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Medical causes of poor performance and their associations with fitness in Standardbred racehorses

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Abstract

Background: Poor performance is a multifactorial syndrome of racehorses, commonly associated with subclinical disorders, which can be diagnosed by exercise testing.

Objectives: Describe the prevalence of medical causes of poor performance in Standardbreds unassociated with lameness, and evaluate their relationships with fitness variables measured by exercise treadmill test.

Animals: Hospital population of 259 nonlame Standardbred trotters referred for poor performance.

Methods: The horses' medical records were retrospectively reviewed. Horses underwent a diagnostic protocol including resting examination, plasma lactate concentration, treadmill test with continuous ECG and assessment of fitness variables, creatine kinase activity, treadmill endoscopy, postexercise tracheobronchoscopy, bronchoalveolar lavage (BAL), and gastroscopy. The prevalence of different disorders was evaluated, including cardiac arrhythmias, exertional myopathies, dynamic upper airway obstructions (DUAOs), exercise-induced pulmonary hemorrhage (EIPH), moderate equine asthma (MEA), and gastric ulcers (EGUS). The associations of these disorders with fitness variables were investigated individually and using multivariable models.

Results: Moderate equine asthma and EGUS were the most common disorders, followed by EIPH, DUAOs, cardiac arrhythmias, and exertional myopathies. Hemosiderin score was positively correlated with BAL neutrophils, eosinophils, and mast cells; increased creatine kinase activity was associated with BAL neutrophilia, DUAOs, premature complexes, and squamous gastric disease. Treadmill velocity at a plasma lactate concentration of 4 mmol/L and at heart rate of 200 beats per minute was negatively affected by BAL neutrophilia, multiple DUAOs, exertional myopathies, and squamous gastric disease.

Conclusions: The multifactorial nature of poor performance was confirmed, with MEA, DUAOs, myopathies and EGUS representing the main diseases involved in fitness impairment.

Abbreviations: BAL, bronchoalveolar lavage; CK, creatine-kinase; DUAO, dynamic upper airway obstruction; ECG, electrocardiogram; EGGD, equine glandular gastric disease; EGUS, equine gastric ulcer syndrome; EIPH, exercise-induced pulmonary hemorrhage; ER, exertional rhabdomyolysis; ESGD, equine squamous gastric disease; HSTE, high-speed treadmill endoscopy; MEA, mild-moderate equine asthma; PC, premature complex; PLH, pharyngeal lymphoid hyperplasia; TB, tracheal bifurcation blunting; THS, total hemosiderin score; TM, tracheal mucus.

KEYWORDS
equine exercise physiology, equine performance, equine sports medicine, poor performance, sport horses, treadmill test

1 | INTRODUCTION

Poor performance is a common and complex condition of racehorses, usually associated with subclinical disorders.[1] In addition to musculoskeletal conditions, medical disorders are common, and their identification can be challenging for the clinician. In fact, poorly performing horses are mostly normal on physical examination, and the identification of underlying medical diseases often requires dynamic tests.[2] Moreover, multiple disorders can concomitantly affect horses,[1,3] and it can be difficult to determine the contribution of each to the impaired racing performance. To investigate poor performance in sport horses, investigators rely either on the subjective expectations of trainers and owners[4-6] or on quantitative data, such as racing placements and earnings.[7-10] Many biases however can interfere with competition results, such as the number of other participants, level of the race and possible handicaps, ground surface, distance, driver interventions, and other factors.[11,12] Objective and measurable information on the fitness of Standardbred racehorses can be obtained by standardized exercise tests, which can be performed either in the field or using a treadmill.[1,13-18] The treadmill allows for control of environment, speed, inclination, and ensures higher repeatability of the results and reproduction of racing conditions. In particular, for nonmusculoskeletal conditions, the treadmill velocity at which a blood lactate concentration of 4 mmol/L (VLa4) is reached and the treadmill velocity at a heart rate of 200 beats per minute (bpm; V200) are considered good indicators of the level of performance.[19-22]

Musculoskeletal disorders are the most common cause of decreased performance in Standardbred racehorses,[2] followed by medical disorders such as respiratory diseases affecting the upper or lower airways,[1-3] including dynamic upper airway obstructions (DUAO),[23-25] mild to moderate equine asthma (MEA),[5,26-28] and exercise-induced pulmonary hemorrhage (EIPH).[29-32] In fact, the respiratory system is considered the main limiting factor to performance even in healthy racehorses (both Thoroughbreds and Standardbreds), and related disorders can substantially impact the aerobic and athletic capacity of the horse.[33] Other reported medical causes of poor performance include exertional rhabdomyolysis (ER) and other myopathies,[18,34] equine gastric ulcer syndrome (EGUS),[35-37] and cardiac arrhythmias.[38-40] Numerous studies have investigated the effects of these conditions on the fitness of Standardbred racehorses, reporting contrasting results, probably because of different inclusion criteria, study design, and the variables chosen to define performance. Moreover, poor performance is rarely caused by a single disorder.[1,3] Therefore, the effects of different diseases should not be considered individually but rather with the perspective of a multifactorial condition.

We aimed to describe the prevalence and distribution of the medical disorders diagnosed in a population of poorly performing Standardbred trotters and their associations with fitness variables measured during a standardized exercise test on treadmill. Moreover, the associations between concomitantly encountered diseases was evaluated, and the contribution of each disorder on changes in VLa4 and V200 in a multifactorial setting was determined.

2 | MATERIALS AND METHODS

2.1 | Study population

In our retrospective study, the medical records of the Standardbred trotters referred to the Equine Sports Medicine Unit of the Veterinary Teaching Hospital, University of Milan (Italy), for poor performance between 2002 and 2021 were reviewed. All horses ($n = 259$) were in full training upon admission and underwent a diagnostic evaluation for poor performance with identification of subclinical causes. Horses presenting with signs of systemic illness, lameness, clinically relevant arrythmias at rest, or clinically relevant valvular regurgitations were excluded from the study. The age of the horses varied from 2 to 9 years (median, 3; interquartile range [IQR], 3-4 years). The population included 93 females (35.91%) and 166 males (146 stallions, 20 geldings; 64.09%).

Ethical approval was waived because only clinical patients undergoing standard diagnostic procedures were included. Informed consent for the use of clinical data was obtained from all owners or holders.

2.2 | Poor performance diagnostic protocol

Because the study included a period of time over 20 years, the poor performance diagnostic protocol varied slightly throughout the years; the number of horses undergoing each clinical procedure is specified in parentheses. The protocol included:

- Collection of history, clinical examination and laboratory analyses ($n = 259$);
- ECG at rest ($n = 259$);
- Resting endoscopy of the upper airway ($n = 259$);
- Incremental exercise test on high-speed treadmill ($n = 259$), during which a continuous ECG was obtained by Holter recorder ($n = 238$);
- Assessment of serum creatine kinase (CK) activity 6 hours after exercise ($n = 255$);
- High-speed treadmill endoscopy (HSTE; $n = 248$);
- Postexercise tracheobronchoscopy (30 minutes after HSTE; $n = 246$);
- Lower airway endoscopy, bronchoalveolar lavage (BAL) collection and cytological examination ($n = 215$); and,
- Gastroscopy ($n = 173$).

2.3 | Fitness variables

Fitness variables were obtained during an incremental exercise test on a high-speed treadmill, performed as described previously.[28] Before performing the test, horses were trained to the use of treadmill by 2 daily sessions; during the test, horses wore the equipment normally used during racing and a heart rate monitor (Polar, Equine Inzone FT1, Steinhausen, Switzerland). A venous catheter was placed into the left jugular vein and connected to an extension tube to allow blood collection during the test. The belt was inclined with a 5% slope, and horses were warmed up at walk (1.5 m/s) for 4 minutes and trot (6 m/s) for 3 minutes. At the end of the warm-up phase, a first sample of blood was collected. Then, 1-minute-long phases followed, and at each phase the velocity of the treadmill was increased by 1 m/s until the onset of fatigue. At the end of each phase, blood was collected. Horses were cooled down at walk with 0% inclination for 30 minutes. At 1, 5, 15, and 30 minutes after the end of maximal exercise, blood samples were collected and used for the measurement of plasma lactate concentration, blood pH, and hematocrit, as described previously.[25]

The obtained variables of fitness included:

- V200 (m/s): velocity at a heart rate of 200 bpm;
- VLa4 (m/s): velocity at a plasma lactate concentration of 4 mmol/L;
- HRLa4 (bpm): heart rate at a plasma lactate concentration of 4 mmol/L;
- Lac_{max} (mmol/L): maximum peak of plasma lactate concentration;
- V_{max} (m/s): maximum velocity until the onset of fatigue;
- Lac_{30} (mmol/L): plasma lactate concentration at 30 min postexercise;
- HR_{30} (bpm): heart rate at 30 min postexercise;
- pH_{min}: minimum pH;
- Hct_{max}: maximum hematocrit.

The exact values of VLa4 and HRLa4 were calculated using specific software (Lactate-E 1.0, Dr. David Higgins), providing precise lactate threshold markers using inverse prediction.[41]

2.4 | Arrhythmias

During the incremental treadmill test and recovery, horses wore a Holter recorder (Cardioline Click Holter, Trento, Italy), which recorded 3 unipolar leads. A modified base-apex configuration was used, and electrodes were placed and fixed as described previously.[40] Obtained data were analyzed using dedicated software (Click Holter Cardioline Prima Manager, Trento, Italy). Because the software is specifically designed for human patients, all ECG recordings were reviewed and analyzed by an experienced operator. Arrhythmias considered clinically relevant in affecting performance were defined either as the presence of at least 2 isolated premature complexes (PCs) during peak exercise (from V200 to the end of maximal exercise) or as the presence of at least 5 PCs or pairs of paroxysms of PCs detected during peak exercise or immediately after.[3] For statistical purposes, horses were divided into affected or nonaffected by PCs.

2.5 | Exertional myopathies

Six hours after the end of the treadmill test, blood samples were collected in plain tubes from the jugular vein, and immediately centrifugated. Serum was isolated and creatine kinase (CK) activity was measured as described elsewhere.[42] Serum CK activity was considered within normal ranges from 44 to 735 IU/L; higher values were considered diagnostic of ER.[42] Moreover, history and postexercise clinical signs of exertional myopathies were recorded.

2.6 | Dynamic upper airway obstructions

After the incremental treadmill test, horses were given 1 day of active rest, during which they were hand-walked twice a day. On the next day, HSTE was performed during a maximum intensity test at constant speed, as described previously.[25] The presence of any DUAO was recorded and classified as mild (medial deviation of aryepiglottic folds, epiglottis entrapment) or severe (dorsal displacement of the soft palate, nasopharyngeal collapse, epiglottic retroversion, dynamic laryngeal collapse).[25] For statistical purposes, horses were divided into 4 groups: no DUAO, mild DUAO, severe DUAO, and multiple DUAOs (if concomitantly affected by >1 type of DUAO).

2.7 | Exercise-induced pulmonary hemorrhage and asthma

Thirty minutes after the end of HSTE, tracheobronchoscopy was performed to assess EIPH, as described previously[32]; the presence of blood in the trachea and bronchi was graded from 0 to 4 based on a recognized scoring system.[43]

Twenty-four hours after the HSTE, horses were restrained in a stock and sedated using 0.01 mg/kg detomidine hydrochloride IV, and a second lower airway endoscopy was performed. Endoscopic scores were assigned to pharyngeal lymphoid hyperplasia (PLH; from 0 to 4),[44] tracheal mucus accumulation (TM; from 0 to 5),[45] and tracheal bifurcation blunting (TB; from 0 to 4).[46] The endoscope was advanced into the bronchial tree until it was wedged within a segmental bronchus, BAL was performed as described previously[28] and the fluid was collected in sterile EDTA tubes. Within 90 minutes of collection, 300 µL of pooled BAL were cytocentrifuged at 26*g* for 5 min (Rotofix 32, Hettich Cyto System, Tuttlingen, Germany). The slides were air-dried, stained with May-Grünwald-Giemsa (for leukocyte differential cell count) and Perl's Prussian Blue (for hemosiderophage count), and observed under light microscopy at 400× and 1000× .

Based on the presence and subtype of lower airway inflammation,[15] horses were classified as:

- Healthy: neutrophils ≤5%, eosinophils ≤1%, and mast cells ≤2%;
- Neutrophilic MEA: neutrophils >5%, eosinophils ≤1%, and mast cells ≤2%;
- Eosinophilic-mastocytic MEA: neutrophils ≤5%, eosinophils >1%, mast cells >2%; and,
- Mixed MEA: neutrophils >5%, eosinophils >1%, and/or mast cells >2%.

For the evaluation of EIPH, 100 macrophages were assessed; a total hemosiderin score (THS) was calculated by multiplying the percentage of hemosiderophages of the total of macrophages by the median 0-4 grade of hemosiderin, as described previously.[32] A score between 0 and 400 was obtained; horses were considered as positive for EIPH when THS was >75.[47]

2.8 | Equine gastric ulcer syndrome

At the end of the diagnostic protocol, gastroscopy was performed as described previously.[37] As recommended by the European College of Equine Internal Medicine consensus statement,[36] the squamous mucosa was scored for equine squamous gastric disease (ESGD) from 0 to 4, according to the Equine Gastric Ulcer Council scoring system,[48] and the glandular mucosa was evaluated for the presence or absence of equine glandular gastric disease (EGGD).

2.9 | Statistical analysis

The data were analyzed using commercially available statistical software (GraphPad Prism 9.5.0 for MacOS; GraphPad Software, San Diego, California). Normality of all data was evaluated using the Shapiro-Wilk test and descriptive statistics were determined. Statistical tests used to evaluate the associations among signalment, fitness variables, and disease-related data are presented in Table 1.

Normally-distributed data are presented as mean ± SD, whereas nonnormally distributed data are expressed as median and IQR. Statistical significance was set at $P < .05$. For the prediction of V200 and VLa4, multiple linear regression models were designed, including only variables that showed statistical association in the previous tests (Spearman's correlation, unpaired *t* test, Mann-Whitney test, Kruskal-Wallis test, 1-way analysis of variance); variables showing collinearity and covariance with other variables were excluded. Moreover, for V200 and VLa4, multiple logistic regression models were designed, with outcomes defined as ≥7.5 and <7.5 m/s for V200, and ≥8 and <8 m/s for VLa4 (cut-offs were chosen based on median values in the study population). Variables were preliminarily screened using univariable logistic regression, and those with a likelihood ratio test $P < .10$ were selected for inclusion in the multivariable model. The model was built using a manual backwards method of elimination of variables based on the likelihood ratio test *P* value and by comparing the corrected Akaike information criteria (AICc).

TABLE 1 Statistical tests used for data analysis in the present study.

Statistical test	Analyzed data
Spearman's correlation	• Age vs fitness variables, BAL leukocyte counts, THS, endoscopic scores, CK values, ESGD grade • Fitness variables vs BAL leukocyte counts, THS, endoscopic scores, CK values, ESGD grade
Kruskal-Wallis and Dunn's multiple comparisons test	• Age vs DUAO groups, MEA groups • Fitness variables vs DUAO groups, MEA groups • DUAO groups vs BAL neutrophils, BAL eosinophils, BAL mast cells, THS, endoscopic scores, CK values, ESGD grade • MEA groups vs THS, endoscopic scores, CK values, ESGD grade
One-way ANOVA and Tukey's multiple comparisons test	• DUAO groups vs BAL macrophages, BAL lymphocytes
Unpaired *t* test	• Sex vs BAL macrophages, BAL lymphocytes • Rhabdomyolysis vs BAL macrophages • Cardiac arrhythmias vs BAL macrophages, BAL lymphocytes • EGGD vs BAL macrophages, BAL lymphocytes
Mann-Whitney test	• Age vs cardiac arrhythmias, rhabdomyolysis, EGGD • Sex vs fitness variables, BAL neutrophils, BAL eosinophils, BAL mast cells, THS, endoscopic scores, CK values, ESGD grade • Rhabdomyolysis vs fitness variables, BAL lymphocytes, BAL neutrophils, BAL eosinophils, BAL mast cells, THS, endoscopic scores, CK values, ESGD grade • Cardiac arrhythmias vs fitness variables, BAL neutrophils, BAL eosinophils, BAL mast cells, THS, endoscopic scores, CK values, ESGD grade • EGGD vs fitness variables, BAL neutrophils, BAL eosinophils, BAL mast cells, THS, endoscopic scores, CK values, ESGD grade
Chi-square test	• DUAO groups vs sex, cardiac arrhythmias, rhabdomyolysis, EGGD, MEA groups • MEA groups vs sex, cardiac arrhythmias, rhabdomyolysis, EGGD
Fisher's exact test	• Sex vs cardiac arrhythmias, rhabdomyolysis, EGGD • Cardiac arrhythmias vs rhabdomyolysis, EGGD • EGGD vs rhabdomyolysis

Abbreviations: BAL, bronchoalveolar lavage; CK, creatine kinase; DUAO, dynamic upper airway obstruction; EGGD, equine glandular gastric disease; ESGD, equine squamous gastric disease; MEA, mild-moderate equine asthma; THS, total hemosiderin score.

TABLE 2 Associations between variables related to different medical disorders in the study population.

	Macrophages	Lymphocytes	Neutrophils	Eosinophils	Mast Cells	MEA group
Premature complexes	ns	ns	ns	ns	$P = .03$	$P = .02$
Serum CK	$P = .01$ $r = .17$	$P < .0001$ $r = -.31$	$P < .0001$ $r = .35$	ns	ns	$P = .0004$
Clinical myopathies	ns	ns	$P = .0001$	ns	ns	ns
DUAO group	ns	ns	ns	ns	ns	ns
EIPH grade	$P = .03$ $r = -.15$	ns	ns	ns	$P = .04$ $r = .14$	ns
THS	ns	$P = .0004$ $r = -.20$	$P = .03$ $r = .15$	$P = .001$ $r = .18$	$P < .0001$ $r = .24$	ns
PLH	ns	ns	ns	ns	ns	ns
TM	ns	ns	ns	ns	ns	ns
TB	ns	ns	ns	ns	ns	ns
Macrophages	–	$P < .0001$ $r = -.65$	$P = .01$ $r = .17$	$P < .0001$ $r = -.26$	$P = .01$ $r = -.18$	$P = .03$
Lymphocytes	$P < .0001$ $r = -.65$	–	$P < .0001$ $r = -.72$	ns	ns	$P < .0001$
Neutrophils	$P = .01$ $r = .17$	$P < .0001$ $r = -.72$	–	ns	$P = .001$ $r = -.22$	$P < .0001$
Eosinophils	$P < .0001$ $r = -.26$	ns	ns	–	ns	$P = .01$
Mast cells	$P = .01$ $r = -.18$	ns	$P = .001$ $r = -.22$	ns	–	$P < .0001$
MEA group	$P = .03$	$P < .0001$	$P < .0001$	$P = .01$	$P < .0001$	–
ESGD grade	ns	ns	ns	ns	ns	ns
EGGD	ns	ns	ns	ns	ns	ns

	THS	EIPH grade	PLH	TM	TB	DUAO group
Premature complexes	ns	ns	ns	ns	ns	ns
Serum CK	ns	ns	ns	$P = .03$ $r = .14$	ns	$P = .02$
Clinical myopathies	ns	ns	ns	ns	ns	ns
DUAO group	$P = .02$	ns	Ns	ns	ns	–
EIPH grade	$P < .0001$ $r = .32$	–	$P = .04$ $r = -.13$	ns	ns	ns
THS	–	$P < .0001$ $r = .32$	ns	ns	ns	$P = .02$
PLH	ns	$P = .04$ $r = -.13$	–	ns	$P = .04$ $r = .13$	ns
TM	ns	ns	ns	–	$P < .0001$ $r = .22$	ns
TB	ns	ns	$P = .04$ $r = .13$	$P < .0001$ $r = .22$	–	ns
Macrophages	ns	$P = .03$ $r = -.15$	ns	ns	ns	ns
Lymphocytes	$P = .0004$ $r = -.20$	ns	ns	ns	ns	ns
Neutrophils	$P = .03$ $r = .15$	ns	ns	ns	ns	ns
Eosinophils	$P = .001$ $r = .18$	ns	ns	ns	ns	ns
Mast cells	$P < .0001$ $r = .24$	$P = .04$ $r = .14$	ns	ns	ns	ns

(Continues)

TABLE 2 (Continued)

	THS	EIPH grade	PLH	TM	TB	DUAO group
MEA group	ns	ns	ns	ns	ns	ns
ESGD grade	ns	ns	ns	$P = .01$ $r = .19$	$P = .02$ $r = .18$	ns
EGGD	ns	ns	ns	ns	ns	ns

	Premature complexes	Serum CK	Clinical myopathies	ESGD grade	EGGD
Premature complexes	–	$P = .01$	ns	ns	ns
Serum CK	$P = .01$	–	$P < .0001$	$P = .001$ $r = .20$	ns
Clinical myopathies	ns	$P < .0001$	–	ns	ns
DUAO group	ns	$P = .02$	ns	ns	ns
EIPH grade	ns	ns	ns	ns	ns
THS	ns	ns	ns	ns	ns
PLH	ns	ns	ns	ns	ns
TM	ns	$P = .03$ $r = .14$	ns	$P = .01$ $r = .19$	ns
TB	ns	ns	ns	$P = .02$ $r = .18$	ns
Macrophages	ns	$P = .01$ $r = .17$	ns	ns	ns
Lymphocytes	ns	$P < .0001$ $r = -.31$	ns	ns	ns
Neutrophils	ns	$P < .0001$ $r = .35$	$P = .0001$	ns	ns
Eosinophils	ns	ns	ns	ns	ns
Mast cells	$P = .03$	ns	ns	ns	ns
MEA group	$P = .02$	$P = .0004$	ns	ns	ns
ESGD grade	ns	$P = .001$ $r = .20$	ns	–	$P = .01$
EGGD	ns	ns	ns	$P = .01$	–

Abbreviations: CK, creatine kinase; DUAO, dynamic upper airway obstruction; EGGD, equine glandular gastric disease; EIPH, exercise-induced pulmonary hemorrhage; ESGD, equine squamous gastric disease; MEA, mild-moderate equine asthma; ns, nonsignificant; PLH, pharyngeal lymphoid hyperplasia; TB, tracheal blunting; THS, total hemosiderin score; TM, tracheal mucus.

3 | RESULTS

3.1 | Diagnosis

In the study population, a single disorder was diagnosed in 30 horses (11.6%), 2 concomitant disorders in 67 horses (25.9%), 3 disorders in 97 horses (37.4%), 4 disorders in 48 horses (18.5%), 5 disorders in 15 horses (5.8%), and 6 disorders in 1 horse (0.4%); in 1 horse (0.4%), no definitive diagnosis was obtained. The associations among all disorder variables are presented in Table 2.

3.2 | Arrhythmias

Among the horses for which continuous EGC was obtained during and after incremental treadmill exercise, 194 showed no or nonclinically relevant arrhythmias (81.5%), whereas 44 experienced clinically relevant PCs (18.5%). No significant difference between groups was detected for age and sex distribution.

3.3 | Exertional myopathies

In the study population, median CK activity was 204 (104-407) UI/L; it fell within normal limits in 227 horses (89%), and was >735 UI/L in 28 horses (11%). Neither positive nor negative association was detected between CK and age. Females showed significantly higher postexercise CK activity (median, 307.5 IU/L; IQR, 172.3-572 IU/L) than males (median, 184 IU/L; IQR, 88-332 IU/L; $P < .0001$; Figure 1).

Clinical signs of exertional myopathies were inferred by CK activity or observed in 42 horses (16.5%), 22 females (52.4%) and 20 males (47.6%), with a median age of 3.5 (IQR, 3-5) years. Conversely, 213 horses showed no clinical signs, and included 143 males (67.1%) and 70 females (32.9%), with a median age of 3 (IQR, 3-4) years.

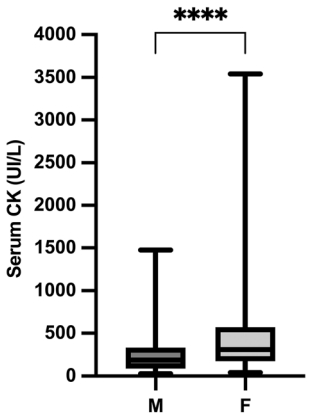

FIGURE 1 Box and whiskers graph representing postexercise serum creatine kinase (CK) activity in males (M) and females (F). The statistical significance is shown as ****$P < .0001$.

TABLE 3 Fitness variables, expressed as median (IQR), in the study population.

Fitness variable	Value
V200	7.5 (6.5-8.5) m/s
VLa4	8 (6.4-9.1) m/s
HRLa4	203.6 (193.1-213.9) bpm
Lac_{max}	22.31 (16.76-27.20) mmol/L
HR_{max}	231 (226-236) bpm
V_{max}	11 (11-11) m/s
HR_{30}	75 (64-88) bpm
Lac_{30}	11.88 (6.97-16.69) mmol/L
pH_{min}	7.14 (7.08-7.21)
Hct_{max}	66 (63-69)%

Abbreviations: Hct_{max}, maximum hematocrit; HR_{30}, heart rate at 30 minutes postexercise; HRLa4, heart rate at a plasma lactate concentration of 4 mmol/L; HR_{max}, maximum heart rate; Lac_{30}, plasma lactate concentration at 30 minutes postexercise; Lac_{max}, peak of plasma lactate concentration; pH_{min}, minimum pH; V200, velocity at a heart rate of 200 bpm; VLa4, velocity at a plasma lactate concentration of 4 mmol/L; V_{max}, maximum velocity.

Age did not differ between groups, whereas females were significantly more affected by clinical myopathies compared to males ($P = .02$).

3.4 | Dynamic upper airway obstructions

At HSTE, no DUAO was observed in 140 horses (56.5%), mild DUAOs in 11 horses (4.4%), severe DUAOs in 64 horses (25.8%), and multiple concomitant DUAOs in 33 horses (13.3%). No significant difference in age and sex distribution was observed between groups.

3.5 | Exercise-induced pulmonary hemorrhage

At postexercise tracheobronchoscopy, median EIPH score was 1 (IQR, 0-2). Specifically, no blood was detected in 98 horses (39.8%), and EIPH score was grade 1 in 57 horses (23.2%), grade 2 in 56 horses (22.8%), grade 3 in 26 horses (10.6%), and grade 4 in 9 horses (3.7%). The median value of THS was 28 (IQR, 8-61), with 37 horses (17.4%) having a THS score >75 and being considered positive for EIPH.

Age was positively correlated with EIPH grade ($P = .002$, $r = .20$) and THS ($P = .002$, $r = .22$). Although median EIPH grade was 1 (IQR, 0-2) in both males and females, higher scores were detected in males ($P = .04$); no differences for THS were observed between males and females.

TABLE 4 Results of Spearman's correlations between different disorder-related variables and fitness variables in the study population.

Fitness variable	Associated disorder-related variables
V200	• CK ($P < .0001$, $r = -.46$) • TM score ($P = .03$, $r = -.14$) • ESGD grade ($P = .03$, $r = -.16$)
VLa4	• CK ($P < .0001$, $r = -.55$) • TM score ($P = .02$, $r = -.15$) • BAL macrophages ($P = .03$, $r = -.15$) • BAL lymphocytes ($P = .001$, $r = .22$) • BAL neutrophils ($P = .001$, $r = -.22$) • ESGD grade ($P = .04$, $r = -.16$)
HRLa4	• CK ($P < .0001$, $r = -.29$) • BAL macrophages ($P = .02$, $r = -.17$) • BAL lymphocytes ($P = .004$, $r = .20$) • BAL neutrophils ($P = .03$, $r = -.15$)
Lac_{max}	• CK ($P < .0001$, $r = .42$) • BAL lymphocytes ($P = .03$, $r = -.15$) • BAL neutrophils ($P = .004$, $r = .20$)
V_{max}	• CK ($P < .0001$, $r = -.25$) • THS ($P = .03$, $r = .15$) • PLH score ($P = .01$, $r = -.15$) • BAL mast cells ($P = .002$, $r = .21$)
HR_{30}	• PLH score ($P = .02$, $r = -.15$)
Lac_{30}	• CK ($P < .0001$, $r = .42$) • BAL macrophages ($P = .02$, $r = .16$) • BAL lymphocytes ($P < .0001$, $r = -.26$) • BAL neutrophils ($P = .001$, $r = .24$)
pH_{min}	• CK ($P < .0001$, $r = -.30$)
Hct_{max}	• EIPH grade ($P = .01$, $r = .24$) • PLH score ($P < .0001$, $r = -.39$) • TM score ($P = .004$, $r = -.26$) • TB score ($P = .02$, $r = -.21$)

3.6 | Asthma

At airway endoscopy, median PLH score was 2 (IQR, 1-2), whereas median TM and TB scores were 1 (IQR, 1-2). Age was inversely correlated with PLH ($P < .0001$, $r = -.52$) and with TM ($P = .01$, $r = -.16$), although it was not associated with TB. Females showed higher PLH scores (median, 2; IQR, 2-2.5) compared to males (median, 2; IQR, 1-2; $P = .01$), whereas no associations were observed between sex and TM or TB scores.

The BAL leukocyte differential cell count consisted of means of 44.9 ± 8.6% macrophages, 36.7 ± 11.6% lymphocytes, and medians of

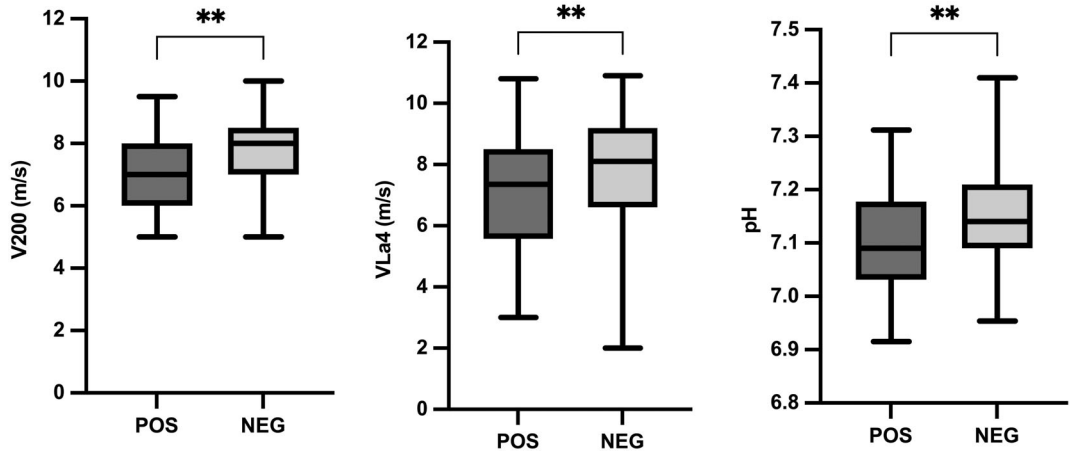

FIGURE 2 Box and whiskers graph representing V200, VLa4 and pHmin in horses with (pos) or without (neg) clinical signs of exertional myopathies. The statistical significance is shown as $^{**}P < .01$.

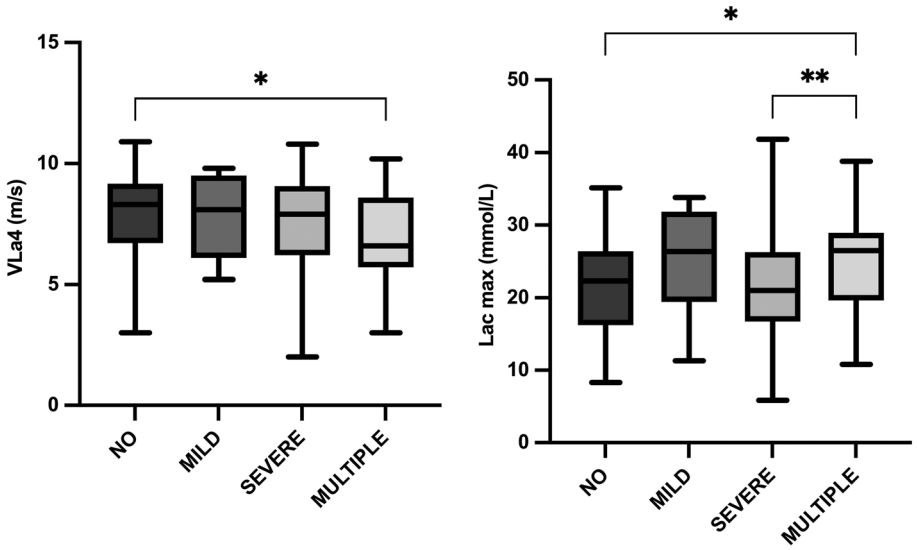

FIGURE 3 Box and whiskers graph representing VLa4 and Lacmax in horses with no DUAO, mild DUAO, severe DUAO, and multiple DUAOs. The statistical significance is shown as $^{*}P < .05$ and $^{**}P < .01$.

8 (IQR, 4.2-17)% neutrophils, 1 (IQR, 0-3)% eosinophils, and 4 (IQR, 3-6)% mast cells. In the population, 6 horses were healthy (2.8%), 23 had neutrophilic MEA (10.7%), 66 had eosinophilic-mastocytic MEA (30.7%), and 120 had mixed MEA (55.8%).

Age was inversely correlated with macrophages ($P = .02$, $r = -.16$) and eosinophils ($P = .02$, $r = -.16$) and positively correlated with mast cells ($P = .01$, $r = .19$). Conversely, age did not differ between healthy and different MEA subtypes. Females had slightly higher macrophages percentages (46.5 ± 8.5%) than males (44.1 ± 8.6%; $P = .05$), whereas no other differences for sex were observed for lymphocytes, neutrophils, eosinophils, and mast cells. No difference in sex distribution was detected between groups.

3.7 | Equine gastric ulcer syndrome

At gastroscopy, the squamous mucosa was healthy in 2 horses (1.2%), grade 1 ESGD was observed in 2 horses (1.2%), grade 2 in 17 horses (9.8%), grade 3 in 32 horses (18.5%), and grade 4 in 120 horses (69.4%). The glandular mucosa was affected by EGGD in 100 horses (57.8%) and healthy in 73 horses (42.2%). Age was not associated with ESGD grade and did not differ between horses with and without EGGD. Median grade of ESGD was equal in females and males and sex distribution did not differ between horses affected and not affected by EGGD.

3.8 | Fitness variables

Medians and IQRs of the fitness variables in the study population are shown in Table 3. Age was inversely correlated with HR_{max} ($P = .02$, $r = -.14$), and positively correlated with V_{max} ($P < .0001$, $r = .35$) and Hct_{max} ($P < .0001$, $r = .48$). The values of V200 and VLa4 were higher in males (V200 median, 8 m/s; IQR, 7-8.5 m/s; VLa4 median, 8.35 m/s; IQR, 6.68-9.3) than in females (V200 median, 7 m/s; IQR, 6-8 m/s; VLa4 median, 7.6 m/s; IQR, 6.2-8.6; V200 $P = .02$;

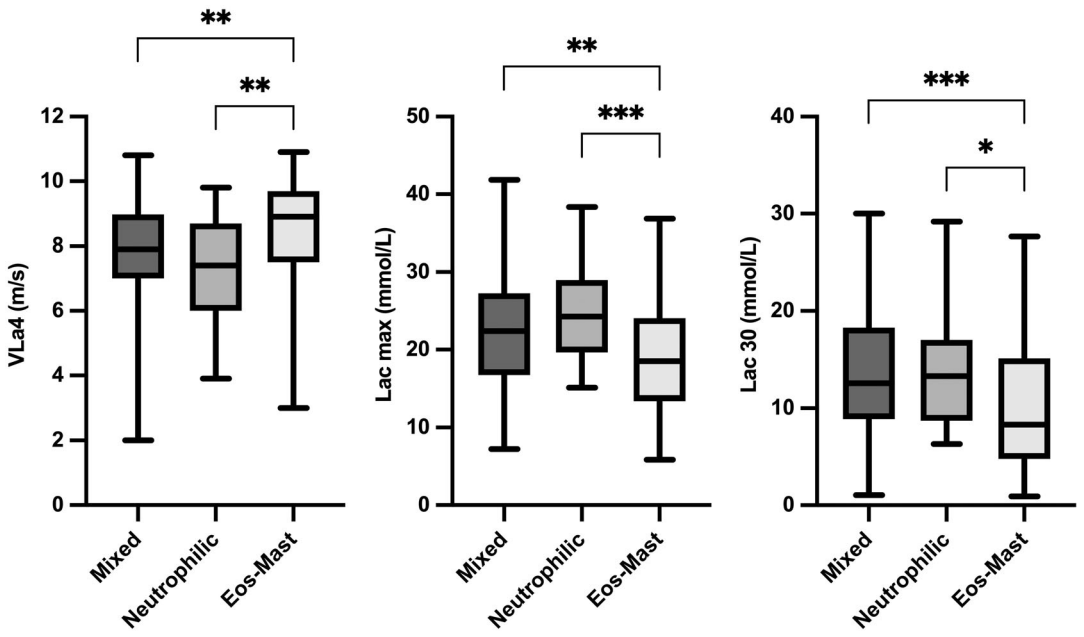

FIGURE 4 Box and whiskers graph representing VLa4, Lacmax and Lac30 in horses with mixed MEA, neutrophilic MEA, and eosinophilic-mastocytic (Eos-Mast) MEA. The statistical significance is shown as $^{*}P < .05$, $^{**}P < .01$, and $^{***}P < .001$.

TABLE 5 Multiple linear regression model for V200, including only variables which showed a significant association.

Variable	Estimate	95% confidence interval	*P* value
Sex (male)	.15	−0.21 to 0.50	.42
TM score	−.14	−0.32 to 0.05	.14
ESGD grade	−.12	−0.33 to 0.09	.25
Serum CK	−.0008	−0.001 to 0.0005	<.0001
Model			<.0001

Abbreviations: CK, creatine kinase; ESGD, equine squamous gastric disease; TM, tracheal mucus.

TABLE 6 Multiple linear regression model for VLa4, including only variables which showed a significant association.

Variable	Estimate	95% confidence interval	*P* value
Sex (male)	0.04	−0.61 to 0.54	0.90
BAL neutrophils	−0.01	−0.04 to 0.02	0.40
TM score	−0.22	−0.52 to 0.08	0.15
Mild DUAO	0.42	−0.85 to 1.68	0.52
Severe DUAO	−0.52	−1.16 to 0.11	0.10
Multiple DUAOs	−0.87	−1.73 to −0.02	0.05
ESGD grade	−0.13	−0.45 to 0.20	0.44
Serum CK	−0.002	−0.002 to 0.001	<0.0001
Model			0.0001

Abbreviations: BAL, bronchoalveolar lavage; CK, creatine kinase; DUAO, dynamic upper airway obstruction; ESGD, equine squamous gastric disease; TM, tracheal mucus.

VLa4 $P = .01$). Moreover, males reached a higher Hct_{max} (66.5 ± 3.8%) than females (64.3 ± 4.6%; $P = .004$). No other associations among age, sex and fitness variables were detected.

3.9 | Associations between disorders and fitness variables

The results of Spearman's correlations between different disorder-related parameters and fitness variables are shown in Table 4. Horses showing clinical signs of myopathies had lower values of V200 ($P = .001$), VLa4 ($P = .004$), and pH_{min} ($P = .004$; Figure 2). Fitness variables that differed among DUAO groups included VLa4, which was lower in horses with multiple DUAOs compared to horses with no DUAO ($P = .02$), and Lac_{max}, which was higher in horses with multiple DUAOs compared to horses with no DUAO ($P = .05$) and severe DUAO ($P = .01$; Figure 3). When dividing horses based on MEA subtype, VLa4 was higher in the eosinophilic-mastocytic MEA group compared to the neutrophilic MEA group ($P = .02$), whereas Lac_{max} and Lac_{30} were lower in the eosinophilic-mastocytic MEA group compared to the neutrophilic (Lac_{max} $P = .003$; Lac_{30} $P = .02$) and mixed (Lac_{max} $P = .01$; Lac_{30} $P = .001$) MEA groups (Figure 4). Horses with EGGD reached a higher Hct_{max} (66.8 ± 4.3%) than nonaffected horses (64.6 ± 3.5%; $P = .01$). The percentage of eosinophils in the BAL and the presence of clinically relevant PCs were not associated with any fitness variable.

Models of multiple linear regression designed for prediction of V200 ($r^2 = .17$) and VLa4 ($r^2 = .21$) are presented, respectively, in Tables 5 and 6. Models of multivariable logistic regression for the prediction of V200 and VLa4 values are shown, respectively, in Tables 7 and 8.

TABLE 7 Multivariate logistic regression model for V200 (equal or higher than 8 m/s vs lower than 8 m/s).

Variable	Odds ratio	95% confidence interval	Likelihood ratio *P* value
Sex (female)	0.70	0.36-1.34	0.07
MEA group (mixed)	0.84	0.33-2.12	0.07
MEA group (neutrophilic)	0.80	0.38-1.68	0.07
EIPH score	0.79	0.62-1.03	0.07
TM score	0.77	0.55-1.08	0.03
Severity of DUAO	0.72	0.55-0.94	0.005
Serum CK	0.998	0.997-0.999	<0.0001

	Likelihood ratio *P* value	AUC (95% confidence interval)	ROC curve *p* value	PPV (%)	NPV (%)
Model	.0001	0.73 (0.66-0.80)	<.0001	69.35	68.75

Abbreviations: AUC, area under the curve; CK, creatine kinase; DUAO, dynamic upper airway obstruction; EIPH, exercise-induced pulmonary hemorrhage; MEA, mild-moderate equine asthma; NPV, negative predictive value; PPV, positive predictive value; ROC, receiver operating characteristic; TM, tracheal mucus.

TABLE 8 Multivariate logistic regression model for VLa4 (equal or higher than 8 m/s vs lower than 8 m/s).

Variable	Odds ratio	95% confidence interval	Likelihood ratio *P* value
Sex (female)	0.77	0.34-1.74	.02
BAL neutrophils	0.99	0.95-1.04	.09
EIPH grade	0.74	0.52-1.02	.10
Severity of DUAO	0.80	0.57-1.12	.03
ESGD grade	0.68	0.39-1.11	.03
Serum CK	0.996	0.994-0.998	<.0001

	Likelihood ratio *P* value	AUC (95% confidence interval)	ROC curve *P* value	PPV (%)	NPV (%)
Model	<.0001	0.78 (0.70-0.86)	<.0001	68.75	75.41

Abbreviations: AUC, area under the curve; BAL, bronchoalveolar lavage; CK, creatine kinase; DUAO, dynamic upper airway obstruction; EIPH, exercise-induced pulmonary hemorrhage; ESGD, equine squamous gastric disease; NPV, negative predictive value; PPV, positive predictive value; ROC, receiver operating characteristic.

4 | DISCUSSION

Our study confirms the multifactorial medical nature of decreased athletic capacity, with the majority of poorly-performing Standardbred trotters being concomitantly affected by multiple disorders. The most commonly diagnosed disorders were ESGD and MEA, followed by EIPH, EGGD and DUAOs. All disorders, except for PCs, were associated with ≥1 fitness variables. In addition to evaluating the effects of each disorder on the athletic capacity of the horses, we investigated the contribution of different disorders to impaired performance in a multifactorial model. In particular, the greatest impairment of fitness, represented by lower values of V200 and VLa4, was related to the diagnosis of exertional myopathies, severe and multiple DUAOs, neutrophilic inflammation of the lower airway, and severe grades of ESGD.

A definitive diagnosis was reached for 254 of 255 horses, which suggests that the diagnostic protocol performed in our study was comprehensive enough to evaluate the most common subclinical non-orthopedic causes of racing poor performance. For the remaining horse, the cause of decreased performance presumably could be inadequate conditioning, training, or management, or nonspecific musculoskeletal pain. The main limitation of our study is the fact that some horses did not undergo the complete diagnostic protocol, and therefore some disorders may have been underdiagnosed. However, our study was retrospective and included horses evaluated over a period of 20 years, during which time the clinical approach and equipment availability varied slightly. Nevertheless, we believe the study's population size may overcome this limitation.

The highest prevalence was observed for EGUS, which was diagnosed in almost all horses. In particular, severe ESGD (grade 3 or 4) was detected in 87% of the horses, whereas the prevalence of EGGD was approximately 58%, similar to that previously reported.[49-51] In fact, intensity and duration of exercise are predisposing factors to the development of gastric ulcers in either the squamous and glandular mucosa, and racehorses are most commonly affected.[49,52,53]

Respiratory diseases represented an equally common cause of poor performance in our population. A diagnosis of MEA was made in 97% of the horses, with the majority of them affected by a mixed inflammatory form. Similarly, previously reported MEA prevalence in

Standardbred racehorses in active training ranged from 58% to 97%,[1,15,54] which may be higher in a selected poorly-performing population. In fact, MEA is universally recognized as 1 of the most common causes of impaired athletic capacity in horses.[1,26,27] Another common disorder of the lower airway is EIPH, which was assessed in our study by postexercise tracheobronchoscopy and calculation of THS in the BAL fluid. Based on endoscopy, approximately 60% of the horses had blood in the trachea, but only 17% were EIPH-positive based on THS and could therefore be considered as bleeders. In the literature, the prevalence of EIPH varies based on the diagnostic method used. Hemosiderophages in the BAL fluid are found in up to 100% of racehorses,[55] and tracheal blood is reported in 87% of Standardbreds when examined postrace on at least 3 occasions[56]; when diagnosed by a single endoscopic examination, the prevalence is 43%-75%.[57] Severity of EIPH seemed to increase with age. Several previous studies have reported such findings,[58] probably because EIPH is a cumulative condition associated with the number of racing starts.[59,60] The presence of DUAOs was observed at HSTE in 44% of the horses, which agrees with previous studies.[3,61,62] In 30% of affected horses, multiple concomitant DUAOs were detected, confirming the findings of others.[63,64]

In our study, clinically relevant PCs were detected in 18% of the horses during treadmill exercise. The prevalence of PCs in Standardbred racehorses ranges from 54% to 78%[39,65] but, similar to our results, it decreases to 16%-19% in studies including only clinically relevant arrhythmias.[3]

Finally, postexercise CK activity was higher than normal in 11% of the horses, whereas clinical myopathies (observed during hospitalization or inferred by history) affected 17% of the horses. Exertional rhabdomyolysis is reported to have a prevalence of 6% among Standardbred racehorses,[66] which may be higher in our study because of the inclusion of only poorly-performing horses. In fact, similar studies reported prevalences of increased CK activity of 15%-18%,[1,3] smilar to our findings. Females were more prone to develop ER in our population, and such predisposition has been widely recognized.[3,66-68]

Two or more disorders were simultaneously observed in almost 90% of the horses, similar to what has been reported previously.[1,3] Among observed associations, those between upper and lower airway disorders are of particular interest. It has been long debated, without consensus, about the possible predisposition of DUAO-affected horses to EIPH or simultaneously to MEA. Some authors, reporting an association between EIPH and DUAO,[23,69] hypothesized that DUAO may cause an increase in transmural pulmonary pressure gradient, leading to pulmonary capillaries rupture.[70,71] Other studies, however, found no association between DUAO and EIPH.[24,72] Similarly, many studies reported associations between DUAO and MEA, hypothesizing that DUAO may predispose to lower airway inflammation or vice versa.[20,23,69,73-75] Conversely, other authors did not find any relationship between MEA and DUAO.[7,72,76] Surprisingly, in our study, the severity of DUAO was associated with lower values of THS, whereas no association with MEA subtype or BAL cell populations was observed. Another widely discussed topic concerns a possible cause and effect relationship between MEA and EIPH.[16,60,77,78] Our results support this hypothesis, because the THS was positively correlated with the BAL percentages of neutrophils, eosinophils, and mast cells, suggesting that horses with EIPH were more likely to suffer from lower airway inflammation. Moreover, tracheal EIPH score was positively correlated with BAL mastocytosis, which has been reported previously in other studies,[32,79] although the underlying mechanisms are still unknown. In our study, high postexercise CK activity was positively correlated with detection of clinically relevant PCs, DUAO severity, BAL neutrophilia, TM accumulation, and ESGD severity. The cause and effect relationships between increased CK activity and these disorders are unclear, but some hypotheses may be proposed. First, high CK activity suggests generalized muscular damage and fatigue, which also could involve the myocardium and muscles responsible of the stability of upper airway structures, with the consequent onset of arrhythmias and DUAOs. However, severe DUAOs and lower airway inflammation may result in decreased oxygenation of the muscles, an early transition to anaerobic metabolism, and the onset of muscular fatigue. Therefore, it is not clear whether muscular damage represents the cause or consequence of other disorders. The association observed between ER and severity of ESGD may be explained by the fact that both conditions seem to be associated with a nervous temperament of horses,[34,36,66,80] although the underlying pathogenetic mechanisms have not yet been determined.

In our study, age was positively correlated with V_{max} and Hct_{max} and inversely correlated with HR_{max}. All of these associations are recognized consequences of conditioning and increased training.[32,81,82] Moreover, similar to previous reports,[32,83] males had higher values of V200, VLa4, and Hct_{max}. Among the diseases affecting fitness, ER was strongly associated with lower values of V200, VLa4, HRLa4, and V_{max}, suggesting a substantial athletic impairment. Moreover, affected horses reached higher peak lactate concentrations and higher lactate concentrations 30 minutes postexercise, and consequently lower values of pH_{min}. Also, neutrophilic inflammation of the lower airways was correlated with lower values of VLa4 and HRLa4, and higher peak lactate concentration and lactate concentration 30 minutes postexercise. Similarly, TM accumulation was associated with lower V200 and VLa4. Another respiratory condition affecting fitness was the presence of multiple DUAOs, which were associated with lower VLa4 and higher peak lactate concentration. Finally, severe forms of ESGD were correlated with lower V200 and VLa4. These results suggest that, as previously reported,[1,7,25,28,37,63,84] these conditions may affect performance by impairing the aerobic capacity of Standardbred trotters, with an early transition to anerobic metabolism and prolonged lactate accumulation. In the case of ER and DUAOs, it is possible that their occurrence may be a consequence, rather than the cause, of the early switch to anaerobic metabolism and onset of fatigue. Interestingly, cardiac arrhythmias were not associated with any fitness variables, suggesting that they do not play an important role in performance impairment. Similarly, previous studies also found a high prevalence of arrhythmias detected during exercise in clinically healthy well-performing horses.[39,65] Finally, in our study, higher grades of EIPH were associated with higher V_{max} and Hct_{max}. These findings may be related to the pathogenetic mechanisms of this condition, rather than fitness status.[32]

The multivariable models highlighted that ER was the condition most likely associated with unsatisfactory values of V200 and VLa4,

followed by severe and multiple DUAOs, BAL neutrophilia, ESGD, and TM accumulation. Moreover, horses with high grades of EIPH were less likely to have satisfactory VLa4. However, the coefficient of determination of the multivariable models was relatively low, suggesting that the values of V200 and VLa4, and therefore the level of fitness, only partially depended on the presence of subclinical diseases. In fact, these values can be influenced by conditioning, trainer, preferred ground, stress, and other factors. Horses included in our study came from different training centers over a period of 20 years. Therefore, different training techniques may have influenced our results. Consequently, the proposed statistical models are not necessarily predictive of V200 and VLa4, but rather may indicate which subclinical disorders play a major role in fitness impairment in a complex and multifactorial condition such as poor performance. Finally, because our study was performed retrospectively and only included poorly-performing Standardbred racehorses, future studies should verify whether or not the identified associations are found in a mixed population, including a control group of well-performing horses.

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CONFLICT OF INTEREST DECLARATION

Authors declare no conflict of interest.

OFF-LABEL ANTIMICROBIAL DECLARATION

Authors declare no off-label use of antimicrobials.

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) OR OTHER APPROVAL DECLARATION

Because the present study was performed retrospectively on clinical records, and the procedures were performed on clinical horses for diagnostic purposes, ethical review and approval were waived for this study. All of the procedures were performed according to relevant guidelines and informed owner consent for the use of clinical data was obtained from the owners or holders of the included horses.

HUMAN ETHICS APPROVAL DECLARATION

Authors declare human ethics approval was not needed for this study.

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