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REVIEW ARTICLE
Systematic review of brucellosis in the Middle East: disease frequency in ruminants and humans and risk factors for human infection

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SUMMARY

A systematic review of studies providing frequency estimates of brucellosis in humans and ruminants and risk factors for *Brucella* spp. seropositivity in humans in the Middle East was conducted to collate current knowledge of brucellosis in this region. Eight databases were searched for peer-reviewed original Arabic, English, French and Persian journal articles; the search was conducted on June 2014. Two reviewers evaluated articles for inclusion based on pre-defined criteria. Of 451 research articles, only 87 articles passed the screening process and provided bacteriological and serological evidence for brucellosis in all Middle Eastern countries. *Brucella melitensis* and *B. abortus* have been identified in most countries in the Middle East, supporting the notion of widespread presence of *Brucella* spp. especially *B. melitensis* across the region. Of the 87 articles, 49 were used to provide evidence of the presence of *Brucella* spp. but only 11 provided new knowledge on the frequency of brucellosis in humans and ruminants or on human risk factors for seropositivity and were deemed of sufficient quality. Small ruminant populations in the region show seroprevalence values that are among the highest worldwide. Human cases are likely to arise from subpopulations occupationally exposed to ruminants or from the consumption of unpasteurized dairy products. The Middle East is in need of well-designed observational studies that could generate reliable frequency estimates needed to assess the burden of disease and to inform disease control policies.

Key words: *Brucella melitensis*, *B. abortus*, human incidence, Middle East, ruminant prevalence, seroprevalence, systematic review.

INTRODUCTION

Brucellosis is a zoonotic disease that affects a wide range of animals including domestic livestock. It is caused by members of the genus *Brucella*; among which; *B. melitensis, B. abortus, B. suis, B. canis* and *B. ceti* have been isolated from human cases in addition to their specific animal hosts [1–4]. Although accurate estimates of human incidence are lacking, largely because of under-reporting and misdiagnosis [5], brucellosis is considered one of the most common bacterial zoonoses worldwide [6].

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In endemic areas, brucellosis is responsible for significant economic losses to livestock production due to abortions, reduced milk yield and infertility in addition to the public health burden [7]. The disease is transmitted to humans via the consumption of unpasteurized milk and dairy products from infected animals and through direct contact with afterbirth and aborted materials. As a result, individuals with occupational livestock contact in endemic areas, including farmers, abattoir workers, shepherds and veterinarians are at high risk [8, 9]. The symptoms of human infection are non-specific, but the majority of patients with the acute form, present with fever, malaise, anorexia, headache, arthralgia, and backache. Persistent and recurrent fever is the most common clinical symptom in sub-acute cases. A small proportion of cases may develop complications including arthritis, endocarditis, spondylitis, sacroiliitis, osteomyelitis and meningoencephalitis [9, 10].

Infected livestock are the source of most human cases; therefore, prevention of human brucellosis is dependent on the control of the disease in livestock. This has been achieved with varying degrees of success using a combination of vaccination, test and slaughter of positive animals and quarantine/animal movement controls [11]. Cattle brucellosis, caused primarily by *B. abortus*, has been successfully eliminated from several countries including Japan, Canada, some European countries, Australia and New Zealand [12]. However, the control of *B. melitensis* in small ruminants appears to be more challenging than that of *B. abortus*, potentially as a result of its higher infectivity [13] as well as the characteristics of the livestock systems where it is endemic including increased mobility of small ruminant populations compared to large ruminants [7, 12]. Different control strategies have been recommended by the Food and Agriculture Organization (FAO) depending on the flock-/herd-level seroprevalence, therefore reliable disease frequency estimates are of great importance to inform and monitor the control programme. In low-prevalence areas (<2%) test and slaughter of positive animals accompanied by sanitary measures is recommended. In settings where prevalence ranges between 2% and 10% the FAO advocates vaccination of young animals, non-compulsory vaccination of adult animals and test and slaughter of infected animals. In regions where prevalence is higher than 10%, mass vaccination of all livestock is proposed as the optimal control strategy until a significant prevalence reduction is achieved and the strategy can be revised [11]. The appropriate strategy also depends on the socioeconomic context, the applied surveillance system, the policy set by the competent authorities as well as the baseline level of infection [14]. Ultimately, decisions on whether to prioritize brucellosis control over other diseases should ideally be informed by estimates of the human health burden expressed as disability adjusted life years (DALYs) and measures of monetary impact, i.e. economic losses due to human illness and decreased livestock productivity [7, 15]. The assumed high burden of the disease, particularly in low-income countries, is not matched by the attention it receives from health systems worldwide and as a result brucellosis has been included in the WHO's list of Neglected Zoonotic Diseases [6] Brucellosis is a major public health problem in the Middle East, Mediterranean region, and parts of Asia, Africa and Latin America [12, 16, 17]. In this paper, we focus on the Middle East, a region where brucellosis is assumed to be among the zoonoses with highest burden [18]. The region includes 15 countries; Bahrain, Egypt, Iran, Iraq, Israel, Jordan, Kuwait, Lebanon, Oman, Palestine, Qatar, Saudi Arabia, Syria, United Arab Emirates, and Yemen [19] (Fig. 1). Most of these countries have many similarities regarding livestock management systems, environmental conditions and culture [18]. Brucellosis is receiving increasing attention in the Middle East; some countries such as Egypt and Oman are implementing vaccination programmes for small and large ruminants whereas others, e.g. Iran, Iraq and Israel are adopting mass vaccination of small ruminants (Fig. 1). The aim of this study was to systemically identify, evaluate and summarize relevant published data on the presence and frequency of ruminant and human brucellosis in the Middle Eastern countries as well as on the strength of association between potential risk factors and *Brucella*. spp. seropositivity in humans.

METHODS

Systematic review protocol

A systematic review was conducted using a predefined protocol based on Cochrane [20] and PRISMA [21] guidelines. The protocol includes four main steps: (i) literature search to identify potential articles of relevance, (ii) screening for relevance, (iii) quality assessment and (iv) data extraction. Figure 2 summarizes the steps of the protocol with the number of papers that fulfilled the necessary criteria at each step.

Search strategy and identification

Eight electronic databases; BioMed Central Journals, CAB Direct (CABI), Cochrane Library – Cochrane

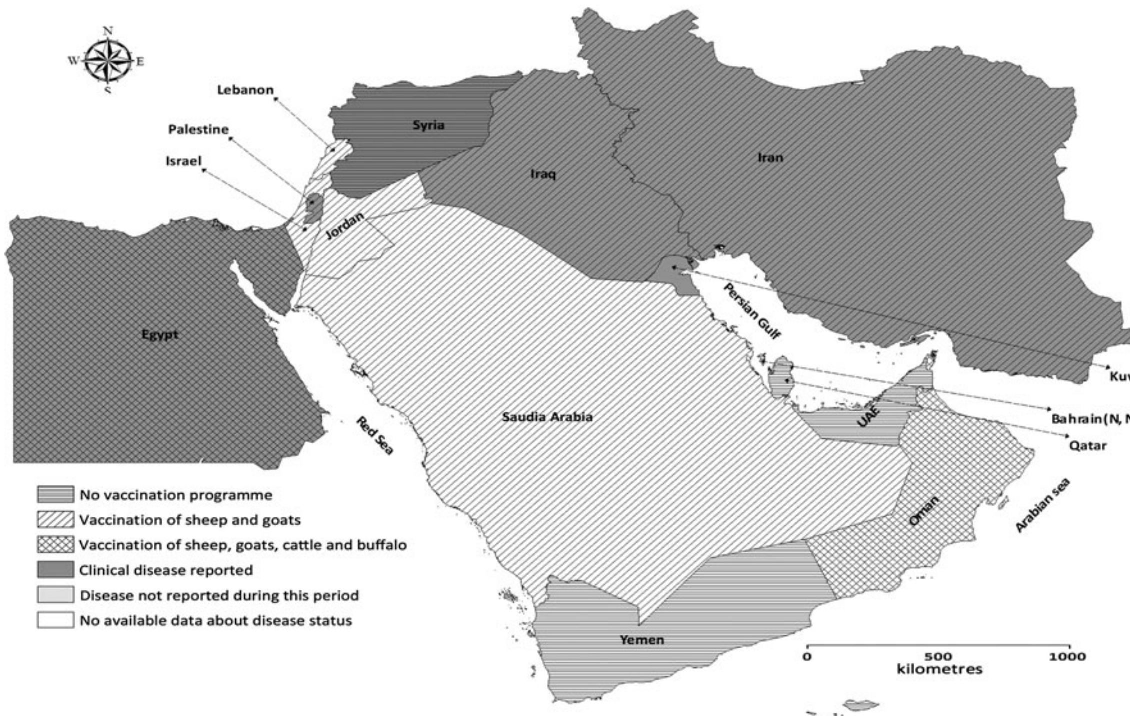

Fig. 1. Map of the Middle East showing ruminant brucellosis infection and vaccination status in different countries of the region, data obtained from OIE, 2013. N, Disease not reported; NV, no vaccination programme.

Database of Systematic Reviews (Wiley), Science Direct, ERIC plus Text (ProQuest), IBSS (CSA), PubMed, and Web of Science (ISI) Zetoc, were searched using the following terms:

(1) Brucellosis OR Malta fever OR *Brucella* OR '*Brucella melitensis*' OR '*Brucella abortus*'.
AND
(2) Middle East OR (Bahrain, Egypt, Iran, Iraq, Israel, Jordan, Kuwait, Lebanon, Oman, Palestine, Qatar, Saudi Arabia, Syria, United Arab Emirates, Yemen).
AND
(3) Humans OR Domestic AND Ruminants OR (Cattle OR Cow OR Bovine) OR (Sheep OR Ovine) OR (Goat OR Caprine) OR (Camel OR Camelidae OR Dromedary).
(4) Prevalence OR Incidence OR Risk AND Factors.

The search was conducted on June 2014 No time limits were set.

Screening process

All references were imported to EndNote (Thomson Reuters) and duplicated articles were excluded. The retrieved abstracts were screened by the primary author for entry into the next stage (quality assessment) based on the following inclusion criteria:

(1) The reported research is original and studies a human or animal population in one or more of the Middle Eastern countries.
AND
(2) The article is published in a peer-reviewed section of a journal.
AND
(3) The article is written in Arabic, English, French or Persian languages.
AND
(4) The research provides:
  (*a*) Estimates of the frequency [(sero)-prevalence and/or incidence] of *Brucella* spp. infection in domestic ruminants and/or humans.
OR
  (*b*) Estimates of the strength of association between *Brucella* spp. (sero)-positivity in humans and potential risk factors.

For articles that met the primary inclusion criteria or articles where the relevance could not be determined by reading the abstract alone; full texts were retrieved and the article was subjected to quality assessment and data extraction.

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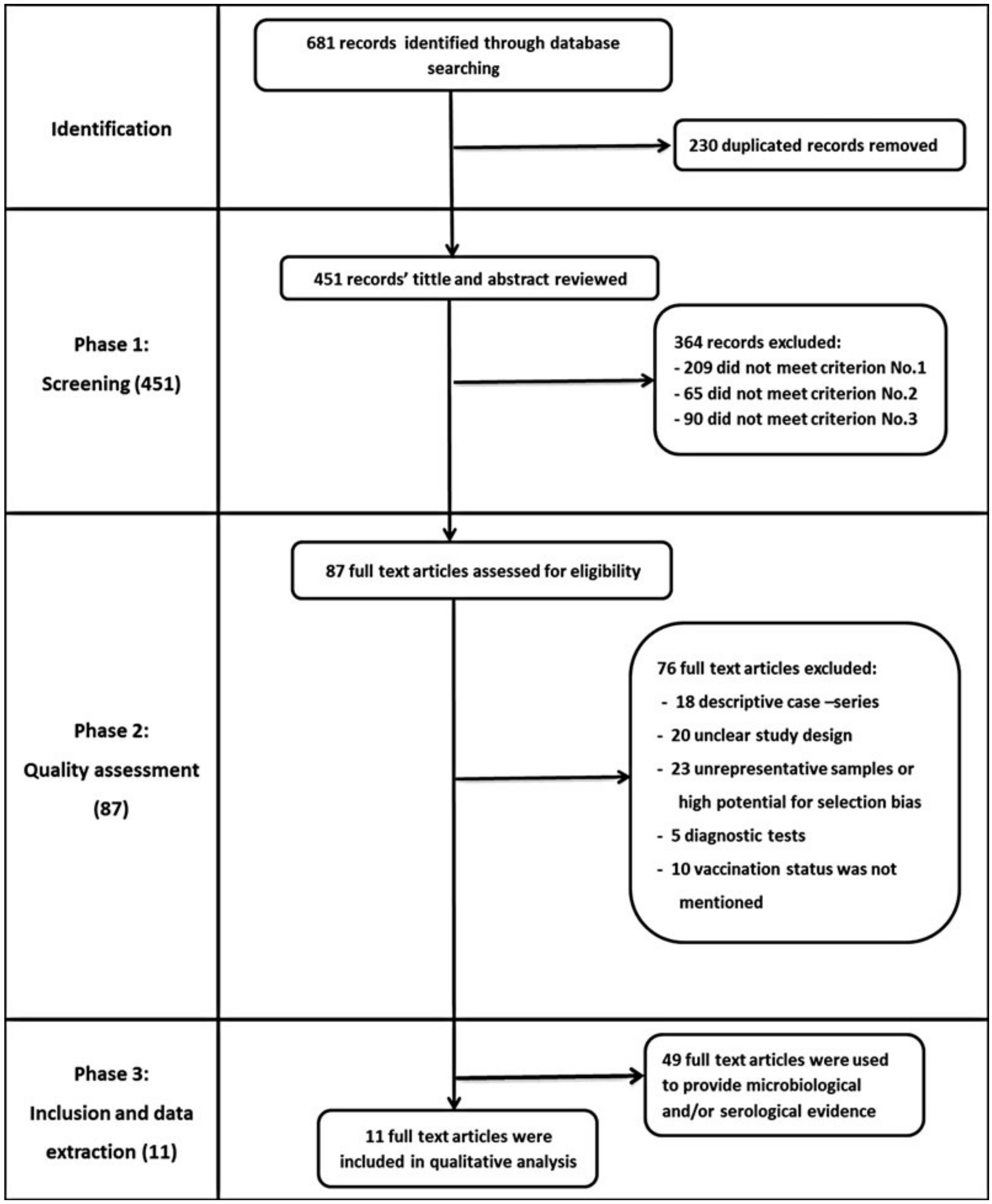

Fig. 2. Flow chart (template provided by PRISMA) showing the numbers of journal papers at each stage of the systematic review.

Quality assessment and data extraction

Two reviewers independently assessed the quality of the articles based on a set of criteria adapted from Cochrane guidelines [20], and Downs & Black guidelines for cross-sectional studies [22]. Reviewers were first asked to specify the type of study, whether it was descriptive or analytical (i.e. involving an element of comparison across groups). Studies that were limited to the description of the characteristics of a series of cases (case reports or case series) were excluded. Reviewers were asked a series of questions to summarize the objectives, study design, study and target population, sampling strategy, diagnostic tests performed, statistical methods used and main outcomes of the study. These general questions were followed by a series of questions specific for each study type and to which reviewers could answer 'yes', 'no' or 'unclear'.

Selected studies were appraised by the two reviewers against the following five criteria and rated as 'high quality' studies when all five criteria were met:

(1) The type of study design was clear from the information provided.

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(2) Sampling strategy was clearly described, study unit was clearly stated (e.g. herd/flock *vs*. individual animal) with consideration of clustering if individual animals were targeted and the study population was considered fairly representative of the target population.
(3) The study was not deemed to have high potential for selection bias.
(4) Diagnostic tests used were those recommended by the WHO for humans and the World Organisation for Animal Health (OIE) for the studied species [23, 24]; given that no time limit was set in the search process, OIE and WHO recommendations at the time when the study was conducted were considered.
(5) The vaccination status of the study population was stated.

When provided, estimates of the frequency of seropositivity (incidence or true prevalence after adjusting for the imperfect performance of the used diagnostic tests) and of the strength of association (relative risks or odds ratios) where extracted.

Each reviewer extracted data independently using a data extraction form prepared by the primary author. Disagreements between reviewers were discussed in detail between them and resolved by consensus. The quality assessment checklist and data extraction forms are available upon request from the primary author.

Data management

Studies considered to be of 'high quality' were grouped according to whether they investigated; the frequency of brucellosis in humans, ruminants or risk factors for human seropositivity. Because of the heterogeneity within each group of studies in terms of study design, geographical areas, human or ruminant subpopulations under study and sample sizes, no statistical tests for heterogeneity or quantitative meta-analysis were performed; instead, data were extracted, summarized and organized in a qualitative manner.

Studies that passed the initial screening but did not fulfil the quality assessment criteria and were therefore deemed not to be of sufficient quality to generate unbiased estimates of frequency of disease or strength of association for human seropositivity were used to summarize available evidence of the presence of *Brucella* spp. in different host populations in the Middle East, where appropriate.

RESULTS

Searching

The initial search revealed 681 research articles, after removing duplicates 451 research articles remained, among these 23, 405, 3 and 20 were written in Arabic, English, French and Persian languages, respectively. Abstract screening was then performed and articles were excluded when they reported studies that were not carried out in one or more of the Middle Eastern countries (95 articles excluded), if they were not original research articles (114 articles excluded), if they were published in non-peer-reviewed journals (65 articles excluded) and if they did not provide estimates of brucellosis frequency in humans or domestic ruminants or potential risk factors for human seropositivity (90 articles excluded). A total of 87 articles (5 Arabic, 77 English, 5 Persian) met the primary eligibility criteria.

Quality assessment

During the quality assessment 76 articles were excluded for not fulfilling all five quality criteria listed in the quality assessment and data extraction part of the methods. Eighteen of studies were excluded because they were descriptive case-series, 43 studies were excluded due to unclear study design or non-representative sampling therefore deemed to have high potential for selection bias. In five studies, the diagnostic tests used were not those recommended by WHO/OIE in the study species. Furthermore, in 10 studies the authors did not mention clearly whether the sampled animals were vaccinated or not, which may lead to inaccurate seroprevalence estimates. Of these 76 articles, 49 were retained and used to provide evidence of the presence of *Brucella* spp. in different ruminant hosts in Middle Eastern countries (Table 1). The range of the years of publication was 1974–2014 with a median of 2005. At least one *B. melitensis* biovar (1, 2, 3) was identified in each country and at least one *B. abortus* biovar (1, 2, 3, 9) was identified in nine of the 15 countries supporting the widespread presence of *Brucella* spp. especially *B. melitensis* across the region. Moreover, *B. suis* biovar 1 was isolation from cattle in Egypt [25]. Only 11 articles were considered of sufficient quality and were used to provide frequency estimates in humans and ruminants or information on risk factors for brucellosis in humans. Table 2 describes the features of the eleven included studies. The number of papers that

Table 1. *Microbiological and serological evidences of the presence of* Brucella melitensis *and* B. abortus *(+) in humans and ruminants in the Middle East identified in a systematic review of peer-reviewed journal articles published in Arabic, English, French or Persian (search conducted on June 2014)*

		Microbiological evidence								
		B. abortus (biovar)					*B. melitensis* (biovar)			
Country	Species [reference]	1	2	3	7	9	1	2	3	Serological evidence
Bahrain	Humans [26]								+	+
	Large ruminants									
	Small ruminants									+
	Camels									
Egypt	Humans [25, 27, 28]								+	+
	Large ruminants [25, 27, 28]	+		+					+	+
	Small ruminants [25, 28]						+		+	+
	Camels [29, 30]	+			+				+	+
Iran	Humans [18, 31, 32]						+		+	+
	Large ruminants [31, 32]	+	+	+		+	+	+		+
	Small ruminants [31, 32]						+	+	+	+
	Camels [33, 34]								+	+
Iraq	Humans [18, 35]								+	+
	Large ruminants [35, 36]		+	+		+	+		+	+
	Small ruminants [18, 35]						+		+	+
	Camels [37]									+
Israel	Humans [38–40]	+		+			+	+	+	+
	Large ruminants [18, 41]	+		+					+	+
	Small ruminants [38, 40, 41]						+	+	+	+
	Camels[39]						+			
Jordan	Humans [42]								+	+
	Large ruminants [43, 44]						+		+	+
	Small ruminants [43, 45–47]					+	+		+	+
	Camels [48]								+	+
Kuwait	Humans [49]					+	+			+
	Large ruminants [18]						+			+
	Small ruminants [18]						+			+
	Camels [50]	+								+
Lebanon	Humans [51, 52]							+		+
	Large ruminants									
	Small ruminants [18]							+	+	+
	Camels									
Oman	Humans [53]						+			+
	Large ruminants [18]					+				+
	Small ruminants [18]						+			+
	Camels [18,53]						+			+
Palestine	Humans [54, 55]								+	+
	Large ruminants									
	Small ruminants [56, 57]								+	+
	Camels									
Qatar	Humans [58]								+	+
	Large ruminants									
	Small ruminants									
	Camels									
Saudi Arabia	Humans [59]								+	+
	Large ruminants [18]									+
	Small ruminants [18]								+	+
	Camels [60]								+	+

Table 1 (*cont*.)

		Microbiological evidence								
		B. abortus (biovar)					*B. melitensis* (biovar)			
Country	Species [reference]	1	2	3	7	9	1	2	3	Serological evidence
Syria	Humans [61]						+		+	+
	Large ruminants [61]					+				+
	Small ruminants [18, 61]						+		+	+
	Camels									+
UAE	Humans [62]						+	+	+	+
	Large ruminants						+			+
	Small ruminants [62, 63]						+	+	+	+
	Camels [63, 64]			+						+
Yemen	Humans [65]								+	+
	Large ruminants [66]									+
	Small ruminants [66, 67]								+	+
	Camels [66]								+	+

Table 2. *Summary of studies on human and ruminant brucellosis in the Middle East deemed as relevant and of sufficient quality to be included in this systematic review describing the country, year, and type of study, diagnostic tests used and the main outcomes obtained*

Country [ref.]	Year	Type	Species studied	Diagnostic tests used	Main outcome
Egypt [5]	2002, 2003	Population-based surveillance	Human	STA	Annual incidence
Saudi Arabia [68]	1988	Case-control	Human	STA, Coombs	Risk factors, OR
Yemen [69]	1991–1993	Case-control	Human	STA	Risk factors, OR
Iran [70]	2005	Case-control	Human	STA	Risk factors, OR
Egypt [71]	2003	Case-control	Human	RBPT, TAT	Risk factors, OR
Egypt [72]	2007	Case-control	Human	STA	Risk factors, OR
Jordan [73]	2013	Case-control	Human	RBPT, ELISA	Risk factors, OR
Jordan [45]	2000–2001	Cross-sectional	Sheep	RBPT, ELISA	TP
Egypt [74]	2008	Cross-sectional	Cattle, buffalo, sheep, goats	RBPT, CFT, iELISA	TP
Egypt [75]	2005–2008	Cross-sectional	Cattle, buffalo, sheep, goats	RBPT, CFT	TP
Egypt [75]	2009–2010	Cross-sectional	Cattle, buffalo	iELISA	TP

CFT, Complement fixation test; ELISA, enzyme linked immnunosorbent assay; iELISA, indirect ELISA; OR, Odds ratio; RBPT, Rose Bengal precipitation test; STA, standard tube agglutination; TAT, tube agglutination test; TP, true seroprevalence.

passed the quality assessment step by country is presented in Figure 3.

Frequency of brucellosis in humans

The frequency of brucellosis in humans was investigated in many of the Middle Eastern countries but only one study [5] fully met the quality criteria. The study described a population-based surveillance for patients with acute febrile illness in an Egyptian governorate and estimated an annual incidence of brucellosis at 64 and 70/100 000 population in 2002 and 2003, respectively.

Seroprevalence of brucellosis in ruminants

A considerable number of studies assessed the frequency of brucellosis in different ruminant sub-populations in the Middle East. Four studies met the inclusion criteria; three in Egypt and one in Jordan. Table 3 summarizes the findings of these studies. Sheep, goat, cattle and

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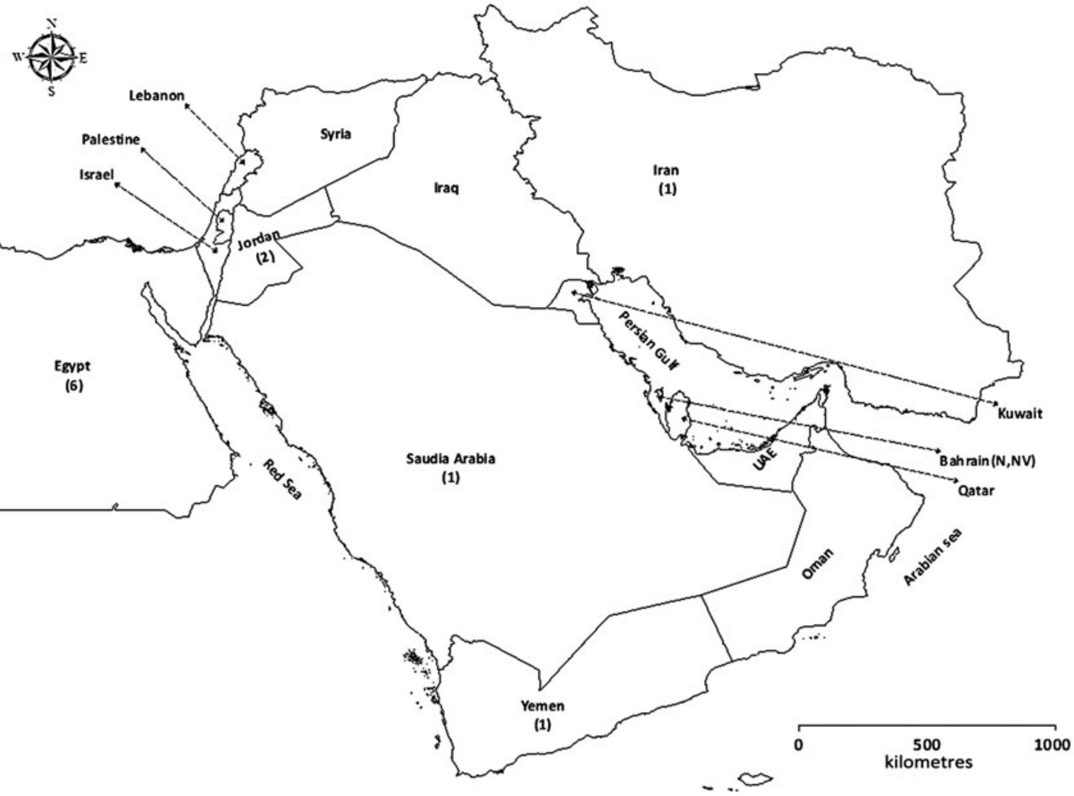

Fig. 3. Map of the Middle East showing countries with the number of studies per country deemed relevant and of sufficient quality to be included in this review in parentheses.

buffaloes were the studied species, and in all cases, frequency of infection was estimated as seroprevalence.

In Jordan, seroprevalence in Awassi sheep in the northern part of the country was estimated at 2·2% [95% confidence interval (CI) 0·5–3·5] and 45% (95% CI 32–58) at individual animal and flock levels, respectively, in 2000/2001 [45]. In Egypt, seroprevalence estimates for different livestock species are available for the Upper Egypt region and for the Kafr el-Sheikh Governorate (the largest governorate of the Nile Delta region). In Upper Egypt, true seroprevalence, after adjusting for imperfect test sensitivity and specificity, was estimated to be 1·16 (95% CI 1·05–1·27) in sheep, 0·44 (95% CI 0·34–0·54) in goats, 0·79 (95% CI 0·71–0·87) in cows and 0·13 (95% CI 0·08–0·18) in buffaloes. These estimates were obtained from a study using secondary data for the period 2005–2008 in seven governorates of Upper Egypt [74]. In the Kafr el-Sheikh Governorate, a study conducted in 2008 [75] estimated true prevalence to be 12·2% (95% CI 8·4–16·0) in individual sheep, 11·3% (95% CI 7·8–14·8) in individual goats, 41·3% (95% CI 26·1–56·7) in 'village flocks', 12·2% (95% CI 7·0–13·3) in milk tanks from cows and 11·3% (95% CI 7·8–14·8) in milk tanks from buffaloes. A small study conducted in one single village in another governorate of the Nile Delta (Menufiya) estimated that 11% (95% CI 3·06–18·4) of unvaccinated individual cows and buffaloes had detectable antibodies in milk and that 15·5% (95% CI 6·61–24·7) of households keeping cows or buffaloes had at least one positive animal [76].

Risk factors associated with human brucellosis in the Middle East

The review identified six studies that measured the strength of association between potential risk factors and human brucellosis in the Middle East. All of them were case-control studies and were conducted in Iran, Saudi Arabia, Yemen, Jordan and Egypt (two studies). Details of these studies are summarized in Table 4 and the studied risk factors are summarized below.

Consumption of dairy products

Generally, the consumption of unpasteurized dairy products was a statistically significant risk factor for seropositivity in the Middle East. In Iran [70] and Jordan [73] sheep-derived, products were posing the

Table 3. *Summary of studies investigating the seroprevalence of brucellosis in different ruminant sub-populations in the Middle East deemed as relevant and of sufficient quality to be included in this systematic review*

			True seroprevalence, % (95% CI)	
Country [ref.]	Level of study	Species	Individual level	Herd/flock level
Jordan [45]	Sub-national	Sheep	2·2 (0·5–3·5)	56 (44–69)
Egypt [74]	Governorate	Cattle	0·79 (0·71–0·87)	0·2 (0·16–0·23)
		Buffalo	0·13 (0·08–0·18)	
		Sheep	1·16 (1·05–1·27)	
		Goats	0·44 (0·34–0·54)	
Egypt [75]	Governorates	Cattle	12·2 (7·0–13·3)	15·1 (4·0–26·2)
		Buffalo	12·0 (7·1–13·0)	15·1 (4·0–26·2)
		Sheep	12·2 (8·4–16·0)	41·3 (26·1–56·7)
		Goats	11·3 (7·8–14·8)	32·2 (17·8–46·7)
Egypt [76]	Village	Cattle, buffalo	11·0 (3·1–18·4)	15·5 (1·4–27·9)

CI, Confidence interval.

Table 4. *Risk factors for human brucellosis: summary of case-control studies deemed as relevant and of sufficient quality to be included in this systematic review*

Country [ref.], study population	Risk factors (OR, 95% CI)
Saudi Arabia [68], 150 cases and 150 controls	Consumption of unpasteurized milk (OR 3·82, 95% CI 2·26–6·46), consumption of buttermilk (laban) (OR 3·1, 95% CI 1·2–7·6) and assisting with animal parturition (OR 3·6, 95% CI 2·2–6·1)
Yemen [69], 235 cases and 234 controls	Drinking fresh milk (OR 2·0, 95% CI 1·3–4·3), drinking laban (OR 2·7, 95% CI 1·7–4·2), occupation as farmer (OR 2·5, 95% CI 1·4–4·5), shepherd (OR 7·8, 95% CI 1·0–61) and microbiologist (OR 24·5, 95% CI 2·9–204)
Iran [70], 150 cases and 150 matched controls	Existence of another case of brucellosis in the home (OR 7·5, 95% CI 3·9–14·6) and consumption of unpasteurized dairy products (OR 3·7, 95% CI 1·6–8·3)
Egypt [71], 149 cases and 298 controls	Having sheep (OR 6·2, 95% CI 1·89–20·40), high-risk occupation (OR 4·4, 95% CI 1·4–14·5) and history of having an aborted animal (OR 3·5, 95% CI 1·3–9·1)
Egypt [72], 72 cases and 144 age-matched controls	Direct contact with goats (OR 3·2, 95% CI 1·2–8·7), occupations dealing with animals (OR 2·4, 95% CI 1·2–4·9) and eating ice cream from street vendors (OR 2·4, 95% CI 1·2–4·6)
Jordan [73], 56 cases and 247 matched controls.	Milking small ruminants (OR 3·5, 95% CI 1·5–8·4), consumption of raw feta cheese (OR 2·8, 95% CI 1·4–5·6), consumption of cows' milk (OR 0·4, 95% CI 0·2–0·8) and the consumption of boiled feta cheese (OR 0·4, 95% CI 0·2–0·8)

OR, Odds ratio; CI, confidence interval.

greatest risk. The study in Saudi Arabia [68] revealed that consumption of unpasteurized sheep and goat milk [odds ratio (OR) 3·8, 95% CI 2·2–6·4] and buttermilk (dairy product locally known as laban) (OR 3·0, 95% CI 1·2–7·6) were significant risk factors for seropositivity. In Egypt eating ice cream from street vendors increased the risk of seropositive status (OR 2·4, 95% CI 1·2–4·6) [71]. In Jordan, the consumption of raw feta cheese (OR 2·8, 95% CI 1·4–5·6) was significantly associated with brucellosis, while the consumption of pasteurized cows' milk (OR 0·4, 95% CI 0·2–0·8) and the consumption of boiled feta cheese (OR 0·4, 95% CI 0·2–0·8) decreased the risk of brucellosis [73].

Occupational exposure

Among exposures not associated with the foodborne route; contact with livestock, especially small ruminants, was a significant risk factor for *Brucella* spp. seropositivity in humans. Contact with sheep in Egypt (OR 6·2, 95% CI 1·9–20·4) and direct contact with goats in Alexandria, Egypt (OR 3·2, 95% CI 1·2–8·7) increased the risk of brucellosis [72].

Similarly, history of having an aborted animal (OR 3·5, 95% CI 1·3–9·1) in Egypt [71], assisting with animal parturition (in Jordan) (OR 3·6, CI 2·1–6·1) and milking small ruminants (in Jordan) were significant risk factors for seropositivity (OR 3·5, 95% CI 1·5–8·4) in humans [73].

Moreover, occupational contact with livestock and biological samples increased the risk of infection. Farmers (OR 2·5, 95% CI 1·4–4·5), shepherds (OR 7·8, 95% CI 1·0–61) and microbiologists (OR 24·5, 95% CI 2·9–204) were the groups at highest occupational risk of acquiring brucellosis in Yemen [69], and occupations dealing with animals in Alexandria, Egypt (OR 2·4, 95% CI 1·2–4·9) had significantly increased risk [72].

Finally, the existence of another case of brucellosis in the home in Iran (OR 7·55, 95% CI 3·9–14·6) was a major risk factor for seropositivity [70].

DISCUSSION

Brucellosis is considered endemic in most Middle Eastern countries where it is assumed to impose a considerable burden in terms of human disease and impaired livestock productivity [7, 10]. Our work aimed to systematically review available data regarding *Brucella* spp. presence and frequency estimates in humans and ruminants and associations between potential risk factors and human seropositive status in the Middle East. Although the primary search revealed 451 studies, after assessing their relevance, only 87 articles met the primary inclusion criteria and 49 of these provided evidence relevant for this review. Using strict quality criteria, only 11studies were deemed of sufficient quality to provide reliable seroprevalence estimates that could eventually be used to quantify the burden of brucellosis in the region or data to inform disease prevention programmes prioritizing populations based on specific risk factors.

Most studies were excluded due to incomplete or unclear description of the design, or a design that was unlikely to generate unbiased estimates, including prevalence studies carried out using non-probabilistic sampling, studies comparing seroprevalence in purposively selected subpopulations without consideration of potential biases and studies where clustering of individual animals within herds was ignored. Most of the articles deemed of sufficient quality were produced in the last 15 years (9 of 11) and the other two articles were produced in the 1990s; moreover, 7 of 11 articles were collaborative work between European or US and Middle Eastern researchers. This reflects the relatively modest and recent development of epidemiological research in the region and the importance of international collaboration.

Another critical issue with some studies was the use of diagnostic tests that are not recommended by the WHO/OIE for the host species being studied making the reliability of the obtained estimates questionable. Uncertainty with regard to the sensitivity and specificity of diagnostic tests being used hinders adjustment of observed apparent seroprevalence to obtain true seroprevalence estimates. Moreover, ignoring clustering of animals within the herd/flock during sampling will result in inaccurate estimates, given the use of imperfect diagnostics; herd specificity can be very low when several animals are tested in the same cluster and only one seropositive result is required for the herd to be classified as positive [77]. Diagnostic specificity may also be low due to cross-reactive bacteria or vaccination with smooth *Brucella* strains [78].

Lack of consideration of the vaccination status of the sampled animals was another reason why some studies were excluded, because vaccination of livestock against *Brucella* spp. is practiced in some Middle Eastern countries and the serological tests used were not able to differentiate between vaccinated and infected animals [22, 23] which can lead to overestimation of disease frequency. For example, two studies estimated the seroprevalence of brucellosis in sheep in Jordan; the first one was conducted in the northern governorates and reported seroprevalences of 2·2% (95% CI 0·5–3·5) and 56% (95% CI 44·0–69·0) at individual animal and flock levels, respectively [45]. The second was conducted in the southern governorates and reported seroprevalences of 37·6% and 47% (95% CI 29–52) at individual animal and flock levels, respectively, the vaccination status of the sampled flocks was not mentioned in the second study. As mentioned previously, they may also have an issue of low herd specificity in these studies.

Although a considerable number of studies did not pass the quality assessment in this review, they provided evidence for *Brucella* seropositivity in all the countries of the region and all host species (Table 1) with a few exceptions: lack of evidence of seropositivity in large or small ruminants in Bahrain (where there is serological evidence in humans) and lack of evidence of seropositivity in large ruminants in Lebanon and Palestine (where there is evidence of seropositivity in small ruminants and humans). However, Lebanon, Palestine and Bahrain have small populations of ruminants compared to other countries in the region [18]. Further, the recent wars and relative instability in Lebanon and Gaza, and the restriction of movement in the Palestinian territories may be related to this. This may also be hindering collaborative work for the control of brucellosis between Middle Eastern countries and research work with international institutions. Data extracted from these studies show that *B. melitensis* biovars 1, 2, 3 and *B. abortus* biovars 1, 2, 3, 7 and 9 were the most frequently isolated *Brucella* spp. in the majority of the Middle Eastern countries and recently, molecular work in Egypt has shown the presence of *B. suis* biovar 1 in milk and lymph node samples from cattle [25]. Frequent isolation of *B. melitensis* from cattle in the Middle East raises questions on the role of cattle in disease maintenance and transmission, which needs further investigation. Moreover, it highlights the role of mixing small and large ruminants which is practices in most of the Middle East countries [18].

Despite the scarcity of sound prevalence or incidence estimates, the review found serological evidence of *Brucella* spp. in humans in all countries (Table 1). Isolation of *B. abortus* from humans only in Israel could be the result of transmission from cattle to humans before it has been eliminated from the country after adapting vaccination programme [38].

Based on our inclusion criteria, only one study provides good quality estimates of the frequency of brucellosis in humans. This was a population-based surveillance implemented in Fayoum Governorate in Egypt in 2002 and 2003 [5]. Most studies concerning human infection consisted of case-series describing cases retrospectively using data from hospital records without a control group, therefore precluding the investigation of risk factors for infection. Other studies have investigated the prevalence among high risk subpopulations such as nomadic people or among patients who suffered manifestations compatible with infection such as women with miscarriage. Such studies were excluded when selection of individuals was not done probabilistically, although the estimates provided by these studies could be of use and in fact, have been included in a recently published review on human brucellosis, commissioned by the WHO [79]; the reason for their inclusion was to fill gaps in some countries to offer frequency estimates to be used in the calculation of DALYs for human brucellosis.

Studies estimating the frequency of brucellosis in humans in the Middle East often rely on the use of records of public hospitals and primary health centres. Such records depend largely on the clinical presentation of the disease rather than laboratory confirmation. Furthermore, a considerable number of cases do not seek medical care or may be referred to private health centres rather than official ones. As a result, such records would result in estimates that are unreliable. There is a need for population-based surveillance combining clinical presentations and laboratory confirmation [5].

Although the seroprevalence of brucellosis in ruminants has been intensively investigated across the Middle East, the current review identified only four studies of sufficient quality reporting seroprevalence in four ruminant sub-populations; sheep, goats, cattle and buffalos in two countries; Egypt and Jordan [45, 74–76].

The reported seroprevalence varied widely from country to country and even between regions within the same country. In Egypt, for example, the true seroprevalence at individual animal level in sheep was estimated at 1·16 (95% CI 1·05–1·27) in seven of Upper Egypt governorates, whereas it was estimated as 12·2% (95% CI 8·4–16·0) in one governorate of the Nile Delta. The results of Upper Egypt study [74] are similar to the results of the Jordanian study which reported a true seroprevalence of 2·2% (95% CI 0·5–3·5) at individual animal level [45]. At flock or village levels the relatively high reported true seroprevalence values for sheep in Egypt at 41·3% (95% CI 26·1–56·7) and in Jordan at 45% (95% CI 32–58) were thought to be the result of free uncontrolled movement of sheep flocks between villages, which facilitates contact between infected and susceptible animals [45, 74] and has implications for the likely success of control programmes. This finding is of high importance and supports the notion that brucellosis is widespread, at least in some Middle Eastern countries, with flock-level seroprevalence estimates which are among the highest when compared with endemic situations reported in other parts of the world. Moreover, animal movement between different countries in the region and the intense animal movement between the Horn of Africa and the Middle East for trading represent a challenge for the control and require more collaboration at the international level.

Available estimates suggest that brucellosis is endemic at high levels not only among small ruminants but also in bovine subpopulations in Egypt, and the reported seroprevalence in Egyptian cattle and buffalo herds varied between governorates [74–76]. This variation in the estimates could result from the heterogeneity of studied populations in terms of husbandry practices and livestock densities as well as different environmental conditions.

Risk factors for human seropositivity with *Brucella* spp. can be grouped into two main categories; direct contact with animals, particularly material from abortion or parturition, and consumption of contaminated milk and dairy products from infected animals. These high risk practices, coupled with lack of sufficient knowledge of the disease and absence of effective prevention strategies result in maintenance of the disease in the region [80]. Due to cultural and livestock management similarities in Middle Eastern countries it may be possible to extrapolate findings with regard to risk factors identified in one country to other countries. In Saudi Arabia and Yemen, consumption of raw milk and other dairy products appeared to be a greater risk factor for human seropositivity compared to direct contact with animals [68, 69]. Conversely, studies from Iran and Egypt identified direct contact with infected animals to be more significantly associated with seropositivity [70–72]. The reason for these differences in the identified risk factors can be attributed to the nature of the populations under study (e.g. urban *vs*. rural). The finding of microbiologists as a high-risk occupational group in a study in Yemen highlights the need of ensuring and promoting biosafety at the same time as diagnostic capacity. Brucellosis is one of the most common laboratory infections, particularly in developing countries, which may not have adequate regulations and enforcements regarding laboratory safety. Transmission can occur via sniffing plates, working with viable organisms outside the safety cabinet, not using protective equipment such as gloves and masks or ingesting suspensions of living organism during mouth pipetting. Developing standard procedures and training staff in good laboratory practices during handling of viable organisms or biological samples and the use of biosafety practices will help mitigate the risk of acquiring the infection [81].

Countries in the region are facing similar challenges: endemicity of one or more *Brucella* spp. in their ruminant populations, inconsistent vaccination practices and difficulties associated with the structure of the production systems and resources (e.g. lack of animal identification, precluding quarantine and movement control implementation). Furthermore, political turmoil, war, human displacement and competing needs for limited resources impede the implementation of national control programmes in some countries. As a result, much needed coordinated action against brucellosis in the region would be challenging.

Although the number of studies that fulfil the quality criteria of this review is small, data presented in these studies indicate that ruminant brucellosis is endemic at high levels in both small and large ruminants in some Middle Eastern countries, such as Egypt and Jordan. Sound epidemiological research is crucial to inform the design of realistic control programmes [82]. Unbiased estimates of frequency of infection are needed to assess whether elimination by means of test and slaughter is a realistic short-term objective or, conversely, large-scale vaccination is advisable to reduce the prevalence of infection to levels that make elimination more feasible. It has been shown that lack of a clear delineation between these two objectives (prevalence reduction *vs*. elimination) official control programmes has contributed to their erratic implementation in some of the Middle Eastern countries [83]. Furthermore, epidemiological evidence on the distribution of infection across geographical areas, production systems and affected ruminant species, ideally accompanied by the identification and characterization of circulating *Brucella* spp., can inform more targeted and effective surveillance and control efforts.

This review highlights the need for more detailed information on the frequency and distribution of infection and its associated burden to identify the most cost-effective options for control. However, based on available evidence it is likely that strategic vaccination of ruminant populations combined with sustained surveillance systems and public health education programmes may be the most appropriate control strategy. The lack of good quality estimates demonstrates the need for more comprehensive and well-designed epidemiological studies to bridge the current gap in brucellosis knowledge in the Middle East; this can be achieved through regional and international collaboration. At the regional level, competent authorities should develop sustainable surveillance systems, apply strict monitoring programmes on livestock movement and provide training programmes for both; veterinarians and provincial doctors in the region.

At the international level, technical and financial support should be directed to endemic areas in the world such as the Middle East.

CONCLUSIONS

Brucellosis remains a major public health problem in the Middle East and available evidence, although limited, supports this belief. Cases are likely to arise from subpopulations directly exposed to ruminants or from the consumption of unpasteurized dairy products from infected ruminants, with some ruminant subpopulations in the region showing among the highest seroprevalence levels compared to other endemic regions. Serological and microbiological evidence supports the widespread presence of *Brucella* spp. across the region. However, there is a lack of reliable estimates of the frequency of disease both in humans and livestock which precludes the formulation of multi-sectorial control policies. There is a need for well-designed observational studies that could generate reliable frequency estimates needed to assess the burden of disease and to inform disease control policies.

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DECLARATION OF INTEREST

None.

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