



Review article

Protective efficacy of liver fluke DNA vaccines: A systematic review and meta-analysis: Guiding novel vaccine development

Rama Jayaraj^{a,*}, Chellan Kumarasamy^b, Luke Norbury^c, David Piedrafita^d, Peter Smooker^e^a Yellow 1.1.05, Charles Darwin University, Ellengowan Drive, Darwin, Northern Territory, 0909, Australia^b University of Adelaide, North Terrace Campus, Adelaide, South Australia, 5005, Australia^c School of Science, RMIT University, Bundoora West Campus, PO Box 71, Bundoora, Vic, 3083, Australia^d Faculty of Science and Technology, Federation University, Northways Road, Churchill, Australia^e Building 223, Level 1, Room 29, School of Science, RMIT University, Bundoora West Campus, PO Box 71, Bundoora, Vic, 3083 Australia

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ABSTRACT

The immunogenicity and efficacy of *Fasciola* DNA vaccines have not yet been comprehensively summarised in the form of a systematic review and meta-analysis. Though multiple vaccine studies with respect to *Fasciola* vaccines exist, the variance in the experimental parameters has made comparison difficult.

We conducted a bibliographic database search in Scopus, PubMed, Science Direct, Cochrane Library, EMBASE and Web of Science databases, limited to publications from 1998 to 2017. The key words: Liver fluke, *Fasciola hepatica*, *Fasciola gigantica*, DNA vaccination, and immunogenicity were used in combination to form search strings. A total of 4760 studies were identified after initial screening, of which 14 qualified for systematic review and 7 for meta-analysis. The mean Odds Ratio (OR) for all studies was 0.565 (95% confidence interval (CI) of 0.293 to 1.087), which means the percentage of protection in terms of decreased fluke burden in animals vaccinated with DNA vaccines was 43.5%. A moderate protective efficacy was observed for cysteine protease and phosphoglycerate kinase vaccine antigen candidates (pooled OR and 95% CI, [0.542; 0.179–1.721] and [0.616; 0.219–1.735], respectively). Vaccine effectiveness was observed in individual studies and cohorts; however, the overall pooled efficacy for all vaccine candidates was found to be non-significant.

Despite multiple individual studies showing promising results for various DNA vaccine candidates against fascioliasis, the pooled studies showed the non-significant effect of the vaccine formulations against fluke burden, and displayed minimal protective efficacy against *Fasciola* infection. Though promising results are observed in isolated studies, further animal trials with standardised experimental parameters are required to develop new vaccine candidates effective against *Fasciola*.

1. Introduction

Helminth parasites cause > 55% of all farm animal diseases in Europe, necessitating strategies for effective disease control, to increase the sustainability of the livestock industry (Morgan et al., 2013; Murphy et al., 2006; Nieuwhof and Bishop, 2005). Particularly, during major seasonal fluke outbreaks, many animal fatalities occur, leading to significant losses (Piedrafita et al., 2010). The losses to the livestock industry as a result of parasitic helminths are multiplied due to the cost and effort of handling helminth infections with regular chemical treatments. In Europe alone, an annual spend of €400 million occurs as a result of trying to control these infections in livestock (Morgan et al., 2013). In particular, fascioliasis, an infection of ruminants and humans caused by digenetic trematodes *Fasciola hepatica* and *Fasciola gigantica*

leads to tremendous financial losses all over the world. The global losses incurred due to *F. hepatica* infection, in particular, are estimated to be around US \$3 billion (Mas-Coma, 2005; Piedrafita et al., 2010; Spithill and Dalton, 1998). An issue that compounds this problem is resistance towards anti-parasitic drugs that has developed and spread among liver fluke to the extent that it is now a global concern for animal health and welfare (Dalton et al., 2013; Fairweather, 2011). Therefore, there is a major push towards the development of vaccines for fascioliasis. This need for research towards vaccination against fascioliasis has been highlighted since 1998, but to date, there are no functional commercially available vaccines providing sufficient protective efficacy against liver fluke infestation (Spithill and Dalton, 1998).

A series of purified, native and recombinant antigens have been

* Corresponding author at: Yellow 1.1.05, Charles Darwin University, Ellengowan Drive, Darwin, Northern Territory 0909, Australia.

E-mail address: Rama.Jayaraj@edu.edu.au (R. Jayaraj).

shown to have immunoprophylactic potential against fascioliasis, including fatty acid binding proteins (FABP), glutathione S transferases (GST), cysteine proteases, cathepsin B (catB) and L (catL) proteases, haemoglobin, leucine aminopeptidase (LAP) and a saposin-like protein denoted SAP-2 as reviewed by Spithill and Dalton (Spithill and Dalton, 1998)

DNA vaccinations have been progressively used as a more attractive vaccine approach because they are capable of directly transfecting dendritic cells, and can stimulate both humoral and cellular immunity. However, the generated specific antibody titres of DNA vaccination are generally observed to be far less than those induced by protein vaccination. DNA vaccinations with *Fasciola* antigens have shown effectiveness of such vaccines in evoking immune responses (Jayaraj et al., 2012).

Previous reviews and studies examining the immunogenicity of individual protein and DNA vaccines have been undertaken (Hillyer, 2005; Knox et al., 2001; Smooker et al., 2004), but a comprehensive systematic review and meta-analysis that examines and compares the efficacy of multiple DNA vaccines has not been reported.

Previous vaccine efficacy studies have been conducted with DNA vaccines based on various antigens obtained from both *Fasciola* species. This heterogeneity between studies, due to the difference in species, vaccine antigen and other study parameters such as animal models, duration of study and initial fluke burden makes comparisons across studies, as well as overall comprehensive inferences from these studies difficult. This also negatively impacts the utility of study data for successful commercial vaccine formulation and testing. Multiple studies on DNA vaccination of animal models with *Fasciola* vaccine candidates have provided highly variable preliminary results. This variability is due in part to the heterogeneity between studies, primarily caused by the differences in the study parameters of the animal studies conducted so far, with the heterogeneity arising from variations in antigenic diversity, dose, frequency, route of administration and testing period of the vaccinated animals. These differences complicate the assessment of the potential effectiveness of vaccination under laboratory conditions, as well as the extrapolation of these results to the clinical sphere.

This study, a systematic review and meta-analysis assessing the protective efficacy of *Fasciola* DNA vaccine candidates in animal models, combines multiple experimental animal studies from the available literature and aims to identify the predictive factors for inducing protective immune responses via DNA vaccination. Subgroup analysis was performed based on available experimental data with regards to vaccine candidates.

The findings of this systematic review and meta-analysis may yield a reduction in unnecessary duplication of experimental vaccine studies and may also help to overcome the limitations of the existing knowledge base with regards to *Fasciola* DNA vaccines. It could also assist in understanding the immunoprophylactic response of DNA vaccination in controlling *Fasciola* infection in animal models and may ultimately help guide future attempts at novel *Fasciola* vaccine development.

2. Materials and methods

This systematic review and meta-analysis was conducted according to Preferred Reporting Items for Systematic review and Meta-Analysis (PRISMA) guidelines (Moher et al., 2009). The protocol for this systematic review and meta-analysis study was previously published (Jayaraj et al., 2018). Two investigators independently performed all the steps involved in the database search, study selection assessment of quality, and data extraction. The identification of articles for analysis was resolved through discussion and consensus to avoid bias and disagreement.

2.1. Search strategy

Literature searches were conducted to identify studies describing

Fasciola DNA vaccines in Scopus, PubMed, Science Direct, Cochrane Library, EMBASE and Web of Science databases (Supplementary Table 1). The searches were conducted for papers published between 1998 to 2017. Numerous searches were carried out to identify the broadest range of manuscripts using one of several terms for *Fasciola* DNA vaccination (Liver fluke, *Fasciola hepatica*, *Fasciola gigantica*, DNA Vaccination, and Immunogenicity). The search strategy used the 'AND' and 'OR' operators to combine the keywords into relevant search strings. Supplementary Table 1 shows the search strings that were constructed and used for the study search in the bibliographic databases. The studies identified via performing the search were then screened using the titles and abstracts by two reviewers independently. No articles were rejected based on study parameters in the initial screening. The initial screening was used to screen-out literature reviews, studies with no clinical data, non-English manuscripts with no official English translations and studies whose focus was not on DNA vaccines and fascioliasis. The citation files of the selected articles were extracted and imported into EndNote to form an initial list of suitable studies, following which duplicates were removed. Though review articles were not selected, the reference lists of review articles, as well as the screened in studies, were thoroughly searched to identify any additional studies suitable for inclusion in the study.

2.2. Eligibility criteria

The following carefully designed eligibility criteria were used for the inclusion or exclusion of identified studies for final analysis.

2.2.1. Inclusion criteria

- 1 Primary research study that was based on a DNA vaccine encoding *Fasciola* vaccine candidates
- 2 Studies that provided a detailed description of vaccination such as delivery, dose, animal model, schedule, and timelines
- 3 Studies that evaluated immune/antibody responses to administration of DNA vaccine
- 4 Studies that reported vaccine protective immune responses in the form of fluke burden and liver damage
- 5 Studies that assessed haematological responses against the vaccines

2.2.2. Exclusion criteria

- 1 Lack of vaccination data regarding time intervals after meta-cercariae challenge infection
- 2 Uncertainty regarding the time of vaccination as well as booster doses
- 3 Vaccination results available in reviews
- 4 Studies containing insufficient data on the animal models, the antigen, the species of liver fluke used, route of administration and other study parameters.

2.2.3. Study selection

The full-text articles of all viable studies that were identified during the initial search were obtained and arranged in the form of a database. These full text articles underwent two phases of screening by two reviewers (co-authors JR and CK working independently in parallel), blinded to each other's selection process, using the predefined set of inclusion and exclusion criteria. All articles selected strictly adhered to the selection criteria. After inspection of all studies by both co-authors, any discrepancies and disagreements with regards to the selection of studies were settled by a mutual discussion facilitated by the involvement of a third reviewer. The articles were included as part of the systematic review and meta-analysis only after complete agreement. The entire selection process has been highlighted in the form of a flowchart (Fig. 1).

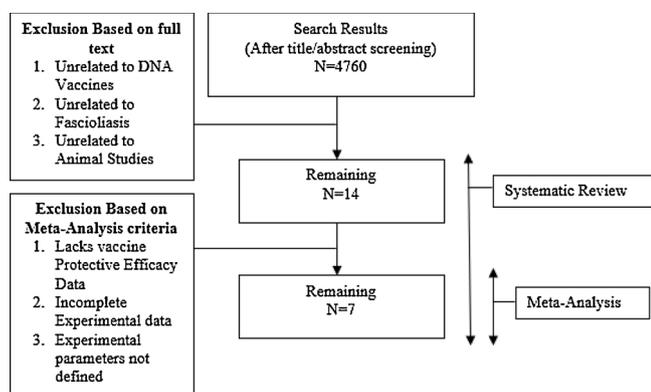


Fig. 1. Flow chart of studies identified for evaluation of the effect of *Fasciola* DNA vaccines. A comprehensive literature search for relevant studies using five online scientific databases (EMBASE, PubMed, Science Direct, Scopus, and Web of Science) was performed to identify studies of interest.

2.3. Data collection

Five reviewers were involved in data extraction. A master Excel spreadsheet was prepared and filled to standardise the data extraction process and to avoid duplication. Details of vaccination, study characteristics, experimental parameters and results were extracted from all selected studies.

2.3.1. Vaccine data items

Collected data included the following characteristics

- 1 Study characteristics (author, publication year, research period, type of study (vaccine efficacy with challenge infection or without experimental infection) and country)
- 2 Vaccine characteristics (*Fasciola* strain, vaccine candidate, design, formulation, route of delivery, vaccine dose, scheduled timeline)
- 3 Subject characteristics (animal type, age, sex, total number and total group size for vaccinated and control)
- 4 Characteristics of challenge infection and necropsy (total number of metacercariae, snail information, route of challenge infection, type of euthanasia and necropsy, and the period between challenge infection and autopsy)
- 5 Characteristics of vaccine efficacy based on parasitological and pathological parameters (fluke burden and liver damage)
- 6 Characteristics of blood cell responses during vaccination and infection based on haematological parameters (leukocyte profile: white blood cell, neutrophil, eosinophil and monocyte counts)
- 7 Characteristics of humoral responses based on antibody parameters (total IgG, IgG subtypes, and IgE) (Jayaraj et al., 2018)

2.4. Outcomes

This study has assessed several outcomes to evaluate the efficacy of *Fasciola* DNA vaccination. The data of the study groups are pooled across different antigens for vaccinated and control groups.

The primary outcome measure was the OR of vaccine efficacy after challenge infection, as defined by fluke number and adult fluke establishment in the bile duct. In addition, this study also examined the association between *Fasciola* DNA vaccination and liver damage score due to challenge infection in vaccinated and control groups. The outcomes were also assessed in the form of percentage of protection, where secondary outcomes aimed to examine the relative effects of variables such as, vaccine antigen, dose, route of administration, and animal model on overall vaccine efficacy.

2.5. Quality Assessment and risk of bias

The quality assessment of the selected studies was performed independently by two authors using the ARRIVE quality guidelines to assess the *in vivo* studies (animal studies) included in the systematic review and meta-analysis (Kilkenny et al., 2012). The quality assessment was based on 20 items described in the ARRIVE guidelines that all scientific publications using animals should include. The parameters included,

- 1 Species, strain, gender and genetic background of the animal model
- 2 Number and groups of animals used
- 3 Descriptive details of housing and husbandry
- 4 Experimental, statistical and analytical methods used.

All the studies included conformed to the ARRIVE checklist's guidelines and were considered high-quality studies providing reliable experimental results. All necessary criteria had to be either mentioned in the study or later represented by the corresponding author, otherwise the study was excluded from the systematic review. This quality assessment also served to reduce the risk of bias and improve the quality of the systematic review and meta-analysis, by validating the studies included.

2.6. Statistical analysis

The protective efficacy of vaccine candidates in each vaccine study was extracted from individual studies as a metric of the fluke burden between the vaccinated and unvaccinated (control) groups (Jayaraj et al., 2009). This data was used to obtain the OR of protective efficacy from summary statistics as an effect size metric using Comprehensive Meta-analysis (CMA) software and was used to generate forest plots depicting pooled OR across all studies.

Results were presented in forest plots as pooled OR and corresponding 95% confidence intervals (CI). A random or fixed-effects model was used to compare the OR between the individual studies depending on between-study heterogeneity (Hooijmans et al., 2014). Heterogeneity between the studies was assessed using the I^2 statistic. The numerical value of the Higgins I^2 statistic was used to categorise between-study heterogeneity into unimportant (0–40%), moderate (30–60%), considerable (50–90%) and substantial (75–100%) levels of heterogeneity (Deeks et al., 2008). The I^2 statistic was used as an indicator of inconsistency in study findings or outcomes and indicated the amount of overlap of the CI between the outcomes of the individual studies (Higgins et al., 2003). The Q value revealed the observed variability within and between vaccine trials (Delgado, 2010). A P value of < 0.01 was considered as statistically significant for the Q test. The z-test was also included in the meta-analysis to indicate the number of standard deviations from the study mean that each study deviated by (Deeks et al., 2008; Hooijmans et al., 2014). Publication bias was estimated visually by the symmetry of funnel plots (constructed using log [OR] and Standard Error), generated using CMA software. Quantitative analysis of publication bias was undertaken using the Egger's bias indicator test, which goes the degree of funnel plot asymmetry as measured by the intercept from a regression standard normal which deviates against precision (Sabarimurugan et al., 2018). Both the classic fail safe and Orwin's fail safe N-test were applied to calculate studies that are missing from the systematic review and meta-analysis (Sabarimurugan et al., 2018). Kendall's Tau-b was to estimate rank order correlation between the vaccine effect and the standard error (Sabarimurugan et al., 2018). The heterogeneity observed between animal studies is caused by differences in vaccine study characteristics and the protective efficacy of the individual vaccine candidates, as evaluated in experimental study groups (Hooijmans et al., 2014).

Table 1
Characteristics of the included Fasciola DNA vaccine studies.

Author	Liver fluke	Vaccine candidate	Vaccine design	Vaccine formulation	Route	Vaccine dose	Scheduled days/ months	Animals	Animal information	Animal groups	Total number	Challenge infection	Total number of meta cercariae	Euthanasia/ Autopsy
Wesołowska et al. (2016)	<i>F. hepatica</i>	Phosphoglycerate kinase	cDNA-FhPGK/pCMV	0.05% bupivacaine	Intramuscular	Rats: (25 + 50µg) Sheep: (100 µg)	4 week interval	Sprague-Dawley Rats; Sheep	n/a	R: 48; S: 16 4G = > (6M, 6F each); S: 2G = > (8M each)	R: 35 meta; S: 250 meta	Yes	12 WPI	
Wesołowska et al. (2013)	<i>F. hepatica</i>	cysteine protease	FhPcW1/pCMV	0.05% bupivacaine	Intramuscular	(20 + 50µg)	4 week interval	Sprague-Dawley Rats	3 months old	3G = > (8M, 8F each)	35	Yes	9 WPI	
Jayaraj et al. (2012)	<i>F. hepatica</i>	cathepsin B	Vr1012 Cat B2; Vr1020 Cat B2; MCP3 Cat B2; CTLA4 CatB2	0.9% saline solution	Intramuscular	100µg	(0,2,4) weeks	BALB/c mice	6-8 weeks old	5G	n/a	No	Bleed sample (W:4,6,8,10)	
Espino et al. (2010)	<i>F. hepatica</i>	FhSAP2	cDNA-FhSAP2	1 mg/ml in saline, PBS	Intramuscular	G1: 20 µg G2: 100 µg G3: 50 µg Basic: 50 µg Booster: 60 µg	G1: 2 week gap; 3 doses G2: 12 week; 3 doses G3: 3 week gap	BALB/c mice	10 weeks old	4G: (10 each)F	40 mice	Yes	45 days (after 1)	
Smitha et al. (2010)	<i>F. gigantica</i>	<i>F. gigantica</i> fatty acid binding protein	(PEI mannose DNA) FhBP	5% glucose	Intra-peritoneal			Swiss Albino mice	6 weeks old	5G: (10 each)	50 mice	No	Bleed sample only	
Jaros et al. (2010)	<i>F. hepatica</i>	Phosphoglycerate kinase	pCMV/myc/EK	G1: 0.05% bupivacaine G2: rFhPGK Fugene 6 G3: Inject Alum	Intramuscular; Sub-cutaneous; Intranasal	G1: 1–50 µg G2: 1–50 µg G3: 1–100 µg	4 week intervals	Rats	3 months old	3G: 65G (6M/6F)	72 mice	Yes	35	
Raina et al. (2009)	<i>F. gigantica</i>	FhBP	PEI conjugate	n/a	Intramuscular	R: 100 µg M: 25–50 µg	4 doses; 3 week gaps	New Zealand White Rabbits, Swiss	M: 6 weeks old R: 300–400 g each	M: 4G = > (10 each) R: 3G = > (10 each)	40 mice; 30 rabbits	Yes	100	Bleed sample only
Espino et al. (2005)	<i>F. hepatica</i>	Saposin like protein	FhSAP -2	1 mg/ml in 0.9% NaCl	Intramuscular; subcutaneous	50 µg	0 weeks and 3 weeks	Albino mice Female BALB/c	6–8 weeks old	4G : (10 each)	40 mice	No	n/a	18 WPI
Wedrychowicz et al. (2003)	<i>F. hepatica</i>	Cysteine protease	CPc DNA	n/a	Intranasal	50 µg + 50 µg lipolectin	2 doses : 4 weeks gap	Sprague-Dawley Rats	3 months old	24 (n/a on number)(exp 1 and exp2)	n/a	Yes	25	exp 1 : 42 days of exp exp 2 : 5 weeks of exp
Smooker et al. (2001)	<i>F. gigantica</i> , <i>F. hepatica</i>	FhBP, Cathepsin - L	Fg FhBP, Fh Cat L5	1 mg/ml, 0.9% NaCl	Intramuscular; intradermal	Intramuscular (100 µg) ; Intradermal (1 µg)	0,5,10,15,20,30 weeks	Female BALB/c mice	6–8 weeks old	G(?) : (5 mice each)	n/a	No	n/a	Bleed sample only
Kofta et al. (2000)	<i>F. hepatica</i>	Cysteine protease	pcDNA - CP	0.05% bupivacaine + saline	Intramuscular	50 µg	single dose	Sprague-Dawley Rats	15 male/ 15 female (12 weeks old)	3G	28	Yes	45	7 WPI
Smooker et al. (1999)	<i>F. hepatica</i>	Glutathione S transferase	VR1012, VR1020	1 mg/ml, 0.9% NaCl	Intramuscular; intradermal	50 µL	4 times; 12 week period	Female BALB/c mice	6–8 weeks old	G(?) : (3-5 mice)	n/a	No	n/a	n/a

(continued on next page)

Table 1 (continued)

Author	Liver fluke	Vaccine candidate	Vaccine design	Vaccine formulation	Route	Vaccine dose	Scheduled days/ months	Animals	Animal information	Animal groups	Total number	Challenge infection	Total number of meta cercariae	Euthanasia/ Autopsy
Kennedy (2006)	<i>F. hepatica</i>	Pro cathepsin B	Fh CatB	Quil A (100 µg) + PBS (2 mL)	Intramuscular	500 µg	0, 4, 8 weeks	Merino crossbreed wethers (sheep) Rats	3 month old	7(G) : (5 sheep each)	35 sheep	No	n/a	Seva samples only
Wedrychowicz (2002)	<i>F. hepatica</i>	Glutathione S transferase	GST cDNA	n/a	Intramuscular	50 µg	2 times; 3 week interval	n/a	n/a	n/a	n/a	No	n/a	10 WPI

3. Results

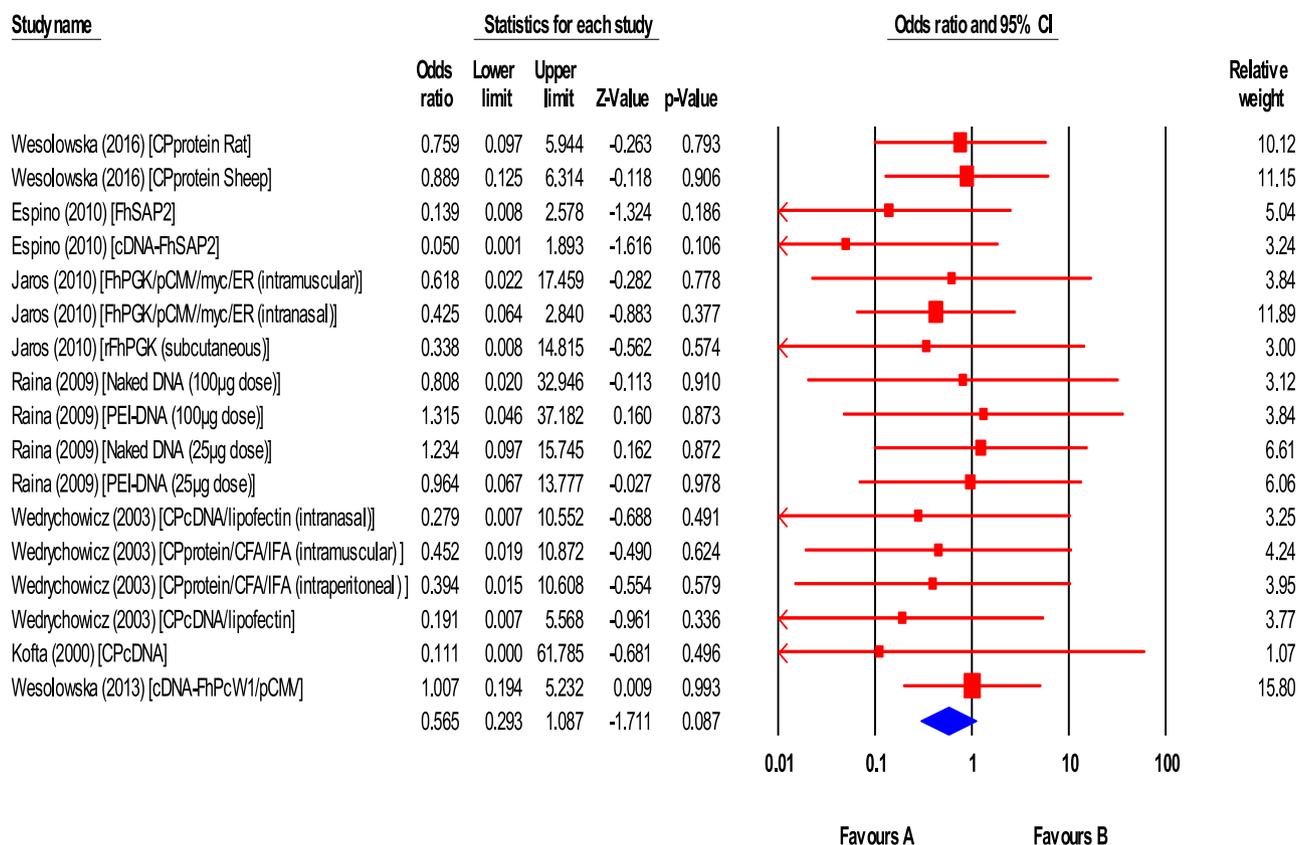
The databases were searched for publications from 1998 to 2017. A total of 4760 studies were identified after initial screening, of which 14 fulfilled the selection criteria. After critical screening, the 14 studies investigating the protective efficacy of various DNA vaccine candidates against liver fluke infestation were considered suitable for the systematic review and meta-analysis. Of the 14 studies, 11 studies used *F. hepatica* as the model for infection, two studies used *F. gigantica*, and one study used both *F. gigantica* as well as *F. hepatica*. Multiple antigens were tested for their protective efficacy in the form of DNA vaccine formulations, such as, phosphoglycerate kinase (studies n = 2), cysteine protease (n = 3), saposin-like protein (n = 2), glutathione S-transferase (n = 2), fatty acid binding p-rotein (n = 3), cathepsin-B (n = 2) and cathepsin-L (n = 1). There were also differences in the host species used in each of these studies, with BALB/c mice (n = 5), Sprague-Dawley rats (n = 4) and Swiss albino mice (n = 2) being most commonly used across all included studies. Only a single study reported using New Zealand white rabbits as the animal model while another study reported using Merino crossbreed weathers (Sheep). Of the 11 studies 2 studies reported using Rats as an animal model but did not specify the breed, and 1 study reported using sheep, but similarly did not report any particular breed. The studies also varied in the dosage and delivery of the vaccine formulations, the frequency of booster doses as well as the observation period after metacercariae infection. The dosage of the antigen varied from 50 to 500 µg, with the frequency of booster doses varying from none (single shot) up to a maximum of three weeks between doses. The test periods also ranged from 3 to 30 weeks. The dosage delivery, however, was relatively consistent among the studies with 11 of the 14 studies opting solely for an intramuscular route of vaccine delivery, with three studies opting for a mix of intramuscular, subcutaneous and intranasal routes of vaccine delivery, while intranasal and intraperitoneal delivery were utilized in a single study. The studies were also consistent in their methods of immunological analysis with 6 studies using ELISA as the exclusive method of analysis, 2 studies used Western Blotting as the only method of analysis, 3 studies used combined Western Blotting and ELISA, 1 study used combined ELISA and Flow Cytometry and 1 study used Flow Cytometry for analysis. The combined comprehensive study data can be found in Table 1 (Espino et al., 2010, 2005; Jaros et al., 2010; Jayaraj et al., 2012; Kennedy et al., 2006; Kofta et al., 2000; Raina et al., 2009; Smitha et al., 2010; Smooker et al., 2001, 1999; Wedrychowicz et al., 2003, 2002; Wesołowska et al., 2013, 2016).

3.1. Meta-analysis of Fasciola DNA vaccines

Out of the 14 studies, 7 studies were found suitable, providing sufficient experimental data for statistical analysis and were thus included for meta-analysis (Fig. 2). These 7 studies investigated a total of 17 different cohorts within the individual studies, based on multiple different factors including group size, antigen, dose, liver fluke species and animal model, and were considered as distinct test cohorts for the meta-analysis. The studies included in the analysis were sampled from the pool of possible studies defined by the inclusion or exclusion criteria as outlined in the methods. For the above reasons, the random-effects model was used for this analysis.

3.2. Methods

The ORs were also calculated from the data obtained from studies in the form of fluke burden differences between vaccinated and control groups. The calculations were assisted in part using CMA Software. The effect size is the OR, and the calculated ORs were then pooled to assess the overall protective efficacy of the antigens against challenge infection.



Meta Analysis

Fig. 2. Forest plot of the meta-analysis of the effect of *Fasciola* DNA vaccination. The study names included in the analysis were shown on the left with their corresponding effect size (shaded diamond), 95% confidence interval (CI) denoted as lower and upper limit and the relative weight to the overall meta-analysis. The dotted vertical line represents an odds ratio (OR) of 1. The overall effect size (0.565; 0.293–1.087) is shown at the bottom.

3.3. Does the DNA vaccination reduce the fluke burden?

The pooled ORs for all 17 cohorts was generated and evaluated after the subsequent construction of a forest plot. The summarised ORs (pooled OR) was 0.565, which means the percentage of protection in terms of the overall decrease in fluke burden in animals vaccinated with DNA vaccines was 43.5%. The 95% confidence interval for the OR is 0.293–1.087, which indicates the odds of occurrence of liver fluke infection in vaccinated groups when compared to non-vaccinated groups falls within the above mentioned range of probability (0.293–1.087). Meta-analysis revealed that most antigenic DNA vaccine formulations had a positive effect, leading to heightened protective efficacies among the vaccinated groups when compared to the controls. Of 17 cohorts, 12 cohorts across four studies showed a positive impact upon the administration of the DNA vaccines, while 3 cohorts across three studies showed little to no impact on protective efficacy, with 2 cohorts across the same three studies showing a negative impact upon administration of the DNA vaccine trea.

3.4. Does the effect size of the percentage of protection vary across the studies?

The Cochran Q test provides a test of the null hypothesis that all *Fasciola* studies in the analysis share a common effect size, where, in

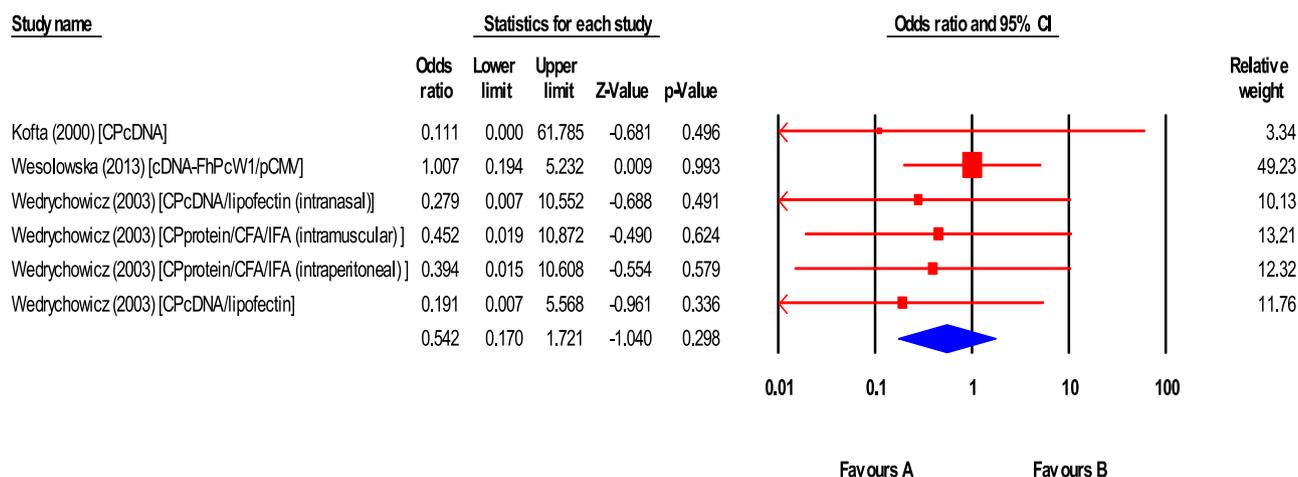
this study, the effect size metric denotes the magnitude of the difference between the effects (protective efficacy) observed in the vaccinated group versus the non-vaccinated group. The effect size in this context is the OR that provides the relative risk for challenge infection (Kumarasamy et al., 2018). If all studies shared the same effect size, the expected value of Q would be equal to degrees of freedom (the number of studies minus 1). The Q value is 5.17 with 16 ° of freedom (df) and a P value of 0.995. Therefore, we reject the null hypothesis that the true effect size is identical in all the DNA vaccine studies.

Here $I^2 = 0.00\%$ tells us the observed variance in the selected studies reflects differences in true effect size rather than sampling errors. The variance of the true effect size (T^2) = 0.00 and the standard deviation of true effects (T) = 0.00.

3.5. Subgroup analysis - cysteine protease

Three studies comprising of six different cohorts assessed the effectiveness of DNA vaccine formulations containing cysteine protease as the antigen of choice against liver fluke infestation and investigated the protective efficacy of this antigen in controlling fluke burden. This variance may be due to the difference in vaccine formulation, dose and dosage frequency as shown in Fig. 3. The pooled OR for these cohorts was 0.542, which means the fluke burden in the vaccinated group with cysteine proteases decreased by 45.8%. The confidence interval for the

Meta Analysis



Meta Analysis

Fig. 3. Subgroup analysis of the differences in vaccine formulation, dose and dosage frequency of cysteine proteases of the pooled DNA vaccine studies.

OR is 0.179 to 1.721. Analysis revealed that five of the six cohorts across two studies showed a moderate protective effect against infestation when compared to control groups. Only a single cohort from a single study showed the negligible impact of cysteine protease formulations against challenge infestation, suggesting no difference between vaccinated and control groups. The Q value is 1.32 with $df = 5$ and $P = 0.932$. We can reject the null hypothesis that the true effect size is identical in all the studies.

3.6. Subgroup analysis - phosphoglycerate kinase

Two studies comprising of five individual cohorts assessed the effectiveness of DNA vaccine formulations containing phosphoglycerate kinase as the antigen of choice against liver fluke infestation and investigated the protective efficacy of this antigen. Meta-analysis showed three of the five cohorts across single studies having a moderate protective effect, due to phosphoglycerate kinase, against infestation when compared to control groups. Two cohorts from a single study showed almost no effect of phosphoglycerate kinase formulations against infestation, suggesting no difference between vaccinated and unvaccinated groups. This variance may be due to the different experimental differences as shown in Fig. 4. The summarised OR for these cohorts was 0.616, which means the fluke burden in groups vaccinated with phosphoglycerate kinase decreased by 38%. The confidence interval for the OR is 0.219 to 1.735. The Q value is 0.41 with $df = 4$ and $P = 0.981$. We can reject the null hypothesis that the true effect size is identical in all the studies.

3.7. Publication bias of DNA vaccine studies

Publication bias was assessed for both the primary meta-analysis and subsequent assessment of the symmetry of funnel plots, where the funnel plots provide a visual representation of the sample size versus the effect size metric (OR) of each included study. These funnel plots are scatter plots that provide a representation of possible publication bias, wherein a lack of symmetry indicates the presence of bias. (Kumarasamy et al., 2018). As shown in Fig. 5, the funnel plot was found to be relatively symmetric. This indicates a certain degree of

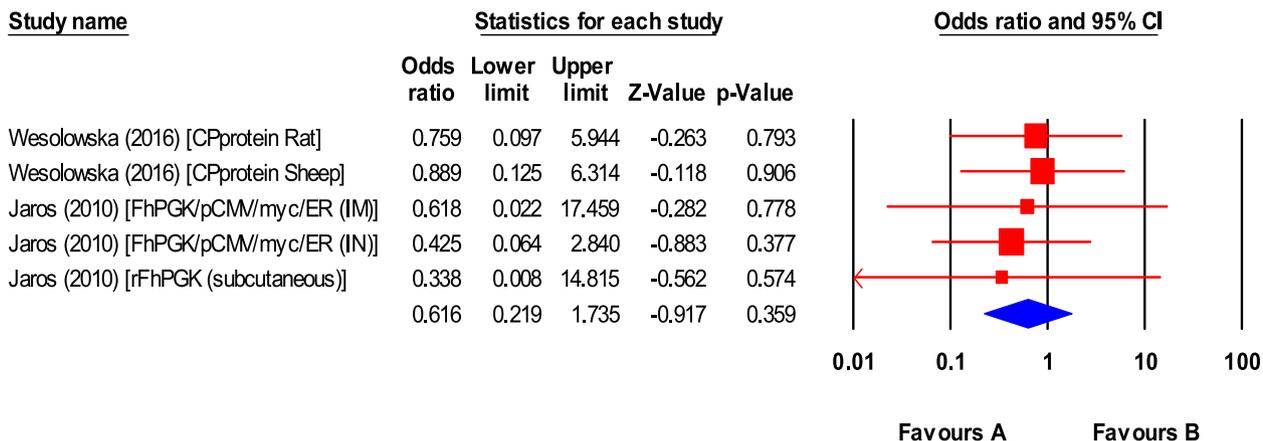
publication bias in the primary meta-analysis. However, considering the paucity of literature in this field coupled with the high variance of experimental and study parameters found between studies, the presence of bias is expected.

The classic fail-safe N is 0.00. There is no need to locate missing studies that would nullify the effect. The Kendall's tau addresses the rank order correlation between vaccine effect and the standard error due to sample size that is 0.323 with a one tailed P-value of 0.034 or 2 tailed P value of 0.069 indicating the non-significant correlation. Egger's regression intercept is -0.254, with 95% confidence interval (-0.579 to 0.070). Using Trim and Fill method, the parameters suggests no studies were missing.

4. Discussion

Due to the potential commercial benefits of endeavours to produce effective DNA vaccines, it is possible that there may be recent advances in the field of DNA vaccines against fascioliasis that may as yet be undisclosed, possibly explaining the paucity of published research in this field. However, the available pool of published research has highlighted some possible antigens for DNA vaccines which we have attempted to verify and validate the significance of, in our systematic review and meta-analysis. The studies in the meta-analysis were identified via an extensive bibliographic search using the inclusion and exclusion criteria as per PRISMA guidelines. These seven studies investigated a total of 17 different DNA vaccines, based on multiple different factors including group size, antigen, dose, liver fluke species and animal model, and were considered as distinct test cohorts for random effects model of the meta-analysis. Though studies suggested phosphoglycerate kinase, cysteine protease, saponin-like protein, glutathione s-transferase, fatty acid binding protein, cathepsin-B and cathepsin-L as antigenic candidates for vaccine production, due to the lack of literature, a meta-analysis could only be carried out for cysteine protease and phosphoglycerate kinase due to the available experimental data. Upon performing the meta-analysis, we observed that cysteine protease and phosphoglycerate kinase exhibited moderate protective effects on challenge infection when comparing the vaccinated and control groups. Despite the overall lack of effect, the results

Meta Analysis



Meta Analysis

Fig. 4. Subgroup analysis of the effectiveness of DNA vaccine formulations containing phosphoglycerate kinase.

of individual studies themselves did suggest a moderate level of protective effect of the vaccine formulations against fascioliasis.

The Q statistic provides a test of the null hypothesis that all DNA vaccine studies in the meta-analysis share a standard effect size. The pooled OR provided sufficient evidence to reject the null hypothesis that the true effect size is identical in all the meta-analysis and the subgroup analyses. The I^2 value is calculated as a measure of heterogeneity and indicates what proportion of the observed variance reflects the difference in true effect sizes rather than sampling error. I^2 indicated a lack of heterogeneity between studies in the meta-analysis. In total, we are confident in presenting the summary of the pooled studies as a relatively reasonable estimate based on the limited available data.

4.1. Limitations

A certain degree of approximation was made during data extraction from figures and graphs in particular, leading to a degree of variance in the meta-analysis' results.

A significant hurdle faced during the study, other than the lack of quantitative data and published studies, was the high variance between the experimental parameters in different studies. These experimental

parameters, including animal model, dosage, dose rate, observational period weeks post infection and vaccine formulation, were different between the studies and could have influenced the results of the meta-analysis. Though not ideal, it was deemed necessary to compare the studies regardless of the existence of variables, to provide a comprehensive summary of the available published literature.

4.2. Future directions

The data does support continued investigation into the highlighted DNA vaccine candidates for *Fasciola* vaccination, especially for phosphoglycerate kinase and cysteine protease. However, looking at the available data, it is observed that the other vaccine candidates indeed require future studies evaluating their protective efficacies using in vivo models. Future studies with comparable study characteristics are necessary to validate the effectiveness of the DNA vaccine candidates analysed in this study. Indeed, trials in field settings and under natural exposure to disease are also needed before proposing the vaccine candidates as sufficiently useful for commercial development. These field trials should provide valuable information regarding vaccine performance (e.g., potency and longevity) at a 'herd' level, due to variation in

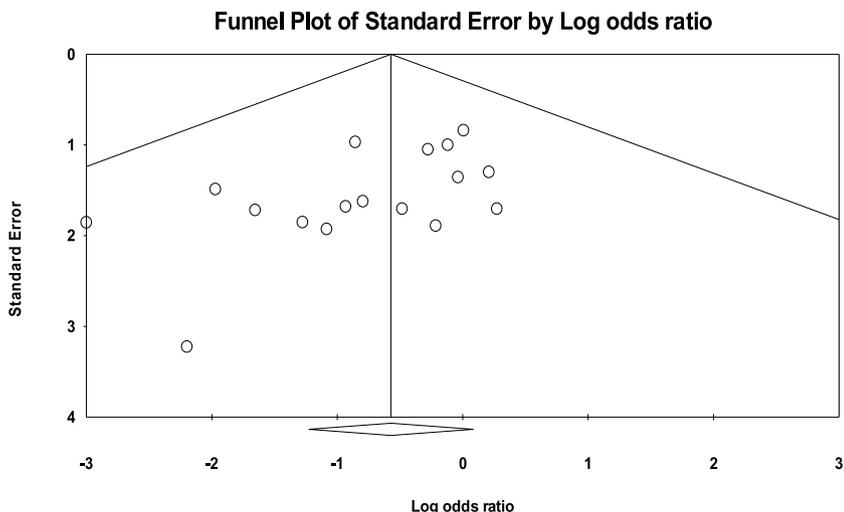


Fig. 5. Funnel plot of protective efficacy and DNA vaccines. This plot measured the study size of *Fasciola* DNA vaccines on the vertical axis as a function of effect size on the horizontal axis. Large studies appeared toward the top of the graph and tend to cluster near the mean effect size. A smaller study (one) appeared toward the bottom of the graph and dispersed across a range of values. The published studies were distributed relatively symmetrically due to the combined effect size.

infection rates between animals and will be able to verify the benefit of vaccination (Molina-Hernández et al., 2015).

5. Conclusions

Although the field of DNA vaccine development could greatly benefit from evidence-based guidance indicating effective vaccine antigen candidates, the available literature is too limited, hampering the ability to provide robust and comprehensive evidence for fellow researchers. However, based on our results, we would like to state that there is potential for DNA vaccines against fascioliasis to be developed. However, further *in vivo* studies, as well as field trials, are required before considering commercial viability.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.vetpar.2019.01.010>.

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