CLÁSICO DEL CARIBE

December 8, 2018

Veterinary Trainers Information

Dr. Robert E. O'Neil, D.V.M

Director of Equine Health & Safety

Table of Contents

Bleeder Certificate

This is to certify that the Horse _____ _____
(Name) (Tattoo)

Exhibited EIPH (Exercise-Induced Pulmonary Hemorrhage) at: _____
(Track/Farm)

On _____ (Date) and it is requested that the horse be placed on the official Florida Salix list.

The mentioned horse was seen bleeding from the nostrils

_____ after a race.

_____ after a workout.

____ an endoscopic examination was not necessary to diagnose EIPH

____ an endoscopic examination was performed by the / Dr._____ to confirm the diagnosis of EIPH.

When utilized as a Bleeder's Certificate, this completed form must be submitted to the Salix Coordinator of the Gulfstream Park.

_____ _____

Veterinary witness date

Salix Coordinator Office 954.648.2087 Racing Office 954-457-6357 Salix Fax (Fax): 954.457.6995

Notification of Salix Administration

Name of Horse:_____ Tattoo: _____

Year Foaled:_____ Color:_____ Sex: _____

In accordance with the provisions of regulation 3 of Salix from the Gulfstream Park, the trainer of record and the attending veterinarian for the horse listed above attest to the following:

- The horse has not exhibited previously Equine Induced Pulmonary Hemorrhaging (*i.e.* External Bleeding)
- It has been determined that it is in the best interest to place the horse on Florida Salix list and to race following Salix administration.

It is understood that a Gulfstream Park veterinarian must administer the Salix no later than four (4) hours before the time of the race, in which the horse listed above will participate.

The trainer of record understands that administration of Salix may not be discontinued without first:

- Provide written verification of the attending veterinarian that it is in the best interest of the horse listed above to discontinue the use of Salix and
- Complete and submit the form required by the Gulfstream Park ("request for discontinuance of Salix") to the Coordinator of Salix in the Gulfstream Park, later than 4:00 p.m. of the day prior to the race in which is scheduled to compete.

This notification must be submitted to the Gulfstream Park Salix Coordinator prior to entry of horse listed above in a scheduled pari-mutual race horse.

Name of the trainer (print): _____
Signature of the trainer: _____ Date: _____
Name of the Attending Veterinarian (print): _____
Signature of Attending Veterinarian: _____ Date: _____

Notification received by:

_____ _____
Coordinator or representative of Salix Date

Salix Coordinator Office: 954.648.2087 Racing office (Fax): 954-457-6357 Salix fax: 954.457.6995

Subject: medication discussion

Per our discussion, Anabolic Steroids Equipoise (Boldenone), Testosterone, Winstrol (Stanozolol), Durabolin, Deca-Durabolin, Etc. should not be used with 6 months of racing in the United States. Also intra-articular steroids have a long withdrawal time. If one should get any of the medication outside of the joint the withdrawal time increases greatly. Intramuscular or extra-articular use of Depo-Medrol (Methylprednisolone acetate) can have a 90 day withdrawal time. Intramuscular Vetalog (Triamcinolone acetonide) can be as long as 30 day withdrawal.

I also caution the withdrawal times listed on the RMTC document are for single dose administration. Again I caution, these are only guidelines and can be influenced by a number of factors, size of animals, training, whether the medication was compounded or it is a Legend medication.

61-6.004 devices, drugs and prohibited procedures; Exceptions.

(1) it is prohibited the Administration, by any means, of any medications, except for furosemide and sodium succinate of prednisolone, an animal of careers within the 24 hours before the officially scheduled time for the race in the that this animal must compete. Prohibits the administration of furosemide or sodium succinate of prednisolone, by any means, an animal of racing during the four (4) hours before the time of a race in which that animal must compete officially scheduled. Removed any animal racing, detected by the Administrators or judges through reasonable evidence that it can be considered reliable, who has received, by any means, any drug that is not furosemide and sodium succinate of prednisolone within 24 hours previous to the officially scheduled time for the race. Nothing in this rule shall be construed as a ban on the use of vitamins, minerals and natural substances, provided that, the day of the race, none of these exceed the normal physiological concentration in the specimen.
(2) (a) Any licensee, within the grounds of a holder of careers where you have hosted or maintained competition animals, must be in or on the premises it occupies or has the right to occupy property or personal effects of rightholder None of the following:

1. traditional drugs dispensed without a valid prescription;
2. any kind of hypodermic needle; injection vial; syringe, capable of accepting a needle hypodermic or with a volume of more than 6 ounces; lubrication for nasogastric intubation device or gastric;
3. except as provided in paragraph (2) (b), any jar, well or device which can be forced to ingested by human resources; o
4. except as provided in paragraph (2) (b), any other device that can be used for the injection, infusion or other administration with a horse or a Greyhound racing from a traditional drug, a patented drug or a medicinal compound (natural or) synthetic).

(2) (b) exempt from the provisions of paragraph (2) (a) are:

1. the possession of a hypodermic needle, a syringe, an injection vial for Administration of a medication for personal use if stewards or judges of the licensed premises which the person is receiving a notice, in writing, of the possession such devices and drugs and is provided a copy of a medical order that documents the need for such devices and medications; and
2. The possession of any of the articles mentioned in the previous paragraph (2) (a) by any veterinarian currently licensed under chapters 474 and 550 of the Florida statutes.
3. the possession of any of the following devices division expressly designated as exempt from the prohibitions contained in the previous paragraph (2) (a):

The possession of a pipe of a length not exceeding 36 inches;
B. the syringes, bulb syringes and other syringes not able to accept a hypodermic needle and not holding one volume greater than 6 ounces;
C. gun; and
D. inhalation devices.

(3) The day of the race is prohibited rectal, oral intubation, nasogastric tube or gastric (commonly known as "intubation") of any animal that is scheduled to run. When Commissioners or judges detected that an animal has been intubated the day of the race, the animal will be removed from it.

Specific authority 120.80 (4) (a) 550.0251 (3). (II). 550.2415 (13) FS. Law Implemented 110.80 (4) (a), 550.0251. 550.115 550.2415 FS. History-New 10-20-96. Amended 1-5-98.

6. ID-6.008 allowed drugs for horses.

(1) prescription medicines defined in this rule will be allowed under the conditions laid down to conserve and protect the health of the horse who has entered a race. These drugs will be purchased and administered by a licensed veterinarian, except when a prescription or dispensing valid occurs in accordance with the requirements of chapter 474, State of Florida.

(2) the following drugs, permitted at concentrations lower or equal to the following scheme, they are not reported by the laboratory of racing to the Division as a violation of section 550.2415, Florida State:

to. Detection of acepromazine: [2 - (1-hydroxyethyl) promacina sulfoxide] to 10 nanograms per milliliter urinary concentration.

b. Detection of albuterol to a urinary concentration of 1 nanogram per milliliter.

c. Detection of betamethasone at a serum concentration of 10 picograms per milliliter.

d. Detection of butorphanol (total) to a (free) or 300 nanograms per milliliter urinary concentration at 2 nanograms per milliliter of serum concentration.

and. Detection of clenbuterol to 140 picograms per milliliter urinary concentration, or a serum concentration in the lower level of detection.

f. Detection of dantrolene (5-hidroxidantroleno) a serum concentration of 100 picograms per milliliter.

g. Detection of detomidine (carboxidetomidina) to a urinary concentration of 1 nanogram per milliliter, or serum concentration in the lower level of detection.

h. Detection of dexamethasone to a serum concentration of 5 picograms per milliliter.

i. Detection of diclofenac to 5 nanograms per milliliter of serum concentration.

j. Detection of dimethyl sulfoxide (DMSO) at a concentration of 10 micrograms per millilitre serum

k. Detection of firocoxib a serum concentration of 20 nanograms per milliliter to.

l. Detection of furosemide to a serum concentration of 100 nanograms per milliliter and an analysis of density of less than 1.010 urine.

m. Detection of glycopyrrolate of serum concentration of 3 picograms per milliliter.

n. Detection of isoflupredona to a serum concentration of 100 picograms per milliliter.

or. Detection of lidocaine to 20 picograms per milliliter of serum concentration.

p. Detection of mepivacaine (hydroxy mepivacaine) to 10 nanograms per milliliter urinary concentration, or a serum concentration in the lower level of detection.

q. Detection of methocarbamol at a serum concentration of 1 nanogram per milliliter.

r. Detection of methylprednisolone at a serum concentration of 100 picograms per milliliter.

s. Detection of omeprazole in urinary concentration of 1 nanogram per milliliter.

t. Detection of prednisolone a serum concentration of 1 nanogram per milliliter.

or. Detection of penicillin procaine at 25 nanograms per millilitre serum concentration.

v. Detection of triamcinolone acetonide to 100 picograms per milliliter of serum concentration.

w. Detection of wellbutrin a serum concentration of 0.01 nanograms per milliliter to.

(3) the collected samples may contain one of the three non-steroidal anti-inflammatory (NSAID) listed below, up to the limit of the primary threshold. The samples may contain two of NSAIDs to a concentration until the secondary threshold limit. No more than two of NSAIDs listed below can be present in any sample.

(a) Flunixin primary serum concentration of 20 nanograms per milliliter, and a high serum concentration of 3 nanograms per milliliter.

(b) Cetoprofeno a primary serum concentration of 2 nanograms per milliliter, and a high serum concentration of 1 nanogram per milliliter.

(c) Phenylbutazone primary serum concentration of 2 micrograms per milliliter, and a serum concentration secondary of 0.3 micrograms per milliliter.

(4) will not be allowed the use of Anabolic-Androgenic Steroids (AAS) in test samples of racehorses, except the major metabolites of stanozolol, nandrolone, and natural substances boldenone and testosterone in lower concentrations to the following thresholds:

(to) Stanozolol or 16ss - hidroxistanozolol - 1 nanogram per milliliter in urine for all horses, regardless of sex.

b) Boldenone - 15 nanograms per milliliter in urine from uncastrated male horses. Boldenone in castrated horses or females will not be allowed.

(c) Nandrolone - 1 nanogram per milliliter in urine of neutered or females; or 45 nanograms per milliliter of metabolite, 5α-strange-3 ss, I7α-diol in urine from uncastrated male horses.

(d) Testosterone - 20 nanograms per milliliter in urine of the geldings, 55 nanograms per milliliter in urine of females. Samples collected from horses that are not castrated males are not tested for testosterone.

(5) all prescribed medicines, regardless of the method of administration, must remain protected under lock and key when they are not actively administered.

Rulemaking Authority 550.0251 (3). 550.2415 (12) FS. Law implemented 550.0251 (11). 550.2415 (1), (7) FS. History-new 10-20-96. Amended 1-5 - 98, 6-6-00, 5-14-02. 6-6-04, 7-6-06. 8-12-07 12-30 - 08, 12 - 29 - 11, 1-10 - 16.

Title XXXIII
REGULATION OF TRADE, COMMERCE, INVESTMENT, AND STRESS

Chapter 550.
PARI-MUTUEL WAGERING

View Entire Chapter

550. 2415 races of animals under certain prohibitions. Sanctions. Exceptions.

(1) (a) prohibits the competition of an animal that has been medicated in unacceptable way or who has determined that you are a prohibited substance found in his body. Is considered that it has violated this section to the person who medicated unacceptably to the animal or the animal that is positive to substances banned in the tests that are made to the samples taken before or immediately after running in a race, applied for the presence of such drugs or substances. The results of the tests and the identities of animals subjected to these test, as well as the identities of their instructors and record owners are confidential and are exempt from paragraphs s.119.07 (1) and s. 24 (a), article I of the State Constitution during the 10 days that take place after the completion of the analysis of all samples collected in one day in particular and of the results of positive tests derived from such samples have been reported to the director of the division, or is there initiated an administrative action.

(b) a violation of this section that the day of the race is a specimen contains a level of natural substance that exceed their normal physiological concentrations. The division may request support from the Department of agriculture and services consumer and adopt standards that specify physiological normal concentrations of natural substances in an animal of its kind, as well as you can adopt standards that specify levels acceptable contaminants environmental and levels of trace amounts of substances in test samples.

(c) the discovery of a substance banned in a specimen the day of the race is evidence prima facie that the substance was administered and transported in the body of the animal while participating in the race.

(2) the division may take administrative action against an occupational licensee responsible in accordance with the rule of the division corresponding to the condition of an animal that has been medicated or drugged way unacceptable, in violation of this section .

(3) (a) to the finding of a violation of this section, the division may revoke or suspend the license or permit to the offender or denial of a license or permit in process; You can fine the offender in an amount not exceeding the amount or draw won by the animal in the race in question or a $10,000 fine, applying the figure that is more than these two; It may require the partial or total return of purse, the drawings and the trophy of the race in question; or to impose against the offender any combination of the penalties referred to in this paragraph (3) (a). The

application of these infractions for a violation of this section does not exclude prosecution for criminal acts committed.

(b) the division, without prejudice to the provisions of Chapter 120, may summarily suspend the license of an occupational licensee responsible in accordance with this section or rule of division due to the State of an animal's career, if the laboratory of the Division reported the presence of a substance banned in the animal or its blood, urine, saliva or other body fluids, either before a race in which the animal participates or after a race that the animal has run.

(c) If an occupational licensee is summarily suspended under this section, the division will offer licensee a hearing prompt post-suspension within 72 hours after the sanction. At such hearing the division will present a laboratory report and the documentation which, in her view, set the suspended licensee responsibilities. To produce documentation, licensee has the opportunity to demonstrate that it has no responsibility.

(d) any administrative proceedings against a licensee or holder of permissions, which is not a procedure under paragraph (c), will be carried out in accordance with Chapter 120.

(4) a prosecution for a violation of this section must begin within 90 days after the violation was committed. Notification of an administrative claim marks the beginning of the administrative action.

(5) the division shall apply a divided sampling procedure for testing animals under this section.

(a) the division shall notify the owner or trainer, Commissioners and Association proper riders on all drug testing results. If the result of a drug test is positive, and if requested by the instructor or the affected owner of the animal from which the sample was obtained, the division will send split sample to an independent laboratory approved so that further analysis. The division shall establish standards and rules for the uniform application of this measure and keep updated a list of at least five approved independent laboratories, which the owner or trainer you can choose to send the result of a drug test that is positive.

(b) if the laboratory of the division are not confirmed by the independent laboratory, no administrative or disciplinary action under this section may be continue.

(c) if the independent laboratory confirms the positive result from the laboratory of the division, the division may initiate administrative procedures as prescribed in this chapter and in accordance with Chapter 120. For the purposes of this subsection, the Department will attempt to in good faith get enough fluid that allows you to make both a primary test as a secondary test animal.

(d) for the test of a Racing Greyhound, if there is an insufficient amount of the secondary sample (divided), to confirm the positive result of the lab division, the division can initiate administrative procedures as prescribed in this Chapter in accordance with Chapter 120.

(e) for the test of a racehorse, if there is an insufficient amount of (divided), secondary sample for confirmation of the positive outcome of the lab division, the division cannot take further action against the owner or trainer and any suspension of the license which has been performed should be lifted immediately.

(f) the division shall require its laboratory and independent laboratories to participate annually in a program of quality assurance administered externally, designed to assess the ability to test on the detection and proper quantification of drugs, drugs and natural substances that can be administered to animals for racing. Quality assurance program manager will report their results and findings to the division and the Department of agriculture and services to the consumer.

(6) (a) is the intent of the legislature that animals involved in races in this State, in which the pari-mutuel betting, is carried out and that the animals that are bred and trained for racing in this State, are treated humanely , both inside and outside the racing, and throughout his life.

(b) the division shall determine, by standard, procedures for euthanasia of greyhounds. However, a Greyhound may not be sacrificed by other means that non-drug lethal injection of sodium pentobarbital. A Greyhound may not be moved outside the boundaries of the State for the purpose of slaughter.

(c) an occupational licensee commits a violation of this chapter if a Greyhound training using live or dead animals. A Greyhound may not be moved outside the boundaries of the State for the purpose of being trained using live or dead animals.

(d) any act committed by a licensee that would constitute an act of cruelty to animals, as defined in s.828.02, involving any animal, constitutes a violation of this chapter. The imposition of any sanction by the division for violation of this chapter or any rule adopted by the division pursuant to this chapter shall not prohibit prosecution for cruelty against animals.

(e) the division may inspect any area of a pari-mutuel facility where animals of competition run, are trained, housed or kept, including any area where food, medicines or other supplies, are kept to ensure the humane treatment of animals of racing and to ensure compliance with this chapter and the rules of the division.

(7) (a) in order to protect security and the welfare of racing animals and the integrity of the events in which they participate, the division shall adopt rules that establish the conditions of use and maximum concentrations of medicines, drugs and substances natural identified in the controlled program of therapeutic medication (Controlled Therapeutic Medication Schedule), Version 2.1, revised on April 17, 2014, adopted by the Association of Commissioners of Carreras international, Inc. (ARCI). Controlled therapeutic drugs include only specific drugs and the concentrations allowed in biological samples approved by the ARCI and recognized as a controlled therapeutic drugs.

(b) the standards of the division should designate appropriate biological specimens that will be used to control the administration of medicines, drugs and natural substances. They will be also used to determine the testing methodologies and the margins in the measurement, which allow the screening of specimens and allowing to confirm the presence of drugs, drugs and natural substances.

(c) the rules of the division should include a system of classification of drugs and substances and a corresponding schedule of penalties for violations. Both the rules and the timetable should incorporate the uniform guidelines of classification for foreign substances, Version 8.0, revised December 2014, by the International Association of racing Commissioners. The division will adopt the laboratory detection limits, approved by the

Commissioners Association of racing international, Inc., for drugs and medications that are not included as controlled, therapeutic drugs and whose presence in a sample It may result in a violation of this section.

(d) the rules of division must include conditions for the use of furosemide in treatment of exercise-induced pulmonary hemorrhage.

(e) the division may request input from the Department of agriculture and services to the consumer on the adoption of the rules required under this subsection. Such rules must be adopted before January 1, 2016.

(f) this section does not prohibit the use of vitamins, minerals or natural substances, provided that the day of the race, these do not exceed the normal physiological concentration in a specimen.

(8) the furosemide is the only drug that can be administered within 24 hours prior to the official schedule of one race, but can not be used within 4 hours prior to the official schedule of one race.

(9) (a) the division you can carry out a test post-mortem of any animal that is injured at a racetrack during training or competition, and that subsequently expire or sacrificed. The division can carry out a test post-mortem of any animal which expires while staying on a race track, a complex of association or a farm or stable licensed. You ask coaches and owners who comply with this paragraph as a condition to keep his license.

(b) the division may take possession of the animal after his death for a test post-mortem. The division may submit to the blood test, urine, other body fluids, or other types of tissue, collected during the test post-mortem to be examined by the laboratory division or his designee. Once completed the test post-mortem, the carcass must be returned to the owner or discarded, at the option of the owner.

(10) a violation of this section the presence of a substance prohibited in an animal, found by the laboratory of division in a sample of body fluid that has been collected after the race or during the test post-mortem of a dead animal during a race.

(11) the cost of the tests post-mortem, tests and deposition of the animal should be borne by the division.

(12) the division shall adopt rules to implement this section.

(13) the division must implement as standard medication for greyhounds from racing levels recommended by the College of veterinary medicine at the University of Florida, same which were developed on an agreement between the College and the betting Division Pari-mutuels. The College of veterinary medicine at the University of Florida may notify the division in writing when you have completed the investigation or review of a medicinal product in particular, in accordance with the agreement and completed a final report of its conclusions and recommendations to the division.

History-s. 27, chap. 92-348; s. 28, chapter 93-120; S. 5, ch. 93-123; s. 1, ch. 95-205; s. 9, ch. 96-364; s. 344, ch. 96-406; s. 1174, ch. 97-103; s. 2, ch. 2002-51; s. 5, ch. 2009-69; S. 11, chapter 2009-170; SS 4, 5, ch. 2010-29; s. 1, ch. 2015-8

PALM BEACH EQUINE CLINIC

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Janet Greenfield BVetMed, MRCVS
Tyler Davis, BVetMed MRCVS
Ryan Lukens, DVM
Selina Passante-Watt, DVM
Samantha Miles, DVM

March 16, 2017

Dear Dr. O'Neil,

Palm Beach Equine Clinic is a USDA approved quarantine facility in South Florida that is available for all emergency surgery and isolation requirements as directed by the Department of Agriculture. Palm Beach Equine Clinic has multiple I SO stalls and an ICU hospital for all critical care medical cases. In addition, we have three Board Certified Surgeons, Board Certified Internist and a Board Certified Radiologist on staff. We are a 90-minute trailer ride from Gulfstream Park to our surgical, ICU and imaging amenities. Palm Beach Equine Clinic is happy to provide veterinary support for The Stronach Group and The Caribbean Classic yearly.

Sincerely,

Scott J Swerdlin, DVM, MRCVS
President of Palm Beach Equine Clinic

13125 Southfields Road • Wellington, Florida 33414
Clinic 561 793 1599 • Fax 561 793-2492 • 1 800-226-1599

Requirements of vaccination of the Gulfstream Park

All racing animals should be vaccinated against equine Influenza and the equine Herpes Virus (vaccine "RhinoFlu") in accordance with Chapter 61 D6.009 (a) 2 of the rules of betting Pari-mutuels. We cannot accept registrations of horses whose trainer has not provided the information of current vaccination. All horses established on the grounds of the Gulfstream Park, GPW and Palm Meadows Training Center should be vaccinated, at least twice a year. Instructors are responsible for keeping them in your stable, and put at the disposal of the office of racing, current records vaccination of all the horses in his care. The claimed horse vaccination records must be transferred by coach previous coach complaining within 24 hours of the official transfer of ownership.

Each horse that seeks entry into the area of stables of the training of the Gulfstream Park, GPW and Palm Meadows Center shall:

(to) Be accompanied by a current Coggins test result

b) Be accompanied by a certificate of veterinary inspection (CVI), which has been signed by a licensed veterinarian and dated within 72 hours of their arrival at the facility. The CVI should contain the following written statement:

to. "Horses which are contained in this certificate of veterinary inspection (CVI) they do not have visible symptoms, or have been diagnosed with Herpes Virus equine (HEV-1) for a period of thirty (30) days prior."

(c) Have been vaccinated against equine Herpes type 1 (HEV-1) virus by a veterinarian licensed within a minimum of fourteen (14) days and no more than ninety (90) days before arrival. The certificate of veterinary inspection (CVI) will also indicate the date of vaccination and the product used to vaccinate each horse.

* Horses arriving at the door of the stable (Stable Gate) without appropriate documentation will not gain access. Only the horses that come directly from GPW and Palm Meadows Training Center to the race are excused of possessing a certificate of veterinary inspection at the time of admission.

ALL horses must have a valid Coggins, a date that will last, at least, until the end of the event in progress. For horses stabled YEAR ROUND at Gulfstream Park, Gulfstream Park West or at Palm Meadows Training Center: every horse must have a valid Coggins with a date that carries through December 31 of the current year. The easiest way to achieve compliance with these regulations is to obtain all Coggins in early January.

Treatment of Equine Piroplasmosis

Abstract

Arthropod-borne apicomplexan pathogens that cause asymptomatic persistent infections present a significant challenge due to their life-long transmission potential. Although anti-microbials have been used to ameliorate acute disease in animals and humans, chemotherapeutic efficacy for apicomplexan pathogen elimination from a persistently infected host and removal of transmission risk is largely unconfirmed. The recent re-emergence of the apicomplexan Theileria equi in U.S. horses prompted testing whether imidocarb dipropionate was able to eliminate T. equi from naturally infected horses and remove transmission risk. Following imidocarb treatment, levels of T. equi declined from a mean of 104.9 organisms/ml of blood to undetectable by nested PCR in 24 of 25 naturally infected horses. Further, blood transfer from treated horses that became nested PCR negative failed to transmit to naı¨ve splenectomized horses. Although these results were consistent with elimination of infection in 24 of 25 horses, T. equi-specific antibodies persisted in the majority of imidocarb treated horses. Imidocarb treatment was unsuccessful in one horse which remained infected as measured by nested PCR and retained the ability to infect a naı¨ve recipient via intravenous blood transfer. However, a second round of treatment eliminated T. equi infection. These results support the utility of imidocarb chemotherapy for assistance in the control and eradication of this tick-borne pathogen. Successful imidocarb dipropionate treatment of persistently infected horses provides a tool to aid the global equine industry by removing transmission risk associated with infection and facilitating international movement of equids between endemic and non-endemic regions.

Guidelines and Procedures for Treatment of Horses Infected with *Theileria equi* APHIS-VS Equine Piroplasmosis Treatment Research Program March 2014

The equine piroplasmosis (EP) treatment research program began in January 2010 in response to the 2009 identification of an extensive *Theileria equi* outbreak in horses on a ranch in south Texas. All EP-positive horses identified in the United States are managed under State quarantine in accordance with the procedures in VS Memo 555.20, which includes enrollment in the EP treatment research program as an option for long-term management of EP-positive horses. The program is jointly conducted by APHIS-VS and USDA-Agricultural Research Service (ARS) and, to date, there have been 197 horses officially enrolled in the program and treated using a published high-dose imidocarb dipropionate protocol.

In February 2013, in response to a U.S. Animal Health Association Resolution, VS evaluated and approved a restricted quarantine release process for domestic horses previously confirmed as infected with *T. equi* that: 1) are enrolled in and treated in accordance with EP treatment research program protocols; 2) have sufficiently met criteria to be designated as permanently cleared of the infection; and 3) have tested negative on applicable EP diagnostic assays. (See details in "Criteria to be Eligible for Quarantine Release" below). This process does not apply to imported horses that test EP-positive during import quarantine or to horses infected with *Babesia caballi*, as there was not enough data on *B. caballi* treatment outcomes to provide an evaluation on clearance.

Responsibilities and Associated Costs

The treatment will be administered by an accredited veterinarian under State/Federal direct oversight (i.e. a State or Federal animal health official should be present for each of the 4 doses of the treatment administered to the horse). It is recommended that the State or Federal animal health official also collect the pre-treatment and post-treatment samples from the horse whenever possible. It is allowable for the accredited veterinarian to collect the pre-treatment and post-treatment samples without direct oversight, however this is likely to incur additional sample collection costs to the owner, whereas collection by State/Federal personnel could be done without further charge. Shipping of pre-treatment and post-treatment samples will be covered by APHIS-VS through the ADD's office. Diagnostic testing costs will be covered by APHIS-VS-NVSL (as non-billable samples submitted under an FAD number) and by ARS-Pullman, WA (as non-billable research testing).

All documentation of the treatment and testing of the horse should be entered into EMRS at the local level in a timely fashion. The Equine Epidemiologist will track the treatment and testing of the horse, provide feedback to State/Federal personnel on the horse's diagnostic progress toward organism and antibody clearance, and will set up calls between State/Federal personnel and research and diagnostic experts if needed throughout the process.

The cost of the drug, the administration of treatment, and any other associated charges due to the accredited veterinarian will be paid for by the owner of the horse. Historically, the total cost of veterinarian's charges to the horse owner has averaged around $1000 per horse.

Managing Owner Expectations

Although identification of a treatment protocol that can provide permanent clearance of *T.equi* from the horse thereby removing all transmission risk is a breakthrough achievement, it is not an outcome that can be guaranteed. Of the 197 horses treated to date, 10 horses thus far have been identified as treatment failures and remained infected after the first attempt at treatment. It is unknown why certain horses may fail to clear the organism after treatment, but likely factors include the innate resistance of some strains of *T. equi* to imidocarb and factors specific to an individual horse's immune system that may help or hinder organism clearance. Retreatment using the same high-dose imidocarb protocol was repeated in 9 of these 10 treatment failures and all but one horse successfully cleared the organism on the second attempt at treatment. Helping the horse owner to understand that treatment success is not guaranteed and that some horses have had to be retreated to achieve clearance is imperative. Additionally, it is possible that horses may fail treatment again after a second attempt and no further treatment option may be available.

Another concept that must be conveyed to owners considering treatment is the length of time that may elapse before a treated horse meets the criteria for quarantine release. Although organism clearance can be confirmed through PCR testing within the first few months post-treatment, the positive antibody status of the horse (cELISA positive status) remains for many months and, in some cases, up to 2 years after successful treatment.

The horse owner must be advised that this process is not a quick method to quarantine release and that, in all likelihood, the horse may not be eligible for release until up to 2 years post-treatment when the cELISA titer finally goes negative (the last criteria for eligibility of quarantine release).

Finally, horse owners must be advised of the potential health risks of administering the high-dose imidocarb therapy to their horse. Firstly, this is an off-label use of imidocarb, as the drug in the U.S. is labeled only for the treatment of babesiosis in dogs. Secondly, while the mechanism of action for how this drug eliminates *T. equi* is unknown, it is known that this drug can have hepatotoxic and nephrotoxic side effects. Thirdly, each injection of imidocarb produces a list of side effects of varying severity in nearly all horses (see "Side Effects of Treatment") which we attempt to temper with the use of premedication. While no horses in the program have died to date from the treatment, it is unknown what the long-term effects of this therapy may be especially in horses with underlying illness or other conditions. It is not recommended to administer high-dose imidocarb therapy to horses with underlying medical conditions or to pregnant mares.

If the horse owner seems unaccepting or cavalier about any of these cautions regarding expected outcomes, then proceeding with enrollment in the EP treatment research program may be contraindicated.

Side Effects of Treatment

Imidocarb dipropionate has anticholinesterase activity and reliably produces the following side effects in nearly all horses with varying levels of severity: agitation, sweating, colic, and diarrhea. These side effects typically begin within minutes following intramuscular administration and can last up to 3-4 hours post-injection. It is recommended that these side effects be managed by premedicating the horse with the label dose (0.3 mg/kg IV) of Buscopan® (N-butylhyoscinium bromide). Buscopan® can also be administered in response to side effects after they have begun with satisfactory results, but pre-medication to prevent the side effects is most effective. Non-steroidal anti-inflammatory drugs should NOT be used to alleviate side effects unless absolutely necessary (emergency situation as determined by the accredited veterinarian), as NSAIDs may have contributed to treatment failures in the past.

Pain and swelling at the injection site is also a common side effect of imidocarb, so the intramuscular injections should be rotated to different injection sites. In some cases, deep nodules in the muscle at the location of injection can persist for months post-treatment and may be unsightly and/or painful. Muscular injection site selection should be established prior to administration and should take into account the use/discipline of the horse. (Example: cutting horses or reining horses that work more off of their hindquarters should have treatment alternated between the right and left sides of their neck to avoid long-term muscle knots/pain that an injection into the hindquarter muscles might cause and that might interfere with the horse's intended use).

Recommendations for the Accredited Veterinarian

Given the off-label use of this drug for the purposes of the program, the unknown long-term effects after administration, and the potential severity of the known side effects that are common after each injection of imidocarb, it is highly recommended that the accredited veterinarian have the horse owner sign a notice of disclosure or waiver indicating that they've been made aware of the potential for negative impacts of this treatment on the health of the horse and have chosen to proceed. The EP treatment research program is a voluntary program and the horse owner has the option to simply maintain the horse under State quarantine with no further resolution of the EP infection required.

The accredited veterinarian should also have a good understanding of the topics presented above under "Managing Owner Expectations" and at any time if he/she determines that the owner may be unable or unwilling to accept the cautions provided, he/she should voice those concerns to the appropriate State or Federal animal health official. The accredited veterinarian is also a voluntary participant in this process and is not required to administer the treatment if he/she has concerns or chooses not to participate. Concerns on receiving full payment from the horse owner for the services planned to be performed are also valid reasons not to proceed. It is recommended that the accredited veterinarian have the owner sign an estimate of treatment costs to be incurred. The accredited veterinarian is responsible for purchasing the FDA-approved U.S. label version of Imizole ® (imidocarb dipropionate) from a reputable veterinary supply company or directly from the manufacturer (formerly Schering Plough, now Merck Animal Health). Be advised that multiple bottles of the drug will be needed to reach the correct volume for each of the 4 doses (see "Protocol for Treatment" below to properly calculate the amount of drug to procure).

It is highly recommended that the horse be premedicated with Buscopan® (N-butylscopolammonium bromide) at the label dose (0.3 mg/kg IV) immediately prior to each dose of imidocarb administered to reduce the severity of the common side effects of imidocarb noted above. Non-steroidal anti-inflammatory drugs (phenylbutazone, flunixin meglumine) should NOT be used to alleviate side effects unless absolutely necessary (emergency situation as determined by the accredited veterinarian), as NSAIDs may have contributed to treatment failures in the past. Additionally, it is recommended that NSAIDS not be administered to the horse before, during or immediately after the 10 day treatment period. While it has not been proven that NSAIDs were the cause of previous treatment failures, it is a concern of the researchers that the inflammatory process may play a key role in elimination of the blood parasite with the administration of chemotherapeutics, so removing the likelihood that this process will be disturbed by administration of other drugs remains an important consideration.

At any time, if the accredited veterinarian has questions on this process or EP treatment in general, additional information can be provided directly or a group conference call with experts can be arranged by the Equine Epidemiologist.

Pre-treatment Testing

Duplicate samples of clear serum (red top tubes; at least 6-10 mL) and whole blood in EDTA (purple top tubes; at least 6-10 mL) from the horse should be collected prior to treatment and submitted simultaneously to the National Veterinary Services Laboratories (NVSL) in Ames, Iowa and USDA-ARS in Pullman, Washington. Each laboratory should receive one serum sample and one whole blood in EDTA sample for the purposes of pre-treatment testing. As long as the horse has been confirmed on previous testing (even years prior) to be PCR positive for *T. equi*, then the pre-treatment samples can be collected on the same day as the first treatment is administered (i.e. immediately before treatment is administered). If there is no history of a previous PCR positive test on the horse, it is recommended that PCR positive results be obtained before proceeding with treatment.

Post-treatment Testing

Samples to collect at 30, 60 and 90 days post-treatment

At each post-treatment sample collection, duplicate samples of clear serum (red top tubes; at least 6-10 mL) and whole blood in EDTA (purple top tubes; at least 6-10 mL) from the horse should be collected submitted simultaneously to the National Veterinary Services Laboratories (NVSL) in Ames, Iowa and USDA-ARS in Pullman, Washington. Each laboratory should receive one serum sample and one whole blood in EDTA sample for each sample collection date. Submission form instructions are the same as indicated under "Pre-treatment Testing" with the exception of the comments section of the submission form which should instead indicate that this is a **post-treatment** sample from a known EP positive horse. Shipping instructions and shipping addresses are the same as indicated under "Pre-treatment Testing"

Information on Results Reporting

Diagnostic test results from NVSL are typically available within 3-5 days after sample receipt at the laboratory and a results report will be issued by email from NVSL to the submitter, the ADD, and the Equine Epidemiologist, any of which can ensure that the NVSL results are forwarded to the interested parties, including the accredited veterinarian. Testing done at NVSL will include cELISA, CFT, and real-time rRT-PCR for *T. equi*.

USDA-ARS is a research laboratory and doesn't have an automatic results reporting system. Additionally, samples are not run on a regular basis and it may be several weeks before results are available on each sample submitted to this laboratory. The Equine Epidemiologist will assist in querying the laboratory on the status of testing and providing results via email to all interested parties. Testing done at ARS will include cELISA, nested PCR, and Western Blot. Currently ARS is the only laboratory providing Western Blot of which one negative result is required (see "Criteria to be Eligible for Release"). However, we are currently working to transition Western Blot testing to

NVSL. Once the Western Blot testing is available at NVSL, all testing for the EP treatment research program can be conducted at NVSL alone without the need for duplicate samples being sent to USDA-ARS.

Interpretation of Post-Treatment Results

If the treatment was successful, then a series of PCR negative results will indicate that the organism has been cleared. A single PCR positive result on the first bleed post-treatment is possible and may not be cause for alarm as the sensitivity of the test can occasionally pick up

nucleic acid remnants from inactivated *T. equi*. The Equine Epidemiologist can provide guidance on interpretation of each set of post-treatment results as they become available. If there is suspicion of treatment failure during the first 90 days post-treatment, the Equine Epidemiologist will organize a conference call with State/Federal officials, the accredited veterinarian, and research experts to evaluate the current status of the horse based on existing diagnostic results and provide recommendations on next steps including the consideration of retreatment on a case-by-case basis.

After a series of negative PCR results is established, the Western Blot clearance test usually goes negative around 4-5 months post-treatment. A negative on the Western Blot is one of the earliest indicators of fading antibody to the organism. The cELISA test is the most persistent indicator of *T. equi* antibody and will decrease in titer relatively slowly throughout the process. It is the dropping of the cELISA titer into the negative range (below 40% inhibition) that will be the last step achieved to qualify the treated horse for quarantine release. Depending on the length of time the horse was infected and the individual response of the horse's immune system, achieving cELISA negative status after successful treatment can take anywhere from 5 months to 2 years post-treatment.

Samples to collect after 90 days post-treatment

After a series of three or more negative PCR results is achieved on samples collected at least 30 days apart and a negative Western Blot result is obtained, post-treatment sample collections should be reduced in frequency to once every 3-6 months thereafter depending on the rapidity in reduction of cELISA titer. Once cELISA titers drop below the 50% inhibition range, they tend to decrease more slowly. More frequent rebleeds than recommended do not allow enough time for the expected titer decreases to be appreciated. Additionally, it should be noted that cELISA titers can vary plus or minus within 10 % inhibition points between bleeds during this time without being considered a significant change. PCR results will remain negative throughout in successfully treated horses despite these slight variations in antibody titer while trending toward the cELISA negative range.

Criteria to be Eligible for Quarantine Release

- The horse is officially enrolled in the VS/ARS treatment research program;
- The horse has completed treatment using the ARS-published high-dose imidocarb dipropionate protocol under State or Federal supervision;
- The horse is permanently identified using an ISO-compliant microchip with the identification number held in a data repository accessible by State and Federal animal health officials;
- The horse has tested negative using either a nested PCR or real-time rRT-PCR assay on a series of three or more post-treatment samples collected at least 30 days apart;
- The horse has tested negative by transfusion to a spelectomized horse OR negative by the ARS Western Blot clearance test;
- The horse has tested negative on the cELISA and CFT at the NVSL

VS further recommends that States establish a compliance agreement with horse owners to conduct the cELISA test annually for 3 years after quarantine release to ensure continued freedom from disease. Under the compliance agreement, the owner would notify the State of the death or permanent movement of the horse within the 3 year period so testing could continue in the horse's new state of residence if needed. If the horse moves to a new state, the owner would enter into a new compliance agreement with that State for the remaining testing period. Samples for annual assurance testing during this 3 year period after quarantine release should be collected by State or Federal personnel and shipped to NVSL only with shipping and testing costs covered by APHIS-VS.

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