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Analytical Clinical Studies

# A systematic review and meta-analysis of the efficacy of furosemide for exercise-induced pulmonary haemorrhage in Thoroughbred and Standardbred racehorses

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## Summary

**Reasons for performing study:** Furosemide is the most commonly used medication for exercise-induced pulmonary haemorrhage (EIPH); however, critical evaluation of the strength of evidence for efficacy of furosemide is lacking and is warranted so that evidence-based treatment decisions can be made.
**Objectives:** To evaluate the efficacy of furosemide to reduce the severity or frequency of detection of EIPH in Thoroughbred and Standardbred racehorses.
**Study design:** Systematic review with meta-analyses.
**Methods:** Primary studies were identified via searches of electronic databases, relevant texts and reference lists of published articles. Studies were not restricted by date or publication status. Only studies published in English were eligible for inclusion. Searches were performed using a predetermined search string. Randomised controlled trials, nonrandomised trials and observational studies were included. Three authors independently assessed each study using the Cochrane collaboration guidelines and Grading of Recommendations Assessment, Development and Evaluation recommendations for rating quality of evidence. Meta-analysis of studies was performed with pooled data to determine whether furosemide reduced the frequency of detection of EIPH (yes or no) as evaluated by tracheobronchoscopy or bronchoalveolar lavage fluid red blood cell number, or if furosemide reduced the severity of EIPH by at least one tracheobronchoscopic grade.
**Results:** Seventeen studies fulfilled the inclusion criteria. The relative risk of detecting any EIPH by tracheobronchoscopy after administration of furosemide was 0.88 (pooled data from 11 studies, n = 5780; 95% confidence interval 0.79–0.97, P = 0.01). When data from only high-quality randomised controlled trials (2 studies, n = 405) were used, the relative risk of detecting endoscopically evident EIPH was 0.68 (95% confidence interval 0.58–0.79, P<0.001). The proportion of horses previously diagnosed with EIPH having a reduction of at least one EIPH grade after furosemide was 68% (2 studies, n = 405; 95% confidence interval 61–78%).
**Conclusions:** There is high-quality evidence, albeit limited, that administration of furosemide reduces the incidence and severity of EIPH in Thoroughbred or Standardbred racehorses.

**Keywords:** horse; frusemide; treatment; racing; Lasix

## Introduction

Exercise-induced pulmonary haemorrhage (EIPH) is an important condition of Thoroughbred and Standardbred racehorses. Approximately 50% of Thoroughbreds develop EIPH during their competitive racing career, and the condition has implications for performance, horse health and welfare [1]. In the short term, horses with EIPH *grades ≥2* are less likely to win or finish in the first 3 race positions and are less likely to be in the 90th percentile or higher for earnings [2,3]. Over the course of their racing career, horses with epistaxis, the most severe form of EIPH, have fewer race starts and a shorter career duration than horses without EIPH [4,5]. Therefore, treatment and management of EIPH could not only be important to continued race performance of horses with the condition, but could also contribute to the overall health, well-being and welfare of equine athletes.

Furosemide, a loop diuretic, has been used for over 30 years to prevent EIPH or reduce the severity of EIPH in racehorses. Studies evaluating the clinical efficacy of furosemide in both laboratory and field conditions have demonstrated conflicting results. Critical evaluation of the evidence for efficacy of furosemide is lacking and is warranted to allow owners, trainers, veterinarians and regulatory authorities to make evidence-based treatment and policy decisions about the administration of furosemide to horses on race day.

The purpose of this systematic review was to critically evaluate the evidence of efficacy of furosemide to prevent EIPH and reduce the severity of EIPH in Thoroughbred and Standardbred racehorses. The objective of the review was to evaluate randomised controlled trials (RCTs), nonrandomised intervention trials and observational studies that assessed efficacy of furosemide administered before strenuous exercise to reduce the frequency of detection or severity of EIPH in racehorses.

## Materials and methods

### Protocol

A systematic review study protocol was developed before beginning the review process, using guidelines provided by the Cochrane collaboration [6] and the evidence grading scheme recommended by the Grading of Recommendations Assessment, Development and Evaluation (GRADE) working group [7]. The protocol detailed the research question, specified important outcomes, outlined a search strategy and the process of data extraction and provided explicit criteria for rating the quality of evidence and performing the meta-analysis (copies of the review protocol are available from the authors on request). The protocol was created using Review Manager 5.2 software[a].

### Criteria for selecting studies for this review

*Types of studies:* Studies included in the systematic review were not restricted by date or publication status in that peer-reviewed articles and articles in conference proceedings were considered for inclusion. Only studies published in English were included. Studies included in the review were RCTs, nonrandomised intervention trials and observational studies. Studies were included in the review only if they included a control or comparator group (either placebo-treated or untreated controls).

*Types of participants:* Eligible participants were Thoroughbred or Standardbred horses aged 2–20 years of either sex, in which EIPH was diagnosed prior to or during the study period.

*Types of interventions:* Studies were included if they investigated use of furosemide at doses of 0.5–2 mg/kg bwt administered by i.v. or i.m.

injection 4 h before exercise. Studies were also included if the dose of furosemide was not specified but was administered according to racing regulatory guidelines of 150–500 mg 4 h before racing. Studies that evaluated multiple treatments were included if furosemide was one arm of the trial and if the trial included an untreated or placebo-treated control group. Trials were not included if furosemide was compared with only another treatment (e.g. furosemide compared with a nasal dilator) or if furosemide was combined with another treatment (e.g. combination of furosemide and clenbuterol compared with untreated controls). Parallel-group trials and trials with a cross-over study design were included in the review. In studies that used a cross-over design, results of each arm of the study were treated as independent observations.

*Types of outcome measures:* The outcomes of interest to the review were as follows: 1) the proportion of horses with EIPH (yes or no), as detected by tracheobronchoscopy within 2 h of the end of exercise; 2) the proportion of horses that experienced a reduction in severity of EIPH of at least one tracheobronchoscopic grade after furosemide treatment; and 3) a reduction in EIPH severity, as determined by a decrease in bronchoalveolar lavage (BAL) fluid red blood cell (RBC) numbers collected after strenuous exercise. Any change in BAL fluid RBC number was considered indicative of a change in severity of EIPH (i.e. no attempt was made by the authors to determine what a clinically important change in BAL fluid RBC number could be).

## Search methods for identification of studies

Literature searches were performed over 3 months between February and April 2012 and then again in December 2013 by 2 investigators (S.L.S. and K.W.H.). Studies were identified from the following electronic search databases: PubMed[b], CAB abstracts[c], Discovery[d], Science Direct[e] and Wiley Interscience[f]. For example, studies were searched in Wiley Interscience using the search string: (horse or equine or thoroughbred or Standardbred) AND (exercise induced pulmonary haemorrhage or exercise induced pulmonary hemorrhage) AND (frusemide or furosemide) using all fields for each category and all date ranges. Bibliographies of referenced textbooks and reference lists of all retrieved studies were hand searched for additional studies. All retrieved bibliographic references were managed in Endnote X5 Reference Manager software[g].

## Selection of studies and data extraction

Two authors (S.L.S. and K.W.H.) selected studies based upon titles, keywords and abstracts. Where uncertainty existed regarding a study's inclusion, full-text manuscripts were retrieved for further evaluation. Differences in opinion were resolved by discussion. One author (S.L.S.) performed data extraction using a customised data-extraction form created during the protocol stage of the systematic review, and one other author (K.W.H.) verified the records for completeness. Disagreements were resolved by discussion. The data items extracted included study design, characteristics of trial participants (number and breed of horses examined), intervention characteristics (dose, route of administration and timing of administration before exercise), the type of control group used, method of exercise used to induce EIPH, outcomes measured (tracheobronchoscopic evidence of EIPH [yes or no], EIPH severity grade 0–4 or 5 or BAL fluid RBC number) and study results. A study was classified as a retrospective study design if data were accessed after the outcome occurred and if the study was designed after the events being studied had occurred.

## Methods of review

*Assessment of risk of bias:* Risk of bias in individual studies was assessed by 3 authors (S.L.S., K.W.H. and T.W.), working independently of each other, who evaluated each trial according to prespecified criteria specific to the types of studies included in the systematic review (RCT and nonrandomised trials that included nonrandomised intervention trials and observational studies; Tables 1 and 2). Risk-of-bias domains and overall risk of bias for each of the studies were primarily decided by one author (S.L.S.). Risk-of-bias decisions were discussed with and agreed upon in consultations with 2 other authors (K.W.H. and P.M.). The risk of bias for each study was summarised as low risk (low risk of bias in all key criteria), moderate risk (crucial limitation for one criterion or some limitations for ≥2 criteria sufficient to lower one's confidence in the estimate of effect) and high risk of bias (crucial limitation for one or more criteria sufficient to lower one's confidence in the estimate of the effect) [6,8].

**TABLE 1: Risk-of-bias criteria for judging individual randomised controlled trials**

| Key criteria for risk-of-bias judgement in included randomised controlled trials |
|---|
| 1) Use of a method of randomisation sufficient to achieve comparison groups that are similar in size and prognostic factors |
| 2) Concealment of the randomised sequence of treatment allocation to investigators |
| 3) Use of a placebo |
| 4) Blinding of investigators for assessment of measured outcomes |
| 5) Completeness of accounting for outcome events (incomplete outcome bias) |
| 6) Completeness of reporting of outcomes, regardless of results (selective outcome reporting bias) |
| 7) Use of a validated outcome measure, shown to be relevant to population of interest and to performance |
| 8) Adequate wash-out periods to prevent carry-over effects in cross-over trials |

*Assessment of publication bias:* Assessment of publication bias was performed when a comparison included more than 10 studies of varying sample sizes. Publication bias was assessed by visually examining funnel plots to determine the precision of the effect estimate.

*Missing data:* Trial authors were contacted by email to provide missing data and to clarify aspects of study methodology relevant to the bias domains (e.g. method of randomisation, use of blinding) not clearly stated in the published report.

*Method of analysis:* A narrative review was performed of all studies, and where sufficient data were available, meta-analyses were performed to pool the results for each outcome using Review Manager 5.2[a] or Medcalc 13.2.2[h]. For dichotomous outcomes, relative risks (RRs) were calculated and reported with the 95% confidence intervals (CIs). For continuous data, the standardised mean differences were calculated and reported with the 95% CIs. Pooled effects for RRs were calculated using the method of Mantel–Haenszel in a random-effects model, and pooled effects for continuous outcomes were calculated using the inverse variance method in a random-effects model. Outcomes that assessed single proportions were analysed in Medcalc 13.2.2[h] using a random-effects model. The random-effects model was chosen because it did not assume a common

**TABLE 2: Risk-of-bias criteria for judging individual nonrandomised intervention trials and observational studies**

| Key criteria for risk-of-bias judgement in included nonrandomised studies |
|---|
| 1) Prospective study design |
| 2) Use of a contemporaneous control group from the population of interest |
| 3) Standardisation of treatment administered and a complete description of the intervention |
| 4) Use of a validated outcome measure shown to be relevant to the population of interest |
| 5) Blinding for assessment of measured outcomes |
| 6) Control of confounding, including accurate measurement of all known prognostic factors and use of appropriate statistical analysis to account for observed differences |
| 7) Other limitations, including incomplete accounting of outcome events and selective outcome reporting |

**TABLE 3: Criteria used for rating of study design type and assessment of quality of each study**

| Study design type | Criteria |
|---|---|
| I | Randomised, placebo-controlled, blinded field or clinical trials (high-quality randomised controlled trials) |
| II | Randomised controlled intervention trials (low-quality randomised controlled trials) |
| III | Nonrandomised controlled trials and prospective observational studies |
| IV | Case series and retrospective observational studies |

treatment effect for all included studies and considered within- and between-study variability.

Studies were assessed for inconsistency using the following 4 main criteria: evaluation of point estimates; overlap of CIs; statistical test for heterogeneity; and the $I^2$ statistic. The $I^2$ statistic quantifies the amount of variation in results across studies beyond that expected by chance [9]; $I^2$ values of <40% were considered to represent low heterogeneity, 40–60% moderate and >60% substantial heterogeneity [10]. Where moderate to substantial heterogeneity was identified, subgroup analysis was planned to explore the impact of methodological quality and clinical diversity on the effect size of the primary outcome measures. Subgroup analysis was planned to evaluate study design and to explore the impact of studies that did or did not use randomisation of treatment sequence, allocation concealment, a placebo treatment or blinding for outcome measurement. Assessment of clinical sources of heterogeneity, in the form of evaluating differences in the intervention dosage, route and timing of administration, were planned, as well as subgroup analysis of studies that used a treadmill vs. racing or simulated racing to induce EIPH.

Sensitivity analysis was performed to determine whether decisions made during the review process influenced the conclusions of the meta-analysis. The main variable we planned to assess was the impact of studies published in peer-reviewed journals in comparison with those published in conference proceedings.

*Grading the evidence across studies:* Assessment of the quality, quantity and consistency of evidence across studies was performed using the GRADE recommendations [11]. Criteria for evaluating quality of evidence across studies for each outcome included study design, risk of bias, imprecision, inconsistency and indirectness (Tables 1–4). Consensus between authors for the overall grade of evidence was achieved by discussion. Randomised controlled trials were initially ascribed as high-quality evidence, and nonrandomised intervention trials and observational studies as low-quality evidence, with 5 possible reasons to rate down the quality of evidence and 3 reasons to rate up the quality [11] (Supplementary Item 1). The strength of evidence for each outcome was then classified into one of the following 4 categories: high (we are very confident that the true effect lies close to that of the estimate of effect); moderate (we are moderately confident in the effect estimate; the true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different); low (our confidence in the effect estimate is limited; the true effect might be substantially different from the estimate of the effect); and very low (we have little confidence in the effect estimate; the true effect of the intervention on the outcome is likely to be substantially different from the estimate of effect) [11]. Evidence profiles and summary-of-findings tables were created in Review Manager 5.2[a] and Grade Profiler 3.6 (GRADEpro Version 3.2 for Windows).

# Results

## Description of studies

*Results of the search:* The search strategy identified 1246 citations, of which 1210 were excluded based on title, abstract, keywords or duplication. Literature was almost entirely identified and retrieved from electronic bibliographic sources. Only one study was identified from hand searching reference lists. Seventeen studies met the inclusion criteria for this review. A flow diagram for inclusion of studies in the systematic review is provided in Figure 1. Excluded studies, with the reason for exclusion, are outlined in Supplementary Item 2. Studies were mostly excluded because they evaluated cardiovascular parameters after furosemide treatment, rather than any effect to prevent or reduce the severity of EIPH as detected by tracheobronchoscopy or BAL fluid RBC number. One study was excluded because 6 of 7 enrolled horses were Quarterhorses.

*Characteristics of included studies:* Summary details are provided in Supplementary Item 3 and are described in general terms below.

Participants: In total, 5653 horses were enrolled in the 17 studies included in this review [1,2,12–26]. The number of horses in each study ranged from 3 to 3539 horses. All studies included horses of both sexes, and horses were aged 2–20 years. Sixteen of 17 studies used Thoroughbreds. One study used only Standardbred horses [19], and one other used Thoroughbred and Standardbred horses [1].

Intervention: Eight studies used furosemide at a dosage of 0.5 mg/kg bwt, and 3 studies used 1 mg/kg bwt. Six studies detailed a range of doses, or did not detail a specific dose but stated that furosemide was administered as per racing regulatory guidelines [12,14,15,22,27]. Two studies [13,17] evaluated several doses of furosemide ranging from 0.5 to 2 mg/kg bwt. One study also examined the effect of furosemide given i.v. 30 and 240 min before exercise and by nebulisation at 30 and 240 min before exercise [16]. Only the arm of the study that used furosemide i.v. 240 min before exercise was included in the meta-analysis.

**TABLE 4: Criteria used for assessing indirectness, imprecision and inconsistency across included studies**

| | |
|---|---|
| Indirectness | |
| No indirectness | Studies that contribute the most to the effect estimate used an intervention that was applicable to the population of interest and used an outcome demonstrated to be relevant to performance |
| Serious indirectness | Some but not all studies that contribute the most to the effect estimate adequately considered these factors |
| Very serious indirectness | None of the studies that contributed the most to the effect estimate considered these factors |
| Imprecision | |
| No serious imprecision | Studies that contribute the most to the effect estimate possess at least 80% power and/or adequate sample size to determine an effect reliably |
| Serious imprecision | Some of the studies that contribute the most to the effect estimate possess at least 80% power and/or adequate sample size to determine an effect reliably |
| Very serious imprecision | None of the studies that contribute the most to the effect estimate possess at least 80% power and/or adequate sample size to determine an effect reliably |
| Inconsistency | |
| No serious inconsistency | The studies that contribute the most to the effect estimate have consistent results, or inconsistent results that are explained satisfactorily |
| Serious inconsistency | Studies that contribute the most to the effect estimate have moderate inconsistency that is not adequately explained by subgroup or sensitivity analyses |
| Very serious inconsistency | Substantial to considerable inconsistency exists, which is not explained by subgroup or sensitivity analyses |

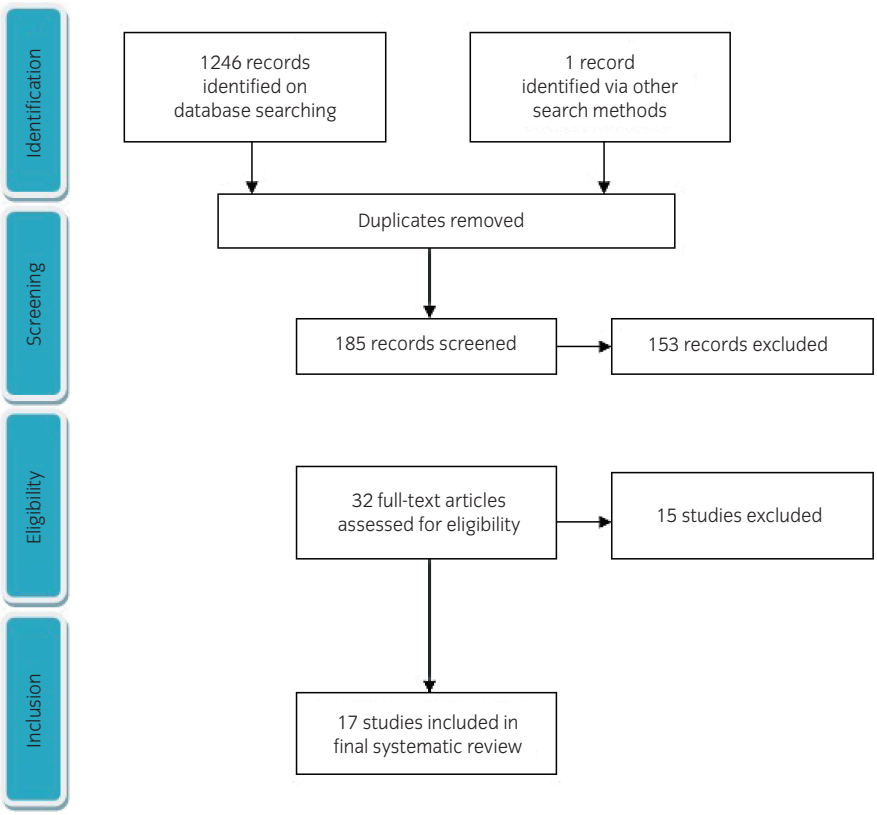


Fig 1: Flow diagram for inclusion of studies in the systematic review.

Outcomes: Ten studies used only tracheobronchoscopy within 2 h of racing to determine presence and severity of EIPH. Four studies assessed severity of EIPH using a 4- or 5-point tracheobronchoscopic grading system. Three of 4 studies used a 0–4 grading system, while one study used a grading scheme of grade 0–3, which combined grades 3 and 4 of a previous grading system of 0–4 (Supplementary Item 4). Six studies determined presence and severity of EIPH by BAL fluid RBC number. One study used both tracheobronchoscopy and BAL fluid RBC number to determine the presence and severity of EIPH.

Study design: Study design and methodology varied widely among the included studies. Included in the review were 11 RCTs [2,16–21,23–25], 6 nonrandomised studies, which included one controlled intervention trial [13], and 5 observational studies [1,12,14,15,22]. Two of the 11 RCTs were considered high-quality randomised, blinded, placebo-controlled field trials [2,23]. Nine studies were paired trials (with ≤9 horses) that used a cross-over design. Eight studies (with 85–3539 horses in each study) were parallel trials (comprising both RCTs and nonrandomised studies). Exercise-induced pulmonary haemorrhage was induced by racing or simulated racing in 8 studies and by exercise on a treadmill in 9 studies.

## Risk of bias in individual studies

Summary risk-of-bias assessments for RCTs and nonrandomised studies are presented in Figures 2 and 3.

*Risk of bias in RCTs:* The method of generation of the randomised treatment sequence was reported in only 4 of 11 studies. Concealed allocation of treatments was reported in 5 of 11 studies. Use of a placebo was reported in 7 of 11 studies. Blinding for outcome measurement was reported in 8 of 11 studies, and blinding was inferred in studies that used an automated haemocytometer to determine BAL fluid RBC number.

Incomplete outcome data were identified in 2 studies [16,18]. In one study [18], the automated haemocytometer did not enumerate less than 30,000 RBCs/μl, and manual BAL fluid RBC count was not performed for the first week of trials. This resulted in 6 missing observations from 2 of 6 horses included in the trial. In another trial [16], 2 of 10 horses did not complete the study. One horse failed to complete 4 of 10 weeks of the study so was eliminated from the analysis, while the other missed 3 of 10 weeks, but information was retained in study.

*Risk of bias in nonrandomised studies (intervention trials and observational studies):* One of the observational studies was retrospective in design, and the other 4 studies did not report whether they were prospective or retrospective. All trials used comparator groups taken from the population of interest (racing Thoroughbreds or Standardbreds); however, no studies reported that horses were randomly selected for inclusion. In one study [22], selection of horses for tracheobronchoscopy occurred in the majority of cases from horses that placed in a race, potentially resulting in a population of horses with grades of EIPH that were not representative of the wider population. Three of the 6 nonrandomised studies did not provide a clear description of the intervention, nor was the route or timing of furosemide reported. Blinding for outcome assessment was performed in only 2 of 6 observational studies.

*Publication bias:* There were sufficient studies to assess publication bias in the evaluation of furosemide for EIPH (yes or no) determined using tracheobronchoscopy. The funnel plot appeared symmetrical and did not reveal evidence of publication bias (Supplementary Item 5).

## Meta-analysis

Not all of the 17 studies provided sufficient information for meta-analysis. This was due to a lack of data reported in the published study or failure of corresponding study authors to provide the information to the reviewers. The total number of studies for which information was extracted is stated in each comparison.

*Tracheobronchoscopic presence of EIPH (yes or no):* Eleven of 17 trials provided data for this comparison, representing 5780 horses, of which 3177 received furosemide (Fig 4). The pooled RR of detecting any EIPH in horses treated with furosemide was 0.88 (95% CI 0.79–0.97), indicating a

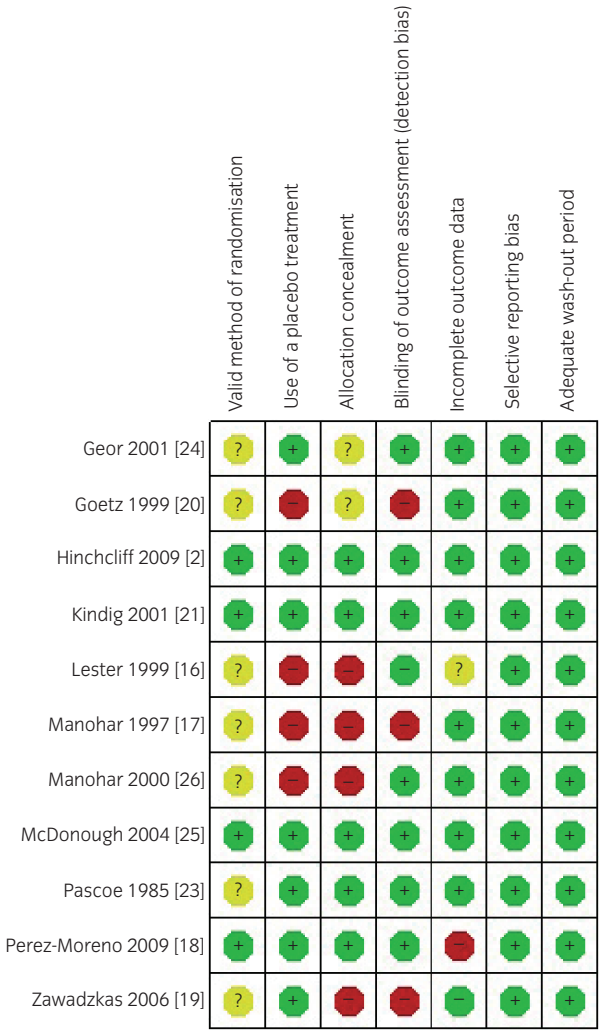


Fig 2: Risk of bias in the 11 randomised controlled trials included in the systematic review of furosemide for exercise-induced pulmonary haemorrhage. Green indicates low evidence of bias, yellow indicates an unclear level of bias and red indicates a high risk of bias.

modest effect of furosemide to prevent or reduce EIPH (P = 0.01). There was substantial heterogeneity among the 11 studies, as indicated by the Chi-square test (P = 0.001) and $I^2$ statistic (65%), thus subgroup analysis was performed.

Subgroup analysis: The effect of study design was explored by categorising trials by design type. The pooled RR in high-quality randomised controlled field trials (2 studies, 405 horses) supported a greater effect of furosemide to reduce EIPH (RR 0.68, 95% CI 0.58–0.79, P<0.0001) and exhibited minimal heterogeneity ($I^2$ 0%, Chi-square P = 0.53) (Figure 5). When low-quality RCTs were evaluated (3 studies, n = 68 horses), the effect estimate was smaller, confidence intervals were wider and the overall P value was not significant (RR 1.01, 95% CI 0.87–1.17, P = 0.92), indicating no effect of furosemide. Likewise, meta-analysis of observational studies (5 studies, n = 5301 horses) resulted in a smaller estimate of effect and wide confidence intervals that encompassed one, indicating no effect of furosemide (RR 0.89, 95% CI 0.78–1.02, P = 0.10). There was only one nonrandomised controlled intervention trial [13], preventing meta-analysis of this category alone.

Trials that used randomisation, allocation concealment or a placebo detected a significant effect of furosemide to reduce or prevent EIPH, whereas studies that did not use these methods found no effect of furosemide (Table 5). Studies that used blinded outcome assessment (4 studies, n = 963 horses) did not detect a positive effect of furosemide to reduce EIPH (RR 0.81, 95% CI 0.64–1.03; P = 0.09). The effect estimate for furosemide was greater in the 8 studies (n = 5712) conducted in simulated or actual racing conditions (RR 0.82, 95% CI 0.79–0.85; P<0.00001). There were insufficient studies assessing differences in the furosemide dosage, route or timing of administration to perform subgroup analysis.

Sensitivity analysis: Inclusion of trials published as conference proceedings proved highly influential in the pooled effect estimate of all the included studies. When only the 10 studies (n = 2247 horses) published in peer-reviewed journals were evaluated, the pooled RR for identifying EIPH after treatment of furosemide was not significant (RR 0.90; 95% CI 0.78–1.03, P = 0.11).

*Improvement in EIPH by at least one tracheobronchoscopic grade:* Two studies provided sufficient data to analyse reduction in EIPH by at least one tracheobronchoscopic grade after furosemide treatment. The pooled analysis revealed that 68% (95% CI 61–76%) of horses with EIPH that received furosemide experienced a reduction in severity by at least one tracheobronchoscopic grade. There was no heterogeneity between the 2 studies ($I^2$ 0%).

*Exercise-induced pulmonary haemorrhage, yes or no, as detected by BAL fluid RBC number:* Five RCTs studies (n = 66) provided data for this analysis. The standardised mean difference was -1.70 (95% CI -2.53 to -0.87,

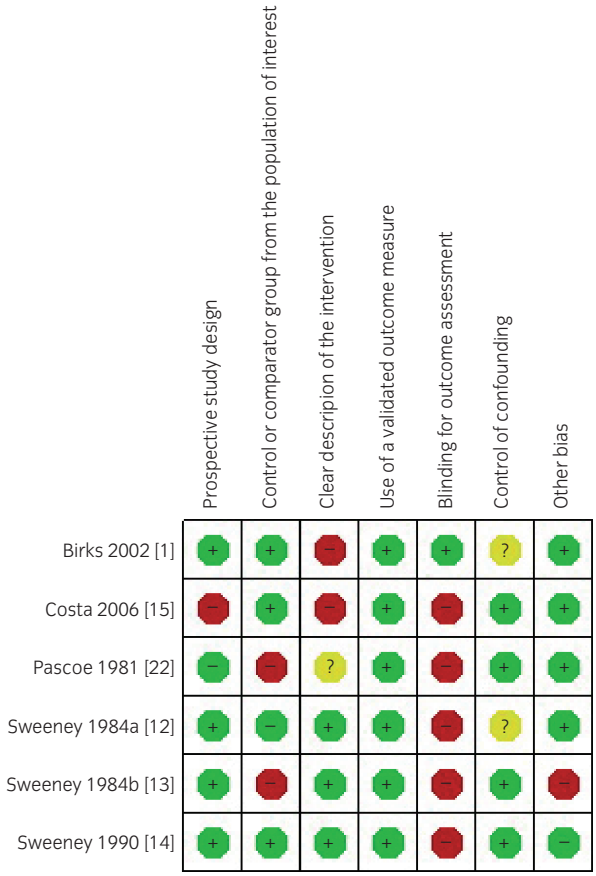


Fig 3: Risk of bias in the 6 nonrandomised studies included in the systematic review of furosemide for exercise-induced pulmonary haemorrhage. Green indicates low evidence of bias, yellow indicates an unclear risk of bias and red indicates a high risk of bias.

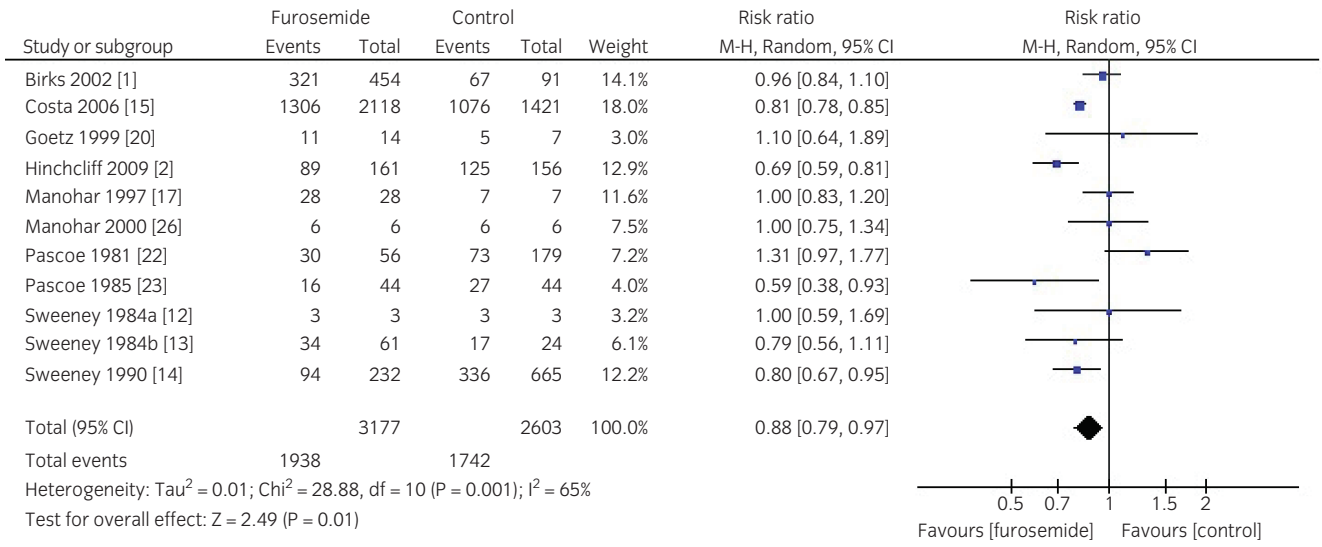


Fig 4: Forest plot of the meta-analysis of control vs. furosemide-treated horses that were evaluated using tracheobronchoscopy performed within 2 h of exercise. M-H, Martel-Haenszel.

P<0.001), indicating a positive effect of furosemide to reduce EIPH. There was significant heterogeneity among these 5 studies ($I^2$ 45%, P<0.001). Although the results indicated a positive effect of furosemide to reduce EIPH, the mean difference in BAL fluid RBC between furosemide-treated and control horses was calculated to be $16.7 \times 10^6$ cells/l (Supplementary item 6). This small decrease in BAL fluid RBC number was considered unlikely to be clinically important, and the overall interpretation of the results was considered by the authors to be severely limited by low number of horses included in each trial. In light of this, no further analyses were performed for this comparison.

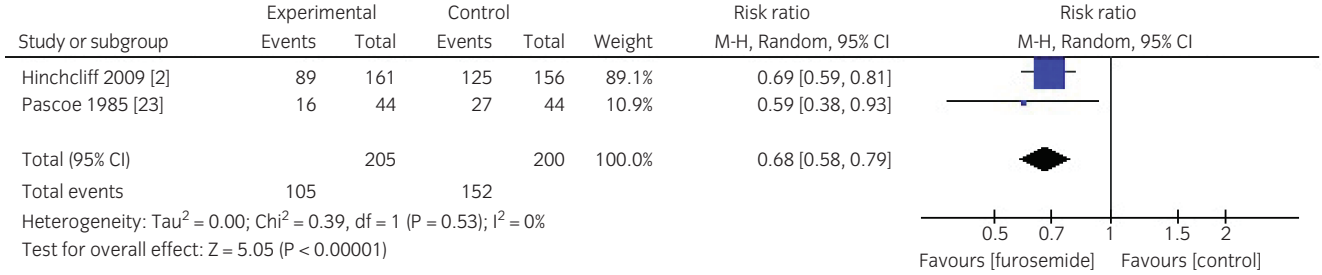


Fig 5: Forest plot of the meta-analysis of control vs. furosemide-treated horses, evaluated by tracheobronchoscopy within 2 h of exercise, in high-quality randomised controlled trials. M-H, Martel-Haenszel.

**TABLE 5: Summarised results of subgroup analyses for the meta-analysis of 11 trials examining the efficacy of furosemide to reduce or prevent exercise-induced pulmonary haemorrhage in Thoroughbred and Standardbred racehorses**

| Subgroup analysis | Number of studies | Number of horses | Relative risk | 95% Confidence interval | P value |
|---|---|---|---|---|---|
| Randomisation | | | | | |
| Yes | 2 | 405 | 0.68 | 0.58–0.79 | <0.0001 |
| No | 9 | 5375 | 0.93 | 0.83–1.03 | 0.16 |
| Allocation concealment | | | | | |
| Yes | 2 | 405 | 0.68 | 0.58–0.79 | <0.0001 |
| No | 9 | 5375 | 0.93 | 0.83–1.03 | 0.16 |
| Blinding for outcome measurement | | | | | |
| Yes | 4 | 963 | 0.81 | 0.64–1.03 | 0.09 |
| No | 7 | 4859 | 0.89 | 0.76–1.05 | 0.16 |
| Type of exercise | | | | | |
| Treadmill | 3 | 68 | 1.01 | 0.87–1.17 | 0.92 |
| Racing | 8 | 5712 | 0.82 | 0.79–0.85 | <0.0001 |
| Type of control population | | | | | |
| Untreated controls | 8 | 5409 | 0.90 | 0.79–1.03 | 0.11 |
| Placebo | 3 | 419 | 0.68 | 0.59–0.79 | <0.0001 |

**TABLE 6: Summary of evidence for the efficacy of furosemide used for exercise-induced pulmonary haemorrhage**

| | Quality assessment | | | | | Number of horses | | Effect | | Strength of evidence |
|---|---|---|---|---|---|---|---|---|---|---|
| Outcome | Design | Bias | Inconsistency | Indirectness | Imprecision | Furosemide | Control | Relative | Absolute | |
| Evidence of EIPH (yes or no), as detected by tracheobronchoscopy | Type I: 2 studies<br>Type II: 4 studies<br>Type III: 4 studies<br>Type IV: 1 study | Low risk: 2 studies<br>High risk: 9 studies | No inconsistency | No indirectness | No imprecision | 1938/3177 (61.0%) | 1742/2603 (66.9%) | RR 0.88 (0.7–0.97) | 93 fewer per 1000 (from 20 to 160 fewer) | High |
| Evidence of EIPH (yes or no), as detected by tracheobronchoscopy in high-quality randomised controlled field trials | Type I: 2 studies | Low risk: 2 studies | No inconsistency | No indirectness | No imprecision | 105/205 (51.2%) | 152/200 (76%) | RR 0.68 (0.58–0.79) | 243 fewer per 1000 (from 160 to 319 fewer) | High |
| Decrease in EIPH by one tracheobronchoscopic grade | Type I: 2 studies | Low risk: 2 studies | No inconsistency | No indirectness | No imprecision | N/A | | | | High |
| Evidence of EIPH (yes or no), as detected by BAL fluid RBC number | Type I: 3 studies<br>Type II: 2 studies | Low risk: 3 studies<br>Moderate risk: 3 studies | Unexplained inconsistency | No indirectness | Imprecision | N/A | | | | Very low |

Abbreviations: BAL = bronchoalveolar lavage; EIPH = exercise-induced pulmonary haemorrhage; N/A = not applicable; RBC = red blood cell; RR = relative risk.

### Rating the quality of evidence by outcome

*Tracheobronchoscopic presence of EIPH (yes or no):* The initial quality of the body of evidence was assessed as being high, because there were relatively more RCTs than nonrandomised studies included in the review. The methodological quality of the 11 studies included in this comparison varied markedly, and there was considerable heterogeneity when meta-analysis was performed for all 11 studies. However, subgroup analysis of the highest quality studies with minimal risk of bias and no evidence of imprecision, indirectness or inconsistency clearly supported a positive effect of furosemide to decrease EIPH. Therefore, the overall rating of the quality of evidence for furosemide to reduce EIPH in Thoroughbred and Standardbred racehorses, as detected by tracheobronchoscopy, is considered high (Table 6).

*Reduction in severity of EIPH by at least one tracheobronchoscopic grade:* The initial body of evidence was assessed as high-quality evidence because both studies included in this comparison were high-quality RCTs. There was no serious bias, inconsistency, imprecision or indirectness across the 2 studies. It was not possible to assess publication bias in this outcome because only 2 studies were included in the comparison. The overall quality of the body of evidence for furosemide to decrease severity of EIPH by at least one tracheobronchoscopic grade was considered high (Table 6).

*Exercise-induced pulmonary haemorrhage, yes or no, as detected by BAL fluid RBC number:* All studies included in this comparison were RCTs; therefore, the initial body of evidence was considered high quality. The authors rated down the body of evidence from high quality to moderate quality for unexplained inconsistency across studies included in this comparison. Furthermore, there was very serious imprecision across studies, thus the quality of evidence was reduced by a further 2 grades to very low-quality evidence (Table 6).

## Discussion

### Summary of the main results

This systematic review provides high-quality evidence that furosemide reduces the frequency and severity of EIPH in Thoroughbred racehorses. The summary estimate from this review suggest that 93 (range 20–160) fewer horses per 1000 horses examined will develop endoscopically visible EIPH after treatment with furosemide. When evidence from high-quality randomised controlled trials only is considered, 243 (range 160–319) fewer horses per 1000 examined will develop endoscopically visible EIPH. Additionally, 68% of horses with endoscopically detectable EIPH experience a reduction in EIPH severity after administration of furosemide.

### Quality of the evidence

The main limitation of this systematic review was the lack of high-quality and sufficiently powered RCTs to evaluate. We explicitly aimed to determine the influence of study design and study bias on the effect estimates. When only the high-quality RCTs were considered, the pooled estimate of effect supporting use of furosemide for EIPH was greater in magnitude than when the evaluation included lower quality studies.

Reporting of the methodological features of studies included in this systematic review was generally poor, making evaluation of bias within and across studies difficult. Because of this, it is possible that studies received a lower individual grading for bias than actually existed. Study authors were contacted by email to verify aspects of study design not clearly reported in the published article, but only 5 of 17 authors responded.

Serious imprecision affected all studies that assessed the presence of EIPH by BAL fluid RBC number and some lower quality RCTs that used tracheobronchoscopy to diagnose EIPH. In studies with presumed low statistical power (due to low sample size, small effect size or both), the chance of identifying effects that are genuinely true is low, and when an effect is identified, the risk of exaggerating the magnitude of the effect estimate is high [6]. The relative contribution of low-powered studies to the effect estimate in studies evaluating the efficacy of furosemide by tracheobronchoscopy was relatively minor; however, all studies that assessed EIPH by BAL fluid RBC number had fewer than 10 horses per study, which is likely to have seriously limited the detection of any effect of furosemide to alter EIPH.

In this systematic review, trials conducted in racing conditions were considered higher quality evidence than those performed on a treadmill. Although experimental trials conducted on a treadmill effectively induce EIPH, they do not reproduce the conditions during a race. Additionally, horses enrolled in treadmill studies are not usually in active training or racing, thus do not represent the 'at-risk' population of horses. The demonstrated efficacy of furosemide in racing conditions was considered higher quality evidence than results of studies undertaken on a treadmill.

Diagnosis of EIPH in the studies included in this review was performed by tracheobronchoscopy or by detection of RBCs within BAL fluid. Randomised controlled trials using tracheobronchoscopy to determine the presence and severity of EIPH were considered higher quality evidence than those using BAL fluid RBC number. The tracheobronchoscopic severity score is the only method of assessing EIPH severity that has been associated with racing performance. Although BAL RBC number is arguably a more sensitive measure of EIPH severity and has the benefit of being objectively quantified, researchers have not determined what constitutes a clinically significant degree of haemorrhage [28], nor have studies detected associations between BAL fluid RBC number and racing performance. Marked disparity in the mean and standard deviations of BAL

fluid RBC counts obtained across studies also challenges the usefulness of this technique for evaluating efficacy of treatments for EIPH.

One of the most challenging aspects of synthesising result data in this review was how best to combine the results of parallel and cross-over studies. Most systematic reviews and meta-analyses in human medicine evaluate RCTs with 2 different parallel treatment groups. In contrast, many of the studies in this review used a cross-over study design. A particular strength of cross-over designs is that variation in repeated responses within a subject is usually less than that between different subjects, which can translate to more precise results with half as many participants. However, one important limitation of cross-over studies is that although trial authors might have analysed paired data, poor presentation often makes it impossible for review authors to extract paired data to perform a meta-analysis [6]. This was the case for this review, where individual subject data were reported in only a few trials, and trial authors did not forward any additional information. Methods of synthesising data from cross-over studies are also inconsistent. Due to the lack of individual subject data available, we elected to treat data generated from cross-over trials as independent observations, because the mean and standard error for each group were reported in most publications. Although a positive effect of furosemide to reduce EIPH was detected, this method of analysing data was very likely to have resulted in some loss of power in the analysis and could partly explain the wide confidence intervals generated in the meta-analysis.

### Overall completeness and applicability of evidence

We attempted to identify all relevant studies by performing a comprehensive, systematic search of electronic and print resources and by including grey literature, such as conference proceedings, in the review. Nonetheless, we accept it is possible that some studies were missed. It is also possible that studies relevant to the review question were published in languages other than English, so were ineligible for inclusion in the review.

Based upon the population of horses included in this review, we are confident that the review can be generalised to the target population of Thoroughbred and Standardbred racehorses. However, conclusions cannot be drawn about the efficacy of furosemide in horses other than adult Thoroughbreds or Standardbreds performing any activity other than racing, simulated racing or strenuous exercise on a treadmill. Although the dose, route and timing of administration of furosemide varied among studies, most horses in this review received 250–500 mg of furosemide i.v. 4 h before exercise. There were too few studies evaluating alternative doses, routes or timing of administration of furosemide to assess how this could affect the overall efficacy of the furosemide for EIPH.

### Potential biases in the review process

Two authors (K.W.H. and P.S.M.) of this systematic review were involved in the design, execution and reporting of one of the studies that was considered a high-quality RCT. All information extracted from the study was readily accessible in the published account of the trial. Neither author was the primary author of this systematic review.

### Conclusions

This systematic review indicates that there is high-quality evidence for the use of furosemide to prevent or limit the occurrence of EIPH in Thoroughbred racehorses. Further studies in this area would benefit from the use of power and sample size calculations prior to starting trials, so that an intervention effect can be detected reliably. The authors of this systematic review recommend that future studies should also be undertaken as randomised, blinded, placebo-controlled trials performed on horses in racing conditions and using outcomes shown to have relevance to meaningful performance measures.

## Authors' declaration of interests

No competing interests have been declared.

## Ethical animal research

Not applicable.

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## Authorship

All authors contributed to the study design and all authors approved the final manuscript. S.L. Sullivan, K.W. Hinchcliff and P.S. Morley contributed to study execution, data analysis and interpretation and preparation of the manuscript. T. Whittem contributed to study design and execution.

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## References

1. Birks, E.K., Shuler, K.M., Soma, L.R., Martin, B.B., Marconato, L., Del Piero, F., Teleis, D.C., Schar, D., Hessinger, A.E. and Uboh, C.E. (2002) EIPH: postrace endoscopic evaluation of Standardbreds and Thoroughbreds. *Equine Vet. J.* **34**, 375-378.
2. Hinchcliff, K.W., Morley, P.S. and Guthrie, A.J. (2009) Efficacy of furosemide for prevention of exercise-induced pulmonary haemorrhage in Thoroughbred raacehorses. *J. Am. Vet. Med. Ass.* **235**, 76-82.
3. Hinchcliff, K.W., Jackson, M.A., Brown, J.A., Dredge, A.F., O'Callaghan, P.A., McAffrey, J.P., Morley, P.S., Slocombe, R.F. and Clarke, A.F. (2005) Tracheobronchoscopic assessment of exercise-induced pulmonary haemorrhage in horses. *Am. J. Vet. Res.* **66**, 596-598.
4. Thomas, A.D., Green, M.J., Morris, T., Bowen, N. and Hallowell, G.D. (2012) Prevalence, recurrence, risk factors and effects on performance of epistaxis in racing Thoroughbreds in the UK: 914,849 starts (2001–2010). *J. Vet. Int. Med.* **26**, 743-745.
5. Mason, B.J., Riggs, C.M. and Cogger, N. (2013) Cohort study examining long-term respiratory health, career duration and racing performance in racehorses that undergo left-sided prosthetic laryngoplasty and ventriculocordectomy surgery for treatment of left-sided laryngeal hemiplegia. *Equine Vet. J.* **45**, 229-234.
6. Higgins, J.P.T. and Green, S. (Eds.) (2011) *Cochrane Handbook for Systematic Reviews of Interventions*, Version 5.1.0 (updated March 2011). Available from: www.cochrane-handbook.org.
7. Guyatt, G., Oxman, A.D., Akl, E.A., Kunz, R., Vist, G., Brozek, J., Norris, S., Falck-Ytter, Y., Glasziou, P., deBeer, H., Jaeschke, R., Rind, D., Meerpohl, J., Dahm, P. and Schünemann, H.J. (2011) GRADE guidelines: 1. Introduction—GRADE evidence profiles and summary of findings tables. *J. Clin. Epidemiol.* **64**, 383-394.
8. Guyatt, G.H., Oxman, A.D., Vist, G., Kunz, R., Brozek, J., Alonso-Coello, P., Montori, V., Akl, E.A., Djulbegovic, B., Falck-Ytter, Y., Norris, S.L., Williams, J.W., Atkins, D., Meerpohl, J. and Schünemann, H.J. (2011) GRADE guidelines: 4. Rating the quality of evidence—study limitations (risk of bias). *J. Clin. Epidemiol.* **64**, 407-415.
9. Moher, D., Liberati, A., Tetzlaff, J. and Altman, D.G. (2009) Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *J. Clin. Epidemiol.* **62**, 1006-1012.
10. Guyatt, G.H., Oxman, A.D., Kunz, R., Woodcock, J., Brozek, J., Helfand, M., Alonso-Coello, P., Glasziou, P., Jaeschke, R., Akl, E.A., Norris, S., Vist, G., Dahm, P., Shukla, V.K., Higgins, J., Falck-Ytter, Y. and Schünemann, H. (2011) GRADE guidelines: 7. Rating the quality of evidence—inconsistency. *J. Clin. Epidemiol.* **64**, 1294-1302.

11. Balshem, H., Helfand, M., Schünemann, H.J., Oxman, A.D., Kunz, R., Brozek, J., Vist, G.E., Falck-Ytter, Y., Meerpohl, J., Norris, S. and Guyatt, G. (2011) GRADE guidelines: 3. Rating the quality of evidence. *J. Clin. Epidemiol.* **64**, 401-406.
12. Sweeney, C.R. and Soma, L.R. (1984) Exercise-induced pulmonary hemorrhage in thoroughbred horses: response to furosemide or hesperidin-citrus bioflavinoids. *J. Am. Vet. Med. Ass.* **185**, 195-197.
13. Sweeney, C.R., Soma, L.R., Bucan, C.A. and Ray, S.G. (1984) Exercise-induced pulmonary hemorrhage in exercising thoroughbreds: preliminary results with pre-exercise medication. *Cornell Vet*. **74**, 263-268.
14. Sweeney, C.R., Soma, L.R., Maxson, A.D., Thompson, J.E., Holcombe, S.J. and Spencer, P.A. (1990) Effects of furosemide on the racing times of thoroughbreds. *Am. J. Vet. Res.* **51**, 772-778.
15. Costa, M.F.M. and Thomassian, A. (2006) Retrospective study of exercise induced pulmonary haemorrhage (EIPH) in Brazilian Thoroughbred racehorses. *Association des Veterinaires Equins Francais*, Versailles, France.
16. Lester, G., Clark, C., Rice, B., Steible-Hartless, C. and Vetro-Widenhouse, T. (1999) Effect of timing and route of administration of furosemide on pulmonary hemorrhage and pulmonary arterial pressure in exercising Thoroughbred racehorses. *Am. J. Vet. Res.* **60**, 22-28.
17. Manohar, M., Goetz, T.E., Sullivan, E. and Griffin, R. (1997) Pulmonary vascular pressures of strenuously exercising Thoroughbreds after administration of varying doses of frusemide. *Equine Vet. J.* **29**, 298-304.
18. Perez-Moreno, C.I., Couëtil, L.L., Pratt, S.M., Ochoa-Acuña, H.G., Raskin, R.E. and Russell, M.A. (2009) Effect of furosemide and furosemide–carbazochrome combination on exercise-induced pulmonary haemorrhage in Standardbred racehorses. *Can. Vet. J.* **50**, 821-827.
19. Zawadzkas, X.A., Sides, R.H. and Bayly, W.M. (2006) Is improved high speed performance following frusemide administration due to diuresis-induced weight loss or reduced severity of exercise-induced pulmonary haemorrhage? *Equine Vet. J., Suppl.* **38**, 291-293.
20. Goetz, T.E., Manohar, M. and Magid, J.H. (1999) Repeated administration of frusemide does not offer an advantage over single dosing in attenuating exercise-induced pulmonary haemorrhage. *Equine Vet. J.* **31**, *Suppl.* **30**, 539-545.
21. Kindig, C.A., McDonough, P., Fenton, G., Poole, D.C. and Erickson, H.H. (2001) Efficacy of nasal strip and furosemide in mitigating EIPH in Thoroughbred horses. *J. Appl. Physiol.* **91**, 1396-1400.
22. Pascoe, J., Ferraro, G.L., Cannon, J.H., Arthur, R.M. and Wheat, J.D. (1981) Exercise-induced pulmonary haemorrhage in racing thoroughbreds: a preliminary study. *Am. J. Vet. Res.* **42**, 703-706.
23. Pascoe, J., McCabe, A., Franti, C. and Arthur, R.M. (1985) Efficacy of furosemide in the treatment of exercise-induced pulmonary haemorrhage in Thoroughbred racehorses. *Am. J. Vet. Res.* **46**, 2000–2003.
24. Geor, R.J., Ommundson, G., Fenton, G. and Pagan, J.D. (2001) Effects of an external nasal strip and frusemide on pulmonary haemorrhage in Thoroughbreds following high-intensity exercise. *Equine Vet. J.* **33**, 577-584.
25. McDonough, P., Kindig, C.A., Hildreth, T.S., Padilla, D.J., Behnke, B.J., Erickson, H.H. and Poole, D.C. (2004) Effect of furosemide and the equine nasal strip on exercise-induced pulmonary haemorrhage and time-to-fatigue in maximally exercising horses. *Equine Comp. Exerc. Physiol.* **1**, 177-184.
26. Manohar, M., Goetz, T.E., Rothenbaum, P. and Humphrey, S. (2000) Clenbuterol administration does not enhance the efficacy of furosemide in attenuating the exercise-induced pulmonary capillary hypertension in Thoroughbred horses. *J. Vet. Pharmacol. Ther.* **23**, 389-395.
27. Birks, E.K., Mathieu-Costello, O., Fu, Z., Tyler, W.S. and West, J.B. (1997) Very high pressures are required to cause stress failure of pulmonary capillaries in Thoroughbred racehorses. *J. Appl. Physiol.* **82**, 1584-1592.
28. Hinchcliff, K.W. (2000) Counting red cells – is it the answer to EIPH? *Equine Vet. J.* **32**, 362-363.

## Supporting information

Additional Supporting Information may be found in the online version of this article at the publisher's website:

**Supplementary Item 1:** A summary of GRADE's approach to rating the quality of evidence.
**Supplementary Item 2:** Table of excluded studies.
**Supplementary Item 3:** Table of included studies.
**Supplementary Item 4:** Summary of tracheobronchoscopic grading systems used in included studies.
**Supplementary Item 5:** Funnel plot of the precision of studies evaluating furosemide for EIPH using tracheobronchoscopy within 2 h of exercise.
**Supplementary Item 6:** Forest plot of the meta-analysis of control vs. furosemide-treated horses that were evaluated using bronchoalveolar lavage fluid red blood cell number. Insufficient data were available to perform analysis for paired observations, so the mean bronchoalveolar lavage fluid red blood cell numbers for control and furosemide-treated horses were considered as independent observations.