Systematic Review and Meta-Analysis of Published Literatures: How Much do We Know about Reliable Prevalence of Leptospirosis in Iran?

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ABSTRACT

Deficiency in knowledge about leptospirosis prevalence in Iran led us to clarify the prevalence of Leptospirosis and its diagnostic tests and increase global awareness of the diseases in this era via systematic review and Meta-analysis. A comprehensive search about the prevalence of Leptospirosis was done in PubMed, ISI Web of Sciences, Cochran Library, EMBAS, Scopus, Magiran, Iranmedex, Scientific Information Database (SID), and Google scholar from 1995 to 2013. Twenty two relevant papers were found and analyzed using R software and comprehensive meta-analysis software Biostat V2.0.

The meta-analysis findings demonstrated that the prevalence of Leptospirosis in Iran was 39% (95% Confidence Interval). Our analysis showed that the highest prevalence was observed in the province of Mazandaran (44%) (95% CI 27-61). While, interestingly, the lowest prevalence occurred in its neighboring province (Golestan, 34%) (95% CI 15-83). Further stratified analysis highlighted that farmers were a population at risk (65% (95% CI 74-57)) and that the disease is more prevalent in males (69% (95% CI 74-56). Based on the information in the current study, we induced that Leptospirosis occurred highly in humid climate areas of northern Iran. Due to the lack of Leptospirosis specific clinical symptoms and differences in sensitivity and specificity of diagnostic tests, we recommend doing further comprehensive studies by reliable tests and establishing national and local controlling programs for public awareness and reduction of human exposure to infected animals and their tissue and fluids.

Keywords: Iran leptospirosis, meta-analysis

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INTRODUCTION

Leptospirosis is known as the most common zoonosis in the world that is caused by spirochetes belonging to the genus Leptospira (1). It is also settled in tropical and subtropical regions (2). Transmission route of *Leptospira spp* to human is debated in different countries (3,4). The majority of researches demonstrated direct or indirect exposure to animals such as rodents and livestock and also poor sanitation, urban overcrowding, poor waste disposal, heavy rainfall and floods are the main risk factors involved in the development of Leptospirosis (3,4). Although, clinical profiles of Leptospirosis have changed in recent years but most of the human infections manifest a wide spectrum of disease from mild flu like and self-limited illness to fulminant multi-system involvement with acute renal failure and hemorrhage leading rapidly to death (5-8). Unfortunately, there is a lack of reliable knowledge on the prevalence of Leptospirosis in Iran, due to mild and self-limited clinical manifestation and/or lack of awareness of physicians about the diseases. So, the current review is an attempt to determine the prevalence of Leptospirosis in Iran based on a comprehensive systematic review and meta-analysis of published literatures.

METHODS

Data Source

The data about the epidemiology of Leptospirosis in Iran as well as period, location, sample size, diagnostic methods, gender, occupation, and age groups were obtained from reputable published articles during the years 1995 to 2012 (table 1). Most of the studies were conducted in Gilan (n=10; 45.4%) and Mazandaran (n=5; 22.7%) and the majority of diagnostic methods for the

detection of Leptospirosis in these studies were MAT (n=10; 45.4%) and ELISA (n=8; 36.4%). The search terms were Leptospirosis; Leptospira, Weil's disease, and Iran. International main databases including ISI web of Knowledge, Medline/PubMed, Scopus, EMBASE and Cochrane Library which were searched by two independent researchers. In order to increase the number of results, Google Scholar and current Persian databases (IranMedex, IranDoc, SID and Magiran) were also searched. The Persian keywords were equivalent to the English keywords and all of the probable combinations were also considered.

Study Selection and Data Extraction

All results were screened and those that were not relevant to the review and/or duplicates were removed (Flowchart 1). All desired information such as study period, location, sample size, diagnostic methods, gender, occupation, and age groups were entered into data collection forms, and then transferred into Microsoft Excel.

Statistical Analysis

According to the main objective of Leptospirosis prevalence in Iran, we estimated variance by binominal distributions. Prevalence and 95% confidence interval was calculated using random effect model for meta-analysis. To pool prevalence reported by different studies, weighting averaging was used. Each study given a weight equal to its inverse variance. Q test and I2 index, at the type I error of smaller than 0.10, were applied in order to evaluate heterogeneity. Whenever the results of the study were heterogeneous, the analysis performed using a random-effects model. When heterogeneity was not significant among the results of the study, the Fix

effects model was used to pool analysis and verses. Publication bias was measured with Begg's adjusted rank correlation test and graphically depicted by funnel plot.

RESULTS

Upon completion of the search, 22 studies with a 10234 sample size were included into the meta-analysis (Flowchart 1). The prevalence of Leptospirosis as well as gender, age and occupation parameters was extracted. Our analysis showed that the prevalence of Leptospirosis in Iran is 39% (95% CI 29-49) (fig 1). It was interesting that while the highest prevalence was obtained from Gilan province (70%; 95% CI 67-73) (with sample size of 995 people); the lowest prevalence was found in Golestan province (10%; 95% CI 9-12) (with sample size of 1025 people) (Table 1). For detailed information about the prevalence of Leptospirosis in Iran, refer to Table 1. According to the analysis based on city and/ or province, the highest prevalence rate 44% with a sample size of 1505 people was seen in Mazandaran province (95% CI 27-61) and the lowest prevalence rate of 34% with a sample size of 1048 people was seen in Golestan province (95% CI 15-83) (fig 2). Generally, analysis based on different geographical regions in Iran demonstrated that the prevalence of Leptospirosis in the northern part of Iran was 41% (95% CI 29-53) with a 9191 sample size. The prevalence rate in central Iran was 49% (95% CI 44-53) with sample size of 480 (fig 3).

Prevalence of Leptospirosis in males was 69% (95% CI 74-56) and in females was 35% (95% CI 44-26). When occupation was subjected for evaluation, the highest prevalence -as expected- was seen in farmers (65%) (95% CI 74-57) (Fig. 4) and the lowest prevalence belonged to students (4%) (95% CI 7-1). The analysis based on age groups demonstrated a 40% (95% CI 34-46) prevalence among the age group of 40-60 years, 16% (95% CI 10-22) among the

age group ≥ 60 years and 9% (95% CI 5-13) among the age group of 0-20 years. The sensitivity for MAT was estimated to be 39% (95% CI 30-48), while for ELISA was 49% (95% CI 27-71) and for IFA was 54% (95% CI 8-100) (Fig. 5).

Furthermore, meta-regression analysis demonstrated that the prevalence of Leptospirosis has slightly decreased from 1995 to 2014 and was statistically significant (p=0124) (Fig. 6), however there was no significant correlation between prevalence and sample size (p=0.58) (Fig. 7). Also funnel plot showed there was no publication bias (Fig. 8)

DISCUSSION

Our meta-analysis demonstrated that the prevelance of Leptospirosis in Iran according to age, gender, and occupation parameters is 39%. Most outbreaks of Leptospirosis, which indicates in the majority of farmers, are occupational and the disease is more prevalent in wet climates. Alayian and colleagues observed a significant correlation between Leptospirosis and occupation and suggested a special control program for susceptible individuals (9). The majority of reports indicated that Leptospirosis is more prevalent among farmers. In agreement with Faraji and colleagues, our results showed that 65% of all studied patients had agricultural occupation (10). Taleei and colleagues showed that 49% of rice farmers were seropositive while only 15% of ranchers were seropositive. In agreement with this meta-analysis results, they indicate a high prevalence of Leptospirosis among farmers and so it seems that this group is at a high risk of developing Leptospirosis (11).

Perret and colleagues reported 72% of farmers have Leptospirosis. Slack *et al* considered type of occupation and exposure as main determinants in development of this disease (12,13). Most of Leptospirosis outbreaks occurred in tropical regions including Asia, Pacific, Latin America, and Caribbean especially after floods and recently it was seen in Nicaragua and Philippines (14).

Leptospirosis was more prevalent among males than females, as showed by previous studies (15-18). Reports from European countries also showed that the risk ratio of developing Leptospirosis in males is four times more likely than females (19). The results of the current study also showed that Leptospirosis was more prevalent among patients aged 20-60 years old. This is confirmed by the other studies in Iran (11, 17, 20-21). In agreement to our results, Fero and colleagues also showed Leptospirosis was observed mostly in patients over 50 years old in Colombia (22).

Despite the deficiency of studies about diagnostic methods for leptospirosis in Iran, it seems that rapid leptospirosis antibody based tests such as ELISA are used frequently (23, 6, 11, 15, 23-28). Similar to the above studies, our results showed higher prevalence of Leptospirosis by ELISA (49%) than MAT (39%) in Iran. The gold standards for diagnosis of Leptospirosis are culture and the microscopic agglutination test (MAT) which needs specific equipments and highly trained staff (29). Culture is not suitable for the diagnosis of the slow-growing *Leptospira spp*, and MAT which detects anti-Leptospira antibodies only in the second week of the disease, cannot detect early stages of the disease (29, 30). Some studies reported that other techniques can detect anti-*leptospira* antibody earlier in the course of the disease than MAT. Although anti-*leptospira* IgM antibodies are not detectable before 4-5 days after the onset of the disease, but appear earlier than IgG and agglutinating antibodies (29, 31). So, although ELISA cannot identify Leptospira serovars but could be applied as a routine diagnostic and primary screening

test especially in endemic regions. According to some researches, it seems that semi-quantitative ELISA is more valuable in Iran because of its high sensitivity and specificity (24-27).

Finally, it could be concluded that Leptospirosis occurred highly in tropical and temperate climates, especially in hot and humid areas of northern Iran such as Gillan and Mazandaran provinces. Although meta-regression analysis shows that during 1997 and 2012 the prevalence of Leptospirosis has decreased slightly in Iran and was statistically significant, but due to lack of Leptospirosis specific clinical symptoms and differences in sensitivity and specificity of diagnostic tests, we cannot conclusively explain this decrease.

Although using rapid diagnostic tests such as ELISA and PCR and conventional methods such as culture and MAT can be very important in diagnosis and starting the treatment, but to greatly reduce Leptospirosis occurrence, we should note that prevention of Leptospirosis must be considered as a basic principle. Leptospirosis is a zoonotic and human disease only. Avoidance of human exposure to infected animals or their tissue, urine and blood, wearing protective clothing such as gloves and boots especially for people at high risk such as veterinarians, farm workers and sewer workers, not swimming or wading in water contaminated with the urine of infected animals, can be very helpful in controlling Leptospirosis in Iran.

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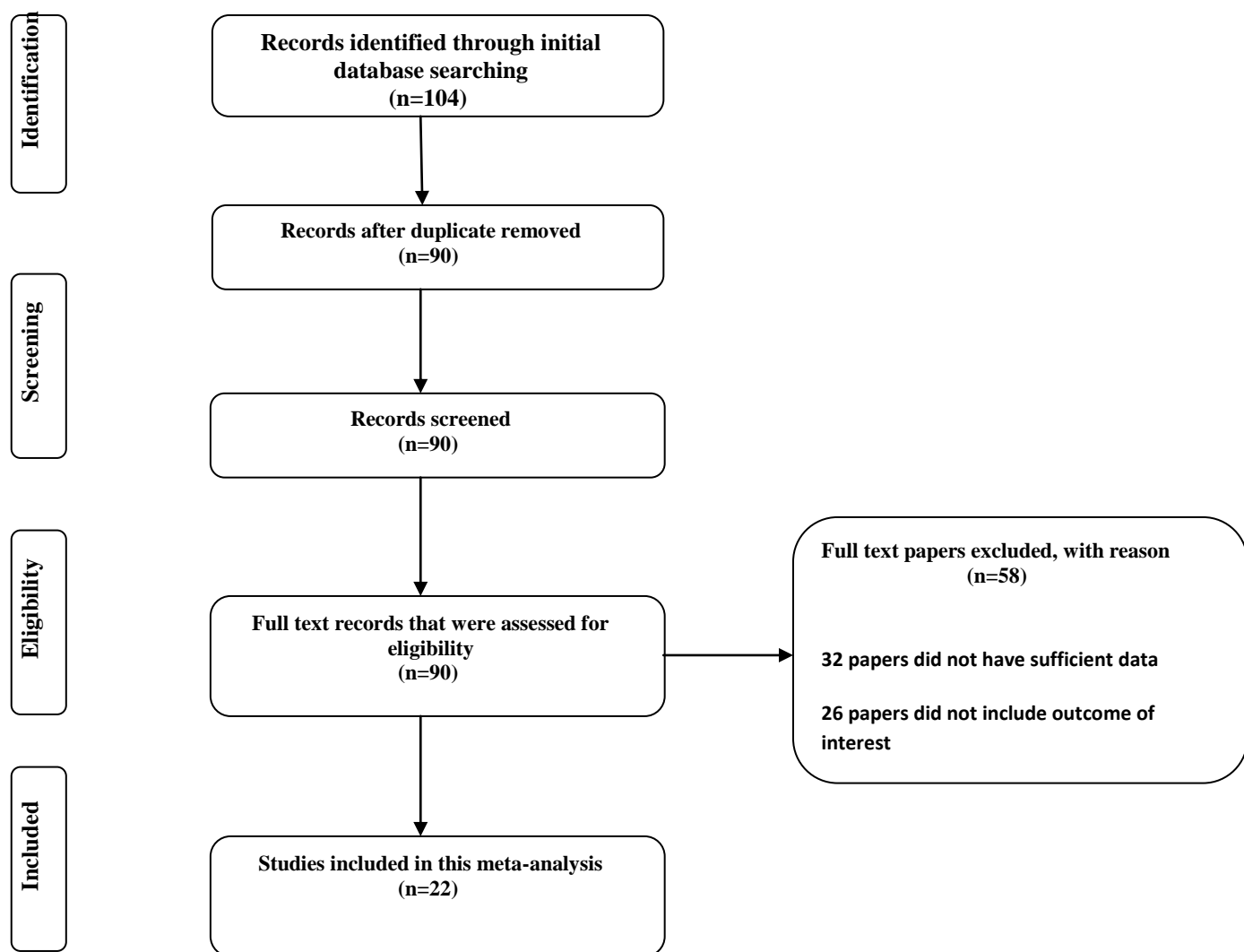
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Table1: Characteristics of papers included in the meta-analysis

Reference	year	Province	Total sample	Positive sample (%)	method
Majd *et al*. (2012)	2012	Zanjan	98	35	MAT
Javid *et al*. (2012)	2008	Golestan	1028	10	ELIASA
Esfandiari *et al*. (2011)	2010	Mazandaran	688	27/5	IFA
Honarmand & Eshraghi (2010)	2010	Guilan	282	25	ELIASA
Ebrahimi & Alijani (2003) (32)	2002	Chaharmahal	400	48/5	MAT
Babamahmoodi *et al*. (2009) (33)	2009	Mazandaran	500	14	IFA
Honarmand *et al*. (2006)	2003	Guilan	282	24	ELIASA
Ziapour *et al*. (2010) (34)	2006	Mazandaran	127	53	MAT
Golsha *et al*. (2007) (35)	2004	Golestan	20	60	MAT
Alian *et al*. (2006)	2004	Mazandaran	63	69	MAT
Faraji *et al*. (2010)	2006	Mazandaran	127	58	MAT
Rahimi *et al*. (2007)	2004	Guilan	552	21	MAT
Hajikolaei *et al*.(2008) (36)	2004	Khuzestan	180	18	MAT
Taleie *et al*. (2007)	2005	Lorestan	80	49	ELIASA
Honarmand *et al*.(2010)	1999	Guilan	3431	60	ELIASA
Ghanaei *et al*. (2008) (37)	2004	Guilan	465	38	MAT
Taghavi *et al*. (2006)	2002	Guilan	87	30	ELIASA
Sakhaee *et al* (2011) (38)	2009	Kerman	285	16	MAT
Mansour-Ghanaei *et al*.(2005)	1999	Guilan	237	31	IFA
Honarmand *et al*.(2008)	2006	Guilan	200	49	ELIASA
Eshraghi *et al*.(2007)	2003	Guilan	995	70	ELIASA
Honarmand *et al*.(2010)	2008	Guilan	107	60	PCR

Identification

Records identified through initial
database searching
(n=104)

Screening

Records after duplicate removed
(n=90)

Records screened
(n=90)

Eligibility

Full text records that were assessed for
eligibility
(n=90)

Full text papers excluded, with reason
(n=58)

32 papers did not have sufficient data

26 papers did not include outcome of
interest

Included

Studies included in this meta-analysis
(n=22)

Flowchart 1. Studies selection for meta-analysis

Study ID	ES (95% CI)	% Weight
Ghanaei (1999)	0.31 (0.25, 0.37)	4.60
Honarmand (1999)	0.60 (0.58, 0.62)	4.67
Taghavi (2002)	0.30 (0.20, 0.40)	4.48
Ebrahimi (2002)	0.49 (0.44, 0.53)	4.62
Eshraghi (2003)	0.70 (0.67, 0.73)	4.66
Honarmand (2003)	0.24 (0.19, 0.29)	4.62
Rahimi (2004)	0.21 (0.18, 0.24)	4.65
Hajikolaei (2004)	0.18 (0.12, 0.23)	4.61
Alian (2004)	0.69 (0.58, 0.81)	4.41
Golsha (2004)	0.60 (0.39, 0.81)	3.84
Ghanaei (2004)	0.38 (0.34, 0.43)	4.63
Taleie (2005)	0.49 (0.38, 0.60)	4.42
Faraji (2006)	0.58 (0.50, 0.67)	4.52
Honarmand (2006)	0.49 (0.42, 0.56)	4.57
Ziapour (2006)	0.53 (0.44, 0.61)	4.51
Javid (2008)	0.10 (0.09, 0.12)	4.67
Honarmand (2008)	0.60 (0.51, 0.69)	4.49
Sakhaee (2009)	0.16 (0.12, 0.20)	4.63
Babamahmoodi (2009)	0.14 (0.11, 0.17)	4.66
Esfandiari (2010)	0.27 (0.24, 0.31)	4.65
Eshraghi (2010)	0.25 (0.20, 0.30)	4.62
Soltani (2012)	0.35 (0.25, 0.44)	4.49
Overall (I-squared = 99.2%, p = 0.000)	0.39 (0.29, 0.49)	100.00

NOTE: Weights are from random effects analysis

-.815   0   .815

Figure 1: The prevalence of Leptospirosis in each of the studies. The segments show a 95% prevalence of Leptospirosis. Diamond mark indicates the estimated overall prevalence rate.
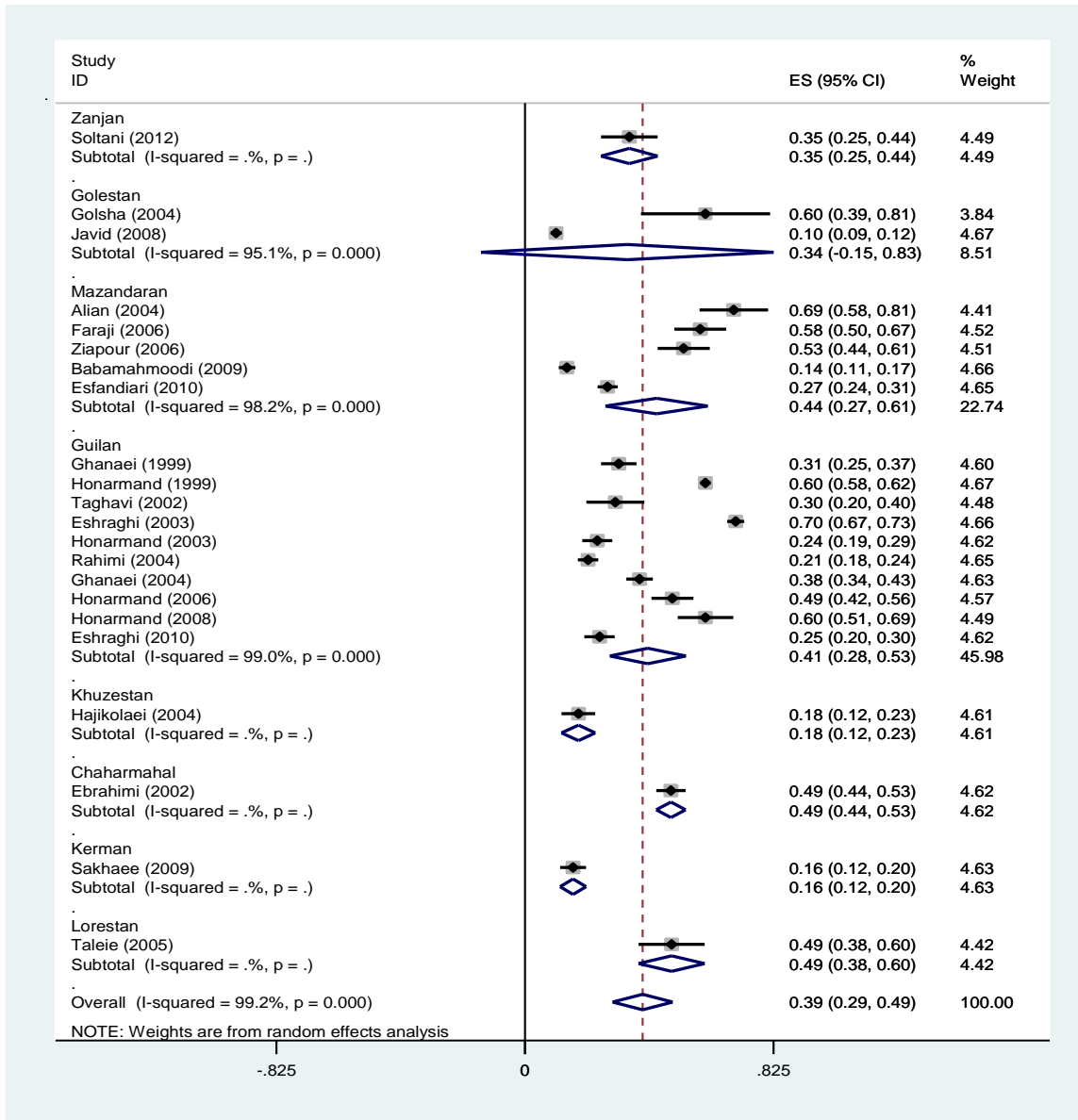
Fig 2: The prevalence of Leptospirosis in different cities. The segments show a 95% prevalence

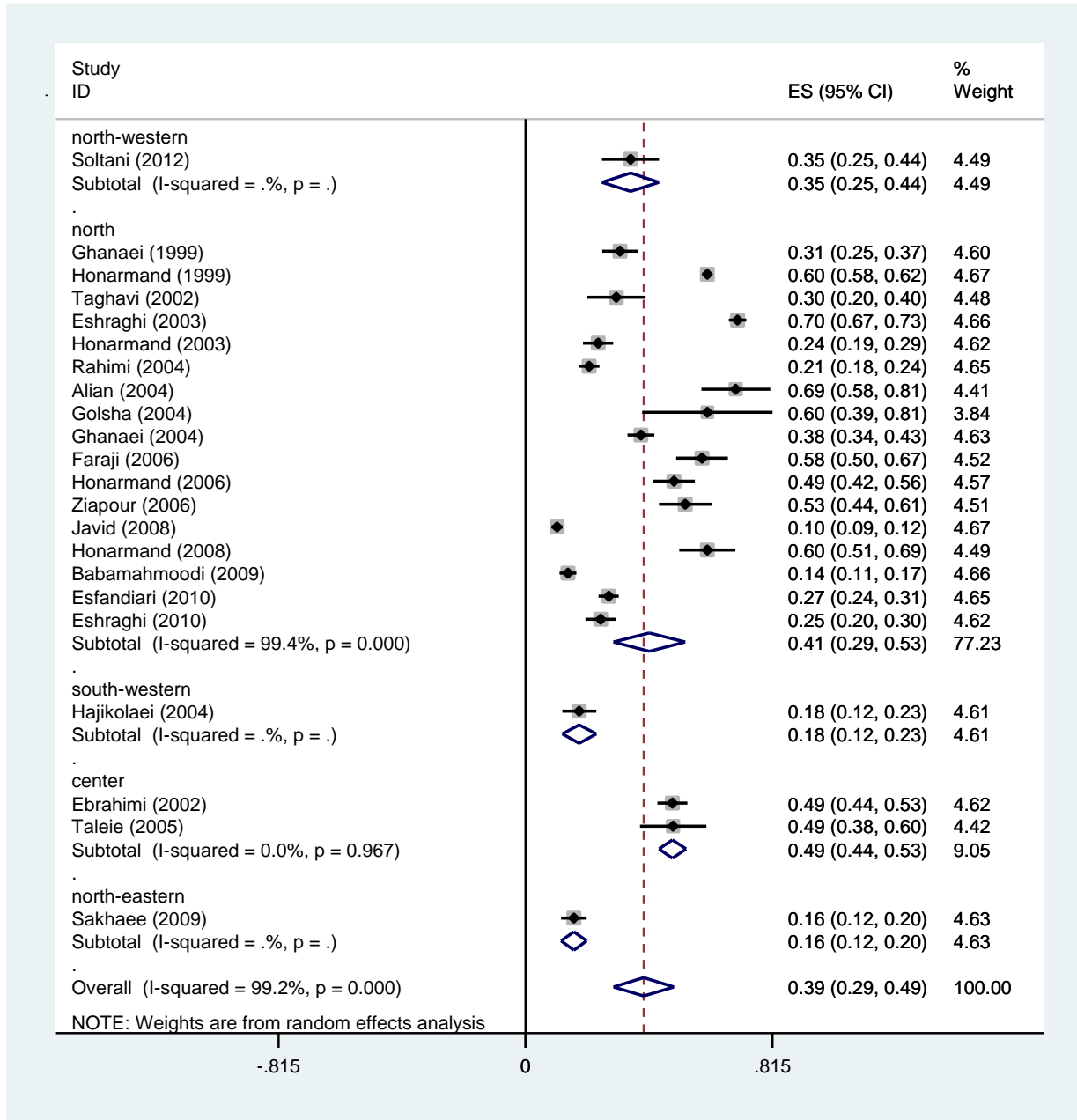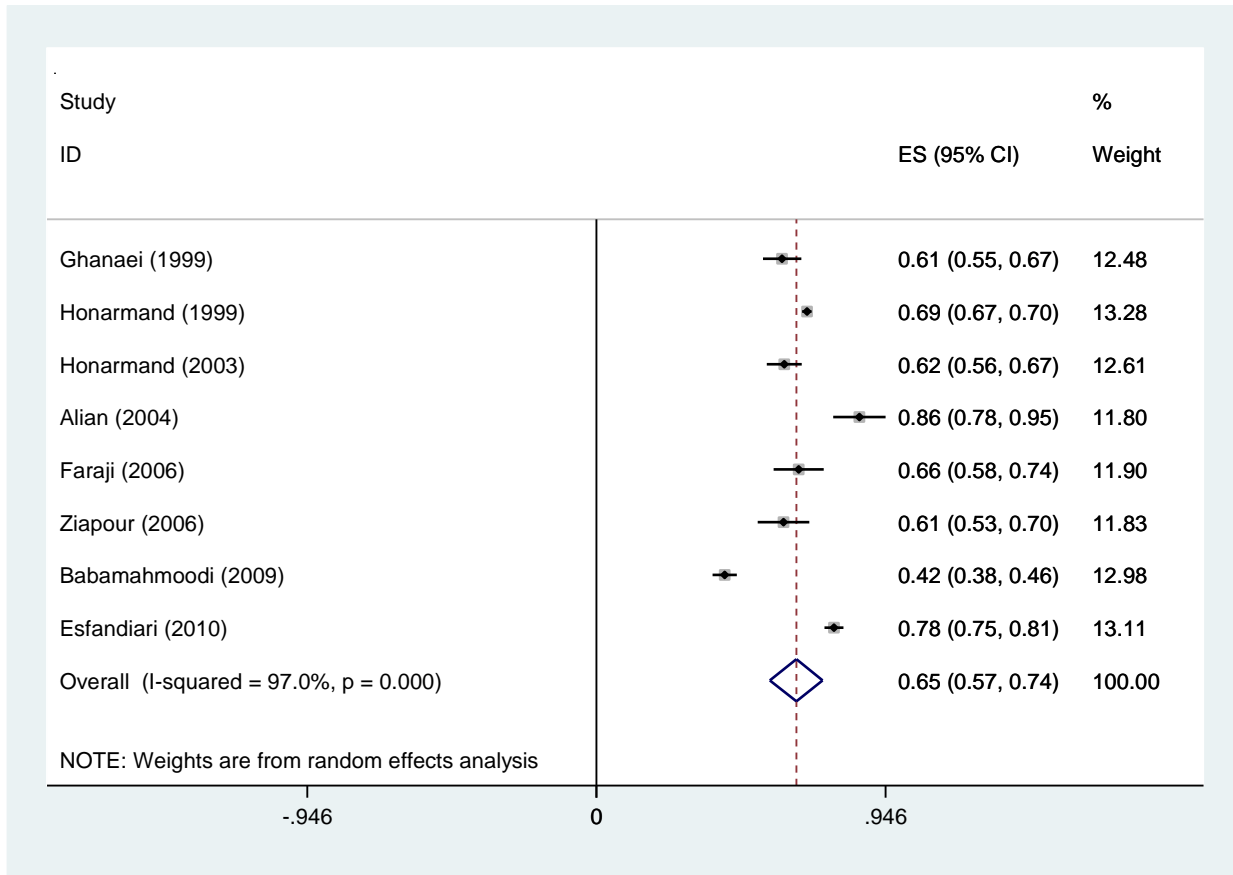of Leptospirosis. Diamond mark indicates the estimated overall prevalence rate.

Fig 3: The prevalence of Leptospirosis in different geographical regions of Iran. The segments show a 95% prevalence of Leptospirosis. Diamond mark indicates the estimated overall prevalence rate.

Fig 4: The prevalence of Leptospirosis among farmers. The segments show a 95% prevalence of Leptospirosis. Diamond mark indicates the estimated overall prevalence rate
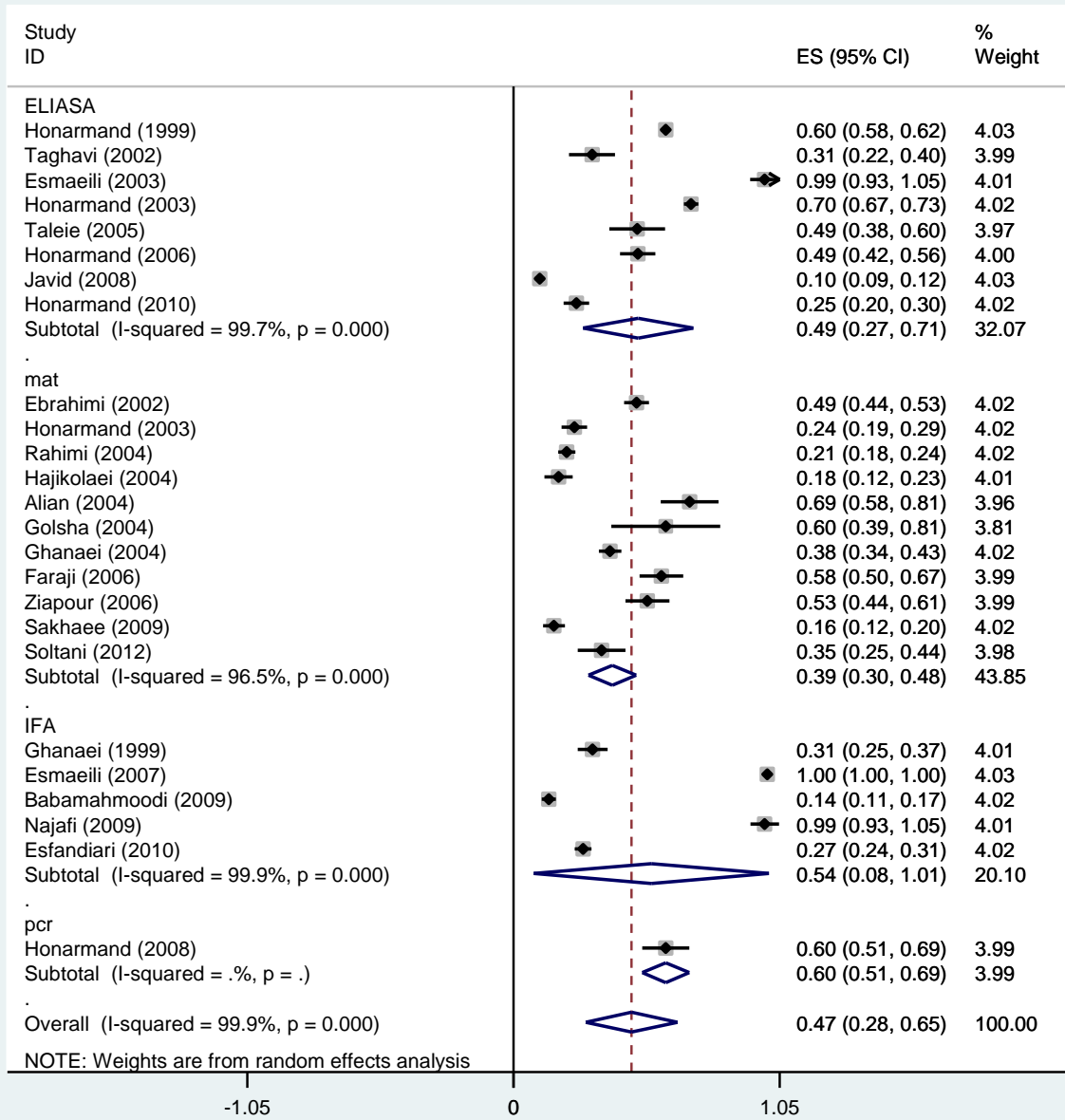
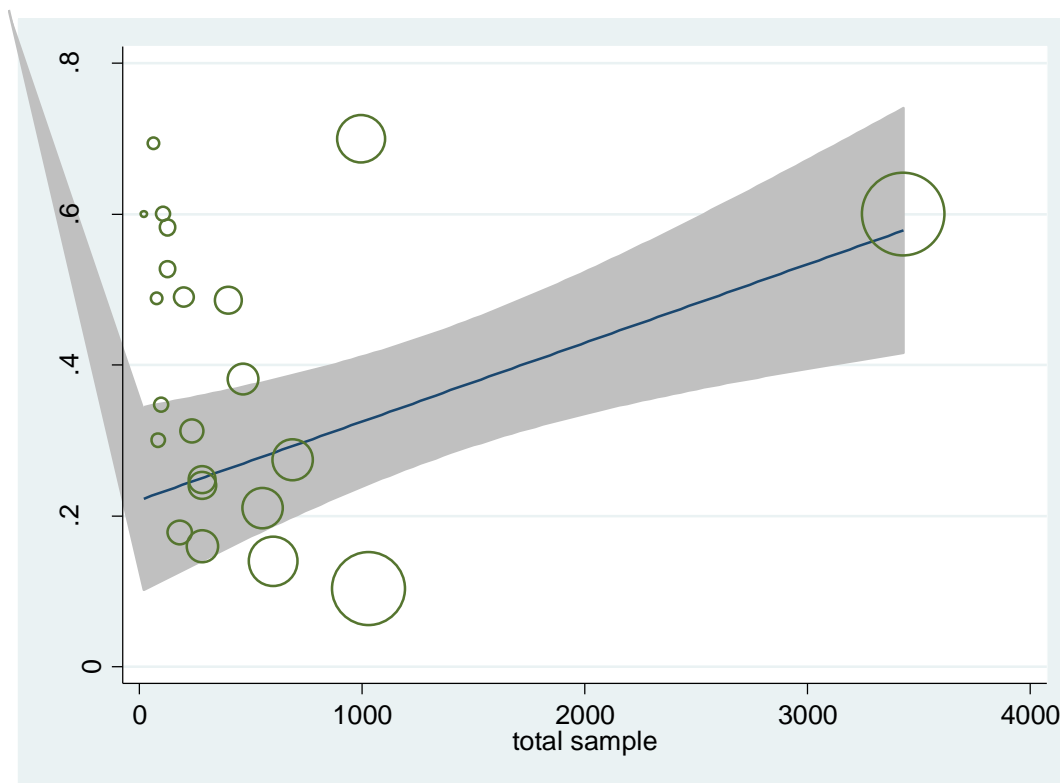Fig 5: Prevalence of Leptospirosis in Iran, according to different diagnostic methods

Fig 6: Meta-regression. The relationship between the prevalence of the disease and the sample size shows that the prevalence also increased with increasing sample size. But there was no statistically significant relationship. P = 0.586. (Size of the circle indicates the sample size and larger circles represent more samples).
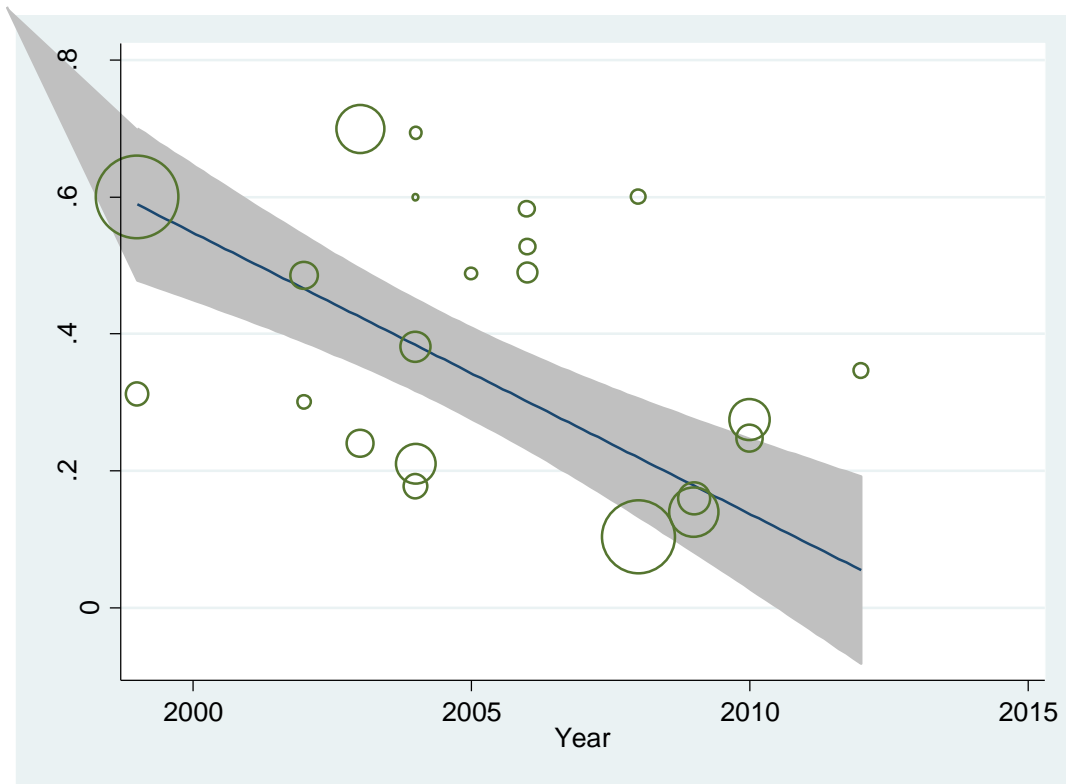
Fig 7: Meta-regression. The relationship between the prevalence of the disease and the time parameter (year) shows that during 1997 and 2012 the prevalence of the disease has decreased slightly and was statistically significant. P = 0.124 (size of the circle indicates the number of samples and more samples are indicative of a larger circle.)
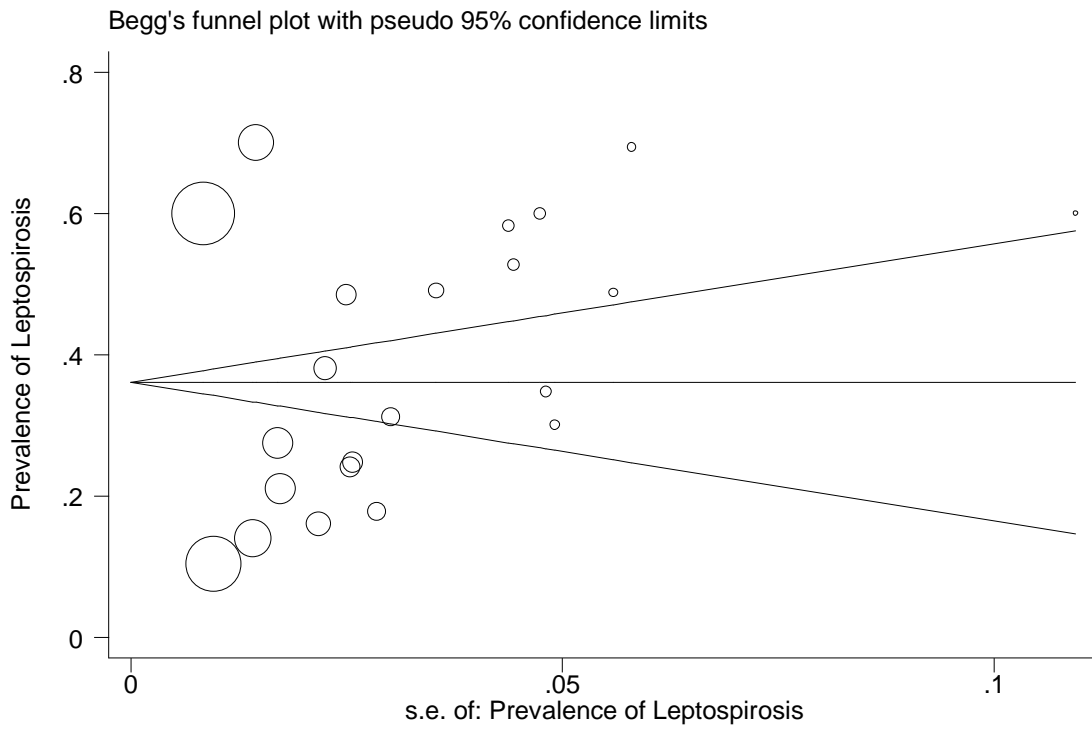
Fig 8: Funnel plot for assessing publication bias