Original Article

Meta-analysis of Johne's disease in the Iranian animal population (1999-2020)

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ABSTRACT

Johne's disease (JD) affects domestic and wild animals across the globe. Paratuberculosis exerts huge economic impacts on the animal industry. Despite significant economic losses, little knowledge is available on the epidemiological status of Paratuberculosis in the animal population of Iran. The present study aimed to evaluate the prevalence rate of this disease in the Iranian animal population with confidence interval (CI) and *p*-value. The search was conducted on and screened the electronic international and national databases. Thereafter, sufficient and relevant data were extracted. Data were analyzed in STATA software (version 14). Prevalence disease rates were determined using random effect models. A total of 52 articles were included in the systematic review. According to the results, the overall disease incidence rate in Iran was 20.39%. The prevalence rate of JD was 22.33% (95% CI, 18.87-25.78) in the cattle population and 25.61% (95% CI, 21.43-29.78) in sheep. This study pinpointed that cattle and sheep were the most commonly infected hosts. The highest prevalence rate of disease was 35.88% in Tehran (95% CI, 16.77-54.99), followed by 32.86% (95% CI, 25.07-40.65), and 20.10% (95% CI, 14.63-25.58) in Khorasan Razavi and Kerman, respectively. The lowest prevalence rate of JD was 2.27% in Ilam (95% CI, 0.84-3.70). Based on this result, molecular-based methods were properly compared to other diagnostic methods. This study reported *Mycobacterium avium* subsp. *paratuberculosis* (MAP) prevalence in dairy herds in the provinces of Iran. The infection transmission from animal sources to humans and the potential role of MAP in human disease highlight a critical need for further study on this issue.

Keywords: JD, Iran, Meta-analysis, *Mycobacterium paratuberculosis*, Prevalence

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1. Introduction

Mycobacterium avium subsp. *paratuberculosis* (MAP) is the causative agent of paratuberculosis or Johne's disease (JD). The symptoms of paratuberculosis are chronic progressive weight loss and intermittent diarrhea (1). This disease has been reported among domestic and wild animals almost worldwide (2). It is responsible for significant annual losses in the livestock industry, such as decreased milk and meat production, reduced reproductive indexes, an increased predisposition to other diseases, high costs of diagnosis, culling of infected animals, and increased mortality (3). Furthermore, researchers have found a potential link between MAP infection and immune system disease, including Crohn's syndrome, Hashimoto's type I diabetes mellitus, blau syndrome, and multiple sclerosis (3). In Iran, JD was first reported in Syndhie and Jersaise cattle, Awassi sheep, and Najdi goats in the 1960s (3). Paratuberculosis has been reported in almost all regions of the country, including Tehran (4), East Azerbaijan (2), Khorasan Razavi (5), Fars (6), Isfahan (7), Markazi (8), and Khuzestan provinces (9). Paratuberculosis is a severe cause of economic losses and financial problems (2); nonetheless, tuberculosis and brucellosis are of concern and challenge in Iran. Although there is no definitive epidemiological data about the status of JD, it has been reported widely in Khorasan Razavi and Tehran provinces (4, 10-12). In 2021, the number of cattle and calves was reported to be 5.6 million. The total population of sheep was reported to be 45.9 million heads, in 31 provinces of Iran. Considering limited data about the epidemiological status of Paratuberculosis in Iran, the present study aimed to evaluate the prevalence of the disease with confidence interval (CI) and *p*-value.

2. Materials and Methods

2.1. Data collection

The study was based on guidelines for the meta-analysis of studies in epidemiology. The search was conducted on the electronic international databases PubMed (http://www.ncbi.nlm.nih.gov/pubmed), Scopus (https://www.scopus.com/home.uri), and CABDirect (http://www.cabdirect.org/) from March 1999 to October 2020 using English keywords "JD OR Paratuberculosis" AND (cattle OR sheep OR goat OR camel OR buffalo) AND (feces OR milk OR semen OR intestinal mucosa OR rectum OR lymph nodes OR blood) AND (molecular OR histopathology OR ELISA OR culture) AND Iran. Furthermore, all relevant manuscripts in Iranian databases, including Scientific Information Database (SID) (www.sid.ir), Iranmedex (www.iranmedex.com), Magiran (www.magiran.com), Iranian National Library (www.nlai.ir), and Irandoc (www.irandoc.ac.ir) as well as conference proceeding and conference papers were searched with Persian keywords. This systematic review was not only limited to abstracts or titles, and the references from these manuscripts were searched for additional information.

2.2. Screening

Studies with insufficient data or details as well as articles not relevant to Iran were not included in the manuscript.

2.3. Inclusion criteria

All manuscripts presenting studies on the prevalence of MAP in Iran were considered. The above-mentioned inclusion criteria were extracted from all papers and listed in table 1.

2.4. Quality assessment

The included studies in the meta-analysis study were estimated for quality by methodological study.

2.5. Statistical analysis

The data were analyzed in STATA software (version 14) using Chi-squared (χ^2) and I-square tests to evaluate heterogeneity. For significant heterogeneity (*p*-value of $\chi^2<0.1$ and I^2 index >75%), the random-effects model was considered with a 95% CI.

3. Results

In this study, 357 articles were included with keywords in databases, of which 305 articles were excluded due to duplication (n=118), irrelevancy (n=169), and the absence of full text (n=118). Finally, 52 articles were included in the systematic review (Figure 1). This procedure is illustrated in figure 1, and table 1 presents all of the research used in this study.

Table 1. Characteristics of studies included in the meta-analysis study

Province	Host	Samples	Method	Year	Ref
Khorasan Razavi	cattle	Stool-Milk	PCR	2012	(12)
Khorasan Razavi	cattle	Stool	Nested-PCR Culture	2010	(13)
Chaharmahal and Bakhtiari	cattle	Stool	Nested-PCR Ziehl-Neelsen staining	2012	(14)
Kerman	cattle	Stool	Culture PCR Nested-PCR	2018	(15)
Tehran	cattle	Stool	Nested-PCR	2014	(16)
Khuzestan	goat	Serum	ELISA	2017	(17)
Tehran	cattle	Stool	ELISA-Culture	2017	(18)
Khuzestan	cattle	Serum-Rectum	ELISA Ziehl-Neelsen staining PCR	2017	(19)
Khuzestan	sheep	Serum	ELISA	2015	(20)
Semnan and Ardebil	camel	Stool	Ziehl-Neelsen staining PCR	2015	(21)
Hamadan	Goat-sheep	Rectum	Ziehl-Neelsen staining	2005	(22)
Ardebil	cattle	Stool	Ziehl-Neelsen staining	2012	(23)
Eastern-Azerbaijan	cattle	Stool-Milk	Ziehl-Neelsen staining PCR	2013	(24)
Eastern-Azerbaijan	cattle	Milk	Culture-PCR	2005	(25)
Eastern-Azerbaijan	cattle	Stool-Milk	PCR-Culture	2013	(2)
Markazi	cattle	Serum	ELISA	2012	(8)
Tehran	cattle	Stool-Milk	Nested-PCR PCR	2010	(11)
Esfahan	cattle	Stool	Ziehl-Neelsen staining Culture	2009	(7)
Chaharmahal and Bakhtiari	cattle	Stool	Nested-PCR Ziehl-Neelsen staining PCR	2009	(26)
West Azerbaijan	cattle	Stool	Culture	2012	(27)
Khuzestan	cattle	ileocecal valve	Ziehl-Neelsen staining	2006	(9)
Khorasan Razavi	cattle	Milk-Stool	Nested-PCR	2010	(5)
Chaharmahal and Bakhtiari	Cattle Sheep camel	Blood-Semen	Nested-PCR	2014	(28)
Chaharmahal and Bakhtiari	cattle	Unknown	Histopathology	2010	(29)
West Azerbaijan	cattle	intestinal tissues	Ziehl-Neelsen staining	2003	(30)
Esfahan	Sheep goat	ileocecal valve	Histopathology	2005	(31)
Tehran	camel	Serum	ELISA	2012	(32)
Khuzestan	buffalo	intestinal tissues	Histopathology	2008	(33)

[a]Loop-mediated isothermal amplification

Unknown	**cattle**	**Semen**	**Nested-PCR**	**2010**	(34)
Fars	cattle	Milk	Nested-PCR	2012	(6)
Alborz	cattle	Serum	ELISA	2016	(35)
Eastern-Azerbaijan	cattle	Stool	PCR	2018	(36)
Tehran	cattle	Stool	LAMP[a] Culture ELISA	2015	(10)
Eastern-Azerbaijan	cattle	Milk	Ziehl-Neelsen staining Culture PCR	2011	(37)
Chaharmahal and Bakhtiari	cattle	Milk	PCR	2009	(38)
Tehran	cattle	Milk	Culture Nested-PCR ELISA Ziehl-Neelsen staining	2012	(4)
Fars	cattle	Milk	Nested-PCR	2008	(39)
Hamadan	cattle	Stool	Ziehl-Neelsen staining Nested-PCR	2018	(40)
Khorasan Razavi	cattle	Stool-Milk	PCR	2008	(41)
Khuzestan	Sheep Goat	ileocecal valve	Ziehl-Neelsen staining	2002	(42)
Fars	goat	Ilium tissue- Mesenteric lymph node	H&E staining Ziehl-Neelsen staining PCR	2018	(43)
Khuzestan	buffalo	Serum- intestine- Liver- lymph node	ELISA PCR Ziehl-Neelsen staining	2020	(44)
Khuzestan	cattle	Liver-Rectum- Serum	PCR Ziehl-Neelsen staining ELISA	2017	(45)
Fars	sheep	Stool-Milk	Culture	2019	(46)
Khorasan Razavi	cattle	Milk	Culture Nested-PCR	2019	(47)
Ardebil	cattle	Serum	ELISA	2018	(48)
Kohgiluyeh and Boyer-Ahmad	cattle	Stool	Ziehl-Neelsen staining	2005	(49)
Khuzestan	Cattle Sheep Goat	Serum	ELISA	2021	(50)
Eastern-Azerbaijan	cattle	Stool	Ziehl-Neelsen staining Culture PCR	2009	(51)
Unknown	sheep	intestine	Ziehl-Neelsen staining Immunohistochemical PCR	2017	(52)
Ardebil	cattle	Milk	PCR	2017	(53)
Ilam	Sheep goat	Unknown	Histopathology	1999	(54)

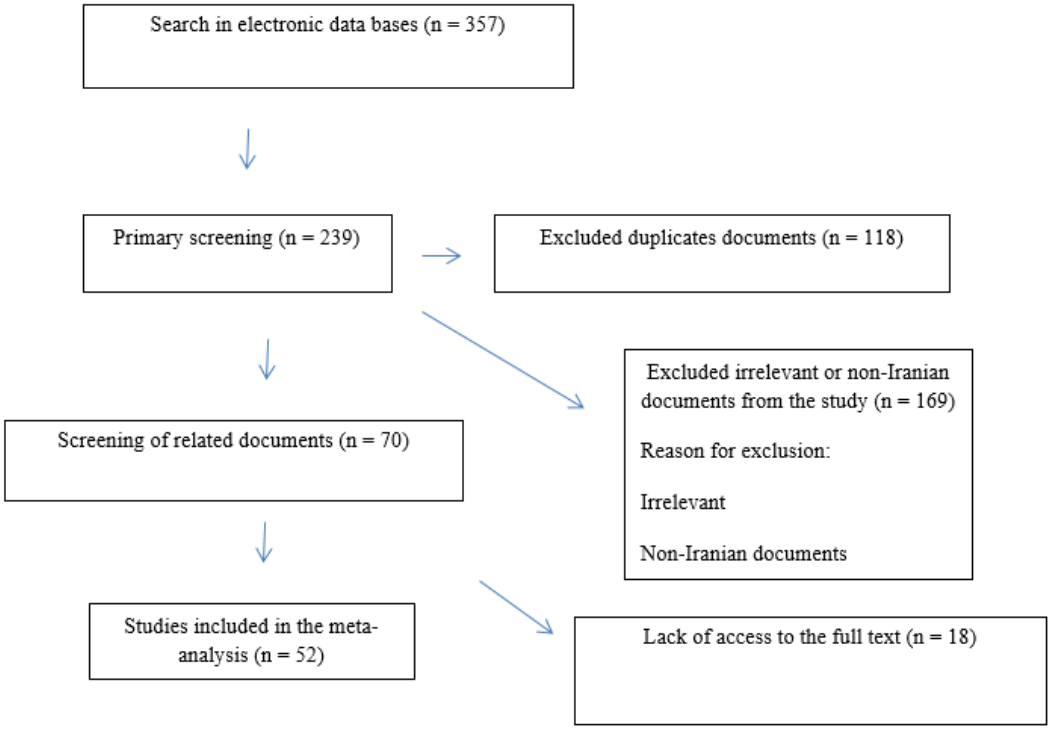


Fig. 1. Flowchart of screening of papers for present study

3.1. Prevalence of Johne's disease

From March 1999 to October 2017, out of 138 animal data, 21,650 samples were analyzed. The overall disease incidence rate in Iran was 20.39% (95% CI, 17.83-22.95). Cattle were the most common host animal used in this study (n=17,205), followed by sheep, goats, buffalos, and camels. Other species (wild mammals) were not found in any paper in Iran. The prevalence rate of JD was 22.33% (95% CI, 18.87-25.78) in the cattle population, 25.61% (95% CI, 21.43-29.78) in sheep, 10.12% (95% CI, 7.60-12.63) in goats, 7.44% (95% CI, 3.66-11.23) in camels, and 14.15% (95% CI, 8.13-20.17) in buffalos. This study pointed out that cattle and sheep were the most commonly infected hosts. The evaluation of the prevalence rate of JD with CI and *p*-value in the Iranian animal population is displayed in table 2 (I^2=100; *P*<0.00). In this study, feces and milk were the most common infected samples (Table 3). The most common diagnostic test used for the detection of MAP was the molecular-based test, followed by enzyme-linked immunosorbent assay (ELISA), histopathology, and culture, respectively. The prevalence rate of disease was 25.62% for the molecular-based test (95% CI, 21.80-29.43), followed by 18.84% (95% CI, 12.21-25.47), 14.15% (95% CI, 11.34-16.96), and 12.13% (95% CI, 2.20-22.07) for culture, histopathology, and ELISA, respectively (Table 4). The distribution of MAP infections in geographical locations is illustrated in figure 2. The highest prevalence rate of disease was 35.88% in Tehran (95% CI, 16.77-54.99), followed by 32.86% (95% CI, 25.07-40.65) and 20.10% (95% CI, 14.63-25.58) in Khorasan Razavi and Kerman, respectively. The lowest prevalence rate of JD was 2.27 in Ilam (95% CI, 0.84-3.70). Variations in JD population structure in the selected papers in Iran are depicted in figure 3. Based on our results, the pooled prevalence rate of JD was calculated in the individual studies of the selected literature, resulting in a pooled prevalence rate of 22.42% (95% CI, 19.04-25.81) using a random-effect model.

4. Discussion

Mycobacterium avium subsp. *paratuberculosis* is an important disease of domestic and wild ruminants, causing worldwide economic losses to the livestock industry. Serious causes for concern are not only economic effects but also zoonotic aspects and public health (3). Iran has old records on infected animals with MAP, especially in cattle (3). A wide array of studies have pointed to the presence of MAP in animals (5-9). There is a paucity of data about the epidemiological status of Paratuberculosis in the animal population and the effects of JD on the animal industry.

Table 2. Meta-analysis of prevalence rate of JD with confidence interval and *p*-value in the Iranian animals' population

Study Population	studies	sample	prevalence, 95% CI	Model
Cattle	**100**	**17205**	**22.33, 18.87- 25.78**	**Random**
Buffalo	**8**	**779**	**14.15, 8.13-20.17**	**Random**
Camel	**6**	**337**	**7.44, 3.66-11.23**	**Random**
Sheep	**12**	**1754**	**25.61, 21.43- 29.78**	**Random**
Goat	**12**	**1575**	**10.12, 7.60 -12.63**	**Random**
Total	**138**	**21650**	**15.93, 8.95- 22.91**	**Random**

p=0.00, I^2 = 100.0

Table 3. Meta-analysis of prevalence rate of JD with confidence interval and *p*-value in samples

Study Population	studies	sample	prevalence, 95% CI	Model
Intestine	**21**	**2624**	**19.48, 16.95- 22.02**	**Random**
Blood &serum	**17**	**6747**	**6.68, 4.75- 8.61**	**Random**
Semen	**2**	**195**	**11.32, 8.02-14.61**	**Random**
Milk	**41**	**5338**	**23.15, 14.22- 32.02**	**Random**
Stool	**46**	**5680**	**26.37, 21.74- 31.00**	**Random**
Liver	**3**	**394**	**11.10, 3.28-18.92**	**Random**
Lymph node	**5**	**392**	**14.74, 7.29- 22.19**	**Random**
Total	**135**	**21370**	**16.12, 9.52-22.72**	**Random**

p=0.00, I^2 = 100.0

Table 4. Meta-analysis of prevalence rate of JD with confidence interval and *p*-value based on diagnostic methods

Study Population	studies	sample	prevalence, 95% CI	Model
Molecular	**64**	**8231**	**25.62, 21.80- 29.43**	**Random**
Histopathology	**37**	**3972**	**14.15, 11.34-16.96**	**Random**
ELISA	**15**	**6498**	**12.13, 2.20- 22.07**	**Random**
Culture	**21**	**2938**	**18.84, 12.21-25.47**	**Random**

p=0.00, I^2 = 100.0

Therefore, the present study aimed to evaluate the status of JD in the population of domestic animals using meta-analysis in Iran. Based on the results of this research, the frequency of positive cases in cattle and sheep was similar to the findings of a study by Chaubey, which demonstrated that the presence of MAP was in 43% of cattle, 41% of sheep, 36% of buffalos, and 23% of goats in India. Chaubey also reported an increased load of MAP in small ruminants (55). Another systematic review in 2014 reported MAP prevalence rates of 73.1% and 11.5% in cattle and sheep in Latin America and the Caribbean, respectively, suggesting that the frequency of the disease was high, especially in large animals (56). The detection methods for paratuberculosis are challenging due to the stage of the disease and the limitations of diagnostic methods (sensitivity, specificity, and accuracy of a diagnostic test). Molecular-based methods, ELISA, and culture are more frequently used to detect paratuberculosis compared to other tests. Ziehl-Neelsen and Hematoxylin & Eosin (H&E) staining (feces, milk, and tissue samples) are the most convenient diagnostic methods; nonetheless, it depends on the experience of the worker. Although the ELISA kit is quick and cheap for screening animals, due to the late detection of antibodies in the serum, it is not suitable in the early stages of the disease. Culture is considered the gold standard for detection (47). On the other hand, molecular-based methods are more sensitive than culture (2). In agreement with the results of a study by Hanifian, in this research, the molecular-based method was more sensitive than the other diagnostic tests (2). Several studies investigated the load of MAP worldwide. In a review article, the load of MAP was 3.3-82.4%, 10.7-33.7%, 1.7-11.2%, 2.5%, 9.4%, and 28% in cattle by ELISA in the United States, Denmark, Ontario, Canada, Chile, and India, respectively (55). Nonetheless, the load of MAP was 2.4-28.6%, 6.9%, 8.3%, 2%, 0.3%, 35%, and 67% in culture in the United States, England, Argentina, Czech Republic, Ireland, Australia, and India, respectively. Except for India, the load of MAP was lower using culture than ELISA. Furthermore, the loads of MAP using polymerase chain reaction (PCR) were 33%, 6-38.8%, and 32% in cattle in the USA, India, and Iran, respectively. In the Indian buffalo population, the load of MAP showed 46.2% and 100% using ELISA and PCR, respectively. In India, MAP appears endemic with high frequency (55). Based on our study, the camel was also reported to be similar to most Arab and Middle Eastern countries (57). Therefore, assiduous attention should be paid to all domestic animals as a source of infection. A study in 2009 investigated the seroprevalence of MAP individually and in herds using ELISA, and the frequency of infection was reported at 3.3% vs. 22%, 2.4-3.5% vs. (0-17%), 5.1% vs. 30%, in France, Italy, and Switzerland, respectively, demonstrating higher rates of herd infections than individual cases (1). Nowadays, Sweden, Norway, Queensland, South Australia, and Japan have regular herd monitoring and control programs. Sweden and some states in Australia eradicated JD in animals (58). Nevertheless, some countries, including South and Central America, Asia, and Africa, have not reported a formal control program for the eradication of JD. Based on our study result, the prevalence of paratuberculosis was estimated at approximately 36% and 33% in Tehran and Khorasan Razavi, respectively, which were the highest prevalence rates in Iran. The pooled prevalence of MAP was reported to be around 20% in domestic animals, similar to India (55). In Iran, despite the high prevalence of JD and the significant economic losses, there is still no regular program to monitor and control the disease. The control of paratuberculosis depends on several factors, such as the culling of infected animals, health issues, the status of farm management, and vaccination. Therefore, we should be planning a national program to control paratuberculosis. Today, an experimental recombinant PTb vaccine has been prepared and is being tested for use in cattle herds in Iran (unpublished data). The pooled estimated prevalence of the Iranian animal population was high; however, some degrees of variability were observed between host sample sizes. Due to the high prevalence of paratuberculosis, systematic training programs and the provision of information to farmers are also beneficial. Molecular tests were found to be highly sensitive to diagnose MAP. Therefore, this diagnostic method could be used for the diagnosis of MAP in laboratories. It is suggested that the screening of animals be performed with more sensitive tests, such as ELISA; nonetheless, we should be careful that no single test can detect all cases of the disease (59).

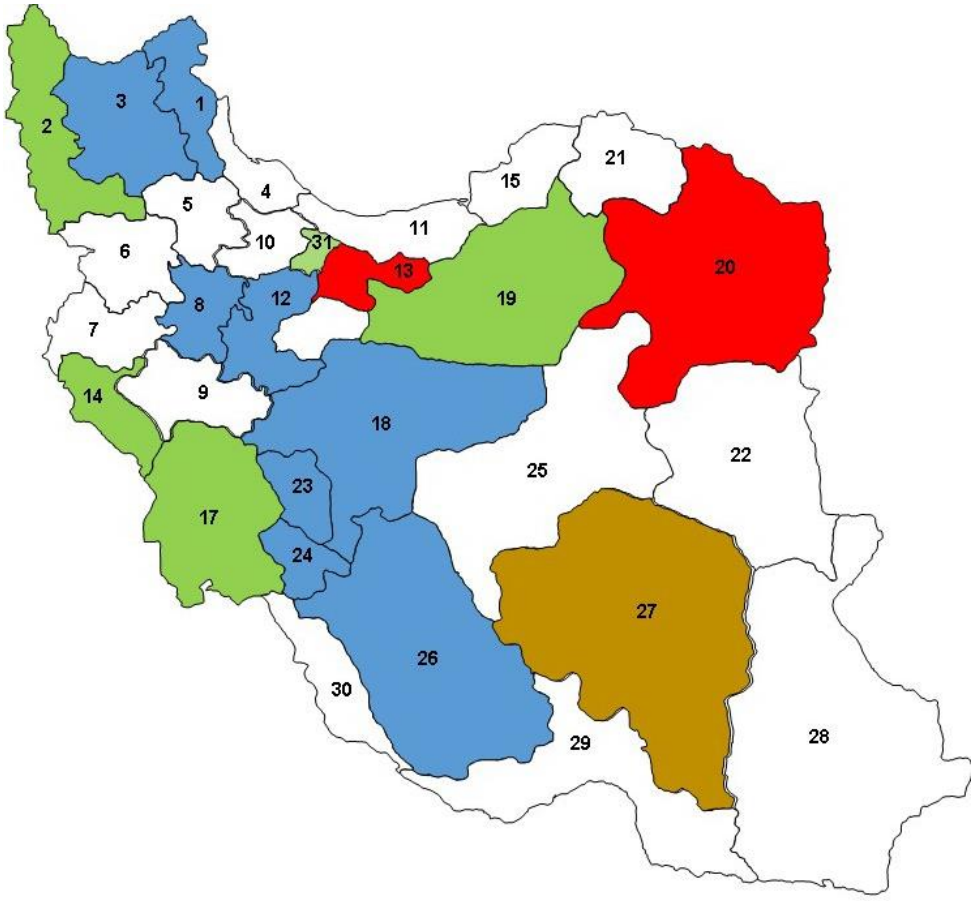
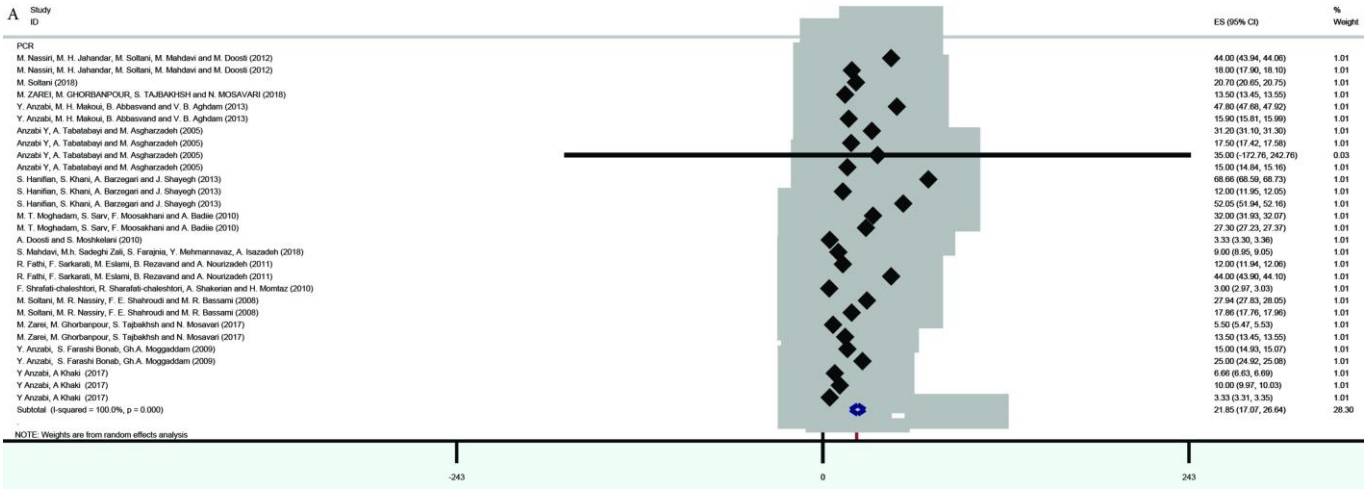


Figure 2. Distribution of *MAP* infections in the geographical region of Iran
1-9% Green 10-19% Blue
20-29% Brown 30< Red
Unknown White



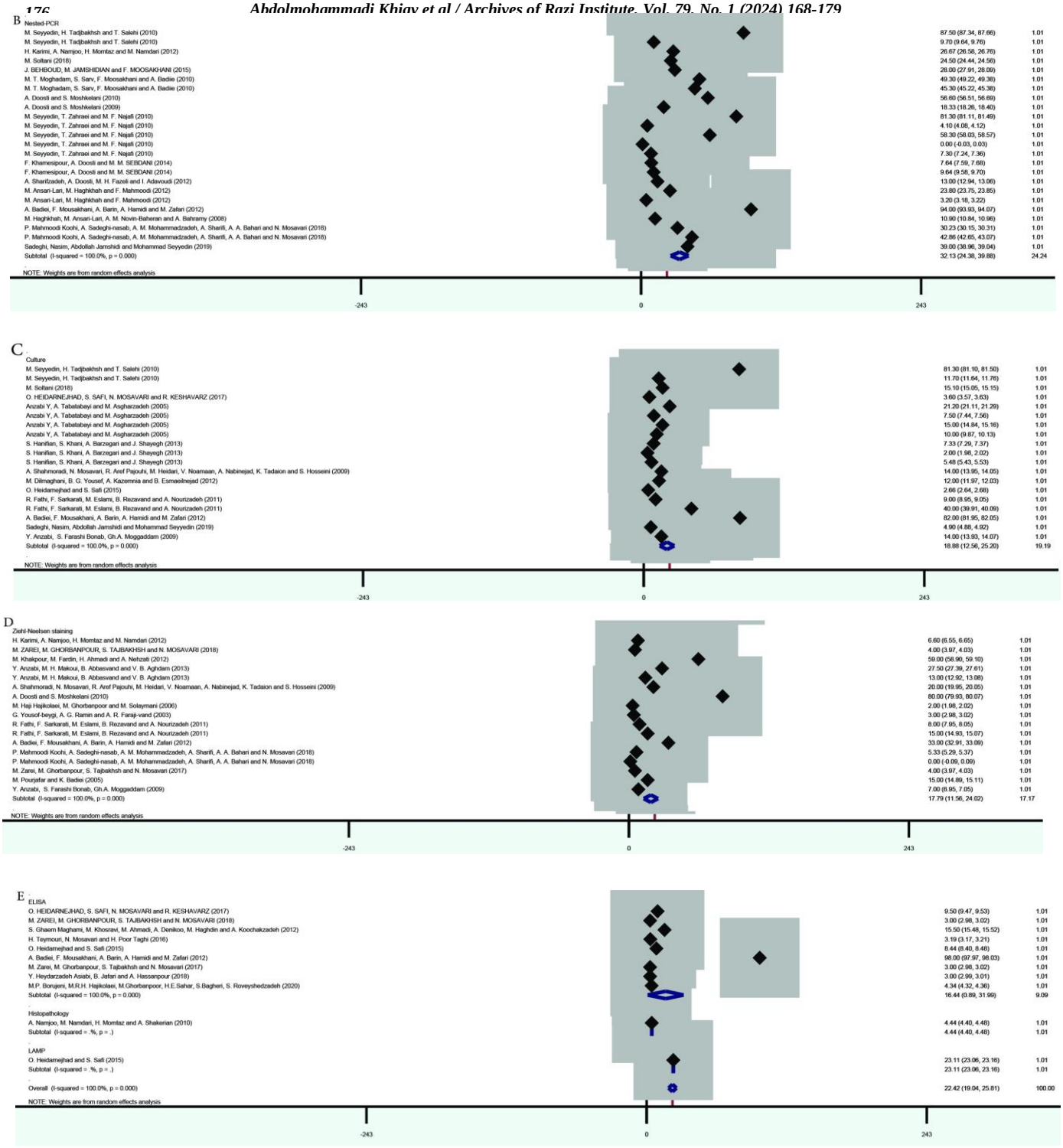


Figure. 3. Variation in JD population structure in the selected papers in Iran: a meta-analysis. A: PCR assay. B: Nested PCR assay. C: Culture. D: Ziehl-Neelsen staining. E: ELISA, and LAMP assay

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Authors' Contribution

Study concept and design: LAK

Acquisition of data: LAK

Analysis and interpretation of data: H.KA. and MH.FM

Drafting of the manuscript: L.AK

Critical revision of the manuscript for important intellectual content: N.M., K.T. and MHFM

Statistical analysis: H.KA. and MH.FM

Ethics

The manuscript does not contain clinical studies or patient data

Conflict of Interest

The authors declare no competing interests.

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