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Brucellosis – Regionally Emerging Zoonotic Disease?

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Aim To gain deeper insight into the seroprevalence of brucellosis, which remains a zoonotic disease of worldwide public health concern, by reviewing studies from countries including North Africa, the Middle East, and India.

Methods Studies on brucellosis performed in countries that are neighbors or important trading partners of the European Union and on trade animals and their products were analyzed. We reviewed 37 seroprevalence studies on brucellosis published from 1948 to 2009 retrieved from Pubmed, Google, and ScienceDirect.

Results The set of studies was heterogeneous in the number of samples and laboratory tests used. We included studies from Algeria (n = 1), Egypt (n = 7), India (n = 3), Iran (n = 3), Iraq (n = 1), Jordan (n = 5), Libya (n = 3), Saudi Arabia (n = 3), Syria (n = 1), Turkey (n = 5), and Yemen (n = 2). The total number of animals in these studies was 116 317 (cattle 75 375; buffalo 9644; sheep 10 550; goats 14 447; camels 6301). The prevalence of brucellosis in different animal species varied widely. Representative surveillance data have not recently been published in any of the countries.

Conclusions Wars in the Middle East, insufficient preventive measures, the lack of adequate control programs in some countries, as well as uncontrolled animal transportation through "open" borders increased the risk that brucellosis will spread in some regions. New seroprevalence data are needed urgently to evaluate the current situation and for continuous monitoring of necessary control programs.

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Brucellosis is one of the most important worldwide zoonoses affecting livestock and humans (1). *Brucellae* are facultative intracellular, Gram-negative coccobacilli that lack capsules, flagelle, and endospores. The genus *Brucella* comprises a group of closely related bacteria. The species *B. melitensis* (which infects sheep and goats), *B. suis* (swine), and *B. abortus* (cattle) cause significant economic losses for animal owners and severe human disease. *Brucella* spp. are also a focus of interest as they are categorized as biological agents due to their high contagiousness and their impact on human and animal health. The zoonotic pathogens *B. abortus, B. melitensis,* and *B. suis* were designated as select agents of Category B by the Centres for Disease Control in Atlanta, USA. This review analyzes studies from North Africa, the Middle East, and India to gain a clear picture of our current understanding of brucellosis and assess threats to transmission into the European Union (EU). The review also identifies areas where research is sorely needed to ensure that brucellosis epidemics are avoided in the future.

TRANSMISSION

Human brucellosis is transmitted by inhalation, animal contact, and consumption of unpasteurized dairy products and undercooked meat products. For example, consumption of traditional delicacies such as raw liver can cause human infection (2). In female animals, the bacterium is localized in the tissues of the udder and is then excreted via milk. In male animals, orchitis and epididymitis can lead to temporary or permanent infertility (3). *Brucella* spp. can survive for long periods in dust, dung, water, slurry, aborted fetuses, soil, meat, and dairy products. As the infectious dose is very low, infections are an occupational risk for farmers, veterinarians, abattoir workers, laboratory personnel, and others who work with animals and consume their products (4). The increase in business and leisure travel to brucellosis-endemic countries has led to importation of the disease into non-endemic areas (3). The prevalence of brucellosis in humans depends upon several factors such as dietary habits, methods of processing milk and milk products, husbandry practices, and environmental hygiene.

DIAGNOSIS

The "gold standard" in the diagnosis of brucellosis is bacterial isolation, which requires long cultivation periods and is often unsuccessful. Although several polymerase chain reaction assays have been developed, serological tests are still frequently used as diagnostic methods. The most commonly used serological screening tests are the serum agglutination test (SAT), Rose Bengal test, complement fixation test, and enzyme-linked immunosorbent assay (5). All tests have limitations concerning specificity and sensitivity, especially when testing individual animals. The SAT appears less sensitive and less specific than any other standard test for all animal species compared (6). Thus, the SAT is no longer recommended as an official screening test for brucellosis within the European Union (7).

EPIDEMIOLOGY

Brucellosis causes more than 500 000 human infections per year worldwide. The disease has a limited geographic distribution, but it remains a major public health problem in the Mediterranean region, western Asia, parts of Africa and Latin America. Brucellosis in animals causes tremendous economic losses due to abortion, premature birth, decreased milk production, and reduced reproduction rate. Despite the advances made in surveillance and control, the prevalence of brucellosis is increasing in many developing countries due to various sanitary, socioeconomic, and political factors (8). Brucellosis in cattle seems to be associated primarily with poor farm hygiene, unrestricted trade and movement of animals, use of local cattle yards and fairs for trading, the practice of returning non-lactating animals to villages for seasonal maintenance, and the use of semen from infected bulls of unknown health status for artificial insemination.

To review the literature on brucellosis seroprevalence, we retrieved studies published between 1948 and 2009 about brucellosis in cattle, buffaloes, camels, sheep, and goats. The studies were retrieved using Pubmed (http://www.ncbi.nlm.nih.gov/pubmed/), Google (http://www.google.com), and ScienceDirect (http://www.sienceddirect). This search was focused on countries in a belt spanning from North Africa to India. These countries are neighbors or important trading partners of the European Union and trade animals and their products among themselves. Table 1 and Table 2 list serological data provided by studies in selected countries.

The studies we retrieved were heterogeneous, especially in the number of samples and laboratory tests. Thus, a meta-analysis or comparison of these data sets is impossible. Nevertheless, all of the studies together indicate that the prevalence of brucellosis in different animal species in Mediterranean countries, the Middle East, and India varies widely. High prevalence appears to be due to insufficient

preventive measures and the lack of adequate control programs in some countries, as well as uncontrolled animal transportation across "open" borders. Due to armed conflicts and political instability in various countries, it is very likely that previously successful eradication programs have had no lasting effect and brucellosis has become a severe hygiene problem again. Therefore, there is an urgent need for the strict implementation of brucellosis eradication programs for cattle and small ruminants in affected regions. Given the huge economic and medical impact such control policies are cost-effective (16).

The impact of control programs and the consequence of their subsequent neglect can be demonstrated by the situation in Iran. The prevalence of animal brucellosis in Iran reached 44% in 1956 and decreased to only 5% following a control program starting in 1958. Because control measures became lax, the prevalence increased to 17.4% in 1977. A new control program was established in 1983 and the prevalence decreased again to 1.25% in 1987 and to 0.85% in 1991. Nevertheless, the number of human cases of brucellosis recorded in 1988 was 710 521 (132.4/100,000), suggesting that the low animal prevalence reported was not representative for the total animal population (45). More recent country-wide data are not available.

In Iraq, several studies on the seroprevalence of brucellosis have been conducted in the recent decades. The Northern provinces of Iraq share an extensive border with Iran, Turkey, and Syria. Other provinces of Iraq share borders with Jordan, Saudi Arabia, and Kuwait. This geographic situation highlights the need for a strategic planning of control measures. In fact, due to its geographical location, the prevalence of brucellosis in Jordan may be an indicator of the status of the disease in neighboring countries in the Middle East region (17). In many countries there is a lack

TABLE 1. Brucellosis prevalence in cattle and buffaloes based on a survey of studies published between 1948 and 2009*

Species/country	Year	Number of animals tested	Number of positive animals (%)	Diagnostic test	References
Cattle					
Algeria	2006	1032	9.7	BPAT	(9)
			8.2	RBPT	
Egypt	2007	1966	5.4	BPAT	(10)
India	1998	23 284	1.9	SAT	(11)
	2000	110	1.81	Culture	(12)
	2007	150	20.7	ELISA	(13)
Iran	1990	6472	3.9	MRT	(14)
	2002-2006	12 113	6.8	RBPT	(15)
Jordan	1973	1064	0.0	MRT	(16)
	1977	250	0.4	MRT	(16)
	2009	671	10.1	RBPT	(17)
	2009	671	10.1	ELISA	
Libya	1985	3753	0.3	RBPT	(18)
	1986	8607	1. 5	SAT	(19)
			1.8	CFT	
Syria	1989	12 554	2.9	RBPT, CFT	(20)
Turkey	2004-2006	407	32.9	RBPT	(21)
	2001-2006	626	35.3	RBPT	(22)
			39.5	ELISA	
Yemen	1992-1993	1645	0.1	ELISA	(23)
Buffaloes					
Egypt	2007	916	1.1	RBPT	(24)
	2007	1337	3.5	RBPT	(10)
India	1998	7153	1.8	SAT	(11)
	2000	43	0.0	Culture	(12)
	2007	195	16.4	ELISA	(13)

*Abbreviations: BPAT – buffered acidified plate agglutination test; RBPT– Rose Bengal plate test; CFT – complement fixation test; SAT – standard tube agglutination test; RIV – Rivanol test; ELISA – enzyme linked immunosorbant assay; MRT – milk ring test; BCT – Brewer card test.

of recent data about the seroprevalence of brucellosis and further studies are required to assess the epidemiological situation.

The prevalence of brucellosis in animal reservoirs is the key to its control in humans. Eradication programs for bovine brucellosis markedly reduce the incidence in humans (46).

TABLE 2. Brucellosis in sheep, goats, and camels*

Species/country	Years	Number of animals tested	Number of positive animals (%)	Diagnostic test	References
Sheep					
Egypt	2007	32	31.3	SAT	(25)
			25.6	RBPT	
	2007	813	5.4	BPAT	(10)
Iraq	1979	2368	0.9	BCT	(26)
India	2000	163	2. 5	Culture	(12)
Jordan	1992	206	16.5	Culture	(27)
	2003	602	14.3	RBPT	(28)
			7.2	ELISA	
Syria	1989	1827	1.8	RBPT, CFT	(20)
Turkey	2002-2004	37	38.0	Culture	(29)
	2007	167	40.1	SAT	(30)
	2008	400	36.7	SAT	(31)
		400	35.5	RIV	
		400	34.8	RBPT	
		400	33.8	CFT	
Yemen	1985	690	0.6	RBPT	(32)
	1992-1993	2045	0.6	ELISA	(23)
Goats					
Egypt	2007	33	3.5	BPAT	(33)
India	2000	115	2.6	Culture	(12)
	2004	54	59.0	Serological test	(34)
	2004	54	88.8	PCR	
Iraq	1979	3156	4.4	BCT	(27)
	2007	184 of which:		RBPT	(35)
		25 vaccinated	72.0		
		17 aborted	52.9		
		142 unvaccinated	20.4		
Jordan	2001-2003	1100	27.7	RBPT	(36)
Iran	2002-2006	7199	3.4	RBPT	(15)
Yemen	1985	538	0.4	RBPT	(32)
	1992-1993	2014	1.3	ELISA	(23)
Camels					
Egypt	1948	200	20.0	SAT	(37)
	1993	360	11.6	SAT	(38)
	2004	766	8.7	RBPT	(39)
Iran	2007	1123	10.5	RBPT	(40)
			8.5	MRT	
Libya	1993	967	4.1	RBPT	(41)
Saudi Arabia	1987	146	1.4	RBPT	(42)
	1992	2536	8.0	RBPT	(43)
	1999-2000	98	7.1	RBPT	(44)
Yemen	1992-1993	105	0.0	ELISA	(23)

*Abbreviations: BPAT – buffered acidified plate agglutination test; RBPT– Rose Bengal plate test; CFT – complement fixation test; SAT – standard tube agglutination test; RIV – Rivanol test; ELISA – enzyme linked immunosorbant assay; MRT – milk ring test; BCT – Brewer card test.

Worldwide reported incidence of human brucellosis in endemic areas varies widely, from <0.01 to >200 per 100 000 inhabitants. However, the true incidence of human brucellosis is unknown due to misdiagnosis, underreporting, lack of proper laboratory facilities in remote areas, as well as poor cooperation and exchange of information between veterinary and public health services (47).

CONTROL MEASURES

The initial aim of surveillance and control programs is the reduction of infection in the animal populations to reduce the effect of the disease on animal health and production, thus minimizing its impact on human health. Within the European Union, measures for the eradication of brucellosis are contained in Directive 2003/99/EC of the European Parliament. The epidemiological situation in its neighboring regions is of great importance for the EU due to the potential risk of importation of infected animals or their products (48).

An effective control of animal brucellosis requires the following elements: 1) surveillance to identify infected animal herds, 2) prevention of transmission to non-infected animal herds, and 3) eradication of the reservoir to eliminate the sources of infection in order to protect vulnerable animals or herds coupled with measures to prevent re-introduction of the disease. In areas where a brucellosis-free status has been established or where such a status is assumed from epidemiological data, the risk of importing the disease by means of animal movement must be eliminated. Movement of infected animals must be prohibited and import permissions should be given only to certified brucellosis-free farms or areas. This is also true for national and international transport of animal products, in accordance with the general principles and procedures specified in the International Zoo-Sanitary Code of the OIE (49). This code also describes the testing procedures for animals and quarantine measures. Control programs should take into account incidental spreading of brucellosis by infected but serologically negative animals originating from inadequately certified sources.

Vaccination of animals practically eliminates the clinical signs of brucellosis and reduces the likelihood that exposure to the infectious agent will cause disease in humans. In small ruminants the initial step in brucellosis control is to vaccinate young animals (kept as replacements) with the *B. melitensis* Rev.1 vaccine. This approach is based on the hypothesis that the Rev.1 vaccine offers life-long immunity and that after implementing the vaccination program for 5-7 years, which is the reproductive life-span of sheep and goats, the whole population will be vaccinated and fully protected against brucellosis. This method is also recommended to minimize postvaccinal diagnostic problems and to prevent abortion (50).

The *B. melitensis* Rev.1 vaccine for small ruminants has not been fully evaluated for use in cattle. *B. abortus* vaccines do not effectively protect against *B. melitensis* infection, meaning that bovine *B. melitensis* infections may pose a serious problem even for vaccinated cattle. In India, culling of cows is a taboo which also complicates eradication efforts.

IMPLICATIONS FOR THE FUTURE

There is a clear need for new studies on the seroprevalence of brucellosis in animals in many countries, as the published studies are scarce, some are more than 20 years old, and they use different laboratory tests, making the data sets impossible to compare. Only the initiation of continuous monitoring programs will allow an evaluation of the current status of brucellosis seroprevalence and the effectiveness of control measures.

In addition, it will be nearly impossible to control this important zoonosis without reimbursement of farmers for their financial losses due to removal of infected animals as part of an effective herd and individual animal registration system. Farmers, the dairy industry, breeding companies, consumers, veterinarians, and politicians in each country must work together to find a suitable eradication strategy. Trade across borders will perpetuate the epizootic unless countries in the same region of the world coordinate their control and eradication programs.

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