard model, Kaplan-Meier survival curves, and a marginal structural model.

Results and Discussion

The models provided no meaningful reliable insight to achieving the aim of the study for the following reasons and are therefore not presented in detail.

Furosemide use in the 2011 cohort was effectively ubiquitous by the early stages of racing participation, leaving no valid untreated comparison group and no clinically meaningful variation in exposure. This limitation arose from historical medication practices themselves and could not be resolved through alternative modeling strategies due to fundamental limitations present in this dataset. 14,202 horses (81.4%) received furosemide in 100% of their starts and 2,767 horses (15.9%) received it in 1-99% of their starts. Only 2.8% of horses (482 of 17,451) never received furosemide during their careers. The few horses that never received furosemide was not a random sample of untreated horses. They were predominantly horses that failed early in their careers, before trainers had the opportunity to place them on furosemide. 35.5% had only one career start. This created severe selection bias. By the third career start, 97% of horses had received furosemide at least once. This near-universal adoption eliminated any meaningful comparison group.

In addition to the near universal adoption of furosemide early in horses' racing careers, the available data lacked critical clinical covariates that simultaneously influenced both furosemide use and career continuation. The most relevant unmeasured confounders were:

1. **Underlying musculoskeletal health:** Horses with subclinical orthopedic issues may have been managed differently, affecting both medication decisions and career duration.

2. **Subclinical injury status:** Early indicators of developing injuries were not captured in race records but likely heavily influenced trainer decisions about medication and racing continuation.

3. **Trainer-driven veterinary decision-making:** Trainers' clinical judgment about when to use furosemide was informed by private information about a horse's condition that was not available to us.

4. **Management intensity:** The overall level of veterinary attention and preventive care a horse received likely affected both furosemide use patterns and career outcomes.

<u>Conclusions and Relevance</u>

1. **Near universal furosemide use (97% by race 3) eliminated valid comparison groups.** With only 2.8% of horses never receiving furosemide, there was insufficient variation to estimate treatment effects.

2. **The 'Never Furosemide' group was systematically different from treated horses.** With 35.5% one-and-done horses (vs. 4.3% in the Always Furosemide group), this comparison group consisted largely of early failures who never had the opportunity to be treated with furosemide.

3. **These limitations cannot be overcome with statistical adjustments.** The problems were structural, not analytical as a valid "no furosemide" comparison group essentially did not exist.

We were unable to determine whether regular pre-race or pre-breeze treatment with furosemide did or did not have a protective effect on horses' health and promoted career longevity.

Study #2

Aim 2: *To compare the effect of pre-race furosemide administration on the number of race starts undertaken in the first 4.5 years of their racing careers by horses that were 2-year-olds or 3-year-olds having their first starts in 2015 and the equivalent cohorts in 2021*.

Introduction

Race-day treatment with furosemide (Lasix) had been used in North American Thoroughbred racing since the 1970s to attenuate exercise-induced pulmonary hemorrhage (EIPH). Despite decades of use, whether routine furosemide administration meaningfully impacted career durability remained contentious. The 2021 implementation of furosemide restrictions for two-year-old horses at select US racetracks created a natural experiment. For the first time, researchers could observe what happened to career outcomes when pre-race furosemide use was prohibited; not as a matter of trainer choice, but as a matter of regulatory policy.

Policy Context and Identification Strategy

The 2021 furosemide ban was applied selectively: race-day furosemide was prohibited for 2-year-olds at select (not all) tracks, while 3-year-old first-time starters remained unexposed.

Methods

Two distinct but related causal questions were asked:

1. Did the 2021 furosemide ban harm the career longevity of 2-yr-old horses exposed to it, based on total race starts up to August 31, 2025? This question related to the *effect of the policy*. The availability of cohorts of contemporaneous control groups (2-year-olds that received furosemide and 3-year-old first-time starters who were never subject to the ban in 2021) facilitated comparisons that provided the answer to this question.

2. Did denying furosemide to a horse *causally* affect its career length? This question pertained to the *effect of the drug;* ie, did furosemide itself (rather than factors correlated with its use) extend racing careers.

Although the Jockey Club data anonymized tracks, the tracks that banned furosemide were identified by determining 2-year-old furosemide usage at the anonymized track. If this usage was 0%, the track was identified as a "ban track".

Statistical Analyses

The question related to the *effect of the policy banning furosemide administration to 2-year-olds* (ie, did the policy banning furosemide administration to 2-year-olds *do any harm*?) was answered using a Difference-in-Differences (DiD) analysis by comparing treated horses (2-year-old starters) to contemporaneous controls (3-year-old first-time starters) who were never subjected to the ban. The 3-year-old first-time starters served as an internal control group. They raced under identical industry conditions, with the same trainers, for the same purses, with same pandemic-era disruptions, *except for one key difference: they were never subject to the 2-year-old furosemide ban*. This structure sharply limited statistical confounding and enabled the policy effect to be isolated.

To answer the question regarding the *drug effect,* ie, whether furosemide itself (rather than factors correlated with its use) extended racing careers, an Instrumental Variables (IV) used track ban status as an instrument that generated exogenous variations in furosemide exposure. This was possible because the partial ban on furosemide also created variation *across tracks*: at ban tracks, furosemide was prohibited for 100% of 2YO races; at non-ban tracks, it was permitted and used in approximately 78% of races. This variation formed the basis of the IV analysis.

Additionally, comparable data was available for 2- and 3-year-olds that had their first race starts in 2015 when there was no ban of any type on the pre-race administration of furosemide. Horses in the 2015 cohort were followed until December 31, 2019. Those in the 2021cohort were tracked through August 31, 2025, providing up to 4 years of post-exposure follow-up when the horses from each cohort that were stilling racing would have been 6- or 7-years old, depending on their ages in 2015 or 2021.

Results and Discussion

Across both cohorts, horses debuting at age 2 consistently accumulated more lifetime starts than those debuting at age 3. (Table 1). This gap likely reflected opportunity and, possibly, biology; ie. an earlier debut provided more time to race and biological factors that might have delayed the start of a horse's racing career until it was 3-years-old may have also impacted the longevity of that career. Put another way, an earlier debut provided more time to race and biological factors that might have delayed the start of a horse's racing career until it was a 3-year-old may have also impacted the longevity of that career.

The gap between 2YO and 3YO starters narrowed by only 0.27 starts, a small, negative change that was not statistically significant (Table 2). If banning furosemide for 2-year-olds

materially harmed durability, the gap should have narrowed substantially as 2-year-old careers shortened relative to those of 3-year-olds. **This did not occur (p = 0.41).**

Table 1. Career Starts by Debut Age and Cohort

Cohort	Debut Age	N	Mean Lifetime Starts	SE
2015	2YO	7,562	**19.0**	0.16
2015	3YO	6,418	**15.2**	0.17
2021	2YO	7,369	**18.0**	0.15
2021	3YO	5,842	**14.4**	0.16

Table 2. DiD Calculation: The 2YO – 3YO Gap by Cohort

Cohort	2YO Mean	3YO Mean	Gap (2YO – 3YO)
2015 (Pre-ban)	19.0	15.2	**+3.89 starts**
2021 (Post-ban)	18.0	14.4	**+3.63 starts**

The first phase of the IV analysis examined whether the instrument (ban-track status) actually affected treatment (furosemide denial) and found that racing at a ban track increased the probability of furosemide denial by approximately 78 percentage points Table 3).

Table 3. Furosemide Denial Rates by Track Type (2YO Races, 2021 Season)

Track Type	Denial Rate	N (races)
Ban Tracks	**100%**	12,331
Non-Ban Tracks	**22%**	9,392

When trainer fixed effects were considered, there was a suggestion that the ban on furosemide may have actually increased career starts by +1.04. However, the 95% Confidence interval was -0.85 - +2.93 with p = 0.28, indicating that this was not a significant finding.

Conclusion

Prohibiting routine race-day furosemide administration to 2-year-olds did not harm their racing careers as it did not have a substantial effect on the number of career starts over a horse's first five years of racing. There was no significant change in the difference in career starts of 2- and 3-year-olds first-time starts in the 2015 and 2021 cohorts. The persistent

advantage of 2-year-old starters over 3-year-old first-time starters in terms of lifetime starts reflected debut timing and opportunity, *not pharmacological protection*.

Study #3

Aim: *To assess the impact of EIPH severity on the number of subsequent race starts and career duration, through August 31, 2025 (a period of 12-18 months), of all horses that were tracheoendoscopically examined after racing in the ≈ 6-month period from February 15 to August 31, 2024.*

Introduction

Pre-race administration of furosemide reduces the severity of EIPH in most racehorses. In Australia, where the use of furosemide is prohibited in all racing, Thoroughbred racehorses with severe EIPH (Grades ≤3) reportedly race less frequently and have shorter careers. Whether this is the case in the United States has not been assessed.

Methods

2,561 videorecordings of post-race tracheoendoscopic veterinary examinations of thoroughbred racehorses were evaluated by 3 veterinarians for evidence of EIPH, and its severity graded on a scale of 0-4 using whole numbers only, with Grade =4 representing EIPH of greatest severity and a Grade =0 indicating that there was no visual evidence of EIPH. Horses were classified by their **maximum (worst) EIPH grade** observed across all endoscopic examinations, not necessarily their first observation. This ensured classification by the true severity ceiling.

The relationship between maximum EIPH grade and a) periods between races and b) declines in class of races, was analyzed for the period between the date the maximum grade was assigned and Augst 31, 2025. Data from 22,873 races were evaluated. Absence durations were calculated from consecutive race dates within each horse's record.

Statistical Analysis

To assess the relationship between the most severe EIPH grade and the number of subsequent race starts, the Poisson regression was used to model racing frequency with an offset for follow-up time (log-transformed years), yielding incidence rate ratios (IRR) comparing each EIPH group to the reference (Grade 0-1). Age at scoping was included as a covariate.

To examine whether EIPH severity, measured by consensus endoscopic grading, was associated with prolonged absence from racing, the outcome variables (>90 days or >120 days to next start) were depicted as binary indicators for whether the horse was absent >90 or >120 days before their next recorded start. Horses in the >120 days cohort were also included in the > 90 days group. Absence variables were calculated from consecutive race dates within each horse's record. Horses with no subsequent race in the dataset were coded as missing, leaving 20,863 race-level observations for prolonged absence analyses. The chi-square (χ^2) test or Fisher's exact test when expected counts < 5 were used with $p < 0.05$ considered significant.

The association between EIPH severity grade and whether the class of race in which a horse competed was also assessed as a negative relationship between the 2 could be interpreted as a negative effect of severe EIPH. Sustained class decline was defined as a drop in race class level from which the horse never recovered and represented functional career deterioration while the horse was still actively racing. Race classes were assigned the following: stakes = 5, allowance/AOC = 4, maiden special weight = 3, claiming = 2, maiden Claiming = 1. A class drop was considered "permanent" if the horse never subsequently raced at the prior class level or higher. Only the first permanent drop was counted as the event.

Results and Discussion

Fifty-three veterinarians provided 2,460 videos of sufficient quality from 2,010 horses for consensus EIPH Grades to be determined (Tables 1 and 2). Fifty-six horses (2.7%) were reclassified to higher severity groups compared to using their first recorded grade. The mean follow-up performance period was 468 days.

Table 1: Consensus EIPH Grade Distribution

Grade	Count	Percentage	Cumulative %
0 (None)	1,118	45.4%	45.4%
1 (Minimal)	704	28.6%	74.1%
2 (Mild)	398	16.2%	90.3%
3 (Moderate)	177	7.2%	97.5%
4 (Severe)	63	2.6%	100.0%

Table 2. Age at Scoping and EIPH Grade

EIPH Grade	2yo	3yo	4yo+	Total
0-1	122	601	724	1,447
2	16	119	204	339
3-4	17	70	137	224
Total	155	790	1,065	2,010

Note: Age distribution: 7.7% 2-year-olds, 39.3% 3-year-olds, 53.0% 4+ years

Career Starts

The number of races subsequent to endoscopy according to maximum EIPH grade is shown in Table 3. After adjusting for variations in follow-up time (log-transformed years), comparing each EIPH group to the reference (Grade 0-1) and age at scoping, racing frequency declined monotonically with increasing EIPH severity (Table 4).

When the Poisson model was adjusted for age at endoscopy, both higher EIPH grade groups showed significantly reduced racing rates compared to horse receiving EIPH grades 0-1. (Table 5).

Table 3. Number of Races Following Assignment of Most Severe EIPH Grade

Grade	N Races	%
0	10,681	46.7%
1	6,469	28.3%
2	3,502	15.3%
3	1,676	7.3%
4	545	2.4%

Table 4. Association between EIPH Grade and Subsequent Career Starts

EIPH Grade	Horses	Mean Starts	Median	Starts/Year
0	914	10.3	9	8.0
1	581	9.8	8	7.7
2	315	9.4	8	7.3
3	148	8.9	7.5	7.1
4	52	8.1	7	6.5

Table 5.

Variable	Rate Ratio	95% CI	p-value
Grade 0-1	ref	-	-
Grade 2	0.91	0.87-0.95	<0.001
Grade 3-4	0.85	0.81-0.89	<0.001
Age (per year)	1.07	1.06-1.08	<0.001

Higher EIPH severity was associated with reduced post-scoping racing frequency. After adjusting for age at scoping, Grade 2 horses raced 9% less frequently than Grade 0-1 horses and Grade 3-4 horses raced 15% less frequently. The most severe bleeders (Grade 4) averaged 6.5 starts per year compared to 8.0 for Grade 0 horses — a 19% reduction. Each additional year of age was associated with a 7% increase in racing frequency. Unlike analyses of single-race performance or binary retirement outcomes, which showed non-monotonic patterns (see below), racing frequency demonstrated a clear severity gradient,

suggesting this metric may best capture the cumulative career impact of EIPH on lifetime starts by a horse and its career duration.

Career Duration

Absences of >90 and >120 days, respectively, from racing had no association with EIPH grade of severity. Despite excellent statistical power (>20,000 observations), absence rates for both periods were virtually identical across all EIPH severity levels (5.45±0.2% for >90 days; p = 0.91 (Table 6); 3.63±0.4% for >120 days; p = 0.10 (Table 7)).

Table 6. > 90-Day Absence

Grade	N	Absent > 90d	%
0	9,769	535	5.5%
1	5,899	311	5.3%
2	3,172	180	5.7%
3-4	2,023	108	5.3%

Table 7. > 120-Day Absence

Grade	N	Absent >120d	%
0	9,769	387	4.0%
1	5,899	214	3.6%
2	3,172	119	3.8%
3-4	2,023	63	3.1%

Class Drop

EIPH severity showed no association with a sustained drop in the class of races that horses competed in regardless of their EIPH grade where drop rates were virtually identical (Table 8) or their age (Table 9).

Table 8. Association between EIPH Grade and Permanent Class Drop

EIPH Group	N	Events	Rate (%)
0-1 (Reference)	1,447	593	41.0
2	339	142	41.9

3-4	224	90	40.2

Table 9. Association between Age, EIPH Grade and Permanent Class Drop

Age Group	EIPH 2 vs 0-1	EIPH 3-4 vs 0-1
≤3yo	1.05 (0.78, 1.42), p = 0.73	1.28 (0.90, 1.81), p = 0.16
>3yo	1.11 (0.88, 1.40), p = 0.39	1.02 (0.77, 1.37), p = 0.88
Overall	1.06 (0.88, 1.27), p = 0.54	1.09 (0.88, 1.37), p = 0.43

Limitations

1. Single EIPH measurement: Horses were assigned a consensus grade from one scoping event. EIPH severity fluctuates between races, so this may not have captured a horse's true bleeding tendency.

2. Absence as a proxy: Prolonged absence may reflect many factors (training decisions, minor injuries, scheduling) beyond EIPH-related pathology.

3. Censoring: Horses with no subsequent race following endoscopy (n = 2,010) were excluded from absence analyses. If horses with EIPH grades ≥ 3 were more likely to be retired, this could bias the results.

4. Confounding: This analysis did not adjust for potential confounders such as age, trainer, track surface, or medication status.

Conclusion:

EIPH severity was clearly associated with racing frequency but not with prolonged (> 90 days or >120 days) absences from racing, or with sustained class decline which was used as an objective measure of competitive performance trajectory. These findings suggested that while EIPH did not appear to impair racing career outcomes when measured by some functional performance metrics, horses with severe EIPH raced less often than horses with either no or only mild endoscopic EIPH grades.

Glossary (in alphabetical order)

A Plain-Language Guide for Understanding Statistical Nomenclature

This glossary explains the statistical and causal inference terminology used in the instrumental variable analysis of furosemide's effect on racing career longevity.

Causal Effect

The change in an outcome that is directly caused by a treatment or intervention, holding all else equal. It answers: "What would happen if we changed only this one thing?"

In this study: The causal effect of furosemide denial is the change in career starts that results specifically from denying furosemide, separate from any other differences between horses.

Cohort

A group of individuals who share a common characteristic, typically defined by time of birth or entry into a study.

In this study: The 2021 cohort includes horses foaled in 2019 who turned 2 years old in 2021 (when the ban was implemented).

95% Confidence Interval (CI)

An interval constructed from the data using a procedure that, under repeated sampling, would contain the true parameter 95% of the time. The interval does not assign a probability to the parameter itself; instead, it reflects uncertainty in the estimate due to sampling variability

In this study: The 95% CI of (−0.35, +0.70) shows the range of effect sizes that are consistent with the data, given the statistical method we used.

Confounding / Confounder

A variable that affects both the treatment and the outcome, creating a misleading association between them. Confounders make it look like a treatment causes an effect when the real cause is something else.

In this study: EIPH severity is a confounder: horses with severe bleeding are more likely to receive furosemide and may have shorter careers due to lung damage. This makes it look like furosemide is associated with shorter careers, when the real cause is the underlying disease.

Covariate / Control Variable

A variable included in the analysis to account for differences between groups.

In this study: We control for trainer (via fixed effects), number of 2YO races, and log sale price.

Fixed Effects

A method that controls for all characteristics (observed and unobserved) that are constant within a group. It compares outcomes within groups rather than between groups.

In this study: Trainer fixed effects control for all stable trainer characteristics—training methods, medication practices, horse selection—by comparing horses trained by the same trainer.

Follow-Up Period

The time window over which outcomes are measured.

In this study: We measure career starts from ages 3-6 (truncated follow-up to ensure comparability across cohorts).

Instrumental Variable (IV)

A variable that affects the treatment but has no direct effect on the outcome except through the treatment. It provides a source of "as if randomized" variation in treatment that can be used to estimate causal effects.

In this study: The 2021 furosemide ban is our instrument. It strongly affects whether horses receive furosemide (first stage) but should only affect career length through its effect on furosemide use (exclusion restriction).

Instrument

Shorthand for instrumental variable. The tool we use to isolate exogenous variation in treatment.

In this study: Track location (ban vs. non-ban) during the 2YO year is our instrument.

Observational Data

Data collected by observing what happens naturally, without researcher intervention. Contrast with experimental data where the researcher assigns treatment.

In this study: Racing records are observational—we observe which horses received furosemide, but we didn't randomly assign it.

Outcome

The result we measure to assess the effect of treatment.

In this study: The outcome is career starts after the 2YO year (ages 3-6).

Percentage Point (pp)

The arithmetic difference between two percentages. If something goes from 20% to 78%, that's a 58 percentage point increase (not a 58% increase).

In this study: The ban increased furosemide denial from 21.5% to 100%, a 78.5 percentage point difference.

p-value

The probability, *assuming the null hypothesis is true*, of obtaining a test statistic as extreme or more extreme than the one observed. A small p-value indicates that the observed data would be unlikely if the null hypothesis were correct, but it does not measure the probability that the null hypothesis is true.

In this study: A p-value of 0.52 means there's a 52% chance of seeing our results even if furosemide denial has no effect—not strong evidence of an effect.

Sample Size (N)

The number of observations in the analysis.

In this study: N = 21,723 races from 7,369 horses in the 2021 cohort.

Standard Error (SE)

A measure of uncertainty in an estimate. Smaller standard errors indicate more precise estimates.

In this study: The standard error of 0.27 tells us how much our estimate might vary if we repeated the study with a different sample.

Statistical Significance

A result is statistically significant if the p-value is below a threshold (typically 0.05) or equivalently if the 95% confidence interval excludes zero. It does not mean the effect is important.

In this study: Our IV estimate is not statistically significant because the CI includes zero and p > 0.05.

Treatment

The intervention or exposure whose effect we want to estimate.

In this study: The treatment is furosemide denial (racing without furosemide).

APPENDIX G: DeNotta Final Report

HISA FUROSEMIDE ADVISORY COMMITTEE
FINAL REPORT

- Grant ID and Title of Project: **Effects of repeated furosemide administration on electrolyte homeostasis and bone density in healthy adult exercising Thoroughbreds**
- Principal Investigator: Sally DeNotta, DVM, PhD, DACVIM; University of Florida College of Veterinary Medicine
- Date of report: February 15, 2026

Study Summary

This study investigated the physiologic effects of chronic furosemide administration on young, fit Thoroughbred racehorses. The primary objective of the study was to examine how long-term furosemide therapy affects extracellular and intracellular electrolyte balance, parathyroid and calcium homeostasis, and urinary electrolyte excretion. The second objective of the study was to determine if horses receiving serial doses of furosemide develop pharmacologic tolerance and reduced physiologic response to treatment. The third objective of the study was to examine the effects of repeated administration of furosemide on bone density and strength using minimally invasive methods of measurement DEXA scan and Bonescore® OsteoProbe® microindentation. The fourth and final objective of the study was to evaluate the effects of chronic furosemide administration on cardiac function before, during, and after exercise.

4-year-old study mare, Sky, exercising on the UF EquiGym treadmill

UF College of Veterinary Medicine
UNIVERSITY of FLORIDA

This work was completed as a prospective, blinded, placebo-controlled study in which 9 horses were administered 250 mg (5 mL) intravenous furosemide weekly for 14 weeks (treatment), while 6 horses were administered a similar volume of weekly saline placebo (control) (Figure 1). Horses were matched by bodyweight and randomly allocated to groups, and study personnel were blinded to group identification throughout the study. Study horses were trained to run on an EquiGym high-speed equine treadmill and maintained fitness throughout the study with 3x/week exercise sessions. All horses were group housed in outdoor paddocks at the University of Florida College of Veterinary Medicine throughout the study, and all were fed the same diet consisting of free choice grass hay, commercial pelleted equine feed, and maintenance dose oral vitamin E supplementation.

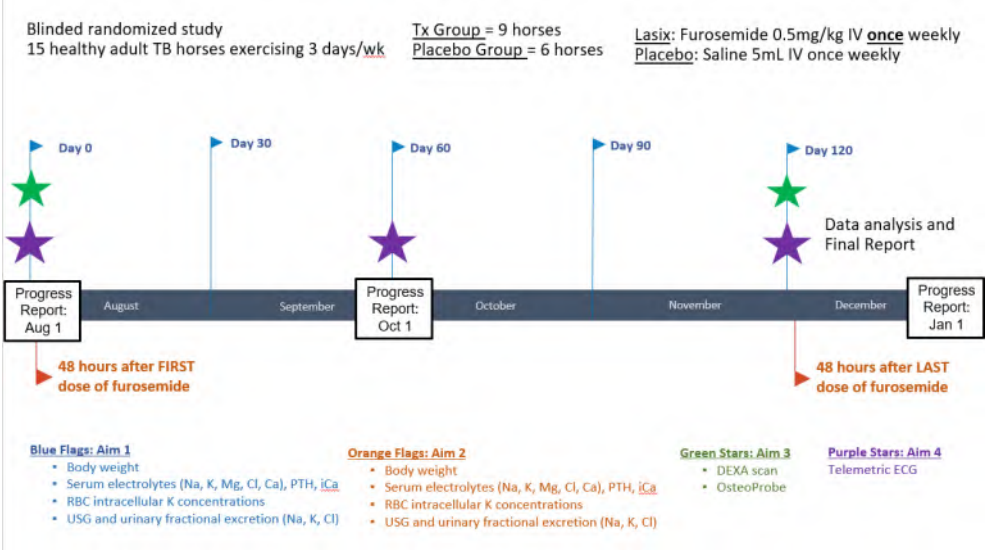


Figure 1: Prospective, blinded, placebo-controlled study design

The study herd consisted of 8 Thoroughbred mares and 7 Thoroughbred geldings 3-5 years of age. To account for study horse attrition and achieve the 12-horse group size identified as necessary by our pre-study power analysis, 16 horses were initially enrolled in this study. One horse was unenrolled during the pre-study fitness period due to chronic intestinal disease non-responsive to standard medical care. The remaining 15 horses remained in the study through completion. During the study period, sporadic lameness issues (hoof abscesses, sole bruises, etc) were treated in routine fashion and horses were returned to work as quickly as medically appropriate. One mare displayed recurrent hind limb lameness and weakness localized to the pelvis when exercised according to study protocol, but was able to maintain adequate soundness and complete the study at a reduced exercise intensity. All horses were screened and confirmed to be negative for bisphosphonates by the UK Equine Analytical Chemistry

Laboratory prior to study start.

Blood and urine samples were serially collected throughout the study, and samples were processed and stored in a -80C laboratory freezer for batch analysis upon study completion. Electrolyte assays were performed in the UF College of Veterinary Medicine Clinical Pathology Lab, while serum PTH and iCa assays were performed at the Michigan State Veterinary Diagnostic Lab. Horse hydration status was assessed via serial body weight measurements as well as packed cell volumes (PCV) and urinary specific gravity (USG). At three timepoints (pre-study, mid-study, post-study), horses underwent telemetric ECG evaluation to assess for any underlying cardiac arrhythmias (Figure 2).

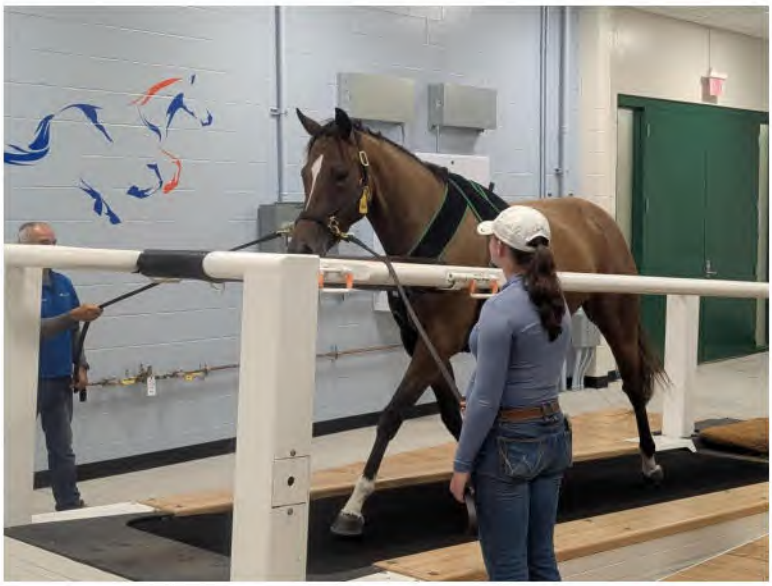
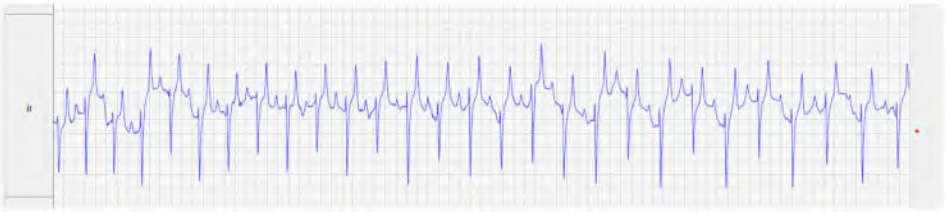
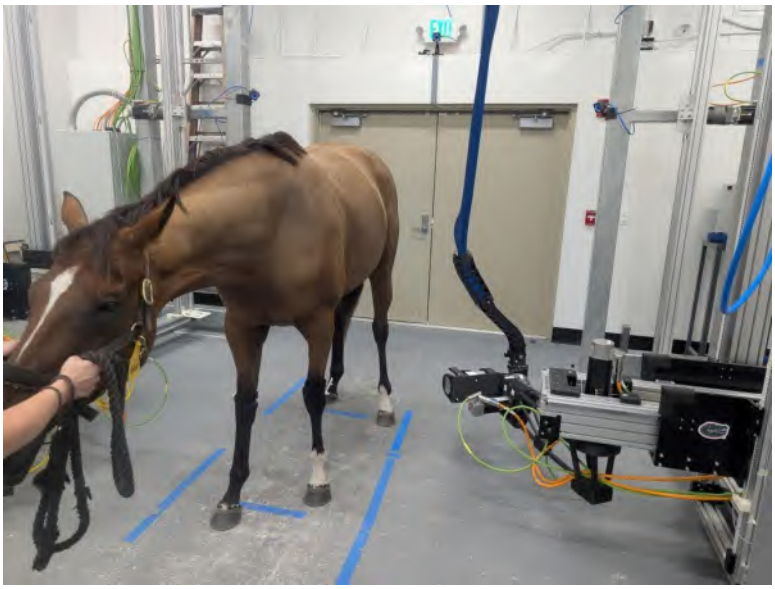


Figure 2: 5-year old study mare, Olive, wearing telemetric device to obtain exercising ECG (below)



To evaluate bone density, DEXA scans were performed immediately before and immediately after study completion (Figure 3). To evaluate bone strength, BoneScore® Osteoprobe® micro-indentation was performed immediately before and after study completion (Figure 4). This novel method for bone strength evaluation is new to the equine veterinary realm, and this study is the first to allow for comparison between traditional DEXA scan bone density and micro-indentation mechanical bone strength.



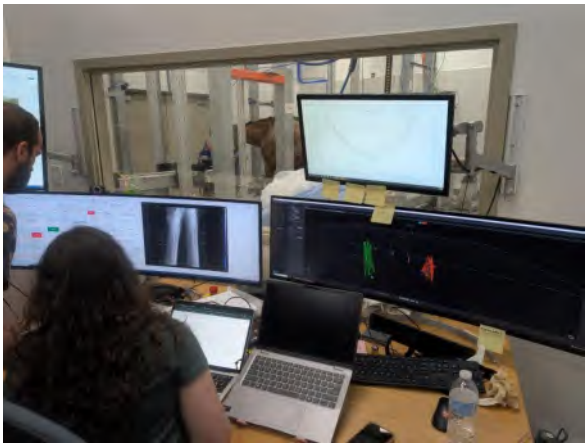


Figure 3: 5-year-old study mare, Olive, undergoing baseline DEXA bone density scan (above) in UF Robotics Imaging Laboratory (left)

UF College of Veterinary Medicine
UNIVERSITY of FLORIDA

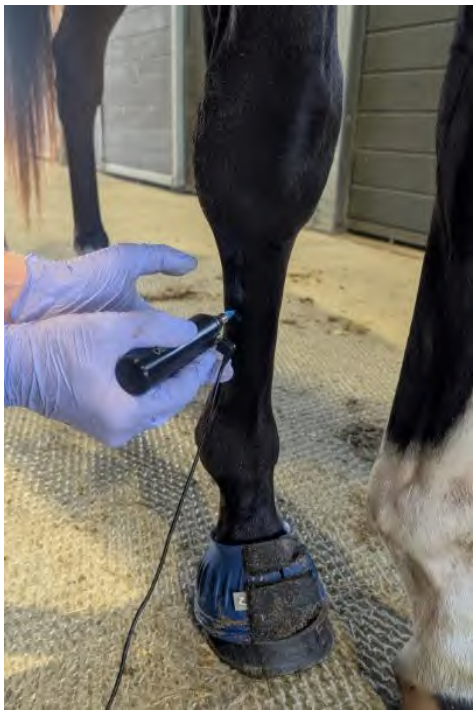


Figure 4: 4-year-old study gelding, Noah, undergoing BoneScore® OsteoProbe® bone strength measurement (left) with corresponding serial measurement data output (below)

Additional Study Period:
Following completion of the study outlined above, an additional study period added to further investigate Aim 2 and the common anecdotal observation that horses develop a tolerance for furosemide after repeated doses, i.e., the medication has less profound physiologic effects in horses that have received the medication previously. To test this hypothesis, body weight, urine and blood samples were collected for PCV, USG, electrolyte analysis, and urinary fractional excretions from all study horses on day 100 (9 horses that had received furosemide weekly for 14 weeks and 6 horses that had received only placebo), after which all 15 horses were administered 250 mg (5mL) of intravenous furosemide. Blood was collected at 7 hours and 24 hours for electrolyte analysis. Body weight, blood and urine samples were then obtained 48 hours after furosemide administration, and the response to treatment was compared between the two groups.

Overall Study Findings and Conclusions:
In this study, only plasma total solids concentrations were found to differ between treatment and control horses at study days 30 and 60, with treated horses having lower total solids concentrations (see Aim 1a below). No statistically significant differences were observed between treatment and control horses in any of the other measured parameters at any

timepoint, including body weight, PCV, USG, plasma K, Na, Cl, Ca, Mg, plasma parathyroid hormone concentrations, plasma ionized calcium concentrations, urinary fractional excretion of K, Na, Cl, and intracellular K concentrations. Horses receiving chronic furosemide treatment did not demonstrate a blunted physiologic response to treatment when compared to previously untreated horses. No differences in heart rate before, during, or after exercise were observed between groups, and cardiac arrythmias were not observed with more frequency in treated horses when compared to controls.

Bone material strength index scores (BMSi) as determined by Osteoprobe micro-indentation were not different between groups at pre- or post-study timepoints. While the mean BMSi increased in both groups over the course of the study, the increase was less pronounced in treatment horses when compared to controls. Although this difference was not statistically significant in this cohort, further investigation to better understand how chronic furosemide affects bone strength is warranted. *DEXA scan bone density measurements are pending at the time of this report. Once available, Osteoprobe and DEXA scan measurements will be compared, the results of which will be supplied as an addendum to this report.*

Overall, the results of this study suggest that weekly administration of 250 mg of intravenous furosemide to young exercising Thoroughbreds for 14 weeks does not affect long term hydration status, electrolyte homeostasis, or bone strength. Furthermore, horses administered weekly doses of furosemide at 250 mg intravenously did not demonstrate a reduced physiologic response when compared to horses that had only received placebo. Detailed results of each study aim are provided below.

RESULTS BY STUDY AIM

Aim 1a: Investigate the effects of repeated furosemide administration on hydration status and parathyroid response in healthy exercising Thoroughbreds.

Hypothesis: Horses administered weekly furosemide for 90 days will experience chronic diuresis resulting in lower body weights and higher PCV and USG when compared to control horses. Chronic calcium wasting via the kidneys will result in lower ionized calcium concentrations and elevated parathyroid hormone production in treated horses when compared to controls.

Key results:

- Total solids concentrations were found to differ between treatment and control horses at timepoint 30 and 60 days (p=0.015; 0.013, respectively), with treated horses having lower total solids concentrations (Figure 5 and 6).
- No statistically significant differences were observed between treatment and control horses in any of the other measured parameters at any timepoint, including body weight (BW), PCV, USG, serum parathyroid hormone concentrations (PTH), serum ionized calcium concentrations (iCa) (Figure 5).

Figure 5:

Comparisons of body weight (BW), packed cell volume (PCV), total solids (TS), urine spec gravity (USG), parathyroid hormone (PTH), ionized calcium (iCA) between groups on Days 0, 30, 60, and 90.

Variable	Control horses n=6				Treatment horses n=9				p
	Day 0	30	60	90	Day 0	30	60	90	
BW kg	532.1 ± 39.8 (490.38, 573.95) 531.1 492.7, 561.7	547.8 ± 35.9 (510.10, 547.83) 543.5 521.0, 575.0	556.5 ± 34.6 (520.17, 592.83) 561.0 518.7, 580.0	558.5 ± 39.3 (517.23, 599.77) 555.0 525.0, 586.2	523.5 ± 38.8 (493.71, 553.40) 524.0 507.0, 555.5	533.6 ± 44.6 (499.60, 568.18) 536.0 510.5, 569.0	535.1 ± 46.4 (499.35, 570.84) 533.0 508.5, 566.0	546.3 ± 46.7 (510.40, 582.27) 550.0 522.0, 570.5	0.529
PCV %	38.1 ± 3.7 (34.27, 42.06) 37.5 35.0, 40.5	40.3 ± 5.2 (34.79, 45.87) 40.0 35.5, 45.0	40.3 ± 7.3 (32.57, 48.09) 38.0 35.7, 43.7	42.3 ± 6.8 (35.19, 49.47) 43.0 36.0, 48.0	37.1 ± 3.2 (34.57, 39.64) 35.0 35.0, 40.5	35.6 ± 1.8 (34.22, 37.10) 35.0 35.0, 36.0	36.8 ± 3.5 (34.18, 39.59) 35.0 35.0, 38.5	41.4 ± 4.3 (38.11, 44.77) 41.0 37.5, 46.0	0.146
TS g/dL	6.6 ± 0.8 (5.79, 7.47) 7.0 5.7, 7.1	7.2 ± 0.2[a] (6.99, 7.50) 7.2 7.0, 7.5	7.2 ± 0.4[1] (6.81, 7.68) 7.2 6.9, 7.4	7.4 ± 0.2 (7.12, 7.74) 7.5 7.2, 7.5	6.4 ± 0.6 (5.90, 6.91) 6.2 5.9, 7.0	6.9 ± 0.1[b] (6.89, 7.07) 7.0 6.9, 7.1	6.7 ± 0.3[2] (6.46, 6.95) 6.6 6.5, 6.9	7.1 ± 0.3 (6.88, 7.47) 7.0 6.9, 7.6	0.008
USG	1.03 ± 0.008 (1.02, 1.04) 1.03 1.02, 1.04	1.03 ± 0.01 (1.01, 1.04) 1.03 1.02, 1.03	1.03 ± 0.009 (1.02, 1.04) 1.03 1.02, 1.04	1.03 ± 0.008 (1.02, 1.04) 1.03 1.02, 1.04	1.02 ± 0.01 (1.01, 1.03) 1.03 1.01, 1.03	1.03 ± 0.006 (1.02, 1.03) 1.03 1.02, 1.03	1.02 ± 0.009 (1.01, 1.03) 1.02 1.01, 1.03	1.03 ± 0.007 (1.02, 1.03) 1.03 1.02, 1.03	0.204
PTH	25.3 ± 36.6 (-13.0, 63.86) 12.4 7.3, 36.8	13.1 ± 11.0 (1.48, 24.71) 8.9 3.9, 25.5	9.7 ± 2.7 (6.88, 12.61) 9.3 8.2, 12.1	8.5 ± 5.3 (2.93, 14.22) 8.1 3.3, 12.4	16.2 ± 15.4 (4.37, 28.13) 8.5 6.5, 24.8	11.4 ± 5.4 (7.23, 15.58) 9.5 8.2, 16.6	19.0 ± 24.3 (0.32, 37.78) 7.6 5.1, 22.4	9.8 ± 6.8 (4.55, 15.09) 6.6 4.8, 14.5	0.990
iCA mmoL/L	1.52 ± 0.04 (1.47, 1.56) 1.52 1.50, 1.55	1.59 ± 0.09 (1.49, 1.68) 1.56 1.52, 1.65	1.56 ± 0.03 (1.52, 1.59) 1.57 1.52, 1.58	1.49 ± 0.11 (1.36, 1.61) 1.53 1.41, 1.56	1.54 ± 0.08 (1.48, 1.60) 1.57 1.47, 1.61	1.54 ± 0.08 (1.48, 1.61) 1.58 1.50, 1.61	1.52 ± 0.05 (1.48, 1.56) 1.54 1.46, 1.57	1.56 ± 0.03 (1.53, 1.58) 1.56 1.54, 1.58	0.852

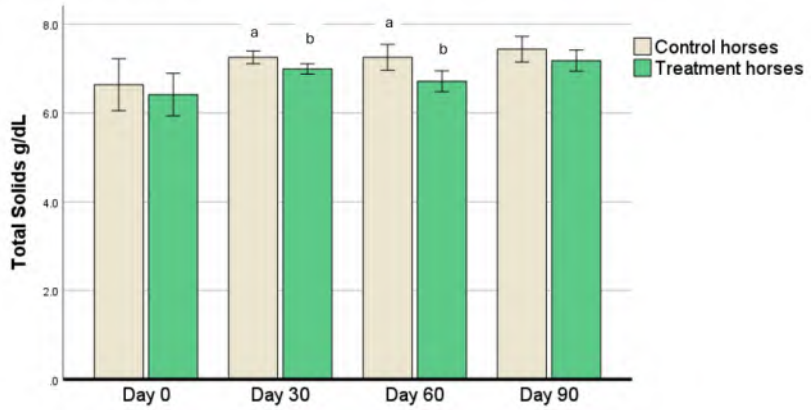
*Data are reported as mean ± SD, 95% confidence intervals, median, first and third quartiles

[ab]Total solids (TS), on Day 30 groups with different superscripts are different (p = 0.015)

[12]Total solids (TS), on Day 60 groups with different superscripts are different (p = 0.013)

Figure 6:

Comparisons of total solids (g/dL) between groups on Days 0, 30, 60, and 90. White bars = control horses. Black bars = treatment horses. Data are reported are mean ± SE. On Day 30 and Day 60, total solids in control horses and treatment horses are different ($p < 0.05$).



Aim 1b: Investigate the effects of repeated furosemide administration on blood electrolyte balance, urinary electrolyte excretion, and intracellular potassium concentrations in healthy adult exercising Thoroughbreds

Hypothesis: Horses administered weekly furosemide for 90 days will experience prolonged elevations in urinary electrolyte fractional excretions leading to chronic electrolyte loss via the kidneys and extracellular depletion of sodium, chloride, calcium, magnesium and potassium, with concurrent intracellular depletion of potassium.

Results:

- No statistically significant differences were observed between treatment and control horses in any of the measured parameters at any timepoint, including plasma K, Na, Cl, Ca, Mg, urinary fractional excretion of K, Na, Cl, and intracellular K concentrations (Figure 7).
- Among control horses, Ca concentrations on Day 0 were different from Day 30 (p= 0.022), Day 60 (p=0.019) and Day 90 (p=0.022). Among treatment horses, Ca concentrations were different on Day 60 from Day 90 (p=0.011) (Figure 7 and 8).
- Ca concentrations were not different between treatment and control groups (p=0.206) (Figure 7 and 8).

Figure 7:

Comparisons of creatinine, K, Na, Cl, Ca, Mg, U Creatinine, U K, U Na, U Cl, Fe K, Fe Na, Fe Cl, RBC K, and RBC K/Cell between groups on Days 0, 30, 60, and 90. Data are reported as mean ± SD, 95% confidence intervals, median, first and third quartiles. Reported p values are for comparisons between groups. Within control horses and within treatment horses, values in Days periods with different superscripts are different ($p < 0.05$). Additional comparisons where $p \geq 0.05$ and $p < 0.10$ are reported on Pg 2-3 below.

Variable	Control horses n=6				Treatment horses n=9				P
	Day 0	30	60	90	Day 0	30	60	90	
Creat	1.47 ± 0.27[a] (1.17, 1.76) 1.44 1.27, 1.60	1.31 ± 0.21[b] (1.09, 1.53) 1.30 1.13, 1.48	1.33 ± 0.17[a,b] (1.15, 1.51) 1.35 1.17, 1.50	1.36 ± 0.14[a,b] (1.21, 1.50) 1.30 1.26, 1.47	1.35 ± 0.14[a] (1.24, 1.46) 1.34 1.28, 1.46	1.20 ± 0.13[b] (1.09, 1.30) 1.22 1.08, 1.31	1.36 ± 0.15[a,c] (1.24, 1.48) 1.39 1.20, 1.50	1.34 ± 0.14[a,c] (1.23, 1.45) 1.27 1.24, 1.49	0.476
K	3.56 ± 0.33[a] (3.21, 3.91) 3.70 3.42, 3.72	3.81 ± 0.14[a,b] (3.66, 3.97) 3.80 3.70, 3.87	3.56 ± 0.55[a,b] (2.98, 4.15) 3.65 3.32, 3.95	3.86 ± 0.26[b] (3.58, 4.14) 3.90 3.70, 4.10	3.94 ± 0.17[a] (3.81, 4.07) 3.90 3.80, 4.10	3.85 ± 0.20[a] (3.70, 4.00) 3.90 3.60, 4.00	3.50 ± 0.44[a] (3.16, 3.83) 3.60 3.20, 3.80	4.00 ± 0.23[a,c] (3.81, 4.18) 4.00 3.85, 4.10	0.231
Na	137.47 ± 1.57 (135.81, 139.12) 137.60 136.43, 138.88	137.43 ± 1.33 (136.33, 139.13) 138.10 136.93, 138.72	138.67 ± 1.84 (136.73, 140.61) 138.65 136.95, 139.72	138.67 ± 2.78 (135.74, 141.59) 138.15 136.70, 140.47	139.08 ± 1.73 (137.75, 140.41) 139.00 138.25, 139.90	138.53 ± 1.91 (137.06, 140.01) 139.80 136.45, 140.10	139.58 ± 2.24 (137.86, 141.30) 140.20 138.55, 141.05	140.28 ± 2.31 (138.50, 142.05) 140.90 138.55, 142.25	0.153
Cl	98.85 ± 1.57 (97.20, 100.50) 99.20 97.20, 100.13	99.05 ± 0.93 (98.07, 100.03) 98.85 98.27, 99.72	99.21 ± 2.30 (96.79, 101.64) 99.55 97.37, 100.97	99.16 ± 2.68 (96.35, 101.98) 98.60 96.97, 101.23	100.90 ± 1.93 (99.41, 102.39) 101.10 99.85, 102.20	100.91 ± 2.35 (99.09, 102.72) 100.30 98.95, 103.10	99.83 ± 3.03 (97.49, 102.17) 110.10 97.95, 102.30	100.28 ± 2.22 (98.56, 101.99) 100.40 98.70, 102.20	0.152
Ca	11.50 ± 0.22[a] (11.26, 11.73) 11.45 11.30, 11.72	11.70 ± 0.27[b] (11.41, 11.98) 11.75 11.45, 11.87	11.88 ± 0.24[b] (11.62, 12.14) 11.85 11.67, 12.12	11.86 ± 0.32[b] (11.53, 12.20) 11.90 11.67, 12.12	11.78 ± 0.17[a,b] (11.65, 11.92) 11.90 11.70, 11.90	11.88 ± 0.20[a,b] (11.73, 12.04) 11.90 11.70, 12.05	11.71 ± 0.34[a] (11.44, 11.97) 11.70 11.45, 12.00	12.06 ± 0.44[b,c] (11.72, 12.40) 12.10 11.75, 12.45	0.206
Mg	1.73 ± 0.10[a] (1.62, 1.84) 1.70 1.67, 1.82	1.96 ± 0.25[b] (1.69, 2.37) 1.90 1.77, 2.17	1.96 ± 0.21[b] (1.74, 2.19) 2.05 1.70, 2.12	1.90 ± 0.22[a,b] (1.66, 2.13) 1.95 1.72, 2.10	1.80 ± 0.08 (1.73, 1.86) 1.80 1.70, 1.90	1.86 ± 0.05 (1.82, 1.90) 1.90 1.80, 1.90	1.91 ± 0.18 (1.77, 2.05) 2.00 1.75, 2.00	1.91 ± 0.18 (1.77, 2.05) 1.90 1.80, 2.05	0.071
U Creat	216.42 ± 100.70[a] (110.75, 322.10) 205.76 126.38, 288.32	153.30 ± 80.88[b] (68.42, 238.19) 162.97 90.96, 215.24	199.44 ± 96.38[a,b] (98.29, 300.59) 181.77 119.17, 287.81	202.97 ± 112.04[a,b] (85.39, 320.54) 154.63 138.72, 277.56	164.33 ± 77.26 (104.94, 223.72) 198.98 99.08, 207.36	146.74 ± 31.50 (122.52, 170.95) 157.07 136.18, 165.91	131.85 ± 59.18 (86.35, 177.34) 117.76 78.80, 178.59	181.26 ± 60.39 (134.84, 227.68) 197.75 126.78, 232.49	0.220
U K	165.27 ± 25.95 (138.03, 192.50)	163.93 ± 57.83 (103.24, 224.63)	146.67 ± 40.19 (104.48, 188.85)	152.27 ± 28.80 (122.04, 182.50)	193.14 ± 132.32[a,b,c] (91.437, 294.85)	244.53 ± 96.76[a,b] (170.15, 318.91)	132.54 ± 79.94[a] (71.091, 194.00)	155.88 ± 33.95[a,c] (129.78, 181.97)	0.405

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	167.25 137.30, 191.63	188.20 139.95, 191.95	166.70 118.18, 170.93	153.80 132.80, 172.80	177.00 95.50, 274.20	198.70 183.40, 325.60	135.20 62.20, 175.95	156.40 128.95, 190.00	
U Na	20.50 ± 8.22 (11.87, 29.12) 16.65 15.72, 25.70	25.80 ± 10.86 (10.51, 35.48) 20.40 18.10, 36.20	44.78 ± 29.11 (7.48, 70.14) 40.50 16.90, 74.80	33.56 ± 30.49 (1.56, 65.57) 21.10 16.35, 50.80	29.58 ± 30.45 (4.77, 49.82) 17.75 15.05, 32.45	16.85 ± 1.81 (15.45, 18.25) 16.30 15.40, 18.75	50.23 ± 62.31 (2.33, 98.13) 18.30 13.15, 73.45	34.03 ± 53.85 (-7.36, 75.42) 15.20 13.85, 21.85	0.800
U Cl	114.20 ± 52.93[a] (58.64, 169.75) 94.20 83.45, 142.85	164.67 ± 67.18[a,b] (94.15, 235.17) 182.15 136.78, 201.00	194.23 ± 58.50[a,b] (132.84, 255.63) 215.80 125.97, 233.82	220.08 ± 92.21[b] (123.31, 316.85) 226.50 129.50, 308.73	176.01 ± 83.08[a,c] (85.26, 230.76) 174.30 99.97, 258.63	237.31 ± 89.52[a,b] (168.49, 306.13) 223.50 179.15, 317.30	149.96 ± 85.34[c] (84.35, 215.56) 151.70 71.65, 198.30	254.12 ± 81.49[b] (191.48, 316.77) 287.00 195.30, 312.55	0.386
Fe K	35.08 ± 9.31 (25.31, 44.86) 37.40 30.86, 41.35	44.66 ± 17.10 (26.71, 62.61) 44.20 26.77, 62.63	33.05 ± 16.81 (15.40, 50.704) 30.23 16.10, 52.57	32.13 ± 16.16 (15.16, 49.10) 28.77 20.03, 46.33	39.27 ± 16.08[a,b] (26.91, 51.63) 33.57 28.54, 48.33	51.80 ± 18.4[a] (37.61, 65.98) 45.31 36.06, 65.48	39.16 ± 18.98[a,b] (24.567, 53.759) 40.14 24.37, 45.57	30.97 ± 8.69[b,c] (24.28, 37.65) 30.71 22.30, 39.65	0.496
Fe Na	0.11 ± 0.06 (0.04, 0.18) 0.09 0.07, 0.16	0.13 ± 0.09 (0.02, 0.23) 0.12 0.05, 0.20	0.24 ± 0.27 (-0.04, 0.53) 0.16 0.03, 0.42	0.20 ± 0.19 (-0.002, 0.40) 0.09 0.06, 0.40	0.32 ± 0.72[a] (-0.23, 0.87) 0.07 0.06, 0.14	0.10 ± 0.03[a,b] (0.07, 0.13) 0.09 0.08, 0.11	0.61 ± 1.04[b,c] (-0.18, 1.42) 0.21 0.08, 0.76	0.26 ± 0.54[a,b] (-0.14, 0.68) 0.08 0.06, 0.13	0.422
Fe Cl	0.86 ± 0.44[a] (0.40, 1.33) 0.69 0.56, 1.25	1.61 ± 0.52[b] (1.05, 2.16) 1.62 1.14, 2.01	1.47 ± 0.50[b] (0.94, 2.00) 1.74 0.92, 1.81	1.85 ± 1.04[b] (0.76, 2.94) 1.80 1.00, 2.89	1.23 ± 0.72[a] (0.67, 1.79) 1.44 0.70, 1.63	1.89 ± 0.63[b] (1.41, 2.38) 1.84 1.29, 2.44	1.62 ± 0.87[a,b] (0.94, 2.29) 1.24 0.99, 2.14	1.92 ± 0.57[b] (1.48, 2.37) 1.93 1.37, 2.48	0.460
RBC K	8.55 ± 1.97[a] (6.47, 10.62) 9.20 6.10, 10.22	9.06 ± 0.91[a] (8.10, 10.02) 8.85 8.32, 9.90	10.66 ± 1.74[b] (8.83, 12.49) 10.05 9.42, 12.70	9.33 ± 1.17[a] (8.10, 10.56) 9.30 8.15, 10.50	10.51 ± 1.51[a] (9.34, 11.67) 9.90 9.15, 11.85	9.03 ± 1.04[b] (8.22, 9.83) 9.30 8.25, 9.65	9.50 ± 1.30[a,b] (8.49, 10.50) 9.50 8.45, 10.35	9.11 ± 1.17[b] (8.20, 10.01) 9.10 8.30, 9.90	0.788
RBC K/Cell	4.52 ± 0.98 (3.48, 5.55) 4.89 3.31, 5.29	4.56 ± 0.50 (4.02, 5.09) 4.50 4.06, 5.01	5.29 ± 0.89 (4.35, 6.23) 5.17 4.43, 6.20	4.76 ± 0.62 (4.10, 5.42) 4.86 4.12, 5.29	5.37 ± 0.82[a] (4.74, 6.01) 5.38 4.88, 5.64	4.56 ± 0.49[b] (4.17, 4.94) 4.49 4.19, 4.84	4.75 ± 0.74[a,b] (4.18, 5.33) 4.68 4.11, 5.43	4.70 ± 0.52[a,b] (4.29, 5.10) 4.78 4.25, 5.17	0.799

Additional comparisons within groups

Creatinine

Control: Day 0 vs. Day 60 (p = 0.065);

K

Control: Day 0 vs. Day 30 (p = 0.060);

Treatment: Dat 30 vs. Day 90 (p = 0.096);

Na

Treatment: Day 0 vs. Day 90 (p = 0.089); Day 30 vs. Day 90 (p = 0.073);

Ca

Treatment: Day 0 vs. Day 90 (p = 0.074);

Mg

Control: Day 0 vs. Day 90 (p = 0.071);

Treatment: Day 0 vs. Day 60 (p = 0.067);

U Na

Treatment: Day 0 vs. Day 60 (p = 0.081); Day 30 vs. Day 60 (p = 0.069);

U Cl

Day 30 vs. Day 90 (p = 0.078);

Treatment: Day 0 vs. Day 30 (p = 0.053);

Fe K

Treatment: Day 0 vs. Day 30 (p = 0.058);

Fe Na

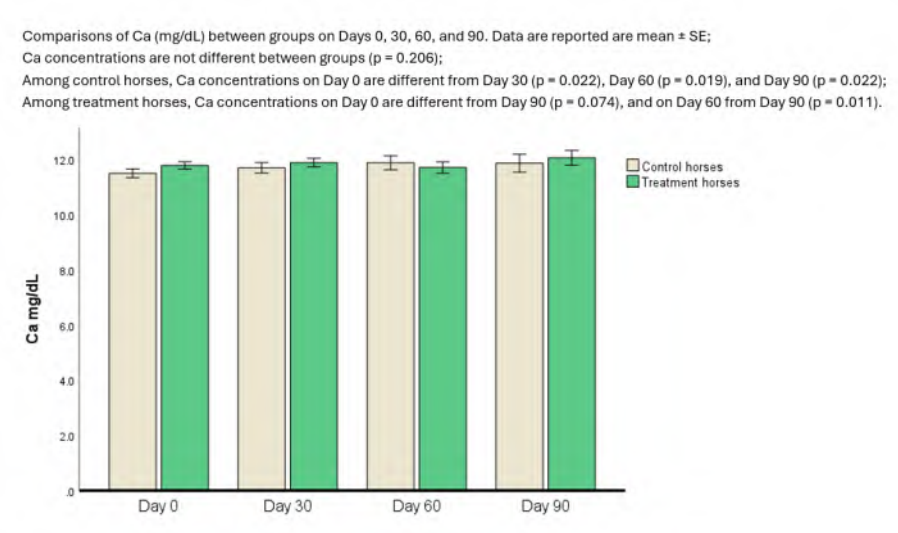
Treatment: Day 30 vs. Day 60 (p = 0.088);

RBC K/Cell

Control: Day 30 vs. Day 60 (p = 0.060), Day 60 vs. Day 90 (p = 0.096);

Treatment: Day 0 vs. Day 90 (p = 0.076).

Figure 8:



Aim 2: Assess the effect of chronic furosemide administration on the therapeutic response at 48 hours post administration

Hypothesis: Horses administered weekly furosemide for 90 days will demonstrate a reduced therapeutic response, e.g. less weight loss and less severe alterations in PCV, USG, urinary fractional excretions, plasma electrolytes, intracellular potassium, PTH concentrations and ionized calcium 48 hours after furosemide administration on days 90-92 compared to the therapeutic response 48 hours after furosemide administration on days 0-2.

Results:

- In the treatment group, the therapeutic response and 48 hours after administration of furosemide was not different after the first dose (Day 2-Day 0) compared to after the last dose (Day 92-Day 90) in any of the variables evaluated (Figure 9 and 10).
- Placebo horses were similarly evaluated to assess for any confounding environmental factors

Figure 9:

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Comparisons of differences observed between Day 0-2 and Day 90-92 for body weight (BW), packed cell volume (PCV), total solids (TS), urine spec gravity (USG), parathyroid hormone (PTH), ionized calcium (iCA), among control horses and among treatment horses. Data are reported as mean ± SD, 95% confidence intervals, median, first and third quartiles.

Variable	Control horses			Treatment horses		
	Day 0-2	Day 90-92	p	Day 0-2	Day 90-92	p
BW kg	-2.1 ± 3.1 (-5.51, 1.17) -2.5 -4.5, 0.0	1.3 ± 3.4 (-2.28, 4.94) 0.0 -1.0, 3.5	0.18	-6.2 ± 8.4 (-12.73, 0.30) -10.0 -11.5, -1.0	-0.1 ± 1.7 (-1.46, 1.24) 0.0 -2.0, 1.0	0.09
PCV %	-1.16 ± 5.03 (-6.45, 4.11) -0.5 -4.75, 3.25	-2.83 ± 8.79 (-12.06, 6.39) -3.5 -11.0, 3.0	0.687	-0.55 ± 3.77 (-3.46, 2.34) 0.00 -4.0, 3.0	2.55 ± 5.27 (-1.49, 6.60) 1.0 -2.0, 7.5	0.203
TS g/dL	0.31 ± 0.59 (-0.31, 0.94) 0.25 -0.3, 0.9	-0.26 ± 0.22 (-0.50, -0.03) -0.35 -0.42, -0.05	0.156	0.34 ± 0.45 (-0.0080, 0.69) 0.5 <0.001, 0.65	-0.03 ± 0.35 (-0.30, 0.24) 0.0 -0.25, 0.3	0.195
USG*	-0.008 ± 6.9 (-0.017, -0.0022) -0.007 -0.015. -0.003	0.006 ± 0.003 (0.0021, 0.01) 0.009 0.003, 0.009	0.062	-0.002 ± 0.006 (-0.0067, 0.0027) -0.002 -0.004, 0.002	-0.002 ± 0.01 (-0.016, 0.0061) 0.001 -0.008, 0.006	0.910
PTH	-15.23 ± 37.53 (-54.62, 24.15) -4.45 -28.27, 5.15	0.53 ± 5.48 (-5.21, 6.28) -1.15 -2.57, 4.15	0.437	-2.23 ± 16.9 (-15.22, 10.76) 0.0 -7.05, 8.5	3.35 ± 11.5 (-5.50, 12.21) -0.3 -3.8, 7.7	0.468
iCA mmol/L	0.03 ± 0.06 (-0.03, 0.10) 0.005 -0.12, 0.10	0.06 ± 0.13 (-0.071, 0.20) 0.04 -0.02, 0.16	0.687	-0.002 ± 0.10 (-0.08, 0.08) 0.01 -0.10, 0.09	-0.002 ± 0.03 (-0.027, 0.22) 0.0 -0.02, 0.02	1.0

*The analysis includes five of six control horses.

MM Data analysis

Outcome comparisons – Differences observed in Day 0-2 vs. those in Day 90-92 for body weight (BW), packed cell volume (PCV), total solids (TS), urine spec gravity (USG), parathyroid hormone (PTH), ionized calcium (iCA) were compared among control horses and among treatment horses by using the Wilcoxon Sign Rank test.

Figure 10:

Comparisons of differences observed between Day 2 minus Day 0 and Day 92 minus Day 90 for CREAT, K, Na, Cl, Ca, Mg, U Creat, U K, U Na, U Cl, Fe, Fe Na, Fe Cl, RBC K, and RBC K/Cell K among control horses and treatment horses. Data are reported as mean ± SD, 95% confidence intervals, median, first and third quartiles.

Variable	Control horses n=6			Treatment horses n=9		
	Day 0-2	Day 90-92	p	Day 0-2	Day 90-92	p
CREAT	0.0016 ± 1.14 (-0.14, 0.14) 0.06 -1.15, 0.11	-0.0066 ± 0.12 (-0.13, 0.11) 0.03 -0.11, 0.08	0.812	0.02 ± 0.08 (-0.04, 0.09) 0.04 -0.04, 0.09	-0.01 ± 0.17 (-0.15, 0.11) -0.06 -0.09, 0.15	0.734
K	0.15 ± 0.28 (-0.15, 0.45) 0.05 -0.02, 0.32	0.16 ± 0.28 (-0.13, 0.46) 0.15 -0.05, 0.37	1.0	-0.16 ± 0.30 (-0.39, 0.06) -0.20 -0.45, 0.15	-0.12 ± 0.31 (-0.36, 0.11) 0.00 -0.40, 0.10	0.742
Na	0.26 ± 1.32 (-1.11, 1.65) 0.3 -0.87, 1.47	2.18 ± 5.48 (-3.57, 7.94) 1.4 -2.02, 8.1	0.687	-0.78 ± 1.83 (-2.19, 0.62) -0.70 -1.80, 0.85	-0.18 ± 3.01 (-2.50, 2.12) 0.80 -2.40, 2.55	0.496
Cl	0.58 ± 1.47 (-0.96, 2.13) -0.15 -0.52, 2.27	2.15 ± 3.48 (-1.50, 5.80) 1.55 -0.87, 6.07	0.312	-0.63 ± 1.53 (-1.81, 0.54) -0.90 -1.85, 0.60	0.50 ± 1.60 (-0.73, 1.73) 1.10 -0.85, 1.95	0.250
Ca	0.18 ± 0.24 (-0.06, 0.43) 0.25 -0.05, 0.4	0.48 ± 0.36 (0.09, 0.86) 0.45 0.15. 0.9	0.312	0.01 ± 0.35 (-0.26, 0.28) 0.20 -0.35, 0.25	-0.04 ± 0.50 (-0.43, 0.34) -0.10 -0.50, 0.45	0.843
Mg	0.11 ± 0.07 (0.03, 0.19) 0.10 0.07, 0.20	0.08 ± 0.11 (-0.03, 0.20) 0.10 -0.02, 0.20	0.843	0.06 ± 0.08 (0.0098, 0.13) 0.10 0.00, 0.10	0.04 ± 0.10 (-0.03, 0.12) 0.10 -0.05, 0.10	0.687
U CREAT	-101.47 ± 50.92 (-164.70, -38.24) -74.42 -146.63, -69.83	39.34 ± 30.58 (1.36, 77.32) 43.92 11.25, 65.16	0.062	-41.53 ± 49.37 (-79.48, -3.58) -28.70 -66.93, -2.08	-7.23 ± 80.80 (-69.34, 54.88) -1.38 -63.08, 48.03	0.496
U K	-18.94 ± 51.49 (-82.88, 45.00) -11.20 -59.25, 17.50	2.24 ± 41.00 (-48.67, 53.15) -18.00 -27.20, 41.8	1.0	-48.82 ± 129.62 (-148.45, 50.81) 4.20 -149.75, 11.40	-15.31 ± 51.90 (-55.21, 24.58) -18.50 -62.20, 29.70	0.910
U Na	-3.12 ± 15.2 (-21.99, 15.75) 0.03 -15.0, 7.35	-15.48 ± 24.65 (-46.08, 15.12) -3.4 -39.65, 2.65	0.437	-0.72 ± 47.08 (-36.91, 35.46) 0.0 -13.25, 5.05	-7.96 ± 58.20 (-52.70, 36.77) 1.80 -4.05, 13.75	0.496
U Cl	24.96 ± 62.80 (-53.02, 102.94) 42.6 -41.35, 82.45	4.86 ± 88.84 (-105.46, 115.18) -47.5 -65.85, 101.75	0.812	-22.10 ± 87.64 (-89.47, 45.27) -31.10 -95.30, 65.60	-73.03 ± 73.05 (-129.19, -16.8) -65.50 -156.15, -3.90	0.300
Fe K	12.97 ± 9.68	-9.04 ± 14.17	0.125	3.74 ± 20.96	-0.53 ± 12.91	0.570

	(0.94, 25.00) 16.29 3.37, 20.91	(-26.65, 8.55) -6.84 -21.34, 2.15		(-12.37, 19.86) 8.14 -8.05, 20.45	(-10.45, 9.38) -6.56 -8.57, 2.25	
Fe Na	0.006 ± 0.13 (-0.16, 017) 0.04 -0.09, 0.09	-0.13 ± 0.16 (-0.33, 0.07) -0.02 -0.30, -0.007	0.312	0.06 ± 0.95 (-0.66, 0.80) 0.02 -0.04, 0.32	-0.11 ± 0.62 (-0.63, 0.41) -0.01 -0.02, 0.20	0.546
Fe Cl	0.76 ± 0.18 (0.54, 0.99) 0.80 0.58, 0.93	-0.43 ± 0.55 (-1.12, 0.24) -0.50 -0.82, -0.02	0.062	0.32 ± 0.68 (-0.20, 0.85) 0.75 -0.45, 0.87	-0.46 ± 0.63 (-0.96, 0.02) -0.46 -1.08, -0.06	0.019
RBC K	1.85 ± 1.96 (-0.20, 3.90) 1.65 0.35, 3.9	-0.08 ± 0.65 (-0.77, 0.60) -0.15 -0.62, 0.62	0.156	0.13 ± 0.99 (-0.63, 0.89) 0.40 -0.30, 1.00	0.05 ± 1.08 (-0.77, 0.89) 0.00 -0.75, 0.95	0.570
RBC K/Cell K	0.58 ± 0.97 (-0.44, 1.60) 0.51 -0.10, 1.39	-0.02 ± 0.50 (-0.55, 0.50) -0.16 -0.48, 0.57	0.437	0.05 ± 0.71 (-0.49, 0.60) 0.34 -0.28, 0.58	-0.03 ± 0.53 (-0.44, 0.38) -0.07 -0.59, 0.37	0.910

MM Data analysis

Comparisons within groups; Wilcoxon Sign Rank test

Aim 3: Investigate the effects of repeated furosemide administration on indices of bone density and strength using minimally-invasive methods DEXA scan and BoneScore® OsteoProbe® microindentation.

Hypothesis: Horses in active training administered weekly furosemide for 90 days will experience a reduction in bone density and strength compared to horses administered a placebo.

Results:

- Pre- and post-study median bone material strength index scores (BMSi) as determined by Osteoprobe micro-indentation did not differ between groups at either timepoint (Figure 11)
- Among control horses, one of six horses (#1) had lower BMSI after treatment. Among treatment horses, four of nine horses (#1, #2, #3, #4) had lower BMSI after treatment (p = 0.580, Fisher's exact chi-square test) (Figure 12)
- While the mean BMSi increased in both groups increased over the course of the study, the increase was less pronounced in treatment horses (mean change = 1.6) when compared to controls (mean change 4.0). Although this difference was not statistically significant (p = 0.381) in this study, further investigation to better understand how chronic furosemide affects bone strength is warranted (Figure 13 and 14)

Figure 11:

Comparisons of bone material strength index between control horses and treatment horses: pre-treatment, post-treatment

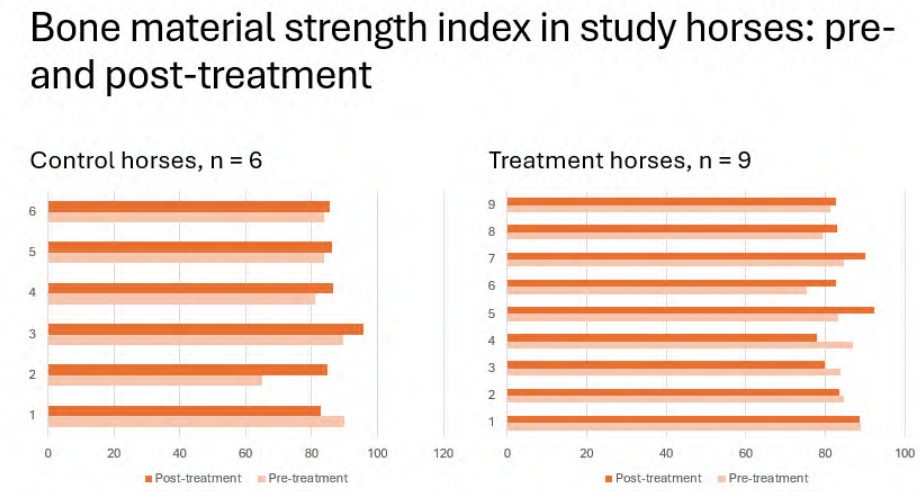
Control horses n=6	Treatment horses n=9	p
Pre-treatment		
82.18 ± 9.19 (72.53, 91.83) 83.75 76.97, 89.77	83.14 ± 4.06 (80.02, 86.26) 83.80 80.30, 85.85	0.886
Post-treatment		
86.90 ± 4.58 (82.09, 91.70) 85.85 84.17, 88.90	84.48 ± 4.74 (80.84, 88.13) 82.90 81.25, 89.25	0.346

*Data are reported as mean ± SD, 95% confidence intervals, median, first and third quartiles

MM Data analysis

Outcome comparisons –The distributions of bone material strength index were compared between groups by using the student t test (post-treatment) or the non-parametric Wilcoxon Rank Sum test (pre-treatment).

Figure 12:



Among control horses, one of six horses (#1) had lower BMSI after treatment. Among

treatment horses, four of nine horses (#1, #2, #3, #4) had lower BMSI after treatment (p = 0.580, Fisher's exact chi-square test).

Figure 13:

Comparisons of bone material strength index difference (post-treatment minus pre-treatment) between control horses and treatment horses

Control horses n=6	Treatment horses n=9	p
4.716 ± 8.841* (-4.56, 13.99) 4.0 -0.5, 9.525	1.344 ± 5.687 (-3.02, 5.71) 1.6 -2.45, 6.25	0.381

*Data are reported as mean ± SD, 95% confidence intervals, median, first and third quartiles

MM Data analysis
Outcome comparisons –The distributions of bone material strength index (difference post-study minus pre-study) were compared between groups by using the student t test.

Figure 14:

AIM 4: Evaluate the effects of chronic furosemide administration on cardiac function before,

during, and after exercise using telemetric ECG.

Hypothesis: Horses in active training administered weekly furosemide for 90 days will have higher heartrates secondary to volume depletion and will be more likely to experience cardiac arrythmias before, during, and after exercise when compared to horses receiving placebo.

Results:

- No difference in heartrate were noted between groups at any timepoint in the study (Figure 15 and 16).
- Heartrates at the start, middle, and end of exercise trended downward in both groups over the course of the study, likely due to increasing levels of fitness (Figure 15 and 16).
- No increase in arrythmia frequency was noted in either group throughout the study (Figure 17)

Figure 15:

Comparisons of Heart Rate (bpm) at start, during exercise, and end of study between control horses and treatment horses

	Start			Exercise			End		
	Day 0	Day 45	Day 90	Day 0	Day 45	Day 90	Day 0	Day 45	Day 90
Control	55.3 ± 8.7 (46.19, 64.47) 54.0 46.7, 64.7	51.0 ± 9.6 (40.82, 61.17) 49.0 43.5, 61.0	49.8 ± 9.0 (40.32, 59.34) 48.0 42.5, 56.7	161.3 ± 10.4 (150.35, 172.31) 162.5 150.0, 169.2	158.1 ± 22.9 (134.05, 182.28) 167.0 137.2, 176.0	148.8 ± 39.9 (106.94, 190.73) 167.0 113.5, 172.2	55.1 ± 7.0 (47.79, 62.54) 55.5 48.5, 61.0	52.1 ± 7.8 (43.89, 60.44) 48.5 47.5, 58.0	48.0 ± 6.2 (41.46, 54.53) 47.0 42.5, 54.5
Treatment	57.5 ± 24.2 (38.91, 76.19) 45.0 42.5, 66.5	47.1 ± 15.1 (35.45, 58.11) 42.0 37.5, 59.0	49.6 ± 14.6 (38.42, 60.91) 47.0 37.5, 61.0	155.2 ± 32.1 (130.50, 179.95) 167.0 135.0, 182.0	164.2 ± 10.6 (156.01, 172.44) 167.0 154.0, 176.0	166.1 + 6.7 (160.93, 171.29) 167.0 167.0, 167.0	51.6 ± 8.3 (45.21, 58.12) 49.0 44.0, 58.0	49.4 ± 12.8 (39.54, 59.34) 44.0 43.0, 49.0	50.7 ± 3.7 (47.88, 53.67) 52.0 47.0, 54.0
p	0.404	0.340	0.886	0.993	0.902	0.574	0.283	0.125	0.370

*Data are reported as mean ± SD, 95% confidence intervals, median, first and third quartiles

MM Data analysis
Outcome comparisons –The distributions of heart rate at start, during exercise, and end of study were compared between control horses and treatment horses by using the non-parametric Wilcoxon Rank Sum test.

Figure 16: Maximum heart rates at the Start of exercise, During exercise, and End of Exercise in

control versus treatment horses. Plot below shows means ± SE bars

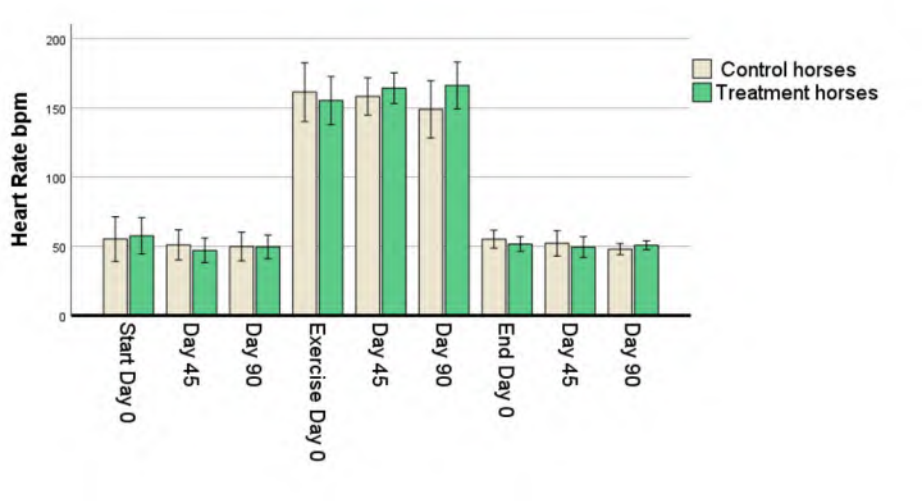


Figure 17:

ID	GROUP	Start Rhythm D0	Start Rhythm D45	Start Rhythm D90	Exercise Rhythm D0	Exercise Rhythm D45	Exercise Rhythm D90	End Rhythm D0	End Rhythm D45	End Rhythm D90
1	0	sinus	sinus	sinus	sinus	sinus +VPC	sinus	sinus	sinus +VPC	sinus
2	0	sinus	sinus	sinus	sinus	sinus	sinus	sinus	sinus	sinus
3	1	sinus	sinus	sinus	sinus	sinus	sinus	sinus	sinus	sinus
4	0	sinus	sinus with 2nd degree AV block	sinus with 2nd degree AV block	sinus	sinus	sinus	sinus	sinus	sinus
5	1	sinus	sinus +VPC	sinus +VPC	sinus +VPC	sinus +VPC	sinus +VPC	sinus +VPC	sinus +VPC	sinus +VPC
6	1	sinus	sinus with 2nd degree AV block	sinus	sinus	sinus	sinus	sinus	sinus	sinus
7	1	sinus	sinus	sinus	sinus	sinus	sinus	sinus	sinus	sinus
8	0	sinus	sinus	sinus	sinus	sinus	sinus	sinus	sinus	sinus
9	0	sinus	sinus	sinus	sinus	sinus	sinus	sinus	sinus	sinus
10	1	sinus	sinus	sinus	sinus	sinus	sinus	sinus	sinus	sinus
11	1	sinus	sinus	sinus	sinus	sinus	sinus	sinus	sinus	sinus
12	0	sinus	sinus	sinus	sinus	sinus	sinus	sinus	sinus	sinus
13	1	sinus	sinus	sinus	sinus	sinus	sinus	sinus	sinus	sinus
14	1	sinus	sinus +VPC	sinus, APC and VPC	sinus	sinus	sinus, APC and VPC	sinus +VPC	sinus	sinus
15	1	sinus	sinus	sinus	sinus	sinus	sinus	sinus	sinus	sinus

ADDITIONAL STUDY PERIOD: Compare the response to a single dose of furosemide at 48 hours in horses administered furosemide weekly for 90 days to horses receiving placebo.

Hypothesis: Horses administered weekly furosemide for 90 days will demonstrate a reduced therapeutic response, e.g. less weight loss and less severe alterations in PCV, USG, urinary fractional excretions, and plasma electrolytes at 48 hours post-administration compared to horses not previously treated.

Results:

- Horses in the chronic treatment group had lower baseline total solids (TS) (Figure 18)
- Horses in the chronic treatment group did not demonstrate a blunted or reduced physiologic response to furosemide when compared to the previously untreated group at timepoints 7 hours and 24 hours (Figures 19 and 20).
- Horses in the chronic treatment group experienced a larger increase in PCV and TS after treatment with furosemide when compared to the previously untreated group ($p = 0.005$, $p = 0.031$, respectively) (Figure 21, 22, 23)

Figure 18

Fresh Samples. Comparisons of body weight, PCV, TS, USG, creatinine, K, Na, and Cl, Ca, Mg, Fe Na, Fe Cl, and Fe K between groups on Day 100

Variable	Control horses n=6	Treatment horses n=9	p
BW	560.67 ± 34.10 (560.67, 596.46) 559.00 531.50, 585.50	544.89 ± 48.98 (507.24, 582.54) 544.00 524.00, 574.50	0.506
PCV	43.66 ± 2.50 (41.04, 46.29) 44.00 41.50, 45.50	39.55 ± 5.12 (35.61, 43.49) 42.00 36.50, 43.00	0.081
TS	7.08 ± 0.18 (6.89, 7.27) 7.00 6.97, 7.25	6.77 ± 0.20 (6.62, 6.93) 6.80 6.60, 6.95	0.011
USG	1.03 ± 0.006 (1.02, 1.03) 1.03 1.02, 1.03	1.02 ± 0.004 (1.02, 1.03) 1.03 1.02, 1.03	0.925
Creatinine	1.45 ± 0.30 (1.13, 1.77) 1.47 1.20, 1.70	1.35 ± 0.18 (1.21, 1.49) 1.33 1.20, 1.51	0.425
K	3.91 ± 0.40 (3.49, 4.33) 4.10 3.57, 4.15	3.57 ± 0.31 (3.33, 3.82) 3.50 3.30, 3.90	0.092
Na	137.03 ± 1.08 (135.89, 138.17) 136.90 136.07, 138.13	138.71 ± 1.14 (137.83, 139.59) 138.80 137.50, 139.45	0.014

Cl	99.88 ± 2.25 (97.51, 102.25) 100.20 97.80, 101.78	99.44 ± 1.59 (98.21, 100.67) 98.90 98.15, 100.65	0.668
Ca	12.33 ± 0.20 (12.11, 12.55) 12.40 12.15, 12.50	12.05 ± 0.32 (11.80, 12.30) 12.00 11.75, 12.25	0.084
Mg	1.95 ± 0.16 (1.77, 2.12) 2.00 1.77, 2.10	1.85 ± 0.24 (1.67, 2.04) 1.80 1.70, 2.05	0.418
Fe Na	0.41 ± 0.36	0.46 ± 0.34	0.793
	(0.038, 0.79) 0.43 0.05, 0.73	(0.20, 0.73) 0.32 0.21, 0.81	
Fe Cl	1.40 ± 0.52 (0.85, 1.96) 1.43 0.94, 1.75	1.23 ± 0.41 (0.91, 1.55) 1.18 0.99, 1.57	0.607
Fe K	24.98 ± 10.05 (14.43, 35.54) 22.17 17.32, 35.24	30.62 ± 7.86 (24.58, 36.67) 28.89 25.56, 33.53	0.244

*Data are reported as mean ± SD, 95% confidence intervals, median, first and third quartiles

MM Data analysis

Baseline comparisons – The distributions creatinine, K, Na, and Cl, Ca, and Mg were compared between groups on Day 100 by using the student t test or the non-parametric Wilcoxon Rank Sum test (Cl, PCV, Fe Cl).

Figure 19:

Fresh Samples. Comparisons of creatinine, K, Na, and Cl, Ca, and Mg between groups on Day 100 7h

Variable	Control horses n=6	Treatment horses n=9	p
Creatinine	1.53 ± 0.33 (1.18, 1.88) 1.48 1.31, 1.77	1.39 ± 0.25 (1.20, 1.58) 1.36 1.18, 1.57	0.361
K	3.56 ± 0.36 (3.18, 3.95) 3.6 3.17, 3.92	3.64 ± 0.38 (3.34, 3.93) 3.6 3.45, 3.95	0.702
Na	131.43 ± 2.72 (128.57, 134.30) 131.2 129.4, 134.25	132.21 ± 2.53 (130.26, 134.16) 132.7 129.95, 134.1	0.581
Cl	94.15 ± 1.84 (92.21, 96.08) 94.9 92.3, 95.5	95.05 ± 2.53 (93.10, 97.00) 96.1 92.7, 96.6	0.172
Ca	12.51 ± 0.7 (11.77, 13.25) 12.3 12.12, 12.85	12.28 ± 0.56 (11.85, 12.72) 12.4 12.2, 12.7	0.752
Mg	2.06 ± 0.12 (1.93, 2.19) 2.05 1.97, 2.2	2.1 ± 0.14 (1.99, 2.20) 2.1 2.05, 2.2	0.644

*Data are reported as mean ± SD, 95% confidence intervals, median, first and third quartiles

Figure 20:

Fresh Samples. Comparisons of creatinine, K, Na, and Cl, Ca, and Mg between groups on Day 101

Variable	Control horses n=6	Treatment horses n=9	p
Creatinine	1.52 ± 0.24 (1.26, 1.78) 1.48 1.33, 1.72	1.47 ± 0.19 (1.32, 1.62) 1.46 1.31, 1.62	0.700
K	2.98 ± 0.58 (2.36, 3.59) 2.95 2.62, 3.37	2.93 ± 0.17 (2.80, 3.06) 3.0 2.80, 3.1	0.885
Na	137.67 ± 0.38 (137.26, 138.07) 137.6 137.35, 138.1	138.49 ± 1.49 (137.34, 139.63) 139.0 137.0, 139.85	0.147
Cl	97.85 ± 1.10 (96.69, 99.00) 97.5 97.2, 98.42	98.66 ± 1.85 (97.24, 100.09) 99.2 96.8, 100.25	0.353
Ca	11.28 ± 0.44 (10.81, 11.75) 11.15 11.02, 11.57	11.28 ± 0.26 (11.08, 11.49) 11.4 11.15, 11.5	0.976
Mg	1.93 ± 0.13 (1.79, 2.07) 1.95 1.85, 2.02	2.0 ± 0.08 (1.93, 2.06) 2.0 1.9, 2.1	0.406

*Data are reported as mean ± SD, 95% confidence intervals, median, first and third quartiles

MM Data analysis

Baseline comparisons – The distributions creatinine, K, Na, and Cl, Ca, and Mg were compared between groups on Day 100 7h and Day 101 by using the student t test or the non-parametric Wilcoxon Rank Sum test (when data were not normally distributed).

Figure 21:

Comparisons of differences observed between Day 102 and Day 100 for body weight (BW), packed cell volume (PCV), total solids (TS), urine spec gravity (USG), Fe K, Fe Na, and Fe Cl between control horses and among treatment horses. Data are reported as mean ± SD, 95% confidence intervals, median, first and third quartiles

Variable	Control horses	Treatment horses	p
	Day 102-100	Day 102-100	
BW kg	-1.0 ± 1.78 (-2.87, 0.87) -1.5 -2.25, 0.5	-0.11 ± 2.31 (-1.89, 1.66) 0.0 -2.0, 2.5	0.496
PCV %	-1.0 ± 1.26 (-2.32, 0.32) -0.5 -2.25, 0.0	4.55 ± 4.82 (0.84, 8.26) 5.0 1.5, 6.5	0.005
TS g/dL	0.05 ± 0.19 (-0.15, 0.25) 0.10 -0.07, 0.15	0.34 ± 0.24 (0.15, 0.52) 0.4 0.15, 0.55	0.031
USG	1.03 ± 0.006 (0.0010, 0.013) 1.03 1.02, 1.03	1.02 ± 0.004 (-0.0012, 0.0077) 1.03 1.02, 1.03	0.119
Fe K	-0.32 ± 8.85 (-9.61, 8.97) 4.33 -10.87, 5.82	0.86 ± 9.84 (-6.69, 8.43) 0.97 -4.22, 5.36	0.688
Fe Na	-0.09 ± 0.24 (-0.34, 0.15) -0.04 -0.25, 0.09	-0.23 ± 0.37 (-0.52, 0.05) -0.09 -0.63, 0.04	0.712
Fe Cl	0.16 ± 0.65 (-0.52, 0.85) 0.4 -0.59, 0.67	0.47 ± 0.42 (0.15, 0.80) 0.34 0.28, 0.70	0.798

MM Data analysis

Outcome comparisons – Differences observed in Day 102 minus Day 100 for body weight (BW), packed cell volume (PCV), total solids (TS), urine spec gravity (USG), Fe K, Fe Na, and Fe Cl were compared among control horses and among treatment horses by using the Wilcoxon Rank Sum test.

Figure 22:

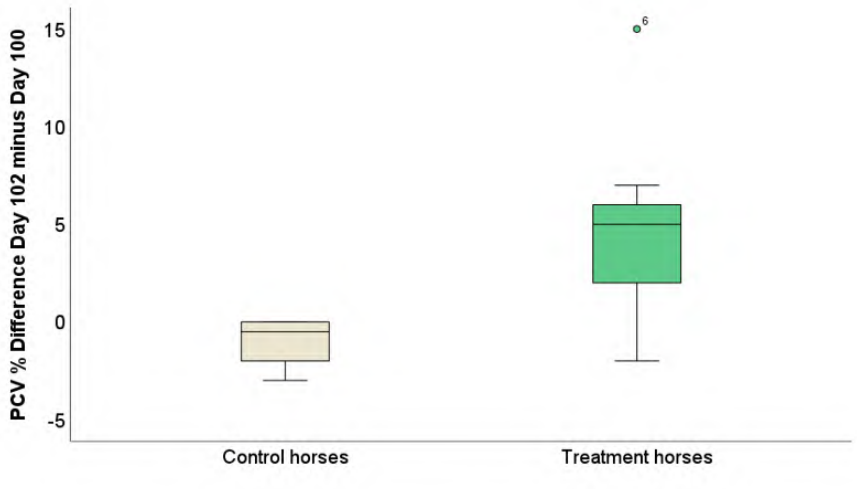
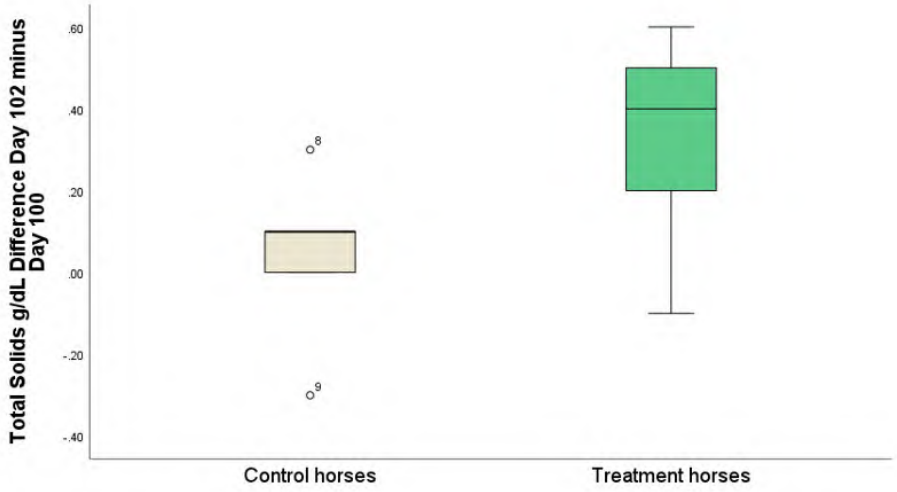


Figure 23:



UF College of Veterinary Medicine
UNIVERSITY of FLORIDA

FINAL REPORT

Report Date	Grant Project	Prepared by (PI)
03/02/2026	N/A	Sarah White-Springer, PhD
Reporting Period	**Grant Period**	**PI Signature**
11/01/25	12/31/25	
Grant Title		
Repeated furosemide administration on mitochondrial energetics in healthy trained Thoroughbreds		

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Methods:

This study was an add-on to Dr. DeNotta's study at the University of Florida, entitled, "Effects of repeated furosemide administration on electrolyte homeostasis and bone density in healthy adult exercising Thoroughbreds." Experimental design, as outlined by Dr. DeNotta, is below.

Mature Thoroughbred horses were matched by bodyweight and randomly allocated to be administered either 250 mg (5 mL) intravenous furosemide (5 mares, 4 geldings; Furosemide) or a similar volume of saline (3 mares, 3 geldings; Control) weekly for 90 days. Study personnel remained blinded to group identification throughout the study and data analysis. All horses were trained to run on an EquiGym high-speed equine treadmill and maintained fitness throughout the study with 3x/week exercise sessions. All horses were group housed in outdoor paddocks at the University of Florida College of Veterinary Medicine throughout the study, and all were fed the same diet consisting of free choice grass hay, commercial pelleted equine feed, and maintenance dose oral vitamin E supplementation.

Muscle samples were collected from the gluteus medius as previously described (White et al., 2016) on day 90 of the study. Before tissue collection, horses were sedated intravenously with xylazine hydrochloride (Rompun, 100 mg/mL; Dechra Veterinary Products, Overland Park, KS) or detomidine hydrochloride (Dormosedan; Zoetis Services LLC, Parsippany, NJ) at doses recommended by the manufacturer. The site of muscle collection was clipped before being cleaned with chlorhexidine scrub and 70% ethyl alcohol. After 1 min, the site was cleaned with 70% ethyl alcohol to ensure lidocaine residues were removed. Tissue was collected using a minimally invasive 12-gauge tissue collection needle (Celero Vacuum-Assisted Breast Biopsy Device, Hologic Inc., Marlborough, MA) inserted to a fixed depth of 5 cm on the left side of the horse. Following collection, the site was rinsed with 70% ethyl alcohol, tissue glue was applied (Vetbond Tissue Adhesive, 3M, St. Paul, MN), and the site was sealed with an aerosol aluminum bandage (AluShield Spray, MWI Veterinary Supply Company, Boise, ID). Muscle samples were flash-frozen in liquid nitrogen and stored at -80°C or placed in cooled biopsy preservation solution (BIOPS; 10mM Ca-EGTA buffer, 0.1µM free calcium, 20 mM imidazole, 20 mM taurine, 50 mM K-MES, 0.5 mM dithiothreitol, 6.56 mM MgCl2, 5.77 mM ATP, and 15 mM phosphocreatine; pH 7.1) and stored at 4°C until being analyzed for mitochondrial capacities within 24 h following collection using an Oxygraph-2k (Oroboros Instruments, Innsbruck, Austria).

Muscle samples were analyzed for activity of the mitochondrial enzyme, citrate synthase (CS) by kinetic colorimetry. Additionally, permeabilized fibers were analyzed for mitochondrial oxidative phosphorylation and electron transfer capacities by high-resolution respirometry and production of ATP was quantified in mitochondria isolated from the same sample by high-resolution fluororespirometry.

Mitochondrial volume density was estimated by CS activity (Larsen et al., 2012). Muscle samples were cryopulverized and sonicated in sucrose homogenization buffer (20 mM Tris, 40 mM KCl, 2 mM EGTA, 250 mM sucrose) with 1 part 5% detergent (n-Dodecyl β-D-maltoside; Sigma D464). Then, samples were centrifuged at 11,000 × *g* for 3 min at 0°C. Using a microplate reader (Synergy H1, BioTek Instruments, Winooski, VT), CS activity was quantified by measuring the initial rate of reaction of free CoA-SH with DTNB at 412 nm at 37°C (Spinazzi et al., 2012). Inter-assay and intra-assay CVs were 5.6% and 1.7%, respectively. CS activity was

normalized to total protein in the homogenate, quantified using the Coomassie Bradford Assay Kit (Thermo Fisher Scientific, Waltham, MA).

Oxidative phosphorylation (*P*) and electron transfer (*E*) capacities in muscle fibers were quantified as previously published in the horse (Artman et al., 2024). After mechanical separation on ice, fibers were chemically permeabilized using a 2% saponin solution for 30 min at 4°C. Following permeabilization, muscle fibers were rinsed in mitochondrial respiration media (MiR05; 110 mM sucrose, 60 mM potassium lactobionate, 0.5 mM EGTA, 3 mM $MgCl_2 \cdot 6H_2O$, 20 mM taurine, 10 mM KH_2PO_4, 20 mM HEPES, 1 g/L BSA, pH 7.1) for 10 min at 4°C. After rinsing, approximately 1.5-2.5 mg (wet weight) of the muscle fibers were added to each chamber of the Oxygraph-2k, which contained 2 mL of MiR06 (MiR05 + 280 U/mL catalase) and 20 mM creatine, and maintained at 38°C for the entirety of the experiment. The substrate-uncoupler-inhibitor titration (SUIT) protocol for the experiment was as follows: 1) complex I substrates pyruvate (5 mM), malate (1 mM), and glutamate (10 mM) as substrates for NADH production, stimulating non-phosphorylating LEAK respiration supported by NADH oxidized through complex I (L_N); 2) adenosine diphosphate (ADP; 2 mM) to quantify *P* supported by complex I through NADH (P_N); 3) cytochrome *c* (10 µM) to evaluate outer mitochondrial membrane integrity (samples exhibiting an increase in respiration over 15% with the addition of cytochrome *c* were excluded); 5) succinate (10 mM), a complex II substrate, to measure maximal coupled *P* (P_{N+S}); 6) uncoupler carbonyl cyanide 3-chlorophenylhydrazone (CCCP, 0.5 µM) to quantify maximal noncoupled *E* (E_{N+S}); and 7) rotenone (0.5 µM), an inhibitor of complex I, to allow for quantification of *E* supported by oxidation of succinate through complex II alone (E_S). Steady state residual oxygen consumption (ROX) was measured after the addition of the sample and prior to the addition of NADH-linked substrates and subtracted from all measures to correct for non-mitochondrial O_2 consumption. Chambers were maintained in hyperoxia (200 to 650 µM O_2) for the duration of the experiment.

For the quantification of ATP production, mitochondria were isolated from approximately 80 mg of wet muscle within 12 hours of collection using a protocol adapted from a commercial kit (MITOISO1; Sigma Aldrich) as previously described (Davis et al., 2024). Isolated mitochondria were immediately analyzed using the Oxygraph-2k with 465-nm fluorescent sensors and Magnesium Green™ (Thermo Fisher Scientific) for the analysis of ATP production. Chambers contained MiR05 without $MgCl_2$ and were maintained at 38°C in normoxia (between 50 and 200 µM O_2) for the duration of the experiment. Before adding 15 µL of sample, $MgCl_2$ (0.1 M) and Magnesium Green (1.1 mM) were added to the chambers and allowed to stabilize. The SUIT protocol for the experiment follows: 1) pyruvate (5 mM), malate (1 mM), and glutamate (10 mM) to measure ATP production during L_N; 2) ADP (2 mM) to quantify P_N; 3) succinate (10 mM) to measure P_{N+S}; and 4) rotenone (0.5 µM) to inhibit complex I and quantify P_S. ROX was measured after the addition of the sample and prior to the addition of NADH-linked substrates and measures corrected as previously described.

Mitochondrial data are presented as integrative (permeabilized fibers relative to tissue wet weight, isolated mitochondria relative to volume), intrinsic (permeabilized fibers relative to CS activity in the sample, isolated mitochondria relative to mg protein in the suspension), flux control ratio (measure of interest relative to maximal noncoupled electron transfer capacity,

E_{N+S}), or phosphorylation control ratio (measure of interest relative to maximal coupled oxidative phosphorylation capacity, P_{N+S}).

Statistical analyses

Differences between group means were determined by unpaired t test with Welch's correction in GraphPad Prism (version 10.6.1). Significance was established at $P \leq 0.05$. Box-and-whisker plots represent the mean (+), median (middle line), interquartile range (25^{th}–75^{th} percentiles as the box), and minimum and maximum (whiskers).

Results:

Mitochondrial volume density, as estimated by citrate synthase activity, was unaffected by 90 d of weekly furosemide administration ($P = 0.83$; Fig. 1)

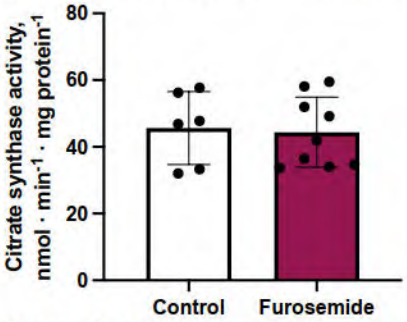

Figure 1. Citrate synthase activity (mean ± SEM) did not differ between control and furosemide horses ($P = 0.83$).

Permeabilized fiber respiratory capacities

Permeabilized fiber integrative (relative to tissue wet weight; $P \geq 0.28$; Fig. 2) and intrinsic (relative to CS activity; $P \geq 0.25$; Fig. 3) respiratory capacities were unaffected by furosemide treatment. The contribution of each respiratory state to maximal noncoupled electron transfer capacity (E_{N+S}), referred to as the Flux Control Ratio, FCR, were similarly unaffected by 90 d of weekly furosemide administration ($P \geq 0.27$; Fig. 4).

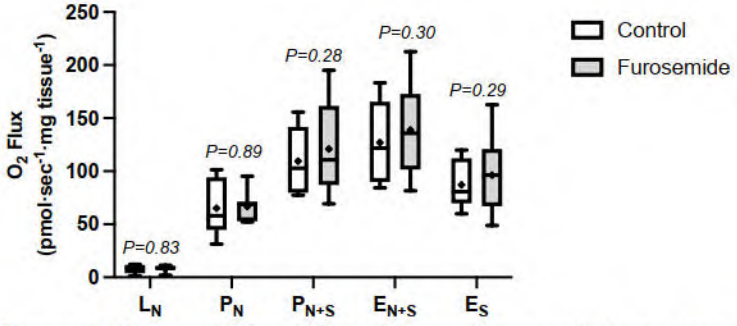

Figure 2. Permeabilized fiber integrative respiratory capacities were unaffected by repeated furosemide administration. L_N, nonphosphorylating leak respiration with NADH-linked substrates (complex I); P_N, oxidative phosphorylation capacity supported by complex I; P_{N+S}, maximal oxidative phosphorylation capacity supported by complex I and II; E_{N+S}, maximal noncoupled electron transfer capacity with NADH-linked (complex I) substrates and succinate (complex II); E_S, electron transfer capacity supported only by succinate (complex II).

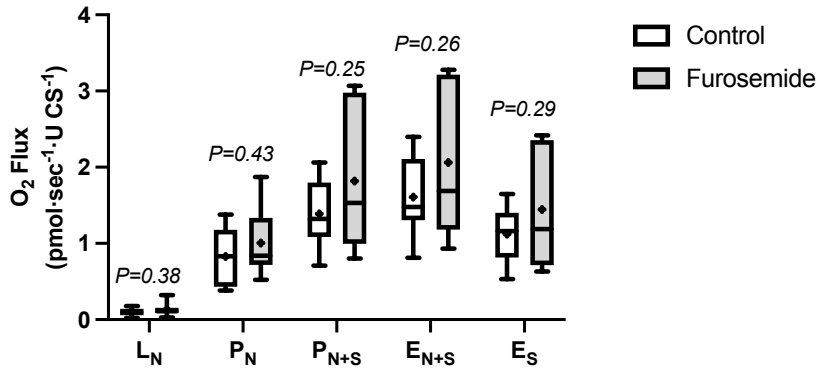

Figure 3. Permeabilized fiber intrinsic respiratory capacities were unaffected by repeated furosemide administration. L_N, nonphosphorylating leak respiration with NADH-linked substrates (complex I); P_N, oxidative phosphorylation capacity supported by complex I; P_{N+S}, maximal oxidative phosphorylation capacity supported by complex I and II; E_{N+S}, maximal noncoupled electron transfer capacity with NADH-linked (complex I) substrates and succinate (complex II); E_S, electron transfer capacity supported only by succinate (complex II).

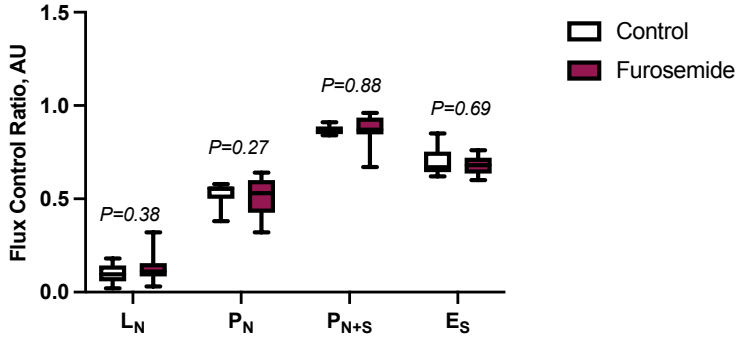

Figure 4. Permeabilized fiber flux control ratio (ratio of measure of interest to E_{N+S}) were unaffected by repeated furosemide administration. L_N, nonphosphorylating leak respiration with NADH-linked substrates (complex I); P_N, oxidative phosphorylation capacity supported by complex I; P_{N+S}, maximal oxidative phosphorylation capacity supported by complex I and II; E_S, electron transfer capacity supported only by succinate (complex II).

Isolated mitochondria ATP production

Given that LEAK is a dissipative state during which no adenylates are provided, ATP is not produced during LEAK respiration. Similarly, no ATP is produced when electron transfer is uncoupled from oxidative phosphorylation. ATP production, then, is only quantified during P_N, P_{N+S}, and P_S.

ATP production relative to sample volume did not differ between control and furosemide horses during any respiratory state ($P \geq 0.62$; Fig. 5). However, when expressed relative to O_2 consumption, providing an indicator of ATP production efficiency, furosemide horses had suppressed ATP production during maximal (P_{N+S}; $P = 0.02$) and complex II-supported (P_S; $P = 0.04$) respiration (Fig. 6).

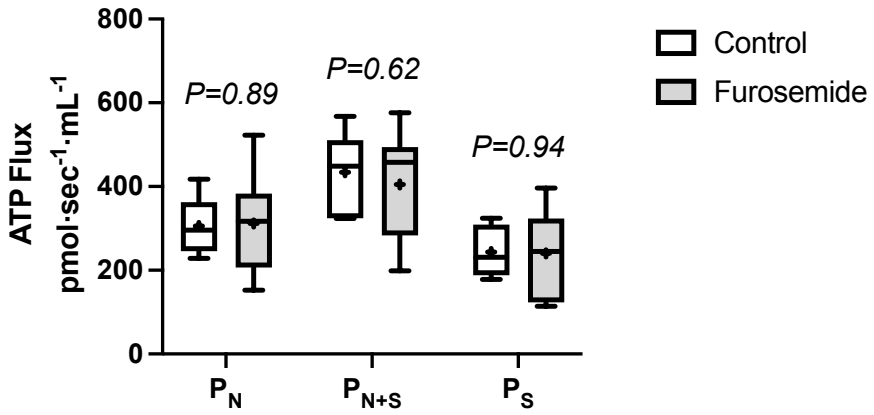

Figure 5. Isolated mitochondrial ATP production capacity was unaffected by 90 d of weekly furosemide administration. P_N, oxidative phosphorylation capacity with NADH-linked substrates supporting complex I; P_{N+S}, maximal oxidative phosphorylation capacity supported by complex I and II; E_S, electron transfer capacity supported only by succinate (complex II).

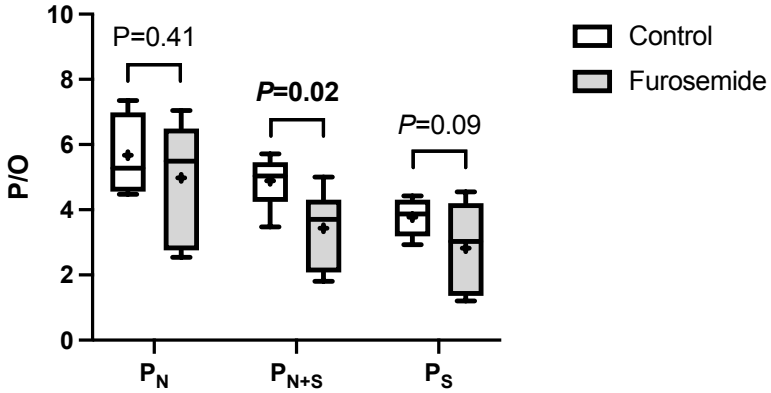

Figure 6. Isolated mitochondria ATP relative to O_2 consumption, an indicator of ATP production efficiency. Control horses had greater ATP production efficiency during maximal oxidative phosphorylation (P_{N+S}; $P = 0.02$) and tended to be more efficient during oxidative phosphorylation supported by complex II alone (P_S; $P = 0.09$).

Conclusions:

Contrary to our hypothesis, 90 days of weekly furosemide administration had no effect on mitochondrial respiratory capacities or maximal ATP production capacities. In contrast, furosemide administration resulted in horses having decreased ATP production efficiency, requiring more cellular input for a similar energetic output. Relationships between these measures and race performance have never been directly investigated in horses.

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APPENDIX I: Johnson Final Report

HISA FINAL REPORT

Prepared December 23, 2025
Sally Johnson and Giovanna Lungu
School of Animal Sciences, Virginia Tech, Blacksburg VA

INTRODUCTION

Furosemide, a diuretic administered to horses with EIPH, may offer advantages beyond control of pulmonary pressure during exercise. Past studies demonstrated that horses treated with furosemide, possess a lower bodyweight (BW) that did not affect speed, VO_{2max}, cardiac output (Q) or arterial and venous blood gases (1). In a second study using a similar design, furosemide treatment did not alter speed or total oxygen consumption during intense exercise but caused a reduced reliance on muscle glycogen metabolism and a lower oxygen deficit (2). These seminal studies argue that furosemide is not a performance enhancing drug (PED) but may offer a competitive advantage during muscle recovery following exercise.

Training causes muscle adaptions that include altered gene expression to meet the metabolic demands for increased shortening velocity. The ability of furosemide administration throughout the training program to affect gene transcription was examined by RNA sequencing. Gluteus medius biopsies (~100 mg) were harvested from racing age Thoroughbreds (4.0 ± 0.17 yr) administered saline (CON, n=6; 3-females, 3-males) or Lasix (n=9, 5-female, 4-male). For details of experimental design, please refer to DeNotta et al, Effects of repeated furosemide administration on electrolyte homeostasis and bone density in healthy adult exercising Thoroughbreds.

MATERIALS AND METHODS

Total RNA was isolated using TRIzol coupled with spin-column purification, as described previously (3). Total RNA (RIN > 8.0) was used for polyA mRNA isolation and cDNA library construction. Adapters and low-quality base reads were removed from the raw sequencing reads using fastp (v0.24.2). The trimmed reads were aligned to the reference genome (EquCab 3.0, GCA_002863925.1) using the STAR aligner (v2.7.11). Quantification was performed using Salmon (v1.10.2) at the transcript level. The R tximport package (v1.30.0) was utilized to import the transcript-level quantification results and to summarize the reads at the gene level. Statistical computation was performed using the Omics Playground (v 3.5.0). Gene-level testing for identification of differences was performed with DESeq2 (Wald test), edgeR (QLFtest), and limma-trend. The Benjamini–Hochberg corrected false discovery rate of $q < 0.05$ was used for all bioinformatic analyses.

RESULTS

The 15 samples were sequenced at an average depth of 20.8±1.9 M reads with exon transcripts representing 79.5±0.22% of the total. Intron reads comprised 14.1±0.18% and intergenic reads representing 6.4±0.10% of the total. Pearson correlation analysis of the sequencing reads (transcripts per million, TPM) ranged from 0.97-1.0 indicating strong similarity amongst the samples. Alignment to the Equus caballus genome demonstrated a total of 12,756 transcripts were expressed within the muscle that fell into 4 major categories with approximately 19% classified as cytochrome family genes (Fig 1). Using the conventional false discovery rate (FDR) of $q < 0.05$, no differences in expression were noted between saline and Lasix treated

horses. Reducing the stringency of the test to $q < 0.10$ also produced no measurable differences between treated and control horse skeletal muscle transcriptomes. Clustering the data into gene sets prior to analysis (GSEA, Fishers exact test) yielded no differences between control and furosemide treatment. Thus, cursory investigation of fit, unexercised Thoroughbred skeletal muscle demonstrates that furosemide has no effect on basal gene expression.
The integrity of the dataset was assessed by examining sex-specific gene expression (DESeq2, edgeR) at $q < 0.05$. Results demonstrate that eukaryotic translation initiation factor 2 subunit 3, Y-linked-like (LOC100066299) is expressed exclusively in males by comparison to females, and LOC102150010, an uncharacterized gene carried on the X-chromosome, is greater in females than males (Fig 2 A, B). Thus, the libraries can identify differentially expressed genes. In addition to these sex chromosome genes, modest differences ($\log_2$ FC > 0.5) in FKBP prolyl isomerase 5 (FKBP5; Fig 2C), malonlyl CoA decarboxylase (MLYCD; Fig 2D) and glutamate decarboxylase like 1 (GADL1; Fig 2E) were noted.

CONCLUSIONS

Library depth and coverage was sufficient to detect changes in sex-linked gene expression validating the approach. Using multiple statistical methods, no differences in gene expression as a function of furosemide administration were detected. These results provide evidence that the drug does not alter basal transcription in gluteus medius muscle of racing age Thoroughbreds. However, the experiment was not designed to directly test the impact of exercise-induced gene expression as a function of furosemide administration. Future analyses should include before and after exercise to ensure that the furosemide does not alter the timing and amount of exercise-responsive gene expression.

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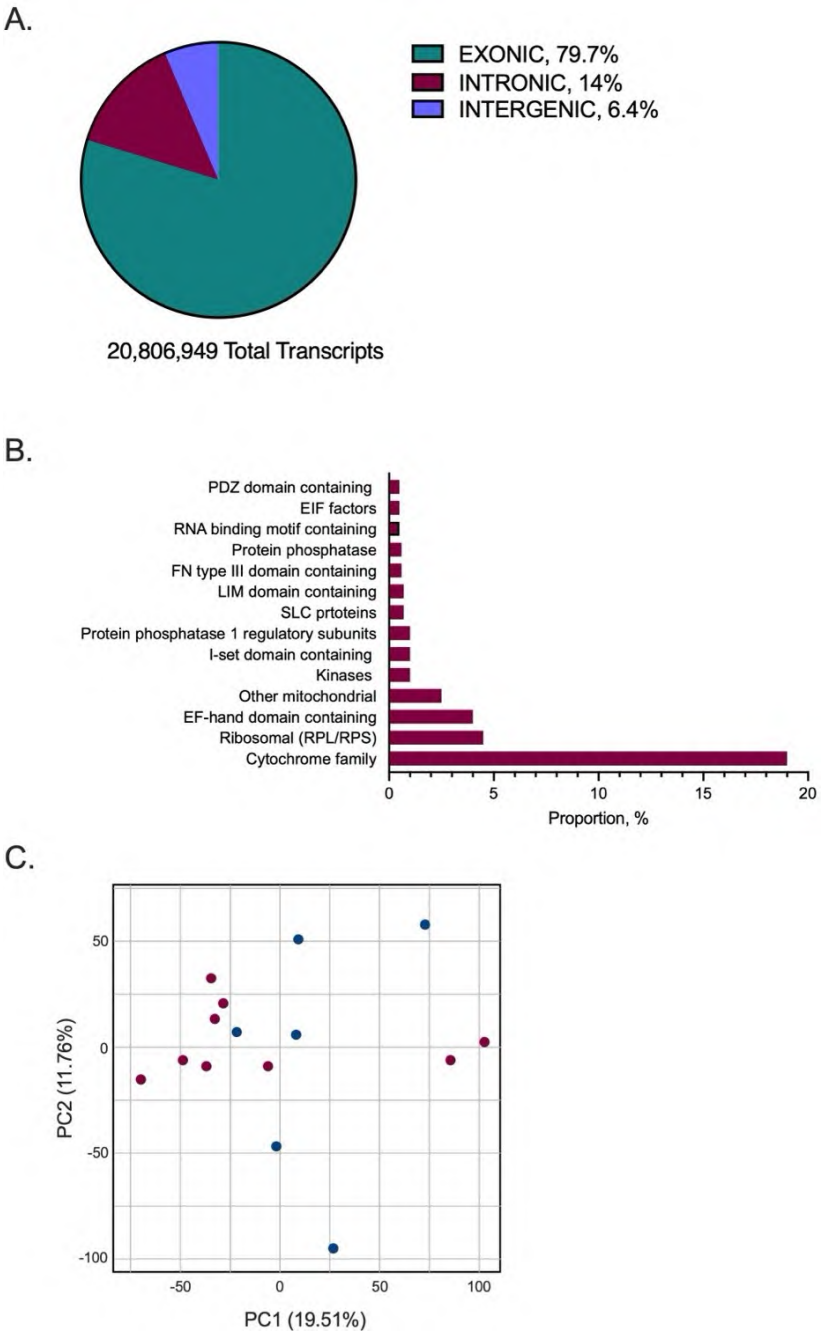

Figure 1. Transcriptome quality control metrics. cDNA libraries prepared from saline-treated and furosemide treated gluteus medius samples were analyzed as indicated in the text. Nearly 80% of the transcripts were sequenced from exons with the remainder from introns and intergenic regions (**A**). The transcripts, independent of treatment, were primarily from cytochrome and ribosomal families (**B**). Principle component analysis (PCA) demonstrates that ~20% of sample varation can be explained by PC1 with approximately 11% of variation between the samples a function of PC2 (**C**). Blue dots indicate a control sample and red dots represent furosemide treated samples.

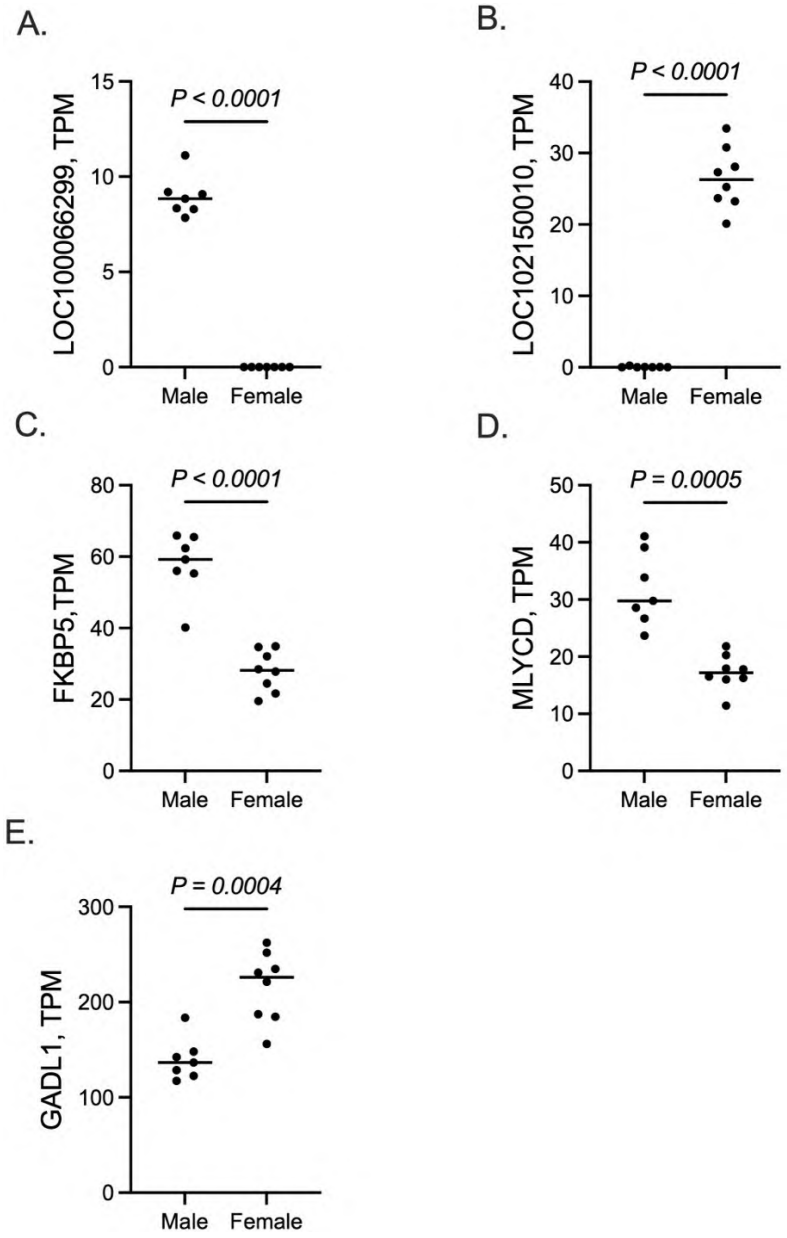

Figure 2. Differentially expressed genes identiPied as a function of sex. DESeq2 and edgR analysis was performed with q<0.05 and $\log_2$ fold change of 0.5 or greater as cutoff points. Five genes were identicied using both programs. LOC100066299 is expressed exclusively in males (**A**) and LOC102150010 in females (**B**); both are sex-chromosome linked. Males contain greater transcript abundance of FKBP5 (**C**) and MLYCD (**D**) than females which contain greater amounts of GADL1 (**E**). TPM, transcripts per million.

APPENDIX J: Waller Final Report

HISA Furosemide Advisory Committee

HISA HORSERACING INTEGRITY AND SAFETY AUTHORITY

FUROSEMIDE STUDY FINAL REPORT

Grant Period	Grant Project	Prepared by (PI)
5/22/24 – 1/10/26	Furosemide Study	Amanda P. Waller, PhD
Date		**PI Signature**
10/20/25		Amanda Waller
Grant Title		
Examining associations between furosemide treatment and racehorse health and welfare		

PI Name	Amanda Waller, PhD
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Abstract

In the U.S. horse racing industry, the administration of race day furosemide as a prophylactic treatment for Exercise-Induced Pulmonary Hemorrhage (EIPH) is both extremely common and controversial. Despite substantial research to date, understanding the associations between furosemide use and racehorse injuries and racing performance remains elusive. Thus, the overall objective of the present study was to examine the effects of race day furosemide treatment on the health and welfare of Thoroughbred racehorses, and the integrity of the sport of horse racing. It was hypothesized that i) the presence of race day furosemide treatment does not significantly alter race performance, and ii) that its absence will increase the risk of race-related injury.

Using publicly available databases, a combination of retrospective observational studies (cohort, case control, case-crossover) were employed to investigate the effects of race day furosemide treatment on the health, welfare, career longevity, and performance in Thoroughbreds racing in North America during the last ten years. The compiled data was stratified by administration of race day furosemide, and horse age was kept constant to mitigate confounding effects. Statistical analysis was performed to compare differences for the presence/absence of race day furosemide, with significance set at $P<0.05$.

Pre-race administration of furosemide was not significantly associated with the risk of racing-related fatality, career longevity, or markers of performance in the observed cohorts. There was a tendency for an increased relative risk of not finishing a race, for horses that did not receive pre-race furosemide treatment. Findings from this study are intended to help guide the development of scientific evidence-based, equine welfare–oriented furosemide medication policies that minimize risk and maximize protection of all Thoroughbred racehorses racing in the U.S.

Summary of Study Goals and Objectives

Horse racing's social license to operate in North America has never been more precarious. For the sport to have any chance of surviving its current existential crisis, rigorous science leading to evidence-based practices/protocols must be at the front leading the field. Additional independent, peer-reviewed research studies related to racehorse health and welfare injuries are desperately needed. And any possible link between racehorse injuries and therapeutic medication use, in particular furosemide, must be fully explored. Findings from such studies will almost certainly have vital implications to society and policy.

Thus, the overall aim of the present study was to examine the effects of race day furosemide treatment on the health and welfare of Thoroughbred racehorses, and the integrity of the sport of horse racing.

Recent retrospective data from the U.S. Thoroughbred racehorse population was analyzed to gain insights towards answering the following questions:

What are the effects of race day furosemide administration (absence or presence) on the health and welfare of Thoroughbreds racing in the USA? (Aim #1)

Is the presence or absence of race day furosemide administration associated with exercise-related sudden death? (Aim #1)

Is the presence or absence of race day furosemide administration associated with an increased risk of race-related fatal musculoskeletal injury? (Aim #1)

What are the long-term social and economic impacts of prohibiting race day furosemide administration to the U.S. Thoroughbred racing industry (using metrics such as frequency of starts, career length, career earnings, average field size, etc)? (Aim #2)

Does the absence of early-career furosemide administration negatively affect overall career performance and longevity? (Aim #2)

What is the effect of race day furosemide administration on racing performance? (Aim #3)

Does race day furosemide treatment adversely affect the integrity of competition? (Aim #3)

The ultimate goal of this research is that the findings from these studies will help guide the development of scientific evidence-based, equine welfare–oriented medication policies that minimize risk and maximize protection of all Thoroughbred racehorses racing in North America.

We anticipate that findings from these studies will help guide the development and implementation of scientific evidence-based, welfare–oriented medication policies that uphold the integrity of competition and maximize protection of all participants in the sport of Thoroughbred racing in North America (human and equine).

Note on Ethics Review- All experiments performed were retrospective studies using secondary data and thus did not involve any interaction with animal subjects. Accordingly, there is no requirement for Institutional IACUC approval included in this report.

Aim #1 - Furosemide & Injury Risk

Background Information

Equine injuries and fatalities have immense negative impacts on the sport of horse racing, from a human and equine welfare, regulatory, and societal standpoint; efforts to reduce horse fatalities is a topic of great importance to the industry. After a spate of breakdowns at Santa Anita racetrack during the 2019 racing season led to unprecedented negative national and international media coverage, a coalition of major tracks across the U.S. announced new safety reforms, including a landmark plan for phasing out race day furosemide treatment.

Presence of Furosemide and risk of injury

Ultimately there has been no evidence to suggest the spike in catastrophic injuries at Santa Anita were related to furosemide use in any way (Lacey 2019, CHRB 2020). Beyond that, there are surprisingly few studies in the scientific literature that specifically investigate the issue (Hitchens et al 2019). A single study by Zambruno et al (2020) examined the association between prerace administration of furosemide and the risk of musculoskeletal injury in Thoroughbreds that raced in Argentina from 2006-2015. In their retrospective analysis of close to 400,000 race starts, Zambruno et al (2020) found that prerace administration of furosemide was not associated with the risk of either musculoskeletal injury or fatal injury. Bimson et al (2022) also attempted to investigate a link between race day furosemide and fatal musculoskeletal injury in Thoroughbreds that raced in Uruguay from 2011-2017. However due to confounding effects of multiple medications (a high proportion of the study population raced on phenylbutazone), their study did not have sufficient power to draw conclusions about injury risk and furosemide use (Bimson et al 2022).

To date the main evidence linking furosemide to fatal injury has come from a recent retrospective study which extracted data from the Equine Injury Database with the aim of identifying risk factors associated with race-related sudden death in Thoroughbred racehorses (Bennet & Parkin, 2022). Their findings suggest that horses medicated with furosemide on race day were at 62% increased odds of sudden death. However, inherent to the methodology are several potential confounders that are known to affect both furosemide use and risk of sudden death (**Figure 1**). Therefore, it is likely the reported findings on furosemide fall under the Table 2 Fallacy (Westreich & Greenland, 2013). Furosemide use was treated as an independent risk factor by univariate analysis, without full consideration of the underlying causal model (**Figure 1**). Importantly, furosemide use in the study cohort is likely to be highly dependent upon age, with 2-year-olds expected to make up the majority of horses racing without furosemide (due to the progressive nature of EIPH along with the aforementioned ban put in place for

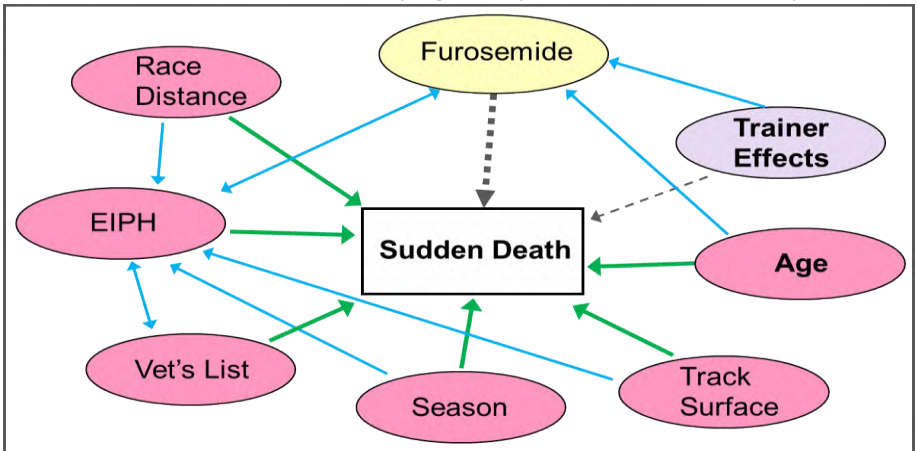


Figure 1: **Casual diagram** for the effect of furosemide on the risk of race-related sudden death, with confounding by horse age, unmeasured trainer effects, previous Exercise Induced Pulmonary Hemorrhage (EIPH) episode, and additional variables

this age group in 2020). Increasing age is a known independent risk factor for sudden death in equines (Lyle et al, 2012) Thus, age, furosemide use, and risk of sudden death are all covariates, and the purported increased risk of sudden death with furosemide use in the Bennet & Parkin (2022) study may in fact be a secondary effect of age.

As additional confounders: i) the study failed to account for the concomitant use of other medications such as phenylbutazone, a previously identified risk factor for fatal injury (Zambruno et al 2020) and ii) EIPH is both a contributing cause of sudden death and a medical indicator for furosemide treatment. Severe acute pulmonary hemorrhage was present in a large proportion (60-70%) of sudden death cases and there was evidence it's due to EIPH type pulmonary capillary pressures (Boden 2008, Lyle et al 2011). And finally, iii) the study fails to adequately account for trainer-level effects, which likely led to group-selection bias. Given that 94.6% of the starts in the dataset were with furosemide, a trainer that chose not to run their horse on Lasix may well have been more conservative when it comes to other risk factors as well, such as exercise training protocols, overall management, or use of other medications.

Absence of Furosemide and risk of injury

Recent epidemiologic evidence could indicate a link between *absence* of race day furosemide and risk of injury. A retrospective examination of data from the Equine Injury Database (Bennet & Parkin 2023) reported an anomalous increase in fatal musculoskeletal injury in North American Thoroughbred racehorses in 2020, that could not be explained by differences in exercise history, training or racing schedule. While it has not specifically been studied, a relationship to the widespread furosemide ban initiated in 2020 is not inconceivable, and there is some mechanistic evidence to support that theory. For example, furosemide treatment results in an acute, transient uresis and accompanying body weight loss of around 2%, and higher body weight at race time is known to be a risk factor for injury in Thoroughbred racehorses (Takahashi et al 2004).

Furthermore, the acute furosemide-induced reduction in blood volume is generally accepted to be the mechanism by which the medication helps to prevent EIPH. Among alternative strategies for preventing EIPH, dehydration via rationing/withholding water during the 24-48 hours prior to racing, is commonly touted. In the U.S. this appears to often include the administration of furosemide 24-48 hours out from the race. Despite the welfare concerns expressly stated by racing authorities themselves (Culberson 2019), this practice of "drawing" has been paradoxically espoused by trainers, scientists, and even racing authorities worldwide (Harris 2021, Poole & Erickson 2016, Knych et al 2020, Villarino et al 2019, Steele 2022). If the common alternative to race day furosemide is to administer it 24-48 hours prior to racing and then restrict water intake, this could potentially result in a greater level of dehydration and/or electrolyte abnormalities by race time compared to the acute administration 4 hours out, in turn increasing the risk for fatigue, arrythmias, post-exertional distress, or heatstroke.

In summary, more research is needed regarding the presence/absence of race day furosemide as an independent risk factor for either sudden death or musculoskeletal injury, before any clear conclusions can be drawn.

Objectives and Hypothesis

The objective of Aim 1) was to examine the effects of race day furosemide administration on equine health and welfare, as evidenced by racing-related injuries or fatalities.

It was hypothesized that prohibition of race day furosemide treatment would be associated with negative consequences to equine welfare, as indicated by increased racing fatalities and/or injuries.

Experimental Methods

The present study used a retrospective observational cohort analysis to assess the association between prerace administration of furosemide and *i)* fatal musculoskeletal injury, or *ii)* exercise-associated sudden death, in two-year old Thoroughbreds that raced in the U.S. from 2020-2024. Importantly, by holding age constant between the two cohorts which raced with and without furosemide, and confining the analysis to the immediate time period before and after the widespread furosemide ban was enacted, the influence of confounding variables that plagued previous studies (ex: age and trainer) were vastly mitigated.

The study cohort consisted of all starts in 2-year old Thoroughbred races in the United States from 2020 through 2024. Data on fatal injuries were taken from a publicly available database previously shown to have excellent accuracy with the Equine Injury Database (von Hippel, 2019). Once relevant fatalities were identified from the database and categorized according to type, further confirmation of the fatal injury and

information on furosemide use during the fatality race was extracted from another publicly available database (Equibase Company LLC, 2025).

The outcome variable of interest was racing-associated fatal injury due to musculoskeletal injury or sudden death. For musculoskeletal, the terms for inclusion were any racing associated catastrophic injuries resulting from fractures or tendon/ligament injury or head/cervical trauma which resulted in death or euthanasia within three days of the race. For sudden death, racing-associated fatality descriptions included the terms sudden death, pulmonary hemorrhage, EIPH, post-exertional distress, collapsed and died, suspected cardiac, etc. Race-related sudden death is defined by cases of sudden death that occur during exercise or within one hour post-race (Lyle et al 2012, Diab et al 2019).

Statistical analysis and graphing were performed using SigmaPlot 12.0 and GraphPad Prism 10 software packages. Chi-squared test was used to test associations between the incidence of racing-related fatality and race-day furosemide treatment, with statistical significance established as $p<0.05$ with a 95% confidence interval. A priori power analysis was conducted using SigmaPlot prior to study commencement to determine the required sample size to detect a projected odds ratio of 0.5 (without/with furosemide; reciprocal= 2.0) for the association between race day furosemide treatment and racing-related fatality., based on a presumed baseline musculoskeletal and sudden death fatality rate prevalence of 1.60 and 0.13, respectively (Hitchens et al 2019, Bennet & Parkin 2022), an alpha of 0.05, and a power of 80%, and using a two-sided chi-square test. A minimum sample size of 19,375 and 123,077 race starts per furosemide group was calculated to be necessary for the musculoskeletal and sudden death outcomes of interest, respectively.

Results

To avoid confounding bias in an investigation of the direct effect of race day furosemide on race-related fatalities requires holding age constant between the two cohorts that raced with and without furosemide. Therefore, the present study examined incidence of sudden death and musculoskeletal-induced fatality in 2-year-old Thoroughbred racehorses competing in the U.S. in the years immediately following the widely enacted furosemide ban for this age cohort.

This study cohort which spanned the years 2020 through 2024 consisted of N= 25.326 and 78,524 total race starts for horses treated with and without furosemide, respectively. Over the five-year study period, pre-race administration of furosemide was not significantly associated with the risk of racing-related musculoskeletal fatality (**Figure 2A**). The incidence of racing-related musculoskeletal fatality per 1000 starts was 0.75 & 0.82 for the presence vs absence of race day furosemide, respectively (P=0.751). A post-hoc power calculation showed that this five-year study cohort had 83.2% and power to detect a Cohen's *d* effect size of 0.38 with 95% confidence.

The association between race day furosemide treatment and racing-related sudden death was similarly investigated in this cohort (**Figure 2B**). Over the five-year study period, the incidence of racing-related sudden death per 1000 starts was 0.076 and 0.072 for the presence and absence of race-day furosemide, respectively (P=0.968). Thus, the relative risk of racing-related sudden death was not significantly different in 2-year-old horses racing with versus without race day furosemide treatment. These findings should be interpreted with caution however, as a post-hoc power calculation showed that the final sudden death study cohort had only 65.3% power to detect a Cohen's d effect size of 0.38 with 95% confidence.

When all-cause racing-related fatalities were examined in total (**Figure 2C**), the incidence of racing-related sudden death per 1000 starts was 0.083 and 0.089 for the presence and absence of race-day furosemide, respectively (P=0.771).

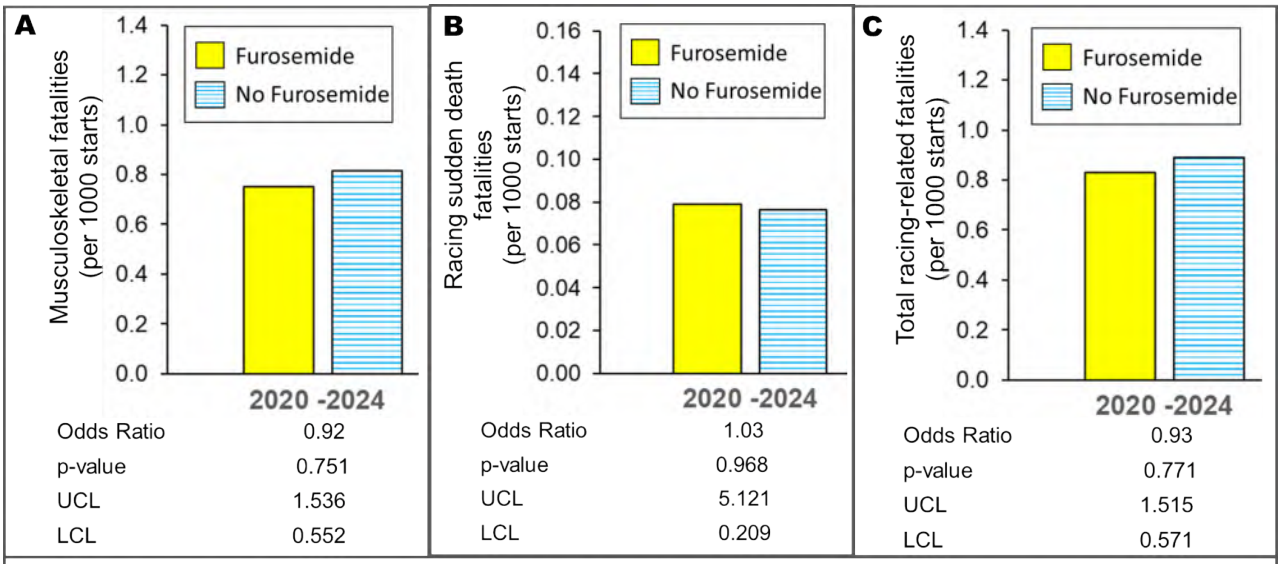


Figure 2: **Race day furosemide treatment is not associated with risk of racing-related fatality in two-year-old Thoroughbreds racing in the United States from 2020 through 2024.**

Graph depicting the rates of racing-related **A)** musculoskeletal fatalities, **B)** sudden death, and **C)** total racing-related fatalities in 2-year-old Thoroughbred racehorses running in the USA during the initial five years following the widespread furosemide (Lasix) ban (2020 through 2024). Data were stratified by race day furosemide treatment and expressed as total fatalities per 1000 starts. Chi-squared test was used to examine associations between fatality rate and furosemide treatment, with statistical significance set at $p<0.05$. N= 25,326 and 78,524 total race starts for horses treated with and without furosemide, respectively. UCL & LCL denote 95% Upper & Lower Confidence Limits, respectively.

Given the lower overall incidence of sudden death compared to musculoskeletal fatalities, the retrospective study period was further extended to a seven-year time frame that encompassed multiple racing seasons both before and after the widespread furosemide ban (2018 through 2024). The final seven-year study cohort consisted of N= 65,770 and 82,889 total race starts for horses treated with and without furosemide, respectively. Over this seven-year study period, pre-race administration of furosemide was not significantly associated with the risk of racing-related musculoskeletal fatality (P=0.932; **Figure 3A**), racing-related sudden death (P=0.936; **Figure 3B**), or all-cause racing related fatality (P=0.916; **Figure 3C**), with an incidence of all-cause racing-related fatality per 1000 starts of 0.90 and 0.88 for the presence and absence of race day furosemide, respectively. A post-hoc power calculation showed that this seven-year study cohort had ≥95.5% power to detect differences in musculoskeletal and all-cause fatalities with 95% confidence, but only 65.3% power to with respect to the sudden death outcome of interest.

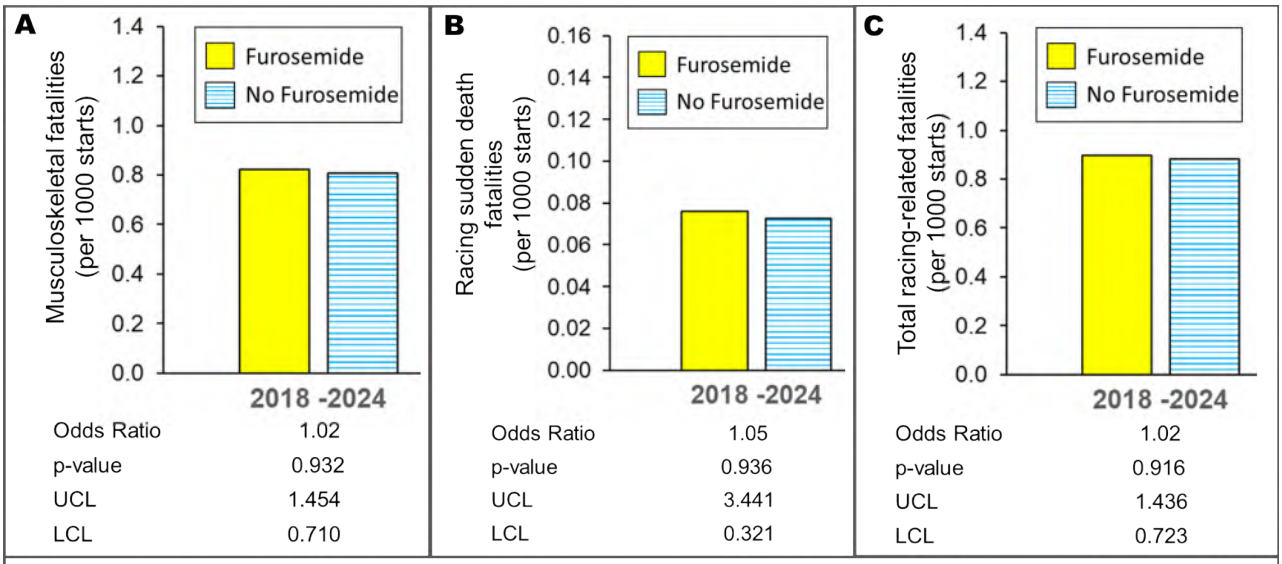
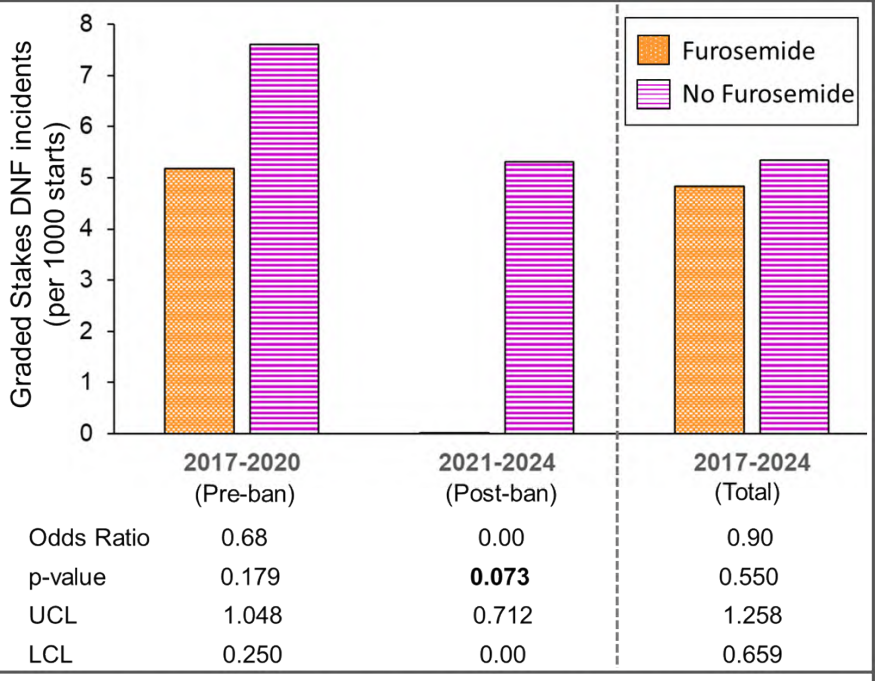


Figure 3: **Race day furosemide treatment is not associated with risk of racing-related fatality in two-year-old Thoroughbreds racing in the United States from 2018 through 2024.**

Graph depicting the rates of racing-related **A)** musculoskeletal fatalities, **B)** sudden death, and **C)** total racing-related fatalities in 2-year-old Thoroughbred racehorses running in the USA encompassing a seven-year time period immediately preceding and following following the widespread furosemide (Lasix) ban (2018 through 2024). Data were stratified by race day furosemide treatment and expressed as total fatalities per 1000 starts. Chi-squared test was used to examine associations between fatality rate and furosemide treatment, with statistical significance set at $p<0.05$. N= 65,770 and 82,889 total race starts for horses treated with and without furosemide, respectively. UCL & LCL denote 95% Upper & Lower Confidence Limits, respectively.

Since furosemide treatment also has the potential to affect non-fatal health and safety outcomes, statistical analysis of an additional dataset was performed. The inclusion criteria for this dataset was all starts in graded stakes races for Thoroughbreds racing in the U.S during the study time period. The predictor variable was presence/absence of race day furosemide treatment. The outcome variable of interest was horse related Did Not Finish (DNF) incidents. DNFs attributed to non-horse factors (jockey falls, equipment failures, gate issues, etc) were excluded.

The incidence of a horse not finishing in a Graded Stakes race was numerically higher for horses racing without race day furosemide, for both the Pre- and Post- ban time periods studied (**Figure 4**). There was a tendency towards this becoming significant for the time period after the furosemide ban however the analysis was underpowered (P=0.073, power 67.9%). When the data was combined into all U.S. Graded Stakes races taking place from 2017-2024, the number of horse-related DNF incidents per 1000 starts was 4.84 & 5.35 for horses competing with/without race day furosemide, respectively (P=0.550; power 53.4%; **Figure 4**).

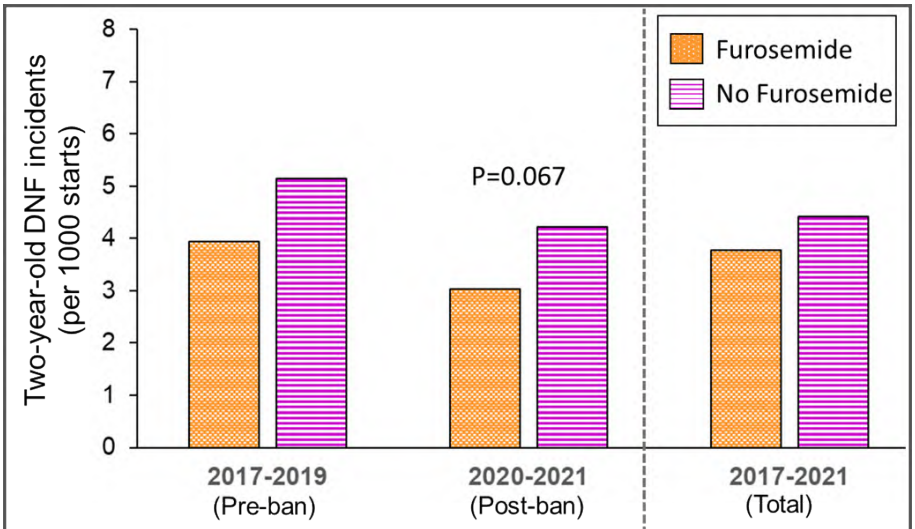


	2017-2020 (Pre-ban)	2021-2024 (Post-ban)	2017-2024 (Total)
Odds Ratio	0.68	0.00	0.90
p-value	0.179	**0.073**	0.550
UCL	1.048	0.712	1.258
LCL	0.250	0.00	0.659

Figure 4: **Horse-related Did Not Finish (DNF) incidents and association with race day furosemide treatment for Thoroughbreds racing in Graded Stakes races in the United States from 2017 through 2024.**

Graph depicting the rate of horse-related DNF incidents in Thoroughbred racehorses running in American Graded Stakes from 2017 – 2024. Data were stratified by race day furosemide treatment and expressed as total DNF incidents per 1000 starts. Left panel depicts data separated into before and after (Pre/Post) the widespread furosemide bans in Graded Stakes races was enacted (in 2020 and 2021 for 2-year old and 3-year olds and older, respectively). Chi-squared test was used to examine associations between DNF rate and furosemide treatment. with statistical significance set at $p<0.05$. N= 14,464 and 13,261 total starts for horses treated with and without race day furosemide, respectively. UCL & LCL denote 95% Upper & Lower Confidence Limits, respectively.

A similar analysis was performed for 2-year-old races separated into the years immediately preceding and following the widespread furosemide ban. In this cohort of 2-year-olds, again the incidence of a horse not finishing in a race as a 2-year-old was numerically higher for horses racing without race day furosemide, for both the Pre- and Post- ban time periods studied (**Figure 5**). There was a tendency for this difference to become significant for the time period after the furosemide ban (P=0.067).
However, when all 2-year-old starts in the U.S. that took place from 2017-2021 are compared, the risk of a horse not finishing a race as a 2-year-old was not significantly different for horses racing without race day furosemide (P=0.123; **Figure 5**). The rate of horse-related DNF incidents per 1000 starts was 3.77 & 4.41 for the presence vs absence of race day furosemide, respectively. A post-hoc power calculation showed that the total 2-year-old study cohort had 80.8% power to detect an effect size of 0.15 with 95% confidence.



	2017-2019 (Pre-ban)	2020-2021 (Post-ban)	2017-2021 (Total)
Odds Ratio	0.76	0.72	0.85
p-value	0.142	**0.067**	0.123
UCL	1.096	1.027	0.973
LCL	0.534	0.500	0.647

Figure 5: **Horse-related Did Not Finish (DNF) incidents and association with race day furosemide treatment for Two-year old Thoroughbreds racing in the United States from 2017 through 2021.**

Graph depicting the rate of horse-related DNF incidents for all 2-year old Thoroughbred races in the U.S. from 2017 – 2021. Data were stratified by race day furosemide treatment and expressed as total DNF incidents per 1000 starts. Left panel depicts data separated into before and after (Pre/Post) the widespread furosemide ban in 2-year old races was enacted (2020). Chi-squared test was used to examine associations between DNF rate and furosemide treatment. with statistical significance set at $p<0.05$. N= 74,858 and 32,428 total starts for horses treated with and without race day furosemide, respectively. UCL & LCL denote 95% Upper & Lower Confidence Limits, respectively. Raw data taken from Angst (2022).

Combining all the data on DNF incidents in races graded stakes & 2-year-olds pre/post furosemide ban, the incidence of not finishing a race was 3.77 and 4.41 per 1,000 starts for the presence and absence of race day furosemide, respectively. (OR=0.89 (0.741 to 1.076) and P=0.235 with power = 81.5%).

Aim #2 - Furosemide & Career Longevity

Background Information

In Thoroughbred racehorses, a single intravenous dose of 250-500 mg of furosemide results in a rapid diuresis and concomitant dehydration, electrolyte losses, alterations in acid-base state, a decreased plasma volume (~10%), and total body weight loss (~2.5%). (Soma & Uboh, 1998; Carlson & Jones 1999; Kline et al 2006; Morley et al 2014; Sullivan et al 2015; Knych et al 2020, Shoemaker et al 2024). Each one of these physiological treatment effects has the potential to alter short- or long-term health or performance (**Figure 6)**, with some being likely to enhance while others have the potential to diminish. As a result, the overall consequences to our equine Thoroughbred athletes are complex, multifactorial, highly situation dependent, and not easily discerned. For example, the fact that EIPH is both a contributor to poor performance and attrition, and also a medical indicator for furosemide treatment is one aspect that adds to the complexities.

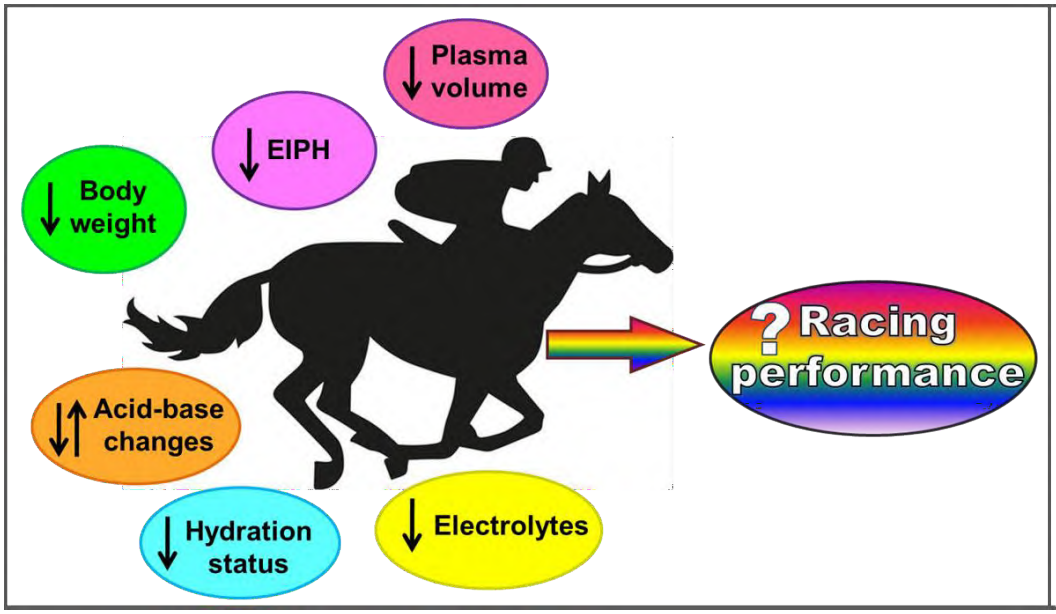


Figure 6: Acute physiological effects of furosemide treatment in Thoroughbred racehorses which could potentially alter exercise performance. EIPH= Exercise Induced Pulmonary Hemorrhage.

As arguably among the most controversial issues in all of North American Thoroughbred horse racing, there exists a plethora of academic and lay articles covering both Exercise-Induced Pulmonary Hemorrhage (EIPH) and furosemide treatment. Interested readers are directed to the following key papers among others (Bayly & Leguillette 2022, Bayly 2021, Poole & Erickson 2016, Hinchcliff et al 2015, Sullivan et al 2015). In attempt to summarize the expansive literature, along with the most recent research presented at the meeting of the world's foremost experts in equine exercise physiology (ICEEP), which took place in Uppsala Sweden in July of 2022, the following key points on furosemide and EIPH in horses are clear: i) EIPH has a high prevalence in race horses and other types of athletic horses, ii) it results from extreme buildup of pulmonary artery intravascular pressures leading to capillary failure, and iii) to date prophylactic furosemide is the only scientifically proven method of reducing its occurrence and/or severity.

In summary, understanding the long-term consequences of race day furosemide treatment remains a complex and multifactorial challenge for the racing industry. Ultimately, any association between race day furosemide administration and lifetime racing career performance and longevity remains to be elucidated.

Objectives and Hypothesis

The objective of Aim 2) was to examine the social and economic impacts of race day furosemide administration to the North American Thoroughbred racing industry, as measured by long-term racing career performance metrics

It was hypothesized that the absence of early-career furosemide administration will have subsequent negative association with a racehorse's overall career performance and longevity.

Experimental Methods

Individual level comparison

A retrospective observational case control study was employed to compare key metrics of career performance in Thoroughbreds that raced exclusively on furosemide as 2-year-olds, versus a matched cohort of controls that did not receive furosemide for any races during their 2-year-old year.

Specific dataset inclusion criteria encompassed Thoroughbreds racing in the USA with three or more starts during their 2-year-old season (2020), at least one of which was a Listed Stakes or Black-Type class of race. Horses in the "Yes" cohort received race day furosemide treatment for all their 2-year-old starts. Horses in the "No" cohort were matched controls such that they were direct competitors to the Yes group horses in at least one Listed Stakes/Black-Type race in their 2-year-old season, and all their 2-year-old starts were conducted without race day furosemide treatment. Thus, matched pairs were matches in racing age, sex, and racing class. Outcome variables of interest included lifetime earnings, career length, lifetime starts, top 3 placings and average lifetime speed figures. Information on furosemide use during each race and measures of racing career performance were extracted from a publicly available database (Equibase Company LLC, 2025).

Group level comparison

To further investigate whether race-day furosemide treatment is associated with career longevity and race participation, an additional group-level analysis of was performed. The cohort consisted of 2-year old Thoroughbred racehorses racing in the North America between 1992 and 2025. Outcome variables of interest were A) 2-year-old starts per total foal crop, B) 2-year-old average starts per horse, and C) 2-year-old starters per total foal crop. A linear regression analysis for each outcome variable vs time was performed to identify the industry trends over time. The outcome values for the most recent years since the furosemide ban were compared to the overall trendline to assess the ban's overall effects to date. Annual racing data from the five years immediately preceding the widespread furosemide ban in 2-year-old races (2015 to 2019) was compared to post-ban seasons (2021 to 2024). The data for annual 2-year-old racing parameters were extracted from a publicly available database (The Jockey Club, 2025), and normalized to each year's respective annual foal crop.

Statistical analysis and graphing were performed using SigmaPlot 12.0 and GraphPad Prism 10 software packages. Linear regression analysis was used to assess trends in two-year old races over time. Welch's t-test or Wilcoxon signed-rank test were used to probe for differences in career metrics between furosemide cohorts. Statistical significance was established as $p<0.05$ with a 95% CI. A priori power analysis estimated a minimum sample size of N=18 horses/matched cohort was required to achieve adequate statistical power (0.80 with alpha= 0.05).

Results

After controlling for age, sex, and racing class level, early career furosemide administration was not associated with outcomes relating to overall racing career performance and longevity. There were no differences in any of the examined metrics of racing career performance and longevity between horses that competed with versus without race-day furosemide treatment for all their 2-year-old races (**Table 1**). Specifically, the presence/absence of early-career furosemide administration did not have subsequent long-term effects on lifetime earnings, racing career length, lifetime starts, number of times placed in the top 3, or average lifetime speed figures.

	Furosemide as 2YO		95% CI	P value
	Yes	No		
2YO starts	4.8 ± 0.4	4.8 ± 0.3	3.9, 5.4	0.832
Career starts	27.0 ± 2.8	22.8 ± 2.9	18.9, 30.9	0.300
Career length (weeks)	155.8 ± 12.6	143.4 ± 15.4	120, 179	0.520
Career earnings (x$1,000)	137.2 ± 23.1	178.2 ± 44.6	99.7, 227.0	0.380
Top 3 finishes	11.9 ± 1.3	9.4 ± 1.8	7.4, 13.9	0.105
Average Speed Figure	65.9 ± 3.6	65.8 ± 4.4	57.5, 74.3	0.991
Earnings/Start (x$1,000)	5.7 ± 1.0	7.0 ± 1.5	4.8, 8.0	0.458

Table 1: **Early career furosemide administration is not associated with differences in racing career performance and longevity metrics.**

Dataset includes Thoroughbreds racing in the USA with three or more starts during their 2-year-old (2YO) season (2020), at least one of which was a Listed Stakes or Black-Type race. Horses in the "Yes" cohort received race day furosemide treatment for all their 2-year-old starts. Horses in the "No" cohort were matched controls such that they were direct competitors to the Yes group horses in at least one Listed Stakes/Black-Type race during their 2-year-old season. Thus, each pair was matched for year of birth (age), sex, and racing class. Horses in the No cohort conducted all their 2-year-old starts without race day furosemide treatment. Welch's t test was used to compare the variables of interest with statistical significance set at $p<0.05$. Data are Mean ± SEM. CI denotes 95% two-tailed confidence interval for difference of means. There were no significant differences between groups for any outcome variables of interest (N=18 horses/group).

To study the effects of the furosemide ban in 2-year-old races at the group population level, the annual racing statistics for 2-year-old North American Thoroughbred racehorses racing from 1992 through 2024 were normalized to each year's respective foal crop data, and linear regression analysis was performed to show the industry trend over time. The results show that, apart from the anomalous COVID-19 season of 2020, these markers of the overall state of 2-year-old racing have been at or above the trendline since the furosemide ban (**Figure 7**). Specifically, **A)** the number of 2-year-old starts per total foal crop, **C)** the number of 2-year-old starts per two-year-old starters, and **E)** the number of 2-year-old starters per total foal crop have all been higher than historical industry trends would predict since furosemide was banned in this cohort.

Excluding the outlier Covid-19 year of 2020, the five-year time periods immediately before (Pre) and after (Post) the furosemide ban were compared by Welch's t-test, and the data indicate higher rates of 2-year-old starters in the Post-ban group. Relative to annual foal crop, the number of 2-year-old starts (**Figure 7B**) and starters (**Figure 7F**) were higher after the furosemide ban ($P=0.03$ and $P<0.001$, respectively), however the average starts/horse (**Figure 7D**) was decreased post-ban ($P=0.002$). Thus, with respect to these key metrics the overall state of 2-year-old North American Thoroughbred racing does not appear to have been negatively affected by the prohibition of race day furosemide treatment.

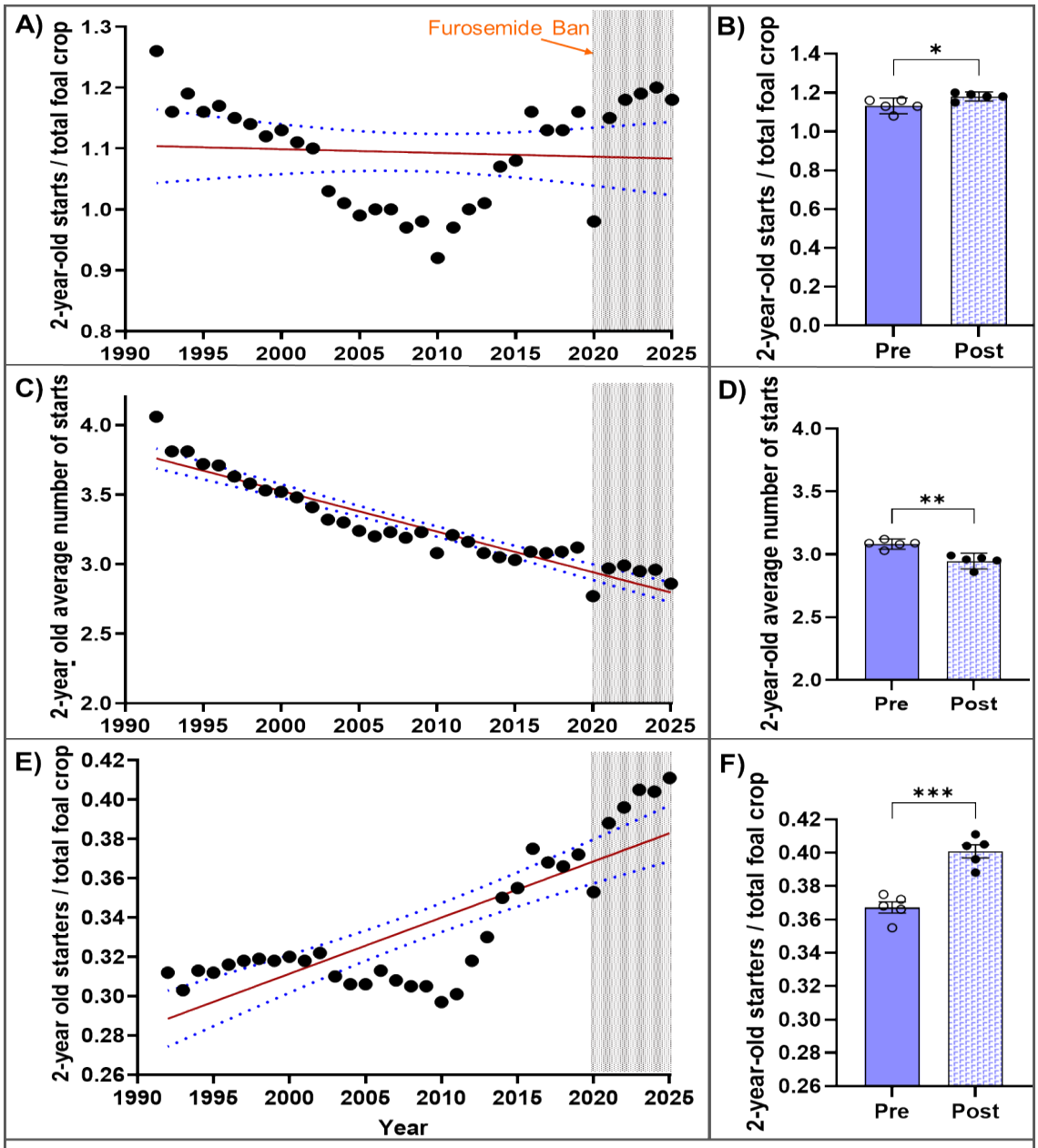


Figure 7: **Graphs of annual racing and foal crop data for 2-year-old North American Thoroughbred racehorses from 1992 through 2025.**
A), B) 2-year-old starts per total foal crop, **C), D)** 2-year-old average number of starts, **E), F)** 2-year-old starters per total foal crop. Linear regression analysis (**A, C, E**) demonstrates that, apart from the anomalous 2020 season, these markers indicative of the overall state of 2-year-old racing have been at or above the historical trend line since the furosemide ban in this age cohort was enacted. Solid and dotted lines indicate the regression trendline and 95% confidence intervals, respectively. Shaded portion indicates the time period after the widespread furosemide ban was enacted (2020 onward). When the five-year time periods immediately before (**Pre**) and after (**Post**) the furosemide ban are compared by t-test (**B, D, F**), the data indicate higher rates of 2-year-old starters in the Post-ban group. Graphs are Mean ± 95% confidence intervals. *, **, *** denotes P<0.05, P<0,01, P<0.001, respectively.

Aim #3 - Furosemide & Racing Performance

Background Information

Race day furosemide treatment and racing performance

Identifying and quantifying true differences in racing performance is incredibly challenging. Field studies involving simulated races on a racetrack or standardized exercise tests on a treadmill generally allow for greater control of experimental variables. However, performance in a race cannot truly be replicated in the laboratory (whether track or treadmill), and thus these studies tend to produce lower quality evidence with little translational comparison to actual racing performance (Hinchcliff et al 2005). Ultimately a race can be won or lost by hundredths of a second, but it is extremely difficult to accurately measure such differences under non- race conditions. Thus, studies conducted under natural race conditions are the 'gold standard' for investigating racehorse performance.

Of course, assessing real-world racing performance also poses its own set of methodological challenges. Consequently, there appears to be only a handful of published studies on the effects of furosemide treatment on natural racing performance of Thoroughbreds competing in North America. The largest study to date is by Gross et al (1999), which involved n= 16.761 and 5,828 Thoroughbreds racing with and without prerace furosemide treatment, respectively. Gross et al (1990) found that horses receiving pre-race furosemide ran 0.56 – 1.09 seconds faster over a distance standardized 6 furlongs, earned more money, and were more likely to win or finish in the top 3 positions than horses that were not administered furosemide. There are a few confounding limitations to the research however, including unknown dose and timing of furosemide treatment, and failure to account for EIPH status, racetrack physical characteristics, or track surface conditions. Finally, the decision to race with/without furosemide was voluntary in this racehorse population. In other words, the trainers essentially chose the inclusion criteria and selected the subjects for the two study treatment groups. which likely led to group-selection bias. Given that 75% of the starts in the dataset were with furosemide, a trainer that chose not to run their horse on Lasix may well have been more conservative when it comes to other performance factors as well, such as exercise training protocols, overall management, or use of other medications.

Another study of furosemide and performance in racing Thoroughbreds did account for EIPH status (Soma et al 1985). When the cohorts were separated into EIPH positive and negative groups, as well as by raceday furosemide treatment, standardized race speed was improved by furosemide treatment only in the EIPH positive horses. EIPH has been shown to impair race performance in Thoroughbreds, and furosemide treatment decreases the incidence and severity of EIPH (Hinchcliff et al 2005). Therefore, the improved racing speeds after furosemide administration seen in both the Soma et al (185) and Gross et al (1999) studies may be a result of treatment-induced EIPH mitigation, rather than evidence of any direct performance enhancing effect of furosemide itself.

Sweeney et al (1990) investigated the effects of raceday furosemide on the racing times of n= 52 and 79 Thoroughbred racehorses with and without prior EIPH diagnosis, respectively. The authors found that geldings both with and without EIPH raced ~0.55 seconds faster when given furosemide before racing. In contrast, there was no effect of furosemide treatment on race performance in the colts or fillies. Ultimately the findings of improved performance in the geldings must be interpreted cautiously however, as there is clear risk of confounding bias, since the geldings in the study were significantly older than the colts and fillies. A horse's age a known factor affecting race speed/racing performance, thus in the Sweeney et al (1990) study the improved racing speeds seen in geldings after furosemide treatment may actually be a secondary effect of age.

A recent study by Shoemaker et al (2024) appears to be the sole publication to date which examines the association of furosemide and performance in the time frame after the widespread furosemide ban was put in place. The authors examined n=830 two-year old Thoroughbreds racing at 15 American racetracks in 2020. Interestingly, the data demonstrate a clear negative effect of furosemide treatment on race performance, such that horses administered race day furosemide were significantly slower (lower speed figures) than horses that did not receive treatment, a relationship that persisted regardless of EIPH status (Shoemaker et al 2024).

In summary, the presently available evidence on the potential performance enhancing effects of furosemide is confounded by methodological limitations, and the research results are conflicting. When the current body of literature is taken together it appears the relationship between furosemide treatment and racing performance still lacks a clear understanding.

Objectives and Hypothesis

The objective of Aim 3) was to examine the effect of race day furosemide treatment on racing performance and the integrity of competition, in Thoroughbred racehorses competing in the United States between 2020-2024.

It was hypothesized that the race day administration of furosemide does not significantly affect racing performance, at either the individual or group population level.

Experimental Methods

Horse racing in North American has undergone a sea change in furosemide regulations over the past few years. It began in 2020 when a coalition of major racetracks banned race day furosemide in two-year old races. This was followed by a similar ban in Graded Stakes races in 2021. And as HISA began its oversight of the sport in 2023, even more race tracks and races joined in these same furosemide prohibitions. This longitudinal, sequential, age- and racing class- based ban on race day furosemide administration has provided an opportune dataset that is ripe for in depth analysis. Specifically, the broad-ranging and compulsory nature of the bans on furosemide treatment have provided a large, highly controlled study population that avoids the risk of selection bias inherent to previous studies. Many of the potential confounding variables seen in previous studies are ameliorated as well. In particular, it eliminates many possible individual trainer effects as covariates. It also mitigates much of the potential for confounding due to the well-known multi-directional casual model effects between i) presence and severity of EIPH, ii) racing performance, and iii) likelihood of receiving furosemide treatment as well. Taken together these factors clearly convey the unique advantages to studying this newly available dataset.

Group level comparison

First the effects of race day furosemide treatment on measures of racing performance was examined at the group level. A retrospective observational cohort analysis was employed to assess the relationship between prerace administration of furosemide and racing performance in a large, controlled population of U.S. Thoroughbred racehorses. The dataset consisted of all American Graded Stakes races which were run every year over a consecutive five-year period from 2019-2023. Graded Stakes that were not run every year or not run under an identical format year-over-year were excluded. Importantly, the influence of confounding variables was vastly mitigated by limiting the inclusion criteria for the study population of interest to graded stakes races that took place annually both immediately before and after a widespread ban on furosemide was initiated. Information on furosemide use during the race and measures of racing performance were extracted from a publicly available database (Equibase Company LLC, 2025).

The official winning Speed Figure, an objective, well-established measures of race performance, was chosen as the primary outcome variable. The Equibase® Speed Figure is a holistic rating of a horse's past performance in a race. Its sophisticated, well- validated algorithms incorporate the actual race time in combination with other factors, such as the racing surface, race distance, the racetrack's inherent physical properties (turn radius, banking, run up distance, etc), and the specific race-day conditions (track surface= dirt/turf/synthetic, fast/firm/sloppy/sealed, etc) (Soma and Uboh, 1998; Shoemaker et al, 2024). Importantly, it is standardized for different tracks, distances and conditions allowing for a truly comparable measure of performance. Therefore, the use of official Speed Figures as the primary outcome variable permits rigorous control of numerous intrinsic and extrinsic factors which would otherwise produce confounding bias.

Individual level comparison

A retrospective, observational, case-crossover study design was used to assess the effect of prerace administration of furosemide on racing performance in individual Thoroughbred racehorses competing in North America from 2020-2024. Information on furosemide use during a race and measures of racing performance were extracted from a publicly available database (Equibase Company LLC, 2025).

In order to reduce variation in performance outcomes due to extrinsic and intrinsic factors, statistical performance comparisons was limited to pairs of On/Off furosemide races run at identical distances, track surfaces, and racing class, under fast/good conditions, and occurring within the same racing season (same racing age /calendar year) and quarter. A random cross-section of individual horses was screened for matched pairs of completed races On/Off race day furosemide that meet the specific race-inclusion criteria.

The well-established, objective outcome variables of interest for each race were the horse's official Speed Figure for that race, along with the placing/ finishing position, and percentage of the field beaten.

Statistical analysis and graphing were performed using SigmaPlot 12.0 and GraphPad Prism 10 software packages. One-way analysis of variance (ANOVA) was used to compare year-over-year race performance outcomes and the treatment effect of furosemide administration. Paired t-test was used to compare individual race performance outcomes and furosemide treatment effect (presence or absence of race day administration). Shapiro- Wilk and Brown-Forsythe tests were used to confirm normality and equal variance, respectively. Statistical significance was established as $p<0.05$ with a 95% confidence interval (two-tailed). At the group level comparison, a priori statistical power calculation using the 2017-2019 graded stakes race data (ie; prior to/absent from any year-over-year modifications in furosemide regulatory status) determined a minimum sample size of n= 20 races per group was needed in order to detect a 1% difference in the race winning Speed Figure with vs without permitted race day furosemide treatment, at a 5% level of significance (two sided) and with 80% power, and assuming an average standard deviation of 2 speed figure points. At the individual level comparison, a priori power calculation determined a minimum sample size of n= 30 races per group was needed to detect a 5% difference in the means of the race performance outcome variables of interest.

Results

To control for racing age and the different time frames of enacting the furosemide ban, the extracted data was separated into four distinct cohorts for subsequent statistical analysis (**Figure 8**): **A)** Two-year old Graded Stakes races, which enacted the furosemide ban starting in 2020. Three-years old and upwards Graded Stakes races were separated by year of eventual furosemide ban into **B)** races which enacted the ban starting in 2021, **C)** races which enacted the ban in 2023, and finally **D)** the races which had no ban and continued to permit race day furosemide through 2023.

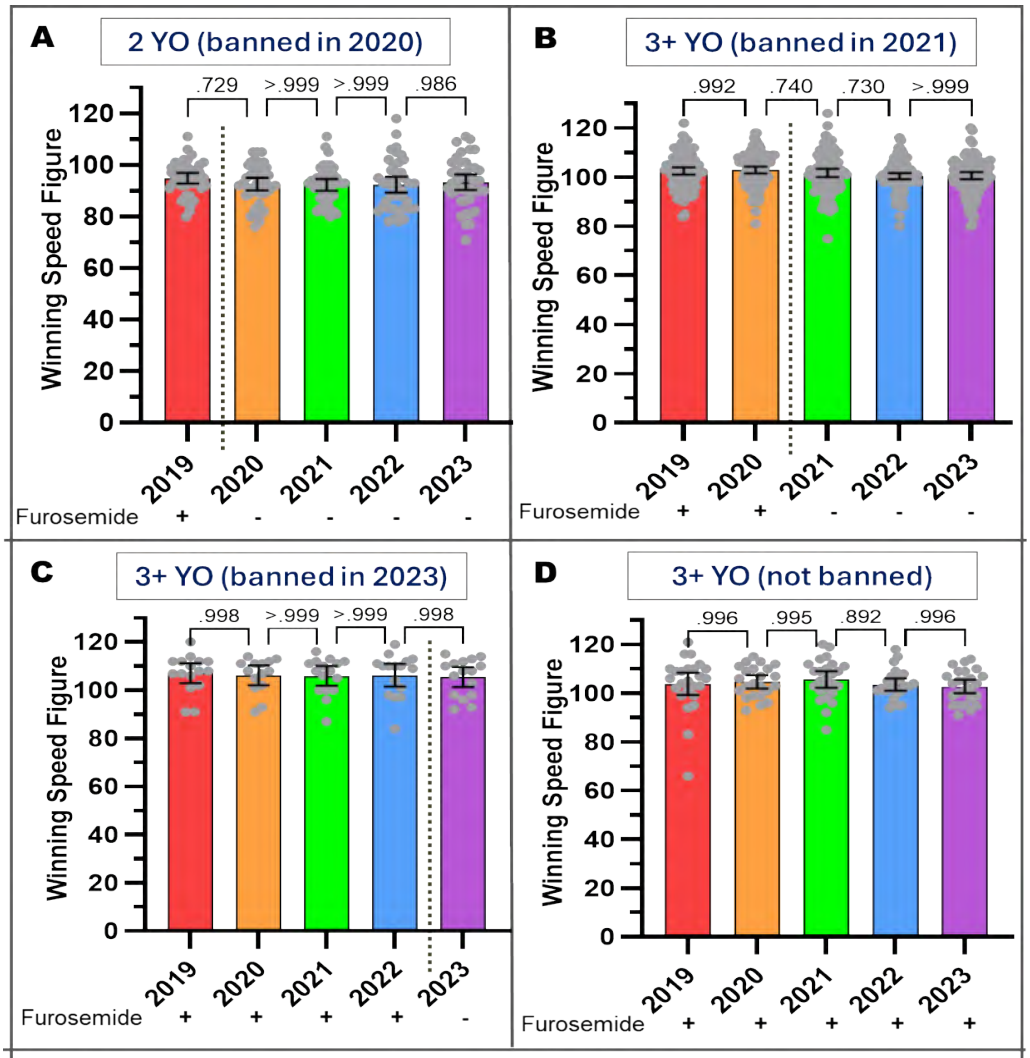


Figure 8: **Race day furosemide treatment is not associated with race performance in a large, controlled population of elite level racehorses.**

Graphs are the race winning Speed Figure for all North American Thoroughbred Graded Stakes races taking place every year from 2019-2023. Data were grouped by racing age and the year in which race day furosemide treatment was banned (indicated on each graph by a vertical dotted line).

Data are Mean ± 95% confidence intervals. p-values comparing year-over-year differences in winning Speed Figure are shown above the graph bars. Statistical significance set at $p<0.05$. **2 YO** = races for two-year olds; **3+YO** = races for three-years old and up;

N= 41, 102, 25, & 20 Graded Stakes races per group for (A) through (D), respectively.

Importantly, since the intrinsic factors of each stakes race remain the same year-over-year, and the race-day conditions are accounted for by the Speed Figure outcome variable, the data are essentially paired year-over-year. This allows for direct comparison of race performance in the year immediately preceding the furosemide ban, and the year following. Furthermore, the since there were no intrinsically or extrinsically applied changes to the independent variables in this cohort year-over-year, the Furosemide Permitted group (**D)** serves as an additional study control accounting for baseline trends over time.
These findings show there were no significant differences in racing performance in Graded Stakes races conducted before vs after permitted race day furosemide, in any cohort group, for any year-over-year period studied (**Figure 8**). Thus, race day furosemide treatment did not significantly affect overall racing performance in a controlled population of Thoroughbreds recently competing in the U.S. at the highest level of racing class. A post-hoc power calculation showed that the final analysis exhibited 97.3% power with 95% confidence.
Finally, a retrospective, observational, case-crossover study design was employed to assess the effects of prerace administration of furosemide on racing performance at the individual horse level. (**Figure 9**). A random cross-section of individual horses was screened for matched (race surface, distance, racing class, racing age and season) pairs of completed races On/Off furosemide. Importantly, the use of the case-crossover design and tightly controlled race matching strategy ensured that the influence of potential confounding variables was minimized.

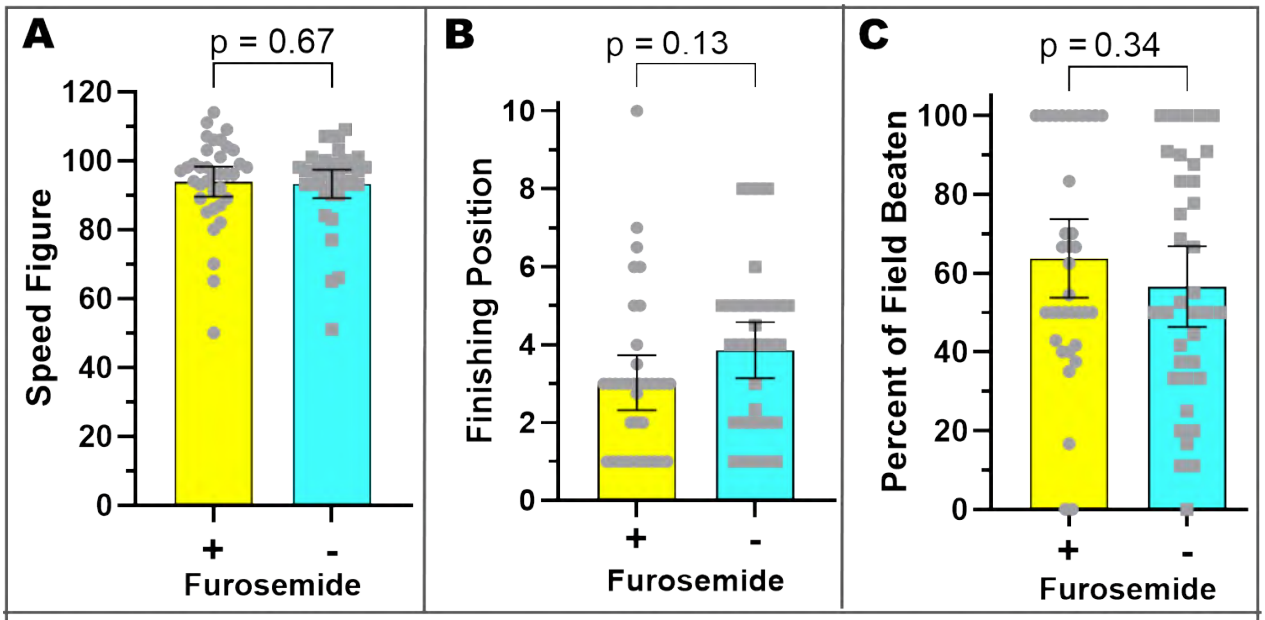


Figure 9: No a**ssociation between race day furosemide treatment and select measures of racing performance in individual Thoroughbred racehorses racing in North America.**

A retrospective, observational, case-crossover study design was used to compare the effect of prerace furosemide administration on racing performance in individual Thoroughbred racehorses racing between 2020-2024. The dataset consisted of races paired 1/1 with/without (**+/-**) race day furosemide, and were matched for race surface, distance, racing class, racing age and season.
Graphs are Mean ± 95% confidence intervals for three race level performance outcome variables of Interest: **A)** race speed figure, **B)** race finish position, and **C)** percentage of the field beaten. Data are from n=36 paired (+/- furosemide) races from 18 individual horses. Statistical significance set at $p<0.05$.

When the race performance of individual race horses was compared over matching pairs of races in which they competed with vs without race day furosemide treatment (**Figure 9**), there was no significant association between a horse receiving race day furosemide and the concomitant **Fig 9A)** speed figure (P=0.670), **Fig 9B)** finishing position (P=0.130), or **Fig 9C)** percentage of the field beaten achieved in that race (P=0.340). A post-hoc power calculation showed that the final analysis obtained
≥83.1% power with 95% confidence.

CONCLUSIONS

The present studies represent a tightly controlled examination of the recent retrospective data from the last 5 years of Thoroughbred racing in North America, with the purpose of determining associations between race day furosemide treatment and racehorse health, welfare, and performance. Importantly, the proposed time frame encompasses the initial years before and after implementation of widespread, mandated and controlled changes to the rules regarding permissibility of race day furosemide treatment. Overall, this research aims to help guide the development of scientific evidence-based, equine welfare–oriented medication policies that minimize risk and maximize protection of all Thoroughbred racehorses racing in North America. Evidence gained from this study can be summarized as the following:

In a large, retrospective cohort of two-year-old Thoroughbreds racing in the U.S., neither the presence nor absence of race day furosemide treatment was associated with risk of racing-related sudden death or musculoskeletal fatality. However, there was a tendency for an increased relative risk of not finishing in a race for 2-year-olds that did not receive pre-race furosemide.

In a retrospective observational case control cohort comparing U.S. thoroughbreds that raced exclusively on furosemide as 2-year-olds versus a matched cohort of controls that did not receive furosemide for any races during their 2-year old year, neither the presence nor absence of race day furosemide showed an association with lifetime starts, racing career length, or additional key metrics of overall career performance and longevity.

Relative to annual foal crop, the number of 2-year-old starts and starters were higher after the furosemide ban was enacted.

In a retrospective cohort of racehorses competing in North American Thoroughbred Graded Stakes during the multi-year time period directly before and after the widespread furosemide ban was enacted, race-day furosemide treatment did not affect racing performance as measured by year-to-year winning Speed Figures.

Using a tightly controlled race matching strategy, a retrospective observational case-crossover study of individual Thoroughbred racehorses competing in North America from 2020-2024 revealed no significant association of pre-race furosemide treatment with race performance outcomes (Speed Figure, finishing position, & percentage of the field beaten).

There are limitations in the present research. The retrospective study design is one example. The lack of a publicly accessible national equine injury database is another. And while not a limitation itself, the present study subjects were restricted to tightly controlled cohorts consisting of 2-year-olds and horses running graded stakes; It remains to be seen whether these findings

are generalizable to other racehorse populations, in particular older Thoroughbreds and those racing at average/lower class levels.
Ultimately, these findings are but one piece of the challenging and fascinating furosemide puzzle. Whether or not any changes are made to current medication policies, ongoing research is crucial to putting all the puzzle pieces together – a goal surely shared by all players in the industry. Thankfully HISA has already shown a keen commitment and high competence in this regard and will surely continue to do so as they collect more and more data.
In conclusion, racing-related fatalities are a topic of great interest to the equine racing industry, owing to their impact from a welfare, regulatory, and societal standpoint. Racing's social license is a major source of debate and is under increasing threat, primarily due to what can be broadly summarized as public perception that racing is overtly harmful to its equine participants. One certainly does not need be a horse racing expert to grasp the critical lens the sport has come under in recent times. As arguably one of the most hotly contested and polarizing of the issues, The Great Furosemide Debate highlights the crucial importance of objective data obtained via rigorous scientific research to inform and guide perspective for all industry stakeholders involved.
Fortunately, HISA is now poised to lead the industry towards creating a consensus for cogent, scientifically justified reforms. A necessary prelude to discussion, consensus, and (hopefully) effective regulation is an objective assessment of all potential consequences and risks of permitting race day furosemide treatment. Thoughtful, diverse, evidence-based contributions engaged from a broad range of the scientific community, and rigorous consideration and evaluation of the findings are expected to contribute towards fulfilling that imperative.

FUTURE DIRECTIONS

The next steps will relate to the eventual goals of sharing these results with interested stakeholders. Work to compile this research into a manuscript for submission to a peer-reviewed veterinary journal will commence shortly, with the aim of submitting sometime in early to mid- 2026 pending final approvals from the Furosemide Study Committee.
In addition, an abstract will be submitted in early February 2026 to present this research at the 12th International Conference on Equine Exercise Physiology (ICEEP) -the definitive symposium for equine exercise physiology researchers, veterinarians, and industry organizations worldwide which takes place in Tokyo, Japan in June 2026.
Many thanks to HISA and The Furosemide Advisory Committee for the opportunity to participate in this important and exciting research venture. Should more information or clarification on any aspects of this study be required, please don't hesitate to contact me at any point in the future (phone calls preferred). I am more than happy to assist if needed.

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APPENDIX K: Boone Final Report
HISA Furosemide Advisory Committee Final Report
Risk Factors for Epistaxis Incidence in United States Thoroughbred Racehorses in 2024

Alana Boone, Sarah White-Springer, PhD; Texas A&M University
January 20, 2026

Objective

This study aimed to apply statistical analysis and modeling techniques to evaluate the relationships between external variables and the likelihood of epistaxis occurrence with assistance from artificial intelligence in conjunction with machine learning.

Materials and methods

Data collection

This dataset is compiled with epistaxis cases documented at Horse Integrity and Safety Authority (HISA) regulated racetracks during the 2024 racing season. Horses presenting with epistaxis, unrelated to physical trauma, were identified via post-race examinations by HISA regulatory veterinarians. Respective information was collected for each confirmed epistaxis case and corresponding data were collected for all other horses participating in the same race to be used as contemporary control horses. The complete dataset includes 202 documented epistaxis cases and 1,286 non-epistaxis cases, yielding a total of 1,488 Thoroughbred horses. A range of variables potentially associated with epistaxis were collected, including racetrack information, race history, race information, environmental information, and horse history. Racetrack information variables include track, surface, distance, and condition. Race history variables include days since last race, previous epistaxis incidences, previous unsound incidences, lifetime starts, lifetime earnings, and furosemide (Lasix) use from the previous race. Race information variables include trainer, time of race, race number, off odds, type of race, finish position, purse, Lasix use, and blinkers. Environmental information includes weather (cloudy, clear, rainy, showery), hourly temperature, moisture content, and barometric pressure. Horse history variables include horse, sex, and age in months.
Finish position was defined as the horse's placing relative to the total number of horses competing in the race. Off odds represented the betting odds assigned to each horse at race start. Lasix use was categorized into four groups based on current and prior race administration: Yes, indicating horses that used Lasix in both the current race and the previous race; On, indicating horses that did not use Lasix in the previous race but did use it in the current race; No, indicating horses that did not use Lasix in either the previous or current race; and Off, indicating horses that used Lasix in the previous race but did not use it in the current race. Data were obtained from veterinary records reported to the Horseracing Integrity and Safety Authority (HISA) and from Equibase horse racing records.

Dataset preparation

Data were explored for missing values and standardized via their respective categorical and numerical values. The inherently categorical variables were converted into numerical codes to be used as quantifiable variables for machine learning. Pairwise correlations among predictors were assessed using Pearson correlation coefficients (r) visualized in a correlation heat map. Variables with correlation coefficients ≥ 0.7 were excluded to mitigate multicollinearity, which can inflate variance in parameter estimates and reduce interpretability of individual predictor effects [1,2]. Based on this criterion, Lasix use last race and lifetime starts were removed due to strong correlations to Lasix and age, respectively. Horse name was excluded as a non-informative identifier and trainer was removed due to excessive variability.

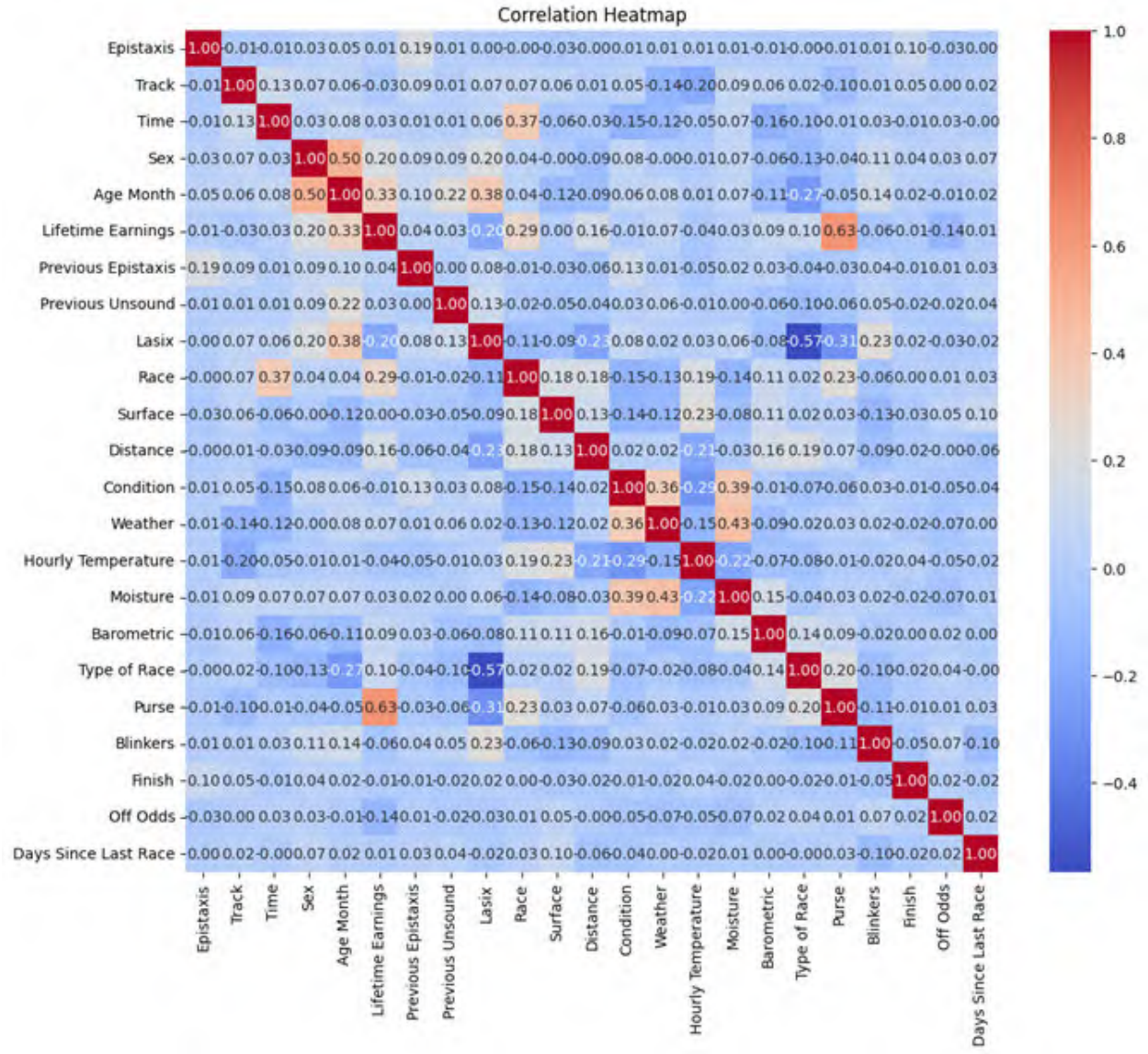

Figure 1: Correlation heatmap between all included variables.

Class imbalance

The dataset used in this study is notably imbalanced, with 202 epistaxis cases and 1,286 non-epistaxis cases. Class imbalance is a common challenge in machine learning classification tasks because models trained on skewed class distributions tend to be biased toward the majority class and can perform poorly on the minority class [3].

To address this issue during training, techniques designed to mitigate class imbalance are used. Synthetic minority oversampling technique (SMOTE) was used to generate synthetic examples of the minority class to produce a more balanced training set [4]. Additionally, 5-fold cross-validation was employed as a resampling approach by using a holdout variation [5].

Machine learning prediction models

Five machine learning algorithms were selected to represent a range of algorithmic structures to evaluate relationships among the collected variables and the occurrence of epistaxis. The models evaluated included logistic regression (LR), random forest (RF), support vector machine (SVM), k-nearest neighbors (kNN), and eXtreme Gradient Boosting (XGBoost) in Python. The

dataset was randomly divided into training (80%) and testing (20%) subsets. Models were trained exclusively on the training data, and predictive performance was evaluated on the held-out testing set. All models were scaled via Z-score normalization.

Feature importance

Feature importance analyses were conducted on the training data to identify the most predictive variables of epistaxis, while avoiding information leakage from the test set. Because k-nearest neighbors does not inherently compute feature importance, permutation importance was used to evaluate variable contributions for this model.

Evaluation metrics

A range of evaluation metrics was used to assess the predictive performance of each model, including accuracy, classification error, area under the curve (AUC), precision, recall, F1-score, specificity, and receiver operating characteristic (ROC) conducted in Python.

Statistical analysis

Using the complete dataset, descriptive statistics were calculated for all variables by comparing mean values between epistaxis and non-epistaxis groups. Variables exhibiting group-level differences were further evaluated by univariable statistical analyses conducted in SAS Version 9.4. A generalized linear mixed model using binomial error distribution was fitted to examine associations. Model estimation was performed using maximum likelihood methods in SAS (PROC GLIMMIX). Statistical significance was defined as $p < 0.05$.

Results

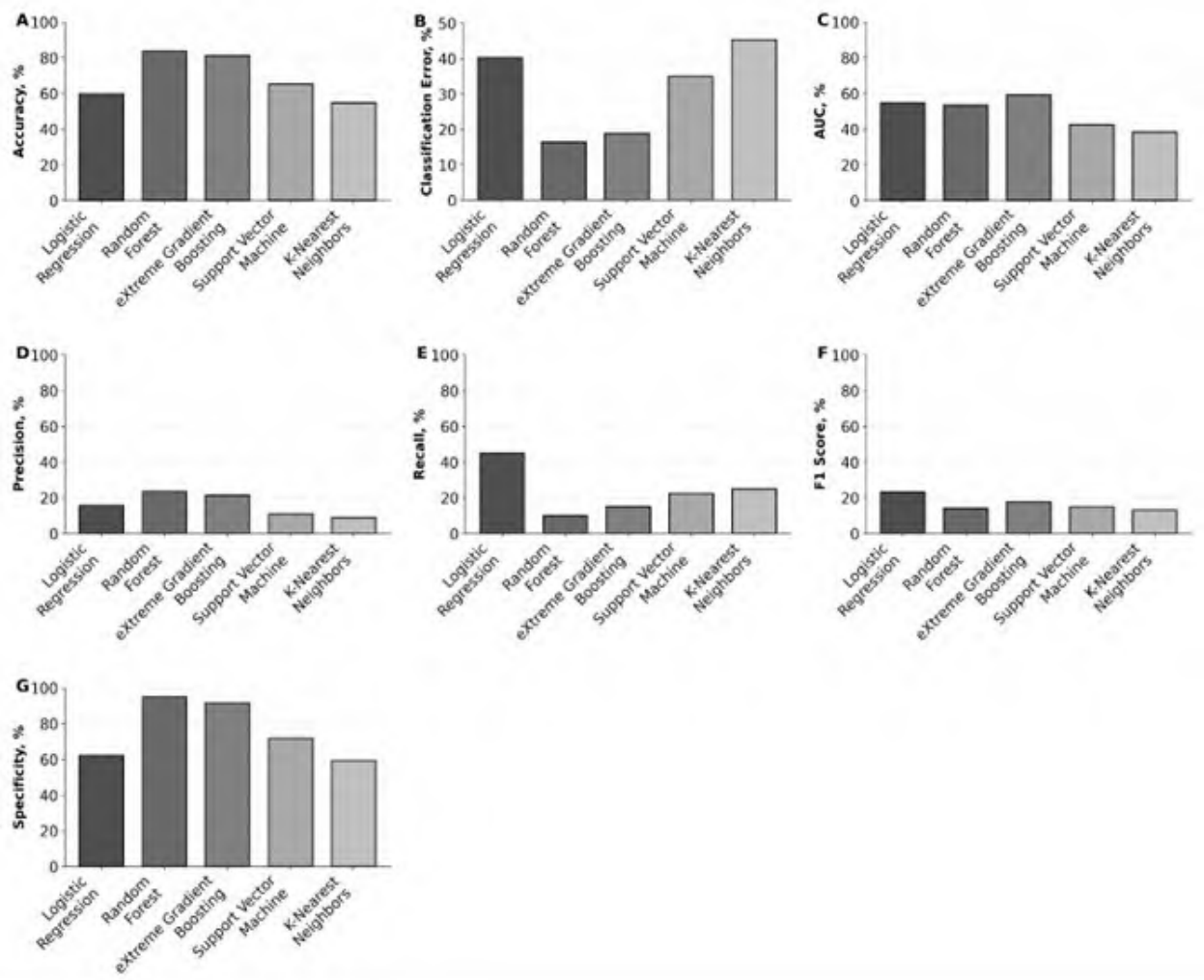

Figure 2: Comparison of seven evaluation metrics for different machine learning models trained to predict epistaxis. Metrics shown are accuracy, classification error, AUC, precision, recall, F1-score, and specificity.

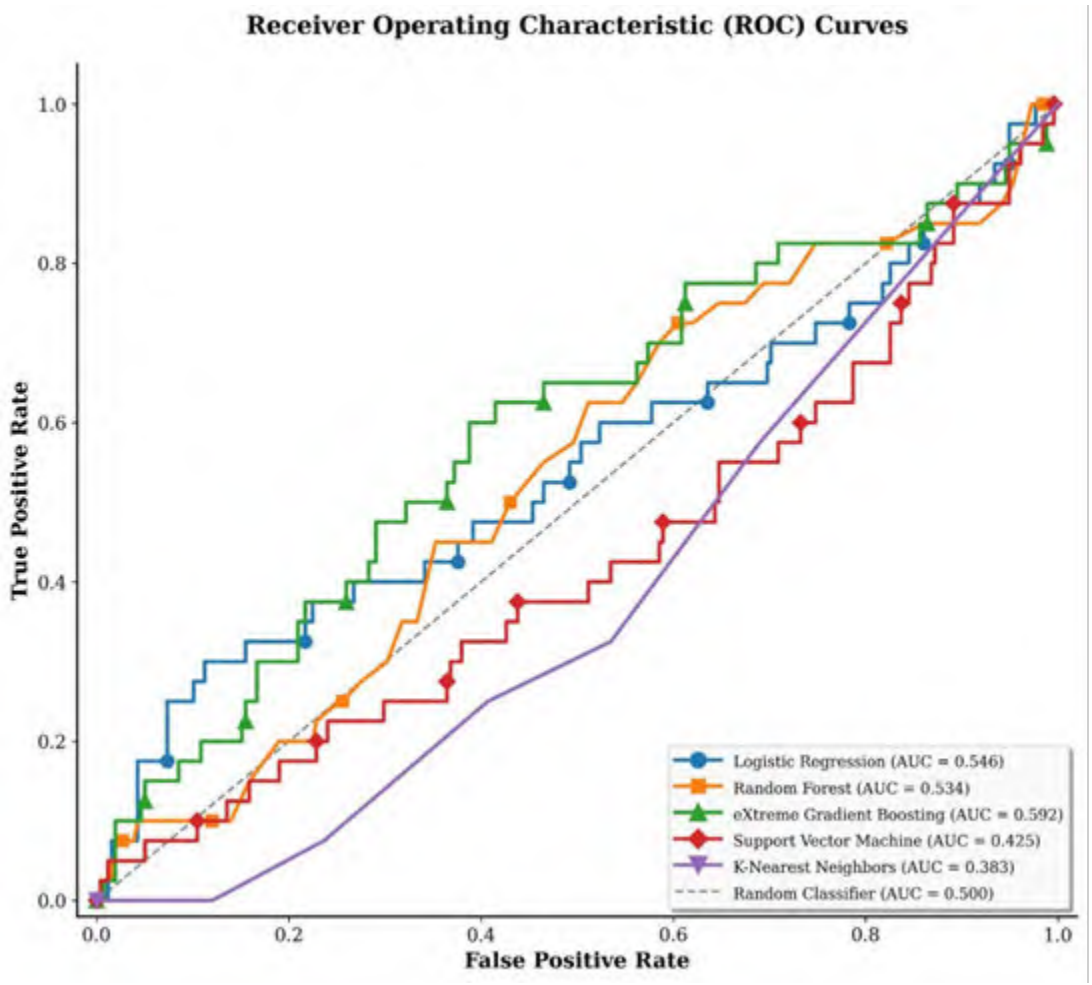

Figure 3: ROC curves illustrating the performance of each machine learning model for predicting epistaxis, with AUC values reported.

P-values were used for exploratory statistical comparisons between epistaxis and non-epistaxis groups, whereas machine learning model performance was evaluated using predictive metrics, including accuracy, precision, recall, classification error, F1-score, specificity, and AUC.

Accuracy

A measure of how often the model's predictions were correct overall, combining predictions of epistaxis and non-epistaxis. Random forest had the highest accuracy, 83.56%, and k-nearest neighbor had the lowest, 54.7% (Figure 2).

Classification error

Classification error quantifies the proportion of incorrectly classified cases. K-nearest neighbor had the highest classification error of, 45.30%, and random forest had the lowest of, 16.44% (Figure 2).

ROC AUC

The ROC and AUC measure the model’s ability to distinguish between classes, specifically epistaxis and non-epistaxis cases. The ROC curve shows the tradeoff between recall or true positive rate, and specificity or false positive rate. Model curves are shown in figure 3. The highest AUC score, 0.59, was from eXtreme gradient boosting and k-nearest neighbor had the lowest of 0.38 (Figure 2).

Precision
Precision, or how precise the model is at predicting epistaxis, varied between models with random forest having the highest, 23.53%. K-nearest neighbor had the lowest precision of 8.7% (Figure 2).

Recall
Recall, or sensitivity, defines the model's performance when solely identifying the positive, or epistaxis, cases. This is also known as the true positive rate. Logistic regression had the best recall, 45%, and random forest had the lowest, 10% (Figure 2).

F1 score
F1 score is used as a measurement averaging precision and recall, commonly used for imbalanced datasets. Logistic regression had the greatest F1 score, 23.08%, while k-nearest neighbor had the lowest of 12.90% (Figure 2).

Specificity
Specificity, or false positive rate, complements recall by assessing the model's ability to correctly identify non-epistaxis horses. Random forest had the highest specificity, 94.96% and k-nearest neighbor had the lowest, 59.30% (Figure 2).

Feature importance
Feature importance analyses were conducted to identify the variables most informative for predicting epistaxis within each model. The k-nearest neighbors model identified previous epistaxis, surface, and weather as the most influential predictors respectively. Logistic regression primarily relied on finish position, followed by previous epistaxis and off odds. In the random forest model, finish position was the most important predictor, with off odds and age contributing subsequently. Support vector machine predictions were driven mainly by finish position, with off odds and lifetime earnings following next. In contrast, eXtreme gradient boosting identified weather, surface, and blinkers as the most influential predictors. Notably, finish position was a dominant predictor across multiple models.

Descriptive Statistics

Table 1: Descriptive statistics of variables showing group-level differences. Non-epistaxis and epistaxis groups represent the average of all inputs within the dataset.

Variable	Non-Epistaxis	Epistaxis
Lifetime Earnings	119466	126902
Previously Unsound	0.13	0.14
Off Odds	14.6	12.9
Purse	66416.5	61414.3
Previous Epistaxis	0.05	0.20
Finish	0.54	0.71

Table 2: Descriptive statistics of lasix use. Lasix divided into Y: Yes, used Lasix and N: No, did not use Lasix. Non-epistaxis and epistaxis groups represent the average of all inputs within the dataset.

Lasix Use	Non-Epistaxis	Epistaxis
N	0.27	0.28
Y	0.73	0.72

Table 3: Descriptive statistics of Lasix use. Lasix divided into: Yes, indicating horses that used Lasix in both the current race and the previous race; On, indicating horses that did not use Lasix in the previous race but did use it in the current race; No, indicating horses that did not use Lasix in current nor previous race. Off, indicating horses that used Lasix in the previous race but did not use Lasix in the current race. Non-epistaxis and epistaxis groups represent the average of all inputs within the dataset.

Lasix Use	Non-Epistaxis	Epistaxis
N	0.21	0.19
OFF	0.06	0.09
ON	0.03	0.02
Y	0.7	0.7

Descriptive statistics were conducted with only the variables listed showing group level differences. These variables were further analyzed by statistical analysis.

Statistical significance

Table 4: Statistical significance of evaluated variables and their associations with epistaxis, with corresponding p-values.

	P-value
Lifetime Earnings	0.5968
Lifetime Starts	0.8108
Previous Unsound	0.6956
Off Odds	0.2016
Purse	0.7049
Hourly Temperature	0.7208
Previous Epistaxis	<0.0001*
Finish	<0.0001*
Track	1.0000
Sex	0.8421
Lasix	0.3607
Lasix (Y/N)	0.7233
Age x Lasix	0.7055
Age x Lasix (Y/N)	0.3052

Surface	0.4167
Finish x Previous Epistaxis	0.3175

Previous epistaxis and finish position were the only variables significantly associated with epistaxis ($p < 0.0001$). All other evaluated variables showed no significant associations, as presented in Table 1. No significant interaction or correlation was observed between previous epistaxis and finish position or between age and Lasix use.

Further Interpretation

The moderate performance of the machine learning models may be attributed to class imbalance and the limited number of variables showing significant univariable associations with epistaxis. While only previous epistaxis and finish position demonstrated statistically significant relationships, the models incorporated all measured variables to reflect real-world racing conditions, where risk is influenced by multiple interacting factors.

Across models, finish position, previous epistaxis, and off odds repeatedly emerged as key features contributing to epistaxis prediction. Based on 2024 data from HISA regulated racetracks, epistaxis was observed in only 0.11% of racehorses.

Previous Epistaxis

Table 5: Previous epistaxis SAS analysis including least square means

prevep Least Squares Means							
prevep	Estimate	Standard Error	DF	t Value	Pr > |t|	Mean	Standard Error Mean
0	-1.9824	0.08205	1483	-24.16	<.0001	0.1211	0.008730
1	-0.6931	0.2315	1483	-2.99	0.0028	0.3333	0.05143
2	2.66E-14	1.0000	1483	0.00	1.0000	0.5000	0.2500
3	13.5661	509.65	1483	0.03	0.9788	1.0000	0.000654

Previous epistaxis and finish position were both strongly correlated to epistaxis. Least squares mean estimates indicated a stepwise increase in the probability of epistaxis with increasing previous epistaxis incidences. Horses with no prior epistaxis had a low predicted probability of epistaxis, 12% (0.1211), whereas horses with one prior episode exhibited a markedly higher predicted probability, 33% (0.3333).

Finish Position

Table 6: Finish position SAS analysis including least square means.

f Least Squares Means							
f	Estimate	Standard Error	DF	t Value	Pr > |t|	Mean	Standard Error Mean
1	-1.9741	0.1948	1483	-10.13	<.0001	0.1220	0.02086
2	-2.5588	0.2076	1483	-12.33	<.0001	0.07184	0.01384
3	-2.4665	0.1841	1483	-13.40	<.0001	0.07824	0.01328
4	-1.1659	0.1068	1483	-10.92	<.0001	0.2376	0.01935

Finish position least square means demonstrated a non-linear association with epistaxis risk, with the highest predicted probability observed among horses finishing in the last quarter of racing positions, 23% (0.2376), followed by those finishing in the first quarter, 12% (0.122). Horses finishing in the middle quartiles exhibited lower predicted probabilities of epistaxis. No correlation was found with previous epistaxis events predicting finish position.

Off Odds

Although off odds were not statistically significant predictors of epistaxis, their frequent selection by machine learning models and comparatively low p-value (0.2016) suggest a potential indirect or contextual role. However, this relationship is not expected to affect decisions regarding race participation.

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APPENDIX L: Sezgin Abstract

Does Furosemide Make Horses Run Faster?

Michele Sezgin† , Carnegie Mellon University, Pittsburgh, PA; Ron Yurko, Carnegie Mellon University, Pittsburgh, PA; Joel Greenhouse, Carnegie Mellon University, Pittsburgh, PA †
E-mail: msezgin@andrew.cmu.edu

In 2023, the Horseracing Integrity and Safety Commission banned the use of furosemide within 48 hours of a race for two-year-old and stakes races. Though furosemide has been used in thoroughbred racing since the 1960s to treat exercise-induced pulmonary hemorrhage in horses, there has been speculation about the drug's performance-enhancing effects. We study a perfectly-matched dataset of 28,617 two-year-old horses that have raced both with and without furosemide to estimate the causal impact of furosemide on race-day performance, as measured by probability of finishing in the money. When controlling for public betting odds, the fraction of total horses using furosemide in a given race, track, post position, and sex of horse, we find that using furosemide increases a two-year-old's odds of finishing in the money by 55% on average. Future work will investigate the race-day performance impacts of furosemide in a larger population of horses matched along relevant characteristics.

DOES LASIX MAKE HORSES RUN FASTER?

Michele Sezgin[1]
Advised by Ron Yurko[1], Joel Greenhouse[1], Joseph Appelbaum[2]
1. Carnegie Mellon University Department of Statistics & Data Science, 2. Waigr

Carnegie Mellon University
Statistics & Data Science

BACKGROUND

- Lasix (furosemide) is a loop diuretic
  - Intended to treat exercise-induced pulmonary hemorrhage
  - Speculation about performance-enhancing effects
- Horseracing Integrity and Safety Authority (HISA) restricts Lasix
  - *No race day Lasix for two-year-old and stakes races*
  - Question: Does Lasix actually *cause* horses to run faster?
- **We aim to quantify the population-level effect of Lasix on race-day performance.**

DATA

- **Response**: Finishing "in the money" (first, second, or third)
- **Treatment**: Lasix indicator
- **Curated sub-dataset**:
  - ~100,000 race results
  - ~28,000 horses
  - 1991 - 2024
  - Two-year-old horses that have raced both with and without Lasix at least once **(perfect matching)**.

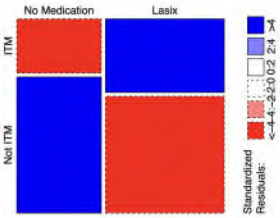


Figure 3: More horses finishing in the money (ITM) than expected when using Lasix.

Figure 1: Each horse has raced roughly two times with and without Lasix on average.

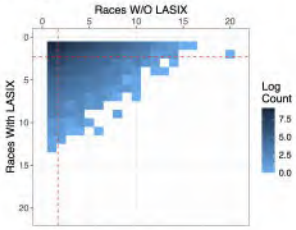
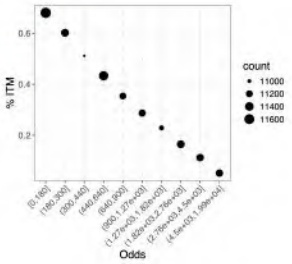


Figure 2: Relationship between finishing in the money and odds is linear and negative.



METHODS

- **Conditional logistic regression** treats each horse as a **strata** with their own intercept:

  For the j^{th} observation from the i^{th} horse, $P(\text{ITM}_{ij} \mid \text{Lasix}_{ij}, X_{ij}) = \frac{1}{1+\exp\left[-(\alpha_i + \tau\text{Lasix}_{ij} + \boldsymbol{\beta}\boldsymbol{X}_{ij})\right]}$

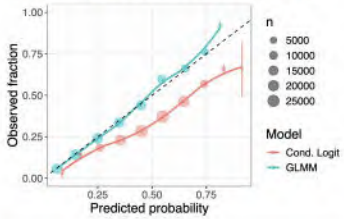
- **Mixed effects logistic regression** treats horse intercepts as random:

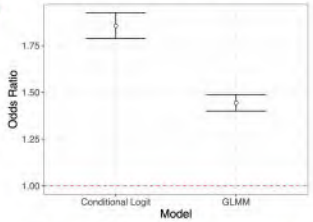
  $P(\text{ITM}_{ij} \mid \text{Lasix}_{ij}, X_{ij}) = \frac{1}{1+\exp\left[-(\alpha_i + \tau\text{Lasix}_{ij} + \boldsymbol{\beta}\boldsymbol{X}_{ij})\right]}$, where $\alpha_i \sim N(0, \sigma^2)$

X_{ij} is a vector with
- Horse sex_{ij}
- Post position_{ij}
- Public betting odds_{ij}
- Fraction of other horses on Lasix_{ij}
- Field size_{ij}

RESULTS

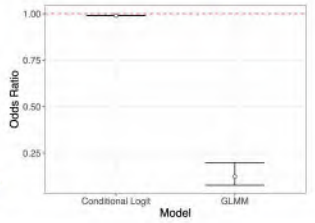
GLMM improves calibration by explicitly modeling between-horse variation.





Both models suggest a positive effect for Lasix on finishing in the money.

Both models suggest a negative effect for race-level fraction of Lasix.



DISCUSSION

- Among two-year-old American thoroughbred race horses, using Lasix corresponds to a marginal effect of a **1.44-fold increase in odds of finishing in the money (GLMM)** (95% CI [1.40, 1.49]), controlling for relevant confounders.
- Among two-year-old American thoroughbred race horses, each percentage increase in the race-level fraction of horses using Lasix corresponds to a marginal effect of a **0.12-factor change in odds of finishing in the money (GLMM)** (95% CI [0.08, 0.20]), controlling for relevant confounders.
- Model diagnostics suggest misspecification in the conditional logistic model, but correct specification of the GLMM, indicating estimation of between-horse variability is important when estimating probability of finishing in the money.

FUTURE WORK

- Fit more flexible hierarchical models, use doubly robust estimators to extend to non-matched settings, and model other performance measures like finishing times and finishing order.
- Analyze GLMM with random slopes for Lasix use and directly model distributional shape/skew parameters as functions of Lasix use to understand its impact on horse performance variability.

APPENDIX M: Regulatory Issues Regarding Drug Treatment of EIPH

REGULATORY ISSUES REGARDING DRUG TREATMENT OF EIPH

R. Sams

Florida Racing Laboratory, 1200 SW 34th Street, University of Florida, PO Box 100117, Gainesville, Florida 32610-0117, USA

ABSTRACT

Furosemide is the drug most commonly used in the treatment of exercise-induced pulmonary haemorrhage (EIPH) with approximately 70–80% of Thoroughbred horses in some US racing jurisdictions receiving pre-race injections of this drug. Furosemide is a high-ceiling loop diuretic that increases urine flow soon after intravenous administration with urine production reaching values that are as much as 40–50 times the normal rate. Diuresis persists for approximately 3 hours with the rate of urine production returning to near or below normal values. The increased rate of urine production causes the urinary concentrations of substances that are not normally reabsorbed in the renal tubules to decrease and thereby interferes with methods to detect them. Analysts recognised this diluting effect of furosemide soon after its introduction and called for restrictions on the time of its administration so that post race urine samples would not be diluted. Regulators responded in 1985 by limiting the dose of furosemide to no more than 250 mg, requiring that it be administered intravenously, and prohibiting its administration within 4 h of the scheduled post time of the race in which the horse was entered. Various regulatory measures were taken to ensure that these requirements were followed. These measures have included a requirement for furosemide to be administered in secure areas by regulatory veterinarians and by imposition of regulatory thresholds for furosemide in plasma. Use of secure areas has been abandoned by most regulatory bodies because of the expense of maintaining the areas. Most regulators now rely on the use of regulatory thresholds and a requirement that the urine specific gravity exceed some limit. The Racing Medication and Testing Consortium (RMTC) and the Association of Racing Commissioners International, Inc (ARCI) recommended regulations establishing a urine specific gravity less than 1.010 as a violation of the rules of racing if the corresponding plasma (serum) concentration is greater than 100 ng/ml. These limits have been challenged by some investigators. One group has demonstrated that urine specific gravity is a poor predictor of time since dose administration and hence is not useful in detecting administration of furosemide within 4 h of race time. Others have pointed out that the purpose of measuring specific gravity is not to detect administration within 4 h of race time but to detect administration that results in collection of a dilute sample. Others have suggested that a plasma concentration in excess of 100 ng/ml is too high and that plasma concentrations 4 h after administration are substantially lower.

INTRODUCTION

The use of furosemide in the prophylaxis of exercise-induced pulmonary haemorrhage is often attributed to veterinarian Alex Harthill. It was suggested that furosemide administration would reduce pressures in pulmonary blood vessels through its antihypertensive effects and that the reduced pressures would result in decreased severity or incidence of pulmonary haemorrhage. Subsequently pre-race administration of furosemide was permitted by a number of racing commissions in the United States as a result of efforts by horse trainers to allow use of the drug to treat their horses.

REGULATORY HISTORY

Pre-race administration of furosemide and certain other therapeutic drugs became widespread in the

1970s (Gabel *et al.* 1977). Analysts working in regulatory laboratories quickly noted that the administration of furosemide to racehorses resulted in the submission of urine samples that were dilute and they suspected that detection of prohibited substances was compromised. Several groups of investigators demonstrated that pre-race administration of furosemide under the conditions that were permitted by the rules of racing at the time caused interference with the detection of certain drugs or metabolites but not others (Tobin *et al.* 1979; Soma *et al.* 1984). These scientific results and pressure from analysts to restrict or curtail the use of furosemide in racing led National Association of State Racing Commissioners (NASRC) to amend its model rules and to recommend that all racing commissions prohibit pre-race administration of furosemide. Several racing commissions soon adopted these recommendations. Horse trainers demanded that the use of furosemide be permitted and threatened to withhold their horses from racing unless the use of furosemide was permitted. Commissioners responded that they would reconsider the ban on furosemide if it could be demonstrated that it would not interfere with drug detection by commission laboratory analysts. After the American Association of Equine Practitioners declared that they would recommend a maximum intravenous dose of 250 mg administered 4 h or more before race time, it was possible to conduct a series of rather basic studies that demonstrated that administration under these conditions results in no appreciable effects on the detection of the analytes that were studied (Sams and Maylin, unpublished observations, 1984). The NASRC responded by recommending that state racing commissions permit the use of furosemide under the following conditions:

1. The maximum permissible dose is not greater than 250 mg;
2. the route of administration is intravenous;
3. the dose is administered 4 or more hours before the scheduled post time of the race in which the horse is entered.

These recommendations were not incorporated into the model rules of the NASRC but were quickly adopted (with some minor modifications) by most racing jurisdictions in the United States. The New York Racing and Wagering Board resisted efforts to allow furosemide use in New York racing until the mid-1990s.

Considerable laboratory efforts were directed toward identifying violations of the rules restricting the use of furosemide (Singh and McArdle 1992; Uboh *et al.* 1992; Soma and Uboh, 1998). These studies revealed that it was difficult, if not impossible, to control the dose, route, and time of administration of furosemide by laboratory analysis. However, it is possible to identify a urine sample that is characterised by a specific gravity that is lower than that produced post race by a horse that has not been administered furosemide and to identify the cause of the dilute urine sample if the corresponding plasma furosemide concentration is elevated above those found 4 h after intravenous administration of permitted doses.

As a result of findings from these studies and based on recommendations from various experts, the Racing Medication and Testing Consortium (RMTC) recommended in 2004 to control the use of furosemide by measuring the specific gravity of the urine to determine whether it is dilute and to measure the corresponding plasma (serum) concentration of furosemide if the urine specific gravity is less than 1.010 and to report a violation if the plasma concentration is greater than 100 ng/ml of plasma. Furthermore, the RMTC recommended testing of the plasma sample if a urine sample was not collected and that any such sample containing furosemide at a concentration greater than 100 ng/mL be considered a violation of the rules. The RMTC also recommended increasing the maximum permitted dose to 500 mg. Many racing commissions have subsequently adopted these recommendations.

Pharmacology and pharmacodynamics

Furosemide (4-chloro-N-furfuryl-5-sulfamoyl-anthranilic acid; Fig 1) is a high-ceiling loop diuretic that inhibits reabsorption of chloride and sodium ions in the proximal and distal tubules as well as the loop of Henle. Inhibition of the reabsorption of sodium and chloride ions decreases the reabsorption of water from the nephrons thereby increasing the volume of urine produced and causing a period of intense diuresis that begins within 15–30 min and continues for 2–3 h after intravenous administration of

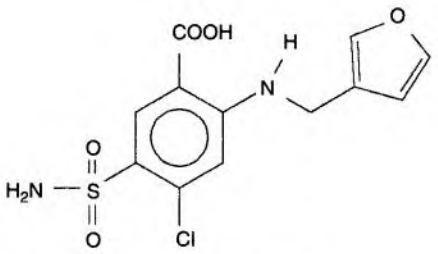


Fig 1: Chemical structure of furosemide.

permitted doses in racehorses (Soma and Uboh 1998).

The disposition of furosemide in horses is characterised by a relatively small apparent volume of distribution, rapid clearance, and short terminal elimination half-life (Chay *et al.* 1983; Dyke *et al.* 1996; Johansson *et al.* 2004). The period of diuresis corresponds to that period during which plasma furosemide concentrations are above approximately 20–40 ng/ml (Chay *et al.* 1983).

The effects of furosemide-induced diuresis on the pharmacokinetics and urinary excretion of other drugs have been investigated and are reasonably well understood. The renal clearances of drugs (metabolites) that are not appreciably reabsorbed from the renal tubules are not affected by diuresis. Consequently, the plasma concentrations of these substances are not altered but their urine concentrations are decreased (Miller *et al.* 1977; Combie *et al.* 1981; Soma *et al.* 1984; Stevenson *et al.* 1990). The horse metabolises many xenobiotics and endogenous substances by conjugation with glucuronic acid and sulfuric acid. These metabolites are highly hydrophilic and are therefore not appreciably reabsorbed. Many of the drugs that are of great interest to racing regulators are drugs that are excreted as conjugated. The effects of furosemide-induced diuresis on their detection in test samples is therefore of great interest. Some of the drugs that are in this group include morphine, etorphine, oxymorphone, hydromorphone, lidocaine, mepivacaine, pyrilamine, and others.

The renal clearances of drugs that are reabsorbed in the renal tubules are increased because these substances are not as extensively reabsorbed during diuresis because the concentration difference between the tubular fluid and the renal blood is diminished thereby reducing the driving force for reabsorption. The concentrations of these substances in urine are not decreased as extensively as those substances that are not normally reabsorbed. Furthermore, the detection of these substances is often enhanced because the concentrations of other substances, including endogenous substances and those of dietary origin, that have the potential to interfere with their detection are reduced.

Regulatory issues

Advances in analytical methods since the early 1980s have lowered the limits of detection for most analytes, particularly for those that are excreted in urine. Therefore, the significance of decreased urinary concentrations of many analytes as a result of furosemide-induced diuresis has been reduced. However, there are still some analytes that cannot be detected adequately and any dilution by diuresis could render them undetectable. Therefore, rules and procedures designed to prevent or detect furosemide-induced diuresis around the time of racing are still necessary and appropriate.

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APPENDIX N: AAEP Position Statement on the use of the Therapeutic Medication in the Racehorse

The AAEP policy on medication in pari-mutuel racing is driven by our mission to improve the health and welfare of the horse. The AAEP policy is aimed at providing the best healthcare possible for the racehorses competing while ensuring the integrity of the sport.

The racetrack veterinarian should be directly involved in the diagnosis and treatment of soundness and health disorders and must advocate with all stakeholders for professional and ethical practice at all times.

Due to the enactment of the Horseracing Integrity and Safety Authority (HISA) and the Horseracing Integrity and Welfare Unit (HIWU) in Thoroughbred racing, there is now consistency across most of the US racing jurisdictions with respect to therapeutic medication control and anti-doping regulations; however, some differences do exist within jurisdictions that have not adopted HISA, and those involved in Quarter Horse and Standardbred racing. It is therefore essential that veterinarians providing care to the racehorse know and carefully follow the rules, especially as they concern the health and welfare of the horse. The AAEP expects its members to abide by the rules of all jurisdictions in which they practice.

Medication usage for non-therapeutic purposes can put horse health at risk and deprives ethical stakeholders of fair competition. The AAEP condemns the administration of non-therapeutic or unprescribed medications to racehorses based on our belief that it is not in the interest of horse welfare and racing integrity. The AAEP believes that all therapeutic medications (prescription or otherwise) should be administered by or under the direction of a licensed veterinarian and based on a diagnosis. Healthcare decisions for individual horses should involve the veterinarian, trainer and owner with the best interests of the horse as the primary objective.

In order to provide the best healthcare possible for the racehorse, veterinarians should utilize scientifically based diagnostic and therapeutic modalities in
accordance with medication guidelines designed to ensure the integrity of the sport. To this end, the following are the essential elements of AAEP policy concerning veterinary care for all horses in competition.

- The AAEP believes the welfare of the horse must be placed above the demands of competition and monetary or social gain.
- The AAEP strongly supports veterinary practitioner adherence to the ethical guidelines of the American Veterinary Medical Association and the American Association of Equine Practitioners.
- The AAEP recommends wherever possible to follow the principles of evidence-based medicine before proceeding with a treatment recommendation. The AAEP believes that treatment recommendations be based on a thorough knowledge of the horse's condition based on previous and current examinations as well as appropriate diagnostic testing.
- The AAEP recommends all medical procedures and treatments be performed in the context of a valid veterinarian-client-patient relationship.
- The AAEP recommends a detailed medical record be completed for each examination, and all records should comply with the veterinary medical board requirements in the state in which one practices, as well as the treatment record requirements of HISA

- The AAEP recommends a horse's history and medical records be shared between the horse's primary veterinarian, consulting veterinarian, trainer and owner, as well as any veterinarian the owner chooses in order to provide a continuity of care.

Additionally, the following are AAEP guidelines specific to the sport of horse racing:

- The AAEP recommends abiding by the rules and regulations of the jurisdiction in which one practices, recognizing that most states are under the purview of HISA. Where HISA does not have a rule, then the individual rules in that state or breed should be followed.
- The AAEP encourages proactive and constructive communication between regulatory bodies and practicing veterinarians, as well as other industry

stakeholders based on the premise that attending veterinarians should be unconditional advocates for the welfare and safety of horses and work closely with regulatory veterinarians to identify and protect horses that are at increased risk for injury.

- The AAEP endorses "rest" as an important component of a comprehensive healthcare program for racehorses and recommends the integration of rest into therapeutic medication programs.
- The AAEP supports the judicious use of compounded medications in strict compliance with FDA regulations and in accordance with the AAEP's Equine Veterinary Compounding Guidelines.
- The AAEP supports the communication of all medical and treatment history to the new trainer and owner of a horse claimed or purchased to ensure continuity of care.
- The AAEP supports a minimum interval of 14 days following an intra-articular injection to a horse's next race, recognizing that differences in risk among varied racing disciplines may warrant consideration.
- The AAEP strongly endorses increased surveillance and out-of-competition testing to facilitate enforcement of the above-mentioned regulations.
- The AAEP currently supports the use of furosemide as a race-day medication to control exercise-induced pulmonary hemorrhage (EIPH) in the absence of a more effective treatment and/or management strategy.
- The AAEP recognizes the ongoing industry initiatives to phase out race-day administration of furosemide. Therefore, it is incumbent upon all racetrack veterinarians to develop an evidence-based comprehensive medical and stable management plan to minimize the incidence and degree of EIPH commonly associated with high-speed exercise, consistent with the medication rules of the jurisdictions in which they practice. The AAEP strongly advocates for research and the development of new strategies including, but not limited to, training methods, nutritional management, genetics, and environmental modifications to assist in improving respiratory health and mitigating the effects of EIPH in training and racing horses.
- The AAEP strongly recommends continued research in determining guidelines for withdrawal intervals that allow for the responsible and ethical use of medication in training and racing horses. The AAEP is aware of the

dynamics of the development of new products, as well as the continuing evaluation of current medications, and will continue to evaluate its policy based upon available scientific research and the best interests of the horse.

Approved by AAEP board of directors in 2025.

EXHIBIT B: COMMENT FROM PATRICIA WONDERLEY

Outlook

Proposed Rule Modifications-Rule Series 5000 and 7000

From Patricia Wonderley <pfwonderley@icloud.com>
Date Wed 2/25/2026 3:41 PM
To Samuel Reinhardt <samuel.reinhardt@hisaus.org>

Dear General Counsel Reinhardt:

I am a thoroughbred racing fan. I am not a thoroughbred racehorse owner (except for micro shares in MyRacehorse); I train no horses, I breed no horses, nor am I involved or related to anyone in the thoroughbred horse business.

It is absolute FOLLY to ban Lasix for thoroughbred racehorses. You will decimate the industry and stop horse handicapping. Horses' breathing systems are like magnificent pistons--they breathe through their mouths and have enlarged capillaries during racing which rupture and they absolutely need Lasix to race at racehorse speed without bleeding.

It is animal cruelty not to administer Lasix.

Further, you cannot handicap horses without Lasix. Who can tell when a horse will stop during a race?

Also, the old adage is true: "once a bleeder, always a bleeder." Untold thousands of young horses who bleed the first time when raced, cannot race again and are effectively "wasted."

We should also allow Lasix in stakes racing. It is crippling stakes races who have really short fields (unbettable) most of the time because Lasix is banned.

It is NOT fair to compare American dirt racing to European grass racing (where Lasix is banned). The Euros basically gallop along on the grass, only exerting themselves really really strenuously in the last couple of furlongs. This is diametrically opposed to American dirt racing, especially sprint racing, where thoroughbreds are asked to go at maximum speed throughout the race. Have you ever wondered why no one ever really looks at or compares European race speeds or quarters/halves/eighths? Because those numbers, unlike American racing, are basically meaningless.

Please, please, we must have Lasix or we will absolutely decimate the industry. And it should be allowed for 2 year olds and stakes horses as well.

Thank you for your consideration,
Patricia Wonderley
6335 Trailhead Rd.
Highlands Ranch, CO 80130
(303) 495-4948

EXHIBIT C: COMMENT FROM THE THOROUGHBRED OWNERS OF CALIFORNIA (TOC), CALIFORNIA THOROUGHBRED TRAINERS (CTT), AND CALIFORNIA THOROUGHBRED BREEDERS ASSOCIATION (CTBA)

Sent via Email

March 20, 2026

Charles Scheeler
Chair of the Board of Directors
Horseracing Safety and Integrity Authority
401 W. Main Street, Suite 22
Lexington, KY 40507-1630

Dear Charles,

This letter from the Thoroughbred Owners of California (TOC), California Thoroughbred Trainers (CTT), and California Thoroughbred Breeders Association (CTBA) states our support for the continuation of the current policy, which prohibits the use of furosemide (Lasix) in all 2-year-old races and all stakes' races.

This policy has served the California racing and breeding industry well, striking a balance that has worked effectively over the past three years. It has also contributed greatly to record results at horse sales across the nation. The increased participation from international buyers at horse sales has helped restore confidence in the quality of our bloodstock and the image of our racing. The current policy has delivered the desired results.

Implementing any changes to the current policy would prove highly disruptive to horsemen in all regions at a time when key economic indicators such as field size are closely monitored. In the absence of any compelling scientific evidence to the contrary, our three organizations strongly recommend maintaining the status quo.

Thank you for your consideration of our collective input.

Sincerely,

TOC

William Nader

CALIFORNIA THOROUGHBRED TRAINERS CTT

California Thoroughbred Breeders Association

Cc: Steve Beshear
Adolpho Birch
Leonard Coleman
Joseph DeFrancis
Terri Mazur
Susan Stover
Bill Thomason
DG Van Clief

EXHIBIT D: COMMENT FROM HISA'S HORSEMEN'S ADVISORY GROUP

201 East Main St., Suite 340
Lexington, KY, 40507

Web: www.hisaus.org

HORSERACING INTEGRITY AND SAFETY AUTHORITY

TO: The Board of Directors of HISA

FROM: The HISA Horsemen's Advisory Group

Subject: Your upcoming vote on the use of Race Day Lasix

Members of the HISA Board,

We, the undersigned, are members of the HISA Horsemen's Advisory Group. Collectively we have spent more than 400 years in the horse racing industry in a variety of diverse capacities including trainers, veterinarians, breeders, and owners. All of us passed HISA's selection process to join this board. Many of us have participated in other horsemen's groups and led various horse racing/breeding organizations regionally and nationally.

We have discussed the topic of Lasix extensively during our monthly meetings and with our fellow horsemen at racetracks, sales, and breeding farms. We acknowledge that the use of race day Lasix may be the single most important topic that horse racing is currently facing. We would like you to know that, among horsemen, this is not a polarizing topic - the overwhelming majority of horsemen favor the continued use of race day Lasix.

We have given this very careful consideration. Out of a deep concern for the health of the horses; doing all we can to continue to support the industry and all of the people it employs both directly and indirectly; and thus for the good of racing in general, the members of the HISA Horsemen's Advisory Group unanimously recommends that the HISA Board of Directors votes to continue the use of race day Lasix along the exact guidelines that currently exist.

Failure to do so will cause an immediate severe and future shrinkage of the industry across the board and likely threaten its overall viability. Conversely, continuation of this policy will go a long way towards improving consistency, planning, and overall cooperation among all horsemen's groups as we move forward.

If you would like to discuss any of this or ask any further questions of us – which we would be pleased to answer - send your inquiries to Ms. Lazarus who will then forward them to us. We are also willing to make representatives available via conferencing when you meet if you would find that beneficial.

Thank you for your understanding, consideration, and support.

Kelsey Danner
John Sadler
Craig Bandoroff
Doug Daniels, DVM
Neil Howard
Ron Moquett
Todd Pletcher
Jon Green
Michael Annechino
Shug McGaughey
Gavin Murphy
Sara Langsam, DVM
Justin Revak
Dale Romans
Michael Levinson
John Piehowicz, DVM
Todd Mostoller

EXHIBIT E: COMMENT FROM THE AMERICAN ASSOCIATION OF EQUINE PRACTITIONERS

American Association of Equine Practitioners
4033 Iron Works Pkwy
Lexington, KY 40511
Main Line 859.233.0147
Fax 859.233.1968
aaepoffice@aaep.org

March 23, 2026

Board of Directors
Horseracing Integrity and Safety Authority
201 East Main St., Suite 340
Lexington, KY 40507

Dear Members of the Board:

On behalf of the American Association of Equine Practitioners (AAEP), I am writing regarding the Board's upcoming May 22 vote on whether to expand the current prohibitions on race-day administration of furosemide.

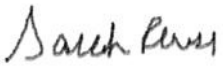
When HISA was established, a three-year pause on a full race-day ban was put in place so that targeted scientific studies could be completed to better understand the effects of furosemide on equine health and the integrity of competition. We understand those studies have now been completed and are under review; however, their findings have not yet been shared publicly.

The AAEP continues to support the use of furosemide as a race-day medication to control exercise-induced pulmonary hemorrhage (EIPH) in the absence of a more effective treatment or management strategy. At the same time, we recognize and support the industry's interest in carefully evaluating the future of race-day medication policies.

Given that the research commissioned to inform this very question has not yet been released, we respectfully ask that the Board continue to allow race-day Lasix with the current prohibitions of two-year-olds and stakes races, until the findings are available for review by the broader industry. Decisions of this significance should be grounded in science that is transparent and accessible to those responsible for implementing and living under these rules.

We believe it would be more appropriate to maintain the status quo while the research is made public and thoughtfully considered. Once the findings are available, we welcome the opportunity for further discussion and collaborative evaluation of any proposed changes.

Sincerely,



Sarah Reuss, VMD, DACVIM
AAEP President