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Original papers

Status of fasciolosis among domestic ruminants in Iran based on abattoir data: a systematic review and meta-analysis

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ABSTRACT. Fasciolosis is considered as an ongoing neglected zoonotic disease in tropical regions of the world, relating to notable financial and public health issues. The current systematic review and meta-analysis was planned to determine the status of fasciolosis among domestic ruminants, including cattle, buffalo, goat, and sheep at the industrial slaughterhouses in Iran, between the years 2000 and 2016. Eight databases, four English and four Persian, were searched. Our findings demonstrated that 2.6% of all inspected livers of slaughtered ruminants were infected with *Fasciola* spp. during 2000–2016. The mean prevalence of fasciolosis for cattle, buffalo, goat, and sheep was 21%, 4.2%, 2%, and 2.4%, respectively. Additionally, most studies were performed on cattle 25 (39.6%), sheep 24 (38 %), and goat 12 (19%), respectively, and just 2 (3.1%) studies were performed on buffalo. The prevalence of animal fasciolosis has significantly decreased among domestic ruminants in Iran except for cattle. In addition, the prevalence of this disease in Northern and Western regions of the country has remained at hypo-endemic level. The results present updated gathered information on the epidemiology of fasciolosis in domestic ruminants in Iran, and will expand the screening strategies to improve health and reduce economic impacts among farm animals.

Keywords: domestic ruminants, fasciolosis, systematic review, Iran

Introduction

Fasciolosis is a neglected foodborne disease caused by at least two genera of *Fasciola* spp., including *Fasciola* (*F.*) *hepatica* and *F. gigantica.* It is habitually considered as an important parasitic disease in farm animals, frequently obtained by metacercaria encysted on leaves that are consumed as forage by animals or as raw vegetables by humans. The disease is considered as one of the important zoonotic helminthic infection of ruminants in endemic countries, since it is associated with socioeconomic issues [1,2].

This parasite is frequently reported from wet lands and temperate regions of the world [3]. A broad range of mammals are well-known as definitive hosts for the parasite such as cattle, buffalos, sheep, goats, camels, and pigs with which human is constantly dealing [4]. Both *F. hepatica* and *F. gigantica* affect almost 250-300 million cattle and sheep and their annual economic losses amount to about $ 3 billion [2,5].

Humans and animals can accidentally ingest the contaminated raw vegetables and become infected [6]. Geographical distribution pattern of the fasciolosis relies on the presence of aquatic snail *Limnea* spp. as an intermediate host in each area.

The disease is frequently diagnosed by the detection of parasite ova in stool as gold standard method in both human and animals. However, animal infections are mostly detected during meat inspection in slaughterhouses. Moreover, immunodiagnostic tests such as enzyme-linked immunosorbent assay (ELISA) and indirect fluorescent antibody test (IFAT) was performed for early diagnosis and/or rule out the ectopic cases of the fasciolosis [7,8].

The prevalence of fasciolosis was reported between range 1 > to 91.4% among ruminants in different provinces of Iran [9]. Despite the presence of several studies concerning fasciolosis among livestock in Iran, the true prevalence of the animal fasciolosis is unknown, thus recognition of the epidemiological aspects of animal fasciolosis in Iran could be useful to estimate the international and national economic and health burden and to manage the preventive programs. The current systematic review aimed to determine prevalence and distribution pattern of fasciolosis among domestic ruminants (cattle, buffaloes, goats, and sheep) in various endemic areas of Iran throughout 2000–2016.

Materials and Methods

Study design and search terms. This review study was designed based on PRISMA guidelines [10]. The search terms that were combined with each other were: "*Fasciola*", "*Fasciola hepatica*", "*Fasciola gigantica*", "fascioliasis", "Iran", "helminthic infection", "epidemiology", "domestic animal", ''ruminants", "cattle", "buffalo", "bovine", "sheep", "ovine", "goat", and "caprine".

Inclusion and exclusion criteria. The published studies in English and Persian languages that reported the prevalence of fasciolosis in domestic ruminants in Iran based on the abattoir survey between the years 2000 and 2016 were evaluated. Furthermore, the literature with only abstract, no safety examination, article not found, book chapter, congress abstract, review articles, no statistical index, inadequate data, or irrelevant (i.e. *Fasciola* spp. not reported) were excluded.

Databases. The search was carried out in English (PubMed, Google Scholar, ScienceDirect, and Scopus) and Persian (Magiran, Elmnet, Barkat Knowledge Network System [Barakatkns], and Scientific Information Database [SID]) databases in December, 2016.

Search strategy and data extraction. The first screening was performed by three independent authors (MS, MF, and SA) through the review of title and abstract of the selected articles. Removing the article duplication was carried out using EndNote X7® software (Thomson Reuters, New York, USA). The articles selected at the screening stage were carefully read by the same three authors. The eligible articles were selected by each of the three authors separately, and disagreement, if any, was resolved by the fourth author. Information from the included articles was extracted by the same three authors (Table 1). There was no disagreement and inter-rater reliability was 100%.

Publication bias and sensitivity assessment. Funnel plot and Egger's test were examined to analyse the publication bias of the included studies. The sensitivity analysis was performed via evaluation of the effect of a study in a total of studies, calculated by removing a study from a total of studies each time. With removing an article, if the total confidence interval (CI) of the studies remained constant within the 95% range, meaning the result is robust and therefore the removed article can be included in the meta-analysis. In addition, to amend the results from possible publication bias and/or selective reporting, we applied the standard method of trim-and-fill analysis.

Heterogeneity assessment. We used the forest plot for estimating pooled effect size and the effect of each study, with a 95% CI, to provide a visual summary of the data. To evaluate heterogeneity among the studies that used common approaches, we performed the Cochran Q-test ($P < 0.1$) and the I-squared index, with I^2 value between 25% and 50%, 50% and 75%, and above 75% as thresholds for low, moderate, and high heterogeneity, respectively. When heterogeneity was present, we used a random effects model (DerSimonian-Laird model); otherwise we applied a fixed effects model (Mantel-Haenszel) to compute overall effects.

Statistical analysis. At first, we examined a primary descriptive analysis of the included studies. Then, for each study the fasciolosis prevalence was calculated. Whilst the estimate for a study tends closer to either 0% or 100%, the variance for that study moves to zero and therefore its weight is overrated in the meta-analysis. Consequently, we

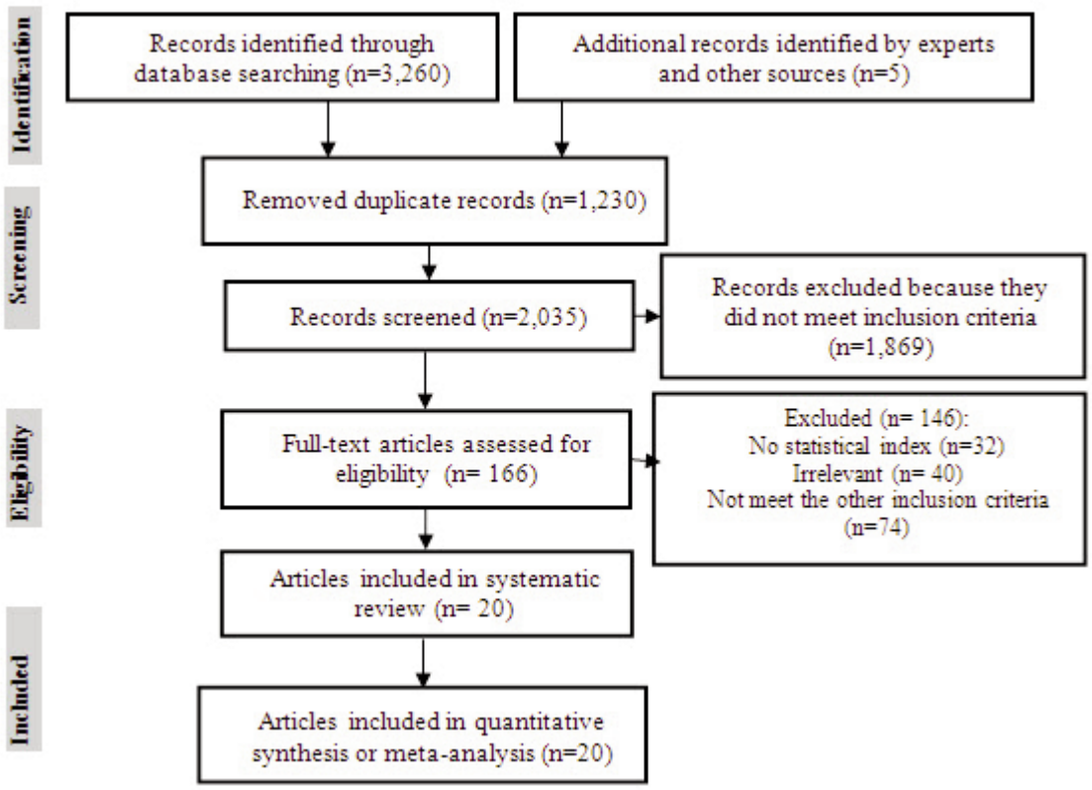

Figure 1. PRISMA flowchart, describing the study design process

carried out the meta-analysis with the prevalence estimates that had been altered using the Freeman-Tukey double arcsine method. The final pooled result and 95% CI were converted for convenience of understanding. We performed all statistical analyses with STATA v11 software (StataCorp LP, College Station, TX, USA).

Results

The included studies

Our systematic search recognized 3,265 possible studies using our search strategy. The 3,099 studies were considered disqualified after removing duplicate records and title and abstract screening. The 166 feasible studies were reviewed via the full-text and 146 studies were excluded because they did not meet the inclusion criteria. The remaining 20 eligible articles were included in the current study (Fig. 1).

The prevalence of fasciolosis

The total number of cases in the 20 included studies was 6,408,202 domestic ruminants (sheep 4,347,898; cattle 1,272,101; goats 1,242,740, and buffalo 95) and overall 170,552 (2.6%) inspected livers were infected. The number of studies on cattle was 25 (39.7%), sheep 24 (38.1%), goat 12 (19.0%), and buffalo 2 (3.2%). For cattle 52,832 (4.2%) of 1,272,101 livers, for sheep 87,093 (2.0%) of 4,347,898 livers, for goats 30,607(2.4%) of 1,242,740 livers, and for buffaloes 20 (21%) of 95 were infected with fasciolosis. According to random effect meta-analysis, the pooled prevalence of *Fasciola* spp. was estimated 3.0% (95% CI: 2.0–4.0) and the highest and lowest prevalence were 32% (95% CI: 29%–36%) and 0.0% (95% CI: 0.0%–0.2%), respectively (Fig. 2).

Subgroups analysis

During the comparison of the prevalence of *Fasciola* spp. a significant difference ($P < 0.001$) was detected in the subgroup of animal species, and most of the difference was related to two studies that were conducted on buffalo (Table 2). After dividing the place of the included studies into four geographic regions, this subgroup study showed the highest prevalence of *Fasciola* spp. was 7.0% (95% CI: 5.0%–10.0%) and observed in the three provinces of the north of Iran, including Mazandaran, Gilan, and Golestan. The lowest prevalence with 0.0% (95% CI: 0.0%–1.0%) was observed in

Table 1. Summary of the main characteristics of included studies in the systematic review and meta-analysis

	Province in Iran	Number of		Reference
		examined cases	infected cases	
Sheep	Kohgiluyeh and Boyer-Ahmad	72,282	8,454	[25]
Goats	Kohgiluyeh and Boyer-Ahmad	100,460	7,580	
Cattle	Kohgiluyeh and Boyer-Ahmad	18119	2,132	
Sheep	Mazandaran	15,952	907	[21]
Goats	Mazandaran	16,372	260	
Cattle	Mazandaran	7,920	364	
Cattle	Gilan	928	13	[26]
Cattle	Fars	131,716	6,533	[27]
Sheep	Fars	577,090	21,871	
Goats	Fars	135,233	3,881	
Goats	Khuzestan	2,473	10	[28]
Sheep	Khuzestan	16,699	309	
Sheep	East Azerbaijan	140	12	[29]
Cattle	Gilan	156	21	[30]
Sheep	Gilan	178	18	
Buffaloes	Gilan	85	20	
Sheep	Hamadan	2,590	109	[31]
Cattle	Hamadan	420	40	
Goats	Hamadan	490	22	
Cattle	Gilan	421	135	[32]
Cattle	Tehran	109,766	2,415	[33]
Sheep	Tehran	457,793	9,218	
Sheep	Khuzestan	2,490,742	23,059	[34]
Goats	Khuzestan	400,695	11,181	
Cattle	Khuzestan	295,318	16,353	
Cattle	North Khorasan	4,933	35	[35]
Sheep	North Khorasan	23,047	81	
Goats	North Khorasan	11,545	23	
Sheep	Isfahan	77,912	538	[36]
Goats	Isfahan	180,824	978	
Cattle	Isfahan	9,066	16	
Sheep	Gilan	640	61	[37]
Cattle	Gilan	600	195	
Sheep	Mazandaran	410	32	
Cattle	Mazandaran	215	26	
Sheep	Golestan	200	5	
Cattle	Golestan	160	5	
Sheep	East Azerbaijan	8,800	15	[38]
Cattle	East Azerbaijan	360	4	
Sheep	Razavi Khorasan	2,400	18	
Cattle	Razavi Khorasan	1,300	10	
Sheep	Khuzestan	540	7	
Cattle	Khuzestan	310	14	
Sheep	Fars	1,700	11	
Cattle	Fars	1,060	5	
Sheep	Mazandaran	950	12	
Cattle	Mazandaran	520	16	
Sheep	Markazi	650	6	
Cattle	Markazi	430	5	

Table 1. Continued

Animal	Province in Iran	Number of		Reference
		examined cases	infected cases	
Sheep	Ilam	17,055	98	[39]
Goats	Ilam	5,703	28	
Cattle	Ilam	4,484	141	
Cattle	Lorestan	150,869	23,004	[40]
Sheep	Markazi	292,797	3,280	[41]
Cattle	Markazi	81,012	1,340	
Goats	Markazi	275,185	3,037	
Sheep	Lorestan	265,692	18,931	[42]
Goats	Lorestan	90,913	3,551	
Sheep	Fars	12,381	41	[43]
Cattle	Fars	6,473	10	
Goats	Fars	22,847	56	
Buffaloes	Fars	10	0	
Total		6,408,202	170,552	

the east of Iran, including North Khorasan, and Razavi Khorasan that there was a significant difference between them (P < 0.001) (Table 2).

Publication bias and heterogeneity

Our result confirmed that no individual studies notably prompted the prevalence of *Fasciola* spp. Furthermore, the shape of funnel plot and consequences of P-value of Egger's test (Coeff = 0.93, t = -0.19, P = 0.85) represented no publication bias existed. Moreover, there was no evidence for publication bias among the studies included in this meta-analysis (Fig. 3). Additionally, Egger's test was analysed for each subgroup (Table 2), in which any potential publication bias was not shown. There was a high heterogeneity (I-square = 99.9%, P < 0.001) among the studies and therefore the analysis was performed in subgroups.

Discussion

In the present systematic review, infection with *Fasciola* spp. was responsible for 2.6% of all inspected livers of the slaughtered ruminants in Iran.

Table 2. Subgroup meta-analysis to compare prevalence of *Fasciola* spp. among domestic ruminants in various geographical areas of Iran

Characteristics		Factors	Number of studies	Prevalence of *Fasciola* spp. (95% CI)	Publication bias	I-square (%)	P-value
Animals		Cattle	25	4.0 (3.0-7.0)	0.78	99.6	P<0.001
		Buffalo	2	19.0 (11.0-28.0)	–	99.3	
		Sheep	24	3.0(2.0-4.0)	0.32	99.5	
		Goat	12	2.0(1.0-3.0)	0.76	99.2	
Geographical areas	Ilam, Lorestan, Hamadan, Kohgiluyeh and Boyer-Ahmad, East Azerbaijan	West	16	5.0(2.0-8.0)	0.55	99.9	P<0.001
	Khuzestan, Fars,	South	15	1.0(1.0-2.0)	0.14	99.9	
	Gilan, Mazandaran, Golestan	North	17	7.0(5.0-10.0)	0.81	98.8	
	Tehran, Markazi, Isfahan	Center	10	1.0(1.0-1.0)	0.27	99.7	
	North Khorasan, Razavi Khorasan	East	5	0.0(0.0-1.0)	0.11	88.4	
Overall		63		3.0(2.0-4.0)	0.79	99.9	

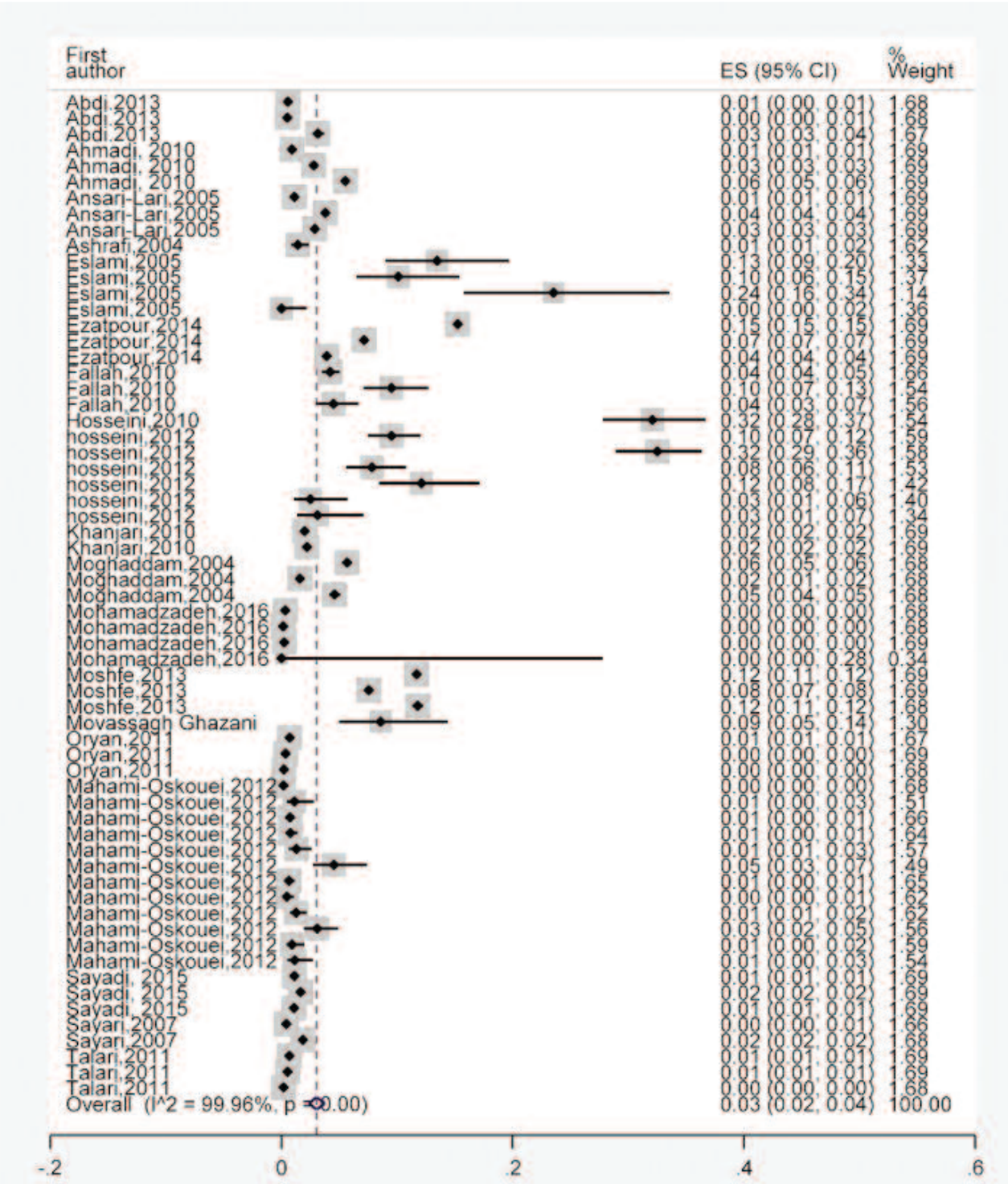

Figure 2. Forest plot of prevalence of *Fasciola* spp. among domestic ruminants in Iran.
The black boxes sizes are proportional to the study weight, with the lines indicating 95% confidence intervals (CIs).

The mean prevalence of the infection in cattle, goats, sheep, and buffaloes were 4.2%, 2.4%, 2%, and 21%, respectively. It has been frequently shown that the distribution of *F. hepatica* and *F. gigantica* in temperate zones, especially in humid areas, in the endemic foci may overlap [1,3,11]. *F. hepatica* has a worldwide distribution and it causes major health problems in Europe (Portugal, France, and Spain), the Americas (Bolivia, Peru, Chile, Ecuador, and Venezuela), Cuba and Oceania and overlaps with *F. gigantica* in many areas of Africa and Asia [1]. In general, the estimated worldwide human infections by *Fasciola* spp. ranged between 2.4 and 17 million of people [12], while the high-risk population is

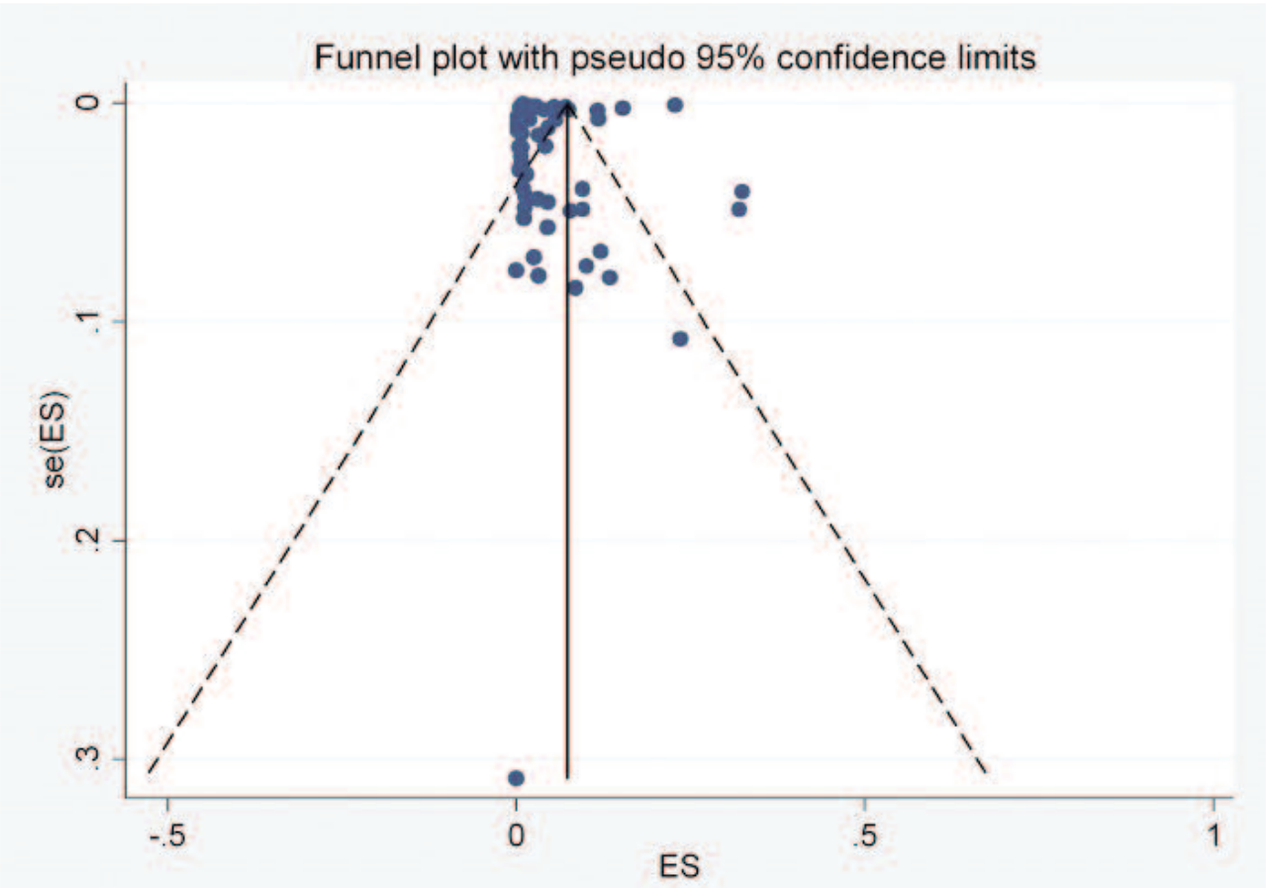

Figure 3. Funnel plot for the studies included in meta-analysis

estimated to be 180 million of people [13].

The published cases from Asian countries are mostly from Iran, Vietnam, and China and less cases have been reported from Turkey, Korea, Japan, Thailand, India, Iraq, Pakistan, Yemen, Israel, and Saudi Arabia [3,14]. Fasciolosis is a zoonotic disease and the cause of important health problems [13] and has been reported from many provinces of Iran such as Kurdistan, Zanjan, Kermanshah, Mazandaran, Tehran, Azerbaijan, Gilan, Fars, and Khuzestan [9].

In Iran, fasciolosis is a major risk for domestic livestock husbandry, including 75 million sheep and goats and six million indigenous cattle. In addition, infections have been reported from wild animals, including wild boar [15] and wild sheep [16]. Because of the high prevalence of human fascioliasis, particularly in Northern Iran, this geographic zone was considered by the World Health Organization (WHO, 1995) as an endemic area of fasciolosis. Mixed infection with both *F. hepatica* and *F. gigantica* has been common in ruminants in many parts of the country, especially in subtropical and wet regions e.g. Gilan Province in the north of Iran [9].

Data concerning *Fasciola* spp. infections among domestic ruminants in the southwest Asia has been reported from some neighbouring countries of Iran such as Iraq [17], Pakistan (Kashmir) [18], Saudi Arabia [19], and Turkey [20]. Furthermore, there are several abattoir-based reports regarding prevalence of animal fascioliasis in several areas of Iran [9,21].

Investigations in the neighbouring countries of Iran have revealed various range of prevalence of fasciolosis in different animals. In Pakistan (Kashmir), infection rate of *F. hepatica* in cattle, sheep, and goats was 85.1%, 51.3%, and 14.8%, respectively [19]. In Turkey, 4% of sheep and 0.5% of cattle were infected with *F. hepatica* [20]. An abattoir-based study in Iraq (Basrah) showed the prevalence rates in cattle, sheep, and gats were 0.13%, 0.72%, and 3.30%, respectively [22]. In Saudi Arabia, 1.2%, 0.04%, and 0.0% of cattle, sheep and goats were found to be infected with *Fasciola* spp., respectively [19].

In Brazil, 10.34% of cattle and 20% of buffaloes were infected with *Fasciola* spp. [23]. In a study in Kenya (during 1990–1999), overall infection rate of

F. hepatica in cattle was estimated 0.8% [23,24].

In contrast to Iran, Pakistan, a neighbouring country, has shown a higher rate of the infection in all ruminants [18]. Moreover, infection with *Fasciola* spp. in livestock of Isfahan province, Iran, (Kashan, Central Iran) was more than that in Saudi Arabia (for all types of livestock) and Turkey (only bovine fasciolosis) [19,20]. Infection rate of fasciolosis in goats of Iraq [17] was comparable to the results of this meta-analysis.

The present systematic review and meta-analysis study describes prevalence rates of *Fasciola* spp. in domestic ruminants in various regions of Iran. Accordingly, the prevalence rate of fasciolosis was indicated by subgroup meta-analysis to compare prevalence of *Fasciola* spp. among animals in Western, Southern, Northern, Central, and Eastern of Iran as 5%, 1%, 7.1%, and 0%, respectively. Most infections were observed in the Western and Northern Iran because these areas have suitable environmental and climatic conditions for circulating of the life cycle of this parasite and also the high density of domestic ruminants there. In addition, the prevalence of this disease in Northern and Western regions of the country has remained at hypo-endemic level.

Our findings were in concurrence with that of researchers who found a higher rate of infection in water buffalo and cattle contrasted to sheep and goat. The observed distinctions could be depicted through the presence of different factors such as climatic variety and husbandry practices. Among the climatic variables, rainfall and temperature were considered as potent factors in the distribution of fasciolosis among livestock so that both factors significantly affect the survival of snails, miracidia, and cercariae of *Fasciola* spp. [9].

Our systematic review and meta-analysis showed a relatively low prevalence and considerable decline of fasciolosis occurrence among sheep, buffalo, and goats, except for cattle, for the period of 17 years in Iran. A wide range of variables have been suggested to influence the distribution of fasciolosis in animals and humans and the impact of climate was shown here too when different parts of Iran were analysed. The results present updated gathered information on the epidemiology of fasciolosis in domestic ruminants in Iran. Abattoir-based studies give helpful information for further management of the infection in a herd or in a certain area. Control programs are encouraged in domestic ruminants in Iran.

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