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Helminth-based therapies for rheumatoid arthritis: A systematic review and meta-analysis

Kane Langdon[a], James Phie[b], Chetan B. Thapa[a], Erik Biros[b], Alex Loukas[c], Nagaraja Haleagrahara[d,*]

[a] College of Medicine and Dentistry, James Cook University, Douglas, 4814 Townsville, Australia
[b] Queensland Research Centre for Peripheral Vascular Disease, Douglas, 4814 Townsville, Australia
[c] Australian Institute of Tropical Health and Medicine, Centre for Biodiscovery and Molecular Development of Therapeutics, Smithfield, 4878 Cairns, Australia
[d] College of Public Health, Medicine and Veterinary Sciences, James Cook University, Douglas, 4814 Townsville, Australia

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ABSTRACT

Objective: Proteins from parasitic worms have been posed as novel therapies for rheumatoid arthritis (RA) and other auto-inflammatory diseases. However, with so many potential therapeutics, it is important that drug discovery be based on the specific phyla or species which show the most promising effects. Therefore, the aim of this systematic review and meta-analysis was to evaluate the reported effects of helminthic secretory proteins and derivative therapy on RA in an animal model.

Methods: Medline, Scopus and Web of Science were searched to identify studies evaluating helminthic therapy in the collagen-induced arthritis model of RA. A meta-analysis was undertaken to determine the overall effect of the proteins. Subgroup analyses were also undertaken to investigate individual treatments.

Results: Seven articles were included in the analysis. Overall, helminthic therapy significantly reduced arthritis score (SMD −1.193, 95% CI −1.525, −0.860). Subgroup analyses found a significant reduction in arthritis score following treatment with helminth protein ES-62 (SMD −1.186, 95% CI −1.633, −0.738) and phosphorylcholine-based treatment (SMD −0.997, 95% CI −1.423, −0.571). Subgroup analyses found ES-62 treatment significantly decreased IFN-γ levels (SMD −1.611, 95% CI −2.734, −0.487) and significantly increased levels of IL-10 (SMD 0.946, 95% CI 0.127, 1.765).

Conclusions: Therapeutics from parasitic worms are a promising avenue for drug discovery, especially with all included studies reporting a significant improvement in arthritis score. Based on pooled data presented in this study, the nematode *Acanthocheilonema viteae* seems to be of particular interest for therapeutics.

1. Introduction

Rheumatoid arthritis (RA) is a progressive inflammatory disease that is associated with significant morbidity and mortality. Primarily localised to the synovium of the joints, unchecked development of this disease can lead to irreversible, erosive damage to joint cartilage and bone [1,2]. RA is estimated to affect 0.24% of the world with Australasia reporting the highest prevalence of 0.46%, closely followed by Western Europe and North America at 0.44%. The lowest prevalence is believed to be in North Africa and the Middle East at 0.16% [3]. However, the disease affects up to 1% of individuals in some population groups [4], and has been estimated to cost the economy > 12,000 USD per patient, per year [5]. This equates to upwards of 9 billion USD from the American economy each year. Despite the huge expenditure, current treatment regimens can only be designed to promote disease remission in patients rather than affecting the upstream pathological factors. This is mainly because the complex interactions between these factors have not been fully elucidated [6]. To better promote disease remission, there have been a number of new potential targets proposed for the treatment of the inflammatory cascades in RA. Spleen tyrosine kinase, choline kinase, hypoxia-inducible factor-1a, galectin 3 and tristetrapolin have all been suggested as novel focusses for treatment [7].

The incidence of RA and other autoinflammatory diseases have increased in developed countries throughout the last century. While this is a multifactorial problem, a major factor is attributed to urbanisation having caused the depletion of environmental organisms with which the human body has evolved. Organisms that may be crucial for

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* Corresponding author at: Building 87, Solander Drive, James Cook University, Townsville, Queensland 4811, Australia.
E-mail address: haleagrahara.nagaraja@jcu.edu.au (N. Haleagrahara).

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immunoregulatory balance [8]. It is suggested that the reduction of these organisms in the environment has removed an element of immune regulation in the population, thus promoting the development of RA and other immune-mediated diseases. The attribution of this recent spike in autoinflammatory disease to the loss of microbes and other immunomodulators has been termed the hygiene hypothesis, or more recently, the old friends hypothesis [8].

The old friends hypothesis lists helminths as one of the major groups of organisms that are implicated in this spike of autoinflammatory disease incidence. Indeed, infection with these parasitic worms or treatment with their secretory proteins have been shown to be protective against immune-mediated diseases in animals and humans [9–12]. Helminthiases and their associated effects on the immune system have been discussed in the literature at length. It is generally recognised that there seems to be some amelioration of Th1-mediated disease by infection with helminths. However, therapeutic uses of helminth infection remain controversial due to the associated morbidity from the infection itself. Furthermore, patients treated with immunosuppressive drugs for diseases such as RA are more prone to poorer outcomes following parasitic infections. Consequently, research is currently focussed around the use of immunomodulatory proteins secreted by helminths as potential treatments rather than helminth inoculation itself; preventing any injury that is associated with helminthiases [13]. However, it is important that the general effectiveness of these proteins is systematically evaluated before more research is undertaken in this area, ensuring that additional research will elucidate further potential treatments for RA. Furthermore, humans alone are home to 300 different species of helminths and each of these have their own secretory repertoire [14], meaning the array of potential therapeutics is expansive. Therefore, it is necessary to focus in on which helminths or species secrete proteins that seem to have the most sizeable effect on the disease to optimise drug discovery. This systematic review with meta-analysis is the first of its kind to evaluate the effectiveness of helminth proteins and their derivatives in general. Subgroup analyses were also utilised to evaluate the effectiveness of treatments from specific helminth species to assist in the direction of future research in this area.

2. Methods

2.1. Literature search and inclusion/exclusion criteria

This review was prepared following the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) statement [15], and was prospectively registered on PROSPERO or the International Prospective Register of Systematic Reviews (CRD42018090626). Medline, Scopus and Web of Science were searched for pre-clinical studies with a search strategy designed around the keywords: experimental rheumatoid arthritis, helminths and mice. These databases were searched from their inception to March 2018. The databases were also searched again in August 2018, just prior to submission, to ensure any recent articles could be included. The complete search strategy can be found in the online Supplementary material (S1). Titles and abstracts were screened by two independent authors (CT and KL) and then the reference list of all included studies was screened for any further studies which fit the inclusion criteria. Any disagreements were resolved by discussion. Articles were eligible for inclusion if they evaluated the effects of a helminthic protein or derivative therapy in the collagen-induced arthritis (CIA) DBA/1 mouse model of rheumatoid arthritis [16]. Only studies that reported clinical arthritis score, cytokine levels or total anti-collagen antibodies as an outcome measure were included. Included articles were restricted to original research publications in English with no concomitant anti-inflammatory therapy.

2.2. Data extraction and quality assessment

Eligible studies were independently assessed by two authors (JP and KL) with final results being agreed upon in a consensus meeting. Data extraction was first attempted from text, figures or tables. If the outcome measures were not reported in this form, the digital ruler tool from Nitro Pro 9 (Nitro Software, Inc. 2013) was used to extract the data from graphs. In cases of missing data, the authors were contacted. One author was contacted for missing data with no reply [17]. The data extracted included mouse age and sex, timing of arthritis induction and date of sacrifice. The evaluated protein and its dose, route of administration, timing and helminthic source were also extracted. Arthritis score, reported serum or cultured cytokine levels (IL-17, IL-1β, IL-22, IL-10, TGF-β, TNF-α, IL-6 and IFN-γ) and total anti-collagen antibodies were extracted as outcome measures. For any outcome measures that were reported as mean ± standard error of the mean (SEM), the SEM was converted to standard deviation (SD) using the equation $\sigma = SEM\sqrt{n}$ where n equals the number of individuals. Study quality was evaluated by using an assessment tool adapted from both SYRCLE's risk of bias tool and the CAMRADES study quality checklist [18,19]. The analysis consisted of 10 yes/no questions with each yes answer worth one point. The number of points accrued by each article was reported as an indication of study quality. The questions asked in the quality assessment included: were the mice the same sex{1} and born within a month of each other{2}? Did the study statistically justify its sample size{3}? Was there randomisation of mouse allocation{4} and housing{7}. Was there blinding in the induction process{5} and assessment of arthritis score{6}? Was there evidence of some sort of environmental control{8}? Did the study comply with applicable welfare regulations{9}? Was a statement of potential conflicts of interest included{10}? Studies were rated as poor (< 4 points), moderate (4–5 points), good (6–7 points), or excellent quality (8–10 points).

2.3. Statistical analysis

All studies which met the inclusion criteria were eligible for pooling in the meta-analysis. Ten separate data points were required for the pooling of data and subgroup analyses required five points. Pre-defined subgroup analyses were each distinct therapy and the specific species from which each protein was sourced. Forest plots were constructed using Hedges' g as a standardised measure of the effect size using the *OpenMEE* statistical package, otherwise a descriptive summary was utilised [20]. Primarily, arthritis score data were pooled as a measure of the clinical effects associated with helminthic proteins. To further measure clinical effects, total anti-collagen antibody levels were summarised. Finally, a more mechanistic evaluation was then carried out by summarising the reported cytokine levels of the studies. No exploratory analyses were undertaken. As the inclusion criteria incorporated any helminth-derived protein therapy in CIA, a random effects statistical model was used for meta-analysis. The degree of this heterogeneity was evaluated using the I^2 statistic, with a result above 50% considered significant. A funnel plot was also produced to assess publication bias. Where a control group was used more than once as a comparator in a study, the n value was divided by the number of times it was extracted from the study when the data points were pooled. A p-value of < 0.05 was considered significant.

3. Results

3.1. Searches and study characteristics

The database searches returned a total of 980 articles (Fig. 1) [15]. After the removal of 75 duplicates, titles and abstracts of the remaining articles were screened against the inclusion criteria. The remaining 15 articles were then assessed in full where two more were excluded. One was excluded because it was not an original research paper [21]. The other paper was assessed in full to make sure there were no subgroups that fit the inclusion criteria. However, the article was ultimately excluded as it did not involve direct treatment with a protein from the

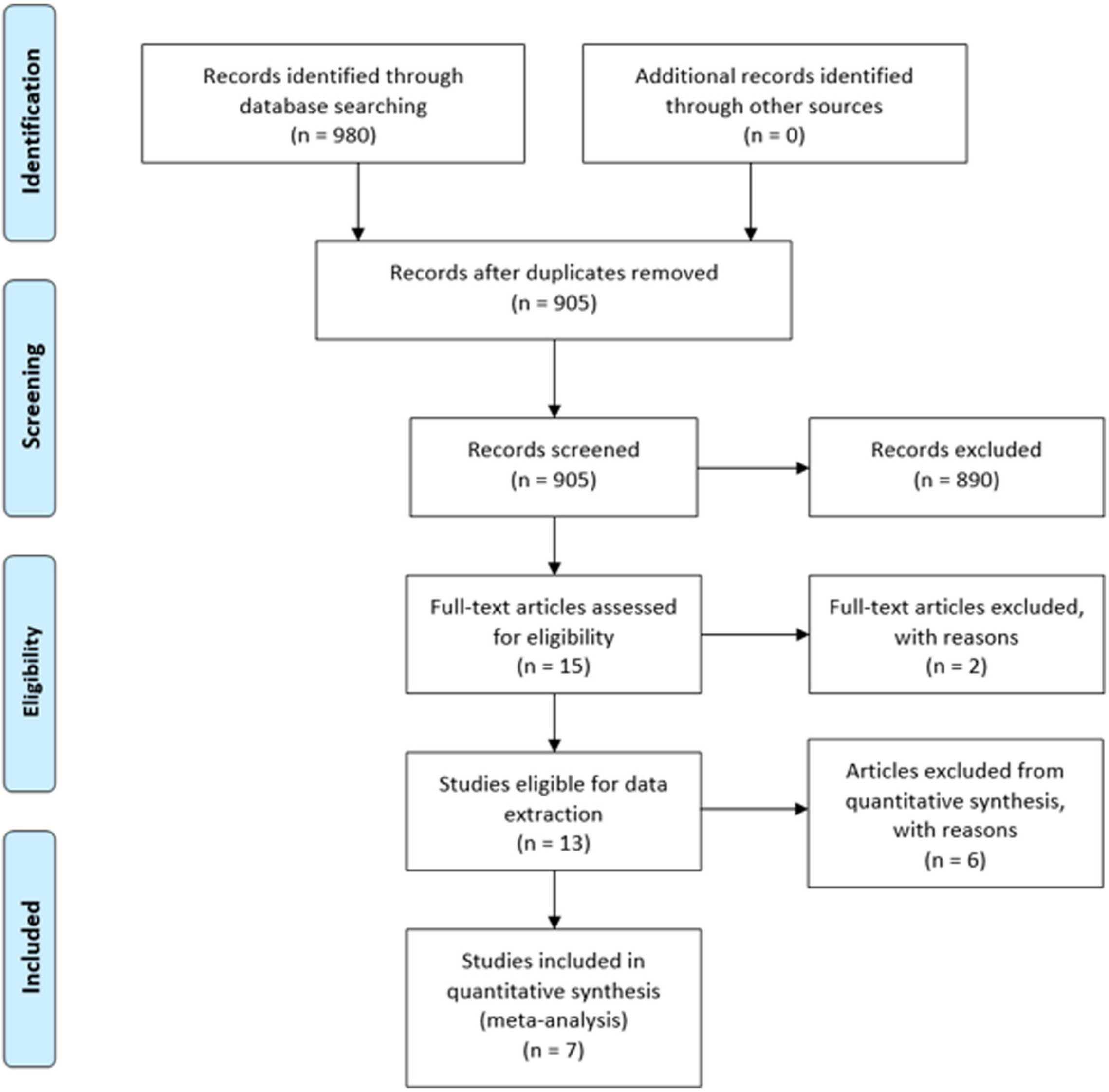

Fig. 1. PRISMA flow diagram for study selection.

helminthic secretome [22]. Data were extracted from a total of 13 articles. However, six of these studies methodologically measured the arthritis score in a manner inconsistent to the majority and as such were removed from the final meta-analysis [23–28]. Some included studies had more than one treatment group that fit the inclusion criteria. This resulted in the extraction of 12 unique data points for the primary outcome measure of clinical arthritis score. The second search for recent publications found one further article which fit the methodological minority [29].

The general study characteristics of the articles included in the meta-analysis can be found in Table 1. Where more than one data point was extracted from an article, the distinguishing feature of that data point was assigned a letter. These letters have been displayed in Table 1 and the forest plots in Figs. 2 and 3 for ease of differentiation. All mice in the included studies were DBA/1 males and had arthritis induced using bovine collagen. Out of the seven studies, five evaluated therapy originally derived from the helminth *Acanthocheilonema viteae* [30–34]; one from *Ascaris suum* [35], and one from *Schistosoma japonicum* [17]. Six studies administered the treatment prophylactically [17,30–34]; three therapeutically [31,32,35]; and one study administered the treatment as mixed prophylactic and therapeutic [32]. At day 0, mice were seven weeks old in one study [17], nine weeks old in five studies [30–34]; and 13 weeks old in one study [35]. The treatment dose was 1 μg/mouse in one study [34], 2 μg/mouse in four studies [30–33]; 10 μg/mouse in one study [17], and 1000 μg/mouse in one study [35]. The treatment was given subcutaneously in five studies [30–34]; intraperitoneally in one study [35], and was not stated in one study [17]. Seven unique treatments were assessed by the included studies with one study each evaluating PC-BSA [30], 11a [30], OVA-PC [31], A. suum extract [35], SMA-12b [34], and SJMHE1 [17]. Three studies evaluated ES-62 [31–33].

3.2. Quality assessment

The results for the quality assessment of included articles can be found in Table 2. The median score for all seven studies was 5 (moderate quality) [30,31,34]; the lowest score was 4 (moderate quality) [32,33,35]; and the highest score was 6 (good quality) [17]. All publications failed to statistically justify sample size. No publications reported randomisation of mouse group allocation or housing. None of the studies noted having blinded the process of arthritis induction. Only one publication indicated that measurement of arthritis score was blinded [17]. Four studies out of the seven included a statement of potential conflicts of interest [17,30,31,34]. All studies indicated that mice were the same age and sex. All included studies also mentioned some sort of environmental control and complied with applicable

Table 1
Characteristics of included studies.

Paper	Treatment	Helminth source	Protocol	Mouse age (weeks)	Dose (μg/mouse)	Route
Al-Riyami (2013) [30]	PC-BSA(a) 11a(b)	*A. viteae*	Prophylactic	9	2	Subcut[a]
Harnett (2008) [31]	ES-62(a) OVA-PC(b,c)	*A. viteae*	Prophylactic(a,b) Therapeutic(c)	9	2	Subcut
McInnes (2003) [32]	ES-62	*A. viteae*	Prophylactic(a) Mixed(b) Therapeutic(c)	9	2	Subcut
Pineda (2012) [33]	ES-62	*A. viteae*	Prophylactic	9	2	Subcut
Rocha (2008) [35]	*A. suum* extract	*A. suum*	Therapeutic	13	1000	IP[b]
Rzepecka (2015) [34]	SMA-12b	*A. viteae*	Prophylactic	9	1	Subcut
Wang (2017) [17]	SJMHE1	*S. japonicum*	Prophylactic	7	10	Not stated

[a] Subcutaneous.
[b] Intraperitoneal.

welfare regulations.

3.3. Arthritis score

All seven included studies, including a total of 12 comparisons, measured the arthritis score out of a maximum of 12. All studies reported a significant reduction in clinical arthritis score with application of a helminthic treatment [17,30–32,34,35]. Overall, helminthic treatment significantly reduced the clinical arthritis score (SMD −1.193, 95% CI −1.525, −0.860; Fig. 2). Subgroup analyses were also performed to evaluate the effects of specific groups of treatment on clinical arthritis score. Subgroups that met the required number of data points include treatment with helminthic protein ES-62 and treatment with a phosphorylcholine-based (PC-based) molecule. ES-62 therapy led to a significant decrease in clinical arthritis score (SMD −1.186, 95% CI −1.633, −0.738). PC-based treatment also saw a significant decrease in arthritis score (SMD −0.997, 95% CI −1.423, −0.571).

3.4. Anti-collagen antibodies

One study reported anti-collagen antibodies [17]. In this study, treatment was found to significantly reduce the total levels of antibodies against collagen.

3.5. Cytokines

In general, measured pro-inflammatory cytokines were reported to significantly decrease after treatment with helminthic therapies. TNF-α levels were reported by three different studies [17,31,32]. Each of these studies measured a significant decrease in this cytokine after treatment. IFN-γ was reported by two studies [17,32]; with both studies recording a significant decrease in cytokine levels following treatment. The four studies which reported IL-17 levels found helminthic therapy significantly reduced the abundance of this cytokine [17,30,33,34]. IL-6 quantities were determined by two studies [17,32]; both finding a significant decrease in the cytokine levels. One study reported on IL-1β and found it to be significantly decreased [34]. One study measured IL-22 levels to be significantly decreased following treatment [17]. Anti-inflammatory cytokines seemed to increase following treatment with helminth proteins or other derived therapies. IL-10 levels were reported by three separate studies [17,31,32]; all of which measured a significant increase in this cytokine after treatment. The one study which reported TGF-β levels found helminthic therapy significantly increased the abundance of this cytokine [31].

ES-62 met the required criteria of five or more data points for mechanistic subgroup analysis and therefore its effects on cytokines IFN-γ and IL-10 were assessed. Treatment with this protein led to a significant decrease in IFN-γ levels (SMD −1.611, 95% CI −2.734, −0.487; Fig. 3A) and a significant increase in the levels of IL-10 (SMD 0.946, 95% CI 0.127, 1.765; Fig. 3B).

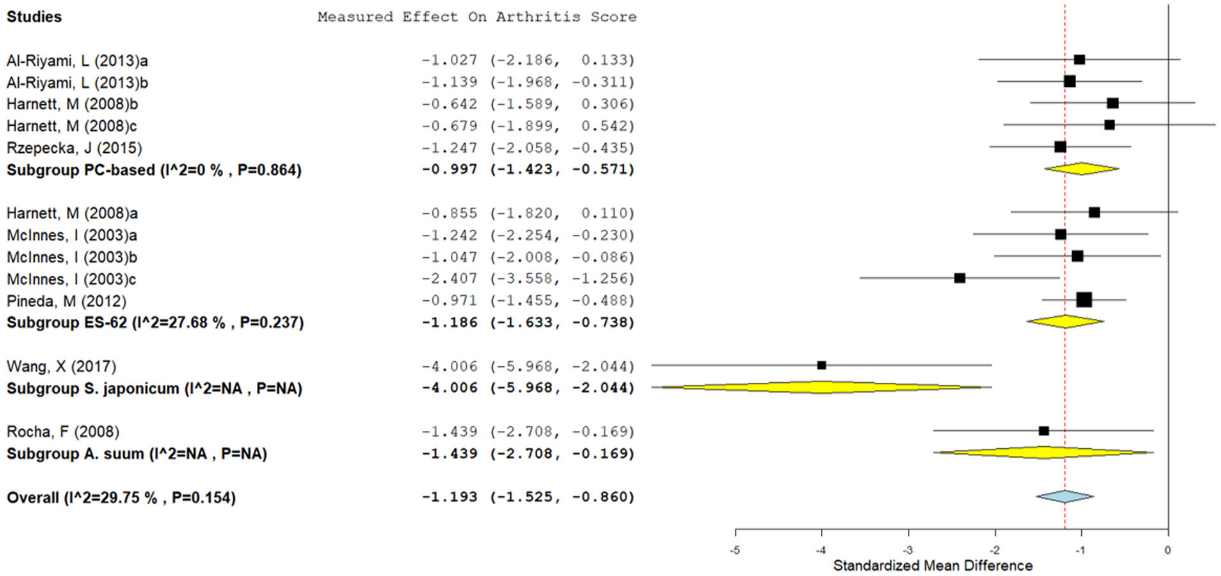

Fig. 2. Forest plot showing the effects of helminthic therapy on clinical arthritis score.

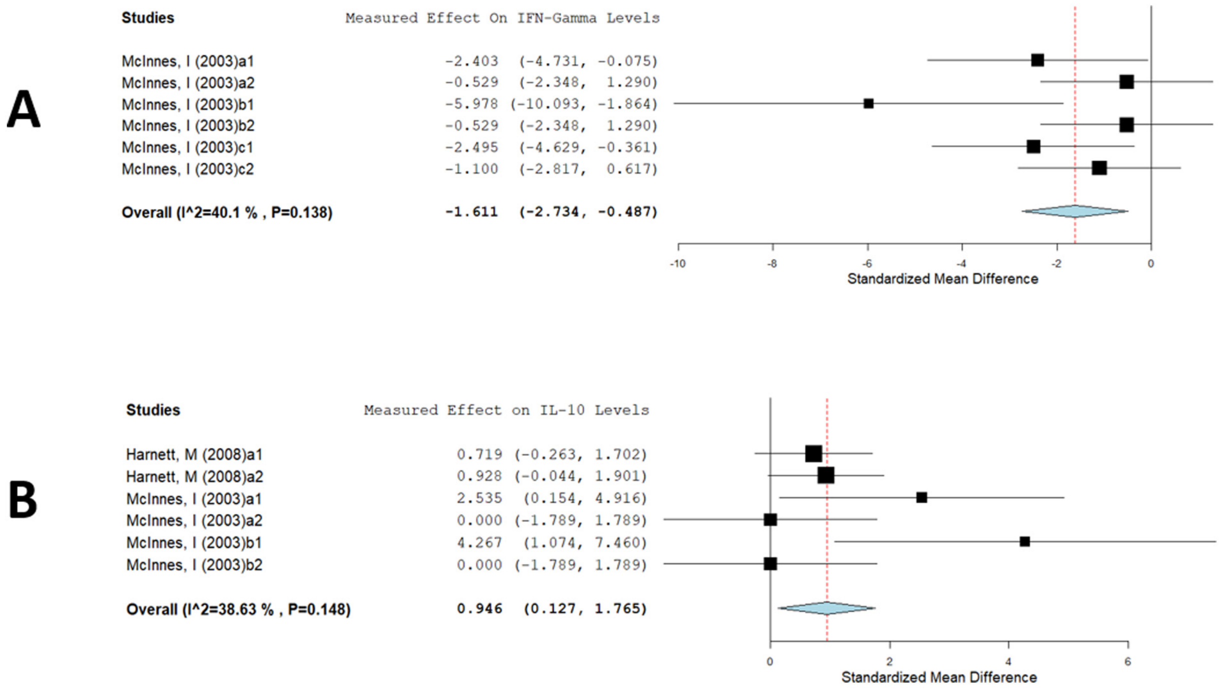

Fig. 3. A. Forest plot showing the effects of the ES-62 subgroup on the measured levels of cytokine IFN-γ. B. Forest plot showing the effects of the ES-62 subgroup on the levels of IL-10.

3.6. Sensitivity analysis and funnel plot

The funnel plot suggests there is publication bias in the included studies (Fig. 4). The leave one out analysis in the online supplementary material (S2) shows the findings of the meta-analysis were derived from all included studies. The removal of any study did not alter the findings.

4. Discussion

The major objective for this systematic review was to evaluate the effects of proteins from the helminthic secretome on the collagen-induced arthritis model of RA. It was found that treatment with these proteins or their derivatives significantly reduced clinical arthritis scores when compared to arthritic controls. Included studies consistently reported a significant reduction in the arthritis score after treatment. Most of the studies that complied with the selection criteria were classified as moderate quality and no significant heterogeneity was found in the pooled data. Furthermore, subgroup analyses found that the phosphorylcholine (PC) moiety derived from the protein ES-62 and the protein itself both significantly reduced clinical arthritis scores.

As each protein naturally affects the immune system in unique ways, it would be futile to pool cytokine data as an attempted mechanistic evaluation. The heterogeneity would be restrictively high. Therefore, forest plots for this mechanistic evaluation was solely used for subgroup analyses. ES-62 treatment was evaluated for its effects on cytokine expression by the immune system. The combined data suggests that ES-62 treatment significantly increased levels of anti-inflammatory cytokine IL-10. This is consistent with the hypothesis that the protein modulates the immune system by increasing the production of IL-10 from B cells [28,36]. IL-10 is an extremely potent cytokine synthesis inhibitor [37]. In line with this, the combined data also suggest a significant decrease in IFN-γ levels is associated with ES-62 treatment.

The methodological strengths of this review include that it follows the PRISMA guidelines (Supplementary materials S3) [15], it was prospectively registered in the PROSPERO database with registration number CRD42018090626 and study quality was appraised with the adapted assessment tool. Furthermore, the statistical heterogeneity in the included studies was consistently low, suggesting that the measured effects were relatively homogenous.

This review does have some associated limitations. Firstly, a majority of the included studies are authored by a similar group of researchers. While this does not extensively limit the study, it does slightly reduce the robustness of the conclusions, especially with regards to subgroup analyses. Secondly, cytokine subgroup analyses could only be undertaken for IFN-γ and IL-10 due to the requirement of five data points to produce a forest plot. This prevented an in-depth mechanistic analysis of the effects of different treatments on the immune system. Another limitation is that the effects of helminthic

Table 2
Quality assessment of included studies.

Paper	Question										
	{1}	{2}	{3}	{4}	{5}	{6}	{7}	{8}	{9}	{10}	Total
Al-Riyami (2013) [30]	✓	✓	–	–	–	–	–	✓	✓	✓	5
Harnett (2008) [31]	✓	✓	–	–	–	–	–	✓	✓	✓	5
McInnes (2003) [32]	✓	✓	–	–	–	–	–	✓	✓	–	4
Pineda (2012) [33]	✓	✓	–	–	–	–	–	✓	✓	–	4
Rocha (2008) [35]	✓	✓	–	–	–	–	–	✓	✓	–	4
Rzepecka (2015) [34]	✓	✓	–	–	–	–	–	✓	✓	✓	5
Wang (2017) [17]	✓	✓	–	–	–	✓	–	✓	✓	✓	6

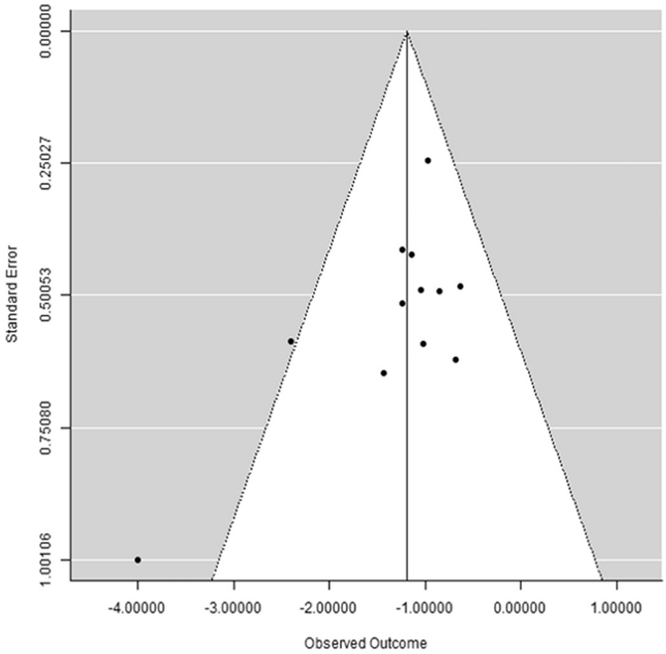

Fig. 4. Funnel plot for primary outcome of clinical arthritis score.

proteins on anti-collagen antibodies was only evaluated by one study [17], which found a significant reduction after helminthic treatment. With only one data point it is difficult to make any conclusions in this area. The inability of the study to compare the effects of different helminthic treatments against each other is another limitation imposed by the small number of subgroup analyses undertaken. Penultimately, the presence of publication bias suggests there may be negative data which has not been published. This is to be expected, as animal models are used to screen potential treatments where many of which may be ineffective, however, the bias may still contribute to the results seeming more favourable. Finally, the level of evidence in this study was only moderate to good quality, slightly reducing the strength of conclusions able to be made from the included studies.

4.1. *Feasibility of helminthic treatment in rheumatoid arthritis*

Although this systematic review evaluated the treatment effects in animals, the research also has applicability to humans. A sizeable component of RA pathogenesis is believed to be Th1/Th17 mediated [1], and CIA has been shown to be a model for this relationship. It was even found that abrogating the IL-17 signalling pathways in these mice suppressed disease development [38]. Therefore, while disease states are not identical in mice and humans, there is enough mechanistic similarity for the success of the included treatments to prompt further investigation. However, the next issue that many of these treatments may face is with regards to immunogenicity. Many of the included treatments consist of whole proteins and while it is feasible to move these onto human trials, it is not ideal. This is because patients' immune systems are likely to mount a response in these trials against the various epitopes on the large protein molecule, potentially hindering trial progression.

Considering the potential issues with the current drug candidates being protein-sized molecules. The question then becomes how to best translate protein-scale efficacy into a treatment that will progress to clinical trials and establish drug therapy itself. This is answered in one manner by identifying and retaining the most active part of the candidate protein and removing all useless and potentially immunogenic extra material. A good example of this drug design methodology can be seen initially in Harnett (2008) [31], where the PC moiety was shown to be the active part of the ES-62 protein. Subsequently, Al-Riyami and co-authors [30], established a library of different small molecule drugs that were designed around the PC moiety. These drugs were then screened in the CIA model. This process of demonstrating the activity of a single moiety and then undergoing drug design has created numerous small molecule treatment candidates with which it would be substantially easier to move into human trials. Furthermore, the pooled data in the subgroup analysis of PC-based treatment suggests a significant improvement in clinical arthritis score. Signifying that these drugs based on phosphorylcholine are well positioned to be trialled further.

With any new potential therapeutic, it is important to elucidate the mechanism by which it has an effect on the body. As this paper evaluates the effects of novel treatments in the CIA animal model, the included studies are in relatively early stages of drug discovery. Consequently, for many of the candidate treatments there is no concrete mechanistic understanding. However, ES-62 has been well characterised in the literature with well-known mechanistic actions. The protein itself and many of its synthetic derivatives have been shown to down-regulate the expression of myeloid differentiation primary response gene 88 (MyD88), a key signalling adaptor involved in the majority of toll-like receptor (TLR) signalling [30]. Downstream from TLR/MyD88 signalling is the activation of nuclear factor kappa light-chain enhancer of activated B cells (NFκB) which eventually causes the release of pro-inflammatory cytokines [39]. Therefore, by down-regulating the MyD88 signalling, ES-62 reduces the production of pro-inflammatory cytokines and minimises the immune-mediated damage in the CIA model of RA.

The helminthic secretome remains a promising potential source of new therapeutics for inflammatory and autoimmune diseases. The majority of the studies included in this meta-analysis evaluated derivatives from the filarial nematode *Acanthocheilonema viteae*, and all studies demonstrated a significant improvement in CIA arthritis score. This warrants further research into the secretome of both *A. vitea* itself and in any of the other 299 parasitic worms that infect humans. Due to the sheer number of proteins secreted by this many parasitic worms, it is sensible to screen these on a worm by worm basis. This was effectively undertaken in the work by Rocha and co-authors [35], where an extract from *Ascaris suum* was administered prophylactically and therapeutically to animal models of arthritis. This methodology of testing the whole secretome of a specific helminth could be used to screen each helminth for potentially immunomodulatory proteins. Subsequently, the helminth extract that demonstrates the best improvement in arthritis score could be further evaluated for specific proteins with immunomodulatory effects.

5. Conclusion

In conclusion, this systematic review and meta-analysis is the first of its kind to substantiate findings that helminthic therapy is generally effective at reducing clinical arthritis score in the CIA model of rheumatoid arthritis. Subgroup analyses also found that both phosphorylcholine-based treatments and ES-62 treatment significantly decreased clinical arthritis score. Drugs derived from parasitic worms have moderate-quality evidence suggesting they would be effective candidates for the treatment of rheumatoid arthritis. The data in this article suggest that further and more detailed research into this area may lead to the elucidation of more effective treatments for rheumatoid arthritis and other auto-inflammatory diseases.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.intimp.2018.11.034.

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