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Review

Prevalence of fasciolosis in livestock and humans: A systematic review and meta-analysis in Iran

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ABSTRACT

Fasciolosis is a re-emerging disease of livestock and rarely human, being endemic in Iran. Herein, we aimed to quantitatively assess the human seropositivity and prevalence of animal fasciolosis in our country. English and Persian databases were searched for online literature. In total, 10 human seroprevalence studies and 49 animal investigations were obtained from January 1999 to March 2019. Only animal studies were included in meta-analysis. The highest human seroprevalence was detected in Guilan province (326/452; 50%). The pooled prevalence of the animal infections was calculated as 6.2% (95% CI = 5.8%–6.5%). In detail, the prevalence ranges in three hosts included as 4.2% (95% CI = 3.8%–4.5%) in sheep, 9% (95% CI = 8.0%–9.9%) in cattle and 3.1% (95% CI = 2.4%–3.7%) in goat. The highest prevalence rate was observed in the north, 11.8% (95% CI = 8.4%–15.1%), while the lowest prevalence was detected in the central Iran with 1.8% (95% CI = 1.3%–2.3%). Egger's regression test revealed no significant publication bias (P = 0.307). Also, there was no remarkable correlation between weighted prevalence and sample size (P = 0.249) as well as year of study (P = 0.172). These findings would be necessary for better preventive strategies in case of human and animal *Fasciola* infections as well as snail intermediate hosts.

1. Introduction

Food-borne trematodiases are zoonotic infections which are principally caused by *Clonorchis*, *Opisthorchis*, *Paragonimus* spp. as well as *Fasciola hepatica* (*F. hepatica*) and *F. gigantica* [1]. With estimated 200,000 morbidity cases and up to 7000 deaths annually, these helminth infections render over 2 million disability-adjusted life-years worldwide [2]. Fascioliasis is a well-known, devastating, food-borne trematode infection of herbivores; although human infection would occur accidentally [3]. It is estimated that 17 million individuals suffer from the liver fluke disease worldwide with 180 million at-risk people [4,5]. In Iran, fascioliasis is of paleo-parasitological significance and dates back to Sassanid Empire (224–651 A.D.), where paleofeces of a donkey was the source of *Fasciola* eggs [6]. At present time, the infection has been considered to be a major issue in Iran since 1971 [7], with most human cases being confined to Northern provinces, alongside the Caspian Sea coast. Moreover, sheep and cattle herds have been considered as the mostly affected livestock populations throughout the country [8]. Despite the ectopic cases [9–11], the parasite frequently dwells in the liver parenchyma and the biliary tree. After embryonic phases in the invertebrate intermediate host, the cercaria come out and develop to metacercariae on aquatic plants. Upon ingestion of encysted parasites, the juveniles get into their final domicile during 7–8 weeks and develop to mature worms, which release 20,000–24,000 eggs/day. Additionally, a possible route of transmission in humans is eating raw infected liver, where the parasite causes Halzoun syndrome, particularly in the Middle East countries [12–14]. The prevalence of infection implicates a proper intermediate host in appropriate environments such

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Table 1
Characteristics of included articles for human fasciolosis seroprevalence in Iran.

Ref.	Province	sample size	female	male	Seropositive cases	female	male	Residence	Method
[124]	Ardabil	458	305	153	9	1.63%	2.60%	no significant difference	ELISA (somatic antigen)
[125]	Chaharmahal and Bakhtiyari	1475	791	684	2	2	0	no significant difference	ELISA (native Cathepsin L antigen)
[126]	Guilan	452	290	162	326	145	181	no significant difference	ELISA (native ES antigen)
[127]	Guilan	1984	1255	729	27	17	10	no significant difference	ELISA (recombinant cathepsin L)
[48]	Ilam	600			4	3	1	no significant difference	ELISA (Commercial kit)
[97]	Isfahan	471	274	197	8	8	0	no significant difference	ELISA (somatic antigen)
[128]	Kohgyluyeh va Boyerahmad	1000	682	318	18	9	9	no significant difference	ELISA (somatic antigen)
[129]	Tehran	206	100	106	51	21	30	no significant difference	ELISA (somatic antigen)
[130]	Lorestan	801	484	317	6	4	2	no significant difference	ELISA (native Cathepsin L antigen)
[131]	Lorestan	1256	679	577	16	10	6	no significant difference	ELISA (somatic antigen)

as proximity to brooks and springs, optimum temperature within 10–25 °C as well as sufficient humidity [15]. Since several decades ago until now, various snail intermediate hosts of *Fasciola* spp. have been found in Iran [16,17]. have always been The infection course in the host involve three principal phases [18]: 1) asymptomatic incubation period, 2) invasive or acute step with fever, gastrointestinal inconvenience, hepatomegaly, ascites, normocytic normochromic anemia and jaundice, and 3) obstructive or chronic stage which causes inflammation, epithelium hyperplasia, abdominal pain as well as cholelithiasis. Seemingly asymptomatic carriage of the *Fasciola* infection is an important condition in livestock husbandry [19], imposing significant financial burden in the field of livestock husbandry, directly by condemnation of infected liver or carcasses and early slaughtering of infected animals, or indirectly through decreased liver function, reduced lactation, loss of appetite and delayed oestrus cycle [20–22]. Several abattoir-based investigations have documented the monetary burden of organ condemnation due to liver parasites including *F. hepatica* in the country [23–25]. Besides, there have been some reports of *Fasciola* co-infection with other pathogens in cattle such as *Salmonella dublin*, *Mycobacterium bovis*, *Mycobacterium avium paratuberclosis* and *Clostridium* spp., hence more consideration should be taken into account in such cases [26,27]. Furthermore, a possible threat would be the emergence of Triclabendazole-resistant parasites in human and animal populations of endemic regions, which poses a major health concern and complicates the *Fasciola* control programs [28–32]. Additionally, there may be a plausible association between *F. hepatica* infection with liver fibrosis and cirrhosis, which may predicate the intricated pathogenesis of this infection [33].

In addition to imaging techniques and necropsy, several serodiagnosis approaches have been used, of which enzyme-linked immunosorbent assays (ELISAs) are the most popular tests [8] in animal and human hosts, particularly using highly antigenic fractions in helminth excretory/secretory products such as cathepsin proteinases, circulating and fecal antigens, either as native or recombinant formulations [13,34]. In addition to standard sedimentation techniques for fluke egg count, a so-called FLOTAC, novel fecal examination system has been recently invented with high rate sensitivity for both fresh and preserved fecal samples, which again accredits the traditional diagnosis of the infection [35]. Despite the fact that fasciolosis infection is considered as a widespread neglected tropical disease, specifically in rustic areas of Asia and Africa, its prevalence and other epidemiologic features are still disputed. During recent years, numerous molecular epidemiology studies using various genetic targets have identified the genetic diversity of *Fasciola* isolates in the country [36–44]. Despite of some information on the prevalence of the liver fluke infection in canids [45] and wild boars [46] in Iran, much of investigations are focused on livestock and human fascioliasis. However, the paucity of classified information on the two latter made us to contrive current systematic review and meta-analysis.

2. Methods

2.1. Information sources and search

From January 1999 to March 2019, we searched the literature for articles that evaluated the prevalence of fasciolosis infection in human and animal hosts in Iran, using five English databases (Scopus, PubMed, Science Direct, Web of Science and Google Scholar) and three Persian databases (Scientific Information Database (SID), Magiran, Iran Medex and IranDoc). Also, the references of included articles were checked for related studies. Keywords that were used for systematic search were including: “Prevalence”, “Seroprevalence”, “Molecular detection” “Human fasciolosis”, “Animal fasciolosis”, “*F. hepatica*”, “*F. gigantica*”, “Sheep”, “Cattle”, “Goat”, “Buffalo”, “Camel”, “Equids”, “Horse”, “Donkey” and “Iran” alone or combined together with “OR” or/and “AND” operators.

2.2. Eligibility criteria, study selection and data collection

The cross-sectional human serological studies were explored and included in our review; however they didn’t undergo meta-analysis due to inadequate records. Animal investigations based on different methods (parasitological, serological and abattoir inspections) were eligible to enter meta-analysis. Exclusion criteria were as follows: 1) lack of full-text accessibility, 2) irrelevant examination method, and 3) other types of study, except cross-sectional. Any possible discrepancy in study selection process was obviated by discussion and consensus. Searching process and data extraction were accomplished by two author (A. Taghipour and N. Asadi), then rechecked by another person (H. Majidiani). The detailed characteristics of each included study for human individuals and animal cases have all been embedded in Tables 1 and 2, respectively. To report our results, the preferred reporting items for systematic reviews and meta-analysis guideline was used [47].

2.3. Analytical approach

Because of insufficient human documentations, only animal studies were analyzed. For each study, the point estimates and their confidence intervals (CIs) for the prevalence of fasciolosis in various hosts were calculated. Random-effects model (DerSimonian or Laird) was used to estimate the effects heterogeneity among chosen records. Representation of included studies based on effect size and CI was illustrated by forest plot diagram. Incoherence and heterogeneity among studies were assessed using I^2 index and Cochran’s Q test, respectively. Furthermore, heterogeneity among subgroups was tested by meta-regression analysis. The relationship between seroprevalence, the year of publication and the sample size were involved in meta-regression. For meta-analysis, included studies were assessed to be a random sample from each study population. All analyses were carried out with STATA statistical software.

Table 2
Prevalence of fasciolosis in livestock populations in Iran.

Livestock species	No. of studies	No. of examined animals	No. of positive cases	Prevalence (95% CI)
Sheep	38	4,470,835	63507	0.042 (0.038, 0.045)
Cattle	31	836,068	32250	0.090 (0.080, 0.099)
Goat	15	1,275,012	26697	0.031 (0.024, 0.037)
Camel	3	725	31	0.053 (0.035, 0.071)
Buffalo	2	44	5	0.147 (0.050, 0.311)
Horse	2	299	46	0.051 (0.029, 0.074)
Donkey	1	45	3	0.067 (0.014, 0.183)
Total	49	6,713,135	122,720	0.062 (0.058, 0.065)

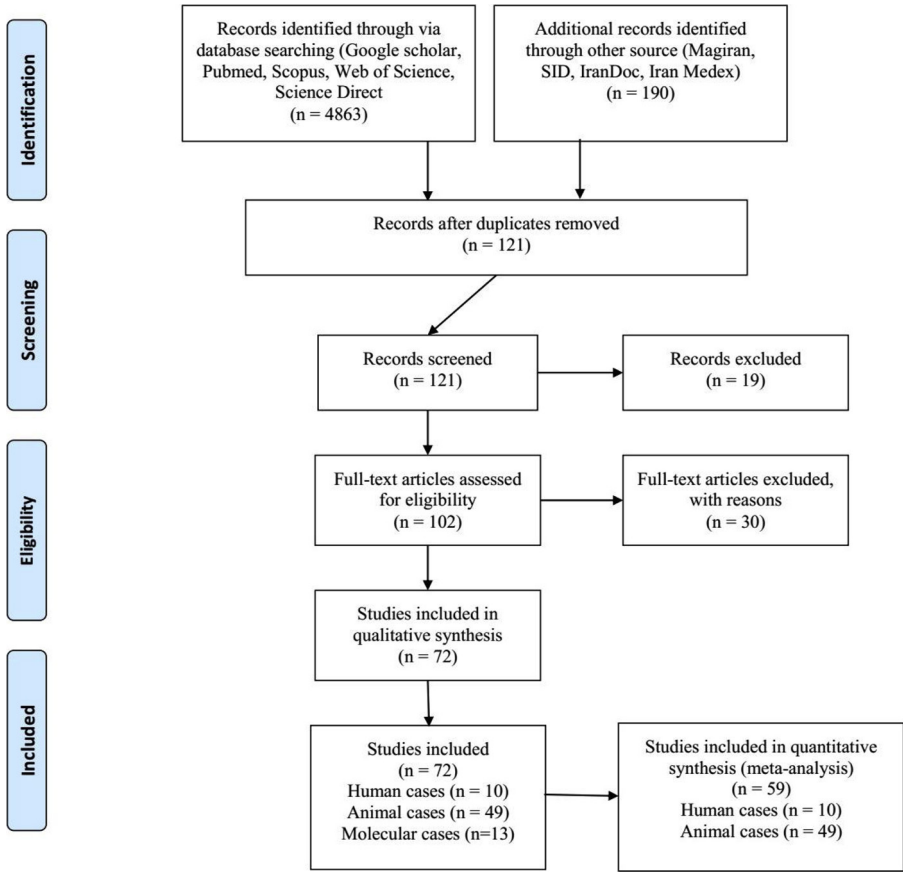

Fig. 1. Flowchart describing the study design process.

Table 3
Geographical distribution and prevalence of included studies for livestock hosts.

Region	No. of studies	Prevalence 95%CI	I^2%	Heterogeneity test		Egger test	
				Q	*P*	t	*P*
North	11	0.118 (0.084,0.151)	99.25	1332.96	< 0.001	8.73	0.071
South	4	0.037 (0.022,0.052)	99.86	2073.29	< 0.001	11.32	0.669
Center	8	0.018 (0.013,0.023)	99.91	7990.12	< 0.001	25.09	0.190
West	20	0.104 (0.087,0.122)	99.92	23336.19	< 0.001	15.66	0.058
East	5	0.027 (0.020,0.035)	99.94	6335.81	< 0.001	30.39	0.263
Iran	1	0.067 (0.014,0.183)					
Overall	49	0.062 (0.058, 0.065)	99.93	37598.82	< 0.001	1.04	0.307

Test for heterogeneity between sub-groups:
Q: 121.91.
P-value < 0.001.

3. Results

3.1. Human individuals

Totally, 10 seroprevalence studies were included (Fig. 1) from eight different provinces. ELISA-based serology test was implemented on a total number of 8703 human individuals; among these, the highest and lowest prevalence rates corresponded to Guilan province with 326/452 (50%) cases and Chaharmahal and Bakhtiari province with 2/1475 (0.13%) cases, respectively (Table 1). Somatic antigens (5 studies) were mostly used in ELISA tests, followed by native cathepsin L antigen (3 studies), recombinant cathepsin L antigen (1 study) and a commercial ELISA kit (1 study).

3.2. Animal cases

In this section, 49 studies in the field of *Fasciola* prevalence were gathered in livestock hosts of Iran (Fig. 1 and supplementary Table 1) [25,48–84]. Altogether, 6,713,135 domestic animals including 4,470,835 sheep, 836,068 cattle, 1,275,012 goats, 725 camels, 299 horses, 45 donkeys and 44 water buffaloes (*Bubalus bubalis*) were examined for fasciolosis (Tables 2 and 3), among which 122,720 animals were considered to be positive. The overall prevalence of infection in animal hosts was appraised as 6.2% (95% CI = 5.8%–6.5%) (Table 2). In detail, the prevalence ranges included as 4.2% (95% CI = 3.8%–4.5%) in sheep, 9% (95% CI = 8.0%–9.9%) in cattle, 3.1% (95% CI = 2.4%–3.7%) in goat, 5.3% (95% CI = 3.5%–7.1%) in camel, 14.7% (95% CI = 5.0%–31.1%) in water buffalo, 5.1% (95% CI = 2.9%–7.4%) in horse and 6.7% (95% CI = 1.4%–18.3%) in donkey. Geographically, the highest prevalence rate was observed in the north, 11.8% (95% CI = 8.4%–15.1%), while the lowest prevalence rate was detected in the central Iran with 1.8% (95% CI = 1.3%–2.3%) (Table 3). Based on Egger's regression test, there wasn't any significant publication bias (P = 0.307) (Table 3). Fig. 2 depicts the forest plot diagram of current meta-analysis. The employed assays to determine infected animals were abattoir-based carcass inspection (38 studies), fecal examination (8 studies) and enzyme-linked immunosorbent assay (3 studies) (Supplementary Table 1). In accordance with meta-regression, no remarkable correlation between weighted prevalence and sample size (P = 0.249) as well as year of studies (P = 0.172) was detected (Figs. 3 and 4).

3.3. Molecular investigations

Totally, 13 reports [40,36,85,86,37,87–90,38,41–43] (2009–2018) assessed the *Fasciola* spp. in Iran by conventional polymerase chain reaction (PCR) (3 studies), PCR-restriction fragment length polymorphism (RFLP) (9 studies) and loop-mediated isothermal amplification (LAMP) (1 study) techniques. Accordingly, a total number of 1057 samples were examined, among which 545 *F. hepatica* and 512 *F. gigantica* were found (Supplementary Table 2).

4. Discussion

Current review points out the prevalence of fasciolosis infection in domestic livestock and human populations of Iran. Being frequent in humid regions, this zoonosis is conveyed to herbivores and rarely humans via green vegetables and contaminated water [3]. Data on this issue was gathered from different provinces of the country by online literature exploration of eight databases. In the case of human individuals, only 10 papers were achieved on the seroprevalence of human *Fasciola* infection, which were not included in the meta-analytical approach, because of insufficient number of studies. Therefore, we couldn't statistically deduce the total estimated seroprevalence of human fasciolosis in Iran. Totally, 467 out of 8703 examined persons were seropositive for the infection by ELISA method. Although cross-reaction may be a consequence of serological assays, the developed diagnostic ELISAs for *Fasciola* have shown good sensitivity and specificity [5,91]. Reportedly, the highest global prevalence rates have been documented in the highlands of Bolivia and Peru, Southern America [92,93]. Furthermore, the Middle East and North Africa (MENA) area [94], encompassing Saudi Arabia [95], Syria [94], Iraq [96], Iran [48,97], Egypt [98,99] and Ethiopia [100] are considered as hyperendemic in terms of human fasciolosis. Since 1990s, human infection reports by *Fasciola* spp. began to rise and showed its significance [95,101]. Nowadays, a considerable rate of global estimation of about 17 million individuals infected as well as ophthalmological and neurological impacts leading to persisting, even fatal sequelae highlights the public health reputation of the disease [10]. Further, the parasite can suppress or modulate the immune machinery of the host during migratory, acute and chronic stages [102,103], leading to subsequent co-infection with other infectious agents in endemic and/or hyperendemic zones [104–106]. Despite of its worldwide distribution, a great heterogeneity exists regarding human fascioliasis concerning complex inter-related epidemiological scenarios and transmission patterns. The following underlying risk factors would influence such heterogeneity: 1) transmission features of fasciolid; 2) capacities for parasite adaptation; 3) ecological characteristics and spreading of lymnaeid vector; 4) involved livestock species and management systems; 5) general human behavior; 6) sources of human infection; and 7) global and climate changes [101,107].

In addition to *F. hepatica* and *F. gigantica* in our country, there also exist a hybrid and/or introgressed morphologic form, which was first described by Sahba and colleagues in 1972 [108], then substantiated by Bargues et al., Periago et al. and Salahi-Moghaddam et al [59,109,110]. The first recorded human case in Iran was an ectopic localization in the thyroid gland in 1956 [111]. Since then, hepato-biliary and cutaneous involvements were demonstrated in various geographical areas, mostly from Guilan, Mazandaran and Kermanshah provinces [8]. So far, two massive outbreak events of human fasciolosis have occurred in Iran, both in Guilan province located at the Caspian Sea littoral involving about 10,000 and 2465 people, respectively, which drew more attentions to this helminthiasis [8]. Still, this endemicity continues, since most human seropositive cases in our review were from Guilan province, which possess optimum ecological milieu for the parasite development. Yet, the human liver fluke infection is mostly overlooked, not only in Iran but also globally, particularly owing to the difficulty in assessment of human infection sources comprising metacercariae identification in consumable products, in-hospital diagnosis of infected fellows after anamnesis and standardization of survey questionnaires in endemic zones [112] as well as the fact that approximately half of the human cases are asymptomatic [5]. Accordingly, hardworking is needed in the field of human fasciolosis to unravel the involved risk factors and other aspects of clinical infection.

Among 49 achieved records from literature review, overall estimated prevalence of fasciolosis in animal hosts was calculated as 6.2% (95% CI = 5.8%–6.5%). Similar to human cases, higher infected livestock dedicated to northern Iran, i.e. Guilan province, in particular, as well as Mazandaran and Golestan provinces, implying optimum circumstances for snail-parasite interplay in this hotspot zone. Despite of various methods of detection for animal cases, most studies in Iran relied upon routine abattoir-based investigations. Ghanimatdan et al. indicated that the hotspots for sheep fasciolosis in Iran are Guilan, Ardabil, Fars, Kohgiluyeh and Boyer-Ahmad, Chaharmahal and Bakhtiari as well as Sistan & Baluchistan provinces. Additionally, goats were most parasitized by *Fasciola* spp. in Northwest and Southeast geographical zones. Also, cattle infection was dominant in Fars, Kohgiluyeh and Boyer-Ahmad provinces, Chaharmahal and Bakhtiari, Khuzestan and Guilan provinces [113]. Globally, the infection prevalence in ruminants has been reported in several continents. Based on published literature in the Americas, sheep (8.87–100%) and goat (24.5–100%) are probably considered as the main animal hosts for

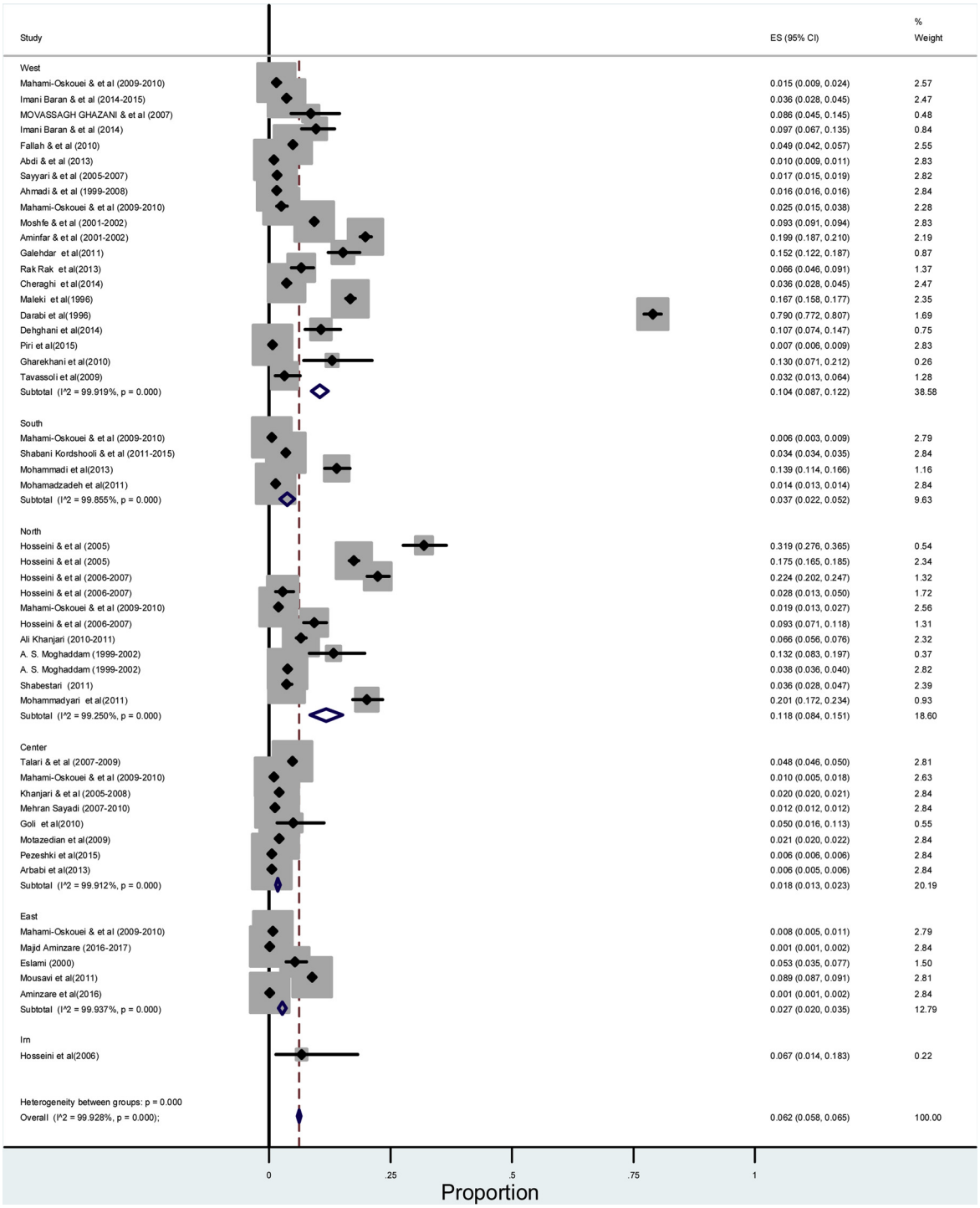

Fig. 2. Forest plot diagram of the current systematic review and meta-analysis based on overall prevalence of animal records.

fasciolosis. Also, the greatest infection rate among production animals in Asia was dedicated to cattle (0.71–69.2%) and goat (0–47%), respectively. Similarly, cattle is the main host in Europe and Oceania region with 0.12–86% and 26.5–81%, respectively. Moreover, in Africa the prevalence rates of infection were enclosing 0.19–73.7% in sheep, 0.28–58.4% in goat and 1.2–91% in cattle. In Pakistan, an important endemic country neighboring Iran which both contribute in highest reports from the continent, the prevalence ranges of the infection were 14.67–39.2% in sheep, 4.08–28.75% in goat as well as 25.46% in cattle [114,115]. *F. gigantica* is more dominant in the tropics such as the Middle East, inducing tropical fasciolosis and transmitted by *Radix* genus in Iran, while *F. hepatica* being prevalent in temperate regions, is mostly transmitted through *Galba/Fossaria* [115,116]. *Fasciola* spp. is well-adapted to bovine species including cattle and water buffalo (*Bubalus bubalis*); such bovids, especially the latter notwithstanding with fewer reports than the former, are principal definitive hosts for *F. gigantica*, particularly due to their susceptibility, behavior and habitat, which bring them into close and regular contact with the parasite. Also, few reports of camel contamination by both *Fasciola* spp. in Iran does exist [51,115,117,118], hence indicating a 5.3% (95% CI = 3.5%–7.1%) weighted prevalence in the country. Despite of findings from some studies [119–121], equids have been shown to be

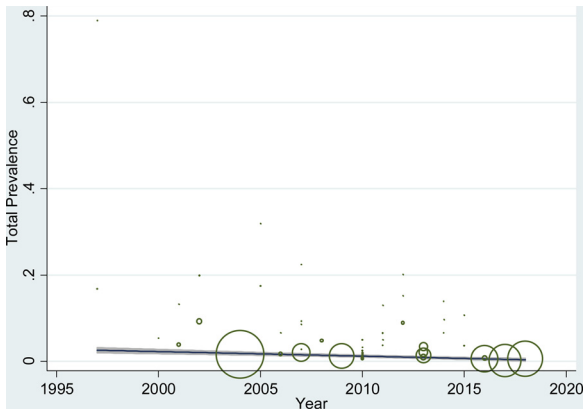

Fig. 3. Meta-regression plot of fasciolosis prevalence in animal hosts, based on year of studies (P = 0.172).

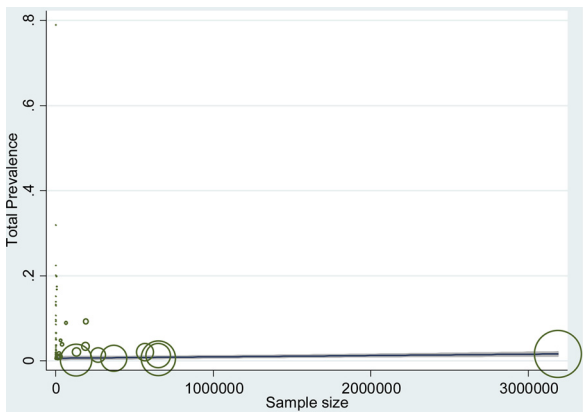

Fig. 4. Meta-regression plot of fasciolosis prevalence in animal hosts, based on sample size (P = 0.249).

tolerable to liver fluke infection [122] and our results regarding horses are certainly influenced by insufficient studies and low sample size. It has been predicted that 3.2 US$ billion per annum is inflicted in animal production industry by liver fluke infection, resulting from anorexia, decreased growth rate, reduced lactation and liver condemnation. Therefore, further considerations should be regarded, particularly in the case of neglected alternative hosts [115].

This review met some limitations, comprising: (1) absence of sufficient studies regarding human cases and animal hosts such as camels, horses and water buffaloes; (2) lack of risk factor evaluation in most studies; and (3) lack of a standard and identical detection method to assimilate the prevalence estimates. These circumscriptions would have biased the reported epidemiologic appearance of fasciolosis infection in Iran.

5. Conclusions

Production farm animals possess a dynamic role in socio-economic evolution and food industries. However, parasitic infections such as fasciolosis are considered as a serious threat for their productivity, particularly in underdeveloped nations [115]. With respect to our findings, a total number of 49 animal records were found for fasciolosis, indicating a 6.2% (95% CI = 5.8%–6.5%) pooled prevalence of infection. Also, after exploring 10 human-based serological investigations, 467 out of 8703 human individuals in Iran were found to be seropositive. Fasciolosis is a cosmopolitan zoonotic infection, being mostly localized in Asia, Africa and America [123]. The animal disease have been prevalent in Iran, at least since 50 years ago with a general decreasing trend, primarily because of the taken measures by veterinary authorities such as livestock treatment and public awareness [8]. Albeit, as previously pointed out by Ghanimatdan et al. [113], three coastline provinces located at northern parts of the country and in the vicinity of the Caspian Sea have demonstrated elevated prevalence rates, which may originate from increased rainfall (four to five folds than the country average), devouring on fresh plant-based food spices, optimum moisture and temperature for both parasite and invertebrate intermediate host, existence of abundant ponds and irrigation canals enriched by aquatic plants, presence of rice culture fields with livestock pasturing around and finally the presence of appropriate snail intermediate host [8]. It is recommended to revisit control programs especially regarding safe water drainage in agricultural fields and combat snail hosts, routinely medicate livestock using trichlabendazole, determination of *Fasciola* spp. isolates and their drug resistance potential.

Author's contribution

H.M. conceived the study; H.M. and S.K. and E.Y. designed the study protocol; A.T. and N.A. searched databases for related literature; A.T. and N.A. extracted the desired information; H.K. analyzed and interpreted the data; H.M. wrote the manuscript draft; S.K., H.M. and E.Y. critically revised the manuscript. All authors read and approved the final manuscript.

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Ethical standards

This study was approved by Ethical Committee of Urmia University of Medical Sciences, Urmia, Iran (no: IR.umsu.rec.1396.363).

Conflict of interest

The authors declare no potential conflicts of interests with respect to the research, authorship and/or publication of this article.

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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.cimid.2019.05.001.

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