BRUCELLOSIS IN IRAQ: EPIDEMIOLOGY, PRESENT STATUS, AND CHALLENGES IN CONTROLLING THE DISEASE

by

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Abstract

Brucellosis is one of the major endemic zoonotic diseases worldwide, and it has history dating back to 1937 in Iraq when it was first isolated by an Iraqi physician. In order to establish a solution for the continuous devastating impacts of the disease in humans and livestock, the Brucellosis Control Program was established in 1995. The main responsibilities of this program were setting and implementing the appropriate strategies for controlling the disease. After the war in 2003, the United Nation organization for Food and Agriculture (FAO) developed a strategic plan to control the disease. The main goal of the project was to improve productivity in the livestock sector and reduce the prevalence of disease in small ruminants (sheep and goats) to less than 2%, and less than 0.2% in cattle and buffalo. Achieving such goals ultimately would reduce the disease incidence among the human population from more than 27.2 cases/100,000 persons in 2002, to less than 4 cases/100,000 people within 15 years. A serological surveillance was conducted and revealed the apparent prevalence of the disease in sheep and goats, cattle, buffalo, and camels was 6.51%, 1%, 1.48%, and 0.02%, respectively in Iraqi governorates except the three northern governorates of Kurdistan province . Based on surveillance results, a vaccination policy was the only appropriate strategy that could be chosen to control the disease. Four vaccination campaigns were implemented in 2006, 2007, 2008, and 2009, with a total number of vaccinated animals each year at 10099972, 4698482, 753153, and 1833482 head, respectively. The primary satisfactory outcome of the program was the apparent decline in livestock abortions leading to obvious increases in productivity. Regarding the incidence of brucellosis among the human population, the apparent decline in the middle and south of Iraq began with the vaccination phase of the control program in 2006. The results represented a

significant decrease in human cases after only four vaccination campaigns of a program that was intended to continue for 15 years.

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It is the time to remember our colleagues who lost their lives while they were doing their best to improve our profession, and the first one is our previous general director, Dr. Dawood M. Shareef who has been kidnapped without any trace until the current moment.

Dedication

I have always believed life is just like a train going in an endless trip as long as we live. During this trip, we will visit a lot of stations and meet uncountable numbers of people. Some of the people will not be forgotten because they will affect our entire life in one way or another. They will influence our pathways either by straightforward decisions or quirky coincidence. I would like to dedicate my work to some of them here.

1) To every sand particle of my valuable and beloved motherland, IRAQ.

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CHAPTER 1 - Literature review

Introduction

Brucellosis, which is also known as "Undulant fever", "Mediterranean fever" or "Malta fever" (WHO, 2006), is a major infectious disease afflicting humans and a wide range of domesticated animals and wildlife. (Robinson, 2003; Eschenbrenner et al., 2002; Laria et al., 2006). It is known to be a worldwide problem and one of the most important among zoonoses in the Mediterranean region, India, and Central and South America. Although there has been continuous progress in controlling Brucellosis, it still remains a major health hazard and of great economic importance (Nicolas, 1998; Carrera et al., 2006).

It is well known that the main sources of Brucellosis in humans are infected animals (sheep, goats, cattle and swine); and in the world, it has been reported there are approximately 1.8 billion sheep and 50 countries with epidemic regions of *B. melitensis*; 1.3 billion cattle, and 101countries with epidemic regions of *B. abortus*; and 0.9 billion swine, and 33 countries with the epidemic regions of *B. suis* (Deqiu et al., 2002).

Etiology

The disease is caused by several species belonging to *Brucella* genus, which are aerobic, non-motile, Gram-negative, facultative intracellular coccobacilli. The genus Brucella belongs to the *alfa*-2 subgroup of the class proteobacteria. It is subdivided, on the basis of its pathogenicity and host preference into seven species *B. abortus*, *B. melitensis, B. suis, B. canis*, *B. ovis,* and *B.neotomae* (Robinson, 2003; Hoover et al., 1997; Eschenbrenner et al., 2002). Recently, a new strain affecting marine mammals isolated and tentatively named (*B. maris*), was first described in 1994 when a bacterial isolate from an aborted fetus of a bottlenose dolphin (*Tursiops truncates*) was characterized as a nontypical *Brucella* species (Forbes et al., 2000). A recent proposal suggested division of *B. maris* into at least two species: *B. pinnipediae* for strains from pinnipeds (seals, sea lions, and walruses) and *B. cetaceae* for isolates from cetaceans (whales, porpoises, and dolphins) (Institute for International Cooperation in Animal Biologics. 2007). *B. abortus* and *B. suis* have been isolated worldwide from a great variety of wildlife species, such as bison, elk, feral pigs, wild boar, European hares, foxes, African buffalo, eland, water buck, reindeer, and caribou, and regarding *B. melitensis*, although it is rarely reported in wildlife, cases were reported in Europe in chamois and ibex (Godfroid, 2002). *B. melitensis* occurs most frequently in the general population and it is the most pathogenic and invasive species of *Brucella*, followed, in order, by *B. suis* and *B. abortus* (Community Health and Disease Surveillance Newsletter, 2002).

Table 1.1: Different *Brucella* species, biovars, and hosts

Species	Biovar/ Serovar	Natural host	Human pathogen
B. melitensis	1 – 3	Goat, Sheep	Yes
B. abortus	1 – 6, 9	Cattle	Yes
B. suis	1 , 3	Swine	Yes
	2	Hares	Yes
	4	Reindeer, Caribou	Yes
	5	Rodents	Yes
B. canis	None	Dogs, Other canids	Yes
B. ovis	None	Sheep	No
B. neotomae	None	Desert wood rat	No
B. maris		Marine mammals	

All *Brucella* species are not host specific and may be transmitted among species under appropriate conditions (Robinson, 2003). International scientific organizations have indicated *B. melitensis* is the most virulent species of all the Brucellae (OIE, 1996).

Transmission

Brucellosis still remains a major zoonosis and an important cause of travel–associated illness. Humans can acquire the disease through consumption of infected, unpasteurized animal milk products (Laria et al., 2006; Carrera et al., 2006; Mendez et al., 2003; Institute for International Cooperation in Animal Biologics, 2005), and individuals who come in contact with infected farm animals or with animal-derived tissues are at risk. In addition, inhalation of infected aerosolized particles including dust, soil and water has been reported (Laria et al., 2006; Fiori et al., 2000; Deqiu et al., 2002). Transmission from human to human is very rare but has been documented after blood transfusion and bone marrow transplantation, and neonatal infection can be acquired transplacentally (Mosayebi et al., 2005) during delivery and postnatally by breast milk (Carrera et al., 2006; Lubani et al., 1988). Transmission through sexual contact usually does not occur, with the exception of very rare cases (Bossi et al., 2004). The disease remains among the most commonly recognized causes of laboratory-transmitted infection; 2% of all brucellosis cases are laboratory acquired (Mazuelos et al., 1994). Accidental human inoculations may occur during vaccination of animals (Ashford et al., 2004). The infection rate of laboratory-associated infections has ranged from 30% to 100% depending, among other factors, on the location of workers, if aerosol–generating procedures have been performed, and the concentration of microorganisms in contaminated media is high (Yagupsky and Baron, 2005).

Initial infection in the reservoir animal species is often followed by abortion and subsequent delay or permanent infertility. Infected animals shed organisms in uterine discharge following abortion and subsequent parturition and also in colostrum and milk (Robinson, 2003; WHO/OIE/FAO/CDC, 2006). Disease is typically transmitted when susceptible animals come into direct contact with tissues or discharges from infected animals and as a result from the ingestion of contaminated material. Once the susceptible animal ingests the organism, the bacteria progress to regional lymph nodes where they reside during the incubation period, which may range from two weeks to over months. After a subsequent brief phase when the bacteria are in the blood stream, the organism localizes in the uterus, placenta, udder, and/or regional lymph nodes (Alton et al., 1988). Transmission via artificial insemination has been reported to occur with relative certainty (FAO, 2000). Venereal infection can occur, but this is mainly seen with *B. suis* infection (Robinson, 2003). Both *B. abortus* and *B. melitensis* can be transmitted from dams to calves, lambs, and kids, and a smaller proportion of lambs and kids can be infected *in utero;* however, the majority of infections are probably acquired by consumption of colostrum or milk (European Commission, 2001). *Bruella ovis* is often transmitted among rams by passive venereal transmission via ewes that can carry this organism in the vagina for at least two months, acting as a mechanical vector. Contamination of pastures does not seem to be an important method of transmission for *B. ovis* (Institute for International Cooperation in Animal Biologics, 2007). *Brucella* has been found in lungworms (*Parafilaroides sp*) in harbor seals where the parasites may act as vectors. Ingestion of infected seals has been suggested as a possible route of exposure for polar bears (Institute for International Cooperation in Animal Biologics. 2007).

Brucella as Bioterrorism Agent

A bioterrorism attack is the deliberate use of viruses, bacteria or other germs (agents) to cause illness or death in people, animal, or plants. These agents are typically found in nature, but it is possible they could be changed to increase their ability to cause disease, to make them resistant to current medication, and to increase their ability to spread into the environment (CDC, 2007). The most likely form of deployment is by airborne release of the agent as an aerosol or powder preparation (White, 2002). Bioterrorism agents can be separated into three categories (A, B, C) according to established criteria, and the common characteristics of each category are:

A: Easily spread or transmitted from person to person.

- Result in high death rate.
- Cause public panic.
- Require special action for public health preparedness.

B: Moderately easy to spread.

- Moderate illness rates and low death rates.
- Require specific enhancements in CDC lab capacity.

C: Easily available.

- Easily produce and spread.
- Have potential for high morbidity and mortality rates. (CDC, 2007a; CDC, 2007b).

Interest in *Brucella species* as a biological weapon stems from the fact that airborne transmission of the agent is possible, via entry through mucous membranes such as the conjunctiva, oropharynx and respiratory tract and skin abrasions. The infectious aerosol dose for humans is considered to be 10-100 organisms (Boosi et al., 2004). Although the causative agents of brucellosis are categorized as incapacitating agents where infections caused by aerosols likely produce large numbers of casualties with little mortality, brucellosis deserves consideration by defense planners because of its extraordinary infectivity (Ciestlak et al., 2000). Both *B. melitensis* and *B. suis* have been developed experimentally as biological weapons by statesponsored programs, and their relative stability in aerosols, combined with low infectious doses, makes them suitable agents for this purpose (WHO/OIE/FAO/CDC, 2006).

Survival of *Brucellae* in the Environment

Brucellae are sensitive to exposure to heat and most disinfectants but can survive in the environment for up to two years under specific conditions, becoming a continuous threat to both humans and animals (Boosi et al., 2004). The ability of *Brucellae* to persist outside mammalian hosts is relatively high compared with most other non-sporing pathogenic bacteria, under suitable conditions. Numerous studies have assessed the persistence of *Brucella* spp. under various environmental conditions. When pH, temperature and light conditions are favorable, including high humidity, low temperature and absence of direct sunlight, *Brucellae* may retain infectivity for several months in water, aborted fetuses and fetal membranes, feces and liquid manure, wool, hay, and on buildings, equipment and clothes. *Brucellae* are able to withstand drying, particularly in the presence of extraneous organic material and will remain viable in dust and soil. Survival can be prolonged at low temperatures, especially below 0^0 C (European Commission, 2001). In countries where traditional hand-made techniques are still used to manufacture dairy products like cheese and yogurt, non-pasteurized fermented dairy products cannot be considered *Brucella*-free after fermentation processes and subsequent storage, especially if the raw milk came from endemic areas (Estrada et al., 2005). Tables 1.2 and 1.3 show the persistence time of *B. melitensis* and *B. abortus* in environmental settings.

Table 1.2: Studies on survival time in the environment (European Commission, 2001)

Environment	Conditions	Survival time
Direct sunlight	< 31º C	4 h 30 m
Water	- 4º C	4 months
Water (Laboratory)	20º C	2.5 months
Water (Lake)	37º C. PH=7.2	< 24 hours
	8º C. PH=6.5	> 2 months
Soil	Dried in Laboratory	< 4 days
	Dried at 18º C	69-72 days
	Wet	< 7 days
	Humid atmosphere	> 2 months
	Autumn (90% humidity)	48-73 days
	February(Rapid drying)	72 days
Urine	37º C, PH=8.5	16 hours
	8º C, PH=6.5	6 days
Raw milk	25-37º C	24 hours
	8º C	48 hours
	- 40º C	2.5 years
Whey	17-24º C	< 5 days
	5º C	> 6 days
Manure / dung	Summer	24 hours
	25º C	1 month
	Winter	2 months
	8º C	12 months
	-3º C	3 months
Manure / Liquid	Summer	3 months
	Winter	6 months
	In tank	1.5 months
	In tank (12º C)	> 8 months
Wool	Warehouse	4 months
Hay	-	Several days – Month
Street dust	-	3 – 44 days
Wooden walls or floors of pens	-	4 months
Pasture	Sunlight	< 5 days
	Shade	> 6 days

Table 1.3: Studies on survival time in dairy products (European Commission, 2001)

Product	Species of Brucella	Temperature (ºC)	PH	Survival time
Milk:	*B. abortus*	71.7	-	5–15 seconds
	B. abortus	38	4.00	< 9 hours
	B. abortus	25 – 37	-	24 hours
	B. abortus	0	-	18 months
Cream:	*B. abortus*	4	-	6 weeks
	B. melitensis	4	-	4 weeks
Ice cream:	*B. abortus*	0	-	30 days
Butter:	*B. abortus*	8	-	142 days
Cheese:				
Various	*B. abortus*	-	-	6–57 days
Various	*B. melitensis*	-	-	15–100 days
Feta	*B. melitensis*	-	-	4 – 16 days
Pecorino	*B. melitensis*	-	-	< 90 days
Roquefort	*B. abortus & B. melitensis*	-	-	20 – 60 days
Camembert	*B. abortus*	-	-	< 21 days
Erythrean	*B. melitensis*	-	-	44 days
Cheddar	*B. abortus*	-	-	6 months
White	*B. melitensis*	-	-	1–8 weeks
Whey:	*B. abortus*	17 – 24	4.3 – 5.9	< 4 days
	B. abortus	5	5.4 – 5.9	> days

Control Policies

It is generally accepted that before a zoonoses control program can be designed and implemented, a well-functioning surveillance system that can be fed with valid data collected from the field must be established. The main purpose of surveillance would be to determine the prevalence of disease so appropriate measures for control can be taken (European Commission, 2001). Over the years there have been a number of guidelines and manuals published on brucellosis, with particular emphasis on *B. melitensis*. Data from the Middle East and Eurasia where the disease is endemic indicate a positive correlation between the level of *B. melitensis* infections in small ruminants and the number of humans infected (FAO, 2009). There are three main control strategies for controlling Brucellosis, each with its own advantages and disadvantages, Table 1.4 (Nicholas, 1998; European Commission, 2001).

1) Mass vaccination:

* Advantages:

- Lower cost
- Easy to manage
- Herd immunity quickly established

* Disadvantages:

- Abortions induced by vaccine post vaccination
- Difficulty to distinguish between Infected and vaccinated animals
- There is some kind of public health hazards

2) Vaccination of young animals and elimination of the infected ones.

* Advantages:

- Minimize abortions

- Differentiation of infected from vaccinated

* Disadvantages:

- Herd immunity slowly established

3) Elimination of infected animals:

* Advantages:

- Elimination of infected animals

* Disadvantages:

- Higher cost
- Requires appropriate legislation
- Need for efficient veterinary services (Nicholas, 1998; European Commission, 2001).

There have been many controversies regarding the most appropriate policy that should be implemented to start control programs in any country intending to control the disease. The controversies generally surround the hazards from using the live vaccines due to the nature of the bacterial strain used in vaccine production.

International agencies such as WHO and FAO have suggested that national control programs should depend on a whole-flock vaccination scheme as a cost-effective strategy until disease prevalence has been reduced; only then would test-and-slaughter be implemented to eradicate the disease (Bardenstein et al., 2002; Carrera et al., 2006). In the meantime, there has been opposition to such policy due to adverse effects encountered in the field and public concern of possible risks to the human population following secretion of the vaccine strain in milk.

Table 1.4: Summary of the advantages of Brucellosis control strategies

Strategy.	Advantages.	Disadvantages.
- **Mass vaccination**	- Lower cost - Easy to manage - Herd immunity quickly established and maintained by vaccinating young animals - Well accepted by owners	- Abortions post vaccination - Difficulty to distinguish between vaccinated and infected animals - Public health hazards. - Infected animals remain on farms along their economical ages
- **Vaccination of young animals and elimination of infected**	- Minimize vaccine induced abortions - Can differentiate between infected and vaccinated	- Herd immunity slowly established - Difficult to manage
- **Elimination of infected animals**	- If successful, will lead to elimination of infected animals - Diagnostic tests are more accurate in non-vaccinated animals but still not optimum	- Cost is very high, and may require a whole herd slaughter to be effective - Requires an efficient and very well organized veterinary service - Suitable for low disease prevalence areas only - Risk of epidemics and subsequent human infection

Brucella vaccines

For most infectious diseases, vaccination of the entire animal population, repeated at regular intervals, is followed by a significant decrease in prevalence, and the first important issue is to choose the safest and the most appropriate vaccine (Blasco, 1997). According to the devastating consequences of the socioeconomic and medical impacts of brucellosis, many studies and much effort have been implemented to control the disease in different ways, and vaccination attempts have been one of the those (Schurig, 2002). There have been different kinds of vaccines used for controlling the disease in different species and many have been discarded because of the low level of immunity produced by them. The effective classical vaccines are S19 in cattle and Rev 1 in small ruminants; however, vaccine-induced antibodies to the O-polysaccharide of the lipopolysaccharide may be difficult to distinguish from those resulting from infection, complicating diagnosis (Moriyon et al., 2004). The most successful vaccines hitherto have been those employing live attenuated derivatives of *Brucella spp.* (Schurig, 2002).

Live *Brucella* Vaccines

B. abortus S19 vaccine

The S19 vaccine has been most widely used to prevent bovine brucellosis (Nicoletti, 1990). This strain was described in 1930 but had been isolated from a bovine milk sample in 1923. The isolate was left at room temperature for more than a year and subsequently was found to be attenuated **(**Schurig, 2002). It has smooth morphology and normally is unable to grow in the presence of erythritol (Jones et al., 1965; Beckett and Mc Darmaid, 1985). The effectiveness of this vaccine depends on several factors, including the age of targeted animals, dose concentration, route of administration, and prevalence of the disease in infected herds (Arenas-Gamboa et al., 2009). Prevention of brucellosis in cattle can be achieved by vaccinating heifers with *B. abortus* strain 19 (S19), which induces production of antibodies to the O antigens of lipopolysaccharide (LPS) (Nicoletti, 1990). Several studies have addressed a wide range of vaccine concentrations administered by different routes, and has been determined that the best protection occurred via subcutaneous inoculation at a concentration of 11.5 x 10^{10} cfu/dose when used in heavily infected areas. The subcutaneous route of inoculation is apparently superior to the conjunctival route (Fensterbank and Plommet, 1979). In an earlier study, the recommended concentration was 6 x 10^{10} cfu/dose (Drimmelen and Steyn, 1958). The immune responses from reduced doses of *B. abortus* (S19) vaccine have been studied by many researchers. It was suggested that starting with a subcutaneous inoculation of 9 x 10^{10} cfu/dose followed by conjunctival inoculation at 5 x 10^{9} cfu/dose six to eight months later would provide the best protection for heifers. However, has been recognized that two vaccinations by the conjunctival route with 5 x 10^{9} cfu/dose, would be simpler, more economical, as effective, and would have the advantage that vaccination could be done at any age without risk of serological response (Fensterbank and Plommet, 1976).

B. abortus RB51

Vaccines based on killed cells of virulent strains administered with adjuvant induced significant protection but also unacceptable levels of antibodies that interfered with diagnostic tests. Development of the rifampicin-resistant mutant *B. abortus,* RB51 strain overcame the problem and provided safe, effective vaccination against bovine brucellosis and exhibited negligible interference with diagnostic serology (Schurig, 2002). Strain RB51 (SRB51) of *B. abortus* is a laboratory-derived rough mutant of the standard virulent strain of *B. abortus*

(S2308). This mutant lacks most of the LPS O-side-chain found in S19 and in naturally occurring field strains of virulent *B. abortus*, and the reduced O-side-chain prevents the bacterium from inducing antibody formation in vaccinated cattle (Schurig et al., 1991). Using this vaccine during the eighth month of pregnancy at a concentration of 1 x 10^9 cfu/dose, did not induce abortion, but it was recovered from milk for up to 69 days post-vaccination (Uzal et al., 2000). The RB51 vaccine is not effective in sheep against *B. melitensis* infection, but it can be used in the replacement of *B. abortus* S 19 vaccine for preventing bovine brucellosis, increasing the efficiency of serologic identification, and removal of infected cattle from herds in the United States (Stevens et al., 1994; OIE, 2009).

***B. melitensis* Rev-1 vaccine**

The *B. melitensis* Rev-1 vaccine is the most widely used vaccine for prevention of brucellosis in sheep and a goat, which is the reference vaccine by which any other vaccine should be, compared **(**OIE, 2009). It is a live attenuated *B. melitensis* strain derived from a virulent *B. melitensis* isolate which became dependent on streptomycin for growth, (Elberg and Faunce, 1957). *Brucella melitensis* Rev-1 vaccine is used as a freeze-dried suspension of live *B. melitensis* Rev-1 strain. It is normally given to lambs and kids between three and six months of age as a single subcutaneous injection. The standard dose is between 0.5 x 10^9 and 4.0 x 10^9 viable organisms. The use of this vaccine administered by the conjunctival route with a minimum dose of 1-5 x 10^8 produced similar protection without a persistent antibody response (OIE, 2000). Subcutaneous vaccination induces strong interference in serological tests and should not be recommended in eradication programs. Because the vaccine produces similar protection without inducing persistent antibody responses when given by the conjunctival route, it can be used in eradication programs combined with vaccination (Martin et al., 1999). The application of the live *B. melitensis* Rev-1 vaccine in whole-flock vaccination programs is the only practical method of controlling *B. melitensis* infection in small ruminants in areas having high prevalence, extensive management systems and low socio-economic levels **(**Blasco, 1997).

B. suis **S2 vaccine**

An orally administered brucellosis vaccine, *Brucella suis* strain 2 vaccine was developed in China. It is effective for oral vaccination of sheep, goats, cattle and pigs and has been widely used for prevention of animal brucellosis in China over the past 15 years, and about 30-40 million doses of the vaccine are produced every year (Xina, 1986). Vaccine strain S2 was found to be protective in different domestic animal species when inoculated orally, especially pigs exposed to wild *B. suis* through conjunctival mucous membranes. The vaccine provided 75% protection when pigs were vaccinated twice two to three months apart. However, protection was absent when vaccinated female animals were challenged by mating them with an infected boar excreting *B. suis* in semen **(**Nicoletti, 1990**)**.

Killed *Brucella* Vaccines

B. abortus **45/20**

Strain 45 was first isolated in 1922 in Great Britain from a cow. It was a rough strain developed by 20 passages in guinea pigs. The strain was shown to have good immunizing properties in guinea pigs and cattle. Its ability to revert to the virulent smooth status was the reason for using it as an inactivated vaccine with adjuvant. The main disadvantages of this vaccine were the marked local reaction at the injection site and the prolonged antibody interference in the serological tests (Bossi et al., 2004).

B. melitensis H38

Brucella melitensis H 38 vaccine was a suspension of formalin-killed cells in incomplete adjuvant used at a concentration of 15 x 10^9 cells/ml. This *B. melitensis* killed vaccine was effective against a *B. abortus* challenge (Renoux and Renoux, 1973). However, the vaccine induced high, persistent titers and caused long lasting unacceptable local reactions at the site of injection (Nicoletti, 1990).

Human Vaccines against Brucellosis

B. abortus 19-BA vaccine

The 19-BA vaccine is a derivative from *B. abortus* strain 19 where dissociated colonies were selected and cultured, called 19-BA by Vershilova to distinguish it from the original seed. The immunogenic dose of this strain was studied and determined by subcutaneous and cutaneous administration in guinea pigs. Immunity was evaluated against virulent cultures of *B. melitensis* and *B. suis*, and the first test of this vaccine was carried out in human volunteers in 1946, who received 20-40 million organisms subcutaneously. The studies were continued and later in 1947-1952, a dried vaccine was made and used to vaccinate 5,000 workers at risk of infection. It was determined that the immunogenic and safe vaccine dose for subcutaneous vaccination was to 300-600 million organisms. It was found that immunological reactivity was maintained for a long time (2-3 years) in the majority (56-63%) of persons working in endemic brucellosis areas or abattoirs. Since that time, the epidemic control services in the former USSR used human vaccination as a main prophylactic measure (Drimmelen et al., 1958).

B. abortus 104M vaccine

The 104M vaccine is one of several *Brucella* vaccines that have been used in China to prevent and control *B. abortus* infection in humans (Dequi et al., 2004).

B. melitensis WR201 vaccine

Rough *Brucellae* mutants have been sought as vaccine candidates because they do not induce seroconversion (Nikolich et al., 2010). The live-attenuated, purine-auxotrophic mutant strain of *B. melitensis,* WR201 was tested for its ability to elicit cellular and humoral immune responses and to protect mice against intranasal challenge with *B. melitensis* 16M. Mice inoculated intraperitoneally with WR201 made serum antibodies to lipopolysaccharide and non-O-polysaccharide antigens, and splenocytes from immunized animals released interleukin-2 (IL-2), gamma interferon, and IL-10 when cultured with *Brucellae* antigens, which led to protection from disseminated infection (Hoover et al., 1999).

In another study, the clearance of orally administered strain WR201 from lungs, livers, and spleens by eight weeks after immunization and the ability of the organism to induce a protective response against intranasal challenge with strain 16M suggest that induction of purine auxotrophy in *B. melitensis* has promise as a strategy for development of a safe, convenient, and effective human vaccine (Izadjoo et al., 2004). These studies suggest that WR201 should be further investigated as a vaccine to prevent human brucellosis.

Brucella Subunit Vaccine

The antigenic fractions extracted from *Brucella* have been utilized in association with a variety of adjuvants to achieve immunity. Some of the preparations include whole killed cells, cell envelopes, outer membrane proteins, insoluble residues of hot sodium dodecyl sulfate (SDS) extract of cell envelopes (PG), soluble SDS extracts, *Brucella* soluble antigens (BASA), periplasmic proteins and salt extractable proteins, chemically modified *Brucella* proteins, smooth and rough LPS, and recombinant Cu-Zn superoxide dismutase (SOD) (Schurig et al., 2002).

Studies that addressed the importance of outer membrane proteins of *B. ovis* revealed that the fraction enriched in outer membrane proteins and rough lipopolysaccharide by using the hot saline extract may represent a useful alternative to *B. melitensis* Rev-1 or *B. abortus* 45/20 as a vaccine against *B. ovis* (Cassataro et al., 2007; Blasco et al., 1993). A genetic vaccine based on the Omp31 gene elicited a strong cellular immune response and the available data suggested Omp31-pcDNA31 was a good candidate for use in future studies of vaccination against brucellosis (Doosti et al., 2009). Moreover, some promising studies showed that outer membrane proteins could be useful for differentiating *B. melitensis* infection from *B. melitensis* Rev-1 vaccinated sheep (Zygmunt et al., 1994). Regarding recombinant vaccines, a bacterium closely related to *Brucella* species, *Ochrobactrum anthropi*, was used as a vaccine vector for the delivery of *Brucella* antigens to mice, leading to protective immunity against brucellosis (He et al., 2002).

Recombinant *B. abortus* L7/L12 ribosomal protein fused to maltose-binding protein (MBP), identified as T-cell reactive, provided protective immunity in mice against brucellosis when immunized with this protein (Oliveria et al, 1996).

A vaccine based on an outer membrane complex from *B. ovis,* encapsulated in poly-epsilon-caprolactone (PEC) microparticles was developed and tested in rams. Homogeneous batches of microparticles were prepared by a new double-emulsion solvent evaporation method called "Total Recirculation One-Machine System" (TROMS). The microparticles had a mean diameter of two microns and displayed antigen loading of about 13 microg HS per mg of microparticles. Subcutaneous vaccination of rams with 800 microg HS (hot saline antigenic extract of *B. ovis*) in PEC microparticles induced an adequate serological response against *B. ovis* antigens and conferred similar protection against challenge with *B. ovis* to that induced by the live attenuated *B. melitensis* Rev 1 reference vaccine (Munoz et al., 2006).

Routes of Administration

A large number of studies have been conducted to determine the most accurate method and dose for administration of different vaccines including subcutaneous (SC), intradermal (ID), intracaudal (IC), conjunctival (C), oral, intravaginal, and intrauterine (Nicoletti, 1990). Regarding the administration of *Brucella* vaccines, the most current practical and commonly used methods are outlined below.

Subcutaneous Route (SC)

Regarding *B. melitensis* Rev-1 vaccine, it can be given in a volume of 1 ml in two different concentrations. The first one is the full dose containing 1-4 x 10^9 cfu/dose, and the second is a reduced dose containing 1 x 10^4 – 1 x10^8 cfu/dose (Blasco, 1997). It has been shown that the use of reduced dose Rev-1 vaccine (1 x 10^5 cfu/dose) protects goats vaccinated in endemic areas for at least five years after immunization. Regarding *B. abortus* S19 vaccine in calves three to eight months old, it can be administered in full dose 60 x 10^9 cfu/dose, and for adult animals, it can be given as a reduced dose 5 x10^9 cfu/dose (Plommet, 1995).

Conjunctival Route

Currently the conjunctival route of vaccine administration is widely used because it overcomes the problematic issues of using the live attenuated vaccines (Rev-1 and S19) that can cause prolonged serological interference and abortions post vaccination (Blasco, 1997; Zundel et al., 1992).

Aerosol Route

In Turkey, aerosol immunization of sheep against brucellosis was successfully performed in lambs and ewes via exposure to 300 ml of 20 x 10^9 cfu/ml aerosol *B. melitensis* Rev-1 vaccine for 20 minutes and then taken to the pasture (Uysal, 1995).

Oral Route

The oral route is mainly used for the administration of *B. suis* S2 vaccine which is usually given via drinking water in a dose of 25-50 x 10^9 cfu/head (Nicoletti, 1990).

Epidemiology of *Brucellosis*

Global Scenario

The infection occurs worldwide in both humans and animals, but is most common in the Mediterranean countries of Europe and Africa, the Middle East, India, Central Asia, and Central and South America, with the highest numbers of cases occurring in Mexico, Argentina, and Peru (Mikolon et al., 1998; Boosi et al., 2004; First International Conference on Emerging Zoonoses, 1997). The disease does not exist in countries where bovine brucellosis (*B. abortus)* has been eradicated, which is usually defined as the absence of any reported cases for at least five years. These countries include Australia, New Zealand, Canada, Cyprus, Denmark, the Netherlands, Finland, Norway, Sweden (except for sporadic incursions from the south) and the United Kingdom as mentioned in the OIE report of 2002 (Robinson, 2003; European Commission, 2001). However, a study for assessment of the risks associated with animals moved from herds infected with brucellosis in Northern Ireland found that 3.1% of animals moved in the six-month period prior to disclosure of infection in the source herd and subsequently tested, were seropositive in their destination herds (Stringer et al., 2008). Brucellosis is a disease of domestic animals and humans in Central America (CA), where bovine and swine brucellosis caused by *Brucella abortus* and *Brucella suis* respectively, have been identified in all CA countries, while ovine and caprine brucellosis caused by *B. melitensis* has been detected in Guatemala. The prevalence of bovine brucellosis is estimated between four and eight percent, with higher prevalence in dairy herds (Moreno, 2002)**.**

Brucella melitensis infection in sheep appears to occur endemically in the Mediterranean region, especially along its northern and eastern shores, stretching through Central Asia as far south as the Arabian Peninsula and as far as Mongolia. The disease also occurs in Africa and India (European Commission, 2001). Sheep, goats and their products remain the main source of infection, but *B. melitensis* in cattle has emerged as an important problem in some southern European countries, Israel, Kuwait, and Saudi Arabia. It has been shown that the same problem exists in some South America countries, like Brazil and Colombia where *B. suis* biovar 1 has become established in cattle (First International Conferences on Emerging Zoonoses, 1997).

Middle East

Although human brucellosis is a notifiable disease in some countries of the Middle East, it is often unrecognized and unreported. In many countries, the awareness of brucellosis by medical specialists is very weak and, in most of the cases, public health laboratories are not performing diagnostic tests. Brucellosis cases very often remain unrecognized and are treated as other diseases. They are often labeled „Fever of unknown causes". For these reasons, the actual number of cases of brucellosis is unknown and is believed to be much more than the officially reported number of cases. Eastern Mediterranean countries experienced an increase in the number of human brucellosis cases in 1982-1990, and in seven countries of the region (Iran, Jordan, Kuwait, Oman, Saudi Arabia and Syria) about 82,000 cases were reported in 1988 as compared to 2,871 in 1985 (Refai, 2003).

In 1977 the incidence of brucellosis in Makah, Saudi Arabia was found to be 0.8% in goats, 0.5% in sheep, 2.8% in camels and 3.6% in cows. In the Asir region, the incidence of brucellosis went up to 18.2% in goats, 12.3% in sheep, 22.6% in camels and 15.5% in cows by 1987. The overall human infection rate was reported in the range of 1.6-2.6 %, including both genders and all ages (El-Eissa, 1999). In another study evaluating milk samples from 120 seropositive milking camels, *B. melitensis* biovars 1, 2, and 3 were isolated from 41 camels (34%) (Radwan et al., 1995). In 1998, brucellosis ranked as the No. 1 reportable communicable disease (22.5%) in Saudi Arabian National Guard communities. Human brucellosis cases increased sharply (4.9 to 69.5/100,000) during the period 1985-1990. The highest rate (79.6/100,000) was recorded in 1988 (Memish and Mah, 2001).

In the Republic of Yemen, the reported prevalence of brucellosis among non-native goats from Africa was 5.6% and among non-native sheep from Africa was 1.7%. The seroprevalence of human brucellosis ranged from 0% to 0.8% (Al-Shamahy, 1999).

In the Kingdom of Jordan, according to a combined cross-sectional and longitudinal design to estimate seroprevalence of *Brucella* antibodies in sheep, the seroprevalence of brucellosis at the individual-animal level was 14.3% by RBT, 7.2% by ELISA, and 2.2% using both tests in series. Moreover, the overall incidence of abortion was 20% and the specific incidence due to brucellosis was 13% (Al-Talafhah et al., 2003). In camels, the incidence of brucella-specific abortion was investigated in 7 camel herds located in different locations in southern Jordan, the true prevalence of *Brucella*-seropositive was 12.1%, and thirteen (35.1%) herds had at least one positive camel (Al-Majali et al., 2008).

Studies performed in different regions of Turkey showed that *B. melitensis* was responsible for approximately 14-31% of the abortion cases in sheep (Fatma and Zabit, 2008). In a study during the period 2004-2006, cattle sera analyzed were 32.92% and 34.64% positive by RBPT and SAT, respectively. Farmer sera were positive at rates of 13%, 14.22% and 17.88% by RBPT, SAT and ELISA, respectively. There was no significant gender difference for *Brucella* seropositivity. Of the 28 sera from veterinarians, 13 (46.42%) were positive by the three serological tests. Moreover, a significant feature in patients with epididymoorchitis was a 9.1% seropositive rate for brucellosis (Yetkin et al., 2005; Otlu et al., 2008).

In Kuwait, a single serum sample was collected from each of 1,836 patients of different nationalities from January 2000 to December 2001. A total of 455 serum samples (24.8%) having a titer of 1:160 were presumptively diagnosed as cases of brucellosis. The peak for positive isolation was during April and May when *Brucella* spp were isolated from 123 blood cultures (74.1%). The blood culture isolation rate was significantly higher in patients with a titer of 1:1,280 than in those with a titer of 1:160 ($p < 0.05$). The study revealed that Kuwaiti and Bangladeshi nationals were most affected. Significant titers on the STA test were detected in 24.8% of the serum samples (Za et al., 1999).

In Iran, a descriptive, cross-sectional study was conducted in north Khuzestan, southern Iran from March 2004 to June 2004. A total of 3,594 persons took part in this study by randomized cluster sampling. Two hundred twenty-eight out of 3,594 were positive for brucellosis (6.3%). The study showed the prevalence of brucellosis among nomads in Iran washigh due to their life style (Alavi et al., 2007). In the town of Baft, located in southeast Iran, home for about 1,350 camels, a study was done to investigate brucellosis in camels. Serological examinations including the Rose Bengal Plate Test (RBPT), MRT and 2ME were performed on 1,123 camel serum samples; positive results were obtained in 118 (10.5%), 96 (8.54%) and 89 (7.92%) camels thus tested, respectively. In another study aimed to determine the seroprevalence of *B. canis,* 102 blood samples from companion dogs were divided into two age groups (1-5 years) and > 5 years. The overall prevalence was 4.90% (5 of 102), and the prevalence in dogs > 5 years was 9.3% (4 of 43), in comparison with dogs < 5 years 1.69% (1 of 59) (Mosallanejad et al., 2009). By using the GIS in explaining spatial distribution of brucellosis in an endemic district in Iran, which was Bardsir district in the Kerman province located nearly to the central part of Iran, the annual incidence of human brucellosis was shown to be 141.6 per 100,000 inhabitants, and most of the high risk villages were seen in the north and south of that district (Haghdoost et al., 2007).

In Israel, *B. abortus* was eradicated from cattle in the 1980s, using vaccination of female calves with strain 19 vaccine and implementation of a test-and-slaughter program. Young (two to six months old) female calf vaccination continues to be mandatory. In 1988, *B.melitensis* emerged as a national problem and a test-and-slaughter program combined with Rev-1 vaccination by the ocular route in young animals was conducted from 1993-97. Over 40,000 sheep and goats were culled before the program ceased due to budgetary constraints. However, the program resulted in a significant reduction in the incidence of human brucellosis (FAO, 2009).

Iraq

Iraq is in both the northern and eastern hemispheres. It is positioned in the Middle East, a recognized geographical region of southwestern Asia. The country is boarded by the Arabian Gulf, and the countries of Kuwait, Saudi Arabia, Jordan, Syria, Turkey, and Iran. Its land area is 432,162 sq km (166,859 sq miles), and consists of 18 governorates, including Anbar, Basra, Muthana, Qadisiyah, Najaf, Arbil, Sulaymaniah, Kirkuk, Babil, Baghdad, Dahuk, Thiqar, Deyala, Kerbala, Missan, Ninava, Salahadin, and Wasit.

Veterinary services in Iraq is controlled and supervised by the State Company for Veterinary Services (SCVS) in Baghdad since being established in 1924. After 1990, the three northern governorates of Arbil, Dahuk, and Sulaymaniah, (the Kurdistan region), had their own administration. The veterinary activities in the other 15 governorates are administered by the

State Company for Veterinary Services (SCVS) in Baghdad. In general, there is a central veterinary hospital in each governorate that controls the work of several related veterinary dispensaries. Accordingly, there are 18 central veterinary hospitals and 273 related dispensaries, including three central hospitals and 45 veterinary dispensaries in the Kurdistan region (Fig. 1.1).

Figure 1.1: The Eighteen Governorates of Iraq

Brucellosis has been considered one of the most endemic diseases in Iraq since 1937 (Al-Zahawi, 1938). A review of serological investigations of brucellosis among farm animals and humans in northern governorates of Iraq for the period 1974 – 2004 showed there were several attempts to determine disease incidence. Paul Nicoletti (1986) reported 10.8%, 4.4%, 3.1% and 1% positive results of samples obtained from human, goat, cattle and sheep, respectively. In 1999, Shareef et al, investigated the prevalence of brucella agglutinins in animals and man in the Qaradagh district, south of Sulaymaniah city and the results showed the percentages of positive goat and sheep by the card test were 3.36% and 1.34%, respectively. According to the annual report of health directorate in Sulaymaniah, Iraq, the positive human sera showed 35% for males and 65% for females, with a male: female ratio of 1 : 1.8, and positive children sera (age range 6–12 years) were only 4.2% of the total positive human sera (Shareef, 2006). In a study of the occurrence and epidemiology of *Brucella* spp in raw milk samples in the Basra province, it was shown that 24.2% of 420 milk samples collected were seropositive by the milk ring test, and the overall prevalence of *Brucella* in milk produced in the Basra province of Iraq was 14.7% by culture isolation results (Abbas and Al-dewan, 2009). It was shown that the prevalence of brucellosis was higher in the suburban semirural area (29.3%) than the rural and urban areas of the Basra region (Yacoub et al., 2006).

According to the personal communications, official data of the Iraqi Ministry of Health (MOH) in Baghdad regarding the annual human infected cases indicated the disease was at minimum levels during the late 1980s. However, the disease incidence among humans began a serious increase during 1990-1995, most likely due to the economical sanction of United Nation resolutions that caused a huge shortage in medical capabilities. The situation improved due to the Oil for Food and Medicine agreement between Iraq and the United Nation in 1996. Table 1.5 and Figure 1.2. show the seasonality of disease, and the data demonstrates an increase in human cases during the season of parturition which is the same season when abortions increase as shown in Table 1.6 and Figure 1.3.

Table 1.5: Human infected cases in Iraq for the period 1988-2003

Year	Cases	Year	Cases
1988	1892	**1996**	7531
1989	2464	**1997**	8911
1990	2819	**1998**	5305
1991	13106	**1999**	7297
1992	14546	**2000**	8030
1993	14989	**2001**	8166
1994	15476	**2002**	7189
1995	19040	**2003**	No Data *

(*) In 2003, the data was not accurate because of the war and the total collapse in all the official governmental foundations in middle and south of Iraq.

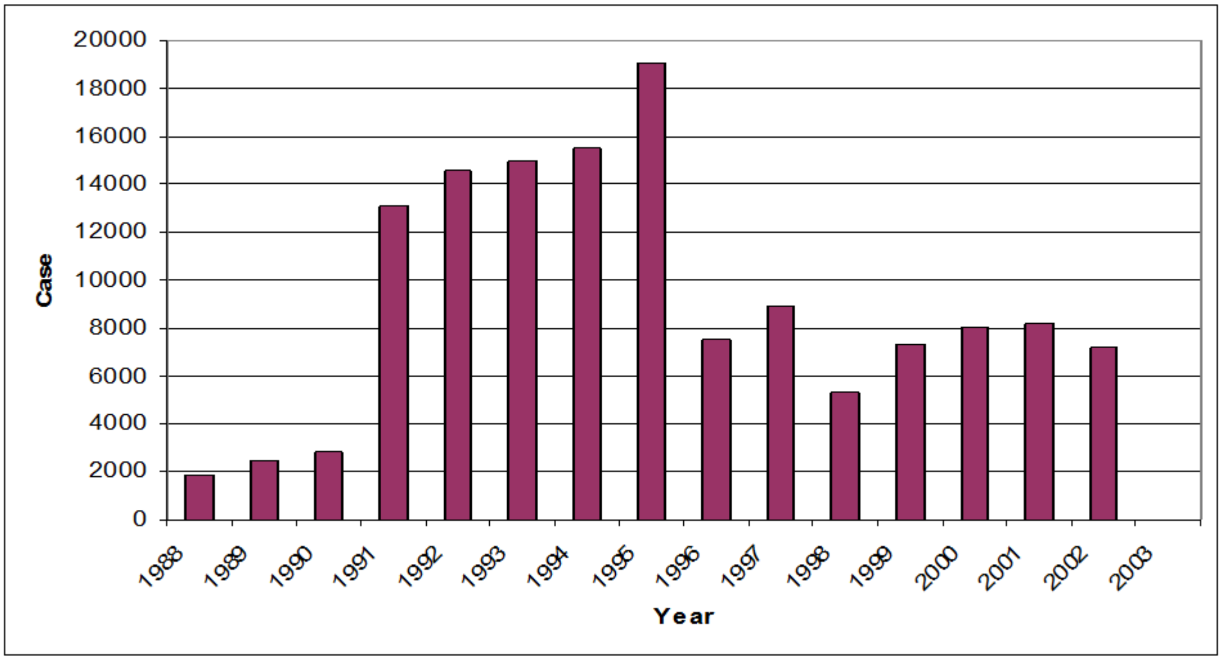

Figure 1.2: The human infected cases in Iraq for the period 1988-2003

Table 1.6: Monthly recorded human brucellosis cases in Iraq for the period 1999-2002

Month	**1999**	**2000**	**2001**	**2002**
January	272	397	486	286
February	404	501	489	401
March	567	463	451	517
April	658	969	968	703
May	972	788	814	689
June	835	892	1006	909
July	850	869	891	855
August	661	1069	837	811
September	564	750	801	654
October	629	443	642	510
November	652	346	434	437
December	423	444	347	417
Total	**7287**	**7958**	**8166**	**7189**

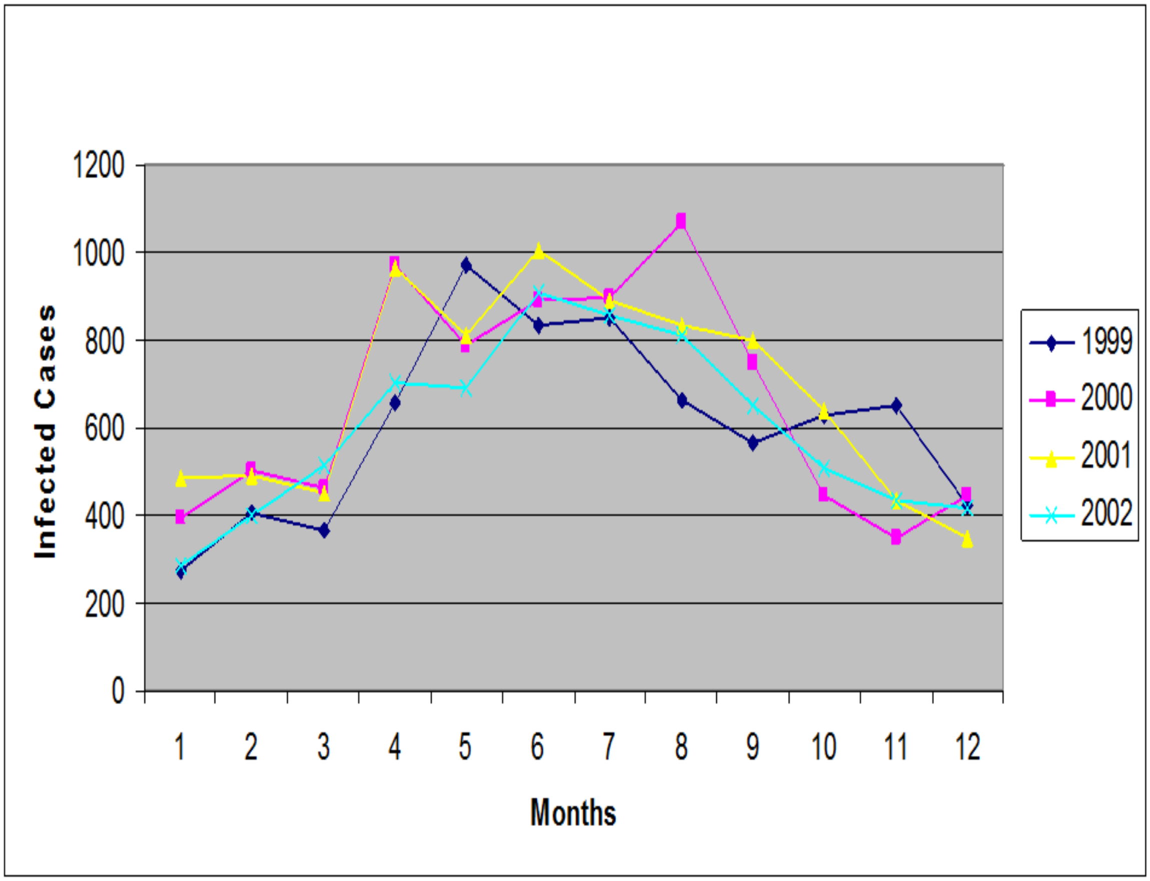

Figure 1.3: Seasonality of Human Cases for the Period 1999-2002

It is important to mention that socio-political issues have had significant influence on administrative and governmental structures. After the second Gulf war in 1991, there were several administrative changes in Iraq. The three northern governorates (Arbil, Duhuk, and Sulaymaniah) established their own administration which covered what recently has been known as the Kurdistan province, representing about 13% of the Iraq population. Accordingly, it was crucial to handle the data inputs as if there were two different veterinary administrations. The first one is in Baghdad which controls the veterinary activities in 15 governorates, and the second one is in Erbil where it controls the veterinary activities in the Kurdistan province. The second administration implemented vaccination 1998-2005 by using the Rev-1 vaccine administered via the conjunctiva route. The targeted animals were lambs and kids of three to eight months age, and the total vaccinated animals were 4,935,313 heads during that period.

There were significant differences between the control strategies of the two administrations, which could not be evaluated by the same parameters. Accordingly, it was more accurate to calculate the incidence of each region separately based upon the data shown below in table 1.7.

During the period between 2001 and 2009, and according to the reported human infected cases in health administration in both Baghdad and Erbil, it was determined that there was significant difference in incidence between the two regions. Infected cases and disease incidence in the middle and south of Iraq are shown in table 1.10 and figure 1.6. Regarding the Kurdistan province, infected cases and incidence are as shown in table 1.8 and 1.9, and figures 1.4 and 1.5.

Table 1.7: Population of Iraq divided into two categories between 1988-2009.

Year	Iraq population	Middle & South population	Kurdistan population
1988	17,814,801	15,498,877	2,315,924
1989	18,349,311	15,963,901	2,385,410
1990	18,899,860	16,442,879	2,456,981
1991	19,409,189	16,885,995	2,523,194
1992	19,932,244	17,341,053	2,591,191
1993	20,469,395	17,808,374	2,661,021
1994	21,021,022	18,288,290	2,732,732
1995	21,587,514	18,781,138	2,806,376
1996	22,249,812	19,357,337	2,892,475
1997	22,932,429	19,951,214	2,981,215
1998	23,635,988	20,563,310	3,072,678
1999	24,361,133	21,194,186	3,166,947
2000	25,108,525	21,844,417	3,264,108
2001	25,748,669	22,401,343	3,347,326
2002	26,405,133	22,972,466	3,432,667
2003	27,078,334	23,558,151	3,520,183
2004	27,768,699	24,158,769	3,609,930
2005	28,476,664	24,774,698	3,701,966
2006	29,202,819	25,406,453	3,796,366
2007	29,947,491	26,054,318	3,893,173
2008	30,711,152	26,718,703	3,992,449
2009	31,501,397	27,406,216	4,095,181

(*) According to the United Nation records, Kurdistan province population represents 13% of total Iraq population.

Table 1.8: **Incidence of Brucellosis in Middle-South of Iraq 2001-2009**

Year	Population of Middle and South	Human infected cases	Incidence/100000 People
2001	22401343	8166	36.45
2002	22972466	7189	31.29
2003	23558151	0	0
2004	24158769	4993	20.66
2005	24774698	5408	21.82
2006	25406453	4234	16.66
2007	26054318	3504	13.44
2008	26718703	4417	16.53
2009	27406216	4659	16.99

(*) In 2003, the data was not accurate because of the war and the total collapse in all the official governmental foundations in middle and south of Iraq.

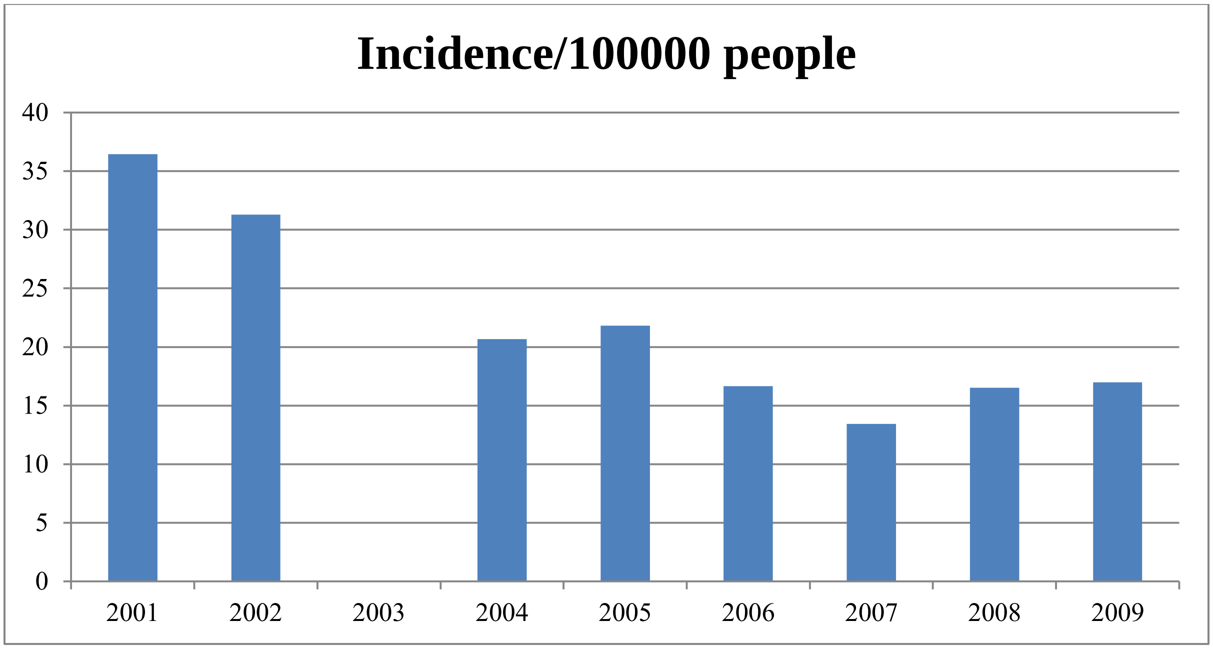

Figure 1.4: The Incidence of Brucellosis in the Middle-South of Iraq 2001-2009

(*) In 2003, the data was not accurate because of the war and the total collapse in all the official governmental foundations in middle and south of Iraq.

Table 1.9: Human Brucellosis Cases in Kurdistan Province 2001-2009

Year	Erbil	Duhuk	Sulaymania	Total
2001	3069	2998	1538	7605
2002	4040	1147	3425	8612
2003	2059	624	2643	5326
2004	900	1113	312	2325
2005	638	499	359	1496
2006	673	401	363	1437
2007	1421	322	857	2600
2008	1111	424	905	2440
2009	772	295	1077	2144

Table 1.10: Incidence of Brucellosis in Kurdistan Province 2001-2009

Year	Kurdistan population	Human infected Cases	Incidence/100000 people
2001	3,347,326	7605	227.19
2002	3,432,667	8612	250.88
2003	3,520,183	5326	151.29
2004	3,609,930	2325	64.40
2005	3,701,966	1496	40.41
2006	3,796,366	1437	37.85
2007	3,893,173	2600	66.78
2008	3,992,449	2440	61.11
2009	4,095,181	2144	52.35

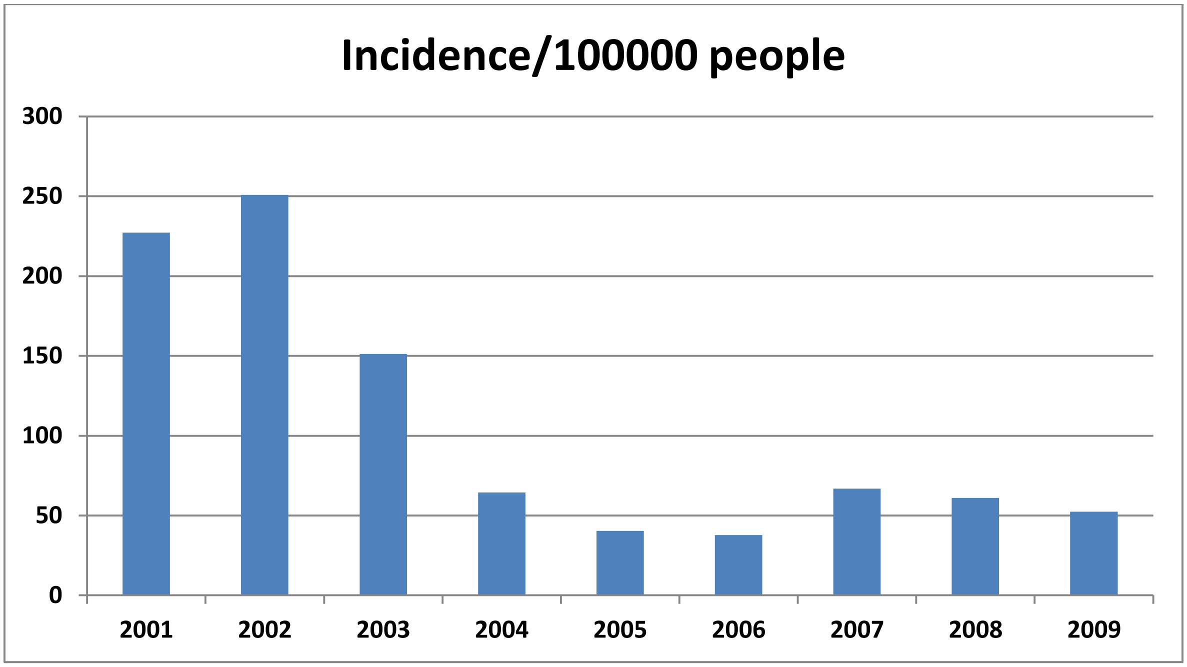

Figure 1.5: Incidence of Brucellosis in Kurdistan Province 2001-2009

(*) According to the stable and safety conditions during the war in 2003, the records can be consider more accurate than that in middle and south of Iraq.

CHAPTER 2 - Serosurveillance Phase

National Project for Controlling Brucellosis and Tuberculosis

The State Company for Veterinary Services (SCVS) established the National Project for Controlling Brucellosis and Tuberculosis (NPCBT) in 1995 due to the endemic status of brucellosis and the severe environmental and economic impacts at both human and livestock levels. The objectives of this project were the following:

- Planning, implementing and supervising epidemiological surveillances.
- Producing protective vaccines against the disease.
- Producing diagnostic kits used for detection of the disease.

The official opening of the project was in July 1995 with limited resources due to the sanctions of the United Nation Resolutions against the ruling regime at that time. The project consisted of four main units:

- *Brucella* diagnostic kits production unit
- *Brucella* vaccines production unit
- Tuberculosis unit
- Laboratory animal unit

The work started with experimental trials for vaccines production such as Rev-1 vaccine which provides protection against *B. melitensis* infection, and S19 vaccine which provides protection against *B. abortus* as shown in Table 2.1. Also, there was production of diagnostic kits used in laboratory for detection of the disease such as Rose Bengal Test antigen

(RBT), Serum Agglutination Test antigen (SAT) and Milk Ring Test antigen (MRT) as shown in Table 2.2.

Table 2.1: Production Quantities of Rev-1 Vaccine

Year	Rev-1
1999	53,000
2000	1,582,700
2001	230,200
2002	262,000
3/2003	30,000
Total	**2,156,700**

* More than 20,000 doses of S19 vaccine was produced according to the requests of the major dairy cattle stations when they intended to vaccinate only the remaining heifers (3–8) months of age.

Table 2.2: Production Quantities of Diagnostic Kits (tests)

Year	RBT	SAT	MRT
1999	34156	1400	-
2000	10824	1750	5700
2001	20900	3700	1500
2002	11616	2600	1200
3/2003	3300	1150	-
Total	**70,796**	**10,600**	**8,400**

After the war in 2003, there was total devastation and collapse in veterinary activities, including the NPCBT. Accordingly, there was great need for restoration and rehabilitation of the veterinary services in Iraq. A series of meetings were held with FAO experts for this objective. The United Nations Development Group (UNDG) Trust Fund project (OSRO/IRQ/406/UDG) was initiated for the restoration of essential and urgent veterinary services in Iraq and a national plan was developed for control and surveillance of animal brucellosis.

One of the most important agreements was consideration of the Brucellosis Controlling Program as a module for controlling other zoonotic diseases in Iraq; therefore, a major surveillance was implemented to monitor the prevalence of diseases in different animal species (sheep, goats, cattle, buffalo and camels).

Objectives of the Control Program

The objectives of the control program were to improve productivity of livestock by conducting the following steps:

1) Determine the seroprevalence and geographical distribution of Brucella infections in sheep, goats, cattle and buffalo by conducting a national serological survey.
2) Design and develop a national brucellosis control program based on the results of the national survey.
3) Employ strategic and active surveillance to monitor changes in prevalence of the disease in livestock with progress in reaching goals and allow management decisions to be made based on scientific judgment of surveillance data.
4) Determine the main abortion causes in small ruminants.
5) Enhancement of an attitude of team work between veterinary services and public health authorities regarding the continuous monitoring of the disease incidence in humans.

The main ultimate goal of the project was to improve the productivity in the livestock sector and reduce the prevalence of the disease in sheep and goats to less than 2% and less than 0.2% in cattle and buffalo. Ultimately, achieving the goals would reduce the disease incidence among human population from more than 27.5 cases/100,000 persons to less than 4 cases/100,000 persons within 15 years.

Serosurveillance Design

A cross-sectional study was performed to investigate some epidemiological aspects related to brucellosis status in different livestock populations in Iraq (sheep, goats, cattle, buffalo and camels). The serological survey was conducted to estimate the prevalence of brucellosis in susceptible animals and to establish the baseline for disease control strategies and options. The serosurveillance included the following topics:

1) Sampling:

Blood samples collection designed according to the population of each animal species in each governorate and based on updated records of livestock held by each owner. The sample size collected for each species was:

- Shoats = 1020 blood samples, from 18 villages in each of 18 included governorates.
- Cattle = 480 blood samples, from 8 villages in each of 18 included governorates.
- Buffalo = 480 blood samples, from 8 villages in each 11 included governorates.
- Camels = 480 blood samples, from 8 villages in a single included governorate.

2) Questionnaire preparation:

A questionnaire was designed, pre-tested and administered to collect information about target animal herd health and management, considering all the required details needed to be collected from randomly selected livestock holders of each species under investigation. The questionnaire was designed in co-operation with FAO experts during a series of workshops held in Amman, Jordan.

3) Database creation:

All information and data collected by the prepared questionnaire were organized in access files.

4) Field work:

- Blood sample collection

Survey field work has been completed with blood sample collected from the targeted species from different governorates. The total blood samples collected from shoats, cattle, buffalo, and camels were 18,360, 8,640, 5,280, and 480 samples, respectively. Samples collected from the four mentioned species totaled 32,760.

- Questionnaire completion.

5) Laboratory work:

All blood samples were tested using the Rose Bengal Test (RBT), and this phase of the work was accomplished in the central veterinary hospitals in each governorate, and then all seropositive samples were confirmed by indirect ELISA testing at the NPCBT in Baghdad.

6) Interactive map system creation:

The true disease distribution status was able to be illustrated by using the Arc View software after entering the field information collected by the questionnaire and the laboratory results carried out by testing the blood samples collected.

Results of Surveillance

The results showed the apparent prevalence of disease in sheep and goats, cattle, buffalo, and camels was 6.51%, 1%, 1.48%, and 0.02%, respectively. In addition, the distribution of disease among the three main animal populations (sheep and goats, cattle, and buffalo) in different Iraqi governorates, except Kurdistan Province in the three northern governorates, was determined in the Figures 2.1, 2.2, 2.3, 2.4, 2.5, and 2.6.

Table 2.3: Apparent Prevalence of Brucellosis Surveillance

Governorate	Shoats	Cattle	Buffalo	Camel
Ninava	4.90%	0.37%	0.56%	-
Kirkuk	16.37%	2.59%	-	-
Baghdad	6.85%	0.69%	2.78%	-
Deyala	9.81%	0.56%	1.30%	-
Wasit	7.93%	0.74%	1.67%	-
Babil	4.59%	0.37%	0.02%	-
Kerbala	8.82%	0.93%	0.74%	-
Missan	3.23%	0.74%	0.37%	-
Thiqar	0.89%	0.02%	0.37%	-
Qadiseya	7.54%	1.11%	2.78%	-
Najaf	5.22%	3.70%	0.18%	-
Muthana	0.20%	0.02%	-	0.02%
Basra	1.67%	0.93%	5.35%	-
Anbar	7.78%	0.74%	-	-
Salahadin	11.86%	1.48%	-	-
Mean	**6.51%**	**1%**	**1.48%**	**0.02%**

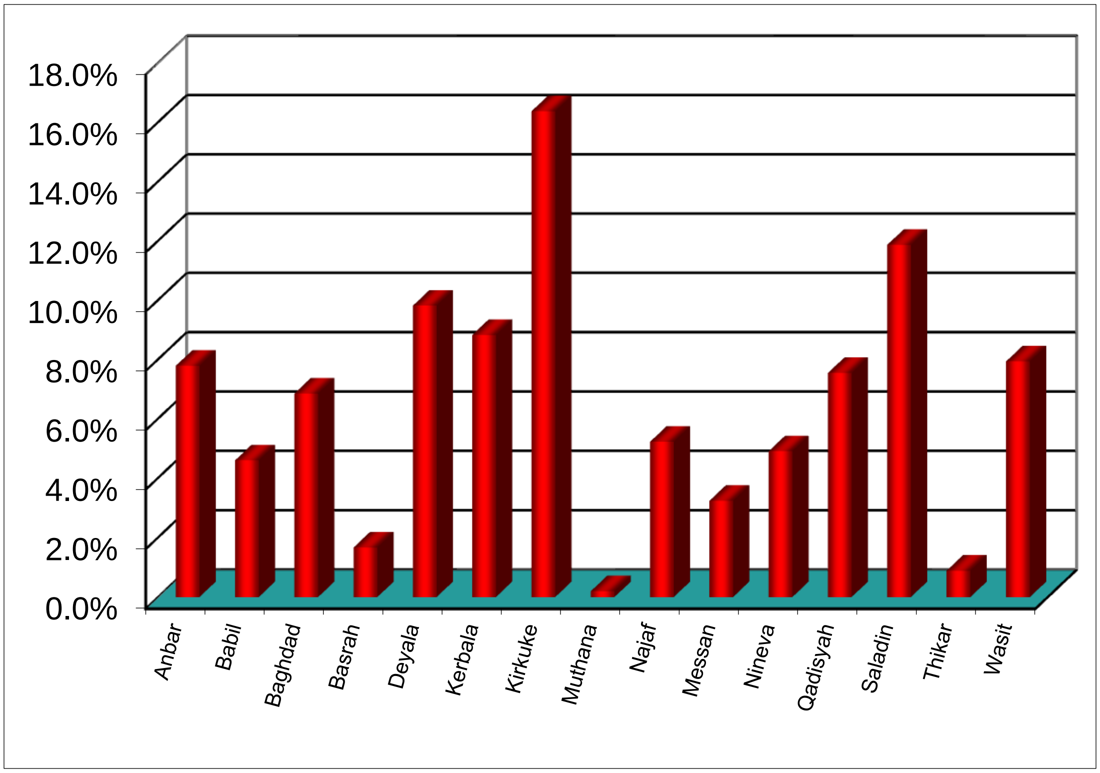

Figure 2.1: Apparent Prevalence of Brucellosis in Sheep and Goats

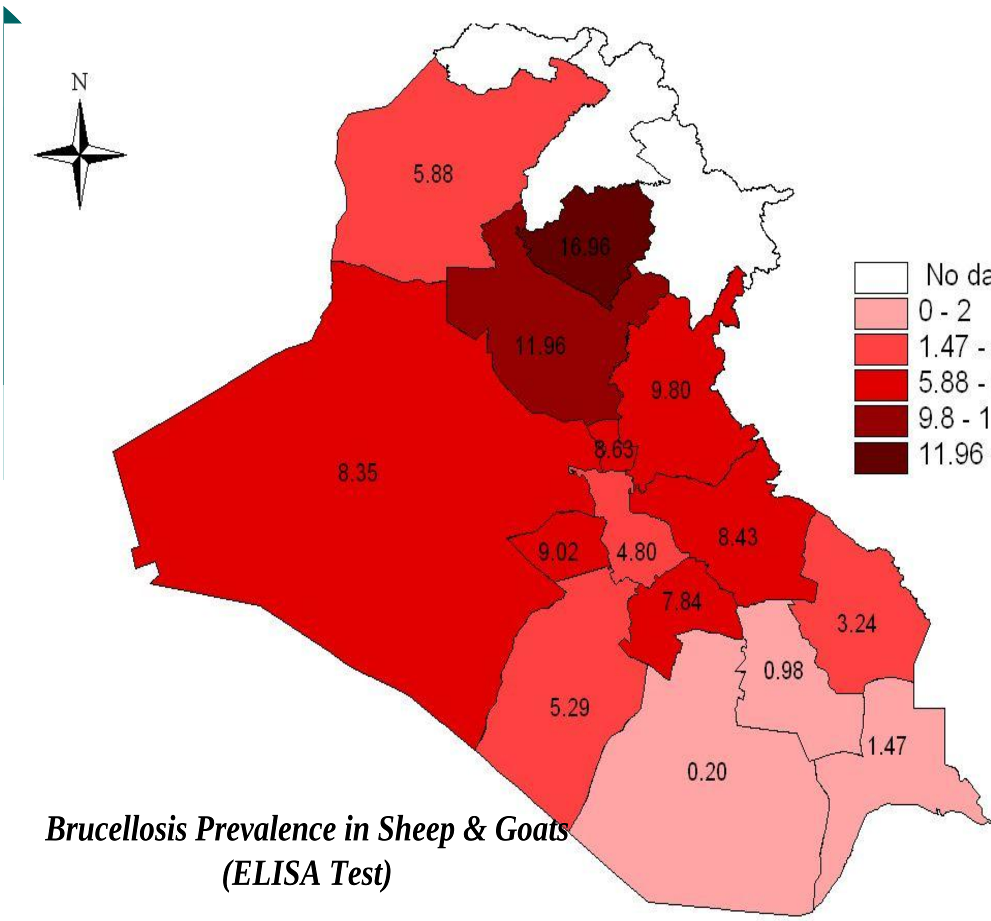

Figure 2.2: Map of Apparent Prevalence of Brucellosis in Sheep and Goats

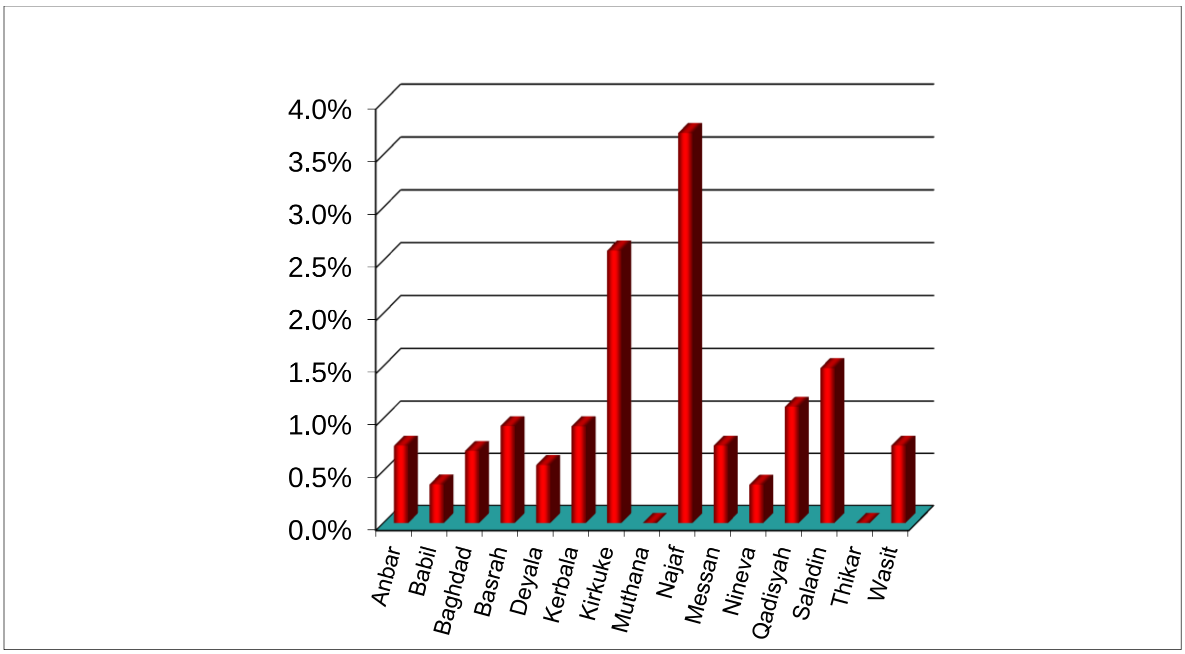

Figure 2.3: Apparent Prevalence of Brucellosis in Cattle

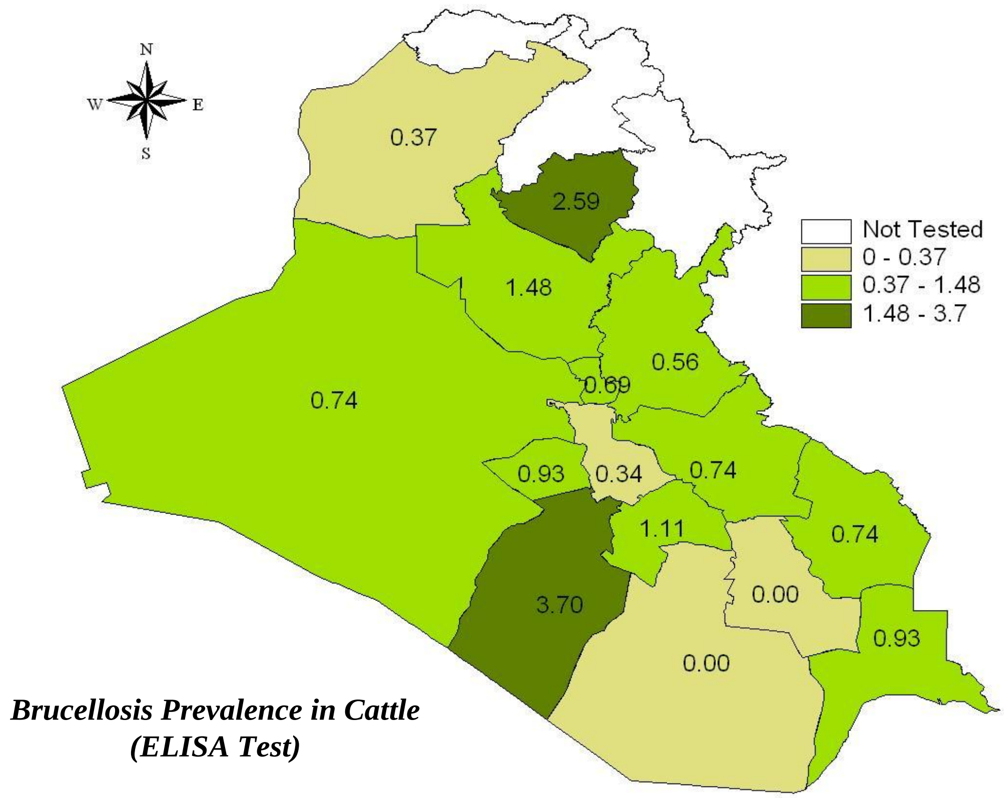

Figure 2.4: Map of Apparent Prevalence of Brucellosis in Cattle

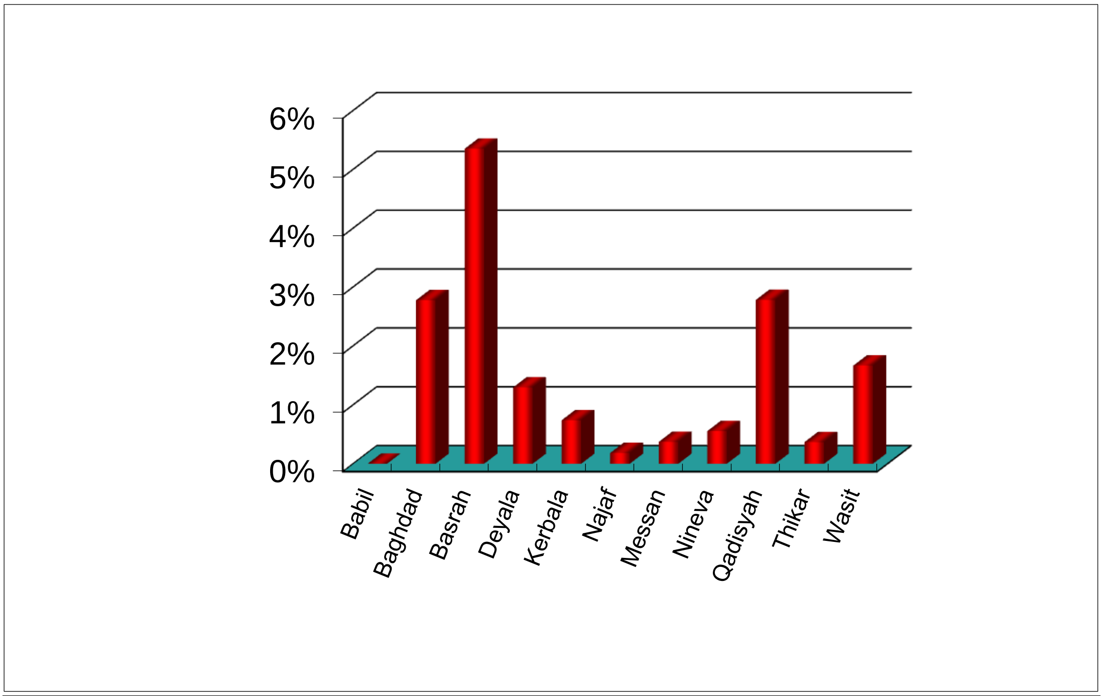

Figure 2.5: Apparent Prevalence of Brucellosis in Buffalo

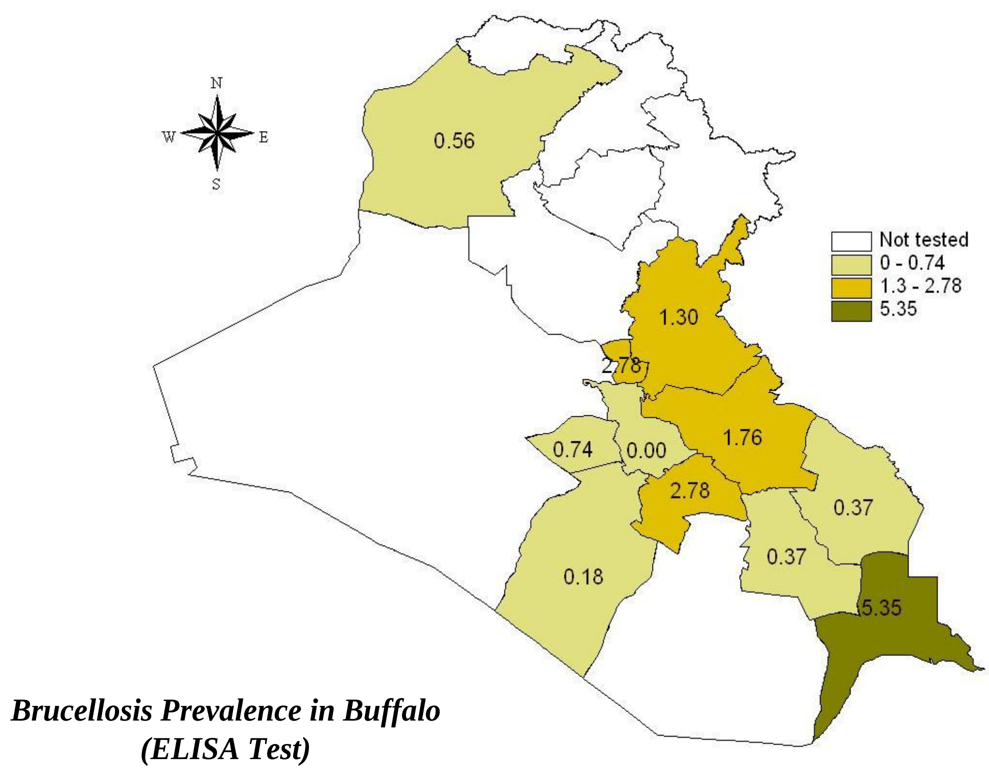

Figure 2.6: Map of Apparent Prevalence of Brucellosis in Buffalo

CHAPTER 3 - Vaccination Phase

The results of serosurveillance indicated sheep and goats were the main source of infection, and there was a decision to implement mass vaccination for controlling the disease by using Rev-1 and S19 vaccines:

1) **Rev-1 Vaccine**

Target animals: sheep and goats.

Route of administration: subcutaneous injection.

Concentration: Full dose of $1x10^9$/dose for young animals 3–8 months of age.

Reduced dose of $1x10^7$/dose for adult animals.

2) **S19 Vaccine**

Target animals: cattle and buffalo female calves 3-9 months of age.

Route of administration: subcutaneous injection.

Concentration: Full dose 50 x 10^9/dose.

Pilot Vaccination Campaign

Since the decision was to implement the mass vaccination policy in the first year of controlling the disease, there was significant need for SCVS to conduct a pilot vaccination campaign to evaluate capabilities and to determine strengths and weaknesses before starting the main mass vaccination campaign at the national level.

The vaccine quantity available was 2,000,000 doses of Rev-1 vaccine provided by the Direct Aid Iraq agency (DAI), after endorsing the proposal of the Royal Netherlands Army for controlling cattle and small ruminants brucellosis in the Muthana governorate. Both SCVS and DAI agreed on the following points:

1) Implement the project in five southern governorates instead of one, including Muthana, Basra, Qadiseya, Missan and Thiqar.

2) The target animals were lambs and kids three to eight months of age, to prevent the risk of abortion that might occur due to the vaccine in adult pregnant females during the implementation.

3) In order to distinguish between vaccinated and infected animals, the SCVS implemented tattooing for identification of vaccinated lambs and kids to avoid revaccinating them during the mass vaccination campaign within the coming few months in 2006.

4) The DAI agency provided SCVS with 1,000,000 doses of Rev-1 vaccine and the remainder was kept in cold storage in the Erbil governorate of the Kurdistan region. The vaccine was considered to be part of the national bulk of vaccine which was supposed to be received from the FAO as scheduled during the series of meetings in Amman, Jordan.

5) Release the vaccine for use after conducting the quality control process in the laboratories of SCVS in Baghdad.

6) Provide temporary work opportunity for 300 unemployed graduated veterinarians to work under the supervision of the governmental veterinary services during the vaccination campaign.

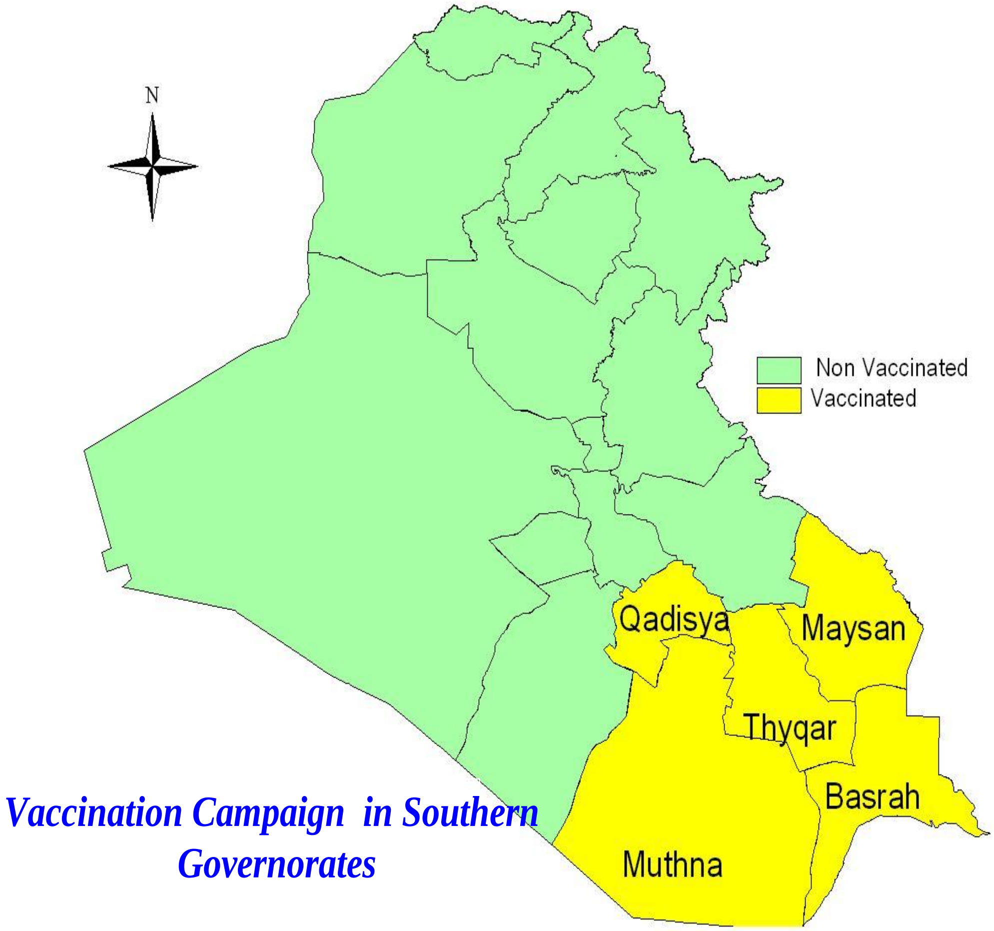

Figure 3.1: Coverage Area of the Pilot Vaccination Campaign in Southern Governorates

Vaccine Specification:

- Manufacturer :CZV
- Origin : Spain
- Kind of vaccine : Rev-1 vaccine for subcutaneous administration
- Concentration : 1-2 x 10^9 /ml
- Quantity : 2,000,000 doses
- Presentation : 25-dose glass vials with diluent

Preparedness:

Before starting the campaign, a meeting was held with directors of the central veterinary hospitals. The meeting was at the veterinary hospital in the Muthana governorate, which was considered to be the headquarters of the campaign because of its very good organization. All technical and logistic issues of the work were presented and discussed in the presence of the DAI agency representative. The estimated number of the target animals in Muthana, Thiqar, Missan, Qadiseya and Basra governorates were 225,000, 75,000, 100,000, 75,000, and 50,000 head, respectively. A total of 97 field vaccination teams were deployed as follows: 26 (Muthana), 20 (Thiqar), 20 (Missan), 20 (Qadiseya) and 11 (Basra). Although one of the aims of organizing this campaign was to provide the work opportunities to unemployed veterinarians, all personnel agreed the work should be conducted under supervision of the SCVS staff located in the governorates and with the work supervised by the SCVS headquarters in Baghdad. Since, the campaign was fully supported by the DAI agency, all technical and logistic issues were discussed with the agency representative.

Achievements:

1) The four governorates started the campaign within the first week of December 2005, while the Qadiseya governorate started December 18th, 2005. All teams accomplished their mission within the 4th week of February 2006, except the Basra governorate which was accomplished on January 27, 2006.
2) The total animals vaccinated (3-8 months of age) were 571,950 head distributed in Muthana with 245,543 head, Thiqar with 85,785 head, Missan with 9, 7000 head, Qadiseya with110, 000 head, and Basra with 33,522 head.
3) The unused quantity of vaccine was 370,000 doses out off the 1,000,000 doses received from DAI, which was stored at the same veterinary hospital in Muthana. The other 1,000,000 doses were kept in cold storage in Erbil.

Table 3.1: Achievements of the Pilot Vaccination Campaign in Southern Governorates

Governorate	Population Sheep & Goats	Estimated Target Animals	Vaccinated Animals
Muthana	1,925,000	225,000	245,543
Basra	94,000	50,000	33,522
Qadiseya	599,500	75,000	110,000
Thikar	735,750	75,000	85,785
Missan	726,100	100,000	97,000
Total	**4,080,350**	**525,000**	**571,950**

Obstacles

The SCVS was expecting delivery of required vaccine quantities and other requirements from FAO as scheduled for the full vaccination campaign. In addition, the national veterinary staff was prepared for the first brucellosis mass vaccination campaign since the veterinary services was established in 1924. The FAO informed SCVS there were financial problems in securing the required funds for the project (OSR0/IRQ/406/UDG,) *Restoration and Development of Veterinary Services in Iraq,* so it was impossible to obtain the necessary vaccine and thus had no chance of implementing the campaign. Although there were several attempts to secure the vaccine, shortage of time and some governmental political issues did not allow resolution of the problem by the Ministry of Agriculture (MOA).

Alternative Plan

There appeared to be only one option to overcome the frustrating situation. The NPCBT suggested an alternative urgent plan based on using the 370,000 doses of Rev-1 vaccine remaining from the pilot campaign implemented in the south. The NPCBT had conducted several studies since 1997 on the locally produced Rev-1 vaccine administered by different routes and in different concentrations. It was determined that 1×10^7 cfu/dose, when administered subcutaneously and timed well (between 1st of May and 1st of July), could produce protective immune responses without causing substantial abortion post vaccination. Since the available vaccine concentration was 2×10^9 cfu/dose, dilution to a concentration of 2×10^7 cfu/dose would increase vaccine doses 100 times and allow the final amount to be more than enough to cover the entire targeted population of approximately 12.5 million head. The reason for choosing the above-mentioned time period was based on two factors. The first one was the well-known fact that abortion occurred 40-60 days post vaccination, and the second factor was the season of parturition for ewes in Iraq. There are two parturition seasons in Iraq, with the first (main) one is between March and May and the second season is between October and December each year. Since, the pregnancy period of ewes is five months and the vast majority of them are delivered during April; the remaining pregnant ewes would be in the last month of pregnancy on May 1st. If pregnant ewes were inoculated with the vaccine during this stage of gestation, they would not be at high risk of abortion. The plan was fully discussed in SCVS headquarters, considering the critical security conditions in the central governorates of Iraq and all the required logistic requirements. The final decision was to endorse the plan implementation in 13 out of 15 governorates. Implementation would not occur in Anbar and Saladin because of critical security conditions in these two governorates.

Preparedness

A general conference was held with the directors and deputies of the governorates central veterinary hospitals to discuss the campaign-related issues as follows:

1) The number of required vaccination and supporting teams in each governorate included a total of 296 teams divided into 267 field vaccination teams and 29 supporting teams.

2) The total number of working staff of 1,047 was divided into: 788 veterinarians, 243 veterinary assistants and 16 follow-up personnel.

3) Endorse and release the required budget allocations of each governorate, regarding logistic needs, transportation of 267 field teams, and cold chain requirements that were needed to maintain the vaccine.

4) Because of shortage in the diluent quantities of Normal Saline 100 ml, the substitution was to use Normal Saline 500 ml used in human fluid therapy for diluting the vaccine in the field just before starting flock vaccinations.

5) There were problems regarding identification of vaccinated animals because of the inability to use tagging or tattooing methods. Tagging and tattooing supplies and instruments were not available and there was a high risk of screw worm infestation following both of these methods. To resolve this problem, NPCBT designed and prepared a card identity of veterinary services which was offered to the livestock owners by field vaccination teams. All details of veterinary activities were listed on the card identity, and the information on the card was organized in records and software by the epidemiological units in central veterinary hospitals in the 15 governorates which were done according to the coding system established by the SCVS.

7) Appendix A shows the procedure for diluting the vaccine from the concentration of $1x10^9$/dose to the concentration of $1x10^7$/dose used by the field vaccination teams.

Achievements

Coverage Percentage

Total vaccinated animals in covered governorates included 9,528,022 head of the estimated 12,693,391 head. The coverage rate was 75.06%, and when the pilot vaccination campaign animals of 571,950 head were added, the total number rose to 10,099,972 head with a coverage rate 79.42% of the total animal population. The reason of low achievement rate in some governorates was due to the military actions in these areas during the vaccination campaign, which was especially noted in Ninava and Deyala where success rates were 56.18% and 49.57%, respectively.

Immune Response

There were 267 field vaccination teams and 25% of the teams were randomly selected according to recommendations of several experts from Australia and USA. Fifty blood samples from animals vaccinated by each team selected were collected and tested using the Rose Bengal Antigen test. In five governorates that conducted the test within three to six weeks post-vaccination, the positive immune response rate was 82.36%. The other governorates implemented the test two to three months post-vaccination, and the results showed a normal decline in humoral immune responses.

Induced Abortion Post Vaccination

Abortion post-vaccination could be one of the disadvantages of using Rev-1 vaccine if it is used without proper timing during the gestation cycle. The total reported abortion cases post vaccination were approximately 5,108 cases. However, these abortion cases were accompanied by outbreaks of sheep pox and foot-and-mouth disease a month after the vaccination campaign, and both diseases can cause abortion in sheep and goats. Thus, it was considered a very real possibility that the 5,108 could have been due to these two diseases. Even if it was presumed that all the abortion cases were due to the vaccination process, the percentage of induced abortions would be < 0.001%. This is a very low level of abortion compared to the abortion cases induced in other countries, such as Tajikistan where 0.8 % of pregnant females aborted post-vaccination with Rev-1 vaccine administered via the conjunctival route.

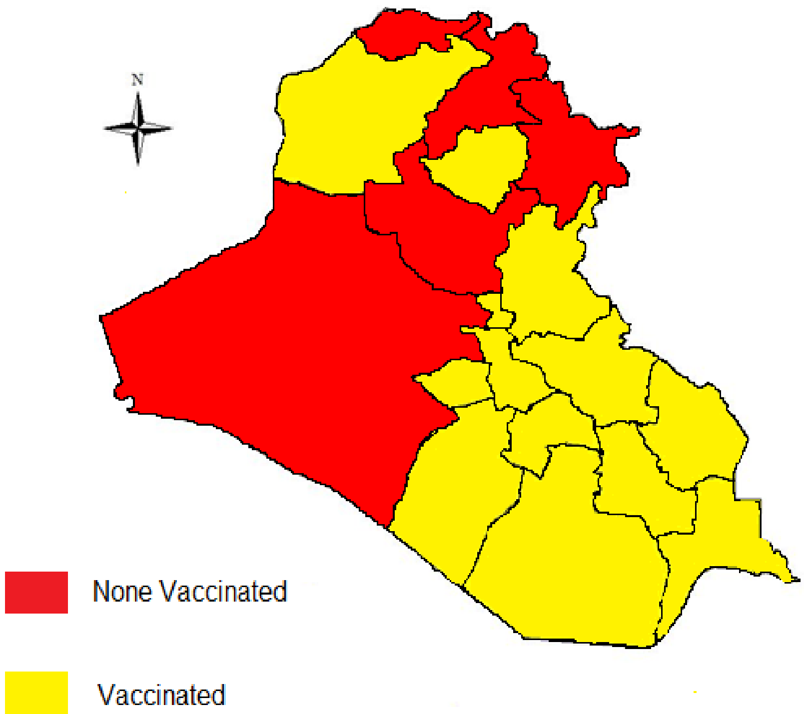

Figure 3.2: Governorates Included in the First-Year Vaccination Program 2006

Table 3.2: Teams Participating in the Mass Vaccination Campaign 2006

Governorate	Field	Support	Total
Ninava	48	3	51
Kirkuk	17	3	20
Baghdad	26	2	28
Deyala	21	2	23
Wasit	23	2	25
Babil	16	4	20
Kerbala	15	1	16
Missan	17	1	18
Thiqar	22	2	24
Qadiseya	16	4	20
Najaf	14	1	15
Muthana	20	1	21
Basra	12	3	15
Anbar	0	0	0
Salahadin	0	0	0
Total	**267**	**29**	**296**

Table 3.3: Total Employees Participating in the Vaccination Campaign 2006

Governorate	Staff	Support Staff	Total
Ninava	113	46	159
Kirkuk	56	6	62
Baghdad	90	0	90
Deyala	57	28	85
Wasit	72	31	103
Babil	78	37	115
Kerbala	45	9	54
Missan	52	0	52
Thiqar	54	33	87
Qadiseya	52	25	77
Najaf	44	9	53
Muthana	33	13	46
Basra	37	6	43
Anbar	0	0	0
Salahadin	0	0	0
Total	**783**	**243**	**1,026**

The supervisory team included 16 personnel raising the total number to **1,042**

Table 3.4: Total Vaccinated Animals in Mass Vaccination Campaign 2006

Governorate	Population	Vaccinated animals	Achievement
Ninava *	4000000	2247346	56.18 %
Kirkuk	921000	868600	94.31 %
Baghdad	264432	231576	87.57 %
Deyala *	1107900	553520	49.96 %
Wasit **	1100000	1120596	101.87 %
Babil **	548900	604352	110.1 %
Kerbala	327159	261989	80.08 %
Missan	700000	523264	74.75 %
Thiqar **	600000	763257	127.21 %
Qadiseya **	550000	577829	105.06 %
Najaf	560000	414236	73.97 %
Muthana	1920000	1283822	66.87 %
Basra	94000	77635	82.59 %
Anbar	0	0	0
Salahadin	0	0	0
Total	**12,693,391**	**9,528,022**	**75.06 %**

- Total vaccinated animals in the five southern governorates by the pilot vaccination campaign were 571,950 head 3-8 months of age.
- Total vaccinated animals after two campaigns = 10,099,972 head.
- Achievement in 13 governorates except Anbar and Salahadin was 79.42%.
- Achievement in governorates denoted with (*) was lower than expected due to military actions and bad security conditions in some of the districts, incapacitating implementation of vaccination activities.
- Achievement in governorates denoted with (**) was >100%, due to the enforced displacement of sheep flock owners from their living places where their flocks were officially registered, to other more safe places because of the civil conflicts during implementation of the vaccination campaign.

Table 3.5: Immune Response after Mass Vaccination Campaign 2006

Governorate	Field teams	Selected teams	Samples	+ ve	-ve	IR%	Post Vaccinations
Kirkuk	17	4	200	145	55	72.5	4 - 6 weeks
Wasit	23	5	250	192	58	76.8	4 - 6 weeks
Thiqar	22	5	250	202	48	80.8	4 - 6 weeks
Qadiseya	16	4	200	169	31	84.5	4 - 6 weeks
Muthana	20	5	254	247	7	97.2	3 weeks

*+ve = positive, -ve=negative, IR=Immune response

Notes:

- Not all governorates conducted serology testing within three to six weeks post-vaccination.
- The mean of positive immune responses in governorates that conducted the test within the recommended period post-vaccination was 82.36%.
- Only Muthana governorate conducted the serology testing within three weeks post-vaccination and the other governorates (Kirkuk, Wasit, Thiqar, and Qadiseya) conducted the test more than four weeks post-vaccination.

Second Year of the Vaccination Phase - 2007

The required amount of vaccine was determined to be 4,000,000 doses, and the animals targeted were the new progeny of sheep and goats three to eight months of age and the adults missed from the previous campaign. The goal was to return to the original plan of using a full dose of Rev-1 vaccine. Unfortunately, the same problem of vaccine shortage was again encountered with the vaccination program because unrealistic rules and regulations were established to regulate the processes of vaccine importation. The only available choice was to conduct a similar alternative plan as the previous year by using available vaccine (1,000,000 doses) from the same manufacturer. The vaccine was located in cold storage of the veterinary services in the Kurdistan province, where the veterinary administration had planned to vaccinate the target animals by using a full dose of the vaccine during the campaign in central and south Iraq. The veterinary administration in Baghdad, with assistance of the US military veterinary service command, was able to secure only 250,000 doses of vaccine, which was enough to vaccinate the targeted animals after diluting the vaccine to $1x10^7$ cfu/dose.

There were 5 to 5.5 million head estimated to be vaccinated including the unvaccinated missed adults from the previous campaign in the 15 governorates except Kurdistan provinces, but unfortunately, the unsafe security conditions existed for the second time in Deyala and Anbar so these two governorates were not able to implement vaccination activities. Total vaccinated animals were 4,698,482 head, as shown in Table 3.6.

Table 3.6: Total Vaccinated Animals in Vaccination Campaign 2007

Governorate	Vaccinated Animals
Ninava *	1480407
Kirkuk *	573619
Baghdad	111410
Deyala	0
Wasit	316777
Babil *	264031
Kerbala	116227
Missan	118156
Thiqar	238563
Kadiseya	371308
Najaf	156545
Muthana *	158118
Basra	36498
Anbar	0
Salahadin	756823
Total	**4,698,482**

(*) Governorates that included the missed adult animals from the previous campaign

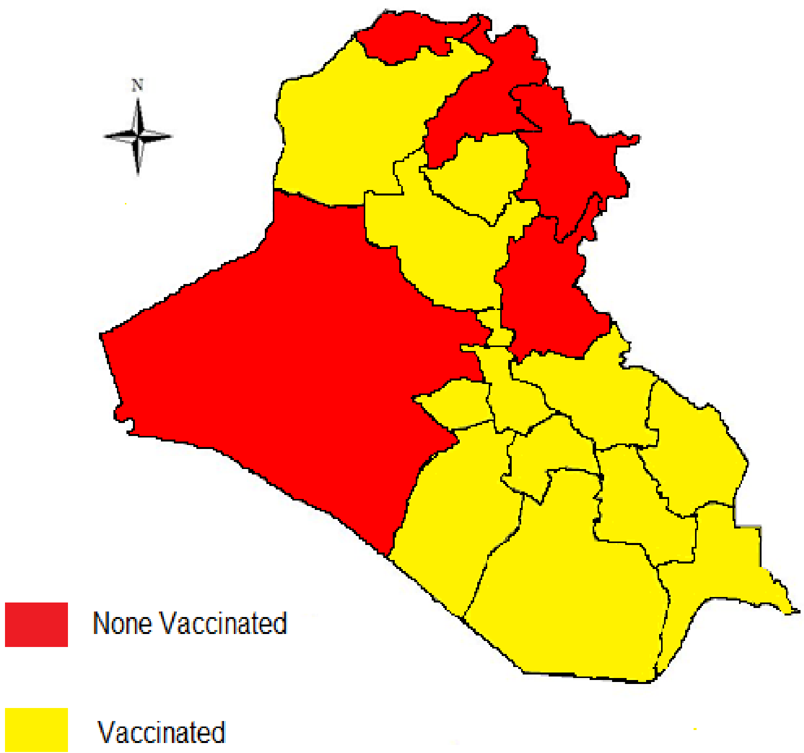

Figure 3.3: Governorates Covered in Vaccination Campaign 2007

Third Year of Vaccination Phase - 2008

The required amount of Rev-1 vaccine was not able to be imported for the third year and for the same reasons. Only 400,000 doses were available from the original inventory provided by the veterinary administration in the Kurdistan region; but there were some remaining amounts of vaccine in some governorates from the previous campaign. The decision made was to continue the vaccination program in Ninava and Kirkuk governorates because of their large population of sheep and goats, and some flocks in Anbar and Deyala were also vaccinated. The targeted animals were lambs and kids about three to six months of age. The full dose (2×10^9 cfu/dose) was used in this campaign, which was the main reason to consider this year as the weakest part of the vaccination phase due to the large gap created by neglecting new progeny (not enough vaccine). New progeny were a significant part of the total animal population in the other 11 governorates, and will remain as potential repository for the disease for at least 6 years among the vaccinated animals. Total vaccinated animals were 578,460, 168,053, 5,140, and 1,500 head in Ninava, Kirkuk, Anbar, and Deyala, respectively and shown in table 3.7.

Table 3.7: Vaccinated Animals in 2008

Governorate	Vaccinated Animals
Ninava	578460
Kirkuk	168053
Anbar	5140
Deyala	1500
Total	**753,153**

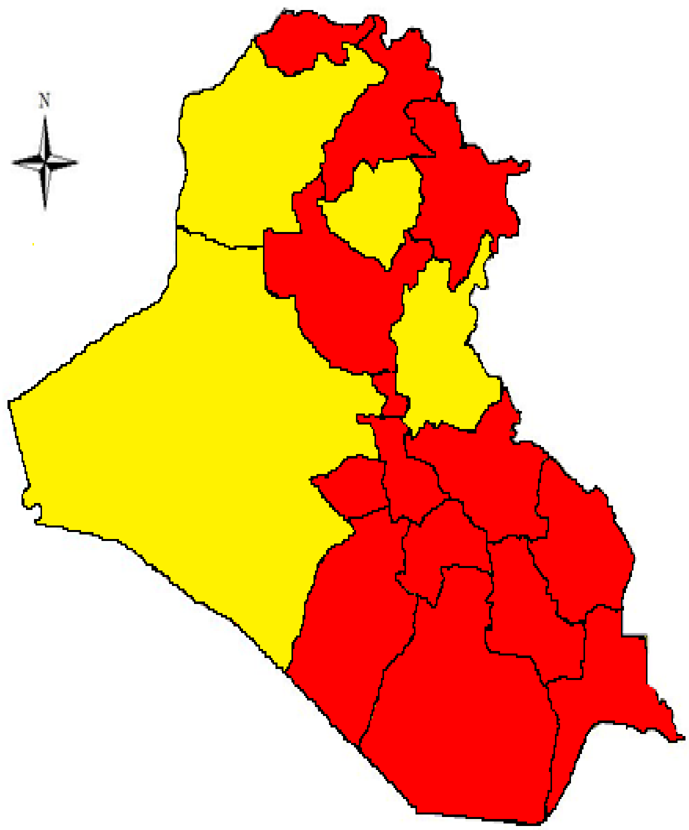

Figure 3.4: Governorates Included in the 2008 Vaccination Campaign

Fourth Year of Vaccination Phase - 2009

Relative improvement in security conditions allowed all 15 governorates in the middle and south of Iraq controlled by the veterinary services in Baghdad to be involved in vaccination activities in 2009. The targeted animals were only lambs and kids three to eight months old. Total animals vaccinated were 1,833,482 head divided into 1,696,699 lambs and 136,783 kids. Serological testing was implemented at the same time in all governorates within three weeks post-vaccination and the mean positive animals was 92.2%. Vaccinated animal numbers are as shown in the Table 3.8.

Table 3.8: Vaccinated Lambs and Kids in 2009

Governorate	Lambs	Kids	Total
Ninava	351881	27723	379604
Kirkuk	107682	7318	115000
Anbar	198958	7666	206624
Deyala	187429	29840	217269
Salahadin *	124000	0	124000
Baghdad	69437	6337	75774
Wasit	149930	19283	169213
Babil	78172	12356	90528
Kerbala	39875	1200	41075
Najaf	95804	3755	99559
Qadiseya	124803	11488	136291
Muthana	45794	4177	49971
Missan	40519	1521	42040
Thiqar	65106	4418	69524
Basra *	20555	0	20555
Total	**1,696,699**	**136,783**	**1,833,482**

(*) Vaccinated animals were not categorized into lambs and kids.

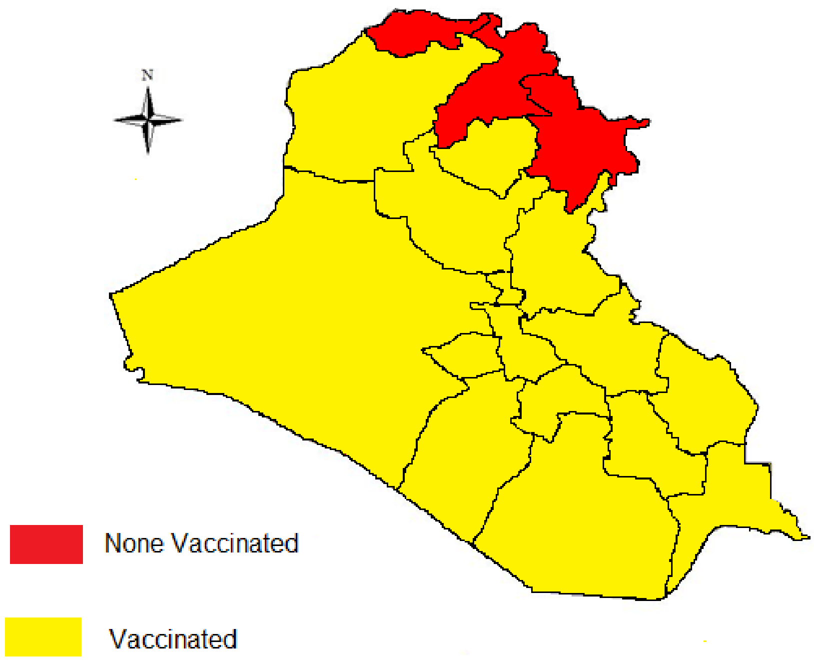

Figure 3.5: Governorates Included in the 2009 Vaccination Campaign

CHAPTER 4 - DISCUSSION

Drawbacks and Pitfalls

1) Animal Identification

Livestock in Iraq are not fully under the control of veterinary authorities because of social, religious and practical considerations. In addition, methods for animal identification such as tagging and tattooing are difficult to use. Screw worm infestations were observed during the 2005 pilot vaccination campaign in southern governorates, and using tattooing applicators for animal identification was a primary cause of infestations, which caused inconveniences for many livestock owners and some refusal to cooperate with veterinary authorities. Moreover, the value of identified animals was reduced due to socio-religious considerations and negligence among poor farmers was another reason for not implementing animal identification.

2) Flock Movement

Generally, the husbandry system of sheep and goats in Iraq is sedentary. However, there is always potential seasonal movement or migration of animal flocks among different Iraqi governorates and also among neighboring countries. This could be considered one of the main causes for endemic spread of brucellosis in the country, which continues to hamper the veterinary authorities in controlling all diseases. The present situation of open borders since the war in 2003 magnifies the problem.

3) Animal Census

The livestock census should play a pivotal role in strategic planning of any national animal health program. However, a census of livestock populations in Iraq has never been done due to various socio-economic and political situations in the country. Something that could be done to rectify this situation in Iraq would be to estimate the census according to the records of periodic vaccination campaigns.

4) Using Different Vaccine Sources

According to the recommendations of the "Round Table Committee on the Use of Rev-1 Vaccine in Small Ruminants and Cattle" held in Alfort, France in 1995, it was highly recommended to use the same vaccine during brucellosis vaccination control programs. The committee recommended using vaccine from the same manufacturer throughout a time course that might last 10-15 years. The main reason for consistent use of a particular vaccine and manufacturer would be to establish sustainable and homogenous immune responses. Another important reason to use one vaccine source would be related to matching field strains with vaccine production strain(s). The vaccine should be from a reference site that can determine residual virulence and immunogenicity to ensure similar levels of immunity from all batches of vaccine produced. This issue was considered during the first, second, and third years of vaccination in Iraq. Unfortunately, in the fourth year (2009) the vaccine was changed to a different source with less quality, and then in 2010 the vaccine will probably be used from another different source.

5) Laboratory Capabilities

Drastic changes in the country due to decades of wars, inappropriate policies and the after-effects of the 2003 war caused significant collapse in all areas of life in Iraq, including the veterinary activities. Moreover, the United Nation sanction due to Security Council resolutions between 1990 and 2003 and beyond caused unlimited devastation on laboratory capabilities. These factors played a major role in bringing down the efficiency of laboratory activities, which were so crucial in providing accurate data regarding various infectious and zoonotic diseases.

6) Animal Health Information System

Animal health information systems are one of the most crucial requirements for successful implementation of any program. During 2005 to 2008, there were many difficulties in transforming and exchanging data and information between different governorates. Currently, serious and promising steps are being taken to build an accurate animal information system that can facilitate transferring data and urgent information between SCVS headquarters and veterinary services in the 15 governorates in the center and south of Iraq.

7) Security Conditions.

Unstable security conditions in the central and western parts of Iraq especially affected implementation of the vaccination campaigns in 2006, 2007 and 2008, which greatly impacted the final achievement of these campaigns. Table 3.4 illustrates the achievement percentages or results of some of the unstable governorates due to military actions and civil conflicts. However, there were relatively high percentages achieved in the stable governorates of southern Iraq.

8) Rules and Regulations of Vaccine Importation.

In any disease control program there should be relevant rules and regulations which facilitate and support implementation of the control and eradication programs of selected diseases. Major factors having a negative impact on the brucellosis control program in Iraq were the rules affecting importation processes of the required amounts of vaccine during the first four years of the vaccination program. In addition, politics, improper personnel techniques and decisions by non-professionals in choosing the vaccine source contributed to a difficult and unstable situation in Iraq.

9) Corporation between different veterinary administrations in different regions.

The lack of transparency and coordination between the veterinary services in both Baghdad and Arbil due to decades of consequences of unstable political conditions, led to big differences in strategies. There should be a full discussed long term plan to control the disease in all 18 governorates simultaneously. Such plan should consider all the related issues including the vaccine origin, route of administration, quantity that should cover at least 90% of the targeted animals, and periodic sero-surveillance that should be implemented to evaluate the efficiency of the control program.

10) Maintenance of official records

Infected human cases detected in either the middle-south of Iraq or the Kurdistan province could not be added cumulatively because the final total number would mislead statistical analysis. This was primarily due to the difference in control strategies between the two regions. Accordingly, the total number of human cases in each region should be separate unless the two veterinary administrations established a plan to similarly manage all 18 governorates. Moreover, the number of infected human cases detected by the private health sector could not be added to the cases reported by official governmental health institutes because it would also mislead the final results. This is due to the different standards and protocols implemented by various private laboratories where they used different diagnostic kits and techniques for obtaining serological results. True incidence should be calculated according to official health documents and records.

Outcomes

After four vaccination campaigns in 2006, 2007, 2008, and 2009, even with the unacceptable faults, derailed steps and difficulties in some areas, several satisfactory outcomes were achieved in livestock productivity and human/public health:

1) The incidence of disease according to official reported cases of the Ministry of Health (MOH) for the period 1988-2004 ranged between 10.6/100,000 and 27.2 cases/100,000 people in the middle and south of Iraq. The highest peak was 88.5 cases/100,000 people in 1995 because it was the most stressful year of sanctions in Iraq as shown in Table 4.1 and Figure 4.1. Regarding the Kurdistan province, the lowest incidence of the disease was 37.8 cases/100000 people in 2006 after conducting the vaccination campaigns in the period 1998-2005 as shown in Table 4.2 and Figure 4.2

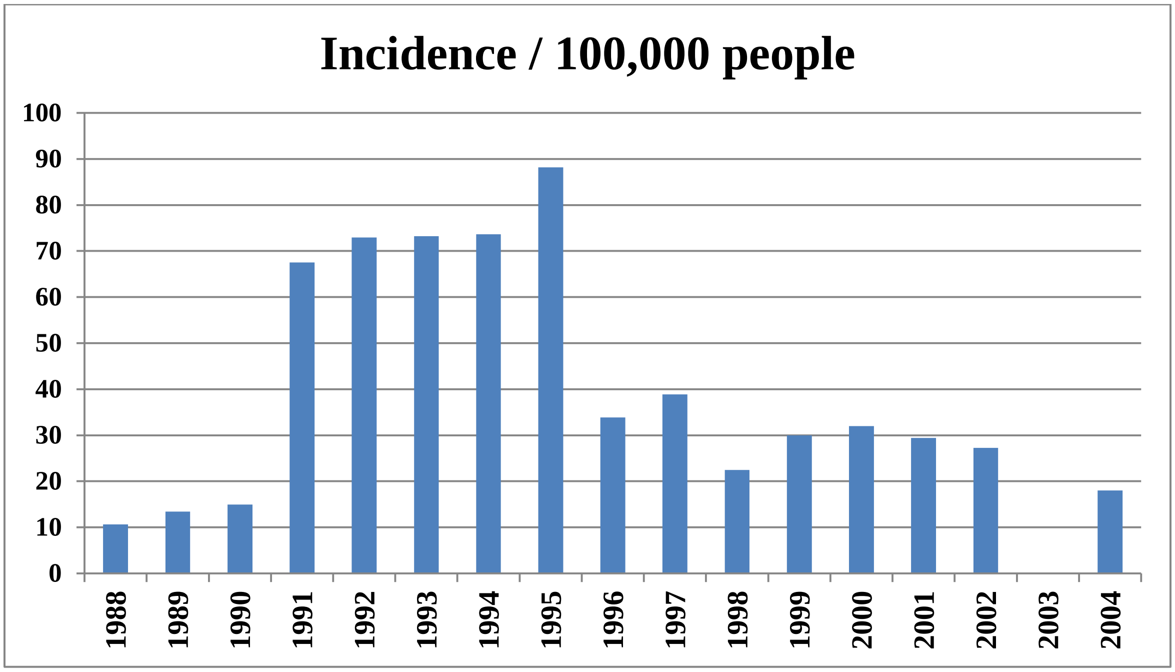

Figure 4.1: Incidence of Human Brucellosis Cases for the Period 1988-2004

After the 2003 war, there were 5347 recorded cases representing 22.7 cases/100,000 people, but there was a lack of appropriate recording in the middle and south of Iraq due to the consequences and devastating impacts of the war.

Table 4.1: Incidence of Human Brucellosis Cases for the Period 1988-2004

Year	Cases	Population	Incidence / 100000 people
1988	1892	17814801	10.62
1989	2464	18349311	13.43
1990	2819	18899860	14.92
1991	13106	19409189	67.52
1992	14546	19932244	72.98
1993	14989	20469395	73.23
1994	15476	21021022	73.62
1995	19040	21587514	88.20
1996	7531	22249812	33.85
1997	8911	22932429	38.86
1998	5305	23635988	22.44
1999	7297	24361133	29.95
2000	8030	25108525	31.98
2001	8166	27748669	29.43
2002	7189	26405133	27.23
2003	0	27078334	0.00
2004	4993	27768699	17.99

2) According to official MOH records, there was an apparent decline in the incidence of brucellosis among humans in the middle and south of Iraq during the last five years (2005-2009). The most recent update of 2009 revealed the incidence to be 16.99 cases/100,000 people in the middle and south of Iraq, and 52.35 cases/100,000 people in the Kurdistan province, as shown in Table 4.2 and Figure 4.2.

Table 4.2: Incidence of Disease in the Two Regions of Iraq 2001-2009

Year	Middle & South of Iraq	Kurdistan
2001	36.45	227.19
2002	31.29	250.88
2003	0	151.29
2004	20.66	64.4
2005	21.82	40.41
2006	16.66	37.85
2007	13.44	66.78
2008	16.53	61.11
2009	16.99	52.35

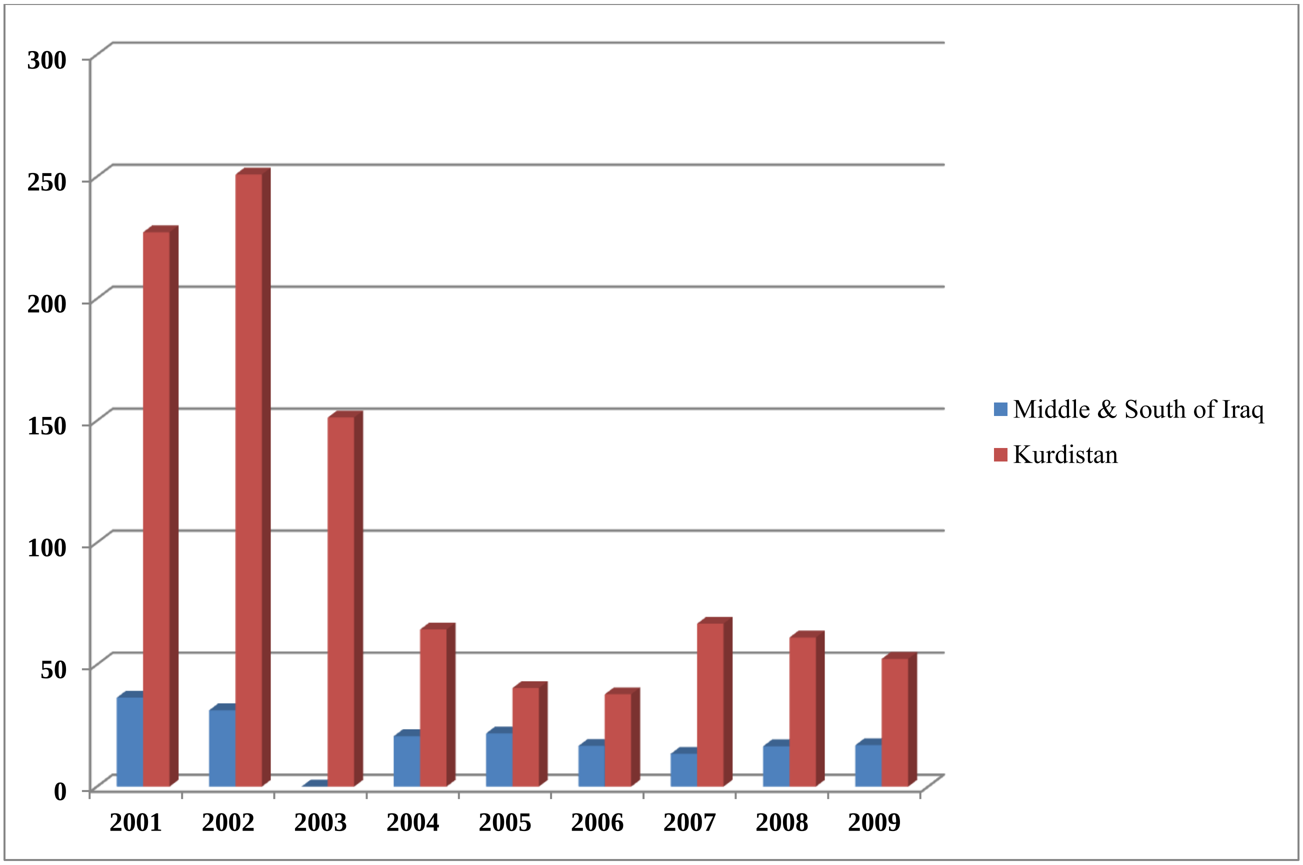

Figure 4.2: Incidence of Brucellosis in the Two Regions of Iraq 2001-2009

2) According to the reports of veterinary hospitals in different governorates, more than 90% of affected flocks showing annual abortion cases no longer suffered from abortions in late-stage pregnancy after vaccination. Moreover, there was field evidence of significant increases in lambing rates among vaccinated flocks.

3) The reduced vaccine dose used during the first and second years of the vaccination program was useful in reducing vaccine-induced abortions post-vaccination.

4) Because the control program yielded significant positive results, there was a beginning to development of new relationships between livestock owners and veterinary services. The success of the control program may be considered a new epoch in Iraqi veterinary services. The program also enhanced the veterinary staff working environment because they were able to develop and use their skills, participate in strategic planning, and receive financial support, all of which had been neglected for decades.

Summary

In Iraq, brucellosis is considered to be one of the most important zoonotic diseases, where it has been discovered and confirmed since 1937. There is controversy regarding total livestock and small ruminant populations in Iraq because accurate census data do not exist. Sheep and goats are estimated to be over 13 million head, and the other species such as cattle, buffalo and camels are about 2.5 million, 285,000, and 58,000 head, respectively.

Decades of unstable socio-economic and security-political conditions that were accompanied by the presence of the disease in livestock caused significant increases in human infections. There was a significant increase in disease incidence during the1990s due to the United Nation (UN) sanction, especially in 1995 when brucellosis reached 88.5 cases/100,000 people according to official records of the Ministry of Health (MOH). Unfortunately, because of the lack in accurate reporting and monitoring processes, brucellosis cases that were not officially reported are estimated to be about 10-12 cases for each officially reported case. Human infections in Iraq represent huge devastating socio-economic impacts.

Regarding the economic impact at the level of livestock, estimates determine the loss to be at least 50 billion Iraqi Dinar from only the annual abortion cases (currently $1 = 1180 Iraqi Dinar, whereas in 1970s, one Iraqi Dinar = $3.1). Moreover, additional losses come from decreased reproduction caused by temporary and permanent infertility of reproductive animals.

Veterinary authorities in Iraq established the National Project for Controlling Brucellosis and Tuberculosis (NPCBT) in 1995 to counter the devastating and continuous

effects of brucellosis. The main objectives of the project were to design and implement plans for controlling the disease, and to produce the required vaccines and diagnostic kits. The project was established during the UN sanction when the various committees of the United Nation Special Commission (UNSCOM) were implementing their duties in Iraq. Thus, there were very limited laboratory resources, but the project was able to achieve several goals regarding disease control. The project conducted several studies on Rev-1 and S19 vaccines that protected livestock against *Brucella* infection in both small and large ruminants. Also, more than 2.1 million doses of Rev-1 vaccine were produced in liquid status and more than 20,000 doses of S19 vaccine were made, as well as production of diagnostic kits.

After the war in 2003 there was a total collapse in NPCBT activities and a great need for rehabilitation and reorganization of the entire Veterinary Services in Iraq. Accordingly, there were a series of meetings with the Food and Agriculture Organization (FAO) experts for this purpose. Brucellosis was considered to be one of the main zoonotic problems in Iraq that needed to be controlled. The first step to control the disease was designing and implementing sero-surveillance to determine prevalence of the disease and then taking appropriate decisions regarding a control program. It was the first organized national surveillance program since establishment of Veterinary Services in Iraq. Surveillance revealed the apparent prevalence of brucellosis in sheep and goats (shoats), cattle, buffalo, and camels to be 6.51%, 1%, 1.48%, and 0.02, respectively.

Based on the sero-surveillance outcomes, the next step was initiating the control phase requiring a long-term commitment to vaccination with a suitable vaccine. Rev-1 vaccine was determined to be the most reliable vaccine that provided protection against

B. melitensis, which represented the main problem in Iraq and the Middle East. The vaccination program encountered several difficulties for many reasons, but ultimately and regardless of difficult situations, field evidence demonstrated success in the vaccination campaigns by reducing infected cases among vaccinated livestock and also decreased the incidence among humans. The vaccination phase began with a pilot vaccination campaign in five southern governorates in Iraq. The pilot was to determine strengths and weaknesses as a preparedness step before the mass vaccination campaign, which was designed to include both genders and all ages of the targeted animals. The total vaccinated animals in four campaigns for the period 2006-2009 was 17,385,089 head of sheep and goats with different coverage rates in different governorates due to security conditions in some areas.

Regardless of the difficulties and derailed steps encountered during the vaccination phase, there were several remarkable achievements attributed to the four vaccination campaigns. The most important output was the apparent decrease in human brucellosis, which declined to almost 17 cases/100,000 people in the middle and south of Iraq in 2009 compared with 27.23cases/100,000 in 2002 and 88.55 cases/100,000 in 1995. The positive results revealed a crucial need to continue vaccination procedures annually in order to reach the lowest possible incidence rate before starting an eradication phase.

Based on the satisfactory outcomes of the "emergency" plan requiring dilution of the vaccine, we conclude the importance of the following points:

1. Isolation, identification, and bio-typing/serotyping of bacteria. The use of proper bacteriological procedure to isolate and identify the bacteria plays a vital role in prevention of disease. This also helps to understand the epidemiological distribution

and dominant serotype of bacteria prevalent in the locality, which in turn aids in mitigating the disease outbreak. While considering isolation and identification techniques followed by biotyping or serotyping of the bacteria, reconsideration must be given to the infrastructure and facilities available in the unit, diagnostic capabilities of the unit, and ability of veterinary services to meet international standards.

2. The appropriate vaccine should be selected and used in vaccination programs. Vaccination is a vital component of both control and prevention, and the use of proper vaccines is always recommended for potential control of disease. The vaccine should be specific against the bacterial pathogen in question to avoid or lessen negative effects of the vaccine. An example is the use of S19 vaccine in Mongolia to control infection caused by *Brucellosis* in cattle without proper isolation and identification of the pathogen(s) in question. However, later isolation of the bacteria causing devastating effects among cattle was found to be *B. melitensis*, so a shift was made to Rev-1 vaccine after years of using S19 vaccine. It is always recommended to systematically evaluate the situation by starting with pathogen isolation and identification, and coupled with effective sero-epidemiological investigations for the most effective control of disease.

3. According to Table 4.2 and Figure 4.2, it could be determined that there was significant importance in continuing the vaccination campaigns so there would be maximum coverage of targeted animals. Such policy would play a positive role in improving the efficiency of the control program, and to maintain a decline in disease incidence. The best example of success was the significant decline in incidence

observed in middle and south Iraq after the vaccination campaigns in 2006 and 2007; when the incidence declined from 21.82 cases/100,000 people in 2005 to 16.66 cases/100,000 people in 2006, and then to 13.44 cases/100,000 people in 2007. However, the incidence began to increase to 16.53 cases and 16.99 cases/100,000 people in 2008 and 2009, respectively due to several factors. One of the factors was the limited amount of available vaccine in 2008 which was only 400,000 doses used as full dose. Consideration should have been given to the importance of conducting vaccination protocols which were consistent with those implemented in 2006 and 2007, to maximize the amount of vaccine coverage of targeted animals. In the Kurdistan province, the positive results of the vaccination campaigns in the periods 1988-2005, showed a decline in incidence to 37.85 cases/100000 people in 2006. However, it increased again in 2007, 2008, and 2009 in when it reached 52.35 cases/100000 people due to the lack of continuing the vaccination processes. These facts reflect the crucial needs for a unique, long-term vaccination program that would cover the entire 18 governorates at the same time and for not less than 10-15 years.

4. Utilization of proper dosages of vaccine during vaccination campaigns is very important. Using a reduced dose of vaccine during the first year (2006) of the vaccination control program was much safer than using the full dose because it overcame the risk of abortion post-vaccination and mitigated potential shed of organisms in milking animals (dilution scheme shown in Appendix A). The result was similar to the outcome of a brucellosis control program implemented in Saudi Arabia during the 1990s when the reduced-dose vaccine at $1x10^6$ cfu/dose was used

to vaccinate animals over eight months of age. There are other important reasons that justify implementing a policy of using reduced doses of Rev-1 vaccine. A key reason may be related to limited resources, such as a shortage in budgets available to acquire the needed vaccine. Many of the developing and under developed countries including countries with limited resources and or budget could utilize these vaccination techniques and dose adjustment protocols to meet their needs for effective implementation and control of the disease. The vaccine provided five years of immune protection among animals.

5. Implementation of surveillance programs is critical. It is important to develop an Animal Health Information System (AHIS) for creating geo-reference maps that reveal the actual status of all infectious diseases. Such a system would play a major role in providing decision makers with accurate inputs regarding several issues related to livestock management, including movement control during disease outbreaks, nomadic movement or migration and animals smuggling across the borders. A system could be considered an instrumental tool to manage other infectious and transboundary diseases. This could be achieved by implementing a well-designed project for monitoring the movement of animal flocks within the country or across the borders by using the GIS and remote sensing technologies. To measure the progress of the control program, a coordinated surveillance program should be developed. Such a program is so important to facilitate monitoring and follow-up processes necessary to determine the prevalence of the disease among livestock.

6. Training programs to increase awareness about the disease would be valuable. Continuous training programs for all staff of the Veterinary Services would develop and increase their capabilities in performing different veterinary activities whether in the field or in laboratories. Developing laboratory and field skills can enhance the accuracy of the data provided from those activities.
7. Training programs targeting livestock owners should be conducted to educate them about the significant zoonotic potential of brucellosis and also to increase knowledge of the vaccination campaign, which would facilitate owner and veterinary staff relationships for effective control of disease. Since the vast majority of livestock owners are of limited educational level, there is great need for continuous education and awareness programs. The programs would enhance and develop cooperation with veterinary authorities that would lead to higher levels of achievement during vaccination and surveillance programs.
8. Establishing a nationwide (Iraq) network that joins all related ministries to enhance cooperation and coordination regarding related issues, and to overcome the difficulties that might be encountered during implementation of annual vaccination campaigns. In addition, it is also crucial to follow-up the disease incidence among humans, which reflects the accuracy of the whole control program. The program related issues must be properly planned for implementation within actual time frames and provide the required capacities as brucellosis is considered as a national/regional priority. Thus, high level organization and cooperation between all related stakeholders would be crucial.

9. Regional control of the disease is needed. Since brucellosis is one of the serious trans-boundary diseases, there is a crucial need for more cooperation between neighboring countries in the Middle East region. A regional control program for disease control is imperative and would require transparency in reporting and exchanging information, including humans and domestic animals.
10. Laws and regulations for disease control should be updated and harmonized with international standards. The national laws and regulations have essentially remained since 1936-1937. Legislation should be initiated that would create regulations consistent with what is known about the disease (organism, epidemiology), the needs and expectations of stakeholder in the country and the rest of the world, and the requirements for eradication of brucellosis. New laws and regulations also should take into consideration how programs will be implemented at the local level. Compensation to livestock owners for animals lost due to the program should be considered as well. Finally, implementation of uniform, nationwide regulatory policies are critical for an effective disease eradication program.
11. Effective eradication of any disease requires cooperation and transparency in exchanging information among all relevant stakeholders, and Veterinary Services in Iraq should be pivotal in that regard, which would increase the Program coverage and data collection of data from all 18 governorates would be enhanced by utilization of Veterinary Services staff that is fully engaged and properly supported. Transparency among stakeholders would be essential for the most effective reporting and information exchange among and species, including humans and domestic animals.

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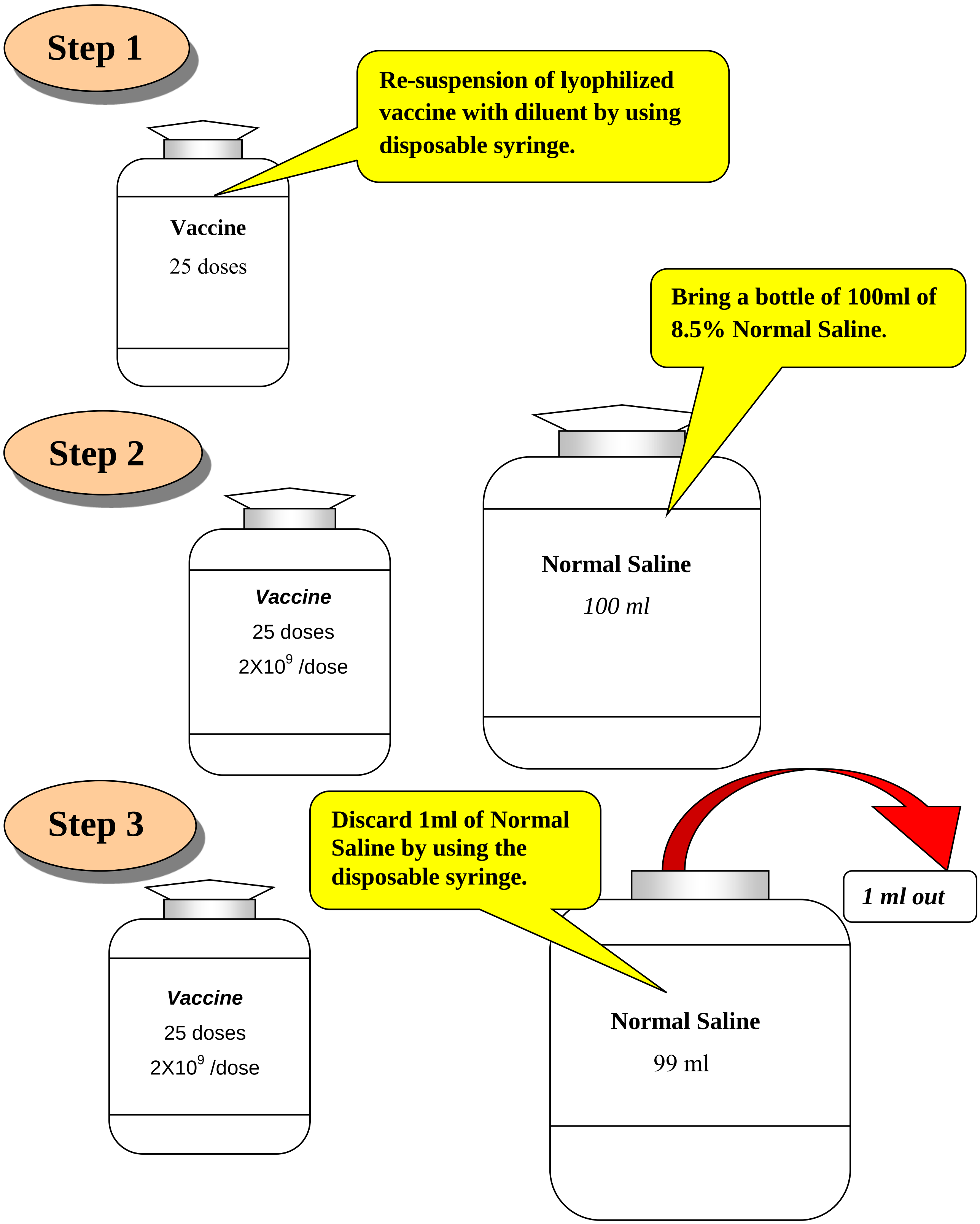
Appendix A - Vaccine dilution procedure



Appendix A- Continue