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Brucellosis in Islamic republic of Iran

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ABSTRACT

Brucellosis is a zoonotic disease that is widely distributed throughout the developing countries. In this review, an overview of the epidemiological and epizootic status of brucellosis in Islamic republic of Iran is presented. In Iran, the disease was first recognized in 1932, which is now endemic in the entire country. The first animal vaccination program was carried out in 1949. Brucellosis has been found in humans, cattle, sheep, goats, camels, horses, buffaloes, dogs and the prevalence of bovine brucellosis is estimated 0.3%. The successful implementation of a national brucellosis control program requires enough compensation, farmers' cooperation, accurate diagnosis of infected animals and impetus of health system is required to overcome the disease.

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Introduction

Brucellae are Gram-negative facultative intracellular pathogens that cause important diseases in humans and various species of domesticated and wild animals. To date, eight Brucella species have been identified: *B.melitensis*, *B.abortus*, *B.suis*, *B.canis*, *B.neotomae*, *B.ovis*, *B.ceti* and *B.pinnipedialis*, most of which, except for *B.neotomae* and *B. ovis*, pathogenic for humans (1). In animals, the disease mainly causes late gestation abortion in pregnant females and orchitis and epididymitis in males (2). Human brucellosis is a disease with a wide range of clinical signs, presenting various diagnostic difficulties because its symptoms are similar to those of many other diseases. According to the reports of World Health Organization, there are more than 500 000 new cases of human brucellosis every year and for each case diagnosed with the disease, there are four undetected cases(3). Since every case in human has an animal origin, animal vaccination appears to be the most effective method for controlling the spread of disease among animals and humans (4). There are some reasons why brucellosis control plans turned out to be inefficient. These include animal trafficking and illegal movements, inadequate attention to livestock health infrastructure in animal husbandry developments, inadequate authority of veterinary services in endemic countries and different human habits in diet and animal husbandry (5). Quality of veterinary service, availability of economic resources, spread of the disease, type of husbandry and the degree of ranchers' involvement are major factors that should be considered in adopting brucellosis control/eradication programs(6). Iran is an endemic area for brucellosis which is a serious public health issue in the country. For the first time, *B.melitensis* was isolated from human blood culture in 1932 and the first vaccination of cattle population was implemented as early as 1949(7).

Swine brucellosis

In Iran, the *Brucellasuis* was first isolated from an aborted swine fetus in 1948 and *B.abortus* and biotypes 1 & 3 *B.suis* were isolated in pig farms (8). The raising of pigs is currently forbidden in Iran due to Islamic law, and no study has been carried out on feral pigs to investigate brucellosis infection. There can be found a lot of feral pigs in rural and nomadic areas which have close contact with domestic animals, thereby raising the odds of *B.suis* infection among livestock raised in such environments. Despite religious restrictions on the consumption of pork in Iran, illegal hunting is still common in some areas. Yet these restrictions are not applied to hunting and religious minorities who can hunt feral pigs. Since the feral pig can carry *B. suis* biovar (bv) 1, 2 and 3, these hunters are at risk of getting brucellosis. Nevertheless, there is no reported data supporting *brucella* infection among the feral pig population.

Canine brucellosis

Some published data indicate the presence of *B.abortus* and *B.melitensis* in sheepdogs and companion dogs through either serology or bacteriology (7,9,10) but *B.canis* has been detected only through serology(11,12).While epidemiology of canine brucellosis is undetermined in Iran, several studies have revealed that the rate of *B.canis* seroprevalence range from 3.5% to 15.8% in different provinces(11,13,14,15). Akhtardanesh *et al* were reported 15.8% seroprevalence based on immunofluorescence assay in southeastern of Iran (11) in compared with another surveys which were 4.9 and 10.34% in urban and rural dogs by immune chromatogeraphy method in the southern part (11,15). Serological tests are not routinely performed in private clinics to detect *B.canis* antibodies. Illegal trafficking of exotic animals not quarantined as required by law contributed to the spread of the disease in Iran. According to Islamic law, consuming testis,

uterus, spleen and lymph nodes is prohibited. Therefore, local butchers would feed these tissues to sheepdogs or stray dogs so that they would be infected easily with *Brucella* spp that may be exist in the tissues. There is, however, no screening plan for the detection of brucella infection in sheepdogs nor is there any vaccination program subjected to them which can be a major source of infection in livestock. Dogs should be included in brucellosis control program.

Equine brucellosis

Behroozikhah reported that *B.abortus* has also been isolated from horses (16). Some surveys using Rose Bengal plate test (RBPT), standard tube agglutination test (STAT) and 2-mercapto ethanol (2ME) tests have revealed the presence of antibody against *Brucella* spp, ranging from 2.5% to 12% in three provinces (17,18,19). There is no vaccination program for horses, asses and mules in Iran. Since horses are kept near small ruminants and cattle in rural and nomadic areas, the risk of infection is very high.

Buffalo brucellosis

Few surveys have been carried out on brucellosis infection among buffaloes (20); however, *B. abortus* was isolated from aborted fetuses (7). Furthers, Nowroozi-Asl *et al* reported infection rates of 19.5% and 11% in STAT and 2ME tests, respectively (21).

Camel brucellosis

Iran is home to two species of camels: Camelusdromedarius and Camelusbactrianus. The populations of dromedary and bactrianus species are estimated to be approximately 150 000 and 2 000, respectively. Camel husbandry is a common practice in deserts of Iran and in some farms camels are raised with cows, sheep and goats for its meat. Some studies revealed that seroprevalence of brucellosis in camels in southern regions are lower than that of brucellosis in eastern and northern regions, due to lower animal movements across illegal borders (22, 23). Based on the serological tests, the prevalence of brucellosis in camel herds is between 1.65 to 8% (22,24,25,23). Abortion is rarely reported in camels whose access is restricted in remote desert environments. Yet, *B. melitensis* biovar 1 and 3 were isolated from milk, lymph nodes and aborted fetuses (7). Camel milk is part of camel keeper diets, yet as it is consumed untreated, it could be a dangerous source of brucellosis.

Bovine brucellosis

Bovine brucellosis is widespread across Iran. In the cattle population, *B.abortus* was isolated from an aborted fetus in 1944 and five years later the first vaccination program for cattle was carried out. In Iran, the national control plan for brucellosis was launched in 1967(26). Because of the high prevalence of bovine abortion due to *B. abortus*, adult cows and 3-8 month old calves were vaccinated using S19 vaccine. Adult vaccination using S19 vaccine was discontinued in 1972, superseded by K45/20A (27). As of 1958, regular file was prepared for each farm and milk ring test was performed on fresh herd milk samples. The main policy to control the disease was to improve herd immunity against *Brucella spp* based on test and slaughter of reactors principles, yet voluntary participation of farmers proved to be the main weaknesses of the program because they could have avoided slaughtering their cattle due to inadequate compensation offered to them. As of 1971, the control program was operational zed in provinces other than the capital, including Fars, Khorasan and Esfahan, which included identifying the entire cattle, slaughtering infected cattle with paying compensation, and vaccinating with S19 & K45/20A. Vaccination with K45/20A was stopped in 1980 (26, 27). In 1988, the program was divided into two groups: group A and group B. Group A included industrial and semi-industrial

cattle farms and animal population around big cities with mandatory control program. In this group, the cattle had to have an individual registration number and a certification for vaccination, test and slaughter, control of animal movement to and from farms, and hygiene education programs had to be implemented for farmers. Group B took in cattle population of rural and nomadic territories with voluntary test and slaughter program. All female cattle between 3 and 6 months of age had to be vaccinated with S19 and hygiene education programs had to be implemented for farmers (Diagram-1). In groups, all female cattle and bulls were subcutaneously vaccinated with S19. In 2004, reduced dose S19 vaccination was introduced to the program, with which adult cattle in rural and nomadic regions were vaccinated. After one year, test and slaughter program for this cattle population was stopped (26, 27). In 2004, some industrial farmers started using RB51 vaccine. As of 2007 S19 was removed from the brucellosis control initiative and since then all cattle were vaccinated with RB51. From 2004 onwards, the vaccination strategy can be summarized as follows: 1) compulsory vaccination of heifers aged 4–12 months with full dose of strain RB51; 2) compulsory vaccination of adult females with reduced dose of strain RB51 repeated every two years. In order to identify the vaccinated animals, they were attached with special ear tags (28).

Test and slaughter

The cattle are screened using RBPT and positive results are confirmed using STAT & 2ME tests. Complement Fixation Test (CFT) is also used occasionally as a confirmatory test in Tehran province. All industrial farms are currently covered by test and slaughter programs; however, this is not the case in rural and nomadic areas. In these regions, screening programs are implemented in the following cases: outbreaks of animal or human brucellosis, in villages which feed milk to dairy factories, in villages in the vicinity of cities, and in villages with more than 200 Holstein cows (26, 29).The cattle suspected in serological tests must be quarantined and retested after 3-4 weeks. As long as there are positive or suspected cattle in farms, farms are forbidden from animal movement because all cattle must be retested every 2 month until freedom from infection is confirmed. When it becomes clear that an animal is infected with *Brucella* spp by serological tests, their owner receive compensation for slaughtering. Compensation of direct losses can either be based on a pre-set animal value or actual market value at the moment of culling. The compensation is 75 percent of the actual value of the cattle. Control of brucellosis in Iran has been divided into four stages since 1950: First stage, 1950- 1970: During this period, control program was carried out in farms around the capital (Tehran) but only in volunteer farms, so the infection rate dropped considerably. On the other hand, from 1970 to 1971, a lot of cattle in this area were slaughtered because of brucellosis. This was due to lack of human resources as a result of involvement in control management of Rinderpest outbreak in 1969. There was a decrease in control program during 1954 to 1957 due to lack of funding and schedule was based on calves vaccination only. Twenty two farms were become free from bovine brucellosis in consequence of control program. Second stage, 1971-1977: The program has also expanded to other provinces. Third stage, 1978-1982: During these years, vaccination and tests were decreased dramatically due to Islamic revolution and Iraq's imposed war against Iran. Fourth stage, since 1983 till now. After *Brucella* vaccination in traditional farms where cattle and sheep are kept close together, Rev-1 vaccine strain was isolated from 2 aborted fetuses of them (30). According to the interpretation guide of serological tests, some cattle have doubtful results which must be retested between 21-28 days. Some studies revealed that after follow up of those cattle, 84% became positive and in some cases, retesting was delayed more than 28 days which is very important because during this period, those cattle were in close contact with the negative animals (31, 32).

A big problem in slaughtering of reactor cattle was the partial cooperation of farmers before 2006 due to the low price they received in compensation for their cattle. Fortunately, after 2006 farmers get 75% of the actual price of their cattle and their cooperation with veterinary organization is much better than previous. According to the study of Esmaeili *et al* prevalence of brucellosis among industrial and semi-industrial dairy cattle was calculated to be 0.3% (26). Strains of *B. abortus* were isolated from cattle, horse, sheep and goat. These isolates were biovars 1, 2, 3, 4, 5, 6 and 9 with bv 3 considered as the dominant one (7, 26, 33, 34). Behroozikhah *et al* determined the genotypic (by RAPD-PCR) and phenotypic relationship among *Brucella spp*. isolated from different animal species in different parts of Iran from 1961-2003. According to this study, thirteen isolates were classified into five groups, each containing 2-4 biovars (Table 1) (16).

Ovine and Caprine Brucellosis

Small ruminant brucellosis has been studied since 1949, when the *B.melitensis* was first isolated from milk of an aborted goat and after 2 years, more studies showed the presence of brucellosis in small ruminant flocks of Isfahan province (35).Early studies about the Rev.1 vaccine production began in Razi Institute in Iran in cooperation with WHO in 1963, and it was produced as a domesticated biological product. After being tested on Iranian goat and sheep, this vaccine was proved by Iranian researchers and cooperation of WHO to be efficient to decrease the epidemic rate of the disease from 45% to 1.8% since 1963(36,37).From 1963 to 1973, kid & lambs and nonpregnant sheep and goats were vaccinated with full dose Rev-1 vaccine. Yet, in the following years, adult vaccination was stopped because of interference with serological test. Since the beginning of 2003, adult vaccination with reduced dose of Rev-1 vaccine was started and testing and slaughtering in small ruminant population were eliminated from control program (Diagram-2) (35, 38).Testing and slaughtering weren't done in all population of small ruminants except breeding centers, industrial farms and areas with abortion outbreaks. Biovars 1, 2 and 3 *B.melitensis* were isolated from sheep, goat, camel, dog, cow and human beings and according to the results of some reports; bv 1 is endemic and widely spread in Iran (7, 35, 39). Esmaeili *et al* revealed that reduced dose of Rev-1 vaccine was the agent of abortion in some regions and this strain was isolated from aborted fetus of small ruminants (35). Animal vaccination has a direct effect on the incidence of human brucellosis. Esmaeili *et al* achieved a negative value for Pearson correlation coefficient of cattle vaccination and human brucellosis($r = -0.48$), sheep and goat vaccination and human incidence rate ($r = -0.44$) in Iran (40).

Rancher's Habits

Rancher's habits have an important role in disease transmission between animals and humans. Farmers customarily consume animal organs such as spleen and testis for nocturia treatment, camel raw milk for treatment of hemorrhoid, joint pain and asthma, uncooked liver in order to anemia treatment and sleeping on freshly slaughtered animal skin for fever fix and joint pain (41). There is a traditional belief among farmers in some areas that probability of female lamb birth will be increase, if they keep old ram (more than 5 years old).This can cause long maintenance of the infected rams and the spread of infection among the flock. Given that milk production decreased dramatically after kid and lamb deaths, farmers buy orphan lamb to prevent milk decline and economic loss. As regards, these neonates that enter this new flock come from flocks with unknown health conditions and could be a source of several infections. They introduce orphan lamb to their ewes with rubbing salt water to the surface of their body.

Wildlife Brucellosis

There have not been major surveys to identify wild-life reservoirs of brucellosis in the Iran.

Table 1. Identification, biovar distribution, and number of RAPD types among Brucella isolates16

Brucellaspp	Biovar	No. of isolate	No. of profile	Animals(n)
B.abortus	A1	8	6	**Pig(3)** **Cattle(1)** **Reference(4)**
	A2	2	2	**Pig (1)** **Cattle (1)**
	A3	29	21	**Pig(21)** **Cattle(7)** **Sheep(1)**
	A5	5	5	**Pig (3)** **Cattle (2)**
B.melitensis	M1	35	28	**Sheep(23)** **Pig(6)** **Cattle(3)** **Reference(2)** **Human (1)**
	M2	2	2	**Sheep** **Cattle**
	M3	1	1	**Pig**
	M1 (Rev.1)	9	8	**Sheep (9)** **Reference (1)**
B.suis	S1	4	3	**Pig (4)**
	S2	6	5	**Pig (6)**
Total		101	72	

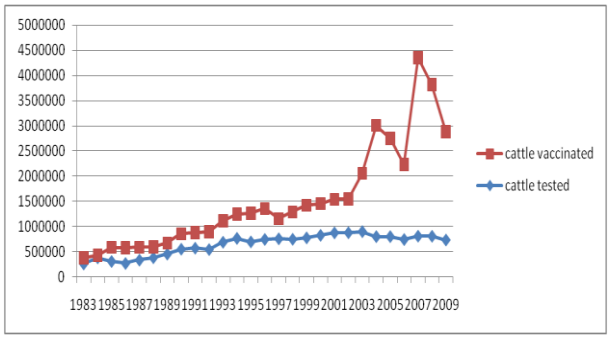

Diagram 1. Bovine brucellosis vaccination and testing during 1983-2009

Human Brucellosis

For the first time, brucellosis was diagnosed in human being in 1932 which *B.melitensis* was isolated from human blood culture (7, 29, 35). In Iran, human brucellosis is prevalent in all seasons yet the majority of cases occur during the spring and summer. The higher prevalence of brucellosis in these seasons is probably due to more contact with livestock and dairy products during the milking process and sheep and goat parturition (42, 43).Brucellosis is prevalent at all ages but, the prevalence of brucellosis was higher in younger age groups than older age groups (20-30 old years). Zeinalian Dastjerdi *et al* reported the median age of cases was 31.3 years due to the chance of contact with infected animals and their products (42, 43). As the health surveillance system in Iran has improved in recent decades, it seems that a number of cases were undiagnosed in the past, particularly in poor and remote provinces. Certainly, by expanding the healthcare system, the number of new cases reported has increased (43). Like other regions, there is no precise information about the incidence of this disease in Iran. According to results of Zeinali's study the average incidence of brucellosis in the Iranian population was 21 cases per 100 000 populations, although this varied between 1.5 and 107.5 per 100 000 population in different parts of the country. In overall, the incidence of brucellosis shows a decreasing trend from 1980 to 1989 and since then till 2010 cases have decreased to 15.9 cases per 100 000 populations (Diagram 3) (43). This disease is more prevalent in rural areas than urban areas due to more contact with livestock in rural occupations. As such, it was concluded that the incidence of brucellosis in rural populations was significantly higher (70%) than in urban populations (30%) (43). According to Zeinalian Dastjerdi *et al*'s study the male: female ratio of cases was >2 and this ratio was slightly higher in urban cases than rural cases. This is due to the fact that women in these areas are more involved in animal husbandry activities than women in urban communities (42). The results of some studies revealed that housewives and farmers were more affected in both urban and rural groups (42-45). It is well known that the main sources of brucellosis in humans are infected sheep and goats and their products. Except one woman and her baby, human cases due to *B. abortus* are rare in Iran (26). The major routes of transmission of brucellosis in Iran include: animal abortion materials, fresh white cheese and raw milk (42, 45). The consumption of fresh white cheese which is made from unpasteurized sheep and goat milk is very favorite in Iran. In the study of Sabbaghian*et al* Brucellas pp were isolated from cheese four weeks after collection from retail shops (46).According to the report of the Ministry of Health and Medical Education in 2009 (43), the four types of provinces for incidence of human brucellosis (Figure-1) are as follows: Type 1: very high incidence regions including East Azerbaijan Hamedan, Markazi, Lorestan, Kermanshah, West Azerbaijan and South Khorasan. The incidence of human brucellosis was about 31-41 per 100 000 populations.Type 2: high incidence regions including Kordestan, RazaviKhorasan and Zanjan. The incidence of human brucellosis was about 21-30 per 100 000 populations.Type 3: moderate incidence regions including Golestan, Ilam, Qazvin, Semnan, Chaharmahal and bakhtiari, Ardabil, Kerman, Mazandaran, Yazd, North Khorasan and Fars. The incidence of human brucellosis was about 11-20 per 100 000 populations.

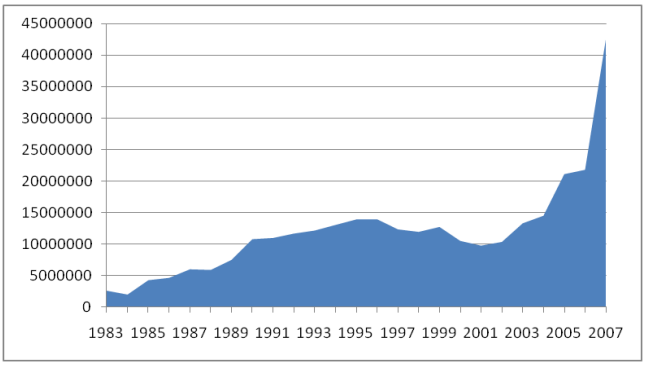

Diagram 2. Small ruminant vaccination in Iran during 1983-2007.

Type 4: low incidence regions including Bushehr, Khuzestan, Kohgiluyeh and boyerahmad, Alborz, Tehran, Gilan, Hormozgan, Sistan and baluchestan and Qom. The incidence of human brucellosis was about 0-10 per 100 000 populations. HasanjaniRoushan *et al* followed 19 pregnant women with brucellosis which 53% of them had spontaneous abortion but became pregnant after treatment. They declared that the brucellosis screening program is required for pregnant women and those with spontaneous abortion, especially in brucellosis endemic regions (44). Epididymoorchitis due to brucella is a focal complication of human brucellosis and has been reported in 2-20% of cases. Najafi *et al* describe the clinical characteristics and treatment of patients with Brucellaepididymoorchitis (BEO) during a 13 year period; their results revealed, epididymoorchitis occurred in 6.7% of all patients and 11.11% of male patients with brucellosis (47). In another study on 96 patients with epididymoorchitis from 1995-1996, BEO was found in 14.6% of the cases. They believed that in brucellosis endemic areas, it is imperative for clinicians to consider the likelihood of brucellosis in epididymoorchitis cases (48). Brucellosis has become an occupational disease and some studies in Iran reported different seroprevalence rates. According to the report of Nikokar*et al*seroprevalence of brucellosis among slaughterhouse workers and the people living in rural areas was 9.8% and 5.5%, respectively. A maximum percentage (30.5%) was seen in the age group above 45 years (49). In the study of Karimi *et al*, seroprevalence rate among slaughter and butchers was 20% and 4%, respectively (50). Rabbani *et al* reported an anti-brucella antibody seroprevalence of about 0.07% in blood donors at blood banks of Bushehr (southern province) with RBPT, STAT, and 2ME tests. They believed that although the detection of brucella infection in blood donors is not recommended as essential, but according to the rate of brucella infection in Iran, it would be sensible to screen blood samples for brucella infection in the regions with a high rate of brucellosis or in blood samples that are denoted to persistent recipients of blood such as hemodialysis, thalassemic, and hemophilic persons (51).

Conclusion

The geographic situation of Iran is always an important risk factor for the propagation of contagious diseases, mainly from the eastern and western neighbors such as Iraq, Pakistan & Afghanistan. The majority of these countries do not have high quality veterinary services for controlling animal diseases and hence brucellosis is endemic in them. Thus, there is a risk of brucellosis transmission within and between these countries. Following are some of the major problems for brucellosis control in Iran:

-Lack of proper law for the punishment of violators in animal health field.

-Weaknesses in border quarantine system and animal trafficking from neighboring countries

-Lack of rural and nomadic livestock identification system

-Nomadic and semi-nomadic conditions of small ruminant husbandry which makes control of animal movement very difficult

-In some areas, farmers keep their sheep as long as they have their incisors which, is more than the immunity period of Rev-1 vaccine

-Veterinarians who fight against the disease in operation teams are not paid handsomely.

This could serve as a demotivating factor in controlling the disease. The quality and organization of the veterinary service is very important in the control program of brucellosis. Experience in countries that have succeeded in eradicating has demonstrated that farmers' cooperation is very significant and that the program will be inefficient if the government doesn't provide enough economic compensation. In addition to veterinarians, sociologists and economists should also be involved in design of every animal disease' control plan given the diversity of factors affecting farmers' habits and behavior. Since farmers' cooperation with veterinary service is based on cost–benefit, thus the control plans must-be affordable for them, and various factors should be calculated in each program. Eradication of brucellosis is more complicated than vaccination and test and slaughter of infected animals. A well designed program with enough compensation, farmers' persuasion for cooperation, accurate diagnosis of infected animals and specially impetus of health system is required to overcome the disease.

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Conflict of interest

None declared conflicts of interest.

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