

Pharmacological treatments in asthma-affected horses: A pair-wise and network meta-analysis

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Summary

Background: Equine asthma is a disease characterised by reversible airflow obstruction, bronchial hyper-responsiveness and airway inflammation following exposure of susceptible horses to specific airborne agents. Although clinical remission can be achieved in a low-airborne dust environment, repeated exacerbations may lead to irreversible airway remodelling. The available data on the pharmacotherapy of equine asthma result from several small studies, and no head-to-head clinical trials have been conducted among the available medications.

Objectives: To assess the impact of the pharmacological interventions in equine asthma and compare the effect of different classes of drugs on lung function.

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

Methods: Literature searches for clinical trials on the pharmacotherapy of equine asthma were performed. The risk of publication bias was assessed by funnel plots and Egger's test. Changes in maximum transpulmonary or pleural pressure, pulmonary resistance and dynamic lung compliance vs. control were analysed via random-effects models and Bayesian networks.

Results: The results obtained from 319 equine asthma-affected horses were extracted from 32 studies. Bronchodilators, corticosteroids and chromones improved maximum transpulmonary or pleural pressure (range: -8.0 to -21.4 cmH₂O; $P < 0.001$). Bronchodilators, corticosteroids and furosemide reduced pulmonary resistance (range: -1.2 to -1.9 cmH₂O/L/s; $P < 0.001$), and weakly increased dynamic lung compliance. Inhaled β_2 -adrenoreceptor (β_2 -AR) agonists and inhaled corticosteroids had the highest probability of being the best therapies. Long-term treatments were more effective than short-term treatments.

Main limitations: Weak publication bias was detected.

Conclusions: This study demonstrates that long-term treatments with inhaled corticosteroids and long-acting β_2 -AR agonists may represent the first choice for treating equine asthma. Further high quality clinical trials are needed to clarify whether inhaled bronchodilators should be preferred to inhaled corticosteroids or vice versa, and to investigate the potential superiority of combination therapy in equine asthma.

Keywords: horse; heaves; therapy; meta-analysis

Introduction

Equine asthma (previously known as recurrent airway obstruction [RAO], heaves or chronic obstructive pulmonary disease [COPD]), is characterised by variable and recurring airflow obstruction, airway hyperresponsiveness (AHR) and airway inflammation following exposure of susceptible subjects to specific airborne agents. Equine asthma is reversible, and removal of the inciting airborne agents may lead to disease remission [1,2]. Current epidemiological data indicate that equine asthma affects up to 20% of mature horses in temperate climates [3]. As with human asthma, equine asthma results from interactions between multiple genes and environmental factors [4], such as *IL4RA* polymorphisms, and changes in the IL-4 receptor function [5,6]. Specific antigens responsible for the onset of clinical signs in susceptible animals may vary according to environmental exposure [2,7].

Equine asthma may lead to irreversible ultrastructural changes in the lung and induce persistent peripheral airway obstruction, even in the absence of neutrophilic airway inflammation when animals are maintained in a low-dust environment [1]. Therefore, since to date there is no known cure for equine asthma, the corner stone of management is environmental control [2]. However, treatment with anti-inflammatory agents (corticosteroids) and bronchodilators (antimuscarinic agents and

β_2 -adrenoreceptor [β_2 -AR] agonists) may provide rapid relief of airway obstruction in horses with severe clinical disease, and where environmental change is problematic [1,2].

Many molecules targeting specific intracellular pathways or mediators have been tested for treating equine asthma, such as the mast cell stabilisers chromones, the nonspecific phosphodiesterase (PDE) inhibitors methylxanthines and specific PDE4 inhibitors [2,7,8]. Furosemide induces a bronchorelaxant effect in asthma-affected horses [1]. Although the mechanisms of action by which this medication improves airway function are not well understood, furosemide may have a local effect on vagal sensory afferent discharge independent of reflex anticholinergic bronchodilation [9], perhaps by damping the responsiveness of sensory epithelial receptors through modulation of their ionic or osmotic environment [10]. This indicates that furosemide's effects on airway responses are not due to a direct modulation of airway smooth muscle contractility [11]. Although it has been suggested that furosemide may act like chromones and prevent release of inflammatory mediators, it has been ruled out that this may be a major mechanism of action in equine asthma [11].

Nonsymptomatic horses may suddenly develop severe AHR in specific conditions, such as sedation/anaesthesia, infections and increased oxidative stress [12–14]. Unfortunately, currently available data on the

pharmacotherapy of equine asthma is derived from studies that, overall, enrolled a small number of animals. In addition, there are no head-to-head clinical trials comparing different inhalant medications with regard to improvement in lung function.

Therefore, in the absence of a pragmatic synthesis of the currently available body of the literature, we performed a meta-analysis of the clinical evidence to assess the impact of pharmacological interventions on the lung function in equine asthma cases, and provide a rank of effectiveness of different therapeutic approaches. The impact of corticosteroids and bronchodilators on challenge-induced AHR was specifically investigated.

Materials and methods

Search strategy

This meta-analysis has been carried out in agreement with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) Statement (Fig 1) [15].

We performed a comprehensive literature search for clinical trials written in English and regarding the impact of different pharmacological treatments on the lung function of mature horses affected by chronic, reversible, inflammatory airway obstruction called either RAO or heaves [16]. The term COPD was previously used [17] and, therefore, the terms “RAO” OR “heaves” OR “COPD” AND “equine” OR “horse” AND “therapy” OR “treatment” were searched to identifying the studies to be included. The search was performed on PubMed and Google Scholar in order to provide for relevant studies published up to 31 August 2016 [18].

Study selection

All studies assessing the impact of drugs on lung function in equine asthma were included. The quantitative meta-analysis was carried out on treatment options investigated at least in two clinical trials. Two reviewers independently examined the clinical trials and any difference in opinion about eligibility was resolved by consensus.

Quality score and risk of bias assessment

The Jadad score, with a scale of 1–5 (score of 5 being the highest), was used to assess study quality of concerning the likelihood of bias related to randomisation, double blinding, withdrawals and dropouts [19]. Two reviewers independently assessed the quality of individual studies, and any difference in opinion about the quality score was resolved by consensus.

The risk of publication bias was analysed by applying the Egger's test to assess funnel plot symmetry through the following regression equation: $SND = a + b \times \text{precision}$, where SND represents the standard normal deviate (treatment effect divided by its standard error), and precision represents the reciprocal of the standard error. Evidence of asymmetry from Egger's test was considered to be significant for $P < 0.1$, and the graphical representation of 90% confidence bands are presented [20].

Data synthesis and analysis

The endpoint of this meta-analysis was to assess the impact of pharmacological therapy on lung function of equine asthma-affected horses, with regard to maximum changes in transpulmonary or pleural pressure ($\Delta P_{l_{max}}$), pulmonary resistance (R_L) and dynamic lung compliance (C_{dyn}) compared with control values detected at baseline and/or in placebo groups.

A pair-wise meta-analysis was carried out to calculate the effect estimates of pharmacological treatments vs. control, and subset analysis was performed by considering exclusively inhaled medications. A network meta-analysis was performed in order to rank the effectiveness of the different classes of drugs administered via inhalation, and for ranking the overall impact of the drugs on pulmonary function with regard to the duration of treatment, defined as ≤ 3 days for short-term treatments and > 3 days for long-term treatments [21]. A further subset analysis was carried out to rank the effectiveness of short-acting vs. long-acting β_2 -AR agonists. The effectiveness of corticosteroids vs. bronchodilators (used either as systemic or inhaled forms) was also assessed with regard to AHR induced by different challenges leading to acute exacerbation.

Researchers were operating independently and, thus, a common effect size cannot be assumed. Therefore, pair-wise meta-analysis was performed

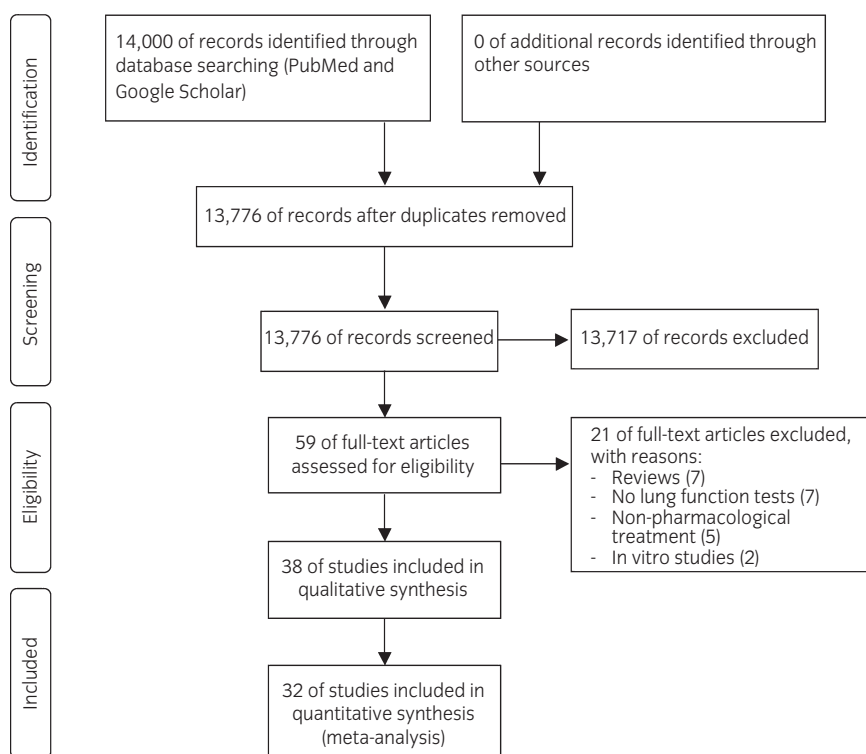


Fig 1: PRISMA flow diagram for the identification of studies included in the meta-analysis concerning the impact of pharmacological treatment of equine asthma-affected horses.

using a random-effects model in order to balance the study weights and to adequately estimate the confidence interval of the mean distribution of drugs effects on the investigated variables [22]. Values are expressed as mean difference and 95% confidence interval. Moderate to high levels of heterogeneity were considered for $I^2 > 50\%$ [23].

Network meta-analysis was performed by using a full Bayesian evidence network (chains: 4; initial values scaling: 2.5; tuning iterations: 20,000; simulation iterations: 50,000; tuning interval: 10) and convergence diagnostics for consistency was assessed by using the Brooks–Gelman–Rubin method. Results of network meta-analysis are expressed as relative effect (RE) and 95% credible interval (CrI). The probability that each intervention arm was the most effective was calculated by counting the proportion of iterations of the chain in which each intervention arm had the highest mean difference, and the surface under the cumulative ranking (SUCRA), that represents the summary of these probabilities, was also calculated. The SUCRA is 100% when a treatment is certain to be the best, and 0% when a treatment is certain to be the worst [24].

The optimal information size (OIS) was calculated as previously reported [25,26], and the statistical significance was set at $P \leq 0.05$. OpenMetaAnalyst [27] was used to perform pair-wise meta-analysis, GeMTC [28] for network meta-analysis and GraphPad Prism^a to graph the data.

Results

Study characteristics

Data obtained from 319 equine asthma-affected horses were extracted from 32 studies, some of which investigated two or more drugs. Ten studies used antimuscarinic agents (eight used a systemic form and one an inhaled form, one used both) [29–38]; nine studies used β_2 -AR agonists (two used a systemic form and seven an inhaled form) [29,39–46]; 13 studies used corticosteroids (nine used a systemic form and one an inhaled form, three used both) [31,32,35,36,47–55]; three used methylxanthines via systemic administration [29,33,53]; three studies used chromones via inhalation [56–58]; two studies used furosemide via systemic administration [11,59]; and one study, including two clinical trials, used a PDE4 inhibitor via systemic administration [36]. Overall, the available inclusion criteria and populations' characteristics of the analysed studies were homogeneous. All studies were published between 1979 and 2014, and the duration of clinical trials ranged from 1 to 44 days.

Only two studies reached a Jadad score ≥ 3 [38,52], 18 studies were of modest quality (Jadad score < 3 and ≥ 1) [30–34,36,37,39,42–46,50,51,53,55,59], and for 12 studies the Jadad score was 0 [11,29,35,40,41,47–49,54,56–58]. Relevant patient demographics, baseline, study characteristics and Jadad score are summarised in Supplementary Item 1.

Pair-wise meta-analysis

Antimuscarinic agents, β_2 -AR agonists, corticosteroids and chromones, but not methylxanthines, significantly improved $\Delta P_{\text{pl,max}}$ with effect estimate values between -8.00 and -21.35 cmH₂O compared to controls ($P < 0.001$; Fig 2a). Subset analysis of inhaled medications indicated increased effectiveness for β_2 -AR agonists ($P = 0.05$), whereas no differences were detected for antimuscarinic agents, corticosteroids and chromones (Fig 2b).

Antimuscarinic agents, β_2 -AR agonists, corticosteroids and furosemide significantly reduced R_L between -1.20 and -1.91 cmH₂O/L/s compared to controls ($P < 0.001$), PDE4 inhibitors worsened the R_L values, whereas methylxanthines had no significant influence on this variable (Fig 2c). Subset analysis did not indicate any increased effectiveness for inhaled medications (Fig 2d).

Antimuscarinic agents, β_2 -AR agonists, corticosteroids and furosemide elicited a weak but significant increase of C_{dyn} compared to controls ($P < 0.001$), and administering antimuscarinic agents and β_2 -AR agonists via inhalation did not significantly modify the effect estimates (Fig 2e, f). Forest plots from the pair-wise meta-analysis are reported in Supplementary Item 2.

Network meta-analysis

The network meta-analysis of inhaled medications showed significantly ($P \leq 0.05$) greater effectiveness of β_2 -AR agonists compared to corticosteroids and chromones, but not compared to antimuscarinic agents ($P > 0.05$), with regard to $\Delta P_{\text{pl,max}}$ improvement. Both corticosteroids and β_2 -AR agonists were significantly ($P \leq 0.05$) more effective than antimuscarinic agents in reducing R_L , and no significant ($P > 0.05$) difference was detected in C_{dyn} changes between antimuscarinic agents and β_2 -AR agonists (Table 1).

SUCRA analysis indicated that β_2 -AR agonists had the highest probability of reducing $\Delta P_{\text{pl,max}}$, followed by corticosteroids, antimuscarinic agents and chromones. Corticosteroids scored highest for improving R_L , followed by β_2 -AR agonists and antimuscarinic agents. β_2 -AR agonists and antimuscarinic agents had the same probability of being the best therapy with regard to C_{dyn} improvement (Table 2).

Long-term treatments were more effective than short-term treatments in improving the overall lung function (SUCRA: 86.0% and 63.5%, respectively). Subset analysis focused on corticosteroids and bronchodilators (either β_2 -AR agonists and antimuscarinic agents) indicated that long-term corticosteroids were more effective than long-term bronchodilators (SUCRA: 87.5% and 68.8%, respectively), whereas short-term corticosteroids were less effective than short-term bronchodilators (SUCRA: 43.8% and 50.0%, respectively). Among bronchodilators, short-term β_2 -AR agonists were significantly ($P \leq 0.05$) more effective than long-term β_2 -AR agonists in improving respiratory mechanics (SUCRA: 98.0% and 50.5%, respectively).

No significant difference ($P > 0.05$) was detected between corticosteroids and bronchodilators (either β_2 -AR agonists and antimuscarinic agents) with regard to the impact on challenge-induced AHR [30–32,44,49,50,54] (corticosteroids vs. bronchodilators: $\Delta P_{\text{pl,max}}$ RE -11.79 , 95% CrI -28.09 to 4.82 ; R_L RE 0.24 , 95% CrI -1.01 to 1.53). However, network meta-analysis indicated greater effectiveness of corticosteroids over bronchodilators in improving $\Delta P_{\text{pl,max}}$ (SUCRA: 96.5% and 51.5%, respectively), whereas corticosteroids were less effective than bronchodilators in improving R_L (SUCRA: 66.0% and 83.0%, respectively) during challenge-induced AHR.

Heterogeneity, publication bias and OIS

Significant levels of heterogeneity were detected for antimuscarinic agents, β_2 -AR agonists, and corticosteroids with regard to $\Delta P_{\text{pl,max}}$ ($I^2 = 87\%$, $P < 0.001$), for antimuscarinic agents and corticosteroids with regard to R_L ($I^2 = 77\%$, $P < 0.001$), and for corticosteroids with regard to C_{dyn} ($I^2 = 60\%$, $P < 0.001$).

No significant heterogeneity was detected for chromones and methylxanthines with regard to $\Delta P_{\text{pl,max}}$ ($P > 0.09$), for β_2 -AR agonists, methylxanthines, furosemide and PDE4 inhibitors with regard to R_L ($P > 0.2$), and for antimuscarinic agents, β_2 -AR agonists and furosemide with regard to C_{dyn} ($P > 0.3$). Detailed results on heterogeneity are reported in Supplementary Item 2.

Subset analysis performed by considering exclusively inhaled medications did not result in any substantial levels of heterogeneity (overall: $I^2 = 5\%$, $P > 0.05$), excluded for corticosteroids with regard to $\Delta P_{\text{pl,max}}$ ($I^2 = 81\%$, $P < 0.001$).

Analysis of heterogeneity was generally confirmed by funnel plots. Egger's test detected a signal ($P < 0.1$) of asymmetry for both β_2 -AR agonists and corticosteroids administered via inhalation, suggesting for potential publication bias (Fig 3).

The cumulative number of enrolled horses in the studies reached the OIS for a binary outcome meta-analysis for either $\Delta P_{\text{pl,max}}$ (OIS: 12; available information: 313; delta: +301), R_L (OIS: 12; available information: 214; delta: +202), and C_{dyn} (OIS: 38; available information: 87; delta: +49).

Discussion

This meta-analysis demonstrates that the majority of pharmacological treatments for equine asthma led to significant improvement of lung function, and that long-term treatments are more effective than drugs administered short-term. Systemic administration of drugs was nonsuperior to the inhalant route, and both antimuscarinic agents and corticosteroids were as effective given systemically as via inhalation,

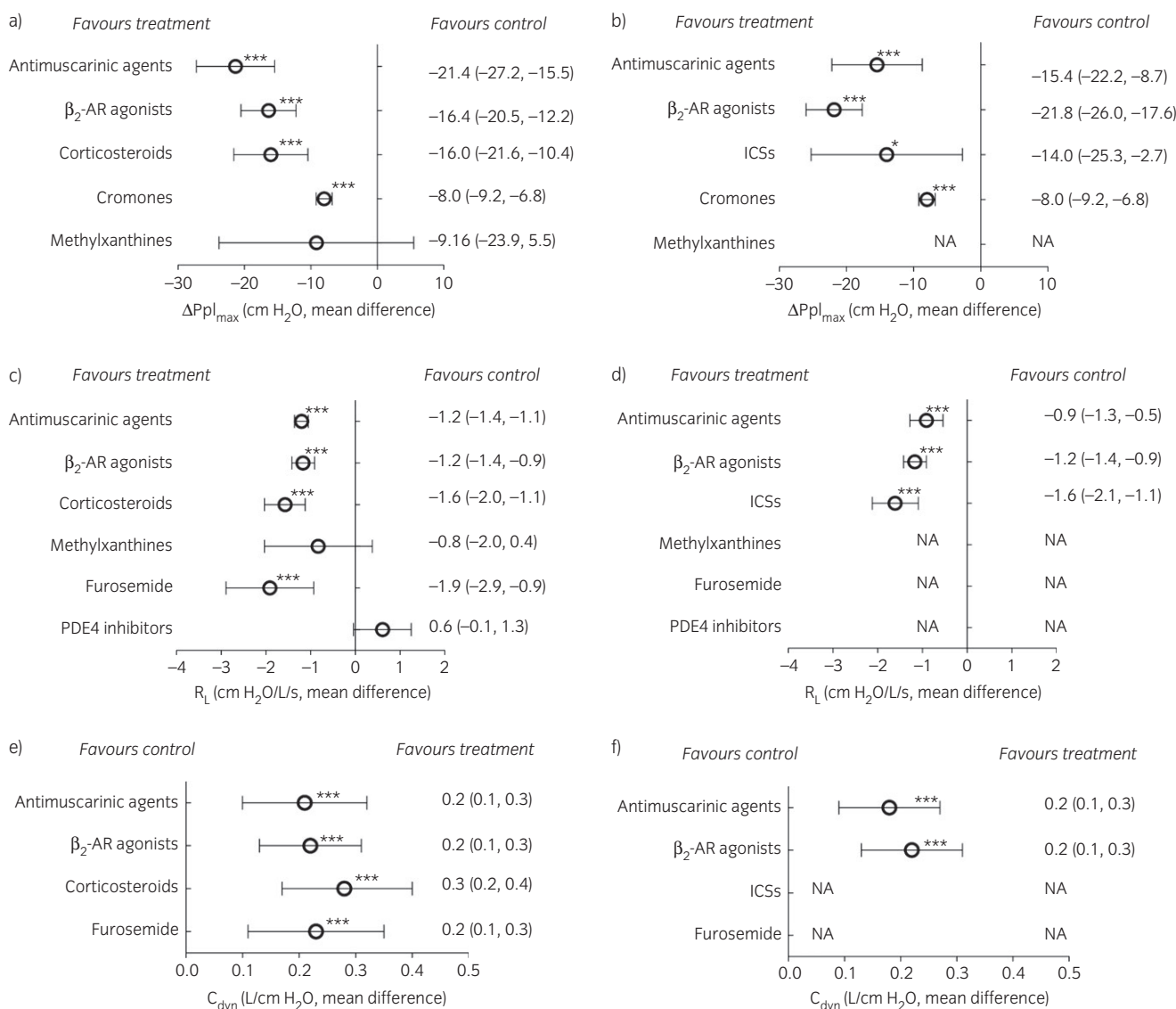


Fig 2: Forest plot pair-wise meta-analysis of the impact of pharmacological treatments on maximum changes in pleural pressure ($\Delta P_{pl_{max}}$, a, b), pulmonary resistance (R_L , c, d) and dynamic lung compliance (C_{dyn} , e, f) of equine asthma-affected horses. Left panels: overall analysis; right panels: subset analysis performed by considering exclusively inhaled medications. Results are expressed as the mean difference vs. control inhaled corticosteroids, inhaled corticosteroids; NA, not available.

whereas β_2 -AR agonists were more effective via inhalation, at least in improving $\Delta P_{pl_{max}}$. Although previous investigations [51,52,54] have demonstrated that systemic corticosteroids may lead to quicker and greater improvement compared to inhaled forms, especially in periods of acute exacerbation [54], our findings indicate that the systemic administration of corticosteroids did not induce a greater improvement of lung function compared with inhalation. Furthermore, inhaled cromones were effective in improving lung function, although less than the other investigated drugs. However, these findings should be interpreted with caution considering the limitations of the studies included in this meta-analysis.

Methylxanthines and PDE4 inhibitors were studied exclusively via systemic administration in the clinical trials we selected for this meta-analysis. Our synthesis also suggests that furosemide given i.v. was as effective as bronchodilators in improving R_L and C_{dyn} . Since inhaled furosemide was investigated in only one clinical trial [11], no meta-analysis of this therapy compared to inhaled bronchodilators or corticosteroids was performed. However, it was reported that the time course and extent of lung function changes were similar following aerosol or intravenous furosemide administration [11].

Although methylxanthines have bronchorelaxant and anti-inflammatory effects in human patients with chronic obstructive pulmonary disorders [60], surprisingly this class of drugs was not effective in improving the lung function in equine asthma. Several PDE4 inhibitors have been investigated for their therapeutic potential in both human asthma and COPD and roflumilast received regulatory approval for use in patients with chronic bronchitis and a history of frequent exacerbations [18,61]. Although the current results indicate that the PDE4 inhibitor L-826,141 did not improve the lung function in horses, further well designed studies are needed before definitely considering PDE4 inhibitors ineffective in equine asthma.

The majority of the analysed studies, excluding those on corticosteroids, investigated acute bronchorelaxant effects of drugs after a single administration. Although this approach may be adequate to study direct bronchodilator agents such as short acting antimuscarinic agents and β_2 -AR agonists, this is not the case for those drugs that may elicit bronchorelaxant effect by reducing lung inflammation, namely PDE4 inhibitors, or long-acting bronchodilators. It is not possible to exclude the possibility that both PDE4 inhibitors and methylxanthines may have a role in the treatment of equine asthma when administered via inhalation. SUCRA analysis indicates the following rank of effectiveness with regard to

TABLE 1: Relative effect (RE) and 95% credible interval (CrI) for comparisons between inhaled medications for the treatment of equine asthma

	$\Delta P_{\text{Pl}_{\text{max}}}$			R_L			C_{dyn}		
	RE	95% CrI	P	RE	95% CrI	P	RE	95% CrI	P
Antimuscarinic agents vs. β_2 -AR agonists	6.15	−3.07, 14.94	NS	0.83	0.17, 1.00	*	0.00	−0.17, 0.17	NS
Antimuscarinic agents vs. inhaled corticosteroids	−3.19	−12.68, 6.44	NS	2.38	0.15, 3.76	*	NC	NC	NC
Antimuscarinic agents vs. chromones	−7.41	−15.56, 0.75	NS	NC	NC	NC	NC	NC	NC
β_2 -AR agonists vs. inhaled corticosteroids	−9.23	−16.80, −1.85	*	1.95	−0.81, 2.76	NS	NC	NC	NC
β_2 -AR agonists vs. chromones	−13.38	−19.37, −7.98	*	NC	NC	NC	NC	NC	NC
Inhaled corticosteroids vs. chromones	−4.22	−11.7, 2.52	NS	NC	NC	NC	NC	NC	NC

$\Delta P_{\text{Pl}_{\text{max}}}$, changes in maximum transpulmonary or pleural pressure; R_L , pulmonary resistance; C_{dyn} , dynamic lung compliance; β_2 -AR agonists, β_2 -adrenoreceptor agonists; NC, not calculable; NS, not significant. * $P < 0.05$.

TABLE 2: Probability of best therapy and surface under the cumulative ranking curve (SUCRA) values for inhaled medications in equine asthma-affected horses

Treatment	Probability of being the best therapy (%)			SUCRA value (%)		
	$\Delta P_{\text{Pl}_{\text{max}}}$	R_L	C_{dyn}	$\Delta P_{\text{Pl}_{\text{max}}}$	R_L	C_{dyn}
Antimuscarinic agents	9	0	50	70.3	16.7	62.5
β_2 -AR agonists	91	25	50	97.8	75.0	75.0
Inhaled corticosteroids	1	75	NC	54	91.7	NC
Chromones	0	NC	NC	28.5	NC	NC

$\Delta P_{\text{Pl}_{\text{max}}}$, changes in maximum transpulmonary or pleural pressure; R_L , pulmonary resistance; C_{dyn} , dynamic lung compliance; β_2 -AR agonists, β_2 -adrenoreceptor agonists; NC, not calculable.

the duration of treatments: long-term corticosteroids > long-term bronchodilators > short-term bronchodilators > short-term corticosteroids.

We have demonstrated that among bronchodilators, long-acting β_2 -AR agonists were less effective than short-acting β_2 -AR agonists in improving lung function. This is an unexpected finding that may be explained by considering that, while the peak activity of short-acting β_2 -AR agonists is reached within a few minutes or hours, long-acting β_2 -AR agonists elicit the maximum broncorelaxant effect after several days or weeks of once or twice-daily dosing [62,63]. Therefore, since the current clinical trials assessed only the acute bronchorelaxant effect of long-acting β_2 -AR agonists in symptomatic horses, it is possible that the maximum effect of long-acting bronchodilators was neither reached nor detected. However, although long-acting β_2 -AR agonists may induce a greater 'pharmacological stenting' of airways compared with short-acting β_2 -AR agonists in long-term treatments [64], short-acting β_2 -AR agonists (namely salbutamol) may represent a valuable rescue medication because of its rapid onset of action and peak effect.

Our network meta-analysis shows that not all the inhaled drugs have the same impact on the lung function in equine asthma. Antimuscarinic agents and β_2 -AR agonists had similar effects on $\Delta P_{\text{Pl}_{\text{max}}}$; β_2 -AR agonists were superior to inhaled corticosteroids and chromones. However, both β_2 -AR agonists and inhaled corticosteroids were superior to antimuscarinic agents in improving R_L , and no differences in changes of C_{dyn} were detected between antimuscarinic agents and β_2 -AR agonists.

Increased R_L indicates diffuse obstruction of both large and small airways, whereas reduced C_{dyn} suggests peripheral airway obstruction associated with uneven distribution of ventilation. Impairment of R_L and C_{dyn} leads to increased $\Delta P_{\text{Pl}_{\text{max}}}$ [65]. Corticosteroids improve R_L suggesting that these drugs are effective in ameliorating inflammation at the level of both large and small airways, with consequent improvement of airflow. However, our results indicate that bronchodilators and furosemide also have a bronchorelaxant effect in small airways.

Since both inhaled corticosteroids and inhaled β_2 -AR agonists had the highest probability of being the most effective medications for improving $\Delta P_{\text{Pl}_{\text{max}}}$, combining inhaled corticosteroids with a bronchodilator may

represent the first-line approach to improve the effort of breathing. Indeed $\Delta P_{\text{Pl}_{\text{max}}}$, but not R_L , was linearly correlated with the worsening of clinical score of horses suffering from equine asthma [65]. However, it is important to recognise that corticosteroids, used either as systemic or inhaled forms, were superior to bronchodilators in improving $\Delta P_{\text{Pl}_{\text{max}}}$ in association with AHR.

The FLAME study has recently demonstrated that the combination of a long-acting β_2 -AR agonists with a long-acting antimuscarinic agent may be more effective than inhaled corticosteroid/long-acting β_2 -AR agonists combination in preventing exacerbations of COPD in human patients [66]. Well-designed clinical trials are needed to assess whether combining an inhaled β_2 -AR agonist with an inhaled corticosteroid may really lead to a greater bronchorelaxant and anti-inflammatory effect, as well as combining an inhaled β_2 -AR agonist with an antimuscarinic agent in horses.

The results of this meta-analysis should be interpreted with caution because information on potential bias particularly in the oldest studies. It is possible that space constraints in most printed journals and the referral of readers to previous publications as the sources for detailed description of the methods could have led to the assumption that a clinical trial was methodologically deficient, even when it was appropriately designed and conducted [19]. Although some of the studies included in the analysis had a Jadad score ≥ 2 , it is evident that in veterinary medicine there is a lack of high quality clinical trials.

In our opinion, veterinary clinical trials should be at least randomised, placebo controlled, double blind, and report withdrawals and dropouts, with a final Jadad score ≥ 3 . Although the funnel plots of clinical trials on β_2 -AR agonists did not show publication bias, the Egger's test indicated a weak but significant asymmetry due to the poor methodological design of some studies [67]. This may be also related to 'small study effects', a tendency for treatment effect estimates in small studies to differ from those in larger studies [68]. Furthermore, both funnel plots and Egger's test confirmed high heterogeneity among the studies on corticosteroids, probably caused by the variability across the doses used. Nevertheless, the overall asymmetry identified by Egger's test was weak, suggesting that this meta-analysis is reliable and free from relevant publication bias.

There is a need for well-designed adequately powered clinical trials for the main pharmacological treatments available for equine asthma, namely bronchodilators and anti-inflammatory agents, lasting at least 4 weeks. The latter point is extremely important, since it is possible that the greater effectiveness of inhaled corticosteroids over bronchodilators in improving R_L may be due to the fact that the studies on inhaled corticosteroids lasted several days or weeks, whereas those on bronchodilators often lasted only 1 day.

In 2001, Robinson and colleagues [17] made the noteworthy effort to achieve consensus on chronic equine airway disease, criteria for definition of the RAO phenotype, and made recommendations to standardise diagnosis and improve research. However, that consensus statement [17] has not been updated recently and our meta-analysis provides a current clinical evidence-based synthesis on treatment of equine asthma. We conclude that both inhaled and systemic therapies are valuable but it remains unclear whether bronchodilator therapy is preferred to inhaled corticosteroids or vice versa. Our meta-analysis indicates that inhaled β_2 -AR

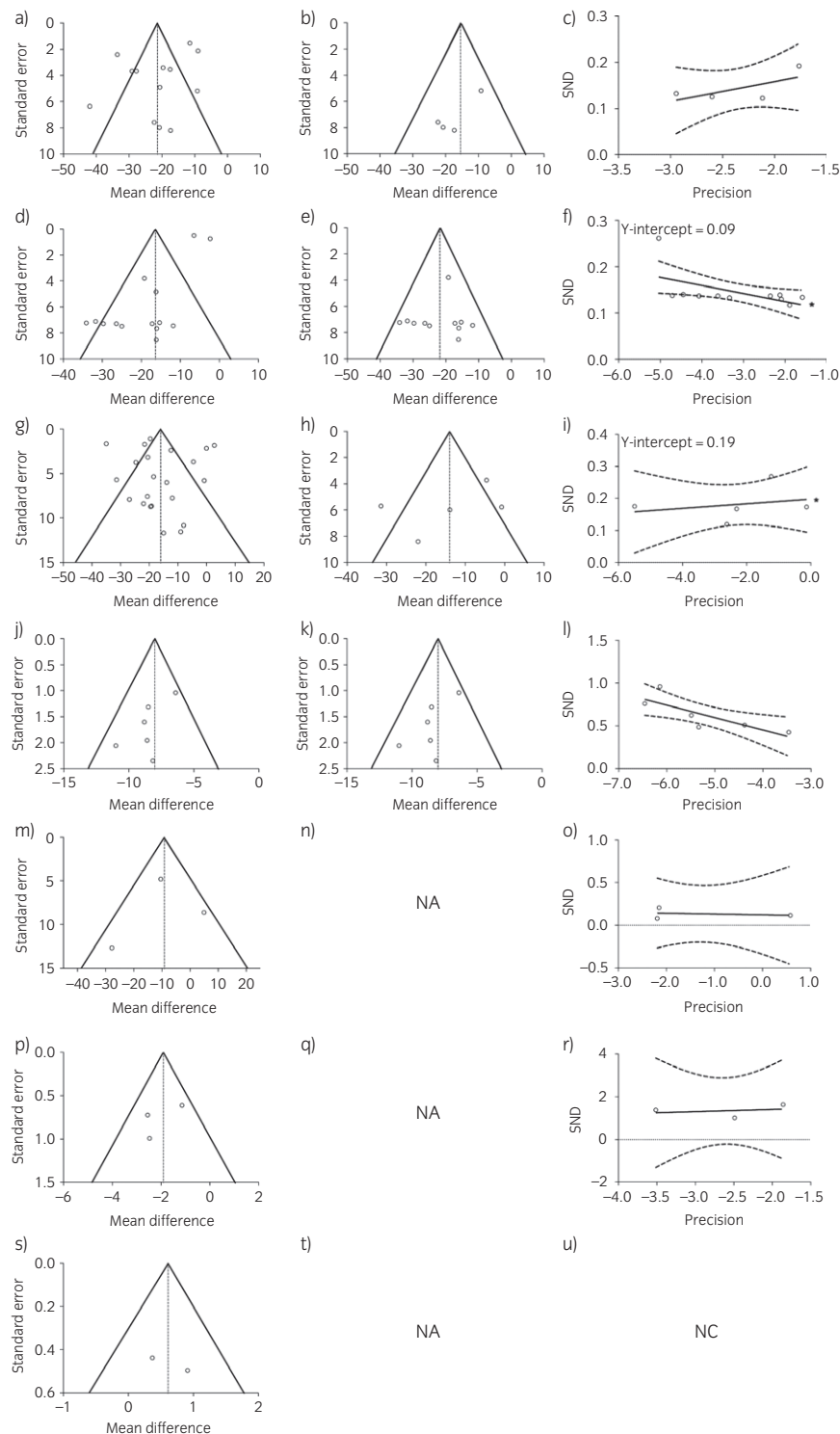


Fig 3: Funnel plots (left panels: all studies; middle panels: studies evaluating exclusively inhaled medications) and graphical representation of Egger's test (right panels) for the impact of antimuscarinic agents a–c, β_2 -adrenoreceptor agonists d–f, corticosteroids, g–i, chromones j–l, methylxanthines m–o, furosemide p–r, and phosphodiesterase 4 inhibitors s, t) in equine asthma-affected horses. NA, not available; NC, not calculable; SND, standard normal deviate. * $P < 0.1$.

agonists are as effective as inhaled corticosteroids in reducing R_L , whereas corticosteroids were more effective than bronchodilators in improving $\Delta P_{l_{max}}$ during acute equine asthma exacerbation. Further studies on long-

term treatment with inhaled corticosteroids/long acting β_2 -AR agonists combinations are needed. Finally, there is a need for more equine-specific information on combining bronchodilators with different mechanisms of

action on emerging drugs used for human chronic obstructive respiratory disorders [69].

Authors' declaration of interests

The authors report no potential conflict of interests.

Ethical animal research

Not applicable.

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Authorship

L. Calzetta designed and executed the study, analysed and interpreted the data, prepared the manuscript and approved the final version of the manuscript. P. Roncada executed the study, analysed and interpreted the data, prepared the manuscript and approved the final version of the manuscript. D. di Cave, L. Bonizzi, A. Urbani, E. Pistocchini and P. Rogliani interpreted the data, prepared the manuscript and approved the final version of the manuscript. M.G. Matera designed the study, interpreted the data, prepared the manuscript and approved the final version of the manuscript.

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Supporting Information

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

Supplementary Item 1: Horse demographics, baseline, study characteristics and Jadad score.

Supplementary Item 2: Forest plots demonstrating the impact on respiratory outcomes of various drugs in equine asthma studies.



Supplementary Item 1: Horse demographics, baseline, study characteristics and Jadad score.

Study and year	Study characteristics	Duration of study (days)	Number of analysed horses	Drugs and regimen of administration	Case characteristics	Age (years)	Body weight (kg)	Jadad score
de Lagarde <i>et al.</i> 2014 [37]	Cross-over	2	8	N- butylscopolammonium (0.3 mg/kg, SA, i.v.), atropine (0.02 mg/kg, SA, i.v.)	Heaves	16	475	2
Couetil <i>et al.</i> 2012 [38]	Randomised, double-blind, cross-over, placebo-controlled	1	9	N- butylscopolammonium (0.3 mg/kg, SA, i.v.)	equine asthma	8 - 29	NA	3
Bertin <i>et al.</i> 2011 [45]	NA	1	10	Salbutamol (900 µg, SA, inh.)	equine asthma	7 - 29	NA	1
Leclerc <i>et al.</i> 2010 [55]	Randomised, cross-over	14	7	Prednisolone (2 mg/kg, q24h, OS), dexamethasone (0.05 mg/kg, q24h, OS)	equine asthma	20	507	2
Robinson <i>et al.</i> 2009 [54]	Cross-over	3	9	Fluticasone (3 mg - 6 mg, q12h, inh.), dexamethasone (0.1 mg/kg, q24h, i.v.)	equine asthma	24,7	502	0
Camargo <i>et al.</i> 2007 [46]	Randomised, cross-over	1	6	Trimetoquinol (100 µg - 1000 µg, SA, inh.)	equine asthma	17 - 29	483 - 570	1
Cesarini <i>et al.</i> 2006 [53]	Randomised, incomplete block	7	10	Dexamethasone (0.05 mg/kg, q24h, i.v.; 0.02 mg/kg - 0.05 mg/kg, q24h, i.v., OS), theophylline (5 mg/kg, q12h, OS)	equine asthma	15	NA	2
Couetil <i>et al.</i> 2006 [52]	Randomised, cross-over, placebo-controlled	10	7	Beclomethasone (500 µg, q12h, inh.), dexamethasone (0.06 mg, q24h, i.m.; 0.02 mg/kg, q24h, OS)	equine asthma	20	431	3
Lavoie <i>et al.</i> 2006 [36]	Parallel groups, blinded, placebo-controlled	22	12	Dexamethasone (0.04 mg/kg, q24h, i.v.), atropine (0.02 mg/kg, SA, i.v.), L-826,141 (PDE4 inhibitor, 0.5 mg/kg - 1 mg/kg, q24h - q48h, i.v.)	Heaves	15	481	1
Couetil <i>et al.</i> 2005 [51]	Randomised, double-blind, placebo-controlled	28	27	Prednisone (200 mg - 500 mg, q12h - q48h, OS), fluticasone (1,100 µg - 1,980 µg, q12h - q48h, inh.)	equine asthma	10 - 25	NA	2
Laan <i>et al.</i> 2005 [44]	Randomised, cross-over, placebo-controlled	4	7	Clenbuterol (0.75 µg/kg, q12h, i.v.)	equine asthma	7 - 10	629	1
Cornelisse <i>et al.</i> 2004 [50]	Randomised, cross-over, placebo-controlled	3	6	Dexamethasone (0.1 mg, SA, i.v.; 0.08 mg, SA, OS; 0.16 mg, SA, OS)	equine asthma	13 - 31	404 - 555	1
Robinson <i>et al.</i> 2003 [49]	NA	7	12	Dexamethasone (0.1 mg/kg, once-daily, i.v.)	equine asthma	20	NA	0

Picandet et al. 2003 [35]	Parallel groups, placebo-controlled	14	12	Isoflupredone (0.03 mg/kg, q24h, i.m.), dexamethasone (0.04 mg/kg, q24h, i.v.), atropine (0.015 mg/kg, SA, i.v.)	equine asthma	12 - 15	NA	0
Bayly et al. 2002 [34]	Placebo-controlled	1	6	Ipratropium (2,400 µg, SA, inh.)	COPD	9	567	1
Leguillette et al. 2002 [33]	Placebo-controlled	14	10	Pentoxifylline (16 g, q12h, OS), atropine (0.02 mg/kg, SA, i.v.)	equine asthma	NA	444	1
Robinson et al. 2002 [32]	Randomised, cross-over, placebo-controlled	10	9	Prednisone (2.2 mg/kg, q24h, OS), dexamethasone (0.1 mg/kg, q24h, i.v.), 0.04 mg/kg, q3days, i.m.), atropine (0.02 mg/kg, SA, i.v.)	Heaves	18 - 30	NA	1
Henrikson et al. 2001 [43]	Randomised, cross-over, placebo-controlled	1	6	Salmeterol (210 µg, SA, inh.)	equine asthma	16 - 28	NA	2
Jackson et al. 2000 [31]	Cross-over, placebo-controlled	44	12	Prednisone (2.2 mg/kg, q24h, OS), atropine (0.02 mg/kg, SA, i.v.)	equine asthma	10 - 28	NA	1
Derksen et al. 1999 [40]	Cross-over, double-blind, placebo-controlled	1	9	Salbutamol (360 µg - 720 µg, SA, inh.)	equine asthma	NA	NA	0
Ammann et al. 1998 [48]	NA	15	6	Beclomethasone (3,750 µg, q12h, inh.)	COPD	10 - 16	400 - 600	0
Derksen et al. 1996 [42]	Randomised, double-blind, placebo-controlled	1	6	Pirbuterol (400 µg - 1,600 µg, SA, inh.)	Heaves	20	447	2
Tesarowski et al. 1994 [41]	NA	1	6	Fenoterol (1 mg, SA, inh.)	Heaves	4 - 20	475 - 550	0
Lapointe et al. 1993 [47]	NA	1	5	Triamcinolone (0.09 mg/kg, SA, i.m.)	COPD	11	442	0
Robinson et al. 1993 [30]	Blind, placebo-controlled	2	8	Ipratropium (1 µg/kg - 31 µg/kg, SA, inh.), atropine (0.02 mg/kg, SA, i.v.)	Heaves	10 - 12	NA	1
Rubie et al. 1993 [59]	Placebo-controlled	1	6	Furosemide (1 mg/kg, SA, i.v.)	Heaves	NA	NA	1
Derksen et al. 1992 [39]	Placebo-controlled	2	6	Pirbuterol (3,200 µg, SA, inh.)	Heaves	21	228	1
Broadstone et al. 1991 [11]	Cross-over, placebo-controlled	4	6	Furosemide (1 mg/kg, SA, i.v.)	equine asthma	18	234	0
Pearson et al. 1989 [29]	NA	1	13	Isoproterenol (0.1 mg - 0.2 mg, SA, i.v.), atropine (0.01 mg/kg, SA, i.v.), aminophylline (12 mg/kg, SA, i.v.)	COPD	11	NA	0
Soma et al. 1987 [58]	NA	3	5	Sodium cromoglycate (520 mg, q24h, inh.)	COPD	8 - 17	NA	0
Thomson et al. 1981 [57]	NA	1	56	Sodium cromoglycate (80 mg, q24h, inh.)	COPD	9	NA	0

Murphy et								
al. 1979	NA	1	2	Sodium cromoglycate	COPD	11	NA	0
[56]				(80 mg, q24h, inh.)				

i.m.: intramuscular

Inh.: inhalation

i.v.: intravenous

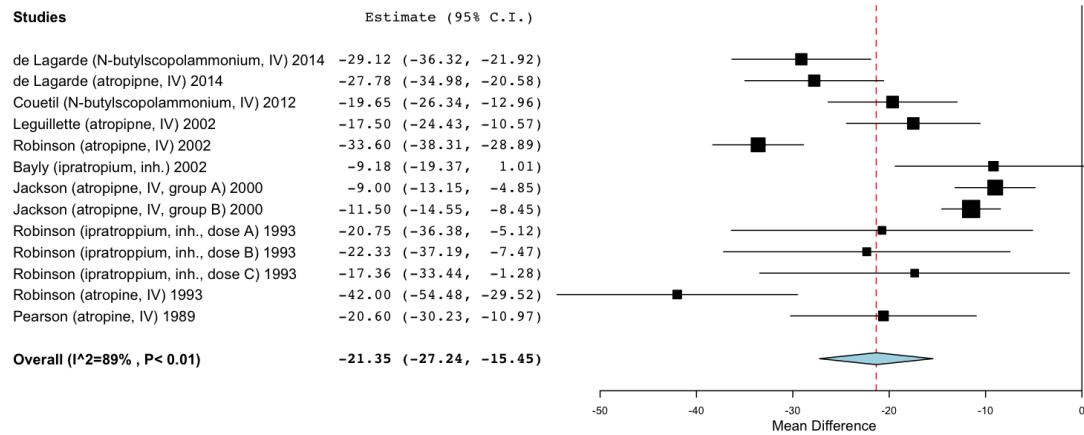
NA: not available

OS: per os

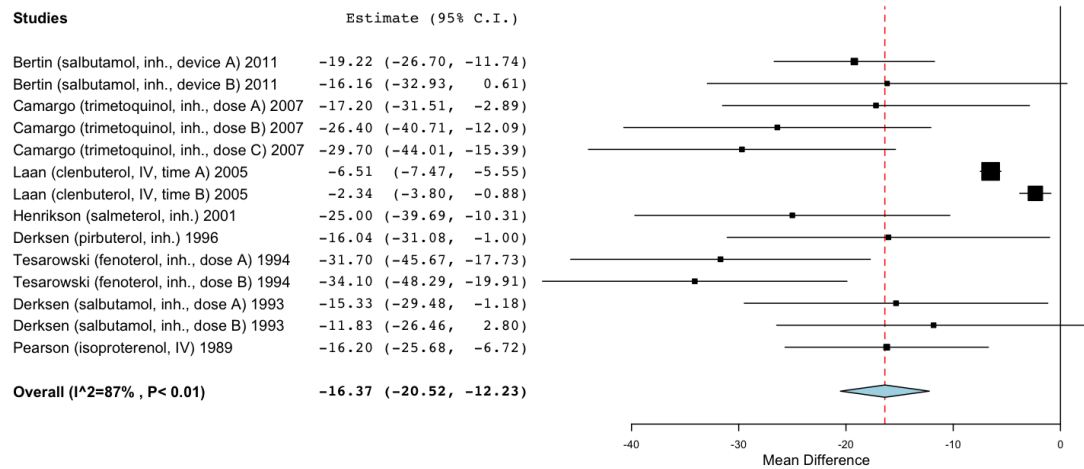
PDE: phosphodiesterase

SA: single administration

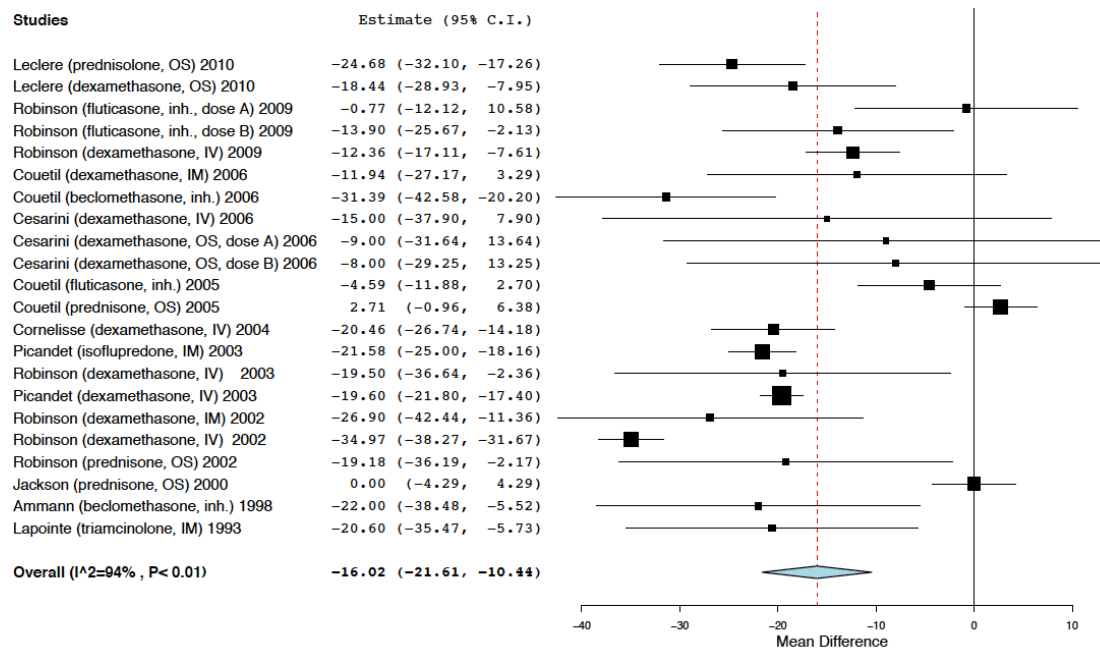
Supplementary Item 2: Forest plots demonstrating the impact on respiratory outcomes of various drugs in equine asthma studies.



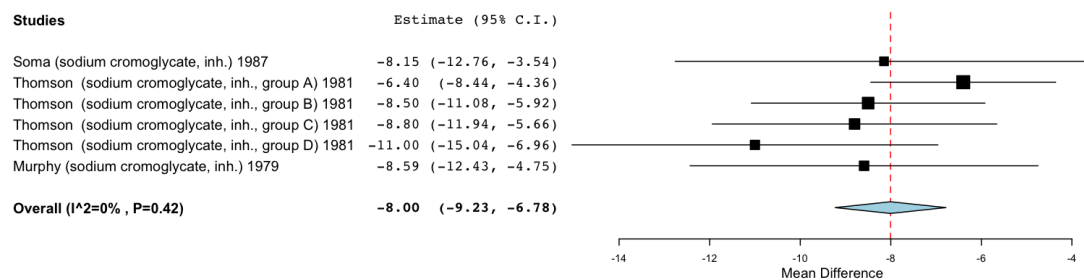
2a: Forest plot from the pair-wise meta-analysis of the impact of antimuscarinic agents on maximum changes in pleural pressure ($\Delta P_{pl_{max}}$) in equine asthma. Results are expressed as the mean difference vs. control.



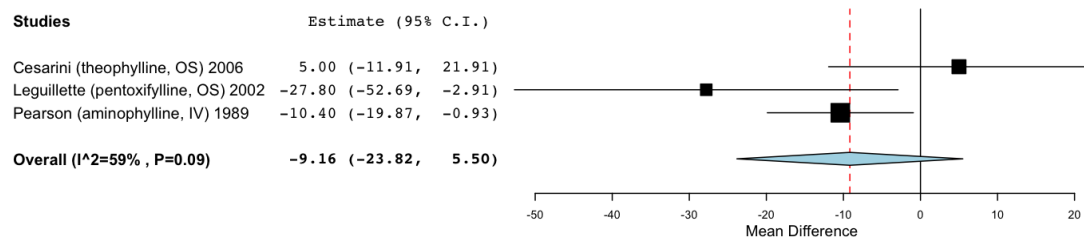
2b: Forest plot from the pair-wise meta-analysis of the impact of β -AR agonists on maximum changes in pleural pressure ($\Delta P_{pl_{max}}$) in equine asthma. Results are expressed as the mean difference vs. control.



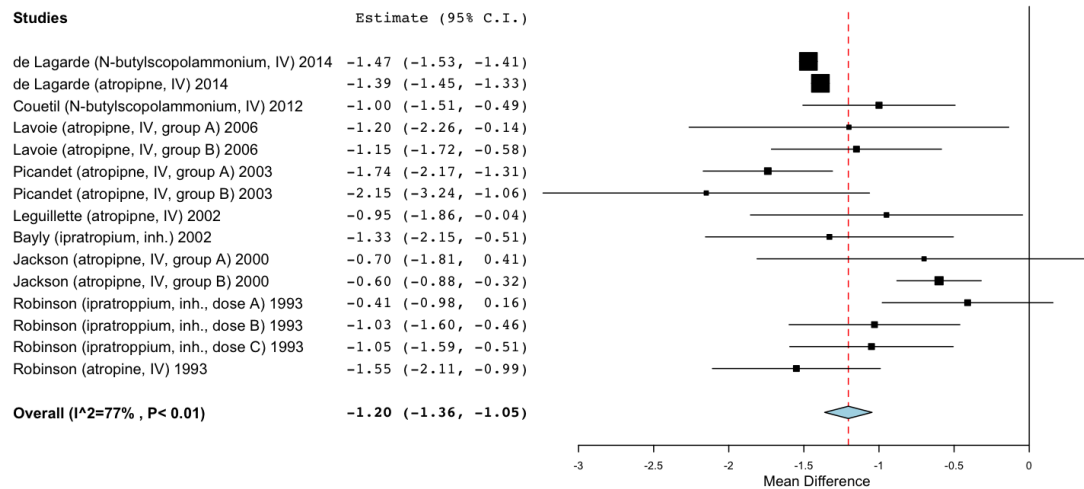
2c: Forest plot from the pair-wise meta-analysis of the impact of corticosteroids on maximum changes in pleural pressure ($\Delta P_{pl_{max}}$) in equine asthma. Results are expressed as the mean difference vs. control.



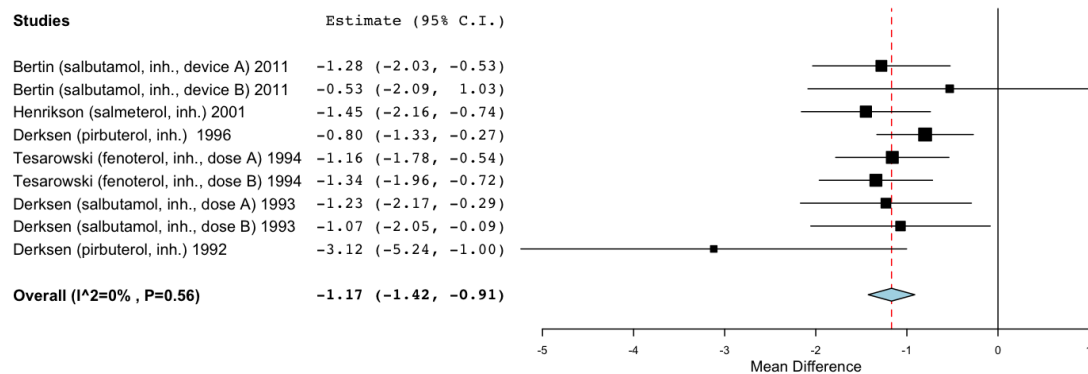
2d: Forest plot from the pair-wise meta-analysis of the impact of cromones on maximum changes in pleural pressure ($\Delta P_{pl_{max}}$) of in equine asthma. Results are expressed as the mean difference vs. control.



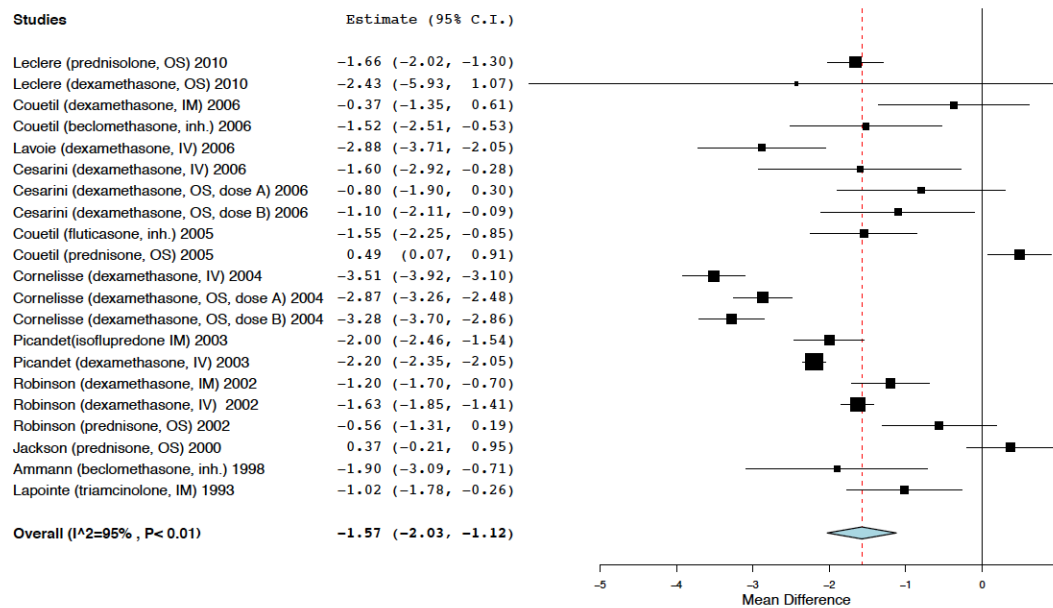
2e: Forest plot from the pair-wise meta-analysis of the impact of methylxanthines on maximum changes in pleural pressure (ΔPpl_{max}) in equine asthma. Results are expressed as the mean difference vs. control.



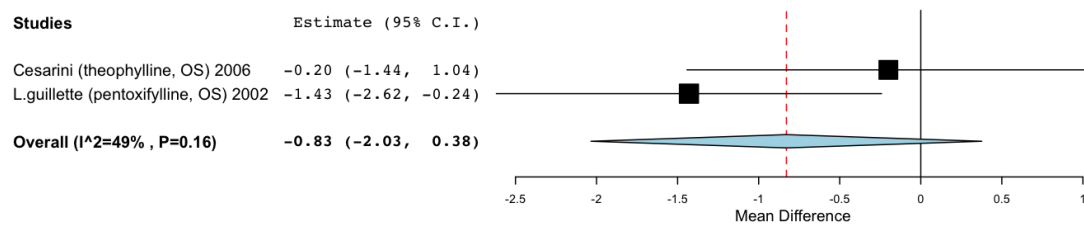
2f: Forest plot from the pair-wise meta-analysis of the impact of antimuscarinic agents on changes in pulmonary resistance (R_L) in equine asthma. Results are expressed as the mean difference vs. control.



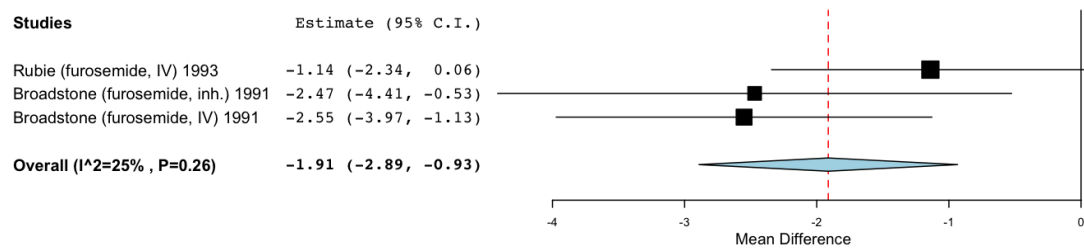
2g: Forest plot from the pair-wise meta-analysis of the impact of β -AR agonists on changes in pulmonary resistance (R_L) in equine asthma. Results are expressed as the mean difference vs. control.



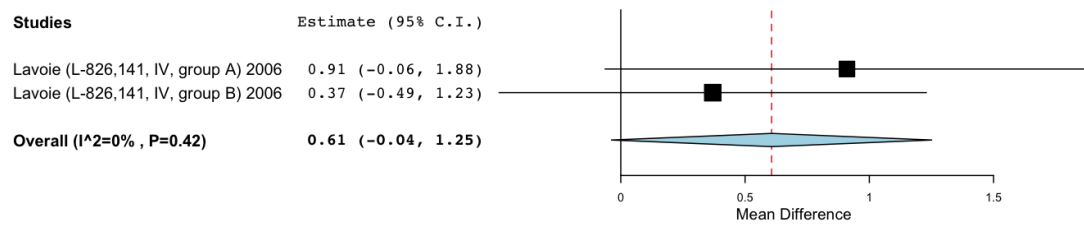
2h: Forest plot from the pair-wise meta-analysis of the impact of corticosteroids on changes in pulmonary resistance (R_L) in equine asthma. Results are expressed as the mean difference vs. control.



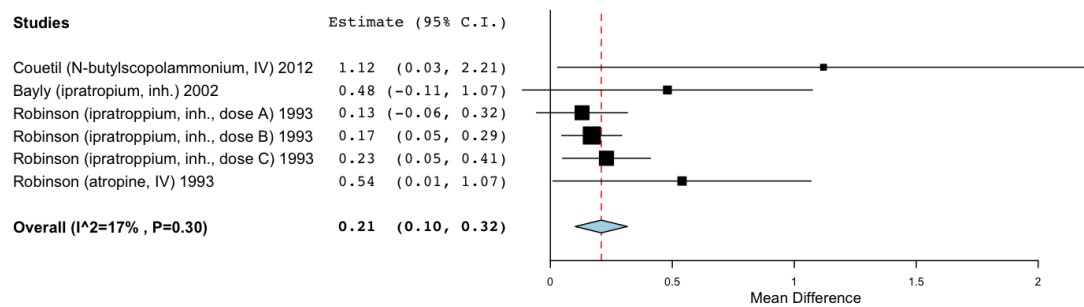
2i: Forest plot from the pair-wise meta-analysis of the impact of methylxanthines on changes in pulmonary resistance (R_L) in equine asthma. Results are expressed as the mean difference vs. control.



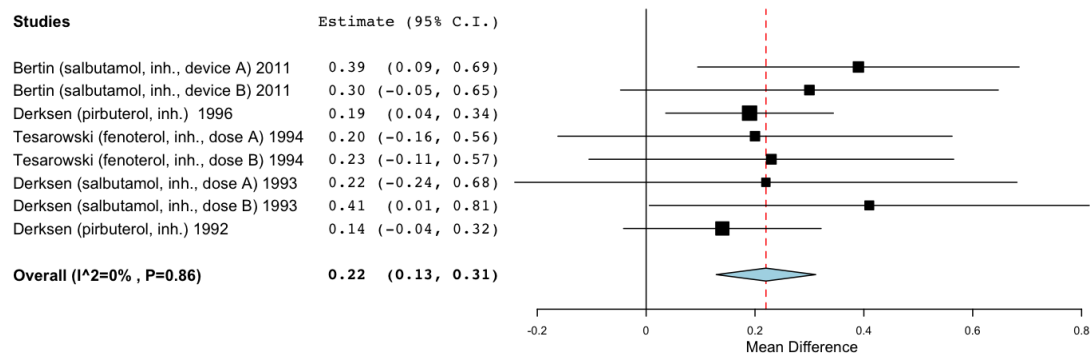
2j: Forest plot from the pair-wise meta-analysis of the impact of furosemide on changes in pulmonary resistance (R_L) in equine asthma. Results are expressed as the mean difference vs. control.



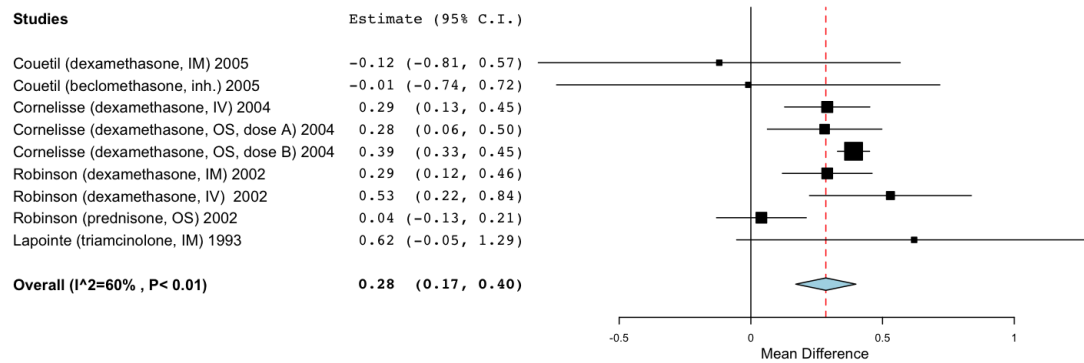
2k: Forest plot from the pair-wise meta-analysis of the impact of phosphodiesterase (PDE) 4 inhibitors on changes in pulmonary resistance (R_L) in equine asthma. Results are expressed as the mean difference vs. control.



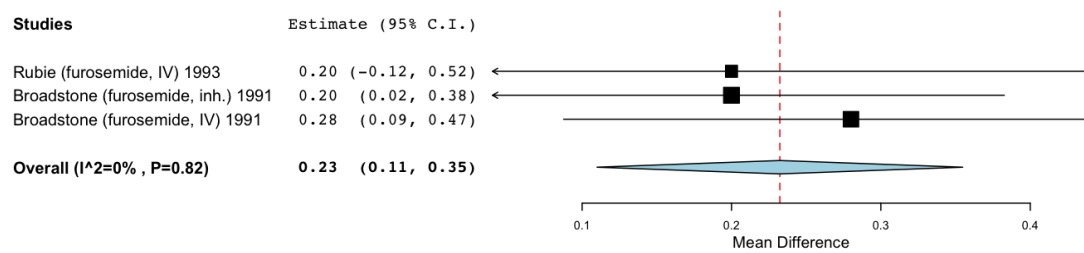
2I: Forest plot from the pair-wise meta-analysis of the impact of antimuscarinic agents on dynamic lung compliance (C_{dyn}) in equine asthma. Results are expressed as the mean difference vs. control.



2m: Forest plot from the pair-wise meta-analysis of the impact of β -AR agonists on dynamic lung compliance (C_{dyn}) in equine asthma. Results are expressed as the mean difference vs. control.



2n: Forest plot from the pair-wise meta-analysis of the impact of corticosteroids on dynamic lung compliance (C_{dyn}) in equine asthma. Results are expressed as the mean difference vs. control.



2o: Forest plot from the pair-wise meta-analysis of the impact of furosemide on dynamic lung compliance (C_{dyn}) in equine asthma. Results are expressed as the mean difference vs. control.