

The relationship between *IFN-γ* and *TNF-α* gene polymorphisms and brucellosis: A meta-analysis

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Abstract

Background. Brucellosis is an infectious disease and one of the major public health problems worldwide. Several current studies have provided data that polymorphisms in the interferon-gamma gene (*IFN-γ*) and tumor necrosis factor-alpha gene (*TNF-α*) are related to brucellosis.

Objectives. The aim of this study was to investigate the relationship between *IFN-γ* +874 A/T, *IFN-γ* UTR5644 A/T, *TNF-α* –308 G/A, and *TNF-α* –238 G/A single nucleotide polymorphisms (SNPs) and brucellosis risk by meta-analysis.

Material and methods. We performed a comprehensive search of the PubMed, MEDLINE, EMBASE, Web of Science, and Elsevier Science Direct databases. Crude odds ratios (ORs) with 95% confidence intervals (CIs) were used to measure the strength of association between *IFN-γ* and *TNF-α* polymorphisms and brucellosis risk.

Results. A total of 17 studies including 1,904 cases and 2,233 controls fulfilled the inclusion criteria. Our pooled analysis demonstrated that the *IFN-γ* +874 AT vs AA genotype in a codominant model may confer an increased risk of brucellosis in the overall population ($p = 0.001$; $OR = 0.51$). Regarding *TNF-α* –308 G/A, our pooled analysis revealed that the AA vs GG + GA (recessive) genotype increased the risk of brucellosis ($p = 0.02$; $OR = 2.00$).

Conclusions. In summary, our pooled analysis suggested that the *IFN-γ* +874 AT vs AA as well as the *TNF-α* –308 AA vs GG + GA genotypes demonstrated a trend for the association with a higher risk of brucellosis.

Key words: *IFN-γ*, *TNF-α*, gene polymorphism, brucellosis, meta-analysis

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Introduction

Brucellosis is a chronic granulomatous infection and the most frequent bacterial zoonotic disease worldwide.^{1,2} The causative agent of brucellosis, *Brucella* spp., is a strain of facultative intracellular bacteria that infect over half a million humans annually.³ Patients with active brucellosis have symptoms such as fever, headache, sweating, weakness, weight loss, persistent joint pain, endocarditis, neurological complications, and testicular or bone abscess formation.^{4,5} *Brucella* spp. invade reticuloendothelial system cells and can reproduce in these cells, and escape from the host's immune system.^{6–8}

Cytokines are key mediators responsible for the regulation of immune and inflammatory responses.^{9,10} *Brucella* spp. can stimulate the secretion of inflammatory cytokines, e.g., interleukins (ILs), interferon-gamma (IFN-γ) and tumor necrosis factor-alpha (TNF-α).^{2,10,11} IFN-γ is a crucial cytokine for the control of *Brucella* infection in hosts and is also a significant mediator in conferring protection against *Brucella* infection. The ultimate result of the activation of macrophages with IFN-γ, which is secreted by T helper-1 cells, is suppressing the reproduction of intracellular *Brucella* organisms and restoring the patient to health.² Tumor necrosis factor-alpha is a proinflammatory and immunoregulatory cytokine that is produced by a variety of cells including neutrophils (polymorphonuclear leukocytes – PMN cells), natural killer (NK) cells, macrophages, lymphocytes, and fibroblasts. Like IFN-γ, TNF-α also is a vital mediator for the clearance of brucellosis infection from a host.¹¹

Genetic polymorphisms in cytokine genes can potentially modify the expression and/or biological activity of cytokines. Studies conducted to date have confirmed

the connection between cytokine gene polymorphisms and brucellosis disease status in various populations.^{2,11–16} The IFN-γ +874 A/T (rs2430561) and UTR5644 A/T polymorphisms, as well as the TNF-α –308 G/A (rs1800629) and –238 G/A (rs361525), are 4 SNP loci which affect the transcriptional regulation of IFN-γ and TNF-α. These 4 SNPs have been investigated for their association with the occurrence of brucellosis in different populations.^{2,11–14,16} However, due to the relatively small sample size of individual studies, the results have been incoherent and contradictory. A wide retrieval of the pertinent literature is required to reach a more precise estimation of the association with disease susceptibility. Therefore, in the current study, we performed a meta-analysis to collect the available data and to examine whether these 4 polymorphisms of *IFN-γ* (+874 A/T (rs2430561) and UTR5644 A/T) and *TNF-α* (–308 G/A (rs1800629) and –238 G/A (rs361525)) genes are associated with susceptibility to brucellosis.

Material and methods

Literature search

We performed a comprehensive literature search using the electronic databases PubMed, EMBASE and MEDLINE. The comprehensive search strategies included the mesh term and keywords (“interferon gamma” or “interferon-gamma” or “interferon γ” or “interferon-γ” or “IFN gamma” or “IFN-gamma” or “IFN γ” or “IFN-γ”), (“tumor necrosis factor alpha” or “tumor necrosis factor-alpha” or “tumor necrosis factor α” or “tumor necrosis factor-α” or “TNF alpha” or “TNF-alpha” or “TNF α” or “TNF-α”),

Table 1. Main characteristics of studies included in a meta-analysis of *IFN-γ* gene polymorphisms and brucellosis

SNP	Author	Year	Country	Ethnicity	Sample size	Allele frequency		Genotype frequency			HWE (p-value)
						A	T	AA	AT	TT	
IFN-γ +874 A/T	Bravo ¹²	2003	Spain	Caucasian	P 83 C 100	P 99 C 100	P 67 C 100	P 28 C 19	P 43 C 62	P 12 C 19	P 0.48 C 0.01
	Budak ¹⁶	2007	Turkey	Caucasian	P 39 C 50	P 43 C 63	P 35 C 37	P 18 C 21	P 7 C 21	P 14 C 8	P 0.00 C 0.48
	Rasouli ⁹	2007	Iran	Asian	P 195 C 91	P 176 C 71	P 214 C 111	P 40 C 9	P 96 C 53	P 59 C 29	P 0.93 C 0.03
	Karaoglan ¹³	2009	Turkey	Caucasian	P 85 C 85	P 92 C 109	P 78 C 61	P 27 C 32	P 38 C 45	P 20 C 8	P 0.35 C 0.16
	Eskandari-Nasab ²	2013	Iran	Asian	P 153 C 128	P 145 C 93	P 161 C 163	P 44 C 17	P 57 C 59	P 52 C 52	P 0.00 C 0.96
IFN-γ UTR5644 A/T	Davoudi ¹⁵	2006	Iran	Asian	P 42 C 161	P 48 C 179	P 36 C 143	P 14 C 60	P 20 C 59	P 8 C 42	P 0.85 C 0.00
	Hedayatzadeh-Omran ¹⁷	2010	Iran	Asian	P 259 C 238	P 341 C 285	P 177 C 191	P 125 C 91	P 91 C 103	P 43 C 44	P 0.00 C 0.12
	Eskandari-Nasab ²	2013	Iran	Asian	P 153 C 128	P 191 C 157	P 115 C 99	P 67 C 53	P 57 C 51	P 29 C 24	P 0.01 C 0.07

IFN-γ – interferon-gamma; SNP – single nucleotide polymorphism; HWE – Hardy-Weinberg equilibrium.

(“polymorphism” or “variant” or “genotype” or “SNP” or “mutations”), and (brucellosis). The search was conducted through July 2, 2016. Eligible studies were retrieved and examined carefully. Review articles and bibliographies of other relevant studies identified were manually searched to find additional eligible studies.

Data collection

The articles were screened by 2 separate reviewers (Mehdi Moghadampour and Ebrahim Eskandari-Nasab) to appraise the fitness of the articles selected by using a standardized protocol and data collection form. The inclusion criteria were: original data; a study which assessed the association of IFN- γ +874 A/T, IFN- γ UTR5644 A/T, TNF- α –308 G/A, and TNF- α –238 G/A and the risk of brucellosis; and a comparison between brucellosis patients and controls. The exclusion criteria were: non-human studies, abstracts only, comments, reviews, editorials or letters, mechanism studies, and studies that lacked controls; family-based design or sibling pair studies; studies with insufficient information for data extraction; and unpublished data.

The following information was extracted from each study: authors, year of publication, country, ethnicity, sample size, allele and genotype frequency distribution, and Hardy-Weinberg equilibrium (HWE). Discrepancies about the inclusion of studies and the interpretation of data were solved by discussion.

Statistical analyses

A quantitative meta-analysis was executed using Review Manager Software, v. 5.3 (the Cochrane Collaboration, Oxford, UK). Crude ORs with 95% CIs were used to measure the strength of association between the IFN- γ +874 A/T, IFN- γ UTR5644 A/T, TNF- α –308 G/A, and TNF- α –238 G/A polymorphisms and brucellosis risk. The significance of the pooled ORs was defined by the Z-test, and $p < 0.05$ was considered statistically significant.

The pooled ORs for IFN- γ polymorphisms (+874 A/T and UTR5644 A/T) and brucellosis risk were assessed for the codominant (AT vs AA and TT vs AA), dominant (AT+TT vs AA) and recessive model (TT vs TA+AA), and for the allele comparison (T vs A). The integrated ORs for TNF- α polymorphisms (–308 G/A and –238 G/A) and brucellosis risk were calculated for the codominant (AA vs GG and GA vs GG), dominant (GA+AA vs GG) and recessive model (AA vs GG+GA), and for the allelic contrast (A vs G). Forest-plot graphs were produced in order to estimate the combined association between the IFN- γ +874 A/T, IFN- γ UTR5644 A/T, TNF- α –308 G/A, and TNF- α –238 G/A polymorphisms and brucellosis risk. Heterogeneity among the studies was assessed by Cochran's Q test and I^2 measurement, which was interpreted as the proportion of total discrepancy among study

variants. A p-value <0.05 and an I^2 value $>50\%$ showed significant heterogeneity. A random-effect model was used in cases of significant heterogeneity; otherwise, a fixed-effect model was applied. Sensitivity analysis was conducted to evaluate the validity and reliability of the primary meta-analysis, and to determine the effects attributed to any particular study.

Results

Study characteristics

In this meta-analysis, a total of 17 studies involving 1,904 cases and 2,233 controls met the inclusion criteria for both IFN- γ and TNF- α SNPs in brucellosis. Five studies assessed the association between the IFN- γ +874 A/T polymorphism and the risk for brucellosis; 3 studies assessed the association between the IFN- γ UTR5644 A/T polymorphism and the risk for brucellosis; 6 studies examined the association between the TNF- α –308 G/A variation and the risk for brucellosis; and 3 studies examined the association between the TNF- α –238 G/A variation and the risk for brucellosis. Baseline characteristics of the included studies on IFN- γ and TNF- α SNPs on brucellosis are presented in Tables 1 and 2, respectively.

IFN- γ +874 A/T polymorphism and susceptibility to brucellosis

Five studies including 555 brucellosis patients and 454 controls assessed the association between the IFN- γ +874 A/T polymorphism and susceptibility to brucellosis. In all studies, the distributions of genotypes in the control subjects were in HWE (Table 1). Figure 1 demonstrates the forest plot and results of the meta-analysis of associations between the IFN- γ +874 A/T polymorphism and the risk for brucellosis, using the codominant, dominant and recessive models. The results showed that the T allele vs A was not associated with the risk of brucellosis with an overall OR of 0.90 ($p = 0.55$; 95% CI = 0.64–1.27). In the codominant model, the pooled evidence suggested that the distribution of the AT vs AA genotypes between the groups was different and that the association was statistically significant ($p = 0.001$; OR = 0.51; 95% CI = 0.37–0.71). In contrast, the general difference between the groups for the TT genotype compared to the AA one did not reach the level of statistical significance, using the codominant model with an overall OR of 0.82 ($p = 0.63$; 95% CI = 0.36–1.87). In the dominant model, the AT+TT genotype vs the AA genotype was not associated with an increased risk of brucellosis with an overall OR of 0.82 ($p = 0.47$; 95% CI = 0.47–1.42). Likewise, in the recessive model, the TT genotype vs the TA+AA one was not associated with an increased risk of brucellosis with an overall OR of 1.22 ($p = 0.47$; 95% CI = 0.71–2.11).

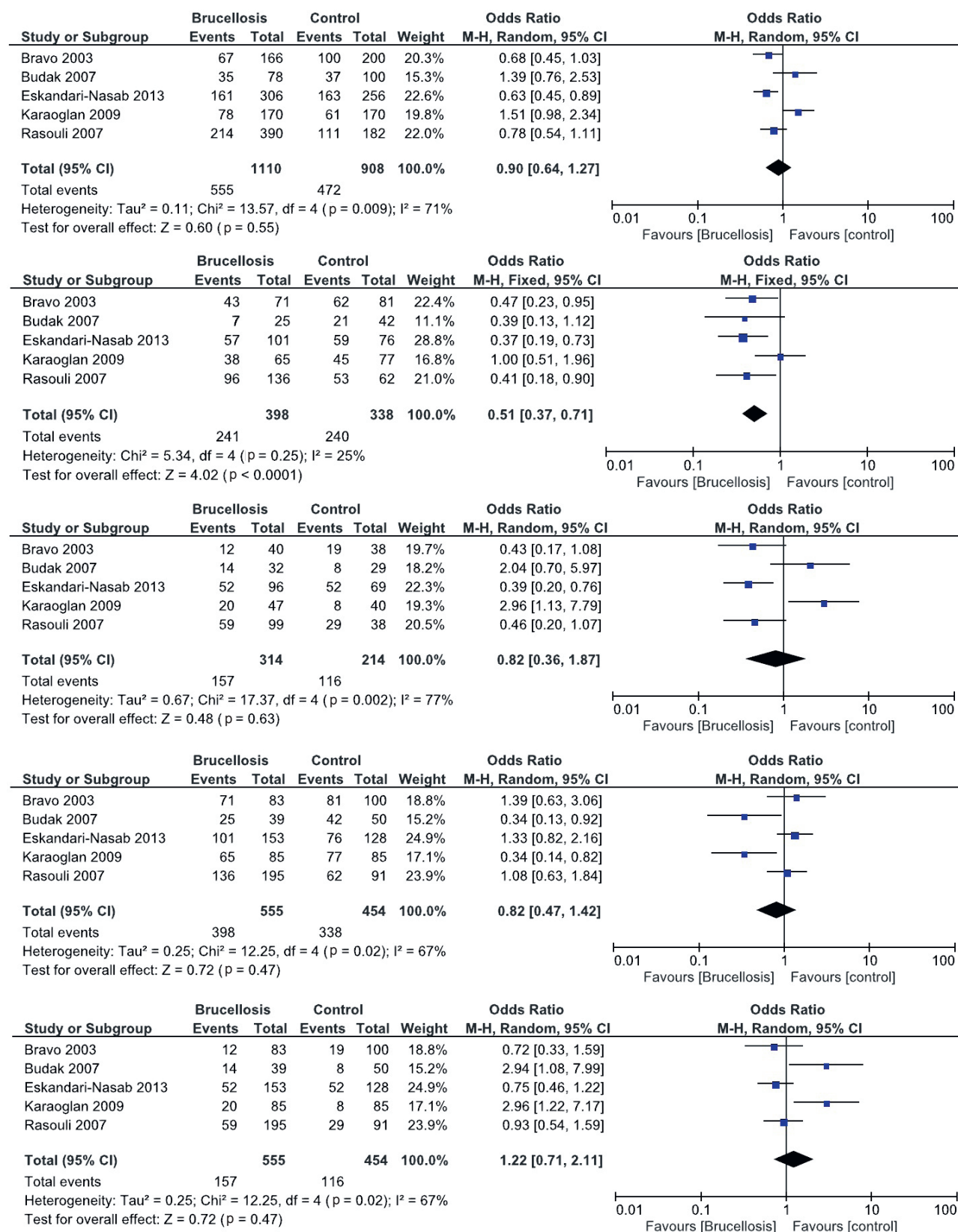


Fig. 1. Forest plot for the association of the IFN- γ +874 A/T polymorphism and brucellosis (T allele vs A allele, AT vs AA, TT vs AA, AT+TT vs AA, and TT vs TA+AA)

IFN- γ UTR5644 A/T polymorphism and susceptibility to brucellosis

Three studies including 454 brucellosis patients and 527 controls evaluated the association between the IFN- γ UTR5644 A/T polymorphism and susceptibility to brucellosis. In all studies, the distributions of genotypes in the control subjects were in HWE (Table 1). Figure 2 presents the forest plot and results of the meta-analysis of associations between the IFN- γ UTR5644 A/T polymorphism and the risk for brucellosis using codominant, dominant and recessive models. The results indicated that the T allele vs the A allele was not associated with the risk of brucellosis with an overall OR of 0.85 ($p = 0.09$; 95% CI = 0.70–1.03). None of the codominant ($p = 0.12$ and 0.22), dominant ($p = 0.09$) or recessive ($p = 0.44$) models showed a significant association between genotype distribution and an increased risk of brucellosis.

TNF- α –308 G/A variation and the risk for brucellosis

Six studies including 640 patients and 802 controls assessed the association between the TNF- α –308 G/A variation and brucellosis. In all studies, the distributions of genotypes in the control subjects were in HWE (Table 2). The pooled analysis revealed that the A allele vs the G allele was not associated with the risk of brucellosis with an overall OR of 1.02 ($p = 0.94$; 95% CI = 0.59–1.77). In the codominant model, the pooled evidence suggested that the AA genotype vs the GG genotype distribution between the groups was not different and that there was no statistically significant association ($p = 0.06$; OR = 1.74;

95% CI = 0.98–3.11). Additionally, the general difference between the groups for the GA genotype compared to the GG one did not reach the level of statistical significance, using the codominant model with an overall OR of 0.98 ($p = 0.96$; 95% CI = 0.48–1.99). In the dominant model, the GA+AA genotype vs the GG genotype was not associated with an increased risk of brucellosis with an overall OR of 0.99 ($p = 0.98$; 95% CI = 0.52–1.91). However, the recessive model suggested that the AA genotype compared to the GG+GA genotype increases the risk of brucellosis with an overall OR of 2.00 ($p = 0.02$; 95% CI = 1.14–3.50). Figure 3 presents the forest plot and results of the meta-analysis of associations between the TNF- α –308 G/A polymorphism and the risk of brucellosis, using codominant, dominant and recessive models.

TNF- α –238 G/A polymorphism and susceptibility to brucellosis

Three studies including 255 brucellosis patients and 450 controls evaluated the association between the TNF- α –238 G/A polymorphism and susceptibility to brucellosis. In all studies, the distributions of genotypes in the control subjects were in HWE (Table 2). Figure 4 demonstrates the forest plot and results of the meta-analysis of associations between the TNF- α –238 G/A polymorphism and the risk of brucellosis, using codominant, dominant and recessive models. The results showed that the A allele vs the G allele was not associated with the risk of brucellosis with an overall OR of 0.66 ($p = 0.36$; 95% CI = 0.27–1.60). In none of the models included – codominant ($p = 0.34$), dominant ($p = 0.34$) or recessive – was there a significant association between genotype distribution and the risk of brucellosis.

Table 2. Main characteristics of studies included in a meta-analysis of TNF- α gene polymorphisms and brucellosis

SNP	Author	Year	Country	Ethnicity	Sample size	Allele frequency		Genotype frequency			HWE (p-value)
						G	A	GG	GA	AA	
TNF- α –308 G/A	Caballero ¹⁴	2000	Spain	Caucasian	P 59 C 160	P 100 C 292	P 18 C 28	P 41 C 134	P 18 C 24	P 0 C 2	P 0.16 C 0.44
	Davoudi ¹⁵	2006	Iran	Asian	P 43 C 162	P 82 C 272	P 4 C 52	P 39 C 110	P 4 C 52	P 0 C 0	P 0.74 C 0.01
	Budak ¹⁶	2007	Turkey	Caucasian	P 40 C 50	P 69 C 80	P 11 C 20	P 29 C 33	P 11 C 14	P 0 C 3	P 0.31 C 0.37
	Karaoglan ¹³	2009	Turkey	Caucasian	P 85 C 85	P 159 C 154	P 11 C 16	P 75 C 71	P 9 C 12	P 1 C 2	P 0.24 C 0.11
	Reza ²²	2009	Iran	Asian	P 260 C 217	P 361 C 311	P 159 C 123	P 138 C 106	P 85 C 99	P 37 C 12	P 0.00 C 0.06
	Eskandari-Nasab ¹¹	2016	Iran	Asian	P 153 C 128	P 266 C 244	P 40 C 12	P 115 C 116	P 36 C 12	P 2 C 0	P 0.66 C 0.57
TNF- α –238 G/A	Caballero ¹⁴	2000	Spain	Caucasian	P 59 C 160	P 106 C 292	P 12 C 28	P 47 C 132	P 12 C 28	P 0 C 0	P 0.38 C 0.22
	Davoudi ¹⁵	2006	Iran	Asian	P 43 C 162	P 80 C 243	P 6 C 81	P 37 C 81	P 6 C 81	P 0 C 0	P 0.62 C 0.00
	Eskandari-Nasab ¹¹	2016	Iran	Asian	P 153 C 128	P 271 C 225	P 35 C 31	P 118 C 97	P 35 C 31	P 0 C 0	P 0.11 C 0.11

TNF- α – tumor necrosis factor-alpha; SNP – single nucleotide polymorphism; HWE – Hardy-Weinberg equilibrium.

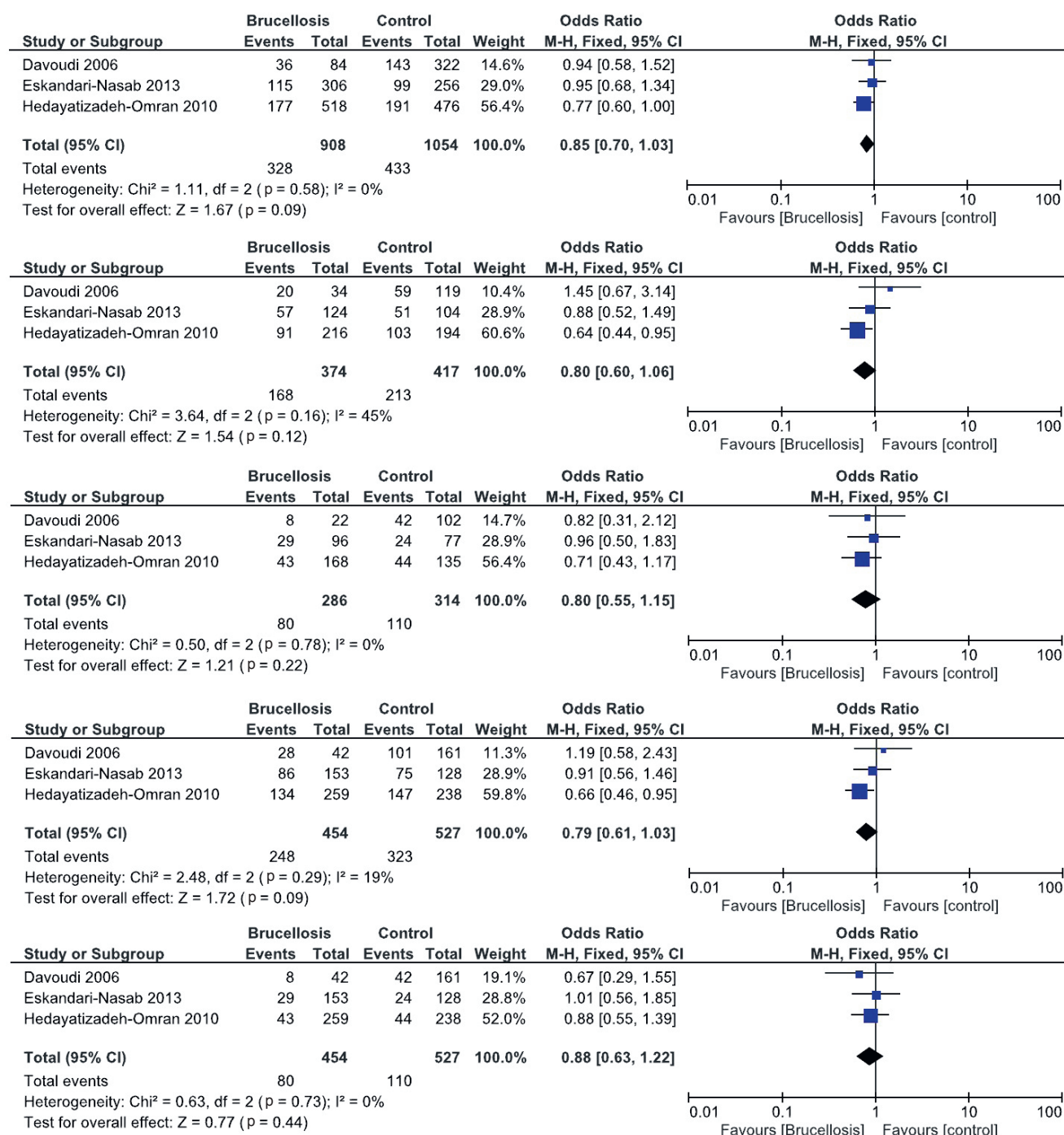


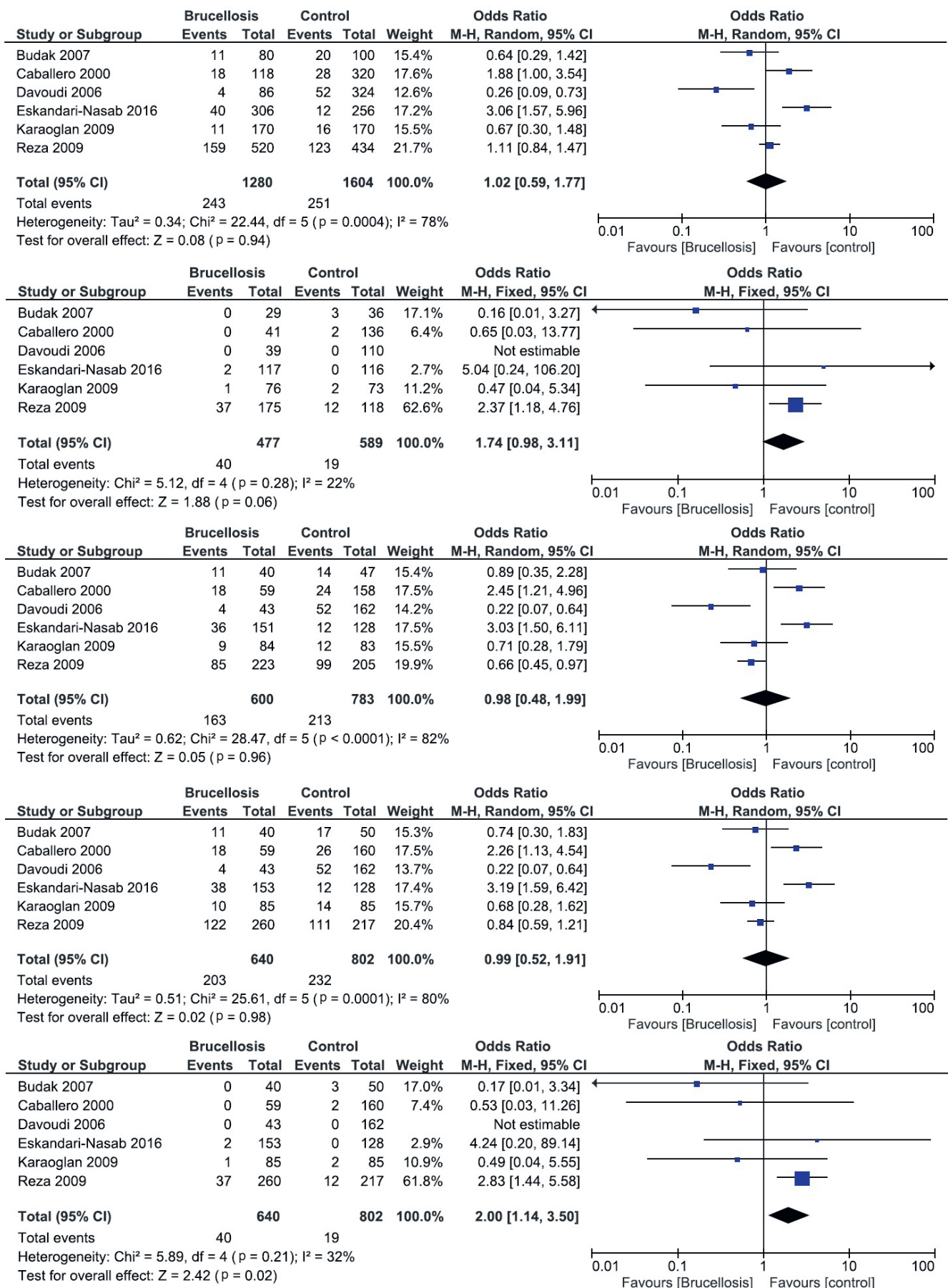
Fig. 2. Forest plot for the association of the IFN- γ UTR5644 A/T polymorphism and brucellosis (T allele vs A allele, TA vs AA, TT vs AA, AT+TT vs AA, and TT vs TA+AA)

Sensitivity analysis and the test for heterogeneity

Our pooled data showed the occurrence of heterogeneity in some genetic models ($I^2 > 50\%$). Sensitivity analyses for both IFN- γ and TNF- α were performed to estimate the stability of the results; specifically, a single study in the meta-analysis was removed each time to observe the impact of the individual data set on the overall OR. Sensitivity analysis indicated that no single study influenced the pooled OR qualitatively, suggesting that the results of this meta-analysis are stable.

Discussion

Seventeen studies were included in the present meta-analysis. Five studies in this meta-analysis were processed for the association between the IFN- γ +874 A/T polymorphism and brucellosis. Our pooled evidence suggests that the AT genotype vs AA genotype was associated with an increased risk of brucellosis overall. Similarly to our findings, Karaoglan et al. suggested that the TT genotype of IFN- γ +874 was associated with an increased risk of brucellosis.¹³ However, Bravo et al.,¹² Rasouli and Kiany,⁹

Fig. 3. Forest plot for the association of the TNF- α -308 G/A polymorphism and brucellosis (A allele vs G allele, AA vs GG, GA vs GG, GA+AA vs GG, and AA vs GG+GA)

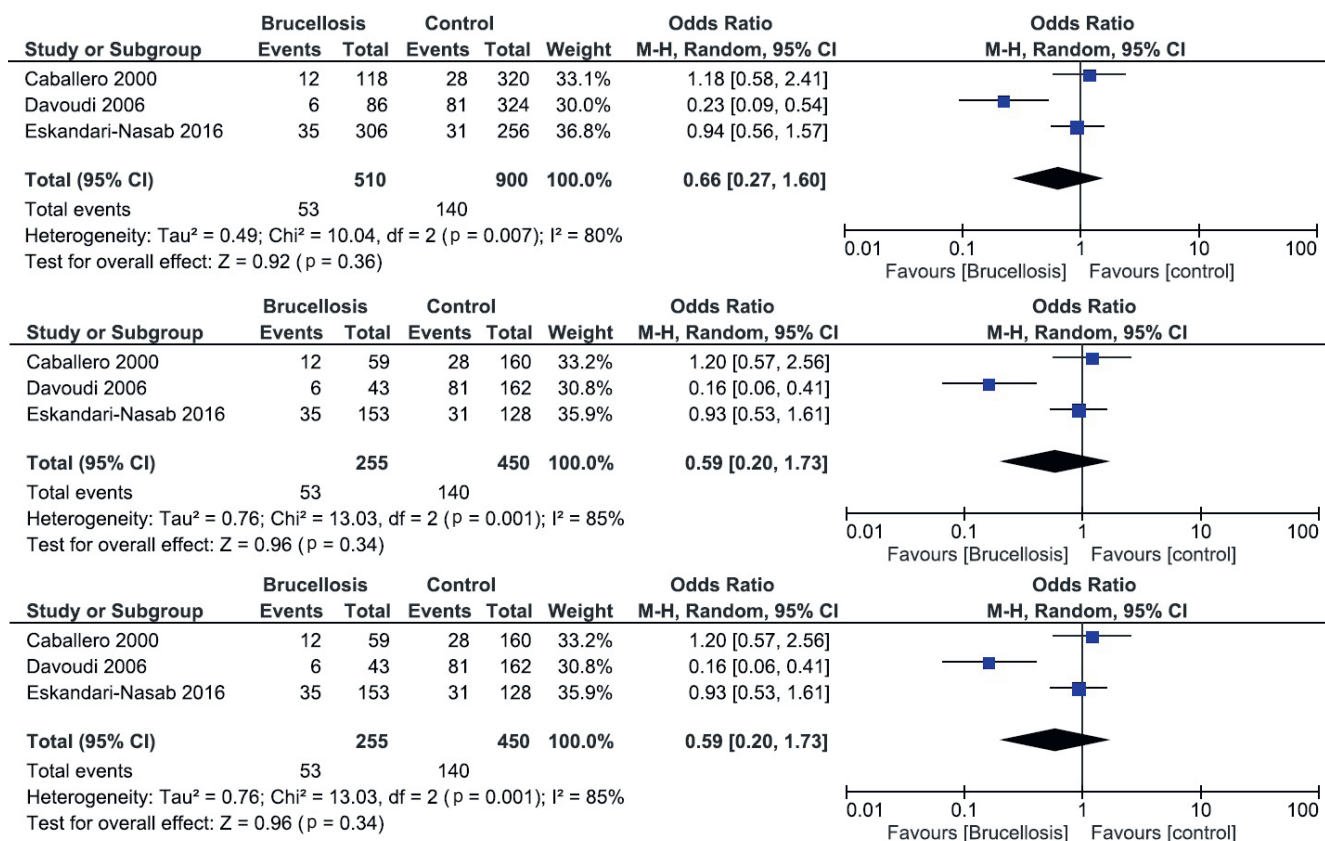


Fig. 4. Forest plot for the association of the *TNF-α* -238 G/A polymorphism and brucellosis (A allele vs G allele, GA vs GG and GA+AA vs GG)

and Eskandari-Nasab et al.² reported that individuals with the wild-type (AA) genotype of *IFN-γ* +874 A/T compared with the mutant (TT) genotype were susceptible to an increased risk of brucellosis.

Concerning the *IFN-γ* UTR5644 A/T polymorphism and susceptibility to brucellosis, 3 studies were processed in this meta-analysis. Our results showed that the UTR5644 polymorphism was not associated with the risk of brucellosis overall, using any models. In agreement with our findings, Davoudi et al.,¹⁵ Hedayatzadeh-Omran et al.,¹⁷ and Eskandari-Nasab et al.² also found no association between this polymorphism and the risk for brucellosis.^{2,15,17}

IFN-γ is an essential cytokine for host control of intracellular pathogens, such as *Brucella* spp. This cytokine increases macrophage activation, promotes cellular immunity responses and contributes to the clearance of brucellosis infection.¹⁸ One of the risk factors that may increase the host's vulnerability to brucellosis is genetic polymorphisms in the form of SNPs in the components of the immune system. Several SNPs, including +874 A/T and UTR5644 A/T in the coding region of the *IFN-γ* gene, have been shown to affect the expression of this cytokine.^{12,19} Previous evidence has indicated that the +874 (A/T) AA genotype correlates with low production, the AT genotype with intermediate production, and the TT genotype with high production of *IFN-γ*.¹⁹ With respect to the *IFN-γ* UTR5644 A/T polymorphism, it has been revealed that homozygosity for the T allele is associated with increased

production of *IFN-γ* compared to other genotypes (AT or AA).¹⁵ Our pooled evidence concurs with several reports demonstrating that *TNF-α* and *IFN-γ* induce cell-mediated resistance against *Brucella* spp. infection.²⁰ *TNF-α* exerts its antibacterial activity against *Brucella* spp. through the stimulation of *IFN-γ* production.²¹

Six studies were analyzed in this meta-analysis of the association between the *TNF-α* -308 G/A variation and susceptibility to brucellosis. Our pooled evidence indicated that in the recessive model, the AA genotype compared to the GG+GA genotype increased the risk of brucellosis overall. However, the A allele, AA or GA vs GG genotype in a codominant model and the GA+AA vs GG genotype in a dominant model were not associated with the risk of brucellosis overall. Our findings regarding the *TNF-α* -308 G/A polymorphism supports those of Reza et al., who found a relationship between -308 AA homozygosity and increased risk of brucellosis.²² In contrast, Davoudi et al. reported that individuals carrying the GG genotype were associated with a higher risk of brucellosis.¹⁵

Three of the studies processed in this meta-analysis concerned the *TNF-α* -238 G/A polymorphism and susceptibility to brucellosis. Our results demonstrated that the *TNF-α* -238 G/A variation was not associated with the risk of brucellosis at both the genotype and allele level, which supports the findings of Caballero et al.,¹⁴ Davoudi et al.¹⁵ and Eskandari-Nasab et al.,¹¹ who reported that this polymorphism was not associated with an increased risk of brucellosis.

Our pooled survey suffers from a few limitations. The first is high genetic heterogeneity among the included studies, which may have resulted from the relatively small sample size of the studies included or from the insufficient amount of data. Our meta-analysis only included published studies, excluding some important relevant abstracts or unpublished studies. Thus, we were aware that these factors might result in high heterogeneity. Further large-scale studies are warranted to confirm the effect of *IFN-γ* and *TNF-α* gene polymorphisms on the risk of brucellosis.

Conclusions

Our meta-analysis demonstrated a significant association between the *IFN-γ* +874 AT and *TNF-α* –308 GG + GA genotypes and a higher risk for brucellosis. However, we found no relationship between the *IFN-γ* UTR5644 A/T and *TNF-α* –238 G/A SNPs and brucellosis.

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