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Prevalence of Avian Influenza H9N2 in human and other mammals in Iran: A Systematic Review and Meta-analysis

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Abstract

Avian Influenza is a contagious zoonotic disease that can be transmitted from birds to mammals. In this study, we conducted a systematic review and meta-analysis to investigate and summarize the occurrence of avian influenza H9N2 in humans and other mammals in Iran. Data were collected systematically until July 1 2023 from four English and two Persian databases. According to the preferred reporting items for systematic reviews and meta-analyses (PRISMA) and inclusion criteria, 14 eligible studies were obtained. Occurrences of AI H9N2 are reported in humans, dogs, and water buffaloes (*Bubalus bubalis*) in Iran. The pooled prevalence of AI H9N2 using a random model among humans was 0.15 (95% *CI*, 0.12-0.18). Pooled prevalence using the HI test was 0.16 (95% CI, 0.13-0.20). The pooled prevalence by different cut-offs for the HI test was equal to 0.34, 0.11, 0.02, and 0.01, respectively for cut-offs 1:20 to 1:160. Pooled prevalence using the HI test, among persons with possible exposure, hospitalized persons and hospital staff (0.20, 0.31, and 0.30) was higher than the prevalence in apparently healthy individuals (0.01). The group of individuals with possible exposure revealed the highest pooled prevalence of 0.25 (95% CI, 0.17-0.33) using the HI test. This was observed predominantly in slaughterhouse workers. The pooled prevalence using the ELISA test was 0.27 (95% CI, 0.23-0.31). These results can help policymakers to create prevention and control programs for possible epidemics in the future and can help researchers to tailor the design and objectives of future studies in this area.

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Introduction

Influenza viruses are divided into four types: A, B, C, and D based on M and NP proteins. According to the antigenic differences between HA and NA, influenza Type A, is divided into 18 HA subtypes and 11 NA subtypes. So far, 16 HA and 9 NA subtypes have been identified in wild birds. These viruses naturally spread among wild birds

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worldwide and can infect poultry and other bird and animal species. Moreover, the viruses are divided into two categories based on their pathogenicity: low pathogenic avian influenza (LPAI) viruses, and highly pathogenic avian influenza (HPAI) viruses. These categories are determined by the virus's molecular characteristics and its ability to cause disease and mortality in poultry/chicken. Only some avian influenza A (H5) and A (H7) viruses are classified as HPAI viruses, while other AIVs usually are considered low LPAI viruses (1, 2).

Avian influenza viruses can occasionally affect mammals, including humans, usually after close contact with infected birds. The severity of the disease in humans will depend upon the virus subtype causing the infection and the characteristics of the infected individual. In other words, the occurrence of the disease in humans has ranged in severity from no symptoms or mild illness (e.g., eye infection, mild respiratory symptoms) to severe disease (e.g., pneumonia) that resulted in death (3, 4).

The H9N2 avian influenza virus, initially isolated from turkey flocks in Wisconsin in America in 1966, has been increasingly detected and reported in birds worldwide during the second half of the 1990s. This has resulted in continuous viral circulation in several countries in Asia, the Middle East, and North Africa. The virus was first isolated and reported in Iran in 1998 from a poultry farm with respiratory disease. Since then, it has been endemic in Iran, causing low pathogenic avian influenza every year and resulting in significant economic losses to the poultry industry (5-7). Additionally, evidence has shown that, in addition to birds, this virus has infected humans and some other mammal species in Iran.

In Iran, several studies have been conducted in different populations of humans regarding the occurrence of avian influenza H9N2, but there is no summary of these results. Indeed there is no available knowledge regarding the pooled prevalence of Avian Influenza H9N2 in humans and other mammal species in Iran. In the present study, we have conducted a systematic review and meta-analysis to determine and summarize the occurrence of avian influenza H9N2 in humans and other mammals of Iran.

Materials and methods

The study was conducted by the preferred reporting items for systematic reviews and meta-analyses (PRISMA) guidelines (8, 9).

Bibliographic search strategy

The appropriate studies were found by searching four English sources, namely PubMed, Springer, Google Scholar, and Science Direct, as well as two Persian databases, Magiran and SID. The search was conducted using the following terms: "Avian Influenza," AND "Influenza," AND "H9N2," AND "Iran" OR "the names of the provinces of Iran", in both Persian (Farsi) and English. Additionally, the references of the studies were checked to ensure comprehensive results. The selection process of the studies is presented in Figure 1 of the PRISMA flowchart. The search was conducted until July 1, 2023.

Inclusion and exclusion criteria

The scientists evaluated studies related to Avian Influenza in Iran by screening their titles and abstracts and then reviewed the full text of papers for quality assessment after eliminating duplicates. Two reviewers conducted separate assessments of the articles' quality. If the two specialists disagreed, a third party was consulted to independently resolve the issue and reach a consensus.

The study selected articles that met the following inclusion criteria: (1) they were conducted on human or other mammal subjects in Iran; (2) they reported the prevalence of avian influenza H9N2.

Exclusion criteria comprised: (1) any studies whose type was non-cross-sectional (experimentally, case report, etc.); (2) studies conducted outside of Iran; (3) studies not performed on mammal populations; (4) studies not reported prevalence of avian influenza H9N2; (5) studies with ambiguous methods for detecting influenza H9N2.

Data collection

An Excel data extraction form was utilized to gather the specified information from qualified studies, including: first author, time of publication, time of the study, place of study, mammal species, diagnostic tests, population characteristics, sample size, total *avian influenza*-positive number and prevalence.

Statistical analysis

We used random and fixed-effects models to estimate the pooled prevalence and 95% confidence intervals (CI). Heterogeneity among the studies was assessed using Cochran's Q test and I2 index. If the I2 index was greater than 50% and the P-value of

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Cochran's Q test was less than 0.1, the random effect model was chosen for estimation. The forest plots presented the proportions of individual studies, pooled prevalence, and the heterogeneity among studies. The meta-analysis was conducted using the trial version of Stata Version 14.

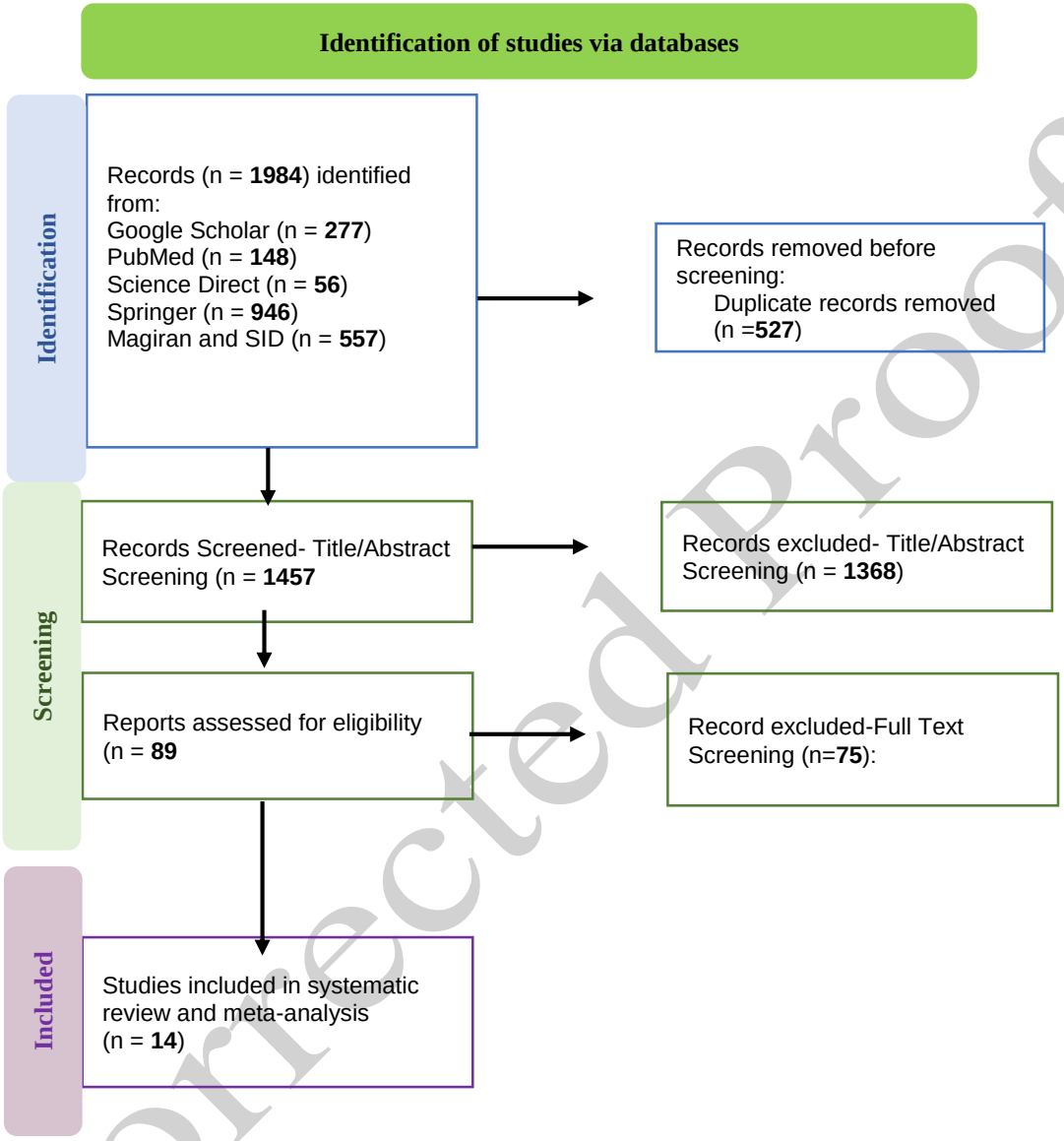

Fig. 1. PRISMA flowchart presenting the selection of articles analyzed in this systematic review and meta-analysis.

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Results

Search results and eligibility studies

In this research, 1,984 papers were initially identified across all databases. After removing 527 duplicates and 1,368 papers that did not meet the inclusion and exclusion criteria based on their captions and abstracts, as well as 75 papers that did not meet the criteria upon full-text review, 14 studies ultimately met the evaluation criteria for this research (Figure 1).

Characteristics of the eligible studies

Among the 14 eligible studies, 4 were published before 2012, and the others were published after 2012. Among them, 9 studies were published in English and 5 published in Persian. Among the studies, 11 studies determined prevalence in

humans, while 3 studies determined prevalence in other mammal species.

Out of the 11 studies, 9 studies were conducted in one province. Also, 9 studies were conducted on poultry workers and 7 studies considered veterinarian and slaughterhouse workers as the studied population. Four studies investigated hospitalized persons in addition to the mentioned population. Among the 11 studies, all of them determined prevalence using the HI Test. Also in two studies, in addition to HI, the Elisa method was used. One study used the MN Test beside the HI Test. Also, one study Used RT-PCR beside the HI test (Table 1).

Among the three studies conducted on other species of mammals, two studies determined prevalence in dogs and one study determined the prevalence in water buffaloes. All three studies described prevalence using the HI test.

Prevalence of Avian Influenza H9N2 in human

Meta-analysis indicated the pooled prevalence of avian influenza H9N2 using random effects was estimated as 0.15 (95% *CI*, 0.12-0.18). There was a high degree of heterogeneity in the prevalence estimates between different observations. It was observed that the Q statistic was 2011.38 (*df*=34), $P<0.01$, and I^2 was 98.31% (Table 2 and Figure 2).

The pooled prevalence of avian influenza H9N2, according to diagnostic tests indicated that the pooled prevalence was 0.16 (95% CI, 0.13-0.20), 0.27 (95% CI, 0.23-0.31), and 0.02 (95% CI, 0.01-0.04) using HI test, ELISA, and MN Test, respectively. One study measured the prevalence using RT-PCR and found no positive case (Table 2 and Figure 2).

Pooled prevalence of AI H9N2 using HI test according to different cut-off values of HI test was as follows: one study reported prevalence of 0.66 using a cut-off 1:2. Pooled prevalence was 0.37 for a cut-off 1:8. One study reported prevalence as 0.42 for a cut-off 1:10. The pooled prevalence for a cut-off 1:20, 1:40, 1:80 and 1:160 was as 0.34, 0.11, 0.02 and 0.01 respectively. Also, two separated studies reported a prevalence of 0.01 for a cut-off 1:320 and a zero prevalence for a cut-off 1:640 (Table 2) (Figure 3).

The pooled prevalence of AI H9N2 using the HI test according to population was 0.20, 0.31, 0.30, and 0.01 among persons with possible exposure, hospitalized persons, hospital staff, and apparently healthy individual, respectively (Table 2 and Figure 4).

The pooled prevalence of H9N2 using the HI test in a group of persons with possible exposure indicated that the prevalence was 0.19, 0.25, and 0.20 among poultry workers, slaughterhouse workers, and veterinarians.

Prevalence of Avian Influenza in other mammals

The prevalence of AI H9N2 has been reported among dogs and water buffaloes in Iran so far. Two studies reported dogs infected with AI H9N2 in Iran. One of the mentioned studies indicated the prevalence to be 0.382 using the HI test (a cut-off 1:16) for AI H9N2 among pet dogs in Kerman province, While reported prevalence to be 0.258 and 0.10 for a cut-off 1:32 and 1:64. Another study investigated dog referred to veterinary clinics in Fars province and reported prevalence to be 0.45 using Elisa Method. Also, one study investigated AI H9N2 among Water Buffaloes (*Bubalus bubalis*) in Khuzestan of Iran and obtained a prevalence of 0.175 using the Hi-Test (3 $\log_2$).

Table 1. Papers met the eligibility criteria of this systematic review and meta-analysis.

Study Number	Author	Year	Province	Population	Avian Influenza Virus	Diagnostic methods	Sample Size Number	Positive Number	Reference
1	Alizadeh	2009	Tehran	Human	H9N2 (A/tky/wisc/1/66)	HI 1:10	152	64	(10)
						HI 1:20	152	48	
						HI 1:40	152	21	
						HI 1:80	152	8	
2	Hadipour	2011	Boshehr	Human	H9N2 (Not Specified)	HI 1:8	300	251	(11)
3	Hadipour	2011	Shiraz	Human	H9N2 (Not Specified)	HI 1:40	600	106	(12)

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4	Goudarzi	2011	East Azerbaijan	Human	H9N2 (A/chicken/Iran/11T/99)	HI 1:20	96	52	(13)
						HI 1:40	96	17	
						HI 1:80	96	7	
						HI 1:160	96	2	
						HI 1:320	96	1	
						HI 1:640	96	0	
5	Fattahi Abdizadeh	2005	Khuzestan	Human	H9N2 (Not Specified)	HI 1:2	100	66	(14)
6	Hadipour	2010	Fars	Human	H9N2 (Not Specified)	HI 1:20	300	176	(15)
7	Azzizpour	2012	Ardabil	Human	H9N2 (Not Specified)	HI 1:20	311	81	(16)
8	Zamani Moghadam	2009	Shahrkord	Human	H9N2 (Not Specified)	HI 1:8	334	70	(17)
						Elisa	90	71	
9	Rahimian	2009	-	Human	H9N2 (Not Specified)	HI 1:40	160	19	(18)
						HI 1:80	160	2	
						HI 1:160	160	2	
						RT-PCR	56	0	
10	Heidari	2016	Fars	Human	H9N2 (A/chicken/Iran/12VIR/9630/1998)	HI 1:40	200	2	(19)
						HI 1:80	200	2	
						HI 1:160	200	2	
						MN 1:40	200	2	
						MN 1:80	200	2	
						MN 1:160	200	2	
					H9N2 (A/chicken/Iran/10VIR/854-5/2008)	HI 1:40	200	14	
						HI 1:80	200	4	
						HI 1:160	200	3	
						MN 1:40	200	20	
						MN 1:80	200	10	
						MN 1:160	200	2	
11	Anvar	2013	Tehran and Qazvin	Human	H9N2 (A/HK/1073/99(07/146))	HI 1:20	182	3	(20)
						Elisa	182	21	
12	Saberi	2019	Kerman	Dog	H9N2 (Not Specified)	HI 1:16	65	170	(21)
						HI 1:32	44	170	
						HI 1:64	17	170	
13	Tajik	2019	Khuzestan	Water Buffalo (*Bubalus bubalis*)	H9N2 (Not Specified)	HI 3 log2	14	80	(22)
14	Abbaszadeh Hasiri	2011	Fars	Dog	H9N2 (Not Specified)	Elisa	182	82	(23)

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Table 2. Pooled prevalence of Avian Influenza H9N2 according to diagnostic tests and population.

Independent Variable				Number of Observation	Sample size	Total number positive	Pooled prevalence (%)	95% Confidence interval	Heterogeneity			Heterogeneity Between Subgroup bias
									Q statistic	I^2 (%)	*P*-value	
Diagnostic Test	**HI**			26	4991	959	0.16	0.13-0.20	1629.83	98.47 %	0.00	193.82 (0.00)
	Elisa			2	272	92	0.27	0.23-0.31	-	-	-	
	MN			6	1200	38	0.02	0.01-0.04	23.18	78.43 %	0.00	
	RT-PCR			1	56	0	0*	0-0.07	-	-	-	
Diagnostic Test	**HI**	Based on Two Fold Dilution	Titer 1:2	1	100	66	0.66*	0.56-0.75	-	-	-	873.54 (0.00)
			Titer 1:4	-	-	-	-	-	-	-	-	
			Titer 1:8	2	634	257	0.37	0.34-0.40	-	-	-	
		Based on Ten Fold Dilution	Titer 1:10	1	152	64	0.42*	0.35-0.50	-	-	-	
			Titer 1:20	5	1041	360	0.34	0.10-0.59	517.08	99.23 %	0.00	
			Titer 1:40	6	1408	179	0.11	0.04-0.18	127.33	96.07 %	0.00	
			Titer 1:80	5	808	23	0.02	0.01-0.04	9.65	58.54 %	0.05	
			Titer 1.160	4	656	9	0.01	0.00-0.02	0.53	0.00%	0.91	
			Titer 1:320	1	96	1	0.01*	0.00-0.06	-	-	-	
			Titer 1:640	1	96	0	0.00*	0.00-0.04	-	-	-	
Diagnostic Test	**HI**	Exposed		25	2907	673	0.20	0.15-0.26	1832.23	98.69 %	0.00	174.22 (0.00)
		Normal		16	1180	39	0.01	0.00-0.002	42.46	64.68 %	0.00	
		Patient		4	560	141	0.31	00.17-0.45	36.44	91.77 %	0.00	
		Hospital Staff		2	344	106	0.30	0.25-0.35	-	-	-	
Diagnostic Test	**HI**	Exposed	Poultry Worker	19	1204	264	0.19	0.15-0.22	1190.44	98.49 %	0.00	2.30 (0.32)
			Slaughterhouse Worker	17	1325	276	0.25	0.17-0.33	812.68	98.03 %	0.00	
			Veterinarian	9	378	133	0.20	0.18-0.22	395.92	97.98 %	0.00	

*Prevalence wasn't obtained by meta-analysis because it was reported in one study

Discussion

In this systematic review and meta-analysis study, we summarized the studies related to the occurrence of avian influenza H9N2 in humans and other mammals in Iran and obtained the pooled prevalence in these species for the first time. Our study revealed that avian influenza H9N2 has been reported in humans, dogs, and water buffaloes (*Bubalus bubalis*) in Iran.

The pooled prevalence of AI H9N2 in humans (persons with possible exposure, hospitalized persons, hospital staff, and apparently healthy individuals) regardless of the diagnostic test was 0.15 up to 2023, whereas the pooled prevalence using the HI test was 0.16. Another meta-analysis study in China reported the pooled sero-prevalence using HI test in a range of all subjects (Influenza-like symptoms person, possibly exposed person, and apparently healthy individual) to be 0.06 as of March 2020 (24). They showed the pooled sero-prevalence using the MN test equal to 0.014 and the molecular prevalence equal to 0.15. In the mentioned meta-analysis, approximately 20% of people were apparently healthy subjects, and in our meta-analysis, nearly 22% of people were as apparently healthy. The two meta-analyses were similar in terms of population composition. However, due to the use of different cut-offs of the HI test in calculating the final pooled prevalence, the interpretation of the results of the comparison of two meta-analyses will probably be challenging. In any case, our results show that there is a history of human encounters with the H9N2 virus, with a sero-prevalence from a range of 0.00 to 0.79 caused by different cut-offs of the HI test from 1998 to 2023 in Iran.

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The results of the pooled prevalence obtained by different cut-offs of the HI test in this meta-analysis showed that with the increase of the cut-off, the sero-prevalence decreased. Therefore, at the 1:160 cut-off, which is also the cut-off recommended by the World Health Organization, the prevalence was equal to 0.01. (25). It is suggested that future studies conducted in Iran or other parts of the world should use the 1:160 cut-off to ensure comparability with other papers and the reference of the World Health Organization.

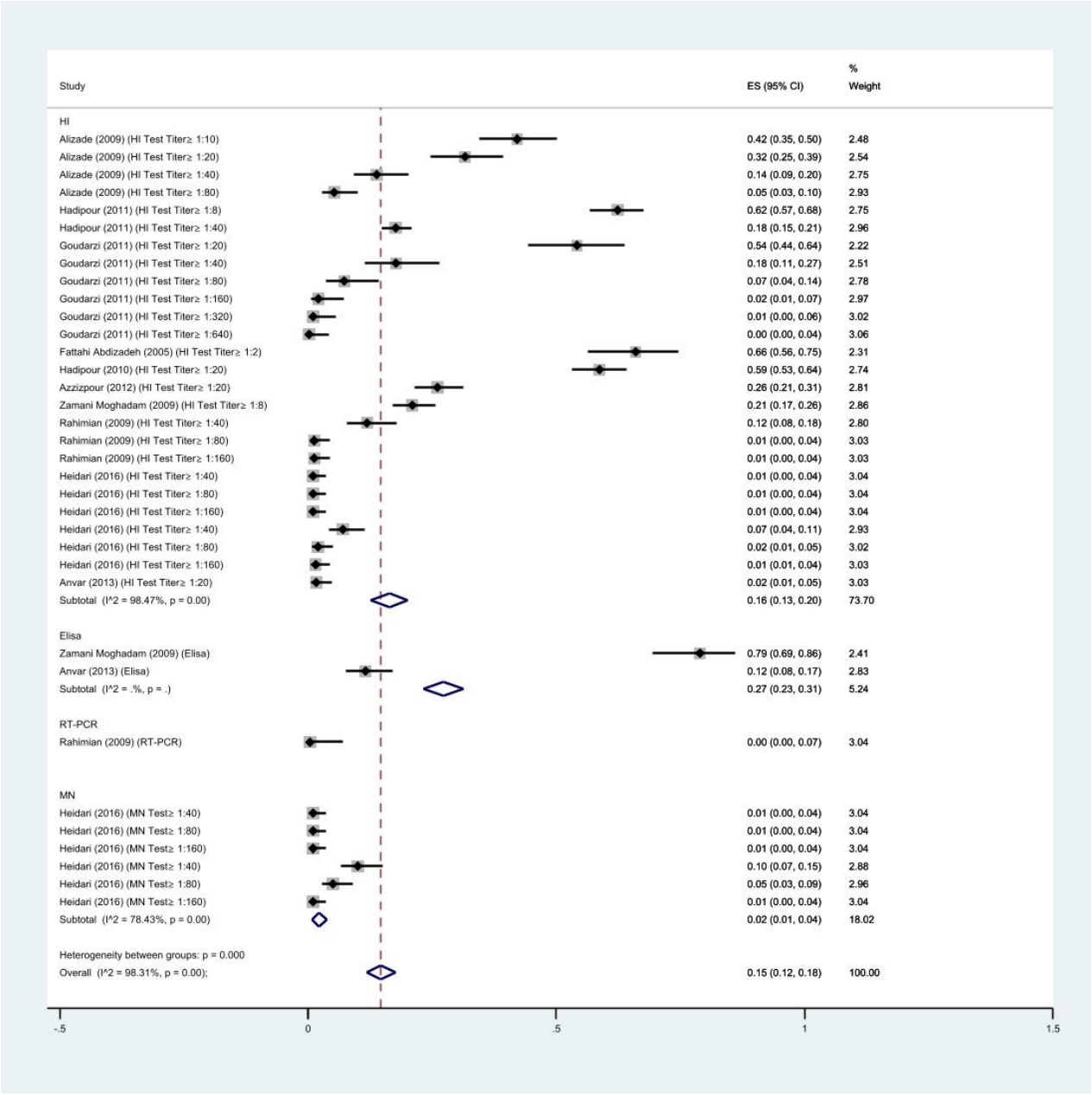

Fig. 2. Forest plots of pooled prevalence of Avian Influenza H9N2 among humans in Iran (first author, year) according to diagnostic tests.

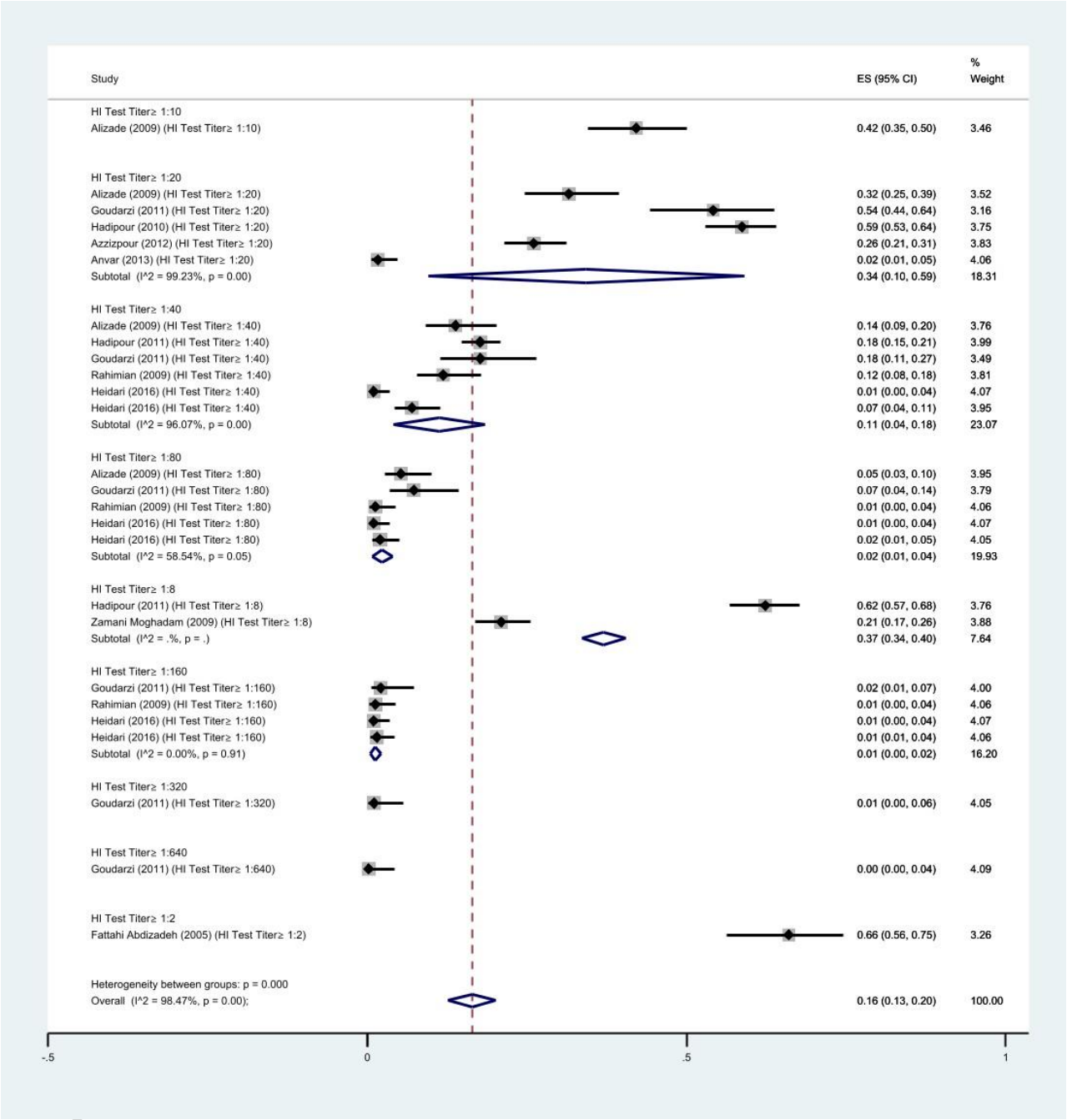

Fig. 3. Forest plots of prevalence of Avian Influenza H9N2 among Humans in Iran (first author, year) according to diagnostic test (Different cut-off of HI Test).

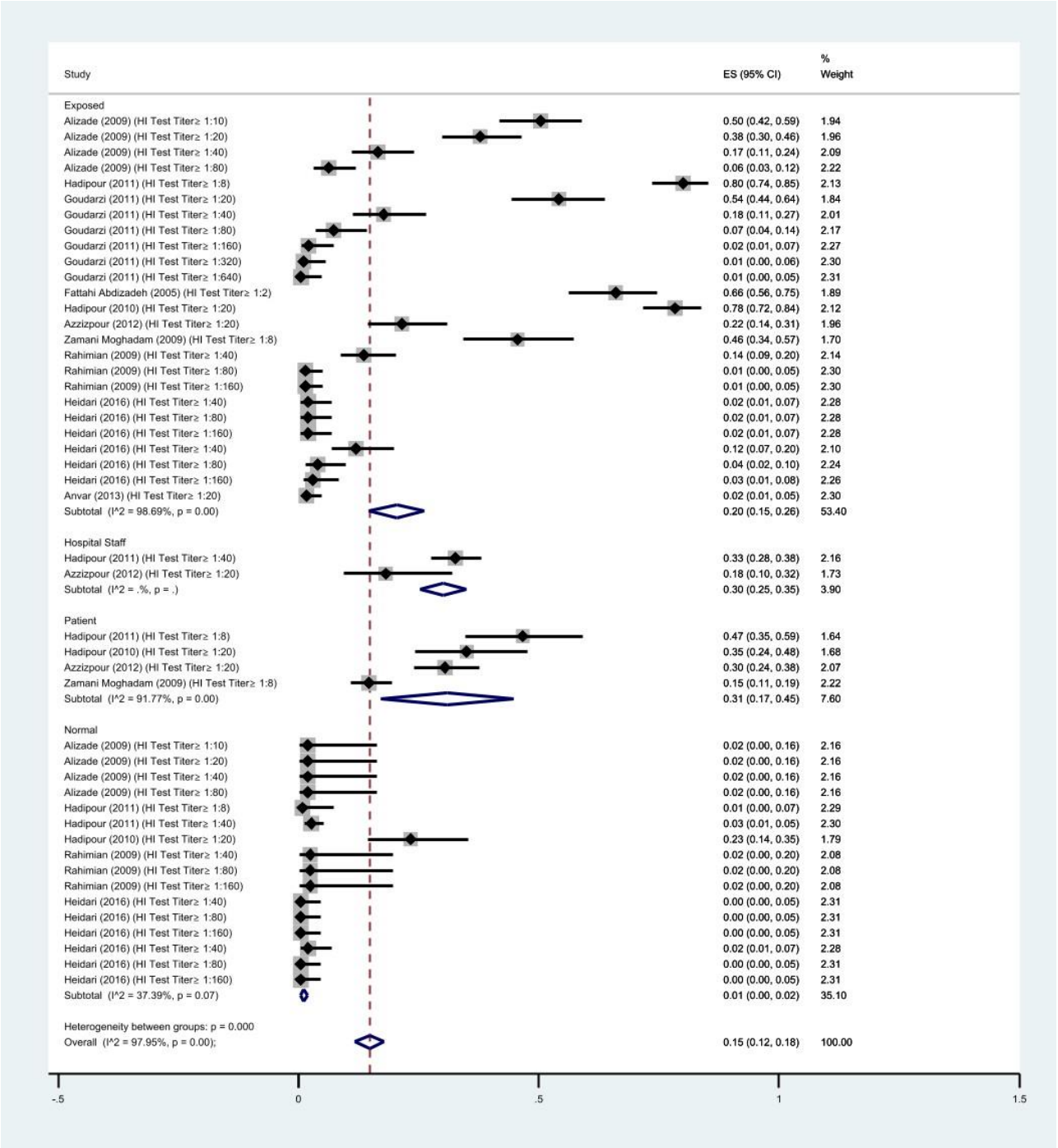

Fig. 4. Forest plots of prevalence of Avian Influenza H9N2 among Humans in Iran (first author, year) according to Diagnostic Test and Population.

H9N2 seroprevalence was measured using the HI test in four populations: individuals with possible exposure, hospitalized patients, hospital staff, and apparently healthy individuals. The seroprevalence rates were 0.20, 0.31, 0.30, and 0.01, respectively. In another meta-analysis conducted worldwide during 1997-2013, the pooled sero-prevalence of H9N2 in people with possible exposure was found to be 9%. (26). Also, another meta-analysis in China up to March 2020, regardless of the type of diagnostic test, had quantified pooled prevalence equal to 35.8%, 2.5-8.8%, and 1.39%, in Influenza-

like symptoms person, possibly exposed person and apparently healthy people respectively (24). In conclusion, similar to the mentioned studies, our results show that the prevalence among people with possible exposure, hospitalized persons and hospital staffs were higher than apparently healthy individuals in Iran. As an explanation, the results obtained in people with possible exposure and hospitalized persons can be justified due to the existence of occupational exposure to birds and the weakness of the immune system. The results obtained from hospital staff should be interpreted with more caution. The two studies that calculated the prevalence in hospital staff mentioned that this group had close contact with infected patients. While the available evidence shows that the virus has a low ability to transmit from human to human (27). Therefore, it is suggested that future studies investigate this group to produce sufficient data for scientists.

The pooled H9N2 influenza prevalence by type of exposure in people with possible exposure showed that the sero-prevalence in veterinarians, poultry workers, and slaughterhouse workers was 0.20, 0.19 and 0.25, respectively. According to the results, the pooled sero-prevalence was higher in slaughterhouse workers and veterinarians compared to poultry workers. It should be noted that veterinarians are not much different from poultry workers. This is probably because some of these veterinarians are veterinary students and had less contact with poultry than graduate veterinarians. In any case, the higher pooled sero-prevalence in these two groups can be because these two groups deal with a wider and more diverse range of poultry at work compared to poultry workers.

In this systematic review, in addition to the studies found in humans, we also found 3 studies in two other mammal species, i.e. dogs and water buffaloes, which proved the existence of antibodies against H9N2 influenza in these two species. In other parts of the world, infection with this virus has been proven in some other species of mammals, especially canines such as cats (28-30). These results show that, in addition to humans, other mammals can also be infected by this virus. Therefore, more studies are needed to understand the epidemiological role of mammal species in the transmission cycle of this virus and its pathogenesis.

Conclusion

This study provides a summary of all the research conducted on avian influenza H9N2 in humans and other mammals in Iran. The findings suggest that dogs, water buffaloes, and humans, particularly those who have been hospitalized or work in healthcare facilities, have been exposed to the H9N2 virus in Iran. In general, this study provided a comprehensive view of the occurrence of avian influenza H9N2 in humans and other mammals in Iran, which can help policymakers create prevention and control programs for possible epidemics in the future. Additionally, the study can assist researchers in tailoring the design and objectives of future studies in this area.

Acknowledgments

Not Applicable.

Conflict of Interest

The authors reported no potential conflict of interest.

Ethical Approval

Not Applicable.

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