Review

Animal brucellosis in Egypt

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Abstract

Brucellosis is a highly contagious zoonosis that affects the public health and economic performance of endemic as well as non-endemic countries. In developing nations, brucellosis is often a very common but neglected disease. The purpose of this review is to provide insight about brucellosis in animal populations in Egypt and help to understand the situation from 1986 to 2013. A total of 67 national and international scientific publications on serological investigations, isolation, and biotyping studies from 1986 to 2013 were reviewed to verify the current status of brucellosis in animal populations in Egypt. Serological investigations within the national surveillance program give indirect proof for the presence of brucellosis in cattle, buffaloes, sheep, goats, and camels in Egypt. Serologic testing for brucellosis is a well-established procedure in Egypt, but most of the corresponding studies do not follow the scientific standards. *B. melitensis* biovar (bv) 3, *B. abortus* bv 1, and *B. suis* bv 1 have been isolated from farm animals and Nile catfish. Brucellosis is prevalent nationwide in many farm animal species. There is an obvious discrepancy between official seroprevalence data and data from scientific publications. The need for a nationwide survey to genotype circulating Brucellae is obvious. The epidemiologic situation of brucellosis in Egypt is unresolved and needs clarification.

Key words: brucellosis; biotyping; Egypt; isolation; seroprevalence.

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Introduction

Brucellosis is caused by bacteria of the genus *Brucella*. Brucellae are small Gram-negative, non-motile, non-spore forming, aerobic, facultative intracellular coccobacilli capable of invading epithelial cells, placental trophoblasts, dendritic cells, and macrophages [1]. The genus includes 10 nomo-species based on their different host specificity [2]. The six classical species are *B. melitensis* biovar (bv) 1–3, mainly isolated from sheep and goats; *B. abortus* bv 1–6 and 9, primarily isolated from cattle and buffaloes; *B. suis* bv 1–3, mainly isolated from pigs, bv 4 from reindeer and bv 5 isolated from small ruminants; *B. canis* isolated from dogs; *B. ovis* isolated from sheep; and *B. neotomae* isolated from desert wood rats [3]. Recently, four new species have been described. Two are of marine origin (*B. pinnipedialis* from seals, and *B. ceti* from dolphins and whales). *B. microti* was isolated from the common vole *Microtus arvalis* [4]. Finally, *B. inopinata* was isolated from a breast implant wound of a female patient [5].

Brucellosis, caused by *B. melitensis, B. abortus, B. suis* (except bv 2) and in rare cases *B. canis*, is a highly contagious and zoonotic disease affecting livestock and humans worldwide. In animals, brucellosis causes tremendous economic losses [6]. The disease provokes abortion, stillbirth, mastitis, metritis, and placental retention in females and orchitis and arthritis in males. Infertility may be seen in both sexes. The true incidence of human brucellosis is not easy to estimate globally, but an estimated 500,000 persons are newly infected every year [7]. The World Health Organization considers brucellosis a neglected zoonosis and classifies Brucellae as risk group III agents because they can be easily transmitted via aerosols [8]. Airborne transmission of *B. melitensis* infection has been previously described [9], and Brucellae have previously been used as biological agents in weapons of mass destruction [7].

Brucella in Egypt

It is likely that brucellosis has been an endemic disease in Egypt for thousands of years. For example, there is evidence in 5.2% of bone remnants from ancient Egyptians (750 BCE) of sacroiliitis in pelvic bones, and evidence of spondylitis and osteoarticular lesions have also been found, both common complications of brucellosis [10]. In 1939, brucellosis was reported in a scientific report from Egypt for the first time [11]. Since then, the disease has been detected at high levels among ruminants, particularly in large intensive breeding farms (Refai, personal communication, 20.07.2013). Consequently, a control program including serological surveys and voluntary vaccination of ruminants was established in the early 1980s [12].

Indirect techniques regularly used in diagnosis of *Brucella* are field tests such as the milk ring test (MRT), serological tests such as the standard agglutination test (SAT) and buffered agglutination test, which are confirmed by the complement fixation test (CFT) and enzyme-linked immunosorbant assay (ELISA) [13]. Serological diagnosis of Brucellae currently relies mainly on the detection of anti-*Brucella* lipopolysaccharide (LPS) antibodies. In *B. melitensis*, *B. abortus*, and *B. suis*, the LPS is smooth (containing an O-polysaccharide); *B. canis* isolates lack the O-polysaccharide and are considered rough. However, these tests cannot differentiate antibodies originating from vaccine or wild-type strains. The tests are also prone to false-negative and false-positive reactions, the latter caused by cross-reactions with LPS of other Gram-negative bacteria [14].

Isolation of Brucellae is still the gold standard for diagnosis; however, this method often fails due to the delays in symptoms, resulting in incorrect sample types and low bacterial loads in specimens such as blood, milk, or tissue. Biotyping of isolates involves evaluation of a combination of growth characteristics (colonial morphology, oxidase, urease, CO_2 requirement, H_2S production, growth in presence of the dyes fuchsin and thionin), lysis by bacteriophage (Tiblisi and R/C), and agglutination with monospecific A, M, and R anti-sera [2,15]. Although various polymerase chain reaction (PCR) assays have been created to diagnose Brucellae at the species level (*e.g.*, the Abortus, Melitensis, Ovis, Suis AMOS PCR), these assays are most useful when applied to DNA extracted from a positive culture.

A comprehensive, evidence-based assessment of current literature and of officially available data on animal brucellosis is missing for Egypt. The aim of this review is to provide insight regarding brucellosis in Egypt over the last 27 years and to assist observers interested in Brucellosis to more fully understand the situation in Egypt.

Literature search and data collection

National and international publications on serological investigations and on typing studies of brucellosis from 1986 to 2013 were obtained through PubMed, Science Direct, Google, and from Egyptian university libraries such as The Egyptian National Agricultural Library (ENAL) and the Federation of Egyptian University Libraries. The following search terms were used: brucellosis in Egypt, *Brucella* infection in Egypt, *Brucella* in animals in Egypt, and animal brucellosis in Egypt. Theses dealing with brucellosis available from Egyptian universities were included in this study (1986–2013). The libraries were personally visited or contacted via e-mail. Reports on brucellosis from the General Organization of Veterinary Services in Egypt (GOVS) from January 2006 through December 2011 were investigated. Studies dealing with human infection were excluded.

A full text analysis of each publication was done by at least two reviewers. Publications describing serological investigations were included even if statistical analyses were not sound to avoid loss of data. Publications on cultivation, bio- and genotyping or PCR analyses were included only if state-of-the-art techniques could be verified by the respective material, and if the methods sections and results were clear. To clarify ambiguities, the authors were first contacted by e-mail or phone. If the authors could resolve those ambiguities, the publications were accepted for further assessment. The following data were extracted from the manuscripts, reports, or theses: seroprevalence for brucellosis in host species populations and regional distribution, prevalence of Brucellae in animals or food proofed, and identification of isolates.

Data acquisition

A total of 25 scientific papers on seroprevalence [6,12,16-38] and 18 on isolation of Brucellae [11,16,17,20,22,25,26,29,31,33-35,38-43] were identified by online search. Local scientific papers and 10 theses were obtained from Egyptian universities; 28 of them dealt with seroprevalence [44-71] and 16 dealt with isolation of Brucellae [44,45,48-51,53-55,58,68,72-77]. The official data collection of the General Organization of Veterinary Services (GVOS) was evaluated for the years 1999 to 2011. Two

publications on serology [31,38] and nine on isolation of Brucellae [17,20,35,38,39,41,48,55,58] were finally excluded from evaluation because ambiguities were identified within the materials and methods sections and the authors could not be contacted to resolve these ambiguities.

Serological investigations

Information on serological investigations was provided by the General Organization of Veterinary Service (GOVS), Cairo, Egypt, as official reports from 1999 to 2011. Screening with the Rose Bengal plate agglutination test (RBPT) and Rivanol test followed by confirmatory CFT in screening test-positive animals is the approved technical procedure of the official control program. This procedure is in accordance with the procedures proposed in the World Organisation for Animal Health (OIE) manual of standard diagnostic tests and vaccines. Serological investigations within the national surveillance program give indirect proof for the presence of brucellosis in cattle, buffaloes, sheep, and goats in 22 of 27 governorates. Ismailia, Red Sea, North Sinai, South Sinai, and Matroh did not report seropositive animals. The total number of animals steadily increased during the reporting time (Figure 1). Sheep and goats had a higher seroprevalence than did cattle and buffaloes (Table 1). Peaks were seen in 2002/2003 and 2008/2009/2010 (Figure 2). The number of animals tested was always very low when compared to the total number of animal stocks in Egypt according to the Food and Agriculture Organization (FAO) registers (Table 1). Sampling plans were not made available. It cannot be excluded that sampling is biased; therefore, only tendencies should be read. Based on this data, it can be concluded that brucellosis is present in all governorates in cattle, buffaloes, goats, and sheep. The lowest total percentage of seropositive animals was recorded in 2011 with 0.33%. In 2011, the riots and civil commotions of the Arab Spring lead to a depletion of state resources, resulting in low numbers of animals tested, a decrease of the reimbursement funds for owners, and increased animal movement within villages and governorates.

A total of 53 scientific publications and theses on serological investigations were selected for review. Serological studies were made in Qalyobia, Menufiya, Gharbia, Behira, Alexandria, Kafrelsheikh, Dakahlia, Sharkia, Giza, Fayoum, Beni-Suef, El-Minia, Assuit, New Valley, Sohag, Qina, Luxor, and Aswan in bovines, small ruminants, camels, and Nile catfish, rendering positive results. Assuit, Menufiya, Kafrelsheikh, Giza, and Behira have been studied very well; they have been included in more than five investigations (Supplementary Table 1). Most studies were made in response to clinical events such as notice of late abortion, elevated levels of insemination, and mastitis. As such, these studies do not comply with the standards for epidemiological investigations concerning study design or biostatistics. However, they show that in infected animal herds, the prevalence rate may be high independent of the animal species (1%–100%). In cross-sectional studies, approximately 15% of households in a study area kept animals and within a herd, up to 15% (cattle and buffaloes) or even more (sheep and goats) animals could be expected to be seropositive [6,19,32].

Figure 1. Total number of animals in Egypt, 1999–2011 (FAO, 2013).

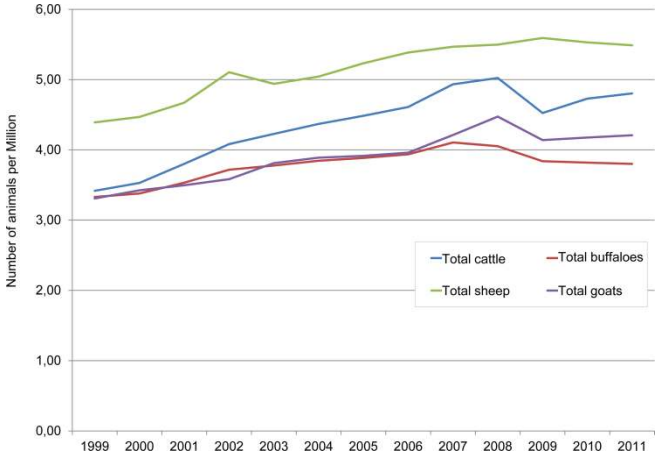

Figure 2. Number of seropositive animals according to the General Organization of Veterinary Service (GOVS, 2012).

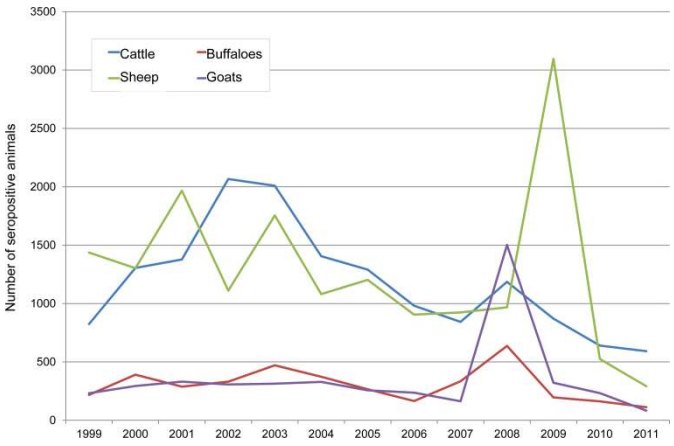

Table 1. Prevalence of brucellosis in Egypt from January 1999 through December 2011 based on reports from the General Organization of Veterinary Services

	Cattle				Buffalo				Sheep				Goat				Total	
Year	Total no. in Egypt	No. tested	No. +ve	% +ve from tested	Total no. in Egypt	No. tested	No. +ve	% +ve from tested	Total no. in Egypt	No. tested	No. +ve	% +ve from tested	Total no. in Egypt	No. tested	No. +ve	% +ve from tested	Total tested	% +ve from tested
1999	3,417,580	108,622	824	0.76	3,329,700	62,900	218	0.35	4,390,730	62,151	1,437	2.31	3,308,150	17,875	232	1.30	251,548	1.08
2000	3,529,720	145,750	1,305	0.90	3,379,410	66,109	391	0.59	4,469,130	68,342	1,303	1.91	3,424,760	16,685	294	1.76	296,886	1.11
2001	3,801,070	152,436	1,378	0.90	3,532,240	81,302	288	0.35	4,671,240	78,310	1,967	2.51	3,497,000	21,912	331	1.51	333,960	1.19
2002	4,081,000	162,309	2,067	1.27	3,717,000	67,802	331	0.49	5,105,000	99,466	1,111	1.12	3,582,000	23,560	307	1.30	353,137	1.08
2003	4,227,000	168,281	2,009	1.19	3,777,000	67,588	471	0.70	4,939,000	79,565	1,755	2.21	3,811,000	29,576	314	1.06	345,010	1.32
2004	4,369,000	154,984	1,406	0.91	3,845,000	56,041	373	0.67	5,043,000	68,122	1,081	1.59	3,889,000	25,719	329	1.28	304,866	1.05
2005	4,485,000	174,673	1,291	0.70	3,885,000	69,931	266	0.38	5,232,000	69,571	1,203	1.73	3,915,000	25,325	257	1.01	339,500	0.87
2006	4,610,000	199,954	982	0.49	3,937,000	61,595	165	0.27	5,385,000	71,929	905	1.26	3,960,000	26,689	237	0.89	360,167	0.64
2007	4,932,660	161,206	843	0.52	4,104,810	68,548	334	0.49	5,467,470	68,171	924	1.36	4,210,710	33,791	163	0.48	331,716	0.68
2008	5,023,160	182,248	1,186	0.65	4,052,650	59,080	637	0.40	5,498,030	106,215	968	0.91	4,473,490	46,703	1502	3.22	294,246	0.99
2009	4,524,950	175,750	871	0.50	3,838,720	51,924	196	0.38	5,591,850	84,798	3,095	3.65	4,139,260	44,023	322	0.73	356,495	1.25
2010	4,728,720	183,490	640	0.30	3,818,240	53,783	162	0.30	5,529,530	66,412	525	0.79	4,174,990	39,143	233	0.60	342,828	0.5
2011	4,803,000	167,188	592	0.35	3,800,000	55,986	112	0.20	5,488,000	65,849	292	0.44	4,207,400	31,772	83	0.26	320,795	0.33

Table 2. Origin of *Brucella* isolates in Egypt

	B. melitensis					*B. abortus*				*B.suis*
Location	*B. melitensis*	bv3	bv2	bv1	rev.1	*B. abortus*	bv1	bv3	bv7	*B. suis* bv1
Cairo		[49,50,73]					[49]			
Qalyobia		[22,49,50,73]					[49]			
Menufiya	[76]	[22,26,33,34,44,49, 73]	[73]		[33]		[49]	[44]	[44]	
Gharbia		[26,34,49,73]	[73]				[49]			
Behira		[20,22,26,34,49,73]					[49]			
Alexandria		[22,49,73,74]					[49]			
Kafrelsheikh		[17,34,44, ,48,50,49,73,74]					[49]	[44]	[44]	
Demiatta		[49,73]					[49]			
Dakahlia		[34,50]					[74]			
Sharkia		[29,41, 49,73]					[49,77]	[77]		
Suez		[49,73]					[49]			
Ismalia	[42]									
Port-Said		[49,73]					[49]			
Matroh		[73]								
Giza	[16,42]	[22,25,49,50,73]		[73]			[25,49]			
Fayoum		[26,44,49]				[54]	[49]	[44]	[44]	
Beni-Suef	[16,40]	[22,44,73]				[40]		[44]	[44]	
El-Minia		[55,73,74]								
Assiut		[22,31,35,49,72,73]					[49]			
Sohag	[16]	[26,73]								
Qina		[73]								
Aswan		[26]								
Different locations in Egypt		[39,43,44,51,53,75]					[53]	[51, 75]	[44,51,53,58,75]	[68]

Data obtained by sampling animals in slaughterhouses have to be considered biased, as brucellosis-seropositive animals ought to be slaughtered by law. Studies on camels (n=12) demonstrated a high seroprevalence in these animals. It should be noted that camels are imported from Sudan, where brucellosis is endemic.

The prevalence of brucellosis in cattle, buffaloes, sheep, and goats was generally higher in Beni-Suef governorate than in other governorates in upper Egypt [11,22]. In the Delta region, the highest prevalence was reported in Behira governorate. Inadequate preventive measures and uncontrolled transport between Egyptian governorates to and from animal markets may play an important role in the incidence of brucellosis.

Culture and biotyping

Isolation of *Brucella* is still the gold standard for brucellosis diagnostics, but it has several drawbacks such as hands-on time and low sensitivity, especially in chronic cases. Handling of culture material poses a high risk of infection to the operator. Our analysis shows that this technique is restricted to a few laboratories in Egypt. A total of 35 publications on isolation or biotyping of Brucellae were selected for review. In general, these studies were done within outbreak investigations. Most authors of theses described the techniques used very clearly and comprehensively so that results could easily be checked for plausibility. Strains isolated were regularly determined by investigating CO_2 requirement, H_2S production, growth in the presence of thionin and basic fuchsin dyes, agglutination test with monospecific A and M antisera, and phage lysis test. In contrast, only 15 articles published between 1986 and 2012 followed the complete method of biotyping. *Brucella* strains were isolated from milk, blood, vaginal discharge, and aborted fetuses of infected cattle, buffaloes, sheep, goats, and camels [22,25,72,73], and also from organs including liver, spleen, lung, kidneys, heart, and lymph nodes [22,40,55]. The rationales for sampling, sampling strategy, or statistics of sampling were missing. Hence, the presence of *B. melitensis* bv 1, 2, 3 and *B. abortus* bv 1, 3, and 7 was unambiguously demonstrated. *B. melitensis* bv 3 is the predominant pathovar isolated independent from the host species and bv 1 and 2 were described in a single study in 2004 only. Isolates of *B. melitensis* originated from all farm animal species and also from rats. Vaccine strain Rev. 1 was isolated from ewes in Menufiya in 2007. Only 12 publications describe the presence of *B. abortus* in Egypt; bv 3 was found by four author groups in 1986, 1987, and 1990. Five publications also mentioned bv 7, which was later on removed from the nomenclature list as being erroneous. The presence of *B. abortus* bv 3 has yet to be confirmed. Isolates were obtained from cattle and buffaloes and the erroneous *B. abortus* bv 7 was obtained from a camel one instance. Human pathogenic *B. suis* bv 1 was isolated from pigs in 1996. No Brucellae isolates exist from Red Sea, New Valley, Luxor, North Sinai, or South Sinai. All data are shown in Table 2.

Isolation of *B. melitensis* from cattle and buffaloes was attributed to mixed rearing of sheep and goats with cattle or buffaloes on holdings or in one flock, contamination of pastures by infected sheep and goats, and spreading of disease by these animals to new areas [22]. However, no proof for this assumption was made via genotyping of strains or tracing back investigations. Alarming is the fact that *B. melitensis* bv 3 was also isolated from 4 out of 65 semen samples from bulls (6.2%) and 3 out of 55 (5.5%) samples from rams, respectively, at the Animal Reproduction Research Institute, Giza [43]. Venereal transmission may be responsible for maintaining a bovine brucellosis cycle based on unhygienic serving methods (*i.e.*, that one bull serves cows of various holdings in different neighboring villages). As a consequence, artificial insemination and semen collection have to be done under strict precautions.

Molecular diagnostics

Because of the shortcomings of culture, the use of new diagnostic techniques for the direct detection of Brucellae was attempted, although no biovar-specific PCR assays exist. Authors of only 15 publications from 1986 to 2012 used PCR. The sensitivity of PCR proved to be higher than cultivation [78], and even small numbers of Brucellae were detected in samples [25]. *B. melitensis* DNA was found in the semen of bulls and rams [43] and in the milk of cattle, buffaloes, sheep, and goats in Menufiya, Gharbia, Behira, Fayoum, Aswan, Beni-Suef, and Sohag governorates [16,26]. Montasser *et al*. and Zahran found DNA of *B. melitensis* in tissue samples of cattle, sheep, and goats in Assiut and El-Minia governorates, respectively [35,55]. *B. abortus* DNA was detected and identified in Fayoum governorate from seropositive cattle [54]. In Menufiya governorate, the use of PCR restriction fragment length polymorphism (PCR-RFLP) identified four strains of *B. melitensis* bv 3 and two strains of *B. melitensis* Rev. 1 vaccine in tissue samples collected

from six seropositive ewes [33]. The first comprehensive report describing the presence of *B. melitensis* DNA in camel milk dates back to 2002 when it was amplified from a milk sample from Giza governorate [25]. *B. melitensis* DNA was found again in Aswan and Sohag governorates in both milk and serum of camels [26]. PCR is a sensitive tool for the diagnosis of brucellosis. Recently, Wareth *et al* identified *B. abortus* and *B. melitensis* DNA in bovine milk collected from apparently healthy animals by species-specific IS711 RT-PCR [79]. These results highlight a special public health hazard for farmers and nomadic peoples who encourage the drinking of raw milk from camels as they believe that it has a soothing and therapeutic effect against digestive tract diseases and liver infections [78].

Environmental contamination with Brucellae

Significant environmental contamination has to be assumed due to local husbandry methods and the lack of effective carcass disposal. Nile catfish have been found to be infected with *B. melitensis*, especially in small tributaries of Nile canals in the governorates of Kafrelsheikh, Menufiya, Gharbiya, and Dakahlia in the Nile Delta region. It was isolated from 5.8%, 4.2%, 5.8%, and 13.3% of liver, kidney, spleen samples and skin swabs, respectively; it was not isolated from samples of farmed fish [34]. It is speculated that disposal of animal waste (carcasses, milk, aborted and parturition materials) into the Nile or its canals plays an important role in the transmission of *Brucella* and is also the reason for the high incidence in these regions. Farmers also wash their animals in these canals or try to reduce the body temperature of diseased animals in the Nile, which may contribute to spreading of Brucellae. Moreover, *B. melitensis* bv 3 was also isolated from rats [44]. Only one study reported Brucellae in fish. This fact is interesting and should be investigated further in the future. The presence of Brucellae in rat and fish indicates high environmental contamination, which is alarming.

Surveillance program

Despite 30 years of work and efforts of the General Organization of Veterinary Services to overcome brucellosis in Egypt by testing female cattle and buffaloes older than six months of age and slaughtering serologically positive animals, the vaccination of calves with *B. abortus* S19 and adults with BR51 vaccines and small ruminants with *B. melitensis* Rev 1 vaccine [11], the results are disappointing and brucellosis is still endemic among humans and ruminants in Egypt. Modeling of the currently applied measures suggests that, at best, 4% of the animal stocks (but not more than 5%) are included in the control program [80]. Our data implies that even this number is overestimated. Several authors proposed that, hotspots are located in the Delta region and in upper Egypt, along the River Nile and south of the Delta containing 32% of the Egyptian large ruminant and 39% of the small ruminant stocks which are often kept in small mixed herds owned by single households [81]. The assumption of hotspots needs further confirmation. A simple sampling bias might be seen. Various authors linked the limited success of the control program to improper diagnosis and spreading of the disease at large animals markets where different animal species of unknown health status from different towns and governorates intermix. Additionally, small ruminant flocks present in high numbers in Egypt are highly migratory [22]. Low compensation for owners results in slaughtering of only 0.2% of seropositive animals [18]. Emotional attachment of owners to animals that they had kept for long time may also be a reason for their unwillingness to slaughter seropositive animals [82].

Summary

In summary, it can only be assumed that brucellosis is prevalent nationwide in all farm animal species, in the environment, and in carrier hosts such as rats. The predominant occurrence of *B. melitensis* bv 3 in bovines is in contrast to Egyptian reports published before 1980 which had described the classic epidemiology of brucellosis with *B. abortus* in cattle and buffaloes and *B. melitensis* in small ruminants, respectively. The question must be raised whether a *B. melitensis* clone was able to cross species barriers and was able to establish a permanent reservoir in cattle and buffaloes. A husbandry system favoring mixed populations of cattle, buffaloes, sheep and goats, limited success of the official control program due to unrealistic high sampling numbers, and poor compliance of livestock farmers has contributed to the emergence of brucellosis in Egypt [18]. The need for a nationwide survey to genotype circulating Brucellae is obvious. Thus, the epidemiologic situation of brucellosis in Egypt is cryptic and needs clarification. Consequently, cultivation and biotyping of *Brucella* isolates has to be made available for all governorates to monitor the effect of control programs and to trace back outbreaks. Future seroprevalence studies must meet scientific standards. The current control program is ineffective and a new strategy to combat brucellosis

has to be developed, tailored for the parlous situation of Egypt farmers.

The need for an efficient animal registration and marking system is obvious. The sale of *Brucella*-infected animals in the open market is increasing in Egypt. The introduction of a *Brucella*-infected animal into a herd can lead to spread of the infection to the whole herd, causing economic losses. Markets should be controlled by veterinarians and compensation for those selling animals should be satisfied to prevent infected animals from being sold [83]. Slaughter has to be replaced by culling and safe disposal of carcasses to avoid human infection or pollution of the environment. The measures of the control program have to be made mandatory, and a reasonable system of compensation has to be implemented to enhance acceptance. The basic tools for a program such as an adequate number of public veterinarians for field work and state laboratories capable of serological techniques are already available. Information technology solutions and further logistic means such as animal identification techniques are in place in many countries and may be adapted to the special needs of a country like Egypt.

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Supplementary Items

Supplementary Table 1. Serology data arranged in tables according to time of publication

Reference	Serology tests	Animals tested	Sample no.	Sample type	Prevalence	Location	Isolates	Inclusion criteria
[16]	BAPAT[*], RBT[**] TAT[***],Riv.T[****] MRT[*****] PCR[******]	Cows Buffaloes Cows Buffaloes	32 18 96 54	Serum Serum Milk Milk	100% 100% 87.5% 83.3%	Sohag, Beni-Suef, Giza	*B. melitensis*	Outbreak investigation
[26]	RBT MRT, ELISA[#] PCR, DBH[##]	Cows Buffaloes Sheep Goats Camels Cows Buffaloes Sheep Goats She-camels	660 482 194 198 151 302 321 73 121 64	Serum Serum Serum Serum Serum Milk Milk Milk Milk Milk	45.8% 66.6% 37.6% 61.1% 42.1% 51% 49.8% 56.2% 36.4% 34.4%	Menufiya, Gharbia, Behira, Fayoum, Aswan, Sohag	*B. melitensis* bv 3	Outbreak investigation and trade (camel)
[12]	STAT RBT	Cattle Buffaloes Camel Mares Ewes Does	305 1,103 381 36 70 40	Serum Serum Serum Serum Serum Serum	7.86% 4.35% 7.61% 2.77% 5.71% 10%	Different localities in lower Egypt		Outbreak investigation
[36]	BAPAT RBT,TAT ELISA LAT[§], ICA[§§]	Cattle Sheep Goats	376 106 158	Serum Serum Serum	5.32% 9.43% 8.86%	Menufiya		Outbreak investigation
[24]	RBT BAPAT Riv.T TAT CFT[###] RBT BAPAT Riv.T TAT CFT	Group 1 cows suspected Group 2 free cows	180 125	Serum Serum	77.2% 79.4% 72.2% 81.1% 72.8% 1.6% 3.2% 0.8% 4% 0.8%		*B. melitensis* bv 3	Outbreak investigation
[45]	BAPAT Brucella card CFT	Cattle Buffalo-cows Sheep Goats Cattle bulls Buffalo bulls	549 338 404 336 217 152	Serum Serum Serum Serum Serum Serum	14.57% 10% 25.4% 30.9% 6.9% 3.9%	Menufiya, Beni-Suef Assuit, Giza, Gharbia, Sharkia, Behira	*B. melitensis* bv 3	Outbreak investigation
[30]	RBT SAT ELISA PCR	Sheep	300	Serum	29.3% 27% 28.3% 39%	Kafrelsheik, Gharbiya		Outbreak investigation
[31]	Positive serum samples	Cattle Sheep Goats	32 69 5	L.N Spleen	28.13% 36.23% 100%	Assuit	*B. melitensis* bv 3	No outbreak investigation
[38]	RBT	Swine	230	Serum	12.61%	Cairo	*B. suis*	No outbreak investigation
[29]	BAPAT RBPT M.P.A.T Riv.T, 2MT ELISA	Cattle Buffaloes Sheep Goats	967 462 591 539	Serum Serum Serum Serum	6.72% 5.62% 7.61% 10.95%	Sharkia	*B. melitensis* bv 3	Outbreak Investigation
[35]	BAPAT RBT,SAT Riv.T	Cattle Sheep Goats	715 1323 100	Serum Serum Serum	4.5% 5.2% 5%	Assiut	*B. melitensis* bv 3	Outbreak investigation
[18]	RBT CFT	Cattle Buffalo Sheep Goats Household	Total 120,077	Serum data from GOVS	0.79% 0.13% 1.16% 0.44% 1.2%	Beni-Suef, El-Minia, Assiut, Sohag, Qina, Luxor, Aswan		Official data

Reference	Serology tests	Animals tested	Sample no.	Sample type	Prevalence	Location	Isolates	Inclusion criteria
[19]	RBT, CFT iELISA	Cattle Buffaloes Sheep Goats	188 173 791 383	Milk Milk Serum Serum	15.1% 15.1% 41.3% 32.2%	Kafrelsheikh		A cross-sectional study was carried out among dairy cattle, buffalos, sheep and goats and a multistage random sampling strategy was used to select cattle milk tanks and individual sheep and goats within the governorate. The first-level sampling unit in this study was the village, the second-level sampling units were the cattle milk tanks and the individual sheep/goat.
[6]	iELISA	Cattle Buffaloes Household	109 46 104	Milk Milk	Total n = 22 14.6% 15.5%	Menufiya		A cross-sectional study was carried out in a village. The village was selected due to convenience. The study population comprised all households with lactating cattle and buffalo in the village. There was no sampling frame in the village and all lactating cattle and buffaloes were sampled.
[34]	RBT, Riv T PCR	Nile catfish	120 from Nile 120 from Farm	Serum Skin Liver Kidney Spleen	8.3% Only from Nile	KafrelsheikhMenufiya, Gharbiya, Dakahlia, Behira	*B. melitensis* bv 3	Samples collected from 17 sites in small tributaries of Nile canals. 120 catfish were collected from 7 fish farms from Kafrelsheikh, Behira and Dakahlia governorates unlikely to be exposed to water contaminated by carcasses and other contaminated animal materials.
[64]	RBT SAT iELISA	Buffaloes	452	Serum	12.83% 11.28% 19.25%		*B. melitensis* bv 3	Outbreak investigation
[27]	RBT, iELISA	Sheep Goats Cattle Sheep herd Goats herd Cattle herd	Total 1670 45 55 26	Serum Serum Serum Serum Serum Serum	21.20% 14.2% 2.16% 26.66% 18.88% 21.6%			A cross-sectional study was carried out on different governorates. In each region, blood samples were taken from herds/flocks with no previous history of vaccination against *Brucella*. The number of samples was collected in simple and/or systemic random sampling as follows: animals from each herd were randomly selected using a table of random digits. Only female cows older than 6 months of age were sampled. The herds were stratified into three herd sizes: small herds (≤ 50), medium herds (50-150) and large herds (>150).
[28]	CFT	Camels	340	Serum	7.35%	Behira	*B. melitensis* *B. abortus*	No outbreak investigation
[48]	BAPAT RBT, Riv T	Cattle Buffaloes	7,102 2,895	Serum	0.20-0.37% 0.11-0.38%	Kafrelsheikh	*B. melitensis* bv 3	Outbreak investigation
[23]	SAT BAPAT RBT Riv T SAT BAPAT	Cattle friesian breed Cattle charolaise	57 43	Serum Serum	8.77% 10.53% 10.53% 8.77% 6.68% 9.30%,	Egypt		Breed

Reference	Serology tests	Animals tested	Sample no.	Sample type	Prevalence	Location	Isolates	Inclusion criteria
	RBT Riv T	breed			11.63% 4.65%			
[22]	BAPAT RBT, SAT Riv T	Cattle Buffaloes Sheep Goats	1,966 1,237 813 366	Milk Tissue	5.44% 4.11% 5.41% 3.55%	Beni-Suef, Assiut, Alexandria, Giza, Behira Qaliobia, Menufiya.	*B. melitensis* bv 3	No brucellosis history
[17]	BAPAT RBPT, TAT Riv T, CFT PCR	Baladi does	577	Serum	3.11% to 5.71%	Kafrelsheikh	*B. melitensis* bv 3	Outbreak investigation
[32]	BAPAT RBT, TAT Riv T	Livestock Cattle Buffaloes Sheep Goats	350 77 35 29 18	Serum Serum Serum Serum Serum		Gharbiya		A cross-sectional survey was conducted in two villages. Criteria for inclusions of the villages were easy accessibility for the study team and a population size of approximately 5,000 in each village. Each village was divided into small clusters from which one house was randomly selected. Members (aged ≥3 years) and their livestock were enrolled until the sample size was achieved.
[63]	MRT, wTAT wRBPT wBAPAT wRiv T	Cattle Buffaloes	210 50	Raw milk Raw milk	12.38% 0.00%	Assiut		No outbreak investigation
[33]	SAT, RBT Riv T, CFT PCR	Ewes native breed	32	Serum	31.25% 25.00% 21.88% 21.88%	Menufiya	*B. melitensis* bv 3 *B. melitensis* Rev 1	No outbreak investigation
[61]	RBPT, BAPAT TAT, Riv T ELISA	Cattle Sheep Buffaloes Dairy cows	197 129 32 41	Serum Serum Serum Milk	3. 6% 11.6% 0.00% 7.3%	Assiut		No outbreak investigation
[71]	BAPAT, RBT SAT, Riv T ELISA	Cattle Sheep Goats Camels Cattle Sheep Goats Camels	180 180 100 100 15 16 36 10	Serum Serum Serum Serum Milk Milk Milk Milk	7.22-10.56% 2.22-3.89% 6-7% 0.00% 6.67% 6.25% 2.78% 0.00%	New Valley		Outbreak investigation
[57]	RBPT, BAPT TAT, Riv T	Ewes Rams Does Bucks Ewes Rams Does Bucks	450 300 220 180 426 210 105 70	Serum Serum Serum Serum Serum Serum Serum Serum	Total 1.26% Total 9.30%	Assiut Sohag		No outbreak investigation
[65]	RBPT STAT ELISA RBPT STAT ELISA	Local camels Imported camels	95 31	Serum Serum	9.47% 5.26% 9.47% 6.67% 9.67% 25.80%	Halaieb, Shalateen, Abo-Ramad triangle		No outbreak investigation
[46]	RBPT, TAT BAPT, Riv T	Camels	300	Serum	3.04% 0.00%	Assuit New Valley		No outbreak investigation
[60]	RBPT, SAT, MET[§§§], Riv T DIA	Camels in closed farm Imported	80 94	Serum Serum	0.0-2.5% 8.5-11.70%	Giza		No outbreak investigation

Reference	Serology tests	Animals tested	Sample no.	Sample type	Prevalence	Location	Isolates	Inclusion criteria
		camels Camels kept with animals	72	Serum	6.94-11.1%			
[54]	TAT PCR	Friesian cattle	124	Serum	29.8%	Fayoum	*B. abortus*	Animals were not subjected to any vaccination.
[37]	RBT BAPT TAT MET Riv T ELISA	Camels	766	Serum	8.74% 9.53% 9.92% 8.09% 8.87% 9.26%	Behira		No outbreak investigation
[34]	RBPT TAT MET Riv T	Camels	430	Serum	7.67% 8.84% 6.97% 6.75%	Assiut		No outbreak investigation
[55]	RBT, SAT Riv T, PCR	Cattle Buffaloes Sheep Goats	1,783 942 1,455 624	Serum Serum Serum Serum	8.5% 7.0% 7.8% 7.0%	El-Minia	*B. melitensis* bv 3	Outbreak investigation
[25]	SAT, RBPT MRT#### PCR	Cattle Sheep Goats Camels	52 21 18 12	Milk	n = 29 n = 10 n = 13 n = 1	Giza	*B. abortus* bv 1 *B. melitensis* bv 3	Outbreak investigation
[20]	SAT, MRT, WRBPT, WRiv T	Cattle	150 150	Serum Milk	10% 8% 4.7% 4%	Behira	*B. melitensis* bv 3	No outbreak investigation
[53]	BAPT, RBPT CFT, SAT	Camels	750	Serum	3.9% 4.9%	Egypt	*B. melitensis* bv 3 *B. abortus* bv 1,7	No outbreak investigation
[56]	RBT, BAPT TAT, Riv 1	Cattle Sheep Goats	6,495 8,457 3,872	Serum Serum Serum	0.46-0.61 0.85-1.15 0.74-1.1	Assiut		No outbreak investigation
[52]	BAPT, RBPT ELISA, CFT TAT, MRT	Milky cattle Dry cows Aborted cows Calves Bulls Milky cattle	238 176 9 6 13 238	Serum Serum Serum Serum Serum Milk	28.51% 28.05% 24.89% 22.85% 21.72% 16.39%	Sharkia	Isolation from milk was negative	Outbreak investigation
[69]	BAPT RBPT TAT Riv T	Sheep	21,776	Serum	1.6% 1.6% 1.33% 1.4%	Assiut		Samples collected officially
[66]	BAPT RBPT TAT Riv T	Goats	16,285	Serum	0.33% 0.33% 0.15% 0.3%	Assiut		Samples collected officially
[67]	BAPT RBPT TAT Riv T	Cattle	8,774	Serum	0.89% 0.87% 0.6% 0.57%	Assiut		Samples collected officially
[70]	BAPAT SAT, MRT	Lactating buffaloes Lactating buffaloes Dry buffaloes Bulls	295 282 44 18	Serum Milk Serum Serum	19.9% 12.3% 19.9% 25%	Giza	*B. abortus*	Outbreak investigation
[68]	SAT MET BAPAT	Swine	288	Serum	29.2% 24.6% 35.7%		*B. suis* bv 1	No outbreak investigation

Reference	Serology tests	Animals tested	Sample no.	Sample type	Prevalence	Location	Isolates	Inclusion criteria
	RBT Riv T				29% 27.4%			
[49]	SAT, MET BAPAT RBPT, Riv T	Cattle Buffaloes Sheep Goats	1,683 1,286 2,257 532	Serum Serum Serum Serum	8.2% 11.4% 5.1% 11.1%	Alexandria, Assiut, Cairo, Giza, Behira, Demiatta, Fayoum, Gharbiya, Kafrelsheik,Qaliobia, Menufiya, Suez, Port-Said, Sharkia	*B. melitensis* bv 3 *B. abortus* bv 1	Outbreak investigation
[59]	RBPT	Cattle Buffaloes Sheep Goats	176 97 169 20	Serum Serum Serum Serum	2.27% 3.09% 4.73% 0.00%	Kafrelsheikh		No outbreak investigation
[58]	TAT MT TAT CFT	Camels	1,500	Serum	5.3% 6.33% 6.4% 7.93%	Egypt	*B. abortus* bv 7	No outbreak investigation
[47]	STA, RBPT 2ME, MRT CFT, Riv T	Friesian cattle Native cattle Buffaloes	533 302 547	Serum Serum Serum	4.48% 6.43% 2.89%	Menufiya	No isolation	Outbreak investigation
[50]	TAT, Riv T BAPAT RBPT, MET MRT	Sheep Goats Sheep Goats	925 560 25 21	Serum Serum Milk Milk	13.3% 7.14% 40% 23.8%	Cairo, Giza, Qaliobia, Kafrelsheik,Dakahlia	*B. melitensis* bv 3	Outbreak investigation
[21]	TAT Riv T RBT TAT Riv T RBT	Cattle Buffaloes 	1,832 118 	Serum Serum 	37.9% 32.8% 61.8% 10.2% 7.8% 22.2%		*B. melitensis* bv 3 *B.abortus* bv 3,7	No outbreak investigation
[44]	CFT, TAT BAPAT, RBPT Riv T, MRT	Cattle Buffaloes Cattle Buffaloes Dogs Wild rats	800 300 800 300 108 130	Serum Serum Milk Milk Serum Serum	3% 4% 2.63% 3.67% 6.48% 10.77%	Menufiya, Beni-Suef, Kafrelsheikh, Fayoum	*B. melitensis* bv 3 *B. abortus* bv 3,7	Outbreak investigation
[51]	TAT, Riv T RBPT, MRT	Cattle Buffaloes Sheep Goats	1,832 118 648 131	Serum Serum Serum Serum	37.99% 10.17% 23.92% 00.00%	Alexandria, Assiut, Cairo, Giza, Demiatta, Kafrelsheik,Qaliobia, Menufiya, Port-Said, El-Menia, Beni-suef, Dakahlia	*B. melitensis* bv 3 *B. abortus* bv 3,7	Outbreak investigation

[*] Buffer acidified plate antigen test (BAPAT)
[**] Rose Bengal test (RBT)
[***] Tube agglutination test (SAT)
[****] Rivanol test (Riv. T)
[*****] Milk ring test (MRT)
[******] Polymerase chain reaction (PCR)
[#] Enzyme linked immunosorbent assay (ELISA)
[##] Dot blot hybridization assay (DBH)
[###] Complement fixation test
[####] Milk ring test
[§] Latex agglutination test (LAT)
[§§] Immunochromatographic assay (ICA)
[§§§] Mercapteoethanol test (MET)