Supplementary Table 4. Baseline characteristics of included studies based on immunization experiments with protein vaccines against *T. gondii* in mouse models (mixed antigens)

Antigen	Adjuvant or carrier	Ag delivery	Mouse strain	Challenge	Immune responses	Brain cyst load	Survival	Conclusions or suggestions	Reference
ROP2+GRA4	Alum	Protein (10 μg each), i.m	C57BL/6 (H-2^b) and C3H (H-2^k)	20 (sublethal dose) or 100 (lethal dose) ME49 tissue cysts, orally	-	20 (sublethal dose) ME49 tissue cysts, orally C57BL/6: reduced ($p<0.01$) C3H: reduced ($p<0.01$)	100 (lethal dose) ME49 tissue cysts, orally There were no significant differences in the survival rates from both strains of immunized mice compared to the control groups	The results reinforce the value of alum as a possible adjuvant to be used in immunization against *T. gondii*, allowing the development of a vaccine for wide application for either humans or animals. We consider that combinations with other effective antigens that generate immunity by different strategies should also be taken into account in the future.	[32]
	CpG-ODN	Protein (10 μg each)+CpG (10 μg), i.m	C3H/HeN (H-2^k)	20 (sublethal dose) tissue cysts, Me49 (type II) strain, orally	Induced a strong humoral Th1-biased response High IgG2a to IgG1 antibody ratio ↑ IFN-γ and IL-10	Reduced (66%, $p<0.001$)	NR	These results indicate that CpG-ODN is an important candidate adjuvant for use in potential multicomponent anti-*T. gondii* vaccines for animals and humans.	[33]
ROP2-LiHsp83 Groups: ROP2 LiHsp83 ROP2+LiHsp83 (mixture) ROP2-LiHsp83 (fused) PBS	*L. infantum* Hsp83	Footpad injections	BALB/c, C57BL/6 and C3H	Acute: 1×10^5 tachyzoites, RH strain, i.p Chronic: 20 cysts of the ME49 strain, orally	Mice immunized with fusion ROP2-LiHsp83 elicited a stronger humoral and cellular response in comparison to mice immunized with ROP2 alone, or a mix of LiHsp83 and ROP2. ↑ IFN-γ secretion and Th1 type response, with predominance of specific IgG2a/IgG2c isotype in mice immunized with fusion protein ROP2 alone or mixed with LiHsp83 induced a Th1/Th2 mixed response (predominance of IgG1 response) ↑ IFN-γ in ROP2-LiHsp83 immunized compared with other groups ($p<0.01$) ↑ IL-4 in mice immunized with ROP2 alone or mixed with LiHsp83, compared with ROP2-LiHsp83 or PBS ($p<0.05$) ↑ Splenocyte proliferation in mice immunized with ROP2-LiHsp83 compared with other groups ($p<0.01$), highest in BALB/c strain	C57BL/6 (reduced in all groups, especially in fusion ROP2-LiHsp83 group, $p<0.05$) C3H (reduced in all groups, especially in fusion ROP2-LiHsp83 group, $p<0.01$)	Increased survival time in C57BL/6 and BALB/c mice immunized with fusion ROP2-LiHsp83 Increased survival time in BALB/c mice immunized with ROP2 alone	In conclusion, here we demonstrate that a member of heat shock protein 90 family, LiHsp83, is a good candidate to carry antigens and develop an adjuvant-free vaccine. This carrier based vaccine system has the capability to produce an immunoresponse that activates antibody secretion, cytokine production and stimulates cellular immune response, all positive features to control parasite infection.	[39]

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Supplementary Table 4. Continued

Antigen	Adjuvant or carrier	Ag delivery	Mouse strain	Challenge	Immune responses	Brain cyst load	Survival	Conclusions or suggestions	Reference
ROP2+GRA4+ SAG1	FIA	Proteins (total amount of each recombinant antigen: 10 μg)+FIA, s.c	C3H/HeJ (H-2^k), C57BL/6 (H-2^b), and BALB/c (H-2^d)	5 tissue cysts, DX strain (type II), i.p	Induced cellular and humoral immune responses	C57BL/6: Reduced (55%, p=0.021) C3H/HeJ: No significant reduction in brain tissue cysts, compared with control BALB/c: Reduced (46%, p<0.001)	NR	This study revealed that immunization with a mixture of recombinant antigens could be a very promising tool in immunoprophylaxis of toxoplasmosis	[40,41]
ROP2+ROP4	FCA and FIA	Protein (10 μg each)+FCA+2 boosters in FIA, s.c	C3H/HeJ (H-2^k)	5 tissue cysts, DX strain (type II), i.p	Predominance of IgG1 over IgG2a Both antigens generated a strong systemic mixed Th1/Th2 response polarized towards IgG1 antibody isotype ↑ Splenocyte proliferation significantly ↑ IFN-γ and IL-2 (p=0.008)	Immunization with rROP2 or rROP2 alone was not sufficient to reduce the brain cysts ROP2+ROP4: Reduced (46%, p=0.003)	NR	Results suggest that, similar to ROP2, ROP4 could be a very good candidate for future anti-*T. gondii* multi-component vaccine based on the recombinant forms of different parasite proteins.	[42]
ROP2+ROP4+GRA4	FIA	Proteins (total amount of each recombinant antigen: 10 μg)+FIA, s.c	C3H/HeJ (H-2^k), C57BL/6 (H-2^b), and BALB/c (H-2^d)	5 tissue cysts, DX strain (type II), i.p	Induced cellular and humoral immune responses	C57BL/6: Reduced (41%, p=0.042) C3H/HeJ: Reduced (59%, p=0.021) BALB/c: Reduced (84%, p<0.001)	NR	This study revealed that immunization with a mixture of recombinant antigens could be a very promising tool in immunoprophylaxis of toxoplasmosis	[40,41]
ROP2+ROP4+SAG1	FIA	Proteins (total amount of each recombinant antigen: 10 μg)+ FIA, s.c	C3H/HeJ (H-2^k), C57BL/6 (H-2^b) and BALB/c (H-2^d)	5 tissue cysts, DX strain (type II), i.p	Induced cellular and humoral immune responses	C57BL/6: Reduced (90%, p<0.001) C3H/HeJ: Reduced (71%, p<0.001) BALB/c: Reduced (77%, p<0.001)	NR	This study revealed that immunization with a mixture of recombinant antigens could be a very promising tool in immunoprophylaxis of toxoplasmosis.	[40,41]
ROP2+GRA5+GRA7	CT	Protein (12.5 μg each)+0.5 μg CT, i.n	BALB/c	50 Cysts from VEG strain, orally	↑ IgG antibody titers ↑ IgA antibody titers in intestinal washes, feces and sera	Reduced (p<0.05)	NR	These results indicate that i.n immunization in BALB/c mice with recombinant proteins rROP2, rGRA5 and rGRA7 associated with CT induced partial protection against tissue cyst formation after oral infection with tissue cysts from *T. gondii*.	[43]

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Supplementary Table 4. Continued

Antigen	Adjuvant or carrier	Ag delivery	Mouse strain	Challenge	Immune responses	Brain cyst load	Survival	Conclusions or suggestions	Reference
ROP5+SAG1	FCA and FIA	Proteins (100 μg each)+FCA+2 boosters in FIA, s.c	BALB/c	1×10^2 Tachyzoites, RH strain, i.p	↑ Level of IgG antibodies ($p<0.01$) The predominance of IgG2a over IgG1 ↑ IFN-γ, IL-2, IL-4, and IL-10 ($p<0.05$) Mixed Th1/Th2 immune response. ↑ Splenocyte proliferation ($p<0.01$)	NR	Prolonged survival time (12.1 ± 3.4 days; $p<0.05$) compared to the control or single-Ag vaccinated groups.	The strategy of using ROP5 protein combined with other antigens appears to be a promising approach to develop a new subunit multi-component vaccine against toxoplasmosis by generating partial, but significant, pro-tective immune responses.	[35]
ROP5+ROP18 ROP5 (full-length ROP5) 1–549 (549 aa) ROP5-C (C-terminal ROP5) 278–549 (272 aa) ROP18 (full-length ROP18) 1–554 (554 aa) ROP18-C (C-terminal ROP18) 316–554 (239 aa) Groups: Blank control Adjuvant control ROP5 ROP18 ROP5+ROP18	poly I:C	Proteins, s.c	BALB/c and C3H/HeOuJ	Acute: 1×10^3 tachyzoites, RH strain (type I), i.p Chronic: 5 tissue cysts, DX strain (type II), i.p	Induced a significant IgG1 and IgG2a production in BALB/c and C3H/HeOuJ mice Induced a mixed type (Th1/Th2) immune response In most cases, the determined titres of Ag-specific antibodies were significantly higher in C3H/HeOuJ mice compared to those in BALB/c mice ($0.00101 \leq p \leq 0.0101$)	BALB/c: Reduced (only in the ROP18-immunized BALB/c mice were significant) C3H/HeOuJ: Reduced (only in ROP5+ROP18 immunized mice were significant)	Increased survival time 25% survival rate in the ROP18-immunized BALB/c mice	The results demonstrated that immunization with ROP5 and ROP18 proteins leads to the activation of both humoral and cellular immune mechanisms, resulting in the partial protection against highly virulent and cysts-forming strains of *T. gondii*. Although the outcomes of the experiments might not be fully satisfactory, these results provide additional evidence that ROP5 and ROP18 proteins may be valuable components of a multi-antigen vaccine against *T. gondii*.	[44]

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Supplementary Table 4. Continued

Antigen	Adjuvant or carrier	Ag delivery	Mouse strain	Challenge	Immune responses	Brain cyst load	Survival	Conclusions or suggestions	Reference
ROP18+ROP38 Groups: Blank control PBS PLG rTgROP38 rTgROP18 rTgROP38+rTgROP18 PLG+rROP38 PLG+rROP18 PLG+rROP38+rROP18	PLG	Proteins+PLG, s.c	Kunming	10 cysts of the PRU strain (genotype II), orally	↑ Specific IgG antibody responses in the mice immunized with various proteins, compared with the control groups ($p<0.01$) ↑ Levels of IgG1 and IgG2a in the mice immunized with various proteins, compared with the control groups ($p<0.01$) Mixed Th1/Th2 immune response Lymphocyte proliferation indexes were similar between the vaccinated and control groups ($p>0.05$) ↑ $CD4^+$ T cells in the mice immunized with different protein vaccines (highest in PLG-rROP38-rROP18 group), compared with the control groups ($p<0.01$) ↑ $CD8^+$ T cells only in the mice immunized with PLG-rROP18 ($p<0.05$) or PLG-rROP38-rROP18 ($p<0.01$) ↑ IFN-γ in the mice immunized with different protein vaccines, compared with the control groups ($p<0.01$) ↑ IL-2 in the mice immunized with different protein vaccines, compared with the control groups (non-significant) ↓ IL-4 and IL-10 in mice immunized with protein vaccines, compared with the control groups ($p<0.05$)	Reduced in the mice immunized with various proteins, compared with the control groups ($p<0.01$) The best was PLG-rROP38-rROP18 (with a cyst reduction of 81.3%)	NR	The findings of the present study indicated that recombinant rhoptry antigens encapsulated in PLG could maintain the protein immunogenicity in an extended period and elicit effective protection against chronic *T. gondii* infection, which has implications for the development of long-lasting vaccines against chronic toxoplasmosis in animals.	[45]

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Supplementary Table 4. Continued

Antigen	Adjuvant or carrier	Ag delivery	Mouse strain	Challenge	Immune responses	Brain cyst load	Survival	Conclusions or suggestions	Reference
ROP18+CDPK6 Main groups: PBS rROP18 rROP18+rCDPK6 rROP18+206 rROP18+rCDPK6+206 rROP18+PLG rROP18+rCDPK6+PLG	206 and PLG	Proteins (10 μg of each), s.c	Kunming	Acute: 1×10^3 tachyzoites, RH strain (type I), ip Chronic: 10 cysts of the PRU strain (type II), orally	↑ Specific IgG antibody responses in the mice immunized with rROP18+PLG or rCDPK6+rROP18+PLG ↑ Splenocyte proliferation significantly (highest in mice immunized with rCDPK6+rROP18+PLG, $p<0.001$), compared with the control groups ↑ $CD4^+$ and $CD8^+$ T cells in mice immunized with the various protein vaccines ↑ Levels of $CD4^+$ ($p<0.001$) and $D8^+$ T lymphocytes ($p<0.01$) in mice immunized with protein–PLG microparticles, compared with controls ↑ Percentages of $CD4^+$ cells in mice immunized with rROP18+206 and rROP18+PLG, compared with the controls ($p<0.05$) ↑ IFN-γ and IL-2 significantly in the mice immunized with various proteins, compared with the control groups ($p<0.05$) ↓ IL-4 and IL-10, compared with the control groups ($p<0.05$) Similar levels of IL-12 between vaccinated and control groups ($p>0.05$) Induced Th1-biased immune responses	Reduced (varied from 47.7% to 73.6%, $p<0.001$) The average survival time of the mice immunized with the various protein vaccines (8.56 days) was slightly longer than that in the controls (8 days) rROP18+PLG (10.9 ± 2.58 days compared with the controls, $p<0.05$) rROP18 (10.1 ± 1.52 days compared with the controls, $p<0.05$)	Increased survival time	These findings suggest that the two recombinant *T. gondii* proteins encapsulated in PLG conferred immunity to *T. gondii* for an extended period, providing the foundation for the further development of a commercial vaccine against toxoplasmosis.	[46]

↑, increase; ↓, decrease; 206, Montanide™ ISA 206 VG; Ag, antigen; CDPK, calcium dependent protein kinase; CpG ODN, oligodeoxynucleotides contained CG motifs; CT, cholera toxin; FCA, Freund's complete adjuvant; FIA, Freund's incomplete adjuvant; GRA, dense granule antigens; HSPs, heat shock proteins; i.m, intramuscular; i.n, intranasal; i.p, intraperitoneally; IFN-γ, interferon-γ; IL, interleukin; *L. infantum, Leishmania infantum*; LiHsp83, *Leishmania infantum* heat shock protein 83; NR, not reported; PBS, phosphate-buffered saline; PLG, polylactide-co-glycolide; poly (I:C), polyinosinic–polycytidylic acid; ROP, rhoptry protein or rhoptry antigens; s.c, subcutaneous; SAG, surface antigens; *T. gondii, Toxoplasma gondii*; Th, T helper.