doi.org/10.1111/evj.12815

Clinical effect of corticosteroids in asthma-affected horses: A quantitative synthesis

L. CALZETTA†*, P. ROGLIANI†, C. PAGE‡, P. RONCADA§, E. PISTOCCHINI#, A. SOGGIU–, C. PIRAS–, A. URBANI¥ and M.G. MATERA††

†Department of Experimental Medicine and Surgery, University of Rome Tor Vergata, Rome, Italy

‡The Sackler Institute of Pulmonary Pharmacology, Institute of Pharmaceutical Science, King's College London, London, UK

§Istituto Sperimentale Italiano "Lazzaro Spallanzani", Milan, Italy

#Laboratorio Analisi Veterinarie, CD Vet, Rome, Italy

–Dipartimento di Medicina Veterinaria, University of Milan, Milan, Italy

¥Istituto di Biochimica e Biochimica Clinica, Universita Cattolica, Rome, Italy

††Department of Experimental Medicine, University of Campania Luigi Vanvitelli, Naples, Italy.

*Correspondence email: luigino.calzetta@uniroma2.it;

Summary

Background: There are limited findings from low-powered studies based on few number of subjects with equine asthma. Furthermore, no studies have been performed to assess a meaningful clinically detectable impact of corticosteroids in equine asthma.

Objectives: To assess and compare the clinical effect of inhaled and systemic corticosteroids in equine asthma and identify a quantitative clinical score suitable to assess the Minimal Important Difference (MID), expressed as the Minimally Clinically Detectable Difference (MCDD).

Study design: Pair-wise and network meta-analysis.

Methods: Literature searches for studies on corticosteroid therapy in equine asthma were performed. The risk of publication bias was assessed by Funnel plots and Egger's test. The effect on changes in clinical scores vs. control was analysed via random-effects models and Bayesian networks.

Results: Corticosteroids significantly improved the clinical condition (Standardised Mean Difference: -1.52, 95% CrI -2.07 to -0.98; P<0.001 vs.control). No difference was detected between inhaled and systemic corticosteroids with regard to the changes in clinical scores (Relative Effect: 0.08, 95% CrI -1.45 to 1.32; P = 0.8). An Improved clinically Detectable Equine Asthma Scoring System (IDEASS) indicated that corticosteroids improved the clinical condition of asthmatic horses by 30% compared with controls (IDEASS value: -2.36, 95% CI -3.39 to -1.33; P<0.001). A one-point change in IDEASS represented the MCDD in equine asthma.

Main limitations: Moderate quality of evidence for systemic corticosteroids.

Conclusions: Inhaled corticosteroids are effective in improving the clinical condition of horses with equine asthma and prevent exacerbations. Systemic corticosteroids should be used only in selected cases with symptomatic airway hyperresponsiveness during exacerbation. IDEASS requires further validation but may represent a suitable approach to rank the level of asthma severity and assess the clinical effect of pharmacotherapy in horses with equine asthma.

Keywords: horse; equine asthma; corticosteroids; meta-analysis; pharmacology

Introduction

Equine asthma, which has formerly been known as broken wind, chronic obstructive pulmonary disease (COPD), heaves, inflammatory airway disease (IAD), and recurrent airway obstruction (RAO), is a nonseptic chronic respiratory disorder of horses with mild (IAD) to severe (RAO) airway disease [1]. Treatment with anti-inflammatory drugs, such as glucocorticosteroids, represents an effective therapeutic approach to control equine asthma and to treat acute exacerbations [1]. A recent Editorial by Muir [2] has highlighted that equine veterinary medicine may benefit from high-quality and well-performed quantitative syntheses of data. A recent meta-analysis reported the results with significance of the effect of pharmacological treatments on respiratory mechanics of asthmatic horses [3]. In particular, glucocorticosteroids ameliorated lung function in symptomatic horses, by modulating inflammation of both large and small airways [3].

Currently, there are no adequately powered trials that assessed the clinical effect of glucocorticosteroids in asthmatic horses. In addition, the current dependence on statistical significance, which is often misinterpreted, has confounded the usefulness of these results to clinical practice [2]. A rigorously conducted meta-analysis on the pharmacotherapy of equine asthma may provide several benefits, such as correcting for low power of available clinical trials and accounting for publication bias in individual studies and across studies. Therefore, we performed a meta- analysis to quantify the clinical effect of inhaled and systemic corticosteroids in asthmatic horses, and identify a quantitative clinical score suitable to assess the minimally clinically detectable effect.

Materials and methods

Search strategy and study eligibility

This meta-analysis was performed in agreement with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) Statement (Fig 1) [4] and satisfied all the recommended items reported by the PRISMA-P 2015 checklist [5]. We undertook a comprehensive literature search for studies written in English and concerning the effect of corticosteroids on the clinical effect of horses affected by equine asthma, previously known as broken wind, COPD, heaves, inflammatory airway disease and RAO [6,7]. The terms

66 ‘equine asthma’ OR ‘broken wind’ OR ‘chronic obstructive pulmonary disease’ OR ‘heaves’ OR ‘inflammatory
67 airway disease’ OR ‘recurrent airway obstruction’ were searched for the disease. The terms ‘equine’ OR
68 ‘horse’ were searched for the species. The terms ‘corticosteroids’ OR ‘glucocorticosteroids’ were searched
69 to identify the pharmacological treatments. The search was further refined using the acronyms for chronic
70 obstructive pulmonary disease (COPD), inflammatory airway disease (IAD), recurrent airway obstruction
71 (RAO), and by searching the specific corticosteroid agents that have been used to treat equine asthma
72 (beclomethasone, budesonide, dexamethasone, fluticasone, isoflupredone, prednisolone and prednisone),
73 in agreement with the Consensus Statements of the American College of Veterinary Internal Medicine
74 (ACVIM), and as reported by Calzetta et al. and Picandet et al. [1,3,8]. The PICO (population, intervention,
75 control and outcomes) strategy for the research question is reported in Table 1. The search was performed
76 on PubMed and Google Scholar in order to provide for relevant studies published up to 30 April 2017 [9].

77 Study selection

78 All studies concerning the clinical effect of corticosteroids in horses suffering from equine asthma using a
79 clinical score system were selected and included in the meta-analysis. Two reviewers (veterinarians with a
80 background in equine respiratory medicine) independently examined the studies, and any difference in
81 opinion concerning the selection of the studies was resolved by consensus.

82

83 Objectives

84 The primary objectives of this study were to assess and compare the effect of inhaled and systemic
85 corticosteroids on the clinical score of equine asthma-affected horses, using values detected at baseline
86 and/or in placebo arms as control. The secondary objective was to identify a clinical score suitable for
87 asthmatic horses and quantify the Minimal Important Difference (MID), expressed as the Minimally Clinically
88 Detectable Difference (MCDD), elicited by corticosteroid therapy.

89 Quality, bias and evidence profile

The Jadad score, with a scale of 1–5 (score of 5 being the best score), was used to assess the quality of the studies, as previously reported [10]. Two reviewers, with a specific background in the field of meta-analysis, independently assessed the quality of the studies, and any difference in opinion concerning the Jadad score was resolved by consensus. The risk of publication bias was analysed by applying the Funnel plot and Egger's test, as previously described [11,12]. The graphical representations of Egger's test have been reported with the 90% confidence bands [11,12]. The optimal information size (OIS) required for our meta-analysis was calculated as previously described [13,14]. The quality of the evidence was assessed in agreement with the Grading of Recommendations Assessment, Development, and Evaluation (GRADE) system [15].

Data synthesis and analysis

Studies characteristics evaluation: Meta-regression analysis was performed to control for potential confounding covariates that may influence the effect size of the outcomes [16]. Pair-wise and network meta-analysis: A pair-wise meta-analysis was performed by applying the random-effect model in order to calculate the effect estimates of corticosteroid treatment vs. control [16–18]. Subset analyses were carried out by considering inhaled vs. systemic treatments, and specific clinical scores. The test for heterogeneity (I2) was performed to quantify the between-study dissimilarity, as previously reported [19]. The overall changes in clinical score are reported as Standardised Mean Difference and 95% CI since this outcome was not always standardised among the studies. The effect estimates of studies performed using the same clinical score are reported as Mean Difference and 95% CI. Large heterogeneity was considered for $I2 \geq 50\%$ [20]. A network meta-analysis was performed in order to compare the effectiveness of corticosteroid administered via inhalation vs. systemic route. Network meta-analysis was performed using a full Bayesian evidence network, and consistency was calculated through the Brooks-Gelman-Rubin method, as previously described [21,22]. Data of network meta-analysis are reported as relative effect (RE) and 95% credible interval (CrI) [21,22]. The analysis of inconsistency was performed as previously described [22].

MID and MCDD assessment: The MID and MCDD were estimated via the distribution-based approach, by focusing on the variance and distributional properties of the clinical score in the corticosteroid-treated vs. corticosteroid-untreated horses with equine asthma. Possible MID and MCDD values were calculated on the

effect estimate resulting from the pair-wise meta-analysis using the methods of the half a standard deviation (HSD), error of measurement (SEM), standardised response mean (SRM), and effect size (ES), as previously described [23–26]. The average of the obtained values identified the final MID elicited by the corticosteroid therapy in equine asthma, by which the MCDD was calculated.

Software and statistical significance: OpenMetaAnalyst was used to perform pair-wise meta-analysis, GeMTC for network meta-analysis and GraphPad Prisma to graph the data [19,27]. The statistical significance was set at $P \leq 0.05$, whereas the asymmetry of Egger's test was considered significant for $P \leq 0.1$ [11].

Results

Search and study characteristics

Overall, 5120 records were found in Google Scholar and 2421 records in PubMed. All the articles found in PubMed were also included in those resulting from Google Scholar. Data obtained from 80 equine asthma-affected horses were extracted from 6 studies that investigated the clinical effect of one or more corticosteroids. Three studies used corticosteroids administered via both inhalation and systemic route [28–30], two studies used exclusively the systemic forms of corticosteroids [31,32], and one study used only inhaled corticosteroids [33]. All studies were published between 2003 and 2016, and the average duration of the clinical trials was 12 days. Only one study reached a Jadad score ≥3 [29], 2 studies were of modest quality (Jadad score <3 and ≥1) [30,32], and for 3 studies the Jadad score was 0 [28,31,33]. Relevant case demographics, baseline, study characteristics and Jadad scores are summarised in Supplementary Item 1. The meta-regression analysis indicated that the effect of corticosteroids on the clinical score of asthmatic horses was not significantly affected by confounding covariates, namely the method for inducing clinical signs (exposure to hay, mouldy grass hay, and straw bedding or clinical occurring cases; P = 0.7), environmental management (housing in environmental dust reduction [EDR] condition or non-EDR condition; P = 0.7), the type of inhaler device used to deliver the drugs (ultrasonic nebuliser or pressurised metered dose inhaler [pMDI]; P = 0.8), and the operator that administered the drugs (veterinary staff or trained personnel/ owners; P = 0.4). In all the studies in which the inhalant drugs were administered via pMDI a spacer was

141 employed. Concomitant medications were excluded from the meta-regression since no concomitant
142 medications were used in all the studies included in this quantitative synthesis. Furthermore details on meta-
143 regression analysis are reported in Figure 2a–c.

144 Meta-analysis

145 Pair-wise meta-analysis: Corticosteroids improved the clinical condition of asthmatic equines by significantly
146 reducing the overall clinical score compared with controls (P<0.001, Fig 3a). Subset analysis indicated that
147 both systemic and inhaled corticosteroids significantly reduced the overall clinical score compared with
148 controls (P<0.001, Fig 3b). Specific analysis of the studies carried out using the same subjective clinical scoring
149 system as proposed by Rush et al. [34] resulted in a significant Mean Difference of -1.89 (95% CI -2.71 to -
150 1.06) for corticosteroids compared with controls (P<0.001, Fig 3c).

151 Network meta-analysis: No significant differences were detected in clinical scores between inhaled and
152 systemic corticosteroids (inhaled forms vs. systemic forms: RE 0.08, 95% CrI -1.45 to 1.32; P = 0.8). The
153 network meta-analysis confirmed the effectiveness of both inhaled and systemic corticosteroids compared
154 with controls in reducing the clinical score of equine asthma-affected horses (inhalation: RE -2.01, 95%
155 CrI -3.29 to -0.94; P<0.001 systemic administration: -2.09, 95% CrI -3.02 to -1.12; P<0.001).

156 Quality score, risk of bias and evidence profile: A significant level of heterogeneity (I2 = 65%, P<0.001) was
157 detected for the overall impact of corticosteroids (both systemic and inhalant formulations) in the changes
158 of clinical scores. The main source of heterogeneity was one of the treatment arms in a study on systemic
159 administration dexamethasone [28]. No significant level of heterogeneity (I2 = 38%, P = 0.09) was found
160 when that arm was excluded from the analysis. High levels of heterogeneity resulted from the subset analysis
161 of systemic corticosteroids (I2 = 77%, P<0.001), but not for inhaled corticosteroids (I2 = 22%, P = 0.3). Analysis
162 of heterogeneity was confirmed by Funnel plots. Egger's test detected a significant (P<0.001) asymmetry for
163 the overall changes in clinical scores and the subset analysis performed on systemic corticosteroids
164 (P<0.001), but not on inhaled corticosteroids (Fig 4). Analysis of inconsistency of the network meta-analysis
165 indicated that no significant discrepancy exists between direct and indirect evidence (inconsistency factor:

0.01, 95% CrI -2.39 to 2.26; P>0.9). The consistency/ inconsistency analysis of the network meta-analysis indicated that all the points fit adequately with the line of equality (R2 0.99; slope 0.96, 95% CI 0.83–1.09, P = 0.5). The cumulative number of enrolled horses in the studies reached the OIS for correctly assessing the changes in clinical scores, with available data

+42.86% greater than the expected OIS. The GRADE approach indicated moderate quality of evidence for the overall effect of corticosteroids on equine asthma (+++). Subset analysis showed high quality of evidence for inhaled corticosteroids (++++), and moderate quality of evidence for systemic corticosteroids (+++).

Discussion

This meta-analysis demonstrates that the treatment with corticosteroids has a significant and beneficial clinical effect in equine asthma, with no significant differences observed between inhaled and systemic corticosteroids. The clinical benefit was directly related with the dose (i.e. fluticasone proprionate was more effective when administered at 6 mg than at 3 mg), and the frequency of administration (i.e. dexamethasone was more effective when administered q12 h than q24 h). The results were not affected by the method of induction of clinical signs, type of inhaler device, environmental management or the professional level of the person administering the inhaled drugs. The few studies performed to assess the effect of corticosteroids using a clinical score recruited exclusively RAO-affected horses [28–33]. Consequently, the current meta-analysis cannot provide specific evidence on the clinical role of corticosteroids in IAD-affected horses. Furthermore, none of the investigated studies evaluated the effect of corticosteroids administered alongside bronchodilators, a clear gap in the research body available and this meta-analysis highlights the need for studies looking at drug combinations, which would be far more applicable to clinical practice [1]. Systemic corticosteroids may induce faster clinical benefits compared with inhaled corticosteroids during an acute exacerbation of equine asthma, as demonstrated in a study comparing the effects of aerosolised fluticasone propionate and i.v. dexamethasone on clinical findings and pulmonary function [28]. That was a small study [28], and one arm was the main source of heterogeneity in this meta-analysis, but it did provide

evidence that inhaled corticosteroids have greater efficacy for prophylaxis than therapy. This was also confirmed in another study [34], which unfortunately was excluded from the current quantitative meta-analysis because the results were expressed as median instead of mean and no error values were reported. However, qualitative evaluation of this study indicated that i.v. dexamethasone was more effective than aerosolised beclomethasone in improving clinical signs of acute airway obstruction induced by exposure to mouldy hay and straw in asthmatic susceptible subjects [34]. On the other hand, Coue€til and colleagues [30] reported that, although the oral administration of prednisone may help reduce airway inflammation, its effect on pulmonary function and clinical signs was marginal, likely the result of poor oral bioavailability of prednisone, and environmental management remains a crucial factor in the management of equine asthma.

This quantitative synthesis shows that inhaled corticosteroids are as effective as systemic corticosteroids in improving the clinical condition of asthmatic horses. Unfortunately, our PICO strategy was not aimed at differentiating between short- and long-term treatments, nor to assess the potential impact of corticosteroid therapy on preventing clinical episodes. It is also important that the choice between inhaled or systemic corticosteroids should also take into account the safety profile of formulations. Conflicting evidence exists on the potential adverse events induced by systemic corticosteroids in horses. Some studies reported that systemic therapy with corticosteroids may induce hypothalamic-pituitary- adrenal axis suppression, hepatopathy, muscle wasting, altered bone metabolism, polyuria, polydipsia, increased susceptibility to infection, insulin resistance, and altered glucose metabolism predisposing to laminitis [39–41]. Conversely, a retrospective case–control study involving a large equine population with various medical conditions documented that oral prednisolone administration did not increase the risk of laminitis [42]. Until further information on the efficacy/safety profile of systemic corticosteroids becomes available, these agents should be administered with caution in subjects with previous laminitis, equine metabolic syndrome, pituitary pars intermedia dysfunction, and concomitant gastrointestinal diseases [41].

This meta-analysis provides high-quality evidence that inhaled corticosteroids are effective in preventing equine asthma, assessed using the GRADE approach. This suggests that no larger clinical trials are needed on inhalant formulations. On the other hand, the GRADE analysis documents only a moderate quality of

evidence for systemic corticosteroids. In fact, while no bias was detected in the subset analysis focused on inhaled corticosteroids, significant publication bias was detected for systemic corticosteroids. This was not only due to the heterogeneity of Jadad score across the studies, but also because one of the treatment group in one study [28] induced the so called 'small study effect', in which the treatment effect was greater than that observed in larger and more robustly designed studies. The high level of heterogeneity detected for systemic corticosteroids limited the GRADE score for systemic formulations. Nevertheless, the overall analysis of risk of bias and evidence profile suggested that the results of this pair-wise and network meta-analysis are robust and reliable. Veterinary research can be affected by financial constraints, especially with regard to the number of enrolled animals. An acceptable Jadad score ≥3 [3] may be easily reached by performing randomised studies (+1 point) that clearly report the randomisation method (+1 point), withdrawals and dropouts (+1 point) [10]. Appropriate blinding further improves the Jadad score. While most of the analysed studies emphasised statistically significant findings, some of the trials included in this meta-analysis [28–31] reported non-significant or poorly significant results that contributed to the final effect estimates, as confirmed by the analysis of forest plot. Thus, the question as to whether a low statistical significance level determines whether or not the experimental outcome has real clinical value remains open [2]. In this context, we have attempted for the first time to quantify the clinical relevance of corticosteroid therapy in asthmatic horses. The anchor-based approach cannot be applied to veterinary medicine since this method compares changes in scores with an 'anchor' as reference, usually the subjective perception of a treatment. The Delphi method, based on a panel of experts who reach consensus concerning a specific outcome, has been recently applied to revise and update the previous ACVIM Consensus Statement on inflammatory airway disease in horses [1]. Currently, scientific evidence on the management of horses with inflammatory airway disease is sparse and therapeutic choices are mainly based on clinical experience [1]. Thus, we used the distribution-based method that focuses on the variance and distributional properties of scores in a population of diseased subjects [43]. In veterinary medicine, subjective interpretation plays a pivotal role in quantifying clinical effects and concept of a Minimally Detectable Difference (MDD) is more appropriate than MID to assess the threshold of detection 25]. Thus, the term MCDD seems adequate for quantifying the magnitude of clinical benefit induced by corticosteroid therapy in symptomatic asthmatic

horses. This approach let us identify IDEASS (deposit number 2017002958, SIAE [Italian Society of Authors and Editors], Rome, Italy), a scoring algorithm that permits the clinical quantification of the level of asthma severity via an eight-point scale. The current quantitative synthesis shows that a one-point change in IDEASS represents the MCDD in equine asthma symptoms. Therefore, we have demonstrated that the treatment with corticosteroids not only improved the clinical condition by a strict statistical point of view, but led to a clinical benefit by reducing 30% the IDEASS value. In addition, since the lower 95% CI of the IDEASS change did not overlap the non- inferiority margin represented by a one-point change, we can assert that corticosteroids have a significant clinically beneficial impact in asthmatic horses. Several different scores have been proposed to assess the clinical effect of corticosteroid treatment on equine asthma [31,33,34,36]. Barton and colleagues [33] have recently proposed a clinical score system that requires the analysis of bronchoalveolar lavage (BAL) fluid. BAL can result in transient improvement in lung function in some asthmatic horses [44] and therefore may alter the results of the score when sequential clinical assessments are made after this procedure. On the other hand, since the sedative drugs used to perform BAL may induce bronchospasm [45–49], this procedure should be avoided in horses presenting with acute severe asthma. Tesarowski and colleagues [36] proposed another weighted clinical scoring system that, although very detailed, was potentially sensitive to the subjective interpretation of clinical signs. Furthermore, the 25-point scale used in this scoring system is not consistent with an optimal response scale, that should include a maximum 10 points [23,24,50]. Finally, there is a mucus score [31] that needs to be associated with other scoring systems to provide adequate information on clinical conditions. IDEASS improves on the scoring approach proposed by Rush and colleagues [34]. Although this meta-analysis found that 0.8 changes in the Rush's score may be a suitable MCDD in clinical trials, this scoring system was based on a point-by-point incremental method. Therefore, Rush's score cannot be applied to assess the MCDD in a single subject. The IDEASS can be used in both clinical trials and single subjects to rank the severity of equine asthma, with one-point change representing the MCDD and IDEASS is consistent with the optimal point response scale [23,24,50]. However, since IDEASS results from an analytical process and not from specific clinical data, this score may be affected by the subjective assessment of the clinical findings. Therefore, before applying IDEASS in clinical practice and research studies, there is a need to validate this system.

In conclusion, the findings of this quantitative synthesis support the use of inhaled corticosteroids to improve the clinical condition of asthmatic horses. We propose IDEASS, a scoring system that, providing it can be validated in future studies, may be useful to rank the level of asthma severity and assess the clinical effect of pharmacotherapy in asthmatic horses.

Authors' declaration of interests

The authors report no potential competing interests.

Ethical animal research

The Ethical approval of this meta-analytical protocol was obtained by the local Board (ID: 0172521/2017 ASL ROMA 2).

Source of funding

This study was supported by institutional funds (University of Rome 'Tor Vergata').

Acknowledgements

The authors thank Prof Diana Lanzellotti, English Lector, Nasa School (Gricignano, Caserta, Italy); Dr Pietro Alfonsi (ASL Roma 2, UOC Igiene degli Allevamenti e delle Produzioni Zootecniche, Rome, Italy) and Dr Giuseppe Cito (ASL Roma 2, UOC Tutela igienico sanitaria degli alimenti di origine animale, Rome, Italy).

Authorship

L Calzetta designed and executed the study, analysed and interpreted the data. P. Rogliani, C. Page, P. Roncada, E. Pistocchini, A. Soggiu, C. Piras and A. Urbani interpreted the data. M.G. Matera designed the study and interpreted the data. All authors prepared the manuscript and gave their final approval.

Manufacturer's address

aGraphPad Prism, La Jolla, California, USA.

292 **References**

293 1Couetil, L.L., Cardwell, J.M., Gerber, V., Lavoie, J.P., Leguillette, R. and Richard, E.A. (2016) Inflammatory
294 airway disease of horses–revised consensus statement. J. Vet. Intern. Med. 30, 503- 515.

295 2Muir, W.W. (2017) Can the quality and clinical relevance of Equine Veterinary Journal publications be
296 improved? A time for reflection and refinement. Equine Vet. J. 49, 135- 137.

297 3Calzetta, L., Roncada, P., di Cave, D., Bonizzi, L., Urbani, A., Pistocchini, E., Rogliani, P. and Matera, M.G.
298 (2017) Pharmacological treatments in asthma-affected horses: a pair-wise and network meta-analysis.
299 Equine Vet. 49, 710- 717.

300 4Moher, D., Liberati, A., Tetzlaff, J. and Altman, D.G.; PRISMA Group. (2009) Preferred reporting items for
301 systematic reviews and meta-analyses: the PRISMA statement. Open Med. 3, e123- e130.

302 5Moher, D., Shamseer, L., Clarke, M., Ghersi, D., Liberati, A., Petticrew, M., Shekelle, P. and Stewart, L.A.;
303 PRISMA-P Group. (2015) Preferred reporting items for systematic review and meta-analysis protocols
304 (PRISMA-P) 2015 statement. Syst. Rev. 4: 1.

305 6Leclere, M., Lavoie-Lamoureux, A. and Lavoie, J.P. (2011) Heaves, an asthma-like disease of horses.
306 Respirology 16, 1027- 1046.

307 7Bullone, M. and Lavoie, J.P. (2015) Asthma “of horses and men”–how can equine heaves help us better
308 understand human asthma immunopathology and its functional consequences? Mol. Immunol. 66, 97- 105.

309 8Picandet, V., Leguillette, R. and Lavoie, J.P. (2003) Comparison of efficacy and tolerability of isoflupredone
310 and dexamethasone in the treatment of horses affected with recurrent airway obstruction (‘heaves’). Equine
311 Vet. J. 35, 419- 424.

312 9Shariff, S.Z., Bejaimal, S.A., Sontrop, J.M., Iansavichus, A.V., Haynes, R.B., Weir, M.A. and Garg, A.X. (2013)
313 Retrieving clinical evidence: a comparison of PubMed and Google Scholar for quick clinical searches. J. Med.
314 Internet Res. 15, e164.

10Jadad, A.R., Moore, R.A., Carroll, D., Jenkinson, C., Reynolds, D.J., Gavaghan, D.J. and McQuay, H.J. (1996) Assessing the quality of reports of randomized clinical trials: is blinding necessary? Control. Clin. Trials 17, 1-12.

11Egger, M., Smith, G.D., Schneider, M. and Minder, C. (1997) Bias in meta-analysis detected by a simple, graphical test. BMJ 315, 629- 634.

12Sterne, J.A., Gavaghan, D. and Egger, M. (2000) Publication and related bias in meta-analysis: power of statistical tests and prevalence in the literature. J. Clin. Epidemiol. 53, 1119- 1129.

13Thorlund, K., Imberger, G., Walsh, M., Chu, R., Gluud, C., Wetterslev, J., Guyatt, G., Devereaux, P.J. and Thabane, L. (2011) The number of patients and events required to limit the risk of overestimation of intervention effects in meta-analysis–a simulation study. PLoS ONE 6, e25491.

14Guyatt, G.H., Oxman, A.D., Kunz, R., Brozek, J., Alonso-Coello, P., Rind, D., Devereaux, P.J., Montori, V.M., Freyschuss, B., Vist, G., Jaeschke, R., Williams, J.W. Jr, Murad, M.H., Sinclair, D., Falck-Ytter, Y., Meerpohl, J., Whittington, C., Thorlund, K., Andrews, J. and Schunemann, H.J. (2011) GRADE guidelines 6. Rating the quality of evidence–imprecision. J. Clin. Epidemiol. 64, 1283- 1293.

15Guyatt, 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 Schunemann, H.J. (2011) GRADE guidelines: 1. Introduction-GRADE evidence profiles and summary of findings tables. J. Clin. Epidemiol. 64, 383- 394.

16Borenstein, M. (2009) Introduction to Meta-Analysis, John Wiley & Sons, Chichester, U.K. pp xxviii, 421 p.

17DeCoster, J. (2004) Meta-analysis notes. http://www.stat-help.com/meta.pdf. Accessed June 14, 2017.

18Turner, J.R. and Durham, T.A. (2014) Meta-methodology: conducting and reporting meta-analyses. J. Clin. Hypertens. (Greenwich) 16, 91- 93.

19Wallace, B.C., Dahabreh, I.J., Trikalinos, T.A., Lau, J., Trow, P. and Schmid, C.H. (2012) Closing the gap between methodologists and end-users: R as a computational back-end. J. Stat. Softw. 49, 1- 15.

20Higgins, J.P., Thompson, S.G., Deeks, J.J. and Altman, D.G. (2003) Measuring inconsistency in meta-analyses. BMJ 327, 557- 560.

21Brooks, S.P. and Gelman, A. (1998) General methods for monitoring convergence of iterative simulations. J. Comput. Graph. Stat. 7, 434- 455.

22Lu, G. and Ades, A.E. (2006) Assessing evidence inconsistency in mixed treatment comparisons. J. Am. Stat. Ass. 101, 447- 459.

23Copay, A.G., Subach, B.R., Glassman, S.D., Polly, D.W. Jr and Schuler, T.C. (2007) Understanding the minimum clinically important difference: a review of concepts and methods. Spine J. 7, 541- 546.

24Revicki, D., Hays, R.D., Cella, D. and Sloan, J. (2008) Recommended methods for determining responsiveness and minimally important differences for patient-reported outcomes. J. Clin. Epidemiol. 61, 102- 109.

25Norman, G.R., Sloan, J.A. and Wyrwich, K.W. (2003) Interpretation of changes in health-related quality of life: the remarkable universality of half a standard deviation. Med. Care 41, 582- 592.

26Middel, B. and Van Sonderen, E. (2002) Statistical significant change versus relevant or important change in (quasi) experimental design: some conceptual and methodological problems in estimating magnitude of intervention-related change in health services research. Int. J. Integr. Care 2, 1- 18.

27van Valkenhoef, G., Lu, G., de Brock, B., Hillege, H., Ades, A.E. and Welton, N.J. (2012) Automating network meta-analysis. Res. Synth. Methods 3, 285- 299.

28Robinson, N.E., Berney, C., Behan, A. and Derksen, F.J. (2009) Fluticasone propionate aerosol is more effective for prevention than treatment of recurrent airway obstruction. J. Vet. Intern. Med. 23, 1247- 1253.

29Couetil, L.L., Art, T., de Moffarts, B., Becker, M., Melotte, D., Jaspar, F., Bureau, F. and Lekeux, P. (2006) Effect of beclomethasone dipropionate and dexamethasone isonicotinate on lung function, bronchoalveolar lavage fluid cytology, and transcription factor expression in airways of horses with recurrent airway obstruction. J. Vet. Intern. Med. 20, 399- 406.

30Couetil, L.L., Chilcoat, C.D., DeNicola, D.B., Clark, S.P., Glickman, N.W. and Glickman, L.T. (2005) Randomized, controlled study of inhaled fluticasone propionate, oral administration of prednisone, and environmental management of horses with recurrent airway obstruction. Am. J. Vet. Res. 66, 1665- 1674.

31Robinson, N.E., Berney, C., Eberhart, S., deFeijter-Rupp, H.L., Jefcoat, A.M., Cornelisse, C.J., Gerber, V.M. and Derksen, F.J. (2003) Coughing, mucus accumulation, airway obstruction, and airway inflammation in control horses and horses affected with recurrent airway obstruction. Am. J. Vet. Res. 64, 550- 557.

32Courouce-Malblanc, A., Fortier, G., Pronost, S., Siliart, B. and Brachet, G. (2008) Comparison of prednisolone and dexamethasone effects in the presence of environmental control in heaves-affected horses. Vet. J. 175, 227- 233.

33Barton, A.K., Shety, T., Bondzio, A., Einspanier, R. and Gehlen, H. (2016) Metalloproteinases and their inhibitors are influenced by inhalative glucocorticoid therapy in combination with environmental dust reduction in equine recurrent airway obstruction. BMC Vet. Res. 12, 282.

34Rush, B.R., Raub, E.S., Rhoads, W.S., Flaminio, M.J., Matson, C.J., Hakala, J.E. and Gillespie, J.R. (1998) Pulmonary function in horses with recurrent airway obstruction after aerosol and parenteral administration of beclomethasone dipropionate and dexamethasone, respectively. Am. J. Vet. Res. 59, 1039- 1043.

35Seahorn, T.L., Beadle, R.E., McGorum, B.C. and Marley, C.L. (1997) Quantification of antigen-specific antibody concentrations in tracheal lavage fluid of horses with summer pasture-associated obstructive pulmonary disease. Am. J. Vet. Res. 58, 1408- 1411.

36Tesarowski, D.B., Viel, L. and McDonell, W.N. (1996) Pulmonary function measurements during repeated environmental challenge of horses with recurrent airway obstruction (heaves). Am. J. Vet. Res. 57, 1214- 1219.

37Traub-Dargatz, J. (1997) Field examination of the equine patient with nasal discharge. Vet. Clin. North Am. Equine Pract. 13, 561- 588.

385 38Erichsen, D.F., Aviad, A.D., Schultz, R.H. and Kennedy, T.J. (1994) Clinical efficacy and safety of clenbuterol
386 HCl when administered to effect in horses with chronic obstructive pulmonary disease (COPD). Equine Vet.
387 J. 26, 331- 336.

388 39Klinkhamer, K., Menheere, P.P. and van der Kolk, J.H. (2011) Basal glucose metabolism and peripheral
389 insulin sensitivity in equine pituitary pars intermedia dysfunction. Vet. Q 31, 19- 28.

390 40French, K., Pollitt, C.C. and Pass, M.A. (2000) Pharmacokinetics and metabolic effects of triamcinolone
391 acetonide and their possible relationships to glucocorticoid-induced laminitis in horses. J. Vet. Pharmacol.
392 Ther. 23, 287- 292.

393 41Leclere, M. (2017) Corticosteroids and immune suppressive therapies in horses. Vet. Clin. North Am.
394 Equine Pract. 33, 17- 27.

395 42Jordan, V.J., Ireland, J.L. and Rendle, D.I. 2017. Does oral prednisolone treatment increase the incidence
396 of acute laminitis? Equine Vet. J. 49, 19- 25.

397 43Cazzola, M., MacNee, W., Martinez, F.J., Rabe, K.F., Franciosi, L.G., Barnes, P.J., Brusasco, V., Burge, P.S.,
398 Calverley, P.M., Celli, B.R., Jones, P.W., Mahler, D.A., Make, B., Miravitlles, M., Page, C.P., Palange, P., Parr,
399 D., Pistolesi, M., Rennard, S.I., Rutten-van Molken, M.P., Stockley, R., Sullivan, S.D., Wedzicha, J.A. and
400 Wouters, E.F.; American Thoracic Society; European Respiratory Society Task Force on outcomes of COPD.
401 (2008) Outcomes for COPD pharmacological trials: from lung function to biomarkers. Eur. Respir. J. 31, 416-
402 469.

403 44Leguillette, R. and Lavoie, J.P. (2006) Effects of the bronchoalveolar lavage procedure on lung function in
404 horses with clinical exacerbation of recurrent airway obstruction. Am. J. Vet. Res. 67, 1929- 1933.

405 45Calzetta, L., Soggiu, A., Roncada, P., Bonizzi, L., Pistocchini, E., Urbani, A., Rinaldi, B. and Matera, M.G.
406 (2015) Propofol protects against opioid-induced hyperresponsiveness of airway smooth muscle in a horse
407 model of target-controlled infusion anaesthesia. Eur. J. Pharmacol. 765, 463- 471.

46Rogliani, P., Calzetta, L., Rendina, E.A., Massullo, D., Dauri, M., Rinaldi, B., Capuano, A. and Matera, M.G. (2013) The influence of propofol, remifentanil and lidocaine on the tone of human bronchial smooth muscle. Pulm. Pharmacol. Ther. 26, 325- 331.

47Matera, M.G., Calzetta, L., Peli, A., Scagliarini, A., Matera, C. and Cazzola, M. (2005) Immune sensitization of equine bronchus: glutathione, IL-1beta expression and tissue responsiveness. Respir. Res. 6, 104.

48Matera, M.G., Calzetta, L., Sanduzzi, A., Page, C.P. and Cazzola, M. (2008) Effects of neuraminidase on equine isolated bronchi. Pulm. Pharmacol. Ther. 21, 624- 629.

49Matera, M.G., Calzetta, L., Rogliani, P., Bardaro, F., Page, C.P. and Cazzola, M. (2011) Evaluation of the effects of the R- and S-enantiomers of salbutamol on equine isolated bronchi. Pulm. Pharmacol. Ther. 24, 221- 226.

50Dawes, J. (2008) Do data characteristics change according to the number of scale points used. Int. J. Market Res. 50, 61- 77.

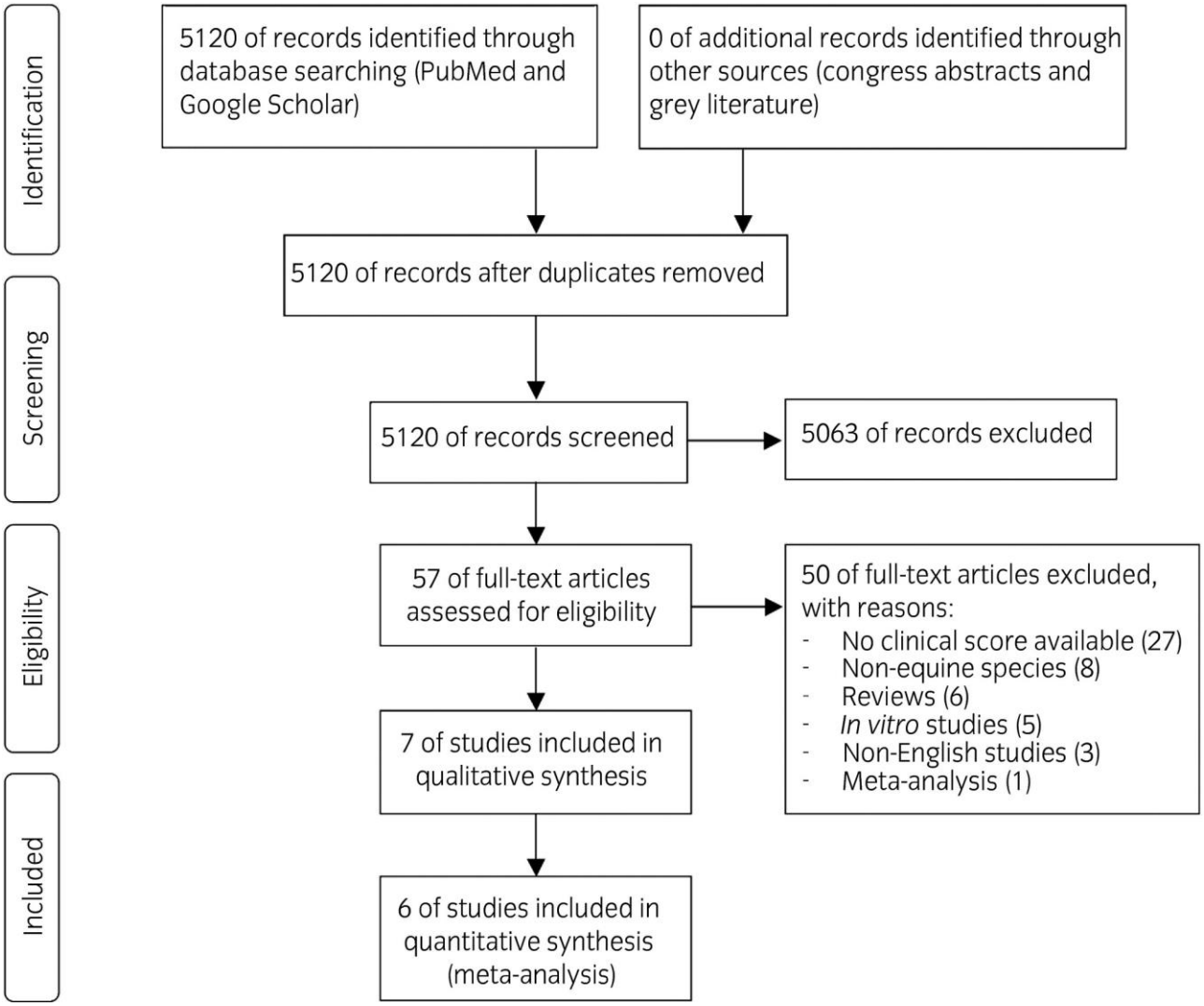
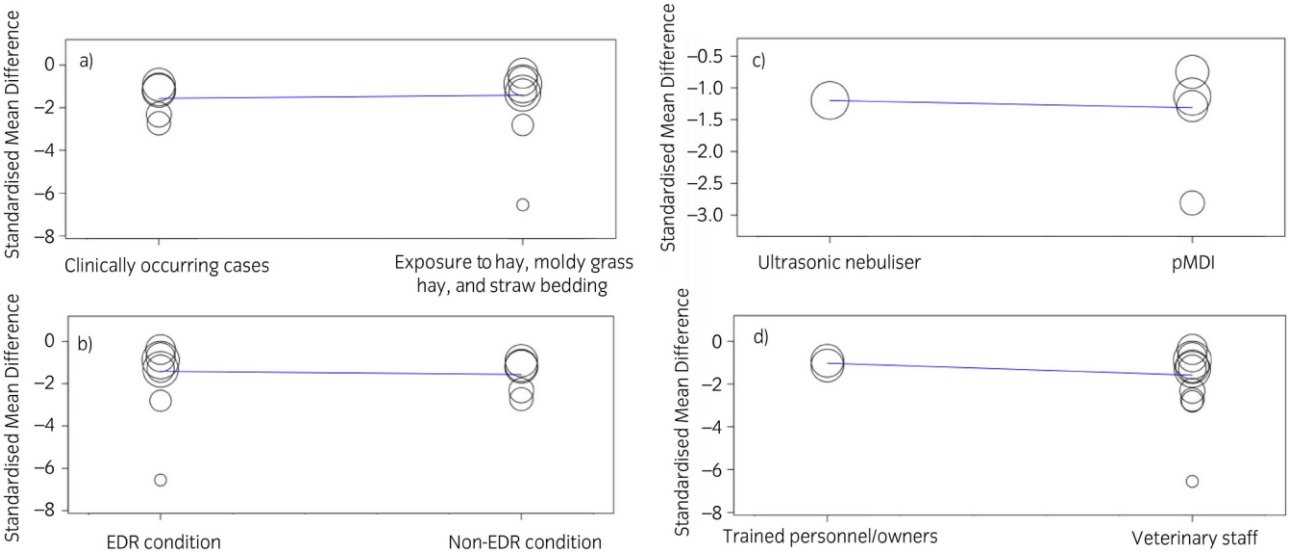


Figure 1. PRISMA flow diagram for the identification of studies included in the meta-analysis concerning the effect of corticosteroid treatment of equine asthma-affected horses.

Acronym	Definition	Description
P	Cases and problems	Horses affected by equine asthma (equine asthma, broken wind, chronic obstructive pulmonary disease [COPD], heaves, inflammatory airway disease [IAD], recurrent airway obstruction [RAO]).
I	Interventions	Corticosteroids, glucocorticosteroids (beclomethasone, budesonide, dexamethasone, fluticasone, isoflupredone, prednisolone, prednisone)
C	Control	Control at baseline and/or placebo
O	Outcome	Effect of corticosteroids on clinical score

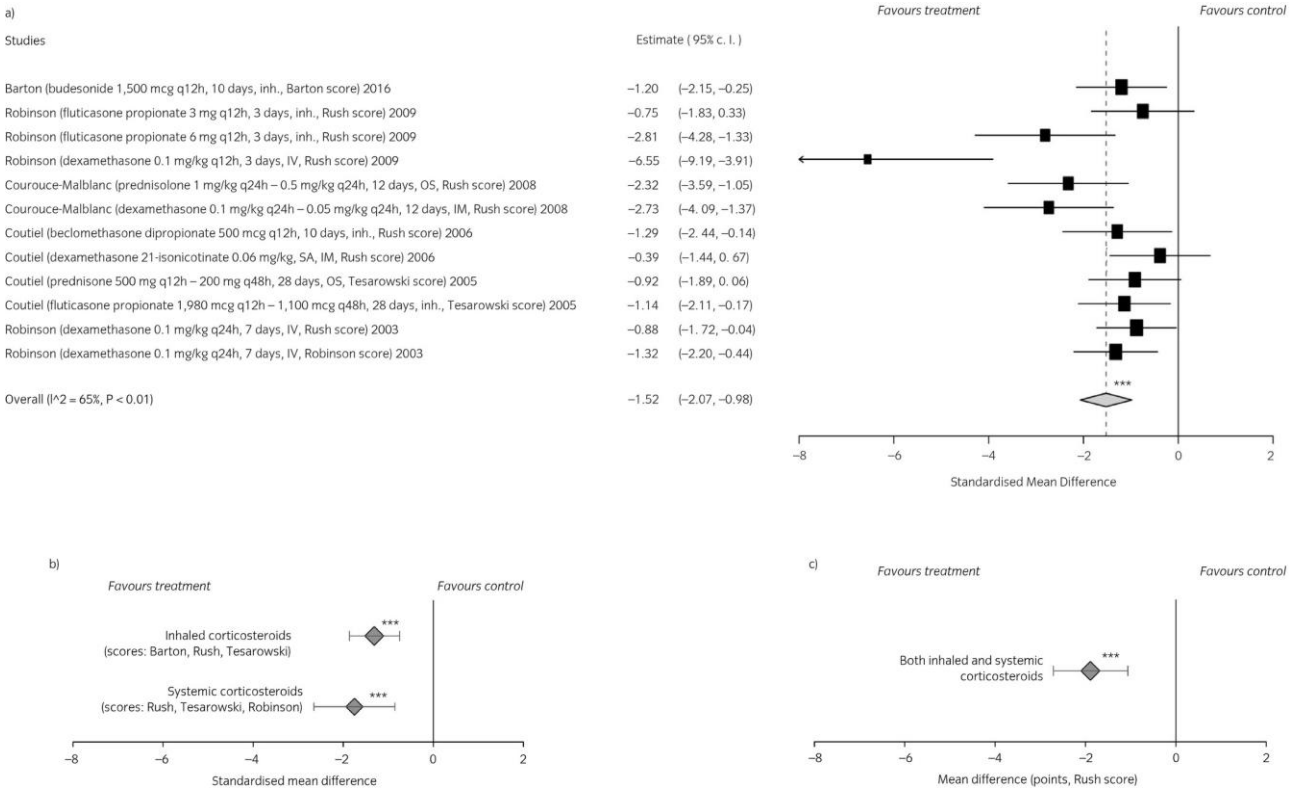
PICO, population, intervention, control and outcomes.

Table 1. Description of the PICO strategy



427

428 Figure 2. Graphical representation of meta-regression models of confounder variables with potential to
429 interact with the size of the effect estimates detected for the changes in clinical score from baseline [a)
430 methods for inducing clinical signs; b) environmental management; c) type of inhaler device; d: operator that
431 administered the drugs]. EDR, environmental dust reduction; pMDI, pressurised metered dose inhaler.



432

433 Figure 3. Overall forest plot pair-wise meta-analysis of the effect of corticosteroid treatment on clinical score
434 in equine asthma (panel a) with subset analysis performed by considering inhaled corticosteroids and
435 systemic corticosteroids (panel b) and subset analysis on studies using the most common clinical scoring
436 system (panel c). Results are expressed as the Standardised Mean Difference (synthesis of studies reporting
437 non-homogeneous clinical scoring scales) and Mean Difference (synthesis of studies reporting consistent
438 clinical scoring scales), vs. control. ***P<0.001 vs. control. Score references: Barton score 33, Rush score 34,
439 Tesarowski score 36, Robinson score 31. IM, intramuscular; Inh., inhalation; IV, intravenous; OS: per os; SA,
440 single administration.

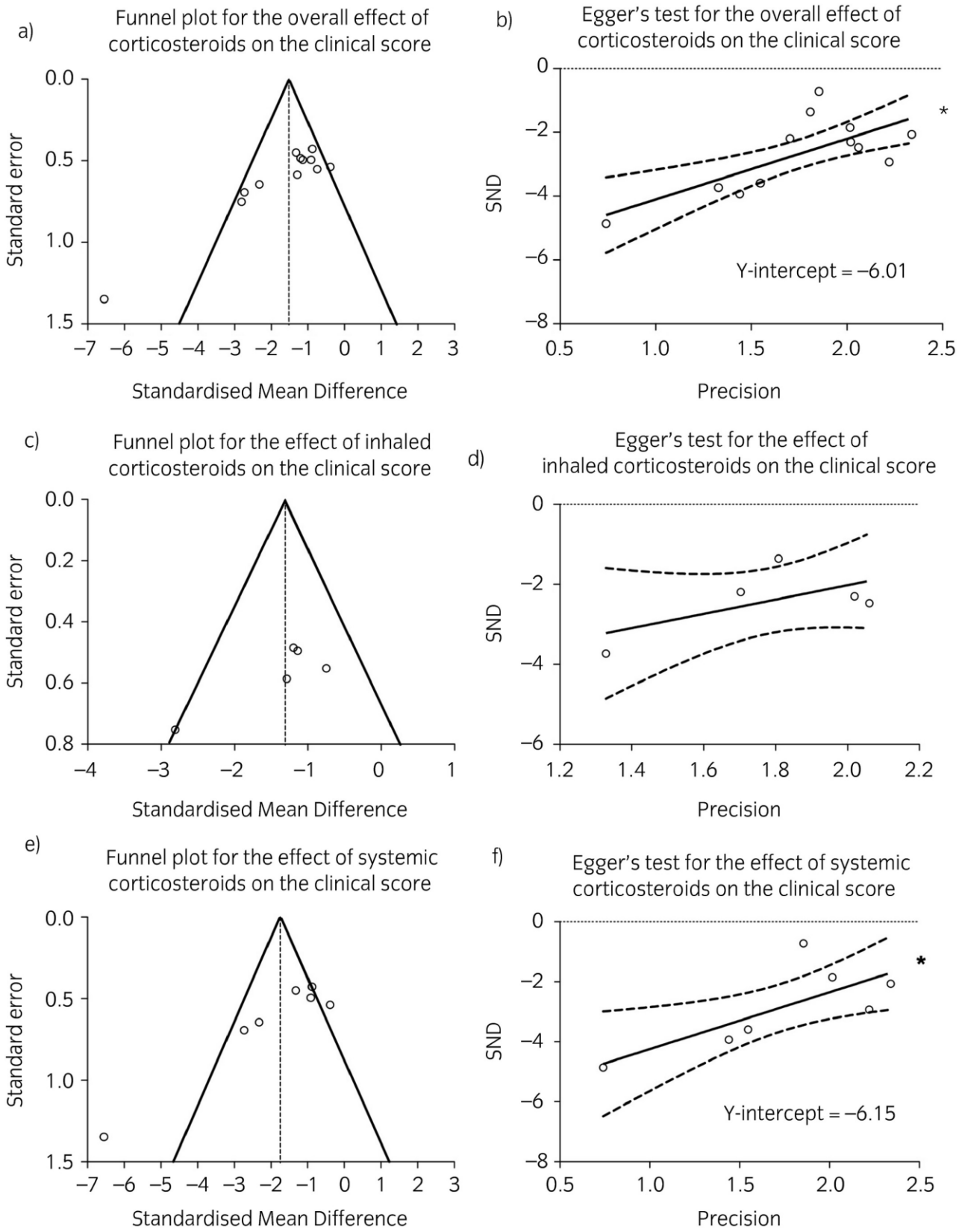
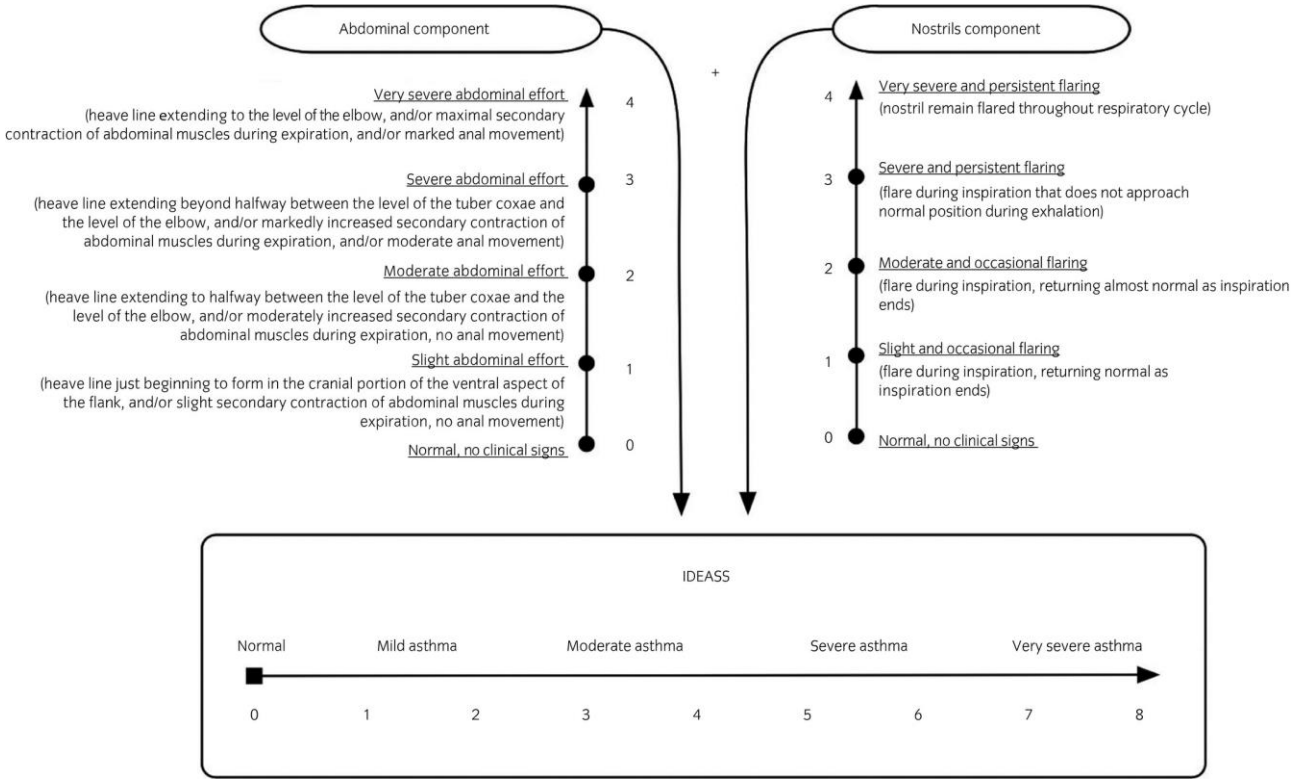


Figure 4. Funnel plots (left panels) and graphical representations of Egger's test (right panels) for the overall effect of corticosteroids on the clinical score of equine asthma-affected horses (a and b), and subset analysis on inhaled corticosteroids (c and d) and systemic corticosteroids (e and f). Funnel plots provide a visual approach to check for publication bias by assessing the symmetry of study distribution. Egger's test quantifies asymmetry in Funnel plots. SND, standard normal deviate. *P<0.1.



447

448 Figure 5. Improved clinically Detectable Equine Asthma Scoring System (IDEASS) for quantifying equine
449 asthma severity. One-point change represents the Minimally Clinically Detectable Difference (MCDD) in
450 clinical signs of equine asthma. Note, for this scoring system, we use the term 'heave line' as the change in
451 muscular definition associated with active abdominal muscular activity during a period of increased effort
452 35. Some clinicians use the same term to refer to abdominal hypertrophy as a result of prolonged or repeated
453 episodes of increased abdominal effort over time which is not its intended meaning here.

454