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Mild-moderate equine asthma: A scoping review of evidence supporting the consensus definition

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ABSTRACT

Current consensus defines mild-moderate equine asthma (mEA; previously inflammatory airway disease) by a hierarchy of indicators of lung pathology: cough, poor performance, increased tracheobronchial mucus, inflammatory bronchoalveolar lavage (BAL) cytology and pulmonary dysfunction. Exclusion criteria include fever, systemic disease, or increased resting respiratory effort. The aim of this review was to inform future research by identifying gaps, strengths and weaknesses in the current body of evidence supporting this consensus-proposed definition. Objectives were to critique evidence supporting the inclusion of each diagnostic indicator in the case definition, by summarising and evaluating evidence for its association with higher-level indicators of lung inflammation. Searches of three databases identified 2275 articles relating to mEA or its diagnostic indicators, from which 298 full-text articles were screened and 45 reviewed in full. Studies (n = 44) had been performed worldwide in clinics, hospitals, racetracks, yards or research herds, in 6092 horses. Studies were predominantly opportunistic observational (n = 13/44: 29.5%) or cross-sectional (n = 11/44; 25%). The median number of horses per study was 74. Where breed and use were reported most were Thoroughbreds (58.2%; 2730/4688) and racehorses (72.8%; n = 3960/5439). Domains rated as high risk of bias in almost 50% of articles were 'study power' and 'masking'. Heterogeneity in clinical and laboratory measures precluded meta-analysis. Evidence was more consistent for certain pairwise relationships (e.g., between cough and tracheobronchial mucus) than others (e.g., BAL cytology and lung function). Findings highlight the need for increased standardisation of diagnostic methods and reporting to facilitate future systematic review and meta-analysis.

Introduction

The current American College of Veterinary Internal Medicine (ACVIM) expert consensus (Couëtil et al., 2016) regards equine asthma as a spectrum, but not a continuum, of disease, ranging from a mild-moderate form previously known as inflammatory airway disease (IAD) to a severe form, previously known as 'heaves' or recurrent airway obstruction (RAO) including summer pasture-associated recurrent airway obstruction. Mild-moderate equine asthma (mEA) is distinguished from severe equine asthma (sEA) by a lack of increased respiratory effort at rest, milder airway inflammation, and milder airflow limitations. The consensus case definition comprises a hierarchy of diagnostic indicators, starting with clinical signs of chronic poor performance or occasional coughing of at least 3 weeks' duration. In horses affected with these signs, increasing evidence of mEA is obtained with identification of increased endoscopically-visible tracheobronchial mucus, mild increases in bronchoalveolar lavage (BAL) fluid neutrophils, eosinophils and/or metachromatic (a.k.a. mast) cells, or abnormal pulmonary function test findings such as airway obstruction or hyperresponsiveness. Exclusion criteria include signs of systemic disease such as fever, lethargy or decreased appetite, or signs of severe asthma. This case definition is regarded by consensus as applicable to horses of all ages and types, including racehorses, other sport horses and leisure horses (Couëtil et al., 2016).

Consensus statements are developed by panels of subject-specific experts with the aim of guiding clinical decision-making based on

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review of published research-based evidence supplemented by expert interpretation and opinion where evidence is lacking or conflicting. Consensus can be reached through formal, transparent methods such as systematic review (Sullivan et al., 2015; Rabe et al., 2018) or the Delphi method (Boulias et al., 2018; van der Lee et al., 2019), or through a less formal nominal group approach. Transparency of methods is valuable to reduce the potential for bias. To date, consensus statements on defining cases of IAD/mEA have been reached by the nominal group method, with no formal, transparent, systematic assessment of the extent and quality of the evidence supporting the consensus case definition.

This scoping review with risk-of-bias assessment aimed to inform future research by identifying gaps, strengths and weaknesses in the current body of evidence supporting the latest ACVIM consensus-proposed case definition of mEA. Objectives were to critique evidence supporting the inclusion of each diagnostic indicator in the case definition, by summarising and evaluating evidence for its association with higher-level indicators of lung inflammation.

Materials and methods

Diagnostic indicators

The five diagnostic indicators contributing to the consensus definition of mEA were regarded as a hierarchy indicating increasing likelihood of lung pathology (Fig. 1). To maximise the chances of capturing all relevant evidence, broader definitions of each indicator than those described in the consensus statement were accepted. Articles reporting any history of cough of any duration or frequency, any indicator of poor performance, any tracheobronchial mucus scoring system, any method of assessing BAL cytology, any pulmonary function testing (blood gas analysis, or lung mechanics with or without bronchoprovocation) or the use of lung pathology as a gold standard for the other diagnostic indicators, were eligible for inclusion.

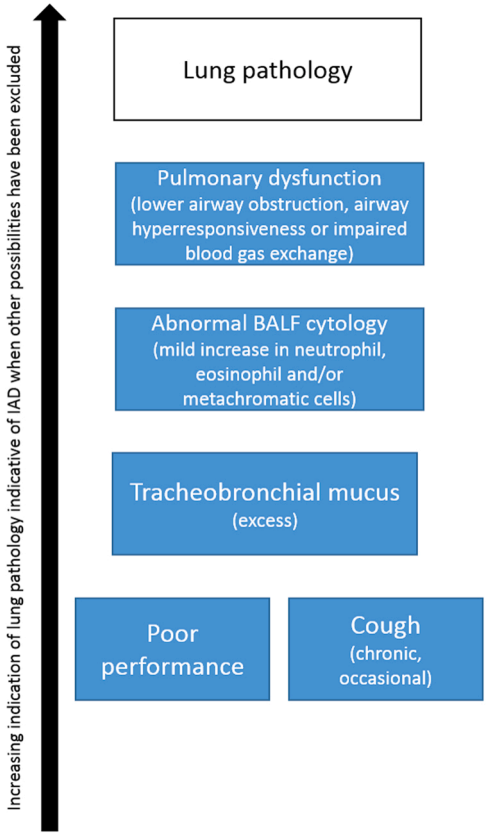

Fig. 1. Hierarchy of indicators of IAD/mEA developed from the revised consensus statement. (BALF, bronchoalveolar lavage fluid; IAD, inflammatory airway disease.)

Inclusion criteria

Studies considered for inclusion were those investigating associations between mEA/IAD (by any definition, as long as the definition excluded sEA/RAO), or any individual consensus indicator, and any other indicator further up the hierarchy including lung pathology. Articles were required to present clearly differentiated results for mEA-affected, healthy or apparently healthy, and sEA horses, where included. All peer-reviewed studies in horses of any breed, use, age, or sex, published in English between January 1987 and March 2021 were eligible.

Search methods

Searches were performed in March-April 2017 in three electronic databases (CAB Direct, PubMed and Scopus) and in March 2021 in CAB Direct and PubMed only. The search string used for CAB Direct was: "(od:(horses) OR ab:(equine OR horse) OR title:(equine OR horse)) AND ((ab:(airway OR "lower respiratory tract" OR pulmonary OR lung OR bronchi* OR trachea*)) OR (ab:(IAD OR COPD OR RAO OR asthma))) AND ((ab:(inflamm* OR performance OR mucus OR mucous OR cough OR "pulmonary function")) OR (ab:(endoscopy OR "bronchoalveolar lavage" OR BAL OR "tracheal wash" OR "tracheal aspirate*" OR cytolog*)))" with refinements of document type (journal article) and language (English). Similar searches were performed in the other two databases with adjustments according to database functionalities. Four review articles (Couëtil et al., 2007, 2016; Cathcart et al., 2012; Barton et al., 2014) were searched for additional references. Retrieved references were managed in Covidence software (covidence.org).

Selection of articles

Two authors (TK and CW) reviewed titles and abstracts of all articles identified in the original search and selected articles for full-text screening, based on the inclusion criteria. Any disagreement or uncertainty was resolved by discussion, or consultation with a third reviewer (JC). Full-text versions of the selected articles were retrieved and further evaluated against the criteria. TK and either CW, JC or KS screened all articles independently. Disagreements were again resolved through discussion, or by consulting a third reviewer (LC). All full texts selected for inclusion then underwent quality appraisal via risk-of-bias assessments. TK and either EGG, JC, SB, CW or KS assessed all articles. TK conducted all data charting, with 10% reviewed by JC. The updated search in 2021 was performed by TM and JC, with both authors screening titles and abstracts as well as evaluating full text articles against the inclusion criteria and performing the quality appraisal via risk-of-bias assessments. TM conducted the data charting, reviewed by JC.

Quality appraisal via risk-of-bias assessment

The Cochrane-style risk-of-bias assessment[2] provided by Covidence was adapted to suit the types of studies included in the review (i.e., typically not randomised controlled trials), and to allow an evaluation of quality without subsequent rejection. Details of risk-of-bias domains (aim, study population, study power, masking, case definition, diagnostic classification, indication of precision, completeness of results, believable conclusions, and consistency) are summarised in Table 1. One

[2] See: Chapter 8: Assessing risk of bias in included studies. https://handbook-5-1.cochrane.org/chapter_8/8_assessing_risk_of_bias_in_included_studies.htm (Accessed 5 July 2022).

Table 1
Details of the 10 domains constituting risk of bias decisions indicating quality appraisal.

Domain		Summary	High risk	Low risk	Unclear
1	Aim	Provision of aims (or objectives/research questions) and whether they were addressed	No clearly focused aim, or an aim which is not then addressed by the rest of the article	Description of a clearly focused aim addressed by the study	Unclear aim/ unclear if addressed
2	Study population	The article: 1. identifies the source of the sample 2. includes a sampling strategy (with either randomisation or inclusion of all horses) 3. identifies the target population (to which results are generalised) 4. has a sample which represents the target population	Explicitly fails in one or more of these points	Documents all four points where appropriate	Incomplete or missing information regarding the four points
3	Study power	Inclusion of a priori sample size calculations or post hoc power tests	Conducted but not met; or not conducted and very small sample size	Conducted and met, establishing power of at least 80%	Not conducted
4	Masking	Masking of measured outcomes to individuals involved in undertaking or analysing indicators	No masking	Documented masking	Masking was likely, but not described
5	Case definition	Definition of a case (e.g., 'increased coughing') and application of this when selecting controls (where appropriate)	Lack of definition of a case, or definitions that are not consistent across cases and controls	Clear definition of a case and similarity across cases and controls	Unclear definition of case/ consistency
6	Diagnostic classification	Information on variables potentially leading to bias for each indicator identified/taken into account. For example, which lung sampled, and stain used during BAL	Variables that may cause bias are not identified/ taken into account in the design or analysis	Variables that may cause bias are identified/ taken into account in the design or analysis	Unclear if variables are taken into account in the design or analysis
7	Indication of precision	Including reporting of specific *P* values and confidence intervals	No description of the precision of the risk of results, or an inaccurate description	Satisfactory description of the precision of the risk of results, including specific *P* values	*P* values etc included, but unspecific
8	Completeness of results	Selection of results reported	Selective reporting of results; incomplete results	Non-selective reporting of results; complete results	Unclear if all results reported
9	Believable conclusions	Believability of conclusions based on interpretations of the results	Inaccurate interpretations of the results, including failure to acknowledge sample size	Appropriate interpretations of results, including acknowledgement of limitations relating to study power or precision	Not possible to make judgement on believability of conclusions due to lack of information
10	Consistency	Consistency of results and/or conclusions with other published findings	Results or conclusions clearly inconsistent with other available evidence according to the article's discussion	Results and/or conclusions consistent with other published findings	Unclear if results and/or conclusions are consistent with other published findings

domain relating to study type (observational vs. experimental) was not included as all studies were observational. Instead, proportions of studies at different levels of the classical 'evidence pyramid' are reported. Guidance was provided by the Critical Appraisal Skills Programme (CASP)[2] tool for case-control studies and other literature (Wylie et al., 2014; Sullivan et al., 2015). In addition, aspects of the 'Grading of Recommendations, Assessment, Development and Evaluation' (GRADE)[3] method of assessing certainty in evidence and strength of recommendations, such as concepts of precision and consistency, were incorporated. To avoid categorising most of the studies as low quality, thus limiting the ability to differentiate between them, the full GRADE approach was not used. Risk-of-bias for each domain was categorised as high, low, or unclear based on the detailed review protocol (available upon request). Results are presented in stacked bar charts for each diagnostic indicator.

Data charting

Data from each article, including horse characteristics, details of diagnostic methods (e.g. BAL processing methods and mucus scoring systems) and key results including effect size and statistical significance, were extracted using a pre-defined form in an online survey tool.[4]

[3] See: The GRADE working group, 2020. https://www.gradeworkinggroup.org/ (Accessed 5 July 2022).

[4] See: SurveyMonkey. https://www.surveymonkey.co.uk (Accessed 5 July 2022).

Results

Review findings are presented here as (i) a summary of search results, (ii) risk-of-bias assessments presented by indicator, and (iii) a narrative synthesis of evidence for associations between indicators, highlighting commonalities and differences across articles reviewed. Detailed results of data charting, including specific diagnostic measures used, significance of associations, and effect sizes (where reported), are presented for each of the 45 reviewed articles, grouped by pairwise relationship, in Appendix A: Supplementary material.

Search results

Numbers of articles identified and subsequently excluded are outlined in Fig. 2. Forty-five articles from 44 studies involving 6092 horses were included. Studies had been conducted in clinics or hospitals, at yards or racetracks, or with research herds. The number of horses per study ranged from 8 to 1005 (median 74). Breed was reported in 30 articles and use of horses in 29 articles. The number of horses for which breed was reported was 4699, with 2730 (58.2%) being Thoroughbreds. Use was reported for 5439 horses, with 3960 (72.8%) being racehorses. Of the 44 studies, 29.5% ($n = 13$) were 'opportunistic observational studies', which examined data collected from groups of horses presenting at clinics, rather than any classical epidemiological study type. Cross-sectional studies made up 25.0% ($n = 11$), case-control studies 13.6% ($n = 6$), longitudinal studies and intervention studies6.8% each ($n = 3$ each), case series 4.5% ($n = 2$) and for 13.6% ($n = 6$) studies the design was unclear. In most articles (80%; $n = 36/45$), multivariable analysis to adjust for potential confounding bias was not used.

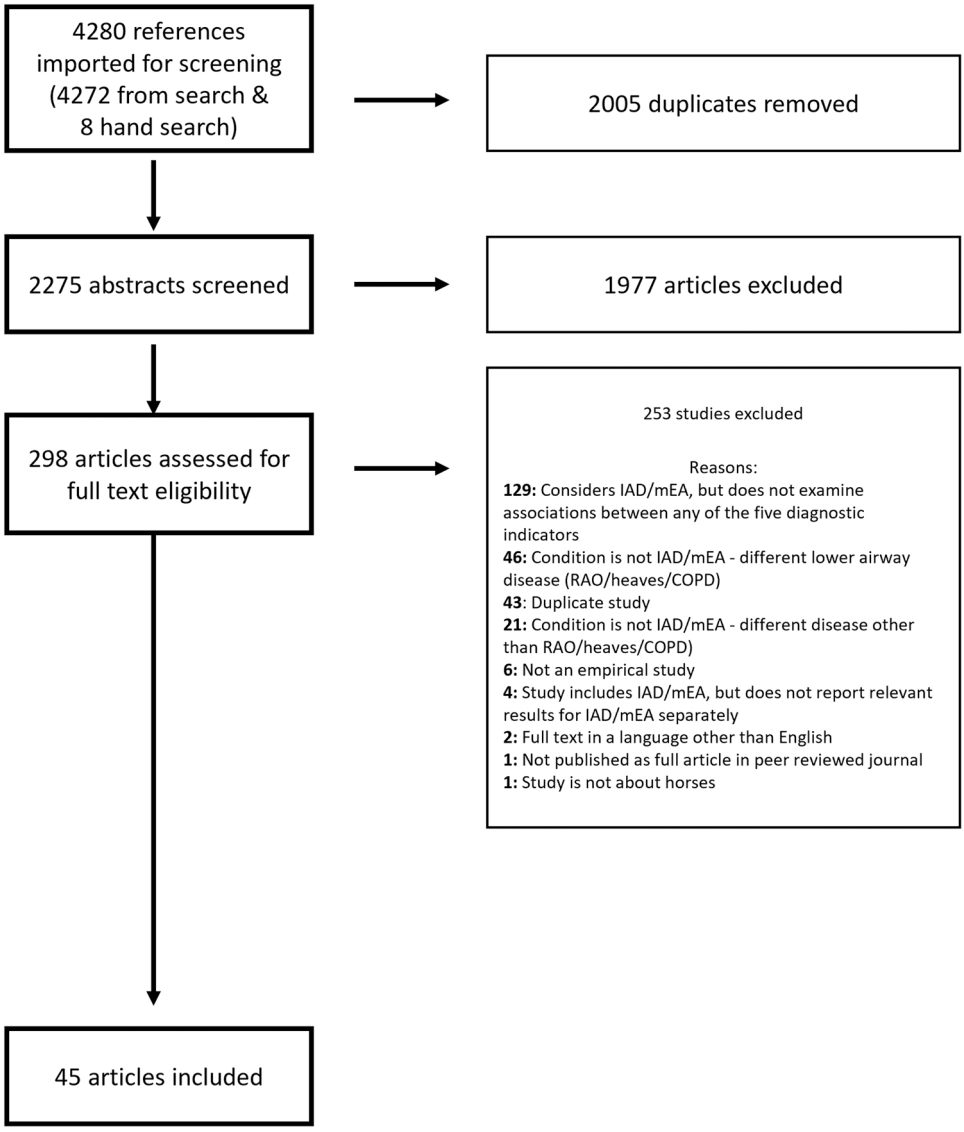

Fig. 2. PRISMA (Transparent reporting of systematic reviews and meta-analyses) flow diagram. (IAD, inflammatory airway disease; mEA, mild-moderate equine asthma; RAO, recurrent airway obstruction; COPD, chronic obstructive pulmonary disease.)

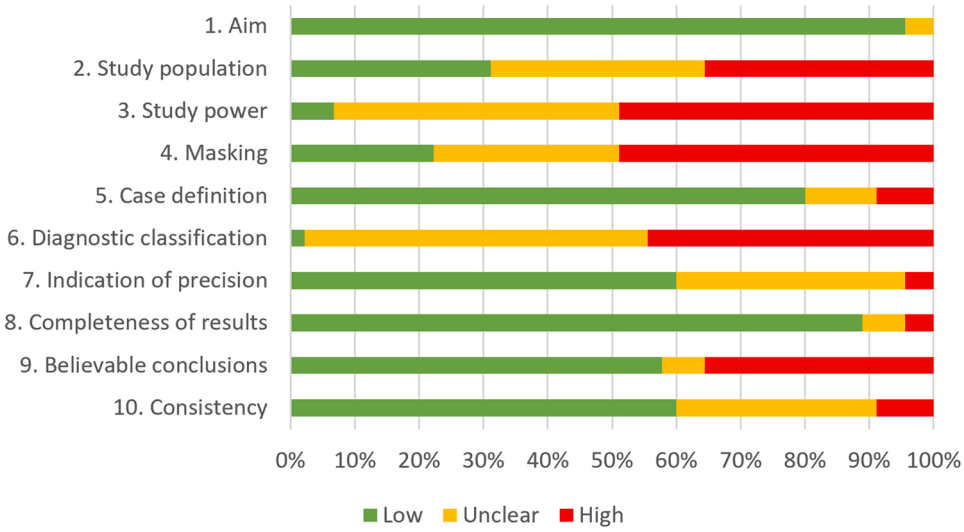

Fig. 3. Summary of the percentage of studies categorised as having low, medium and high risk of bias for each risk of bias domain, across all 45 reviewed articles.

Quality appraisal (risk-of-bias)

Overall

Proportions of articles with low, unclear or high risk-of-bias for each of the 10 domains across all 45 articles are summarised in Fig. 3. High risk of bias related to 'study power' and 'masking' in almost 50% of articles, and 'diagnostic classification' in over 40%.

Common factors leading to this level of risk included non-random, small convenience samples, and lack of information on recruitment and diagnostic procedures. Risk of bias for each of the indicators under review is outlined in detail below and summarised in Fig. 4.

Cough

Associations with coughing were examined in 10 articles (22.2%) from nine studies. Most (60%; $n = 6$) did not specify cough duration or frequency, describing only a history of cough or presence of cough at exercise. Two studies required coughing to be chronic, defined by a duration of more than three weeks (Bedenice et al., 2008) or at least three weeks (Wichtel et al., 2016). Two articles from one study defined a case by at least four coughs in 10 min of exercise (Christley et al., 2001a, 2001b). High risk of bias related predominantly to diagnostic classification (70% of articles) and lack of masking (60%; Fig. 4a).

Poor performance

Associations with poor performance were examined in 14 articles (31.1%), with poor performance defined by one or more of the following: trainer, rider or owner opinion ($n = 6$ articles), race place ($n = 7$), stake earnings ($n = 1$), speed figures ($n = 3$), velocity producing a lactate level of 4 mmol/l (VLA4; $n = 1$) and recovery time ($n = 1$). High risk of bias related predominantly to diagnostic classification (50% of articles) and study power (<40%; Fig. 4b).

Fig. 4. Summary of the percentage of studies categorised as having low, medium and high risk of bias, for each risk of bias domain, by diagnostic indicator. (BALF; Bronchoalveolar lavage fluid).

Tracheobronchial mucus

Associations with tracheobronchial mucus were examined in 28 articles (62.2%). Just over half (53.6%; $n = 15$) recorded mucus quantity using a 0–5 scale based on or equivalent to that described by Gerber et al. (2004). Otherwise mucus was recorded as present/absent ($n = 3$ articles) or using a scale of 0–3 ($n = 5$), 0–4 ($n = 1$) or 1–4 ($n = 1$). One study used a 0–5 score in the lower trachea and a 0–3 score at the bifurcation, and in one article mucus was described as 'excessive', without reference to a scale. High risk of bias related predominantly to lack of masking (>50% of articles) and 'diagnostic classification (50%; Fig. 4c).

BAL cytology

Thirty-one articles (68.9%) examined associations with BAL cytology, reporting a variety of sample collection and processing methods, including different volumes of lavage fluid, different cytological stains and number of cells counted (Table 2). Two studies collected BAL samples from the left lung, three from the right, two from both and 24 did not specify. High risk of bias related predominantly to study power (>60% of articles), masking and study population (both >40%; Fig. 4d).

Pulmonary dysfunction

Lung function testing was conducted in 18 studies (40%). Most ($n = 11$) used a combination of bronchoprovocation and lung mechanics testing. Lung mechanics tests included forced oscillatory mechanics ($n = 5$), flowmetric pleth ($n = 4$), impulse oscillometry ($n = 2$), inductive pleth ($n = 1$) and other methods ($n = 2$). Four studies used gas exchange analysis alone and one used gas exchange analysis in combination with lung mechanics. High risk of bias related predominantly to study power (>60% of articles; Fig. 4e).

Lung pathology

Assessment of histological lesions in lung tissue was reported in two articles (4.4%), for which risk of bias is summarised in Fig. 4f. Both articles were judged as having high risk of bias in relation to study power, case definition and believability of conclusions.

Associations between indicators

Detailed results of data charting are reported in Appendix A: Supplementary material to accompany the narrative synthesis below.

Cough and poor performance ($n = 2$)

Léguillette et al. (2016) reported correlations between cough at rest and owner-reported exercise intolerance, increased recovery time, pattern length and average run speed, and between cough at exercise and owner-reported exercise intolerance. However, it is unclear how to interpret reported correlation coefficients, as both cough variables were binary (present/absent) only. Bedenice et al. (2008) reported an unexpected lower occurrence of cough in association with poor or reduced performance in horses visiting private practices.

Cough and tracheobronchial mucus ($n = 8$)

All eight articles reporting on seven studies examining cough and mucus reported an association (Christley et al., 2001a, 2001b; Robinson et al., 2006; Kusano et al., 2008; Cardwell et al., 2014; Secombe et al., 2015; Léguillette et al., 2016; Wichtel et al., 2016), with cough identified as a specific but insensitive indicator of increased mucus (Robinson et al., 2006; Cardwell et al., 2014). Odds ratios ranged from 3.5 (95%CI: 1.3–9.3; Cardwell et al., 2014) to 82.0 (95%CI: 14.5–463.4; Christley et al., 2001b).

Cough and BAL cytology ($n = 3$)

All three articles examining this relationship (McKane et al., 1993; Bedenice et al., 2008; Secombe et al., 2015) reported significant associations between cough and BAL cytology, but involving different cell populations. Secombe et al. (2015) reported that horses with sole or mixed eosinophilic responses were more likely to present with cough, but detected no associations with neutrophilic responses. Bedenice et al. (2008) reported higher neutrophil percentages in coughing (for >3 weeks) than in non-coughing horses, but no association with eosinophils. Both McKane et al. (1993) and Bedenice et al. (2008) reported lower macrophage percentages in coughing than in non-coughing horses but had conflicting observations of higher (McKane et al., 1993) and lower (Bedenice et al., 2008) lymphocyte percentages in coughing than in non-coughing horses.

Cough and pulmonary dysfunction ($n = 1$)

Only one study (Bedenice et al., 2008) investigated a relationship between coughing and lung function, identifying just one significant association (higher frequency dependence of respiratory system resistance in coughing horses) among multiple comparisons and concluding that there was little evidence of an association overall.

Poor performance and tracheobronchial mucus ($n = 7$)

Two studies reported associations between tracheobronchial mucus and subjective measures of performance in different sport horse populations. Fraipont et al. (2011) identified an association between mean mucus score and trainer opinion of performance in the previous 3 months, and Widmer et al. (2009) reported an association between increased mucus and rider-judged poor performance in show horses.

Of five studies using race place as a more objective indicator of performance, three detected associations. MacNamara et al. (1990) observed mucopurulent exudate in the trachea of 10% of horses finishing first or second ($n = 558$) compared to 39% of horses finishing seventh or eighth ($n = 407$), reporting this as significant but without reporting a P-value or confidence intervals, while Richard et al. (2010) identified significantly higher mucus scores in intermediate/poor performers (finishing 5th-8th or >8th) than in horses performing well (finishing 1st-5th). Holcombe et al. (2006) detected significantly higher race places in horses with high compared to low mucus scores. Two studies (Saulez and Gummow, 2009; Salz et al., 2016) did not detect associations between mucus scores (using different scales) and race place (placed or not). However, most horses in the Salz et al. (2016) study (97.4%; 189/194) had a mucus score of zero and no horses had a score higher than 2 (0–4 scale).

Poor performance and BAL cytology ($n = 6$)

In four studies examining this relationship, higher BAL neutrophil percentages were associated with poor performance, based on trainer opinion (Fraipont et al., 2011), trainer opinion and race place (Richard et al., 2010), speed figures (Ivester et al., 2018) and velocity at 4 mmol/L of lactate (VLA4; Stucchi et al., 2020). Richard et al. (2010) also reported significantly lower macrophage percentages in intermediate/poorly performing horses. Ivester et al. (2018) reported a reduction in speed figures for each percent increase in both neutrophils and mast cell proportions. Maximal speed achieved and duration of final step in treadmill tests were significantly lower in horses with increased BAL neutrophils, mast cells or eosinophils than in controls (Richard et al., 2012). Stucchi et al. (2020) reported a significant reduction in VLA4 with increasing neutrophil proportions and a significant increase in VLA4 with increasing lymphocyte proportions. However, Mazan and Hoffman (2001) did not detect associations between BAL cell percentages and exercise capacity during standardised exercise tests when

Table 2
Summary of bronchoalveolar lavage (BAL) sampling and analysis methods used in the 31 studies examining relationships with BAL cytology.

Time sampled after exercise	**30–60 min**	**> 60 min - < 24 h**	**Not specified**	**Time varied**	**Not conducted after exercise**	
n (%)	7 (22.6)	3 (9.7%)	1 (3.2)	2 (6.4)	18 (58.1)	
Lung sampled	**Both**	**Right**	**Left**	**Not specified**		
n (%)	2 (6.4)	3 (9.7%)	2 (6.4)	24 (77.4)		
Stain	**Wright-Giemsa stain (including modified)**	**May-Grunwald Giemsa**	**Wright's stain (including modified)**	**Diff-Quik**	**Other**	**Not specified**
n (%)	10 (32.2)	1 (3.2)	3 (9.7)	2 (6.4)	11 (35.5)	4 (12.9)
Cells considered	**Neutrophils**	**Eosinophils**	**Mast cells**	**Macrophages**	**Lymphocytes**	
n (%)	30 (96.8)	26 (83.9)	25 (80.6)	21 (67.7)	22 (71.0)	
Number of cells counted	**200 or lower**	**300**	**400**	**500**	**800**	**Not specified**
n (%)	8 (25.8)	4 (12.9)	7 (22.6)	6 (19.4)	2 (6.4)	4 (12.9)

measured by, for example, number of steps completed.

Poor performance and pulmonary dysfunction (n = 2)

Two studies compared lung function between horses performing well and poorly as judged by trainers (Couëtil and Denicola, 1999; Fraipont et al., 2011) and race place (Couëtil and Denicola, 1999). Couëtil and Denicola (1999) identified more severe exercise-induced arterial hypoxaemia and hypercapnia in poor performers compared with good performers, while Fraipont et al. (2011) did not detect any significant differences in lung mechanics measured by impulse oscillometry between poor, intermediate and good performers. In both studies, the relationship between poor performance and lung function was confounded by tracheal mucus.

Tracheobronchial mucus and BAL cytology (n = 14)

Fourteen studies assessed the relationship between tracheobronchial mucus and BAL cytology in different horse populations using different approaches to mucus scoring and cytological case definition and mostly without reporting specific effect sizes or significance. Most (64.3%; $n = 9$) did not detect an association (Holcombe et al., 2001; Gerber et al., 2003; Allen et al., 2006; Richard et al., 2010; Fraipont et al., 2011; Hughes et al., 2011; Depecker et al., 2014; Secombe et al., 2015, 2019), including when examining BAL from both lungs (Depecker et al., 2014). Specifically, six studies found no association between a specified mucus score (Gerber et al., 2004) and BAL neutrophil percentages or absolute numbers (Gerber et al., 2003; Depecker et al., 2014), > 5% neutrophils or mast cells (Hughes et al., 2011), or multiple definitions of abnormal BAL cytology (Richard et al., 2010; Fraipont et al., 2011; Depecker et al., 2014; Secombe et al., 2019). Allen et al. (2006) found no association between an undescribed mucus score and neutrophil percentages and Secombe et al. (2015) found no association between presence of mucus and various definitions of abnormal BAL cytology. Koblinger et al. (2011) reported an association between tracheal mucus score (Gerber et al., 2004) and severe BAL inflammation, but not moderate BAL inflammation likely to be mEA. Overall weak but significant correlations were reported between mucus score and neutrophil, mast cell and macrophage percentages. Elsewhere, increased mucus, defined by a score > 2 on a 0–5 scale (Gerber et al., 2004) was associated with higher mean neutrophil percentages (Sad et al., 2013) and with > 20% neutrophils (Wichtel et al., 2016). Increased mucus defined as moderate (continuous streak along the ventral trachea) or large amounts (secretions adherent around the inner tracheal surface in addition to a continuous streak) was associated with higher mean neutrophil count, neutrophil percentages, and lower mean macrophage percentages (Couëtil and Denicola, 1999). One study using the Gerber et al. (2004) 0–5 mucus score for the lower third of the trachea and a 0–3 score at the tracheal bifurcation identified correlations between BAL neutrophil percentage and both scores, macrophage percentage and both scores, and between lymphocyte percentage and the six-point score (Wysocka and Kluciński, 2015). No correlations were detected between lymphocyte percentage and the four-point score or between eosinophil percentage and either mucus score (Wysocka and Kluciński, 2015).

Tracheobronchial mucus and pulmonary dysfunction (n = 5)

Two studies identified associations between abnormal arterial blood gas values and presence of (Durando et al., 2006) or moderate to large amounts of (Couëtil and Denicola, 1999) mucus in the trachea. The majority (10/13; 76.9%) of horses with increased mucus in the latter study were also defined as poor performers. Two further studies did not detect associations between airway hyperresponsiveness and the Gerber et al. (2004) tracheal mucus score (Wichtel et al., 2016) or a score of 2 or greater on the same scale (Secombe et al., 2019). Pires et al. (2017) reported a significant association between elevated intrapleural pressure and a mucus score (Gerber et al., 2004) of 2 or greater.

Tracheobronchial mucus and lung pathology (n = 1)

One study (Winder et al., 1989) detected no significant difference in amount of mucus (prior to, or immediately after, slaughter) between normal horses ($n = 17$) and those with histological signs of mild chronic small airway disease ($n = 23$).

BAL cytology and pulmonary dysfunction (n = 13)

Of the 13 studies that examined relationships between BAL cytology and lung function, seven (Hare and Viel, 1998; Hoffman et al., 1998; Mazan and Hoffman, 2001; Mazan et al., 2005; Richard et al., 2009; Houtsma et al., 2015; Secombe et al., 2019) identified some associations and six (Davidson et al., 2011; Nolen-Walston et al., 2013; Pacheco et al., 2014; Wichtel et al., 2016; Léguillette et al., 2017; Cullimore et al., 2018) did not. Hoffman et al. (1998) and Mazan and Hoffman (2001) identified significant associations between mast cell percentages and lung mechanics following bronchoprovocation and Mazan et al. (2005) and Houtsma et al. (2015) detected significant associations between both mast cell and neutrophil percentages and lung mechanics following bronchoprovocation. Secombe et al. (2019) found that horses with mixed cell responses or sole mast cell responses (>5%), but not sole neutrophil responses, were more likely to have airway hyperreactivity compared to horses with normal cytology. Richard et al. (2009) reported significantly higher respiratory resistance and significantly lower reactance at certain frequencies in horses with mEA/IAD (defined by >10% neutrophils, >2% mast cells or >1% eosinophils) compared with control horses. Significant correlations between lung function measurements and both mast cell and eosinophil counts, but not neutrophil counts were detected in mEA/IAD horses only. Hare and Viel (1998) examined only eosinophils, detecting significant differences in lung mechanics both with and without bronchoprovocation between horses with BAL eosinophil count > 5% and controls, and an overall correlation between eosinophil count and lung mechanics following bronchoprovocation.

Of the six studies that did not detect an association between lung

function and BAL cytology, three used blood gas values to assess lung function but different BAL cytological thresholds. Nolen-Walston et al. (2013) detected no significant differences in exercising blood gas values between horses with mEA and those with normal BAL cytology. Davidson et al. (2011) reported no significant associations between low PaO2 or high PaCO2 before, during or following exercise and mEA/IAD, but only two horses were defined as having normal BAL cytology and data relating to a comparison between IAD and non-IAD horses were not reported. In both studies, all horses had presented with poor performance and the majority (~80%) were cases of mEA/IAD, defined by the reported thresholds. Pacheco et al. (2014) detected no association between proportions of all examined cell types and PaO2 and PaCO2 but only examined blood gases at rest.

Pacheco et al. (2014), Wichtel et al. (2016), Léguillette et al. (2017) and Cullimore et al. (2018), detected no associations between lung mechanics following bronchoprovocation and numbers (Wichtel et al., 2016) or percentages (Pacheco et al., 2014; Wichtel et al., 2016; Cullimore et al., 2018) of neutrophils, macrophages, eosinophils, mast cells or lymphocytes (Pacheco et al., 2014; Wichtel et al., 2016) or neutrophils, mast cells, eosinophils (Léguillette et al., 2017; Cullimore et al., 2018).

BAL cytology and lung pathology (n = 1)

One study reported a 'good qualitative correlation', based on subjective judgement rather than statistical testing (Fogarty, 1990) between > 10% pre-slaughter BAL neutrophils and neutrophil infiltration of lower airways and alveoli, as well as between pre-slaughter BAL mast

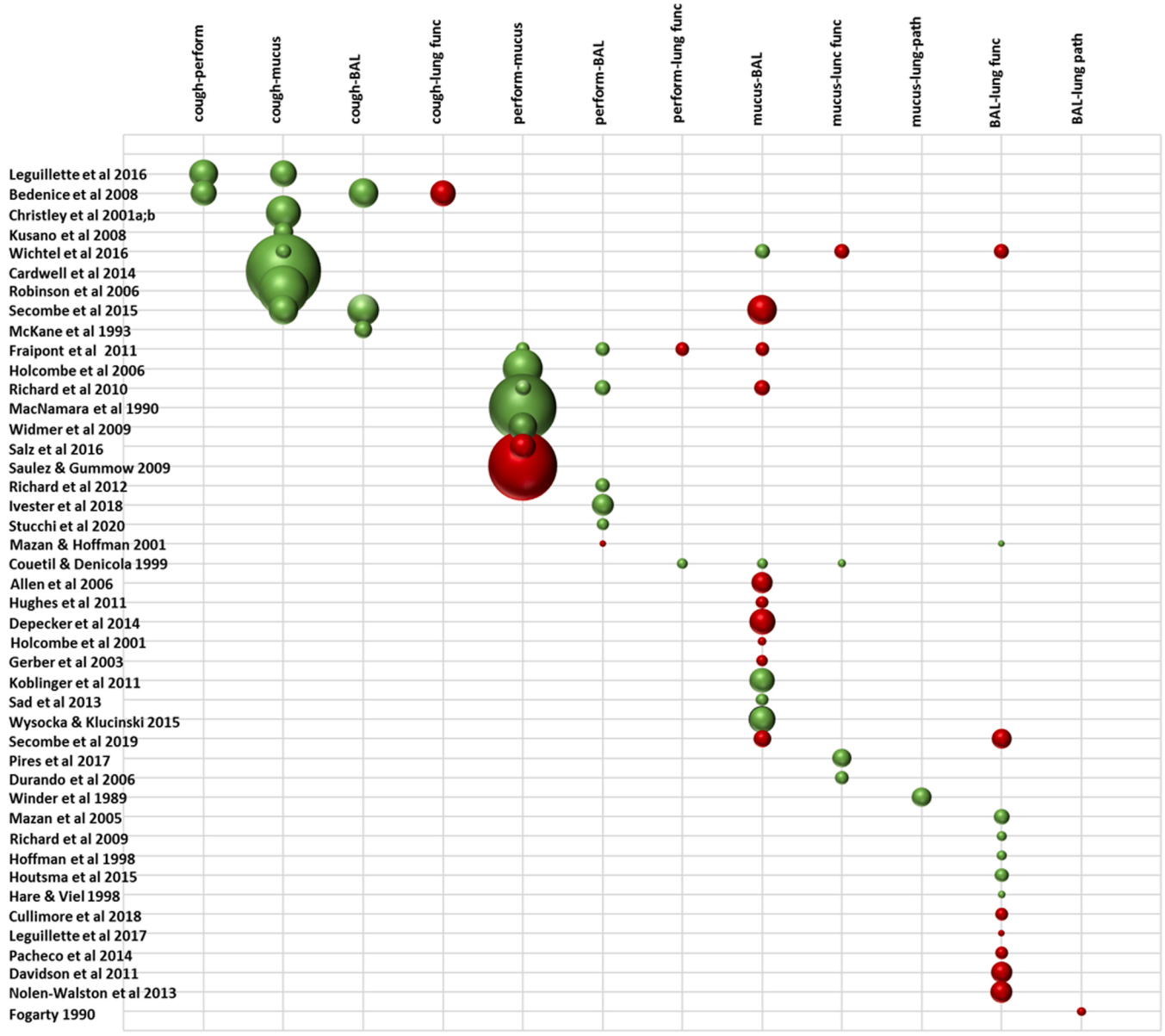

Fig. 5. Bubble chart summarising associations between diagnostic indicators identified in each of the 45 reviewed articles. Refer to text and supplementary material for further detail. Size of bubble represents study sample size. Colour indicates the significance (green, significant*; red, not significant) but not the direction of the association – note that both studies examining associations between cough and poor performance detected a significant association, but with different directionality. The number of horses evaluated for tracheal mucus (179 vs. 170) was unclear in one study (Secombe et al., 2015) and is represented here assuming $n = 179$. (Perform, poor performance; BAL, bronchoalveolar lavage; lung func, lung function; lung path, lung pathology.) *On the basis of reporting a P-value < 0.05 or confidence intervals not containing the null value, except one study that reported a significant association without reporting statistical parameters (MacNamara et al., 1990).

cells and mast cells in bronchiolar tissue, in 15 horses. BAL eosinophils were reportedly detected in only 40% of lungs in which histopathology subsequently identified eosinophilic infiltration.

Results summary

Relative study sample sizes and significance of associations for each relationship are summarised in Fig. 5. This highlights that there was consistent evidence across studies for associations between cough and both mucus and BAL cytology, but less consistent evidence for associations between performance and mucus, performance and BAL cytology, and mucus and lung function. There was conflicting evidence for associations between performance and lung function, mucus and BAL cytology, and BAL cytology and lung function. Relationships between mucus and lung pathology (association detected) and between BAL cytology and lung pathology, and cough and lung function (associations not detected) were examined in single studies. No studies examined associations between lung pathology and pulmonary dysfunction, poor performance or cough.

Discussion

This review aimed to complement the latest ACVIM consensus statement on mEA (Couëtil et al., 2016) and inform future research by assessing the scope and evaluating the quality of the evidence base supporting the consensus mEA case definition. Inconsistencies in clinical and diagnostic methods and varying measures of the key clinical indicators presented a challenge to the evaluation of evidence across studies and would preclude meta-analysis. We identified 45 articles that examined relationships between relevant diagnostic indicators of mEA and fulfilled our inclusion criteria. Articles that did not present results for IAD/mEA alone, or where these relationships were clearly confounded by other unrelated conditions, were rejected.

The current case definition is broadly relevant to sport and leisure horses of any breed and age, with acknowledgement that determining the prevalence of different equine asthma phenotypes in different equine populations is required. The evidence base comprised studies of various breeds, uses and ages of horses, but with an overall predominance of racehorses, particularly Thoroughbreds. There was also a predominance of small, opportunistic studies of clinical populations, mostly examining univariable relationships, with few larger population-based or classical epidemiological studies; this means that the overall body of evidence for independent associations is distorted by confounding to an unknown extent. Risk-of-bias assessments indicated that high risk of bias most often related to low study power, lack of masking, and potential diagnostic misclassification. Some of this risk of bias related to lack of detail in reporting. As publication dates of studies reviewed spanned over 30 years, this might partly reflect less stringent reporting standards at the time of publication; it is likely that manuscript checklists now required by many journals have improved reporting transparency.

There was conflicting evidence for all the examined relationships except for that between coughing and tracheobronchial mucus, for which all seven studies reported an association, and between cough and BAL, for which all three reported an association. We did not explore evidence for these relationships in the RAO/sEA-related literature because until the relationship between mEA and sEA is better understood it cannot be assumed that evidence from sEA studies can be extrapolated to mEA.

The consensus case definition regards the presence of a chronic (>3-week duration), occasional cough as a key baseline indicator of mEA. However, our review did not identify evidence supporting the requirement for a > 3-week duration of coughing. One study (Bedenice et al., 2008) reported a significant association between chronic cough, but not cough of unspecified duration, and BAL neutrophil percentage, but most of the reviewed studies examining cough did not specify the duration. Pressure on veterinarians to investigate and treat coughing before it becomes chronic can preclude adoption of the consensus case definition in some contexts (Kinnison and Cardwell, 2020) and further investigation of cough duration as an indicator of mEA would be valuable.

Another key baseline indicator of mEA is poor performance, which, as acknowledged in the consensus statement, is difficult to define objectively. In the studies reviewed, poor performance was frequently defined based on subjective measures. When relatively objective measures such as race place were used, variations across studies (e.g., recording placed versus not placed, or first/second versus seventh/eighth place) prevented meaningful comparisons.

Having been excluded from the first consensus statement on IAD case definition (Couëtil et al., 2007), tracheal mucus was incorporated into the most recent consensus on defining mEA (Couëtil et al., 2016) on the basis of reported associations with poor performance (MacNamara et al., 1990; Holcombe et al., 2006; Widmer et al., 2009) and cough (Christley et al., 2001a; Cardwell et al., 2014) and with reference to a 0–5 point scoring system (Gerber et al., 2004). Although the scoring of mucus was not consistent across studies we reviewed, the scale proposed by Gerber et al. (2004) predominated. This scale has been validated as a reproducible measure in a study of only nine horses (two healthy, four IAD/mEA-affected and four RAO-affected), suggesting that validation in a larger study would be advantageous. Although mucus was consistently associated with cough evidence for a relationship between mucus and poor performance was slightly less consistent, with five of seven relevant articles reporting an association (MacNamara et al., 1990; Holcombe et al., 2006; Widmer et al., 2009; Richard et al., 2010; Fraipont et al., 2011). However, findings from MacNamara et al. (1990) and Holcombe et al. (2006) suggested that it is increased mucus, rather than small amounts of mucus, that is associated with poor performance and as one of the studies that did not detect an association (Salz et al., 2016) had no horses with increased mucus, a lack of association could not be inferred, leaving just one study that did not detect an association (Saulez and Gummow, 2009). An association between mucus and pulmonary dysfunction was identified in three of the four relevant studies (Couëtil and Denicola, 1999; Durando et al., 2006; Pires et al., 2017), but in one of these (Couëtil and Denicola, 1999) this relationship was confounded by poor performance.

Most studies investigating relationships between tracheal mucus and BAL cytology, however, did not detect an association. The studies not detecting associations tended to be larger than those that did, making low power less likely to be responsible for the lack of association. Whether mucus accumulation and BAL cytology abnormalities are indicative of the same underlying disease process and, if not, what the underlying cause of increased mucus is, remains to be determined. Given that assessment of tracheal mucus is relatively non-invasive, well tolerated and is a focus of concern in some contexts (Kinnison and Cardwell, 2020), improved understanding of its relevance in relation to lung function and performance, and as a diagnostic indicator of mEA, would be valuable.

Airway secretion cytology is the primary method of confirming a diagnosis of mEA in cases suspected on clinical grounds, with BAL widely considered to be the preferred tool (Couëtil et al., 2016). Considerable variations in BAL collection and cytological methods across reviewed studies included variations in timing of collection following exercise, lung sampled, staining agent used, number of cells counted and cytological thresholds for defining a case. Overall, most studies counted around 200 cells with higher proportions of more recent studies having counted 400 or more cells, perhaps reflecting updated practices, with the counting of at least 500 cells now recommended as part of a minimum database in a recent review (Couetil et al., 2020). Some degree of consensus on standardisation of BAL sampling and analysis would be useful. However, although there was consistent evidence for a relationship between BAL cytology and poor performance across reviewed studies, and athletic performance is a key concern for owners or trainers of sports horses, there was considerably more conflict across studies examining associations between BAL cytology and lung

function, with just under half reporting an association. Studies detecting and not detecting associations were of similar size, suggesting that the absence of significant associations was not simply because of low study power. None of the three studies using blood gases as an indicator of lung function detected an association with BAL cytology, although one of these only examined blood gases at rest. However, except for examination of blood gases, lung function testing is not readily available for many veterinarians, other than in some specialist centres. A recently developed portable modality is no longer being marketed (Couetil et al., 2020) and thus one of the consensus statement's recommended future research directions, to develop portable sensitive lung function testing devices, is still highly pertinent.

BAL sampling is widely accepted in many, but not all contexts. On British racing yards, investigation of lower airways instead relies on tracheal endoscopy and tracheal wash sampling (Kinnison and Cardwell, 2020). Tracheal wash cytology was not considered a sufficiently reliable indicator of lower airway inflammation for inclusion in the consensus case definition of mEA and was therefore beyond the scope of this review of case definition components. A recent article reported that, using neutrophil percentage, tracheal wash cytology provided greater sensitivity and specificity than BAL cytology in the diagnosis of equine respiratory disease (Rossi et al., 2018). However, the study included a variety of respiratory diseases, of which only a small number were classed as mEA; the diagnostic accuracy of TW compared with BAL for mEA is therefore still unknown. Further work to review evidence supporting, or otherwise, the use of tracheal wash cytology as a diagnostic indicator of mEA would be valuable.

Research data and outputs are more comparable when more specific case definitions are used, but it is inevitable that a variety of methods will be used in different contexts worldwide. The performance of any diagnostic test will vary in different contexts and the trade-off between test sensitivity and specificity must be optimised through the choice of context-specific thresholds. The consensus statement mitigates this through its broad recommendations for diagnosing mEA. For example, specific cut-offs for cytological parameters are not defined, allowing for different thresholds to be used for different populations in varying environments. Given the wide variation in environments, uses and management of horses worldwide it is likely to be unfeasible to devise a consensus case definition that is relevant for all contexts, internationally applicable and accepted. However, the use of a broad definition and the wide variety of methods used preclude meaningful direct comparisons or meta-analyses and the building of a robust evidence base. Reaching some degree of consensus on standardisation of diagnostic approaches, and on the minimum information required for reporting results would facilitate direct comparisons of future studies.

The aim of a scoping review is to synthesise all available evidence and identify weaknesses or knowledge gaps (Peterson et al., 2017). Despite a systematic approach to literature searches in formal reviews, inclusion of all relevant articles is not guaranteed, as a manageable balance between comprehensiveness and precision of searches must be achieved according to the Cochrane Handbook for Systematic Reviews.[5] Additionally, the detailed evaluation that the review requires can result in identification of presumed errors and lack of clarity in the original articles. This creates difficulty in the interpretation and reporting of some results and conclusions. We aimed to be comprehensive by including all relevant studies of mEA/IAD published over a long timeframe. However, a large proportion of these preceded the latest consensus statement and research published subsequently did not necessarily adhere to consensus guidelines. This meant accepting a variety of case definitions, including some composite definitions for which results relating to specific components were not reported. Because of strict inclusion criteria, it is unlikely that we have included studies of cases that would now be classified as sEA/RAO, but we may have missed studies of cases classified as sEA/RAO that would meet current IAD/-mEA case definition criteria. Our requirement for articles to present separate results for IAD/mEA cases led to the exclusion of some articles (e.g. Fogarty and Buckley, 1991; Couroucé-Malblanc et al., 2002) in which IAD/mEA diagnostic components were explicitly confounded by other conditions. Conversely, we will have included studies in which confounding conditions were present but not identified.

[5] See: Cochrane Handbook for Systematic Reviews of Interventions Chapter 4: Searching for and selecting studies. https://training.cochrane.org/handbook/current/chapter-04 (Accessed 5 July 2022).

Conclusions

Overall, this review provides a foundation on which subsequent reviews can build and indicates where further research is required to refine the mEA case definition. In particular, evidence for a relationship between mucus and BAL cytology was conflicting, and neither was consistently associated with lung function. This means that the functional relevance of these measures remains unclear. As highlighted recently by Couetil et al. (2020), the use of standardised methodological approaches and reporting would be beneficial to improve the evidence base and facilitate future systematic review and meta-analysis.

Conflict of interest statement

None of the authors of this paper has a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.tvjl.2022.105865.

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