



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

Abdolmohammadi Khiav, L¹, Khaneghahi Abyaneh, H^{2,3}, Fallah Mehrabadi, MH⁴, Mosavari, N⁵, Tadayon, K^{5*}

1. Department anaerobic bacterial vaccine Production and Research, Clostridia Research laboratory, Razi Vaccine and Serum Research Institute, Agricultural Research, Education and Extension Organization (AREEO), Karaj, Iran.
2. Iran veterinary organization, Tehran, Iran.
3. Animal and Animal Products-borne Diseases Research Center, IVO, Tehran, Iran.
4. Department of Poultry Diseases Research, Razi Vaccine & Serum Research Institute, Agricultural Research, Education and Extension Organization (AREEO), Karaj, Iran.
5. Bovine Tuberculosis Reference Laboratory, Razi Vaccine & Serum Research Institute, Agricultural Research, Education and Extension Organization (AREEO), Karaj, Iran.

How to cite this article: Abdolmohammadi Khiav L, Khaneghahi Abyaneh H, Fallah Mehrabadi MH, Mosavari N, Tadayon K. Meta-analysis of Johne's disease in the Iranian animal population (1999-2020). *Archives of Razi Institute Journal*. 2024;79(1):168-179. DOI: 10.32592/ARI.2024.79.1.168



Copyright © 2023 by



Razi Vaccine & Serum Research Institute

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

Article Info:

Received: 7 July 2023

Accepted: 27 August 2023

Published: 29 February 2024

Corresponding Author's E-Mail:
mmb093@gmail.com

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)

^aLoop-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)

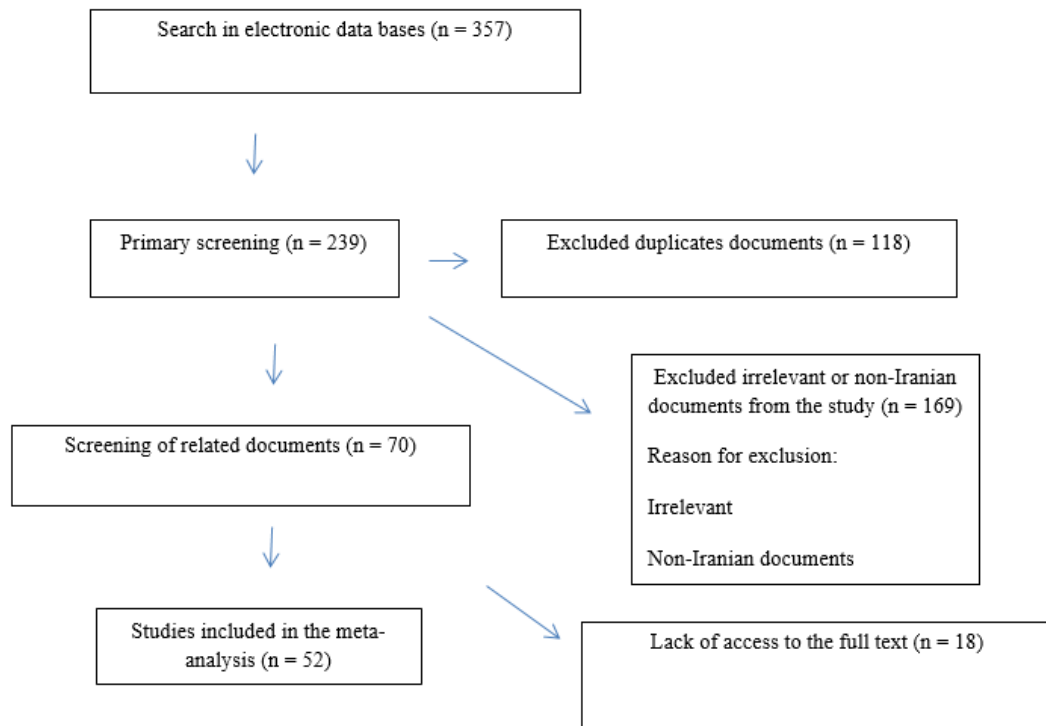


Fig. 1. Flowchart of screening of papers for present study

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).

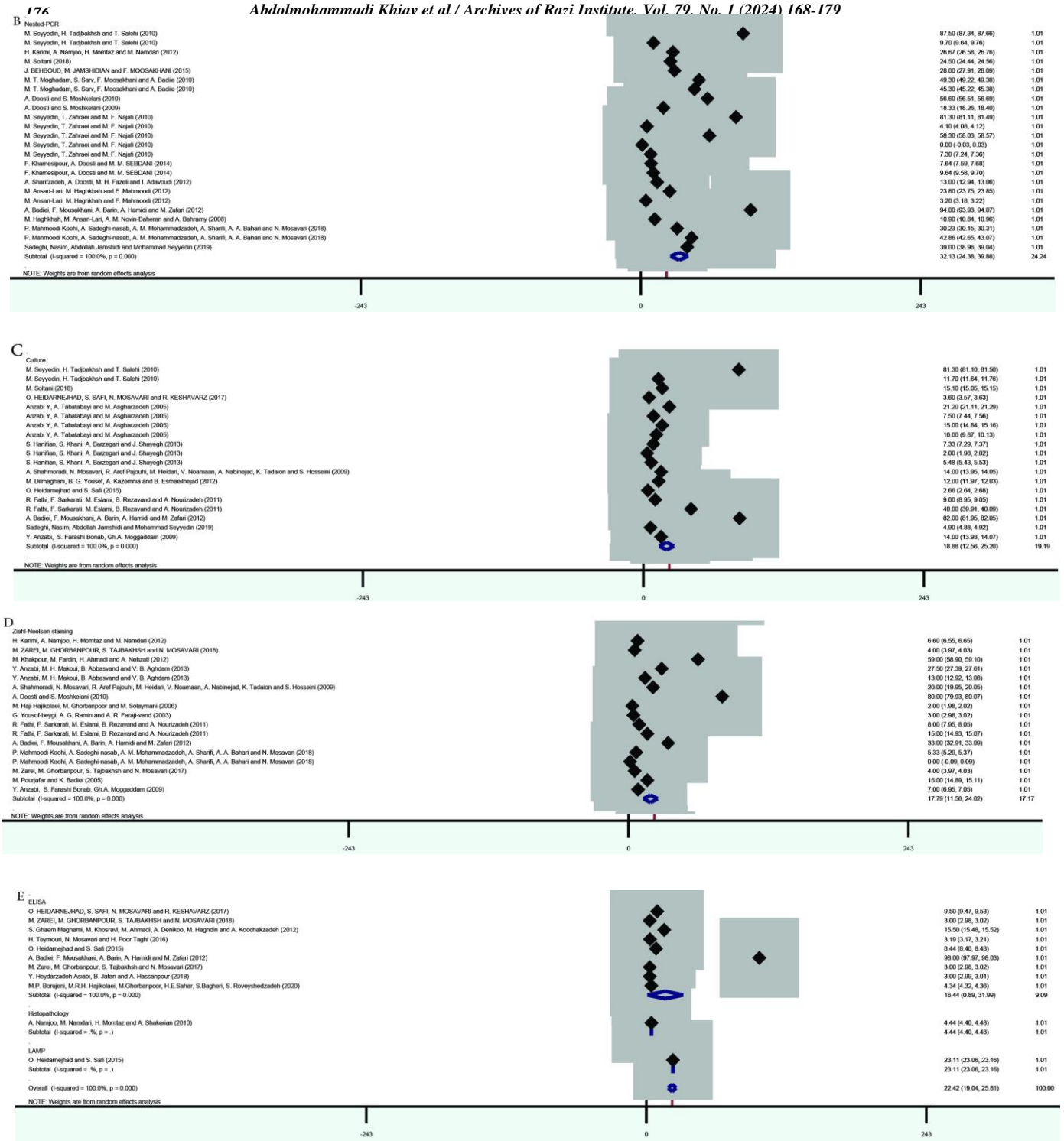


Figure 3. Variation in JD population structure in the selected papers in Iran: a meta-analysis. A: PCR assay. B: Nested PCR assay. C: Culture. D: Ziehl-Neelsen staining. E: ELISA, and LAMP assay

Acknowledgment

We would like to thank from all the people who contributed in this research

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.

References

- Nielsen SS, Toft N. A review of prevalences of paratuberculosis in farmed animals in Europe. *Prev Vet Med.* 2009;88(1):1-14.
- Hanifian S, Khani S, Barzegari A, Shayegh J. Quantitative real-time PCR and culture examination of *Mycobacterium avium* subsp. *paratuberculosis* at farm level. *Vet Microbiol.* 2013;162(1):160-5.
- Haghighat M, Shahmoradi A, Tadayon K, Keshavarz R, Ghaderi R, Sekhavati M, et al. Molecular identification of *Mycobacterium avium* subspecies *paratuberculosis* 316F and III & V strains by a multi-approach strategy. *Veterinary Research & Biological Products.* 2017;30(2):89-100.
- Badii A, Mousakhani F, Barin A, Hamidi A, Zafari M. Comparison of direct microscopic examination, Enzyme Linked Immunosorbent Assay (ELISA), culture and Nested-PCR for diagnosis of herds bulk tank milk infection with *Mycobacterium avium* subspecies *paratuberculosis*. *Vet. J. of Islamic Azad Uni. Tabriz Branch.* 2012;5(4):1369-78.
- Seyyedini M, Zahraei T, Najafi MF. Comparison of isolation frequency of *Mycobacterium avium* subspecies *paratuberculosis* from different types of samples. *Pak Vet J.* 2010;30(3):143-9.
- Ansari-Lari M, Haghkhah M, Mahmoodi F. Association of *Mycobacterium avium* subspecies *paratuberculosis* infection with milk production and calving interval in Iranian Holsteins. *Trop Anim Health Prod.* 2012;44(5):1111-6.
- Shahmoradi A, Mosavari N, Aref Pajouhi R, Heidari M, Noamaan V, Nabinejad A, et al. Study of John's disease in industrial & semi industrial cattle husbandry of Esfahan province. *Vet Res Biol Prod.* 2009;22(1):13-7.
- Ghaem Maghami S, Khosravi M, Ahmadi M, Denikoo A, Haghdin M, Koochakzadeh A. Study of the prevalence rate of John's disease in markazi province and evaluation of absorbed elisa for adoption as a diagnostic method. *Iran Vet J.* 2012;8:54-9.
- Haji Hajikolaei M, Ghorbanpoor M, Solaymani M. The prevalence of *Mycobacterium paratuberculosis* infection in ileocecal valve of cattle slaughtered in Ahvaz abattoir, southern Iran. *Iran J Vet Res.* 2006;7(2):77-80.
- Heidarnejhad O, Safi S. Comparison of culture, ELISA and LAMP-PCR for diagnosis of *Mycobacterium avium* subsp. *paratuberculosis*. *J. Bio. Env. Sci.* 2015;6(3):279-88.
- Moghadam MT, Sarv S, Moosakhani F, Badii A. Detection of *Mycobacterium avium* subspecies *paratuberculosis* in Milk and fecal samples in dairy cattle by PCR and Nested-PCR. *J Anim Vet Adv.* 2010;9(24):3055-61.
- Nassiri M, Jahandar MH, Soltani M, Mahdavi M, Doosti M. Identification and strain determination of *M. paratuberculosis* (MAP) by PCR and REA methods based on IS900 and IS1311 insertion segments. *Agric Biotechnol J.* 2012;4(1):83-96.
- Seyyedini M, Tadjbakhsh H, Salehi TZ. Identification of *Mycobacterium avium* subsp. *paratuberculosis* in fecal samples of Holstein-Friesian cattle using molecular and cultivation methods. *J Vet Res.* 2010;65(2):135-71.
- Karimi H, Namjoo A, Momtaz H, Namdari M. Identification of *Mycobacterium avium* subsp *paratuberculosis* in samples tissue ileum of cattle slaughtered in shahr-e kord with ziehl neelsen taining and nested PCR method. *J Comp Pathobiol.* 2012;8(4):697-703.
- Soltani M. Detection of *Mycobacterium avium* subsp. *paratuberculosis* in Kerman province;s dairy cows using microbial culture, PCR and nested PCR methods. *Iran J Anim Sci Res.* 2018;10(2): 263-273.
- Jafari B, Jamshidian M, Mousakhani F. Phylogenetic analysis of *Mycobacterium avium* subspecies *paratuberculosis* isolated from dairy cattle of Tehran province. *Vet Clin Pathol.* 2014;8(3):574-86.
- Bagheri S, Pourmahdi Borujeni M, Hajikolaei H, Rahim M, Ghorbanpoor M. Seroprevalence of *Mycobacterium avium* subspecies *paratuberculosis* infection in goats of Khuzestan

- province. *Vet Clin Pathol (Veterinary Journal Tabriz)*. 2017;10(4):297-305.
18. Heidarnajhad O, Safi S, Mosavari N, Keshavarz R. Seroprevalence of subclinical paratuberculosis (Johnes disease) in dairy farms of Tehran-Iran using absorbed ELISA assay. *J Com Pathobiol*. 2017;14(3):2239-46.
 19. Zarei M, Ghorbanpour M, Tajbakhsh S, Mosavari N. Comparison of ELISA method, PCR and Ziehl-Neelsen staining of the rectal mucosa for detection of *Mycobacterium avium* subsp. *paratuberculosis* infection in cattle. *Iran Vet J*. 2017;13(2):29-37.
 20. Roveyshedzadeh S, Hajikolaei MRH, Pourmahdi M, et al. Seroprevalence of *Mycobacterium avium* subspecies *paratuberculosis* infection in sheep in Khuzestan province. *J Vet Microbiol*. 2015;11(2):119-28.
 21. Haghkhah M, Derakhshandeh A, Jamshidi R, Moghiseh A, Karimaghaei N, Ayaseh M, et al., editors. Detection of *Mycobacterium avium* subspecies *paratuberculosis* infection in two different camel species by conventional and molecular techniques. *Vet Res Forum*. 2015; 6(4): 337-41.
 22. Azimi MR, Gholami MR, Rashidi J. A study on the rate of *Mycobacterium paratuberculosis* infection in sheep and goats with pathologic signs in slaughter house of hamadan. *agris.fao.org*. 2005.
 23. Khakpoor M, Fardin M, Ahmadi H, Nehzati A. The infection status of *Mycobacterium avium paratuberculosis* in traditional dairy cattle farms in Moghan region. *Food Hyg*. 2012;1(4):45-52.
 24. Anzabi Y, Makoui MH, Abbasvand B, Aghdam VB. Evaluation of contamination of cattle's raw milk to Johnes's disease in farms of Kaleybar region. *Life Sci J*. 2013;5(10):87-92.
 25. Anzabi Y, Tabatabayi A, Asgharzadeh M. A survey on the infection status of *Mycobacterium avium paratuberculosis* in dairy cattle using PCR of Tabriz. *J Iran Vet Sci*. 2005;4:125-31.
 26. Doosti A, Moshkelani S. Detection of *Mycobacterium paratuberculosis* by Nested PCR in dairy cattles suspected to john's disease. *JMW*. 2009;2(1):19-22.
 27. Dilmaghani M, Yousefbeygi G, Kazemnia A, Esmailnejad B. Detection of *Mycobacterium avium* subsp. *paratuberculosis* in cows from Urmia region. *Vet Res Biol Prod*. 2011;24(4):13-7.
 28. Khamesipour F, Doosti A, Sebdani MM. Survey for the Presence of *Mycobacterium avium* subsp. *paratuberculosis* in the bull frozen semen samples and blood samples of cattle, sheep and camel by Nested-PCR. *Kafkas Univ Vet Fak Derg*. 2014;1(2):3.
 29. Namjoo A, Namdari M, H M. The abattoir study of johnes disease in cattle in shahrekord histological study. *J Mod Vet Res*. 2010;2(6):7-12.
 30. Yousof-Beygi G, Ramin A, Faraji-vand A. Study on the prevalence of subclinical cattle Johnes Disease in the Urmia abattoir. *Arch Razi Inst*. 2003;55(1):63-70.
 31. Sharifzadeh A, Momtaz H, F H. An abottior survey of paratuberculosis infection in sheep and goat in Isfahan and coparision some diagnostic methods. *Iran Vet J*. 2005;2(4):317-22.
 32. Raoofi A, Hemmatzadeh F, Ghanaei AM. Serological survey in camels (*Camelus dromedarius*) to detect antibodies against bovine herpesvirus type-1 and *Mycobacterium avium paratuberculosis* in Iran. *J Camel Prac Res*. 2012;19(1):65-8.
 33. Haji Hajikolaei M, Ghorbanpoor M, Amirsoleimani M. The abattior study of *Mycobacterium avium paratuberculosis* infection in buffalo in Ahwaz. *J Faculty Vet Med Shahrkord Uni*. 2008;2(1):55-60.
 34. Sharifzadeh A, Doosti A, Fazeli MH. Nested PCR on semen samples for the detection of *Mycobacterium avium* subsp *paratuberculosis*. *Afr J Microbiol Res*. 2010;4(24):2787-9.
 35. Teymouri H, Mosavari N, Taghi HP. Detection of *Mycobacterium avium* subsp. *paratuberculosis* in Cattle by using Indirect Absorbed ELISA (enzyme-linked immunosorbent assay) system and culture in Alborz province, Iran. *Int J Mycobacteriol*. 2016;5:S220-S1.
 36. Mahdavi S, Zali MS, Farajnia S, Mehmannaavaz Y, Isazadeh A. The comparison of bovine fecal and buffy coat samples for diagnosis of Johnes's disease based on PCR. *Gene Cell Tissue*. 2018;5(2): e79745.
 37. Fathi R, Sarkarati F, Eslami M, Rezavand B, Nourizadeh A. Detection of *Mycobacterium avium* subsp. *paratuberculosis* in Cow Milk Using Culture and PCR methods. *Arch Razi Inst*. 2011; 66(2): 95-100.
 38. Shrafati-chalesshtori F, Sharafati-chalesshtori R, Shakerian A, Momtaz H. Detection of *Mycobacterium paratuberculosis* using polymerase chain reaction (PCR) in cow raw milk samples in shahre-kord. *Med Lab J*. 2009;3(1): 15-19.
 39. Haghkhah M, Ansari-Lari M, Novin-Baheran AM, Bahramy A. Herd-level prevalence of *Mycobacterium avium* subspecies *paratuberculosis* by bulk-tank milk PCR in Fars province (southern Iran) dairy herds. *Prev Vet Med*. 2008;86(1-2):8-13.
 40. Mahmoodi Koohi P, Sadeghi-nasab A, Mohammadzadeh AM, Sharifi A, Bahari AA, Mosavari N. Identification of *Mycobacterium avium* subsp *paratuberculosis* infection in industrial dairy farms of Hamedan. *Iran Vet J*. 2018;14(1):55-61.
 41. Nassiri M, Eftekhari Shahrودي F, Bassami MR. Detection of *Mycobacterium paratuberculosis* in feces and milk samples from holstein dairy cows by PCR. *Biotechnol*. 2008;7(3): 582-585.
 42. Haji Hajikolaei K, Ghorbanpour M, Fardbijar KA. A study on the prevalence of *Mycobacterium avium paratuberculosis* in sheep and goat slaughtered at Ahwaz abattoir. 2002.
 43. Derakhshandeh A, Namazi F, Khatamsaz E, Eraghi V, Hemati Z, editors. *Goat aratuberculosis in Shiraz:*

- Histopathological and molecular approaches. *Vet Res Forum*. 2018; 9(3): 53-57.
44. Etesami M, Mohammadian B, Esmailzadeh S, Ghorbanpoor M, Haji Hajikolaee MR, Pourmehdi Borojeni M. Histopathological, molecular and serological study of Johnes disease in buffaloes of Ahvaz. *Iran Vet J*. 2020;15(4):24-34.
 45. Zarei M, Ghorbanpoor M, Tajbakhsh S, Mosavari N. Comparison of rapid diagnostic tests to detect *Mycobacterium avium* subsp. *paratuberculosis* disseminated infection in bovine liver. *Trop Anim Health Prod*. 2017;49(6):1195-200.
 46. Abdolmohammadi Khiav L, Haghkhah M, Tadayon K, Mosavari N. Isolation of *Mycobacterium avium* subsp. *paratuberculosis* and confirmation of cases by Nested-PCR. *Vet Res Biol Prod*. 2019;32(1):41-7.
 47. Sadeghi N, Jamshidi A, Seyyedini M. Detection of *Mycobacterium avium* sub sp. *paratuberculosis* in pasteurized milk samples in northeast of Iran by culture, direct nested PCR and PCR methods. *Iran. J. Chem. Chem*. 2020;39(6):251-8.
 48. Heydarzadeh Asiabi Y, Jafari B, Hassanpour A. Investigating serumic levels of antibodies against *Mycobacterium avium* subspecies *paratuberculosis* using the ELISA method in Holstein dairy cattle. *Vet Clin Pathol* 2018;12(2):157-66.
 49. Pourjafar M, Badiei K, editors. Presence and trace element analysis in cattle affected by paratuberculosis in Yasouj, Iran. 8th international colloquium on paratuberculosis, Spain; 2005.
 50. Borujeni MP, Hajikolaee MRH, Ghorbanpoor M, Sahar HE, Bagheri S, Roveyshedzadeh S. Comparison of *Mycobacterium avium* Subsp. *paratuberculosis* (MAP) infection in cattle, sheep and goats in the Khuzestan Province of Iran: Results of a preliminary survey. *Vet Med Sci* 2021;7(5): 1970–1979.
 51. Anzabi Y, Farashi Bonab S, Moggaddam G. Efficiency of direct microbial diagnosis, IS900 PCR and microbial culture for detection of *Mycobacterium avium* subsp. *paratuberculosis* in the feces of apparently healthy cattle. *Vet. J. of Islamic Azad Uni. Tabriz Branch* 2009;2:309-317.
 52. Kheirandish R, Sami M, Khalili M, Shafaei K, Azizi S. Diagnosis of paratuberculosis in fresh and paraffin embedded samples by histopathology, PCR and immunohistochemistry techniques. *Bulg J Vet Med*. 2017;20(4):339–347.
 53. Anzabi Y, Khaki A. Detection of *Mycobacterium avium paratuberculosis* in pasteurized milk by PCR according to the search of IS900 gene sequence. *Med J Tabriz Univ Med Sci Health Serv*. 2017;39(3):7-14.
 54. Irai M. Abattoir study of paratuberculosis prevalence in sheep in ilam, Iran. In proceedings: 11Th Conference Iran Veterinary. 1999; 180-183.
 55. Chaubey KK, Singh SV, Gupta S, Singh M, Sohal JS, Kumar N, et al. *Mycobacterium avium* subspecies *paratuberculosis*—an important food borne pathogen of high public health significance with special reference to India: an update. *Vet Q*. 2017;37(1):282-99.
 56. Fernández-Silva JA, Correa-Valencia NM, Ramírez NF. Systematic review of the prevalence of paratuberculosis in cattle, sheep, and goats in Latin America and the Caribbean. *Trop Anim Health Prod*. 2014;46(8):1321-40.
 57. Alhebabi AM, Alluwaimi AM. Paratuberculosis in Camel (*Camelus dromedarius*): The diagnostic efficiency of ELISA and PCR. *Open Vet Sci J*. 2010;4(1): 41–44.
 58. Kennedy D. International efforts at paratuberculosis control. *Vet. Clin. North Am. Food Anim*. 2011;27(3):647-54.
 59. Zare P, Tolooie M, Rahmani Y, Fakhri O, Farshi Y, Shadi S. Comparison of serological evaluation (indirect ELISA) and clinical manifestations in detection of Johnes's disease in industrial cattle farms of Tabriz. 2nd International Congress of Large Animal Practitioners. 2013.