The Epidemiology of Brucellosis in Sheep, Goats and Humans in the Iraqi Kurdistan Region

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Declaration

I declare that this thesis is my own account of my research and contains as its main content work which has not previously been submitted for a degree at any tertiary education institution.

Emad Alshwany

Dedication

I dedicate this Doctoral Thesis to the most precious people in my life, for their everlasting love and strong support in my study:

My parents, wife, daughters, son, sister and brothers.

Abstract

Brucellosis is a disease affecting a wide range of domesticated animals and wildlife as well as humans. The disease remains a major zoonotic problem in many regions including the Middle East. In Iraq, where brucellosis is endemic, the disease is a major economic and production limiting disease for livestock owners and the community. Impacts on production arise from reduced milk production, abortions, decreased reproduction rate and premature births. The aim of this project was to investigate the seroprevalence, risk factors and economic impact of brucellosis in sheep and goats in the Kurdistan Region. Also a retrospective study of human brucellosis in Iraq was conducted to describe the historical distribution of the disease and its impact on the population.

Fifty one (39 sheep and 12 goats) of 1,050 sera samples were positive on both an RBT and ELISA (overall seroprevalence of 4.9%; 95%CI 3.6 - 6.3). Although there were no significant differences between groups, the highest seroprevalences were reported in sheep, male animals (sheep and goats) and animals (sheep and goats) older than 6 months compared with goats, female animals and animals younger than 6 months of age, respectively. A multivariable logistic-regression analysis was undertaken to identify risk factors for infection in flocks. This analysis indicated that farmers who introduced (purchased) new sheep (OR: 4.24, 95%CI 1.0, 17.3) and who introduced (purchased) new goats in the 12 months preceding the survey (OR: 15.2, 95%CI 3.0 - 76.36) were significantly more likely to have seropositive flocks. In contrast, flocks that used water sourced from a well (OR: 0.27, 95%CI 0.09 - 0.84) and had goats vaccinated against brucellosis in the 12 month period preceding

the survey (OR: 0.31, 95%CI 0.12 - 0.75) were significantly less likely to have seropositive flocks.

Based on the data available, the total economic impact of brucellosis in sheep and goats in 2015 was estimated to be US$6.14 million (95%CI 4.48 - $7.96 million) ($2.56 per adult female) in the Kurdistan Region. By adopting a mass vaccination control program for 10 years the economic losses arising from abortions and decreasing milk production were estimated to decrease to US$1.83 million (95%CI 1.33 - $2.39 million) (US$0.76 per adult female). The median cost of the mass vaccination program over the ten-year period was estimated at US$7.18 million (95%CI 7.11 - $7.25 million) and the total median benefit in present day dollars was estimated at US$18.42 million (95%CI 13.43 - $23.83 million). The abortion rate had the largest effect on the outcome (regression coefficient = 0.74) followed by the prevalence of the disease (0.63).

Based on the official records, the average annual incidence of brucellosis in Iraq, for the period from 1988 to 2002 was 41.88 cases per 100,000 people. There were significant differences between years (overall P value < 0.0001) with the highest annual incidence of 88.2 cases per 100,000 people occurring in 1995. The average annual incidence over this five-year period (2004 to 2008) was 54.11 per 100,000 people in Kurdistan which was significantly higher than the 17.82 per 100,000 people in the rest of Iraq (RR 3.0; 95%CI 1.76 - 5.11). The average annual incidence of brucellosis per 100,000 people for the period 2009 to 2014 in four different provinces of Kurdistan was 36.74. The median cost per patient diagnosed with brucellosis was estimated to be US$321.78 (95%CI 259.53 to $388.72) in

the Iraqi Kurdistan region in 2014. The median annual DALYs due to the disease was estimated to be 27.17 (95%CI 15.81 - 42.65) per 100,000 people per year.

It is recommended that to effectively control brucellosis in small ruminants in the Iraq Kurdistan region, an integrated approach should be implemented including adopting risk-based control measures, mass vaccination and education.

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Publications, Conference (Oral) and Poster Presentations arising from this research

Alshwany, E.A. and I.D. Robertson. 2018. "Epidemiology of brucellosis in sheep and goats in the Iraqi Kurdistan Region." The Online Journal of Science and Technology-October no. 8 (4):33-37.

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List of Acronyms and Abbreviations

B.	*Brucella*
E. coli	*Escherichia coli*
S.	*Salmonella*
Y.	*Yersinia*
V.	*Vibrio*
spp.	Species
S19	*Brucella abortus* strain 19
Rev-1	*Brucella melitensis* Rev-1
CO_2	Carbon dioxide
pH	Stands for 'potential of Hydrogen'
°C	Celsius
H_2S	Hydrogen sulphide
S-LPS	Smooth lipopolysaccharides
IgG	Immunoglobulin G
IgM	Immunoglobulin M
cfu	Colony-forming unit
UI	Uncertainty interval
CI	Confidence interval
OR	Odds ratios
RR	Relative risk
BCR	Benefit-cost ratio
NPV	Net present value
PV	Present value
IRR	Internal rate of return
WHO	World Health Organization
FAO	Food and Agriculture Organization of the United Nations
OIE	World Organization for Animal Health
VMC	Veterinary medical centers
PCR	Polymerase chain reaction
BAPAT	Buffered acidified plate antigen test
RBT	Rose Bengal plate test
CFT	Complement fixation test
SAT	Serum Agglutination Test
ELISA	Enzyme-linked immunosorbent assay
iELISA	Indirect ELISA
cELISA	Competitive ELISA
MRT	Milk ring test
ME Test	Mercapto-ethanol test
γ IFN	Gamma interferon assay
SDTH	Skin delayed-type hypersensitivity
SC	Subcutaneous

CHAPTER ONE

Introduction

1.1 Background

Brucellosis, also known as Malta fever, Mediterranean fever and undulant fever (Corbel et al. 2006), is a zoonotic disease which can infect a wide range of domestic and non-domesticated animals (Pappas et al. 2006). It is recognised as a significant problem throughout the world, particularly in the Mediterranean Region, north and east Africa, the Middle East, South and central Asia, India, and central and South America and is considered one of the most important zoonotic diseases internationally (Arroyo Carrera et al. 2006, Pappas et al. 2006). Despite the fact that many countries have implemented control programmes against the disease, it still remains a major health problem for humans and a disease of economic importance in livestock (Seleem 2010), even though the causative agent was first recognised by David Bruce over 130 years ago (Bruce 1887).

Infected animals, in particular cattle, sheep, goats and pigs, are the main source of brucellosis for humans. According to Deqiu et al. (2002) there are approximately 1.8 billion sheep in 50 countries in regions where *B. melitensis* is endemic, 1.3 billion cattle in 101 countries with endemic regions for *B. abortus* and 0.9 billion pigs in 33 countries where *B. suis* is endemic. There are only a few countries in the world that are officially free of the disease in some species, including Japan, Canada, Australia and New Zealand (OIE 2019), however human cases still can occur in these countries when people acquire the infection during international

travel or through contact with animal species that are reservoirs for the pathogen (Robinson 2003).

Brucellosis in humans results in an acute or sub-acute intermittent fever with malaise, prostration and anorexia, and without treatment the disease may continue for weeks or months progressing into a chronic form. Because of the non-specific clinical signs of infection, diagnosis should be confirmed by laboratory tests (Corbel et al. 2006). Brucellosis in animals results in significant economic losses because of abortions, reduced milk production, decreased reproduction rate and premature births (Seleem 2010). Although surveillance and control programmes have been implemented, the prevalence of brucellosis is increasing in some countries due to political, socioeconomic and sanitary factors (Gwida et al. 2010, Pappas et al. 2006).

1.2 General information on the Iraqi Kurdistan Region

1.2.1 Location

Kurdistan “Land of the Kurds” is a defined geo-cultural region which includes the north-western Zagros and the eastern Taurus mountain ranges. The contemporary use of Kurdistan includes large parts of eastern Turkey (Turkish Kurdistan), northern Iraq (Iraqi Kurdistan), north-western Iran (Iranian Kurdistan) and north-eastern Syria (Syrian Kurdistan) (Figure 1.1) and these regions are predominantly populated by the Kurdish people or Kurds (Tasie 2015, O'Shea 2004). The Iraqi Kurdistan Region borders Syria to the west, Iran to the east

and Turkey to the north. It is located in the northern part of the Republic of Iraq known as Kurdistan.

The region lies between latitudes 34-42° and 37-22° north and between longitudes 42-25° and 46-15° east, and is traversed by the Sirwan River and the Tigris and its tributaries, the Great Zab and the Little Zab. The mountains have an average height of 2,400 meters above mean sea level (amsl) with the lowest point in the region is Kifri district, which has an elevation of 140 meters-amsl, and the highest point is the peak of Hasarost mountain in Erbil province, measuring 3607 meters amsl.

In 1970 Iraqi Kurdistan gained autonomous status by agreement with the Iraqi Government and in 2005 its status was re-confirmed as an autonomous entity within the Federal Iraqi Republic (O'Leary et al. 2006). The Kurdistan Region is located in the north of Iraq and officially includes the three provinces of Erbil (Capital City of Kurdistan and also spelt Arbil), Sulaymani (also spelt as Sulaiminiyah) and Dohuk (Figures 1.2 and 1.3). However, Kirkuk province and some districts in Nineveh and Diyala provinces are also controlled by the Iraqi Kurdistan Government's forces (Peshmarga), as the majority of the population in these locations are Kurds and these are unofficially included within the Kurdistan Region. The provinces are subdivided into districts, sub-districts and villages. The Kurdistan Region is approximately 80,000 km² in size and forms 18% of the total area of Iraq. The total human population of Iraqi Kurdistan is estimated at 7 million, representing 17% of the total population of Iraq (BBC 2018, Kurdish-Institute 2007). Erbil, the capital of Kurdistan, is estimated to have a human population of 2 million people.

Figure 1.1: Map of Kurdistan
https://ekurd.net/mismas/articles/misc2007/12/independentstate1825.htm
(Accessed 23rd May, 2019)

Figure 1.2: Location of the Iraqi Kurdistan Region.
https://www.institutkurde.org/en/info/iraqi-kurdistan-does-independence-beckon--1189163374.html (accessed 23rd May 2019)

Figure 1.3: Location of the Provinces of Iraq
https://en.wikipedia.org/wiki/Governorates_of_Iraq#/media/File:Iraqi_Governorates.svg
(accessed 23rd May, 2019)

1.2.2 Economy

The Kurdistan Region has an expanding economy built upon progressive economic policies and growing government transparency. With an abundant amount of proven natural resources and a large labour force, the Kurdistan Region has the potential to become a regional economic powerhouse. Investment opportunities span every sector, including oil and gas, electricity, energy, agriculture and the service industries (Soderberg and Phillips 2015, KRG 2012). According to Iraqi Kurdistan Regional Government's data, Iraqi Kurdistan is estimated to contain around 45 billion barrels of oil, making it the sixth largest reserve in the world, and extraction of these reserves began in 2007. Other mineral resources that exist in significant quantities in the region include coal, copper, gold, iron ore, limestone, marble and zinc. Despite the total capital invested in agriculture related projects was only 1.43% of the total investment in Kurdistan’s economy in 2012, there are strong indicators that the industry will play a prominent role in shaping the future of the region (Abdullah 2013).

1.2.3 Climate

The climate of Kurdistan is considered to be continental and semi-tropical. It has wet, cold winters and dry, hot summers, except for the mountainous regions which have moderate summers with snow cover on the high mountains during the winter months. The rainfall pattern is influenced by the Mediterranean climate and the region is divided into three areas in terms of annual rainfall, which ranges from 350 to 1,200 mm (MAWR 2015, INSAM 2003). The hottest months are from June to September (summer) with mean temperatures of

39-43°C and maximum temperatures of nearly 50°C. The autumn months of October and November are dry and mild with average temperatures of 24-29°C. The winter months are cold and wet with mean maximal temperatures of 7-13°C. In spring the mean temperatures range from 13-18°C in March to 27-32°C in May (MAWR 2015), and this season is the time when Kurds celebrate Nawroz, the Kurdish New Year (Katzman 2010). Water resources are largely associated with the quantity of rainwater and melting snow supplying the main water basins, along with water sourced from dams and reservoirs built upstream on rivers shared with Turkey, Syria and Iran (KRG 2012). However, the absence of international water sharing agreements between these countries results in a lack of certainty of available water resources from one year to the next.

1.2.4 Livestock

A range of livestock species are present in the Kurdistan Region of Iraq, however sheep, goats and cattle are the most numerous and important, providing meat and milk, as well as skin and wool. There are approximately five million head of livestock and these are mainly found in the villages, sub-districts and districts of Kurdistan (KRG 2012, Personal Communication Dr Ali, Kirkuk Hospital). Sheep (3.25 million), goats (1.25 million) and cattle (0.42 million) represent 66.1%, 25.5% and 8.5% of the region's livestock, respectively. Most livestock are in Sulaymani province (43.4% of the total livestock), followed by Dohuk (21.8%), Erbil (17.5%) and Kirkuk (17.3%) (Table 1.1).

Table 1.1: Number of livestock in the different provinces in the Kurdistan Region in 2010

Provinces	**Sheep**	**Goats**	**Cattle**	**Total**
Erbil	416,410	356,636	86,432	859,998 (17.5%)
Sulaymani	1,423,383	525,786	179,990	2,130,659 (43.4%)
Kirkuk	681,500	75,500	97,650	854,650 (17.3%)
Dohuk	724,822	293,869	52,152	1,072,343 (21.8%)
Total	3,246,115	1,251,791	416,224	4,914,130
Percent of total	66.06%	25.47%	8.47%	100%

(KRG 2012, Personal Communication Dr Ali, Kirkuk Hospital)

1.3 Statement of the problem

Brucellosis is a well-known, worldwide-distributed contagious zoonotic disease, which infects animals, including humans, through direct or indirect contact. Brucellosis remains a major zoonotic problem in the Middle East, and is a major economic burden to Iraq where the disease is endemic. The main source of human infection is through the consumption of raw milk and unpasteurised home-made white cheese (Seleem 2010), which is popular in Kurdistan.

The first study on brucellosis conducted in Iraq was undertaken in humans, cattle, sheep and goats by Al Zahawi (1938) and brucellosis was recognised as an endemic disease in Iraq in 1937. Between 1974 and 2004 several studies were undertaken in the northern provinces of Iraq in an attempt to determine the disease's prevalence in livestock and humans. Nicoletti (1986) reported a seroprevalence of 1.0, 4.4, 3.1 and 10.8% in sheep, goats, cattle and humans, respectively in Iraq. Shareef et al. (1999) also investigated the seroprevalence in

animals and humans in Sulaymani City in the district of Qaradagh and reported that 1.34 and 3.36% of sheep and goats were seropositive, respectively. Furthermore, in the City of Sulaymani, 65% of seropositive humans were females, although overall only 4.2% of the seropositive individuals were children (aged between 6 and 12 years) (Shareef et al. 1999). In another study 24.2% of 420 raw milk samples from the province of Basrah in the south of Iraq were seropositive to the milk ring test and overall 14.7% of samples were positive on culture (Abbas and Aldeewan 2009). The prevalence of human brucellosis has been shown to be higher in semi-rural areas (29.3%) than in rural or urban areas in Basrah (Yacoub et al. 2006).

Based on personal communications with representatives from the Iraqi Ministry of Health in Baghdad, the lowest number of cases of brucellosis in humans was in the late 1980s. However, during the 1990s, due to the economic sanctions resulting from the United Nations resolutions leading to reduced medical capability, there was a significant increase in the disease's incidence. This, along with decades of unstable socio-economic and security-political conditions, resulted in an increase in the disease in both animals and humans. In 1995 the annual incidence of brucellosis was reported to have reached 88.5 cases/100,000 people (Salih 2010). Subsequently the situation improved due to the Oil for Food and Medicine agreement between Iraq and the United Nations (Salih 2010).

1.4 Aims of the current study

The main aim of this project was to further our understanding of the epidemiology of brucellosis in sheep and goats in the Iraqi Kurdistan Region. This information is critical for the future development and implementation of effective control programmes.

The specific aims of this study were to:

1. Determine the seroprevalence of the disease in sheep and goats in the Iraqi Kurdistan Region.
2. Identify risk factors for infection of sheep and goat flocks by administering a questionnaire to farmers in the Kurdistan Region whose small ruminants had been sampled.
3. Conduct an economic analysis to determine the impact of the disease on productivity and evaluate the economic benefit in implementing a control programme that focused on vaccination.
4. Conduct a retrospective study of human brucellosis in Iraq to describe the historical distribution of the disease and its impact on the population.

1.5 Significance of this study

The data analyses and information acquired from this study will provide beneficial information on brucellosis to allow the development and instigation of preventive measures against the disease and implementation of suitable targeted surveillance programmes by the Iraqi Kurdistan Regional Government.

1.6 Hypothesis

The main hypothesis of this study is that brucellosis is endemic in Kurdistan. It was also hypothesised that certain management and husbandry factors (such as purchasing new animals or water sources) increase the risk of infection, and control or elimination of these factors would help reduce the disease's transmission within the area.

CHAPTER TWO

Literature review

2.1 Definition and history

Brucellosis is a well-known, worldwide distributed, zoonosis (Memish and Balkhy 2004). The disease is endemic in many animal species around the world and humans are infected primarily through the oral or percutaneous routes after contact with infected animals or their products (Doganay and Aygen 2003). It is caused by infection with bacteria belonging to the genus *Brucella* and was first recognised as a zoonotic disease by David Bruce when he cultured *B. melitensis* from the spleen of four soldiers who had died after displaying fever in Malta (Bruce 1887). *Brucella abortus* was subsequently isolated in 1897 from a cow that aborted in Denmark by Bang and consequently the disease was initially known as Bang's disease (Meador 1988, Williams and McKusick 1954). Other members of the genus *Brucella* were subsequently discovered including *B. suis*, *B. canis*, *B. ovis*, *B. neotomae*, *B. microti*, *B. ceti*, *B. pinnipedialis* and *B. inopinata* (Scholz and Vergnaud 2013).

Although *Brucella* species are not truly host specific (Robinson 2003), they do have a host preference, which is evident in their ability to establish a chronic infection and be transmitted within populations of specific animal species (Glynn and Lynn 2008).

2.2 Microbiological characteristics

Brucella is small Gram-negative coccobacilli, 0.6 to 1.5 µm long by 0.5 to 0.7 µm wide. They are not truly acid-fast bacteria but resist decolourisation by weak acids, thus they stain red by the Stamp's modification of the Ziehl-Neelsen method (Corbel and Banai 1984). The morphology of *Brucella* is relatively constant, except in old cultures, where pleomorphic forms may be seen. They are arranged singly and less frequently in pairs or small groups. *Brucella* spp. are facultative intracellular, non-spore-forming and non-capsulated bacteria. Although they are considered to be non-motile, they carry all the genes, other than the chemotactic system, necessary to assemble a functional flagellum (Fretin et al. 2005).

Brucella are aerobic, but some strains require an atmosphere containing 5-10% CO_2 to grow, especially on primary isolation (Jensen et al. 1995). The optimal pH for growth varies from 6.6 to 7.4 and culture media should be adequately buffered near pH 6.8 for the best growth. Although the optimal growth temperature is 36 to 38°C, most strains can grow between 20 and 40°C (Corbel and Banai 1984). *Brucella* grow best on trypticase, soy-based or other enriched media with a typical doubling time of two hours. Species and biovars are differentiated by their CO_2 requirements, ability to use glutamic acid, ornithine, lysine, and ribose; hydrogen sulphide production; growth in the presence of thionin or basic fucshin dyes; agglutination by antisera directed against particular lipopolysaccharide epitopes; and susceptibility to lysis by bacteriophages (Jensen et al. 1995).

After 48-72h of incubation at 37°C, *Brucella* colonies are 0.5 to 1.0 mm in diameter with a convex and circular outline and a smooth, shiny surface (Alton et al. 1988). Smooth strains are transparent and pale yellow, resembling droplets of honey with a shiny surface when observed in transmitted light. Smooth colonies produce a yellow uniform suspension whereas rough colonies produce granular agglutinates (Padilla Poester et al. 2010, White and Wilson 1951). Colonies were visible on nutrient agar after 3-5 days of incubation and they appear transparent or pale honey coloured when grown on serum dextrose agar (Alton et al. 1988).

2.3 *Brucella* species

There are 12 *Brucella* species currently recognised. The species that have been isolated from terrestrial animals and/or humans are: *B. abortus, B. melitensis, B. suis, B. ovis, B. canis, B. neotomae, B. inopinata, B. vulpis, B. papionis* and *B. microti* (Corbel 1997, Scholz et al. 2016, Whatmore et al. 2014), while *B. ceti* and *B. pinnipedialis* have been isolated from marine mammals (Cloeckaert et al. 2001). The first six species are considered classical *Brucella* and within these species, seven biovars are recognised for *B. abortus*, three for *B. melitensis* and five for *B. suis*. The remaining seven species have not been further differentiated into biovars (Verger et al. 1987). The species of *Brucella* were named based on the host animal preferentially infected (Corbel and Banai 1984) with *B. abortus* primarily affecting cattle, *B. melitensis* sheep and goats, *B. ovis* sheep, *B. suis* pigs, *B. canis* dogs and *B. neotomae* desert woodrats (*Neotoma lepida*) (Blasco 1997, Corbel 1997, Corbel 1989). In addition, four more species have been identified from different animal types: *B. ceti* -

cetaceans; *B. pinnipedialis* - pinnipeds; *B. microti* – vole; and *B. inopinata* – humans (Blasco 2011, Godfroid et al. 2011, Scholz et al. 2008a) (Table 2.1).

Table 2.1: The species, biovars and natural hosts of *Brucella*.

Species	Biovars	Natural host	Zoonotic pathogen?
B. abortus	1 – 6 & 9	Cattle	Yes
B. melitensis	1 - 3	Sheep and Goats	Yes
B. suis	1 & 3	Swine	Yes
	2	Hares	No
	4	Reindeer, Caribou	Yes
	5	Rodents	Yes
B. canis	None	Dogs and other canids	Yes
B. ovis	None	Sheep	No
B. neotomae	None	Desert woodrat	No
B. microti	None	Vole	Unknown
B. ceti	None	Cetaceans	Unknown
B. pinnipedialis	None	Pinnipeds	Unknown
B. inopinata	None	Unknown	Unknown
B. vulpis	None	Red foxes	Unknown
B. papionis	None	Baboons	Unknown

(Godfroid et al. 2011, Scholz et al. 2016, Whatmore et al. 2014)

2.3.1 *Brucella melitensis*

Brucella melitensis was the first species of *Brucella* to be described (Seleem 2010). It causes abortions and orchitis in goats and sheep and Malta fever in humans (Megid et al. 2010). This is considered to be the most virulent species for humans, resulting in the highest morbidity with severe complications, including endocarditis, following infection (Corbel et

al. 2006). It is common in Latin America and the Middle East and in the latter region is considered a re-emerging pathogen (Seleem 2010, Pappas et al. 2006, Samartino 2002). *Brucella melitensis* also results in significant economic losses to the livestock industries through abortions (Radostits et al. 2000).

Brucella melitensis is transmitted more readily from animals to humans than other members of the genus (Seleem 2010). Most cases of infection in humans are related to direct or indirect exposure to infected sheep or goats or their products (Sofian et al. 2008, Husseini and Ramlawi 2004). The best effective vaccine for control of brucellosis from infection with *B. melitensis* in small ruminants is Rev-1. This is an attenuated smooth strain of *B. melitensis* which also gives heterologous protection against infection with other *Brucella* species (Estein et al. 2009, Marin et al. 1990).

2.3.2 *Brucella abortus*

Brucella abortus was initially named *Bacillus abortus* by Bang in 1897, however it was subsequently renamed *B. abortus* in 1920 (Vassallo 1992). It is the aetiological agent of brucellosis in cattle, which is associated with premature calving, abortions and infertility in cattle, potentially resulting in significant economic losses. Although most species of *Brucella* are host specific, *B. abortus* can affect multiple species, including humans, particularly in situations where there is close contact between the animal species (Corbel et al. 2006). The disease is found in most cattle-raising regions of the world except for Japan, Canada, Australia, New Zealand, Israel and some European countries (Pal et al. 2017).

The primary hosts for *B. abortus* are cattle, elk (*Cervus canadensis*), bison (*Bison spp*.), African buffalo (*Syncerus caffer*), water buffalo (*Bubalus bubalus*) and camels (*Camelus dromedarius*) and a variety of other species, including sheep, horses, goats, raccoons, Rocky Mountain bighorn sheep (*Ovis canadensis*), dogs, foxes, wolves and opossums can become "spill-over" hosts in areas where the bacterium is endemic (Diaz Aparicio 2013, Corbel et al. 2006, Alton et al. 1988, Corbel and Banai 1984). *Brucella abortus*, along with *B. melitensis* and *B. suis*, are recognised as potential military, civilian and agricultural bioterrorism agents (Valderas and Roop 2006).

2.3.3 *Brucella suis*

Brucellosis in pigs is primarily caused by the bacterium *B. suis*. The disease was first described by Traum in 1914 in swine herds in Indiana and was initially considered to be as a result of infection with pathogenic *B. abortus*, however the affecting organism was later named *B. suis* by Huddleson (Conger et al. 1999, Alton 1990). There are five biovars recognised, with biovars 1 to 3 affecting swine (Timoney et al. 1988). Biovars 1 and 3 have been detected in both domesticated and wild/feral pigs (Molin 2004). Biovar 2 currently occurs mainly in wild boar; however, this biovar can be transmitted to domesticated pigs and spreads readily in these herds. Biovar 4 is maintained in caribou and reindeer and can also infect moose, cattle, arctic foxes and wolves. Biovar 5, which is still poorly characterised, is believed to infect only murine species (Molin 2004, Timoney et al. 1988). *Brucella suis* have occasionally been reported in dogs, cattle, small ruminants, horses and other spill-over hosts.

Biovars 1 and 3 are both very pathogenic for humans (Conger et al. 1999); and, although biovar 2 and 4 have also been isolated from humans, infection occurs very rarely.

Human infection is primarily limited to those exposed to the bacterium through their occupation, such as farmers and abattoir workers (Robinson 2003). In addition, recreational hunting of wild boars and consumption of meat from wild boar/feral pigs provides sufficient opportunity for the transmission of *B. suis* to humans. The potential for contact between wild boars/feral pigs and domestic swine also increases the likelihood of infection in domesticated pigs (Gibbs 1997, Meng et al. 2009).

Abortion is the primary indicator of disease in pigs and this can occur at any stage of pregnancy (Al-Rawahi 2015), as well as clinical signs associated with atrophy of the epididymis, unilateral orchitis and infertility. There are reports of infection resulting in lameness associated with swollen joints, bursa and tendons and paralysis arising from abscess formation near the spine (Alton 1990). *Brucella suis* have been isolated from horses with septic bursitis, aborted equine foetuses and the internal organs of a mare with no obvious clinical signs of disease (Megid et al. 2010). *Brucella suis* was the first biological warfare agent developed by the USA in 1952 and was field-tested in organism-filled-bombs (M33 cluster bombs) (Okutani 2007). However, because many infections in humans are asymptomatic with a low mortality, the agent was not considered an ideal biological weapon, although it could be used to target military personnel, civilians or food supplies (Christopher et al. 2005).

2.3.4 *Brucella ovis*

Brucella ovis causes a genital infection in sheep resulting in epididymitis, increased lamb mortality and occasional abortions (Júnior et al. 2012, Ficapal et al. 1998, Blasco 1990). The bacterium was first isolated in New Zealand and Australia (Blasco 1990), and has also been reported in North and South America, South Africa, parts of Asia and European countries and probably occurs in most sheep-rearing regions of the world (Blasco 1990).

Ewes rarely display clinical signs and only a small percentage of them abort; however, some ewes may develop placentitis that may result in the birth of weak lambs (Grilló et al. 1999). In sexually mature rams, *B. ovis* causes epididymitis, orchitis and infertility (West et al. 2002). Venereal transmission via the ewe appears to be a frequent way of infection, but transmission from one ram to another ram by direct contact also occurs (Bushra et al. 2017, Blasco 1990). Infected ewes may also excrete *B. ovis* in milk and vaginal discharges and accordingly ewe-to-lamb transmission and ewe-to-ram transmission is also possible (Bushra et al. 2017). Although rams play a major role in the spread of the disease, eradication or control of *B. ovis* is only possible if both rams and ewes are included in any control programme (Blasco 1990). Until now no human cases have been reported, and consequently *B. ovis* is considered to be non-zoonotic. Although goats, deer and cattle have been experimentally infected with *B. ovis*, other than in sheep natural infection has only been reported in deer (Bushra et al. 2017).

2.3.5 *Brucella canis*

Brucella canis was first described in 1966 in the USA when abortions in beagles were documented (Carmichael and Kenney 1968). It has since been reported in several countries in central and South America, along with Mexico and the southern states of the USA. In addition, it has been reported in commercial or research breeding beagle kennels in several other countries, including China and Japan, and has been reported sporadically in Europe (Wanke 2004, Carmichael 1990, Flores-Castro and Segura 1976).

Brucella canis is mainly transmitted via sexual contact. The organism survives in the vaginal and uterine tissues of the bitch and can often be excreted for the life of the bitch. In male dogs, the bacteria resides in the testicles and seminal vesicles and can be shed in the semen or urine (Hollett 2006). Semen from infected males usually contains large numbers of inflammatory cells and abnormal sperm, especially during the first three months of infection. Infection can result in azoospermia in chronic infections (Carmichael 1990).

Brucella canis is rarely a zoonotic organism (Hollett 2006, Carmichael and Kenney 1968). Canine brucellosis rarely is fatal, although it does result in reproductive failure. Clinical signs include infertility in males due to development of antibody against the sperm (Lucero et al. 2010). Infected males often display no clinical signs, except in advanced cases where epididymitis, scrotal dermatitis, testicular atrophy, and infertility may be observed (Carmichael 1990). Infected bitches may abort, although otherwise appear clinically normal (Carmichael 1990).

2.3.6 *Brucella neotomae*

Brucella neotomae has only been isolated from desert rats (*Neotoma lepida*) in Utah, USA (Godfroid 2002). It has no known pathogenicity in any other animal species, including humans. This was recognised as a new species of *Brucella* on the basis of conventional genus speciation, including the organism's behaviour on differential dye media, H_2S production and CO_2 requirements (Tiller et al. 2010).

2.3.7 Marine mammal species

The first marine mammal isolations of *Brucella* came from harbour seals (*Phoca vitulina*), a porpoise (*Phocoena phocoena*) and a common dolphin (*Delphinus delphis*) in Scotland (Ross et al. 1994), as well as from walruses (*Odobenus rosmarus*), whales and a broad range of marine mammal species from many parts of the world (Foster et al. 2002). The *Brucella* isolated (the Scottish strains) had biochemical properties which did not closely correlate with the descriptions of other recognised *Brucella* species, although it is possible that some or all of these strains will be identified as atypical cultures of existing species or biovars. Identification of these species to date has been based on staining, cultural characteristics, serology, metabolic phenotype and phage type (Vizcaíno et al. 2004, Clavareau et al. 1998, Jahans et al. 1997).

These bacteria appear to be widespread in marine mammal populations with seropositive animals detected in the Mediterranean Sea, North Atlantic Ocean, Arctic Ocean including the Barents Sea, and along the coasts of Peru, Australia, New Zealand and the Solomon Islands and the Atlantic and Pacific coasts of North America (Godfroid et al. 2012, Godfroid 2002). Successful experimental infections of sheep and cattle have been reported (Rhyan et al. 2001) and several human infections have been documented, including three individuals with no occupational exposure to marine mammals. One marine mammal isolate was cultured from a laboratory worker with acute brucellosis (Brew et al. 1999).

There is little information on the effects of brucellosis in marine mammals, although *Brucella* have been isolated from the reproductive organs of some marine species (Lopes et al. 2010) suggesting a potential to impact on fertility. *Brucella* isolates from marine mammals are genetically different from the terrestrial species. The name *B. maris* was originally suggested for all marine mammal isolates of *Brucella* based on the traditional naming system, with the division into two or more biovars based on host specificity (Moreno et al. 2012, Jahans et al. 1997). Later on two new species names were proposed, i.e. *B. cetaceae* for isolates from cetaceans (whales, porpoises and dolphins) and *B. pinnipedialis* for strains from pinnipeds (seals, sea lions, and walruses) instead of *B. maris* (Cloeckaert et al. 2001).

2.4 Brucellosis in humans

Brucellosis in humans in some areas are usually associated with the consumption of unpasteurised milk or soft cheeses made from the milk of infected animals (Corbel et al.

2006). Brucellosis is also considered an occupational disease because of the higher incidence in people working with animals, such as farmers, veterinarians, laboratory workers and slaughterhouse workers. In these cases infection results from direct or indirect contact with infected animals and the bacterium enters via skin wounds or mucous membranes (LeJeune and Kersting 2010).

The most common *Brucella* species to infect humans are *B. melitensis*, *B. abortus* and *B. suis* (Godfroid et al. 2011). *Brucella canis* has occasionally been reported to cause human infection; however human infection by *B. neotomae* and *B. ovis* has not been reported, and little is known about the capacity of *B. inopinata* to cause infection in humans, even though it was initially isolated from a human. Brucellosis is a serious zoonosis and results from direct or indirect contact with infected animals or their products. Although person-to-person transmission is rare, it may occur through sexual contact, tissue transfer (such as bone marrow and blood transfusion) and breastfeeding of infants. In addition, laboratory acquired *Brucella* infections due to accidental ingestion, inhalation, and mucosal or skin exposure to infected tissue specimens or cultures of virulent or attenuated *Brucella* species are potential health hazards (Thakur et al. 2012, Pike 1978). Brucellosis induces undulant fever, sweating, miscarriage, headaches, weakness, depression, anaemia and muscle pain (Al Dahouk et al. 2003).

Human brucellosis is a very old disease being first reported in 1887. It has minimal mortality and is one of the commonest zoonotic diseases worldwide (Pappas et al. 2006) with an estimated 833,000 (95% uncertainty interval (UI) 337,929 - 19,560,440) cases of brucellosis

due to foodborne routes each year (Kirk et al. 2015). Brucellosis is also an important cause of travel-associated morbidity (Gautret et al. 2013). Despite being controlled or eradicated from some developed countries, the disease remains endemic in many parts of the world, including Latin America, the Middle East, Spain, parts of Africa, and western Asia (Memish and Balkhy 2004). The Middle East has traditionally been considered an endemic area for the disease, with five Middle Eastern countries (Syria, Iraq, Turkey, Iran and Saudi Arabia) being ranked in the top ten countries in the world for the highest incidence of human brucellosis (2^{nd}, 4^{th}, 5^{th}, 6^{th} and 7^{th} respectively) (Hotez et al. 2012, Pappas et al. 2006).

The incidence of brucellosis in humans varies widely, not only between countries but also within countries. There is an obvious lack of high quality scientific data relating to the incidence of brucellosis in humans globally, with the majority of data coming from north Africa and the Middle East (Dean et al. 2012a). A lower disease incidence is seen in developed countries when compared to low and middle income countries. However, brucellosis can still target specific sub-groups of developed countries, including Hispanic communities of low socioeconomic status in the USA (Doyle and Bryan 2000), Turkish immigrants in Germany (Al Dahouk et al. 2007) and Australian pig hunters who are at risk of infection from *B. suis* (Massey et al. 2011, Young 1995). These findings indicate that brucellosis remains a disease of potential public health importance, even in developed countries. It is well accepted that nearly every case of human brucellosis has an animal origin and, therefore, control is primarily a veterinary problem (Nicoletti 2002).

2.5 Sensitivity and survival of *Brucella* in the environment

Several studies have commented on the relatively high persistence of *Brucella* spp. under suitable conditions compared with most other non-spore forming pathogenic bacteria (Salih 2010, Bossi et al. 2004). Thus when temperature, pH, and light conditions are favourable (high humidity, low temperature and absence of direct sunlight), *Brucella* may retain their infectivity for several months in water, faeces, liquid manure, aborted foetuses and foetal membranes, hay, wool, building construction materials, clothing and equipment (Salih 2010). Furthermore, *Brucella* can withstand drying, particularly in the presence of extraneous organic material, and will remain viable in dust and soil for up to two months. Survival is prolonged at low temperatures, especially below 0°C (EC 2001). The reported survival times of *Brucella* are summarised in Tables 2.2 and 2.3.

Brucella are sensitive to exposure to heat and most disinfectants, however they can survive in the environment for up to two years under ideal conditions. This long survival can result in them being a serious threat to both humans and other animals (Bossi et al. 2004). *Brucella* may be killed at temperature of 60°C for 10 minutes, although the large numbers present in some heavily contaminated environments and laboratory cultures can require more drastic heat treatment to ensure their inactivation (Barer and Irving 2018). Fortunately infected milk is rendered safe by pasteurization at 72°C for 1 minute (EC 2001). *Brucella* are very sensitive to direct sunlight, and moderately sensitive to acid, so that they tend to die in sour milk and in hard cheeses that have undergone lactic acid fermentation (Barer and Irving 2018). Treatment with 2.5% sodium hypochlorite, 2-3% caustic soda, 20% freshly slaked lime

suspension or a 2% formaldehyde solution will destroy *Brucella* within one hour on contaminated surfaces (EC 2001).

Table 2.2: Survival time of *Brucella* under different environmental conditions and in different media.

Medium	Condition	Survival time
Ice	-4ºC	4 months
Water (laboratory)	20ºC	2.5 months
Lake water	37ºC, pH 7.5	< 1 day
	8ºC, pH 6.5	> 57 days
Soil	autumn, 90% humidity	48-73 days
Urine	37ºC, pH=8.5	16 h
	8ºC, pH=6.5	6 days
Manure	Summer	1 day
	Winter	53 days
Manure (liquid)	Summer	108 days
	Winter	174 days
Slurry (animal waste)	Tank	> 8 months
Abdominal fluid, sheep	-	10-30 min
Wool	-	110 days
Hay	-	Several days to a month
Street dust	-	3-44 days
Raw milk	25-37ºC	24 h
	8ºC	48 h
	-40ºC	2.5years

(EC 2001)

Table 2.3: Duration of survival of *B. melitensis* and *B. abortus* in various dairy products.

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

(Memish and Balkhy 2004, EC 2001)
NA: not-applicable or no information

Fermentation of unpasteurised milk into products such as cheese or yogurt does not destroy *Brucella* (Estrada et al. 2005) and Iraqi yogurt may contain viable *Brucella* organisms (Personal Communication Dr Ali, Kirkuk Hospital). It has been hypothesised that homemade cheeses in many rural areas may lead to infection of humans if they are made from *Brucella* contaminated milk (Yumuk and O'Callaghan 2012).

2.6 Clinical signs of brucellosis

Although there is no standard classification of brucellosis in both animals and humans, some authors have classified the disease according to the duration and severity of illness as subclinical, acute, sub-acute and chronic (Ulu Kilic et al. 2013, Doganay and Aygen 2003, Pappas et al. 2005). In contrast Ulu Kilic et al. (2013) classified the disease into acute, chronic and localised forms, while Goldbaum et al. (1992) classified the disease as either active or inactive. It is necessary to isolate the causative agent or demonstrate some type of specific serological response in order to mention these terms.

Brucellosis in animals results in significant economic losses because of reduced milk production, abortions, decreased reproduction rate, intrauterine infection with foetal death and premature births (Seleem 2010). In cattle, brucellosis is generally a disease of females but entire males can also be infected with the bacteria localizing in the testicles resulting in orchitis (Diaz Aparicio 2013). Infected cows usually abort only once, and subsequent calves may be born either weak or healthy, although some infected cows will not exhibit any clinical signs. Because of the non-specific clinical signs of infection, diagnosis should always be confirmed by serological tests (Corbel et al. 2006).

Brucellosis in humans is a systemic infection with a range of clinical signs ranging from asymptomatic disease to severe or very rarely a fatal disease. The incubation period is usually one to four weeks; although occasionally, it may be as long as several months. The primary manifestations are acute febrile disease (with or without signs of localization) and chronic

infection with clinical and laboratory features that vary widely (Ulu Kilic et al. 2013). Symptoms in affected humans include intermittent or irregular fever of variable duration (the most common symptom), profuse sweating, chills, diffuse or localised arthralgia, weight loss and generalised pain (Bossi et al. 2004). Fever can be spiking and accompanied by rigors, if bacteraemia is present, or may be relapsing, mild, or protracted. Chronic brucellosis can develop from acute infection, or it may develop directly without a prior acute phase (Bukharie 2009). Infection among children is usually more benign than in adults with less severe complications and a better response to treatment (Akhvlediani et al. 2010). Jennings et al. (2007) reported that the majority of patients delay seeking medical attention and, because of the non-specific nature of the symptoms, diagnosis is often delayed.

Infection in humans with *B. melitensis* results in a more severe infection than *B. abortus*, however infection with *B. suis* can be as severe as *B. melitensis*. *Brucella canis* is infrequently associated with human disease and reported cases have usually been mild. *Brucella melitensis* generally results in an acute infection whereas the infections with other species are usually sub-acute and prolonged (Dean et al. 2012b, Young 1995).

2.7 Pathogenesis and transmission

Infection with *Brucella* usually occurs following ingestion or inhalation of the organisms (Corbel 1989). The bacteria are transported through the mucosal epithelium either free or within phagocytic cells to the regional lymph nodes (Paixão et al. 2009). The subsequent multiplication and spread of *Brucella* in the lymph nodes, bone marrow, spleen, mammary

glands, liver and sex organs occurs via macrophages. After replication, organisms are released with the help of haemolysins and induced cell necrosis. *Brucella* survive within neutrophils following phagocytosis and also can replicate in macrophages and phagocytes (Ko and Splitter 2003). Survival in macrophages is considered to be a key factor in the establishment of chronic infections, allowing the bacteria to escape the extracellular mechanisms of the host defences, such as complement and antibodies (Ko and Splitter 2003).

Brucella can survive for several days in milk, for weeks in ice cream and months in butter, consequently it is recommended that these products are made from pasteurised milk (Table 2.3) (Memish and Balkhy 2004). The sale of dairy products from unpasteurised milk is a key cause of infection in urban populations and travellers visiting areas where the disease is endemic (Makita et al. 2010, Al Dahouk et al. 2005). In addition, meat from infected animals may also be a source of infection if not cooked adequately (Corbel et al. 2006). Some particular food habits, such as eating aborted foetuses as occurs in Ecuador, is also likely to increase the risk of brucellosis in humans (Godfroid et al. 2005).

Infected livestock, such as cattle, sheep, goats and pigs, are the primary source of brucellosis for humans with transmission occurring through contact with infected animals or their materials (Corbel et al. 2006). *Brucella* is an occupational disease that mainly affects butchers, veterinarians, and slaughterhouse workers through contamination of skin wounds (LeJeune and Kersting 2010). Most cases of *B. melitensis* infection can be related to direct or indirect exposure to infected sheep or goats or their products; with occasional transmission from other animals, such as members of the *Bovidae* and *Camelidae* genera (Corbel 1989).

Mothers who are breast-feeding may transmit the infection to their infants, and sexual transmission has also been reported, but these are of minor importance compared with other routes of transmission (Arroyo Carrera et al. 2006, Corbel et al. 2006, Lubani et al. 1988).

Many factors can influence the prevalence of brucellosis in livestock, such as the prevailing environmental conditions, sex, age, species, diagnostic tests used and geographical location of the study (Crawford et al. 1990). *Brucella* spp. are usually transmitted between animals by direct or indirect contact with an infected animal through aborted foetuses, placenta, vaginal discharges and foetal fluids. In addition, dogs can play a role in the contamination of the environment by feeding upon infected aborted foetuses and/or dragging them to "clean" areas (Baek et al. 2003). The main route of entry for *Brucella* spp. is oral arising from the ingestion of food or water contaminated with secretions or aborted foetal remains from infected animals (Samartino and Enright 1993, Crawford et al. 1990). While semen can be contaminated with the bacteria in infected bulls, artificial insemination is of less importance unless the semen is sourced from an infected bull (Corbel et al. 2006). Infected cows shed *Brucella* in their milk and this is key in the transmission to calves. In dairies, milking is another mode of transmission that must be considered because the bacteria are highly likely to be transmitted from cow-to-cow if the same teat cups are used for milking. For this reason, it is recommended that healthy cows be milked first and infected cows last (Samartino 2003, Samartino and Enright 1993). In extensively managed goat and sheep farms, it is common practice for flocks to share pasture and watering points. Such mixing of animals is a risk factor for the spread of disease from infected to free flocks, making control more challenging (Samadi et al. 2010). The purchase of infected animals and introduction to a disease-free herd is the most common means of disease introduction (Mee et al. 2012). In contrast to terrestrial

animals, the transmission of *Brucella* in marine mammals is poorly understood, however it has been hypothesised that the pathogen may be acquired via the food chain (Godfroid et al. 2011, Godfroid 2002).

2.8 Epidemiology of brucellosis

Although brucellosis has a worldwide geographical distribution, it remains a particularly important disease of livestock and a public health problem in the Mediterranean Region, Africa, the Middle East, Asia, India and South America (Figure 2.1 and Table 2.4) (Benkirane 2006, Corbel et al. 2006, Pappas et al. 2006). The geographical distribution of brucellosis is always changing, with new foci emerging or re-emerging (Seleem 2010). The epidemiology of human brucellosis has changed dramatically over the last 25 years because of socioeconomic and political reasons and the various sanitary control measures implemented, in addition to increased international travel by a larger population of people (Pappas et al. 2006). New foci of human brucellosis have emerged, particularly in central Asia, while the situation in certain countries of the Middle East has rapidly worsened (Pappas et al. 2006). Brucellosis is an important disease of humans and domestic animals in central America (CA), where swine and bovine brucellosis caused by *B. suis* and *B. abortus*, respectively, have been identified in all CA countries, while caprine and ovine brucellosis caused by *B. melitensis* has been detected in Guatemala along with other countries in the region (Moreno 2002).

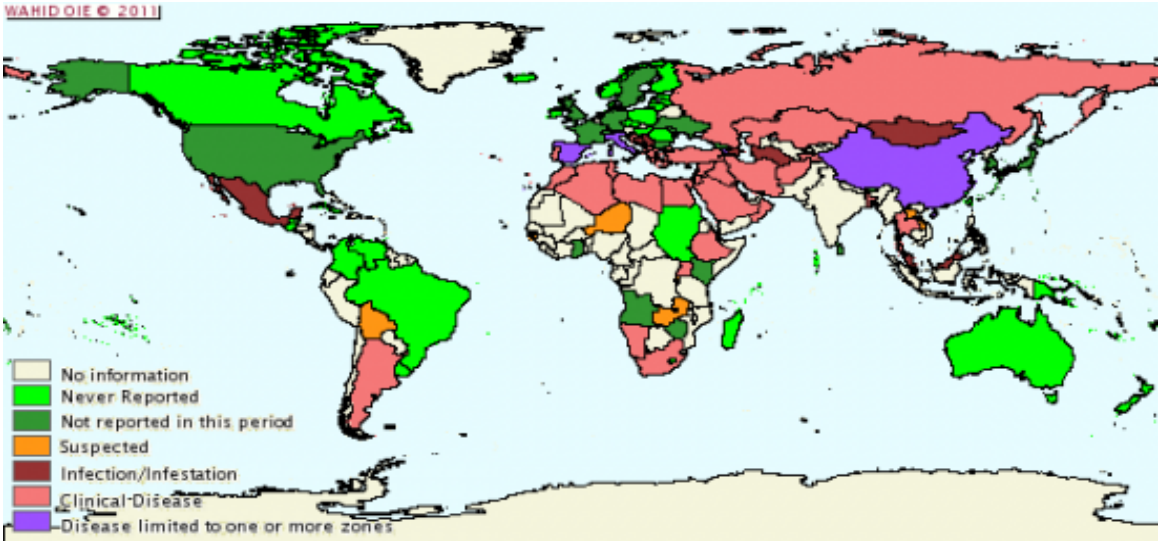

Figure 2.1: Distribution of *B. melitensis* of domestic and wild animals between July and December 2010.
http://www-old.caribvet.net/fr/diseases/brucellosis/distribution-g%C3%A9ographique
(Accessed 26 February, 2019)

Traditionally the Middle East has been considered an endemic area for brucellosis, and although human brucellosis is a notifiable disease in some countries of the Middle East, it is often under-reported and/or unrecognised (Refai 2002). However, five of the 10 countries with the highest incidence of human brucellosis in the world are located within the region (Pappas et al. 2006). The estimated incidence of infection in humans varies widely between countries from <0.03 to >160 per 100,000 people per year (Pappas et al. 2006, Taleski et al. 2002) (Table 2.4); however, the actual number of human cases of brucellosis is unknown and is believed to be much more than the officially reported number (Refai 2003). In Iraq brucellosis has been recognised in humans, cattle, sheep and goats since the 1930's (Al Zahawi 1938).

Although brucellosis occurs worldwide in animals, some countries are free of the disease in specific species (Seleem 2010, Corbel 1989), although disease may occur in people returning

from countries where the disease is endemic (Al Dahouk et al. 2005). *Brucella abortus* in animals has been eradicated from Sweden, Japan, Finland, The Netherlands, Cyprus, Norway, Denmark, Canada, United Kingdom, Australia and New Zealand (OIE 2019, Seleem 2010). While *B. melitensis* has never been detected in some countries (Robinson 2003).

Table 2.4: The incidence of brucellosis in humans per 100,000 people per year by country.

Country	Study level	Incidence per 100,000 people per year
Egypt	Local regions	0.3 – 70.0
Iran	Local regions	0.7 – 141.6
Iraq	Local regions	52.3 – 268.8
Jordan	National	25.7 – 130.0
Kuwait	National	3.4
Lebanon	National	5.0
Oman	Local regions	11.0
Palestine	Local regions	8.0
Saudi Arabia	National	137.6
Syria	National	160.3
Turkey	Local regions	11.9 – 49.5
United Arab Emirates	National	4.1
Kyrgyzstan	National	88.0
Chad	Local regions	34.9
Germany	National	0.03
Greece	Local regions	4.0 – 32.5
Italy	National	1.4
Argentina	Local regions	12.8
Mexico	Local regions	25.7

(Dean et al. 2012a, Pappas et al. 2006)

Infection of sheep with *B. melitensis* is endemic in the Mediterranean region, particularly along the eastern and northern shores. It is found throughout central Asia, south to the Arabian Peninsula and as far as Mongolia, India and Africa (EC 2001) (Figure 2.1). Although the main sources of infection are sheep, goats and their products, *B. melitensis* has emerged as an important problem in cattle in Saudi Arabia, Kuwait and some Southern European countries (Álvarez et al. 2011, Benkirane 2006). Similar cross-species transmission has been reported in Paraguay and some South America countries, where *B. suis* biovar 1 has become established in cattle (Baumgarten 2002, Samartino 2002).

Table 2.5: Seroprevalence of brucellosis in small ruminants in some Middle Eastern countries.

Country	Animal host	% Seropositive	Source
Egypt	Sheep & goats	2.4 & 8.2	Benkirane (2006)
Iran	goats	10.18	Gul and Khan (2007)
Iraq	Sheep & goats	1.3 & 3.4	Shareef et al. (1999)
Israel	Sheep	8.2	Benkirane (2006)
Jordan	Sheep	2.2	Al-Talafhah et al. (2003)
Kuwait	Sheep	2.4	Benkirane (2006)
Oman	Sheep & goats	0.14 & 0.42	Al-Rawahi (2015)
Saudi Arabia	Sheep & goats	6.5 & 9.7	Gul and Khan (2007)
Syria	Sheep	12.87	Darwish and Benkirane (2001)
Turkey	Sheep	11.5	Yumuk and O'Callaghan (2012)
UAE	Sheep & goats	8.4	Mohammed and Shigidy (2013)
Yemen	Sheep & goats	1.7 & 5.6	Al-Shamahy (1999)

2.9 Diagnosis of brucellosis

The diagnosis of brucellosis on presenting clinical signs alone is difficult because of the variety and non-specific nature of the signs (Lulu et al. 1988) and therefore diagnosis must be confirmed through the use of laboratory tests (Corbel et al. 2006). Fast and accurate diagnosis of brucellosis in humans and other animals is critical as misdiagnosis or a delay in diagnosis may result in treatment failure, disease spread, the disease progressing to a chronic nature, relapses or focal complications (Seleem 2010, Al Dahouk et al. 2007). Diagnostic tests can be applied with different goals: confirmatory diagnosis; screening or prevalence studies; certification; and in countries where brucellosis is eradicated, surveillance in order to avoid the reintroduction of brucellosis through importation of infected animals or animal products. However the validation of such diagnostic tests can be challenging, particularly in wildlife (Godfroid et al. 2010).

2.9.1 Laboratory diagnosis

A number of techniques have been developed to diagnose brucellosis in humans and other animals. Definitive diagnosis of the disease is based on cultural or serological techniques or both, and a range of serological tests are widely used for the diagnosis of brucellosis (Etman et al. 2014). There are several challenges facing the diagnosis of brucellosis using serological tests: firstly antibodies from a range of other microorganisms, such as *Yersinia enterocolitica* O:9 and *Escherichia coli* O157:H7, *Stenotrophomonas maltophilia* and *Salmonella* spp., can cross-react with diagnostic tests resulting in false positive results (Matope et al. 2010, Nielsen

et al. 2004, Alton et al. 1988); secondly most serological tests are not capable of detecting antibodies during the early stage of infection or in chronic or latent carriers (Al-Rawahi 2015); thirdly most serological tests cannot differentiate between naturally infected and vaccinated animals (Godfroid et al. 2010); and finally some tests are expensive and require experienced technicians and expensive equipment and consequently are not suitable for routine use in developing nations (Dauphin et al. 2009). Although these factors can limit the usefulness of serological tests in control and eradication programmes, serological tests do offer the advantage of speed, large numbers of samples can be tested and they are relatively inexpensive (WHO 2009). Many studies have highlighted the advantages of using PCR tests and culturing for individual animals (Gwida et al. 2010); however these are not practical for control programmes or for testing large populations of animals (Ghodasara et al. 2010).

2.9.1.1 Isolation of *Brucella* by culture

Isolation of *Brucella* from aborted foetuses, still-births, uterine discharges, blood, lymph nodes, cerebrospinal fluid or bone marrow from both humans and animals remains the gold standard for the diagnosis of brucellosis (Alton et al. 1975a). However, culture cannot practically be used as a screening test, due to slow growth of the organisms and the technique's low sensitivity (Franco et al. 2007). The sensitivity is influenced by: the quantity of pathogen in the clinical samples; the methods used for culturing; the individual laboratory practices; the use of antibiotics prior to culture of the media; the cultured strain and species (*B. abortus* is harder to culture from clinical samples than *B. melitensis*); and the stage of infection. The sensitivity can range from 15 to 70% in human cases; however, higher values

have been reported when using the lysis centrifugation technique (91%) (Seleem 2010, Glynn and Lynn 2008). Although some authors believe that culture is the only definitive method of diagnosing infection (Gotuzzo et al. 1986), it is expensive, time-consuming, has low sensitivity and can be influenced by the presence of contaminants (Blasco et al. 1994). In addition, the pathogenic nature of some *B.* species means that laboratory and field staff must take adequate precautions to prevent infection. Hence, in the laboratory, *Brucella* spp. or potential infectious materials should always be handled in biosafety level 3 cabinets (Yagupsky and Baron 2005, Staszkiewicz et al. 1991). Due to these reasons the diagnosis of brucellosis in most control and eradication programmes has been based on the results of serological assays (Corbel et al. 2006).

2.9.1.2 Diagnosis through the use of serological assays

Several serological tests can be used to detect *Brucella* specific antibodies in individual animals or herds including the buffered acidified plate antigen test (BAPAT), Rose Bengal plate test (RBT), the complement fixation test (CFT), buffered plate agglutination test and enzyme-linked immunosorbent assay (ELISA) (Weynants et al. 1996a). One major disadvantage with serological tests is the occurrence of false-positive and false-negative reactions due to the tests specificities and sensitivities being less than 100% (Nielsen and Yu 2010).

The most widely used tests for the serological diagnosis of brucellosis in small ruminants are the RBT and the CFT. However, both tests lack specificity when used for testing sera from

goats and sheep vaccinated with Rev-1, although this problem is reduced if the vaccine is administered via the conjunctival route (Díaz-Aparicio et al. 1994, De Bagüés et al. 1992, Fensterbank et al. 1982). The sensitivity of the RBT and the indirect ELISAs are better than that of the CFT, and these tests detect antibodies raised against smooth lipopolysaccharides (S-LPS) (Blasco et al. 1994). Mizanbayeva et al. (2009) reported that the immuno-chromatographic *Brucella* specific immunoglobulin lateral flow assay (LFA) was a simple, rapid test for the detection of specific antibodies in a variety of human body fluids. Moreover, it is highly specific and sensitive, and the application of it does not need specific equipment, experienced personnel, electricity or a refrigerator, making this test ideal for use in poor countries (Anumolu 2015). Serological tests developed for detecting antibodies to *B. abortus* in cattle have also been used to detect *B. melitensis* in small ruminants as no serological test has been developed only for *B. melitensis* (Godfroid et al. 2010).

2.9.1.2.1 Rose Bengal Plate Test (RBT)

The RBT is a single dilution serum agglutination test and is often used to screen entire herds of ruminants for evidence of infection (Kaltungo et al. 2014, Ali 2012). The test was developed for the diagnosis of bovine brucellosis, and is internationally recommended for the screening of brucellosis in small ruminants (Blasco et al. 1994, MacMillan 1990). The principle of the test depends on the development of an antigen-antibody reaction leading to agglutination, however the quality of the antigen can affect the sensitivity of this test (Alton et al. 1988).

Sharma (2016) reported that the RBT was capable of detecting both IgG and IgM. However the test can result in false positive reactions up to 6 months after animals have been vaccinated, although these cross-reactions decrease with time. Such false positive reactions result in challenges in the diagnosis of brucellosis, especially in endemic areas (Kaltungo et al. 2014). False positive reactions with the RBT may also arise due to cross-reactions with antibodies against *Salmonella* spp., *Y. enterocolitica* and *V. cholera* (Kaltungo et al. 2014, Nielsen et al. 2004). The test is easy to run with little equipment required and has been widely used in the field as a pen-side test (Corbel et al. 2006). However its sensitivity may be affected by high ambient temperatures, and in such situations the test is best run under standardised conditions in a laboratory (Blasco et al. 1994).

2.9.1.2.2 Complement Fixation Test (CFT)

The CFT has been widely used in control and eradication programmes for brucellosis (MacMillan 1990, Alton et al. 1975b). It is of particular value in differentiating active infection from a vaccine response arising from the use of a live vaccine and the CFT mostly identifies IgG antibodies in the later stages of infection (Alton et al. 1975). Although the sensitivity of the RBT is sufficient for the surveillance of free areas at the flock level, the CFT should be used in conjunction (in parallel) with the RBT in infected flocks to improve the sensitivity of diagnosis (García-Bocanegra et al. 2014). The CFT has a higher specificity compared with the RBT, Indirect ELISA and SAT (serum agglutination test) (Dohoo et al. 1986) and has been reported to have a higher sensitivity and specificity in adult cows vaccinated with S19 than the rivanol and milk ring tests (Huber and Nicoletti 1986). However

the CFT does have several disadvantages including the need to use an extremely labile reagent (complement), the occurrence of anti-complementary activity, technical demands, a failure to detect a response in early stages of the disease and subjectivity of the interpretation of low titres (Lucero et al. 1999). False negative reactions may also occur due to the test only being able to detect antibody at least two weeks after infection (Sutherland 1980).

2.9.1.2.3 Serum Agglutination Test (SAT)

The simple agglutination test was developed more than 100 years ago to diagnose brucellosis (Nielsen 2002) and was the standard test used before the RBT was developed. The SAT detects IgM more efficiently than IgG and consequently is ideal for the detection of early infection. However the SAT can generate false positive reactions if animals have other illnesses including tularaemia, salmonellosis, cholera and myeloma (Allan et al. 1976). False negative in humans may also occur early in the course of the disease [cited by Al-Attas et al. (2000)]. In endemic areas, a *Brucella* antibody dilution of 1:320 or 1:640 is considered significant with this test, while in areas where the disease is not endemic a lower antibody dilution of 1:160 is considered to be the cut-off point for positivity (Pappas et al. 2005). The SAT has some other disadvantages including the potential for prozone phenomena that can result in false negative reactions when the IgG concentration is high (MacMillan 1990).

2.9.1.2.4 Enzyme-linked Immunosorbent Assay (ELISA)

ELISAs have been used to detect serum antibodies following vaccination and for naturally infected animals, can detect antibody in milk and can detect all isotypes of antibodies (Godfroid et al. 2010, McGiven et al. 2003, Al-Shamahy and Wright 1998). These tests have several advantages including high test sensitivity and specificity, they are not affected by haemolysis or anti-complementary effects, and they are commercially available and are not complicated to perform (Memish et al. 2002, Reynolds 1987). However there is a need for an ELISA plate reader and the test is not cheap compared to other tests, such as the RBT (Sutherland et al. 1986).

Most ELISAs used for the diagnosis of brucellosis are iELISAs (indirect ELISAs); however the competitive ELISA (cELISA) is gaining prominence for the diagnosis of brucellosis. This ELISA is a multi-species assay that can differentiate between antibodies induced by natural infection from those induced by vaccination in cattle (Nielsen et al. 1996, Nielsen et al. 1995). The cELISA is quick to perform, but does require specific monoclonal antibody to the S-LPS, however it is commercially available (Marin et al. 1999). According to Nielsen et al. (1995), the ELISA is the most suitable assay for the diagnosis of brucellosis in individual animals because of its ease of performance and its high sensitivity. Hornitzky and Searson (1986) reported that the usefulness of the ELISA was highlighted in cattle that were culture positive, had a low CFT titre or were non-vaccinated RBT negative reactors animals. False positive reactions can occur due to an immune response of the animal to another

microorganism which shares epitopes with *Brucella* spp. for instance, *Y. enterocolitica* O:9 [cited by Lucero et al. (1999)].

In humans the most important factor affecting immunoglobulin titres was the duration of the disease at hospital admission. Patients with a longer duration of illness before hospitalization had relatively low ELISA IgM titres, whereas IgG titres were relatively high. An ELISA is the most sensitive serological test in humans and is useful to monitor antibodies in patients undergoing treatment (Clavijo et al. 2003). The test is commonly used in endemic areas to detect antibody in affected animals and people prior to the development of clinical signs or symptoms (Seleem 2010). In the first days of infection, IgM antibodies to the S-LPS predominate, after which there is a switch to IgG isotype synthesis in individuals who have not received treatment. This different timing of antibody production allows discrimination between patients (people) with acute or chronic brucellosis (Marrodan et al. 2001, Reddin et al. 1965). In contrast the RBT, SAT and CFT cannot discriminate between the two classes of antibodies because, although IgM antibodies specific to the S-LPS are efficient agglutinins, IgG antibodies can behave as either agglutinating or non-agglutinating (incomplete) antibodies and both classes are active in the CFT. Consequently, supplementary tests such as the iELISA with S-LPS and anti-IgM and anti-IgG conjugates are used for these latter tests (Marrodan et al. 2001, Diaz and Moriyón 1989).

2.9.1.2.5 The Mercapto-ethanol Test (ME Test)

The mercapto-ethanol test (ME Test) is not as sensitive as the SAT; however the results correlate better with the activity of the disease, and it is considered superior to other tests in determining the efficacy of antimicrobial therapy in humans (Madkour and Kasper 2001a). Mercaptans (2-mercaptoethanol) or dithiothreitol cleave disulphide bonds of IgM resulting in loss of agglutination activity. This can be used to distinguish between persistent and early infection in human brucellosis (Young 1991, Buchanan and Faber 1980). A titre of 1/20-1/40 of the ME test is indicative of active *Brucella* infection (Al-Shamahy and Wright 2001). However the test requires experienced technicians and the results can be difficult to interpret and therefore it is rarely used today (Brinley-Morgan 1967).

2.9.1.2.6 Milk Ring Test (MRT)

The milk ring test is a simple test using antigen of whole cell haematoxylin stained killed *Brucella* that can detect antibodies attached to the fat globules of the milk of infected cows (Huber and Nicoletti 1986, Sutra et al. 1986). The antigen-antibody complex that forms when the antigen is added to the milk rises to the surface to form a ring in the cream layer (Nielsen and Yu 2010). Corbel et al. (2006) confirmed that the sensitivity of the test was reasonable; however false-negative reactions have been reported arising from a variety of conditions affecting the milk including mastitis, colostrum and milk at the end of the lactation cycle (Nielsen and Yu 2010). Because of these concerns the MRT is recommended as a screening test for bovine brucellosis (OIE 2000). In addition, in areas where the prevalence of

brucellosis is low, where animals have been vaccinated with strain 19 or in animals with mastitis, the MRT has been found to be less useful (Thoen et al. 1995). However, the MRT does have the advantages that it is a cheap, simple test that can be used for screening dairy herds by non-skilled personnel, and it is usually used in conjunction with other tests (Nielsen and Yu 2010).

2.9.1.2.7 Rivanol Test

The rivanol test depends upon the precipitation of serum protein by rivanol dye and can help differentiate naturally infected from vaccinated cattle (Alton et al. 1988). Huber and Nicoletti (1986) reported the presence of false-negative reactions with this test in cattle, especially when they had been vaccinated with *B. abortus* S19 at a young age. According to Mikolon et al. (1998) the test had a high specificity (99%) and a good sensitivity (90%) (1:25 dilution) and as a result of the low false positive rate it was concluded that the test was useful for detecting infected goats experimentally challenged with *B. melitensis*.

2.9.1.2.8 Coombs Test

The Coombs test, which is also called the antihuman globulin test (AHG), has mainly been used to detect infection in people. However it has also been used to confirm the results of the CFT in cattle (Sutherland 1980). This test can have a high percentage of false positive

reactions in vaccinated animals; however, it has been demonstrated to be effective in detecting chronic carriers (Brinley-Morgan 1967).

2.9.1.3 Molecular detection and identification of *Brucella* spp.

2.9.1.3.1 Polymerase chain reaction (PCR)

The PCR is a technique used widely in medical and biological research laboratories throughout the world (Rahman et al. 2013b). It has been used to study the epidemiology of brucellosis and to differentiate between species and strains through differences in *Brucella* DNA (Allardet-Servent et al. 1988). Scholz et al. (2008b) confirmed that PCR methods based on the 16S rRNA amplified a DNA fragment common to all *Brucella* species, although cross-reactions with members of the closely related genus *Ochrobactrum* were reported. For general identification purposes the preferred target is the IS711, as it has a restricted occurrence in *Brucella* and is present in multiple copies resulting in high sensitivity and the ability to directly test clinical samples (Halling et al. 1993). The AMOS PCR was developed to differentiate between the *Brucella* species and is based on the insertion site of the IS711 element resulting in unique profiles for strains of *B. melitensis*, *B. abortus*, *B. suis* and *B. ovis*. In contrast, *B. neotomae*, *B. canis*, biovar 4 of *B. suis*, *B. abortus* biovar 3 and the *Brucella* species isolated from marine mammals cannot be detected by this PCR (Bricker and Halling 1994).

The PCR has several advantages over traditional microbiological techniques for the identification of *Brucella* species. Firstly, the results are available within a few hours compared with several days when conventional microbiological methods are used (Matar et al. 1996). Secondly, it can be automated with a subsequent reduction in cost, and only a small volume of sample is required (Bricker and Halling 1994). Thirdly, it minimises the need to handle potentially infectious samples, as live organisms are not necessary for this test (Matar et al. 1996, Bricker and Halling 1994). Finally, contamination with other microbes that might be present in tissue samples do not affect the test (Bricker and Halling 1994). However, there are some challenges using the PCR in a laboratory including contamination of the DNA, as the assay has to be performed under strict standardized conditions which are not always available in laboratories (Costa et al. 1996).

2.9.1.3.2 Gamma Interferon Assay (γ IFN)

The gamma interferon test has been developed as an *in vitro* alternative to the Skin Delayed-Type Hypersensitivity test (Weynants et al. 1995). The γ IFN involves using a mixture of cytoplasmic protein from *B. melitensis* B115 with whole blood culture as a specific antigenic stimulus for cattle. Weynants et al. (1995) confirmed that this test offered the advantage of being able to distinguish between false positive and true positive results; however, as with many other tests, it was not able to distinguish between naturally infected and vaccinated animals.

2.9.1.3.3 Skin Delayed-Type Hypersensitivity (SDTH)

The SDTH test has been widely used for the diagnosis of brucellosis in ruminants, and it is a useful addition to serological tests (Bercovich 2000). The principle of this test is similar to the tuberculin test for tuberculosis, and the test evaluates the cell-mediated immunity after the intradermal injection of 0.1 ml of brucellin into the caudal tail fold or the skin of the neck (Bercovich et al. 1993). Within 24 to 72 hours after injection, a hypersensitivity reaction is detectable at the site of injection in diseased animals. The intensity of the reaction is determined by the degree of skin swelling and an increase in skinfold thickness of 2 mm or more is considered a positive reaction (Bercovich et al. 1993).

Measuring cell-mediated immunity has significant benefits in resolving some of the diagnostic dilemmas associated with other serological tests, due to the intracellular nature of this bacteria (Bercovich et al. 1989). The SDTH test offers the advantage in being able to confirm the status of false negative results from serological tests, and can detect latent carriers. Bhongbhibhat et al. (1970) reported that the test could distinguish between infections with *B. melitensis* and *B. abortus* or *B. suis*. However, a study undertaken by Bercovich (2000) confirmed that the test was unable to distinguish between naturally infected and vaccinated animals. Weynants et al. (1995) reported that if the SDTH was repeatedly used in an animal then the animal's immune status could change, interfering with subsequent serological tests. In a study undertaken by Bercovich (2000) it was suggested that the test should not be used in brucellosis-free areas to prevent the confusion of interpreting positive

readings and they reported that the benefit of the SDTH was reducing the number of false negative results arising from some serological tests.

2.10 Treatment of brucellosis

The treatment of brucellosis in animals is a challenge because of the organism's intracellular nature (Metcalf et al. 1994). However treatment of livestock with brucellosis is rarely undertaken because of the expense associated with the treatment, and the priority to eliminate infection from the herd or flock (Robinson 2003). Several antibiotics have been used to successfully treat brucellosis in livestock, with the commonly used ones being oxytetracycline and streptomycin with sulphadiazine (Radwan et al. 1995, Radwan et al. 1993). However incomplete or inadequate treatment can result in the development of chronic infection. In conclusion, treatment of infected animals is not practical or feasible from an economic point of view unless the animals have significant value.

In humans two treatment regimens have been recommended: a combination of rifampicin and oral doxycycline twice a day over a 6-week course (Al-Tawfiq 2008); or a combination of three or four antibiotic drugs such as doxycycline, rifampicin, streptomycin and aminoglycoside for a prolonged course (> 45 days). These regimes have been found to successfully treat disease in humans (Ariza et al. 2007, Corbel et al. 2006). Ariza et al. (2007) found that using a combination of at least two antibiotics such as streptomycin, rifampin, doxycycline or trimethoprim-sulfamethoxazole improved the efficacy of treatment in humans, although they must be used for prolonged periods. Tetracyclines are generally

contraindicated for pregnant patients and children <8 years old. Rifampicin 900mg once daily for 6 weeks is considered the drug of choice for treating brucellosis in pregnant women. In children <8 years old the preferred regimen is rifampicin with cotrimoxazole (trimethoprim-sulfamethoxazole) for 45 days. An alternative regimen consists of a combination of rifampicin for 45 days with gentamicin 5 to 6 mg/kg/day for the first 5 days (Solera et al. 1997). Historically, 2% of humans infected with *B. melitensis* and who aren't treated will die from endocarditis or meningitis (Ko and Splitter 2003, Madkour 2001b). Although all patients will have some response to antibiotic treatment, clinical symptoms may last for weeks or months, although most patients recover within a year (Bossi et al. 2004). The World Health Organization (WHO) recommended antibiotic treatment for humans is 100 mg doxycycline twice daily for six weeks combined with either 600 to 900 mg of rifampicin daily for six weeks, or 1 g of streptomycin once daily for 2 to 3 weeks (Ariza et al. 2007). Treatment of humans with brucellosis is costly and time consuming, and often requires long periods of hospitalisation (Del Pozo and Solera 2012).

2.11 Prevention and control of brucellosis

Vaccination is a key component of disease control and, as outlined previously, a range of vaccines are available for the control of the disease, although only *B. melitensis* Rev-1 vaccine has been shown to be effective in preventing brucellosis in small ruminants (sheep and goats) (Blasco 1997). The disadvantage of vaccination is the induction of antibodies which can potentially interfere with the interpretation of results from diagnostic tests; however the advantages of vaccination far outweigh this disadvantage (Blasco 1997). When

administered by the classic subcutaneous route, a long-lasting serological response is induced, which makes an eradication program based on test and slaughter impractical. When the same vaccine is administered by the conjunctival route, the immunity conferred is similar to that induced by the subcutaneous route, although the serological response is significantly reduced, making it suitable for use in an eradication program (Corbel et al. 2006, Blasco 1997). Many vaccines have been produced to protect sheep, goats, cattle and pigs from *Brucella* infection. However, the most common vaccine to control *B. melitensis* is Rev-1 vaccine, with S19 and RB51 commonly being used to control *B. abortus* infection in endemic areas. There are three different strategies for controlling brucellosis: vaccination of the entire at-risk population (mass vaccination); vaccination of young animals and removal of infected animals; and test and slaughter (McDermott and Arimi 2002). In many high-income countries, brucellosis has been successfully controlled or eliminated in livestock populations through a range of strategies (McDermott et al. 2013). The advantages and disadvantages of these are summarised in Table 2.6 and in the following sections.

2.11.1 Test and slaughter of infected animals

Whole flock/herd testing and removal of infected animals is usually the most efficient method for the rapid elimination of an introduced exotic disease, such as brucellosis or other emergency diseases. It is also often the most cost-effective, although it can still be very expensive and requires the availability of accurate diagnostic test(s) (Corbel et al. 2006) and significant infrastructure for the safe disposal of the positive animals. As the remaining

animals do not have protective antibody there is the potential for an outbreak if disease is reintroduced to the population (Smits 2013).

Table 2.6: Summary of the advantages of brucellosis control strategies

Strategy	Advantages	Disadvantages
Mass vaccination	Lower cost Easy to implement and manage Herd/flock immunity quickly established and maintained by vaccinating young animals Well accepted by owners	Abortions post vaccination Potential public health hazards from the process of vaccination Difficulty in distinguishing between vaccinated and infected animals Infected animals remain on the farms
Vaccination of young animals & elimination of infected animals	Minimises vaccine induced abortions	Herd/flock immunity established slowly
Test and slaughter 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 & may require whole herd/flock culling to be effective but the most efficient method for the rapid elimination of the disease	Requires an efficient and very well-organised veterinary service Suitable for low disease prevalence areas only Risk of subsequent epidemics in animals & human infection (the disease may re-emerge)

(EC 2001).

2.11.2 *Brucella* vaccines

Vaccination has been an important step in controlling brucellosis in many countries, regions and individual herds (Schurig et al. 2002). Routine and sustained vaccination has been shown to result in a significant decrease in the disease's prevalence with time (Godfroid et al. 2011, Blasco 1997). However, Alton et al. (1980) highlighted that the cessation of vaccination could result in a susceptible cattle population and other measures were also needed to minimise the likelihood of reintroduction of the bacterium. There have been numerous vaccines used for controlling brucellosis in different species; however, many of them have been discarded due to the low level of immunity induced.

Nicoletti (2010) reported that live attenuated vaccines were the most effective vaccines and several studies have been undertaken to determine the most effective dose and route of administration of different vaccines. The routes trialled have included intradermal, subcutaneous (SC), oral, conjunctival, intra-vaginal, intra-caudal and intrauterine (Nicoletti 1990). The most commonly used and practical route is SC, however the conjunctival route is also used widely, although the serological response after vaccination with Rev-1 via this route in rams has been shown to be of a lower intensity and a shorter duration than that induced when the vaccine was administered SC (Muñoz et al. 2008).

2.11.2.1 Live *Brucella* Vaccines

2.11.2.1.1 Vaccines for small ruminants

The most widely used vaccine for the prevention of brucellosis in sheep and goats is *B. melitensis* Rev-1 (Blasco 1997). It was developed in the 1950's and is a live attenuated strain of virulent *B. melitensis* which is dependent upon the presence of streptomycin for growth (Herzberg and Elberg 1955). The vaccine is efficacious in adult animals, as well as lambs and kids, and induces a high and durable immune response (Blasco 1997, Alton and Elberg 1967).

The vaccine can be administered via the conjunctival or subcutaneous route in both adult and young animals. In young animals, vaccination via the conjunctival route confers adequate protection without interfering with serological assays (Blasco 1997). Although the vaccine is usually given to kids and lambs between the ages of 3 to 6 months via a subcutaneous or conjunctival injection; Blasco (1997) recommended that in eradication programmes this vaccine should not be administered by this route as it results in a high level of interference with serological tests. When the vaccine is administered by the conjunctival route, protection is induced without the issue of persistent antibody levels. Use of the Rev-1 vaccine in whole-flock vaccination programmes has been considered the only practical method to control infection with *B. melitensis* in small ruminants in areas with low socio-economic levels, extensive management systems or where there is a high prevalence of infection (Montiel et al. 2015, Blasco 1997).

There is no entirely safe strategy for mass vaccination because of the risk of abortion in pregnant animals; therefore it is recommended that Rev-1 should not be used in animals that are over half way through their gestation, as this is the critical period for abortions (Gonzalez et al. 2008). There are also other disadvantages of this vaccine including the bacteria's potential to develop resistance to streptomycin which is used to treat infection with *B. melitensis* in humans. Furthermore Rev-1 has the potential to infect humans, and consequently its use as a vaccine for humans is not recommended (Blasco and Diaz 1993).

2.11.2.1.2 Vaccines for other animal species

Brucella abortus strain 19 (S19) has been the most widely used vaccine to prevent bovine brucellosis (Nicoletti 1990) and it is considered the reference vaccine to which other vaccines are compared against (Nicoletti 1990). This strain was isolated in 1923 from a bovine milk sample and then sub-cultured 19 times (Nicoletti 1990). The effectiveness of S19 is influenced by a range of factors, including the route of administration, the age of the vaccinated animals, the prevalence of infection in the herd and the dose (number of bacteria) administered (Arenas-Gamboa et al. 2009, Schurig et al. 2002, Nicoletti 1990). Vaccinating calves with S19 prevents infection through an increased production of antibodies to the O antigens of LPS (Nicoletti 1990). According to Sangari et al. (1996) and Jones et al. (1965) S19 is unable to grow in the presence of erythritol, is less virulent than field strains, has high immunogenicity and retains its viability during lyopholisation.

Many studies have investigated the dose required through different routes, and it is recommended that 11.5 $x10^{10}$ cfu/dose are administered subcutaneously in heavily infected areas. Although more protection can be offered through the conjunctival route, this method is usually not practical in field situations (Fensterbank and Plommet 1979). Fensterbank and Plommet (1979) reported that two vaccinations with 5 x 10^{10} cfu/dose by the conjunctival route would be more economical, effective and without risk of inducing a serological response as well as offering the advantage that vaccination could be performed at any age, however this is not practical in most grazing herds.

The RB51 strain of *B. abortus* is a laboratory-derived O antigen deficient mutant of a virulent strain of *B. abortus* (S2308) (Schurig et al. 1991). This vaccine was developed to overcome the problems of S19 and has been used in several countries to protect against bovine brucellosis (Schurig et al. 2002). The advantages of this vaccine is that abortions are rarely induced by administering the vaccine and higher levels of protection against infection with *B. abortus* are induced, compared with S19 (Palmer et al. 1996). Furthermore the immunity induced in cattle is mostly cellular, leading to fewer false positive reactions on serological testing (Stevens and Olsen 1996). However, the organism has been detected in the milk of vaccinated cows, it is not effective against *B. melitensis* in sheep, and it can produce infection in humans (Moriyón et al. 2004).

Brucella suis S2 vaccine is a live attenuated brucellosis vaccine that was developed in China (Avila-Calderón et al. 2013). This vaccine has the advantage that it can be administered orally, as well as via subcutaneous injection to pigs, cattle, sheep and goats (Xin 1986). It

can initiate strong protection in pigs exposed to wild *B. suis* and has the advantage that it can be used in different domestic animal species (Xin 1986).

2.11.2.2. Killed *Brucella* vaccines

2.11.2.2.1 *Brucella melitensis* H38

Brucella melitensis H38 killed vaccine has been shown to induce effective immunity against challenge with *B. abortus* (Gonzalez et al. 2008). This vaccine contains a suspension of formalin-killed cells at a concentration of 15 x 10^{10} cells/ml in incomplete adjuvant (Renoux and Renoux 1973). However, Meyer and Gibbons (1978) reported that the vaccine induced high, persistent titres and caused long-lasting unacceptable local reactions at the injection site.

2.11.2.2.2 *Brucella abortus* strain 45/20 vaccine

This vaccine is not widely used because it may result in a reaction (lesion) developing at the injection site, it can cause abortions in vaccinated cattle if the organism reverts to a smooth form (Schurig et al. 2002, Hall et al. 1976) and non-agglutinating immunoglobulins (IgG) can act as blocking antibodies, delaying bacterial clearance and increasing the likelihood of chronic infections (Stevens and Olsen 1996, Parma et al. 1987). However, this vaccine does have good immunogenicity and can be used at any age or pregnancy status in cattle (Alton 1978).

2.11.2.3 Vaccines against brucellosis in humans

The 19-BA vaccine is a derivative from *B. abortus* S19 from which dissociated colonies were selected and cultured. In 1945, Dr Pelagea Vershilova from the Gamaleya Research Institute for Epidemiology and Microbiology in Moscow was able to select a sub-clone of *B. abortus* S19, which possessed both minimal reactogenicity and high immunogenic properties for humans (Feodorova et al. 2014, Sumarokov et al. 1984, Vershilova et al. 1982). The vaccine was first trialled in human volunteers in 1946 and 5,000 at risk workers were vaccinated (Feodorova et al. 2014). It was considered that 2.5–8.0 x 10^8 organisms was a safe dose to induce an immunological protective response when administered via the subcutaneous route. The Epidemic Control Services of the former USSR subsequently used this vaccine in humans as a primary prophylactic measure against the disease (Aleksandrov et al. 1961).

The *B. abortus* 104 M vaccine has also been used in the former USSR and in China to control and prevent *B. abortus* infection in humans (Deqiu et al. 2002). The T and M strains of vaccine were isolated from the foetus of an aborted calf in 1950 by a Russian scientist and tests indicated that the M strain had low virulence, high stability and high immuno-antigenicity. A skin scratch vaccination method was used to introduce 5 x 10^9 bacteria and achieved 90% protection with 12 months duration. This vaccine has been used in humans since 1965 (Deqiu et al. 2002).

WR201 is a live attenuated purine auxotroph, which has experimentally been shown to protect mice against infection with virulent *B. melitensis* 16M. Protection is related to the

production of IFN-γ by antigen-stimulated immune spleen cells and production of anti- LPS antibodies (Hoover et al. 1999). Izadjoo et al. (2004) suggested that induction of purine auxotrophy in *B. melitensis* has the potential to develop into a convenient, safe and efficient human vaccine and they recommended that further work should be undertaken on this as a potential vaccine candidate against brucellosis in humans.

2.11.3 Other preventive measures

Brucellosis is usually introduced into a flock/herd through contact with infected animals and/or semen of infected males. To prevent its introduction new animals should be purchased from *Brucella*-free herds and new animals should be isolated and screened before they are added to the herd. However managing the disease in endemic areas where animals co-graze can be difficult unless a vaccination programme is also implemented (Al-Rawahi 2015). Improvements in: awareness about brucellosis; movement restrictions; diagnostic capabilities; and surveillance of the disease in livestock, humans and wildlife are beneficial in the effective control of the disease (Corbel et al. 2006). In order to increase awareness among the farmers it is important to conduct public education programmes on the clinical signs and transmission routes of the diseases and methods to prevent and control its introduction and spread (Chen et al. 2016). In addition, other biosecurity measures that could help to reduce transmission include: isolating flocks on common grazing land and avoiding shared watering points; developing a strong quarantine and border control system; disposing of placenta, and non-viable birth tissue by incineration or deep burial; using personal protective equipment (wearing protective glasses and gloves) when milking ruminants and

when assisting livestock giving birth; and boiling or heating milk before consumption or using it to prepare other dairy products (Islam et al. 2013).

Understanding the epidemiology of brucellosis in a country, in particular the distribution of the disease, risk factors for infection and the disease's impact on local communities are key components of the local prevention and control of the disease. In the following chapter the results of a cross-sectional study are presented to highlight the distribution of brucellosis in small ruminants in the Kurdistan Region of Iraq.

CHAPTER THREE

Serological Survey of Brucellosis in Sheep and Goats in the Iraqi Kurdistan Region

3.1 Introduction

Brucellosis is an infectious bacterial disease of worldwide importance in livestock and people (Hadush and Pal 2013). The disease is widespread, particularly in some Mediterranean and Middle East countries (Abo-Shadi et al. 2014), however a thorough study on the seroprevalence in animals has not previously been undertaken in the Iraqi Kurdistan Region. A cross-sectional serological study was undertaken to better understand the distribution and frequency of brucellosis in sheep and goats in this region and the results of this study, in particular the disease's seroprevalence, are reported in this chapter.

3.2 Materials and Methods

3.2.1 Study population and sampling

A cross-sectional study was conducted in the Iraqi Kurdistan Region to determine the seroprevalence of brucellosis in sheep and goats. Field sampling was carried out from March to May 2015 throughout the Iraqi Kurdistan Region using two different sampling plans. The sample size was calculated in the program EpiTools (Sergeant 2017) using a test sensitivity of 92% and a test specificity of 99% (Rahman et al. 2013a, Blasco et al. 1994), to be 95%

confident of estimating an expected prevalence of 10% with a precision of 2%. In Sulaymani and Dohuk Provinces a multi-stage sampling protocol was adopted. Six districts were randomly selected from the 22 districts in Sulaymani Province and two were similarly randomly selected from the six districts in Dohuk Province for sampling. Within each selected district one sub-district was randomly selected. Two villages were then randomly selected from each selected sub-district and within each village five farmers were randomly selected from those who owned at least 50 sheep and/or goats. Finally, five animals (sheep and/or goats) were randomly selected from the selected farmers with the first animal being selected randomly and then every 10th animal was selected when the animals were run through an open gateway. In total 300 blood samples (216 sheep and 84 goats) were collected (6 districts × 1 sub-district × 2 villages × 5 farmers × 5 animals = 300 samples) from Sulaymani Province and 100 blood samples (82 sheep and 18 goats) were randomly collected from Dohuk Province (2 districts × 1 sub-district × 2 villages × 5 farmers × 5 animals = 100 samples). The number of sheep and goats sampled in the provinces (Table 3.1) was in proportion to the number of animals in that province.

In Erbil and Kirkuk Provinces, blood samples were collected from sheep and goats by cooperating with the Veterinary Medical Centres (VMC) in these provinces. In Erbil Province there are 27 VMCs in 10 districts, of which 18 agreed to participate and collected 25 blood samples each from sheep and goats (total 450 blood samples - 236 sheep and 214 goats). In Kirkuk Province eight of 13 VMCs in 4 districts agreed to participate and collected 25 samples each for a total of 200 blood samples from 40 flocks (160 sheep and 40 goats). The two different sampling methodologies used in this study are summarised in Figure 3.1.

A total of 694 blood samples were collected from 137 flocks containing sheep (flocks either only contained sheep or both sheep and goats) and 356 samples from 113 flocks containing goats (flocks either contained only goats or contained both sheep and goats). In total, 1,050 blood samples from sheep and goats were collected from 166 flocks in the four sampled provinces. Five ml of blood was collected from the jugular vein directly into vacutainer tubes from each animal. All sampling had been approved by the Animal Ethics Committee of Murdoch University - R2698/14. After collection, blood samples were transported to one of four laboratories (Erbil Veterinary Hospital, Kirkuk Veterinary Hospital, University of Sulaymani and Semel Veterinary Hospital), where they were centrifuged at 4,000 rpm for 5 minutes, after which the sera was separated and stored in Eppendorf Tubes prior to testing.

In the first phase of testing all samples were tested within 24 hours of collection with the RBT at room temperature and the results interpreted within 2 to 4 minutes of mixing, as per the manufacturer's recommendations (Figure 3.2). In the second phase of testing, the 65 positive samples on the RBT and an equal number of randomly selected negative samples (to see if there are any false negative samples) collected from animals located in the same villages as the test-positive animals were tested with an enzyme-linked immunosorbent assay (ELISA) (Nova Tec Immundiagnostica GmbH Technologie & Walpark/Germany www.novaTec-ID.com) to confirm their brucellosis status. An animal was classified as seropositive if it tested positive to both the RBT and the ELISA (tests interpreted in series). Positive and negative controls were used in both the RBT and ELISA tests to confirm that the tests were working. The real prevalence (RP) was estimated using the formula:

RP= (test prevalence + specificity – 1) ÷ (sensitivity + specificity – 1) (Reiczigel et al. 2010)

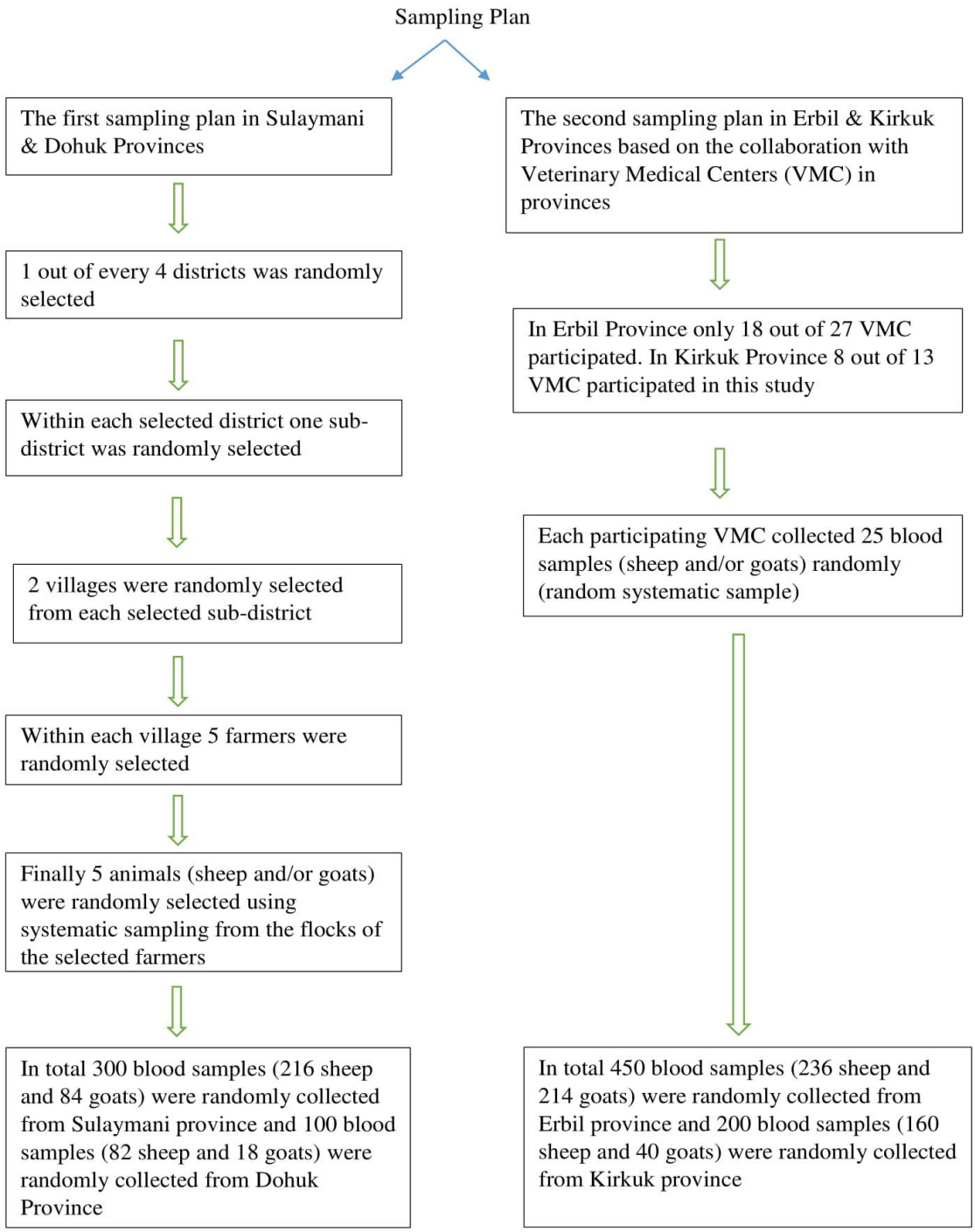

Figure 3.1: The sampling plan illustrating the two different sampling methodologies used in this study

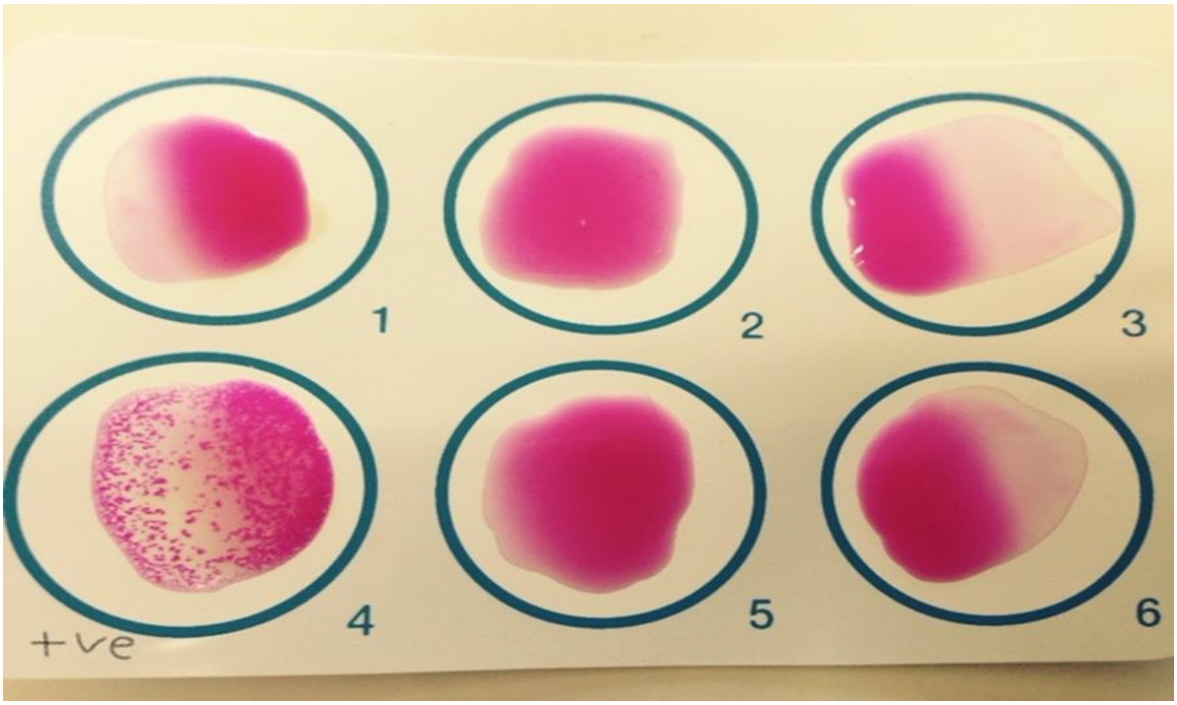

Figure 3.2: Positive and negative results of the Rose Bengal Test
Number 4 is a positive result and numbers 1, 2, 3, 5 and 6 are negative results

Table 3.1: Distribution of samples (sheep & goats) collected for the study on the seroprevalence of brucellosis in the Provinces of Kurdistan, and percentages in each province.

Province	Animal	Number of males tested (%)	Number of females tested (%)	Total number tested (%)*
Erbil	Sheep	81 (11.7)	155 (22.3)	236 (34.0)
	Goats	47 (13.2)	167 (46.9)	214 (60.1)
Sulaymani	Sheep	59 (8.5)	157 (22.6)	216 (31.1)
	Goats	13 (3.7)	71 (19.9)	84 (23.6)
Kirkuk	Sheep	29 (4.2)	131 (18.9)	160 (23.1)
	Goats	11 (3.1)	29 (8.1)	40 (11.2)
Dohuk	Sheep	46 (6.6)	36 (5.2)	82 (11.8)
	Goats	7 (2.0)	11 (3.1)	18 (5.1)
Total	Sheep	215 (31.0)	479 (69.0)	694 (100)
	Goats	78 (21.9)	278 (78.1)	356 (100)

* Percentage of the total tested in each province for each species.

3.2.2 Data management and analysis

Data were entered into a Microsoft Excel spreadsheet and analysed in Excel and SPSS (IBM SPSS Statistics 24 for Windows®, IBM Corporation, Route 100 Somers, New York, USA). Descriptive statistics were calculated for each variable of interest (species, sex, provinces, age and flock type). Odds ratios and their 95%CI were calculated using Woolf's method (Kahn and Sempos 1989). The Fisher's exact test and chi-square test for independence were also used to determine statistical associations. The real and test prevalence were calculated and their 95%CI estimated using Ross's method (Ross 2003).

3.3 Results

3.3.1 Seroprevalence of brucellosis in sheep and goats

Sixty five of the 1,050 (6.2%) samples were RBT test-positive (51 sheep & 14 goats) (95%CI 4.8 - 7.8). Of the 130 samples tested with both the RBT and ELISA, 51 (39 sheep and 12 goats) were positive to both tests (overall seroprevalence of 4.9%; 95%CI 3.6 - 6.3). Fourteen samples (12 sheep and 2 goats) were positive on the RBT and negative on the ELISA test; three samples (2 sheep and 1 goat) were negative on the RBT but positive on the ELISA test; and 62 samples were negative on both the RBT and the ELISA.

The test seroprevalence in sheep (5.6%; 95%CI 4.0 - 7.6) was similar to that in goats (3.4%; 95%CI 1.8 - 5.8) (OR 1.67: 95%CI 0.86 - 3.22) (Table 3.2). After adjusting for the sensitivity and specificity of the tests, the overall real prevalence was estimated at 5.8% (95%CI 4.5 - 7.4).

Table 3.2: Seroprevalence to brucellosis based on seropositivity to both the Rose Bengal test and the ELISA Test in sheep and goats

Species	Number positive on RBT & ELISA	Number negative on at least one test	Test seroprevalence (95%CI)	Real prevalence (95%CI)	Odds ratios (95%CI)
Sheep	39	655	5.62% (4.0 - 7.6)	6.72% (5.0 - 8.8)	1.67 (0.86 - 3.22)
Goats	12	344	3.37% (1.8 - 5.8)	4.02% (2.2 - 6.6)	1.0
Total	51	999	4.86% (3.6 - 6.3)	5.81% (4.5 - 7.4)	-

3.3.2 Gender specific seroprevalence for brucellosis

Overall 757 female animals were sampled in this study, representing 72.1% of the sample population. For sheep, 69% (479) were females and 31% (215) males, whilst for goats 78.1% (278) were females and 21.9% (78) were males. The test seroprevalence in female sheep (5.2%; 95%CI 3.4 - 7.6) was similar to that of male sheep (6.5%; 95%CI 3.6 - 10.7) (OR 0.79; 95%CI 0.40 - 1.55) (Table 3.3). Similarly, for goats the test seroprevalence in females (4.0%; 95%CI 2.0 - 7.0) was not significantly different to that of males (1.3%; 95%CI 0.03 - 6.9) (OR 3.17; 95%CI 0.40 - 24.96) (Table 3.3).

Table 3.3: Seroprevalence to brucellosis (positive on both RBT & ELISA Tests) in male and female sheep and goats.

Species	Sex	Number positive on RBT & ELISA tests	Number negative on at least one test	Test seroprevalence (95%CI)	Odds ratios (95%CI)
Sheep	**Female**	25	454	5.2% (3.4 - 7.6)	0.79 (0.40 - 1.55)
	Male	14	201	6.5% (3.6 - 10.7)	
Goats	**Female**	11	267	4.0% (2.0 - 7.0)	3.17 (0.40 - 24.96)
	Male	1	77	1.3% (0.0 - 6.9)	
Total	**Female**	36	721	4.8% (3.4 - 6.5)	0.93 (0.50 - 1.72)
	Male	15	278	5.1% (2.9 - 8.3)	

3.3.3 Seroprevalence to brucellosis in sheep and goats from different provinces

There was no overall significant difference (P = 0.76) in the test seroprevalence in sheep between provinces based on the RBT and ELISA results interpreted in series (Table 3.4). The test seroprevalence was highest in sheep from Erbil (6.8%) and lowest in Kirkuk (4.4%). Similarly, there was no significant difference in the test seroprevalence in goats between provinces (P = 0.56) (Table 3.4). For goats the test seroprevalence was highest in Sulaymani (6.0%), and no seropositive goats were detected in either Kirkuk or Dohuk.

Table 3.4: Seroprevalence of brucellosis (based on positivity to both the RBT & ELISA) and comparisons between different provinces of Kurdistan.

Species	Province	Number positive on RBT & ELISA tests	Number negative on at least one test	Test prevalence (95%CI)	Odds ratios (95%CI)
Sheep	**Sulaymani**	12	204	5.6% (2.9 - 9.5)	0.81 (0.37 - 1.75)
	Kirkuk	7	153	4.4% (1.8 - 8.8)	0.63 (0.25 - 1.57)
	Dohuk	4	78	4.9% (1.3 - 12.0)	0.71 (0.23 - 2.17)
	Erbil*	16	220	6.8% (3.9 - 10.8)	
Goats	**Sulaymani**	5	79	6.0% (2.0 - 13.3)	1.87 (0.58 - 6.07)
	Kirkuk	0	40	0.0% (0.0 - 8.8)	-
	Dohuk	0	18	0.0% (0.0 - 18.5)	-
	Erbil*	7	207	3.3% (1.3 - 6.6)	

* Erbil is the comparison province.

3.3.4 Seroprevalence of brucellosis in different age groups

The test seroprevalence in sheep ≤ 6 months (4.1%) was similar to that of sheep older than 6 months of age (5.7%) (OR 0.70; 95%CI 0.16 - 3.0) (Table 3.5). No test seropositive goats ≤ 6 months were detected compared to 3.5% of goats older than 6 months of age (P = 0.55) (Table 3.5).

Table 3.5: The influence of age on test seroprevalence in the sampled animals.

Species	Age	Number positive on RBT & ELISA tests	Number negative on at least one test	Test seroprevalence (95%CI)	Odds ratios (95%CI)
Sheep	**≤ 6 months**	2	47	4.1% (0.5 - 14.0)	0.70 (0.16 - 3.0)
	> 6 months	37	608	5.7% (4.1 - 7.8)	1.0
	Total	39	655	5.6% (4.0 - 7.6)	-
Goats	**≤ 6 months**	0	17	0.0% (0.0 - 19.5)	P = 0.55*
	> 6 months	12	327	3.5% (1.8 - 6.1)	
	Total	12	344	3.4% (1.8 - 5.8)	-

* Results from Fisher's exact test

The association between demographic factors and seropositivity are summarised in Table 3.6. Univariable analyses for the seroprevalence of individual animals revealed that overall older animals (> 6 months of age) had a slightly higher seroprevalence (5.0%) than lambs and/or kids aged ≤ 6 months (3.0%) (OR 1.68; 95%CI 0.40 - 7.1) and no seropositive sheep or goats ≤ 6 months were detected in flocks containing sheep only or goats only. The overall seroprevalence in female animals (sheep and goats) (4.8%) was similar to that of males (5.1%) (OR 0.93; 95%CI 0.50- 1.7). Similarly, the seroprevalence in female animals in mixed flocks (containing sheep and goats) (5.4%) was not different to that of males (7.1%) (OR 0.75; 95%CI 0.15 - 1.6) compared with flocks containing sheep only or goats only (Table 3.6).

Table 3.6: Influence of flock composition on animal level seroprevalence

Flock type	Variable	Seropositive animals		Odds ratio	P value
		Yes (%)	No (%)	(95%CI)	
Sheep only	**Female**	10 (4.3)	222 (95.7)	1.22 (0.37 - 4.0)	0.499*
	Male	4 (3.6)	108 (96.4)	1.0	
Goats only	**Female**	4 (3.4)	115 (96.6)	1.36 (0.15 - 12.5)	0.630*
	Male	1 (2.5)	39 (97.5)	1.0	
Mixed sheep and goats flock	**Female**	22 (5.4)	384 (94.6)	0.75 (0.15 - 1.6)	0.233
	Male	10 (7.1)	131 (92.9)	1.0	
Total	**Female**	36 (4.8)	721 (95.2)	0.93 (0.50 - 1.7)	0.403
	Male	15 (5.1)	278 (94.9)	1.0	
Sheep only	**> 6 months**	14 (5.1)	260 (94.9)	-	0.286*
	≤ 6 months	0 (0.0)	25 (100.0)	-	
Goats only	**> 6 months**	4 (2.8)	137 (97.2)	-	0.869*
	≤ 6 months	0 (0.0)	5 (100.0)	-	
Mixed sheep and goats flock	**> 6 months**	31 (5.4)	538 (94.6)	0.98 (0.22 - 4.3)	0.599*
	≤ 6 months	2 (5.6)	34 (94.4)	1.0	
Total	**> 6 months**	49 (5.0)	935 (95.0)	1.68 (0.40 - 7.1)	0.364*
	≤ 6 months	2 (3.0)	64 (97.0)	1.0	

* Results of analysis with a Fisher's exact test

3.3.5 Flock based seroprevalence of brucellosis

Overall 18.7% (95%CI 13.1 - 25.4) of flocks contained at least one animal that was positive to both the RBT and ELISA tests. The lowest flock test prevalence was in flocks only containing goats (6.9%; 95%CI 0.8 - 22.8). In contrast, 16.7% (95%CI 7.9 - 29.3) of flocks only comprising sheep contained at least one test positive sheep. The highest flock test

prevalence (24.1%; 95%CI 15.4 - 34.7) was in flocks containing both sheep and goats (Table 3.7).

Table 3.7: Flock level seroprevalence in sampled flocks.

Flock type	Number of flocks containing at least 1 seropositive animal	Total number of flocks tested	Flock level seroprevalence (95%CI)	Odds ratios (95%CI)
Sheep only	9	54	16.7% (7.9 - 29.3)	0.63 (0.26 - 1.51)
Goats only	2	29	6.9% (0.8 - 22.8)	0.23 (0.05 - 1.07)
Mixed flock containing sheep and goats	20	83	24.1% (15.4 - 34.7)	1.0
Total	31	166	18.7% (13.1 - 25.4)	-

3.4 Discussion

In this study 4.9% of animals were classified as test seropositive (positive to both the RBT and ELISA tests). This was lower than the 14.5% reported by Jabary and Al-Samarraee (2015) in unvaccinated flocks in Sulaymani, based on the RBT alone, and that reported by Al-Naqshabendy et al. (2014) of 39.1% in non-vaccinated ewes in Dohuk, using the ELISA test. The differences in the results between the current survey and previous surveys may be the result of ongoing vaccination control programs of lamb and kids from 3 to 6 months of

age in the Kurdistan region (MAWR 2015). In contrast, the test seroprevalence observed in this study in sheep (5.6%; 95%CI 4.0 - 7.6) was higher than the 2.2% (95%CI 0.5 - 3.5) reported in a study of sheep (using the RBT and ELISA tests in series) in Northern Jordan (Al-Talafhah et al. 2003). Also the test seroprevalence in sheep was higher than a study (4.2%; 95%CI 2.9 - 5.9) in Sarab City (East Azarbayjan province) in Iran (Akbarmehr and Ghiyamirad 2011). These differences were not unexpected given differences in husbandry and management practices between countries.

In the current study the test seroprevalence in sheep (5.6%; 95%CI 4.0 - 7.6%) was similar to that in goats (3.4%; 95%CI 1.8 - 5.8%) (OR: 1.71, 95%CI 0.88 - 3.3). This similarity is not unexpected given the similar traditional husbandry practices for handling both species. Hosie et al. (1985) in Yemen also reported a similar test seroprevalence in sheep and goats (0.6%, 95%CI 0.2 - 1.5; and 0.4% 95%CI 0.1 - 1.4, respectively). However, it is likely that the differences in the prevalence between studies arose because of different management and husbandry practices adopted as outlined earlier, although the method of selecting animals could also account for these differences.

In this study there was no significant difference in the animal-level test seroprevalence between provinces. Again this was not unexpected due to the similar management and husbandry systems adopted between provinces. However, surprisingly no seropositive goats were found in Kirkuk and Dohuk Provinces. This could be due to the sample size, as the number of sheep and goats sampled in Erbil and Sulaymani provinces was larger than that

for Kirkuk and Dohuk (the probability of not getting a positive goats in Kirkuk and Dohuk provinces were 25.38 and 53.39%, respectively).

The test seroprevalence in males and females was also similar in the current study for both sheep and goats. This finding was again expected because of the similar management practices male and female sheep and goats are subjected to. These findings concur with those previously obtained by Jabary and Al-Samarraee (2015) who reported a test prevalence of 14.3 and 10.1% in female and male small ruminants (sheep and goats), respectively in Sulaymani and the study of Al-Hankawe and Rhaymah (2012) who reported a test prevalence (ELISA) of 16.1 and 15.2% in females and males, respectively in Nineveh province.

In the current study the seroprevalence increased with age, although not significantly. This rise is most likely due to the increased number of contacts between animals increasing the likelihood of contact with an infected animal, as well as mating after puberty resulting in a greater chance of infection, as has been described by others (Zeng et al. 2017, Teklue et al. 2013, Dinka and Chala 2009).

Almost one-fifth (18.7%) of the flocks tested in this study contained one or more animals test positive for brucellosis. A higher, although not significant, proportion of flocks comprising both sheep and goats contained at least one seropositive animal (24.1%) compared with flocks that only contained sheep (16.7%). Generally in Iraq, small ruminants (sheep and goats) are usually run as one large flock. Other studies conducted in the region prior to this study have unfortunately not separated out the test prevalence for the two species

for comparative purposes so it was not possible to compare the results of the current study with other local studies.

Differences in test seroprevalence between studies can arise from: the sample size; the tests used; the method of interpreting seropositivity ie tests interpreted in series or in parallel; the study location; the associated management and husbandry practices adopted within those locations; and the control methods adopted (biosecurity measures and vaccination) (Haileselassie et al. 2011). Further research is needed on the incidence, the *Brucella* species affecting small ruminants, risk factors for infection, and manifestations of brucellosis in other regions of Iraq, as well as in Kurdistan. Control or elimination of risk factors for disease will reduce disease transmission and in the following chapter risk factors for infection are investigated further.

CHAPTER FOUR

Questionnaire Survey of Farmers in the Kurdistan Region: Husbandry Practices Adopted and Risk Factors for Brucellosis in Sheep and Goats

4.1 Introduction

Throughout the Middle East, the main *Brucella* species affecting sheep and goats is *B. melitensis* (Seleem 2010, Pappas and Memish 2007). Brucellosis has been an endemic disease in livestock in the Kurdistan Region of Iraq, probably at least since the time of the Ottoman Empire in the 1800's (Obi et al. 2000). Brucellosis can have a considerable impact on the economy through direct and indirect effects on livestock production and productivity and the associated human-health effects (Al-Majali et al. 2009, Perry and Grace 2009). Prevention of brucellosis in humans is dependent upon control of the disease in animals, however brucellosis in the Iraqi Kurdistan Region is a widely spread disease and remains a challenging public health problem (Jaff 2016), and as with other regions continues to be a significant disease impacting livestock productivity (Roth et al. 2003).

The factors influencing the epidemiology of brucellosis in livestock can be divided into those associated with the transmission of the disease between flocks and those influencing the maintenance and spread of infection within individual flocks (Crawford et al. 1990). The demographics and distribution of livestock, management and husbandry systems adopted and

environmental factors are all important determinants of disease spread (Al-Majali et al. 2009, Salman and Meyer 1984). Understanding these factors is critical for the control and eradication of brucellosis. However there is a lack of baseline quality epidemiological data on the occurrence and impact of many zoonotic infections, including brucellosis (McDermott and Arimi 2002), in the Kurdistan Region of Iraq. Consequently the research outlined in this chapter was designed to investigate the role of potential risk factors that could affect the seroprevalence of brucellosis in sheep and goats in the Kurdistan Region. The information arising from this study will help to improve our understanding of the epidemiology of brucellosis, particularly the flock-level factors that are associated with seropositivity. These findings can be used to help develop an evidence-based brucellosis control strategy in livestock in Kurdistan, as well as other regions of Iraq.

4.2 Materials and methods

4.2.1 Questionnaire design

At the time of blood sampling (Chapter 3) data on the sampled animals were collected (age, gender and species). After blood samples were collected from the selected flocks a questionnaire was administered in a face-to-face setting with the owners/managers of the sampled livestock to identify risk factors associated with flock-seropositivity. Owners or managers of 146 (79 farms owned both goats and sheep, 24 owned goats only and 43 owned sheep only) of the 166 sampled flocks were available for surveying (Table 4.1) (20 owners/managers were not available for surveying when the questionnaire was administered). The questionnaire (Appendix 1) included both closed and open questions and

was developed in English and then translated into Kurdish, the local language, prior to administering to farmers. This questionnaire was designed to collect information from the farm owner/manager on their flock including its location, animal species kept and their sex, age, and type and flock size, management and husbandry practices adopted (grazing practices, methods for disposing afterbirth, source of stock-water, sale and purchase of animals, and vaccination history), and incidence of abortions. This questionnaire and study was approved by the Human Research Ethics Committee of Murdoch University (approval number 2014/190) and was sponsored by the Ministry of Higher Education and Scientific Research in Kurdistan Regional Government of Iraq.

Table 4.1: Number of flocks sampled and farmers surveyed in the Kurdistan Region.

Province	**Number of flocks blood sampled (Chapter 3)**	**Number of farmers surveyed**	**Percentage response**
Erbil	86	70	81.4
Sulaymani	30	28	93.3
Kirkuk	40	38	95.0
Dohuk	10	10	100.0
Total	166	146	88.0

4.2.2 Statistical Analyses

Data on the individual animal and flock seropositivity (Chapter 3) and results from the questionnaires were entered into an Excel spreadsheet and analysed using SPSS (IBM SPSS Statistics 24 for Windows®, IBM Corporation, Route 100 Somers, New York, USA). A farm/flock was classified as positive if at least one animal (sheep and/or goat) was positive to both the RBT and ELISA tests out of the 5 animals sampled from that farm. The analyses were conducted in two stages. Firstly, univariable analyses were performed to quantify the strength of association between the exposure variables and flock *Brucella* seropositivity. The Chi-square test for independence or the Fisher's exact test were used to determine if the flock seroprevalence was influenced by different husbandry or management practices. Odds ratios (OR) and their 95% confidence intervals (95%CI) were also calculated to determine the association between factors and seropositivity to brucellosis. In the second stage of the analyses, factors in the univariable analyses with a p-value ≤ 0.25 were offered to a multivariable logistic regression model. A backward stepwise (conditional) selection approach was used to arrive at a final logistic regression model with factors with a $p < 0.05$ retained in the final model. The Hosmer-Lemeshow test was calculated to assess the goodness of fit of the model for the data.

4.3 Results

4.3.1 Univariable analyses for determining risk factors for seropositivity in sheep and goats flocks

The association between management and husbandry practices with seropositivity at the flock level are summarised in Table 4.2. Farmers who had introduced sheep into their flock in the 12 months preceding the survey were significantly more likely to have one or more seropositive (60%) animals in their flock (OR: 5.67, 95%CI 1.5 - 21.8; P value 0.013) than those who hadn't introduced sheep (20.9%). Farmers who introduced goats into their flock in the 12 months preceding the survey were also significantly more likely to have a flock containing seropositive (46.2%) animals (OR 3.7, 95%CI 1.1 - 12.5; P value 0.026) than those who hadn't introduced new goats (18.7%) (Table 4.2).

Farmers who had vaccinated their sheep against brucellosis in the 12 months preceding the survey were significantly less likely to have seropositive flocks (OR: 0.14, 95%CI 0.02 - 0.79; P value 0.028) than farmers who did not vaccinate their sheep. Similarly farmers who vaccinated their goats against brucellosis in the 12 months preceding the survey also were significantly less likely to have seropositive flocks (OR: 0.19, 95%CI 0.05 - 0.79; P value 0.026) than those who did not vaccinate their goats (Table 4.2).

Table 4.2: Univariable risk factors for seropositivity to brucellosis in flocks[#]

Variable			Infected Farms (%)	Number of farms	Odds ratio (95%CI)	P value
Grazed with other flocks		**Yes**	2 (40.0)	5	2.57 (0.41 - 16.1)	0.287*
		No	29 (20.6)	141	1.0	
History of abortion in sheep (last 12 months)		**Yes**	27 (27.6)	98	4.2 (0.92 - 19.0)	0.061*
		No	2 (8.3)	24	1.0	
History of abortion in goats (last 12 months)		**Yes**	10 (23.8)	42	1.05 (0.41 - 2.7)	0.919
		No	14 (23.0)	61	1.0	
Aborted foetuses / Threw away		**Yes**	29 (26.4)	109	-	0.335*
		No	0 (0.0)	6	-	
Aborted foetuses / Gave to dog		**Yes**	23 (25.3)	91	1.01 (0.36 - 2.9)	0.978
		No	6 (25.0)	24	1.0	
Aborted foetuses / Burnt		**Yes**	0 (0.0)	0	-	-
		No	29 (25.0)	116	-	
Purchased sheep in the preceding 12 months		**Yes**	6 (60.0)	10	5.67 (1.5 - 21.8)	0.013*
		No	23 (20.9)	110	1.0	
Purchased goats in the preceding 12 months		**Yes**	6 (46.2)	13	3.7 (1.1 - 12.5)	0.026
		No	17 (18.7)	91	1.0	
Source of water	**River**	**Yes**	9 (24.3)	37	1.27 (0.52 - 3.1)	0.595
		No	22 (20.2)	109	1.0	
	Spring	**Yes**	19 (27.9)	68	2.13 (0.95 - 4.8)	0.064
		No	12 (15.4)	78	1.0	
	Well	**Yes**	23 (19.3)	119	0.57 (0.22 - 1.5)	0.237
		No	8 (29.6)	27	1.0	
Sheep vaccinated against brucellosis		**Yes**	25 (21.6)	116	0.14 (0.02 - 0.79)	0.028*
		No	4 (66.7)	6	1.0	
Goats vaccinated against brucellosis		**Yes**	18 (19.4)	93	0.19 (0.05 - 0.79)	0.026*
		No	5 (55.6)	9	1.0	

Yes = present, No = absent

* Results from a Fisher's exact test

[#] Not all farmers answered every question

4.3.2 Multivariable analysis to identify factors influencing the seroprevalence of brucellosis in sheep and goats flocks

Of the 13 variables analysed in the initial univariable analyses (Table 4.2), abortion history in sheep, introduced (purchased) new sheep, introduced (purchased) new goats, water sourced from a spring, water sourced from a well, sheep vaccinated against brucellosis and goats vaccinated against brucellosis had p values ≤ 0.25 and were offered to the initial multivariable logistic regression model.

In the final model farmers who introduced (purchased) new sheep in the 12 months preceding the survey (OR: 4.24, 95%CI 1.0 - 17.3) and introduced (purchased) new goats in the 12 months preceding the survey (OR: 15.2, 95%CI 3.0 - 76.36) were significantly more likely to have seropositive flocks (Table 4.3). In contrast, flocks that used water sourced from a well (OR: 0.27, 95%CI 0.09 - 0.84) and had goats vaccinated against brucellosis in the 12 month period preceding the survey (OR: 0.31, 95%CI 0.12 - 0.75) were significantly less likely to have seropositive flocks (Table 4.3). The Hosmer-Lemeshow Test (P value 0.67) demonstrated that the final model was a good fit of the data.

Table 4.3: Multivariable analysis to identify factors influencing the brucellosis flock status in sheep and goats in the Kurdistan Region, Iraq

Variables	β	S.E.	P value	Odds ratios (95%CI)
Purchased sheep in the 12 months preceding the survey	1.45	0.72	0.044	4.24 (1.0 - 17.3)
Purchased goats in the 12 months preceding the survey	2.72	0.83	0.001	15.20 (3.0 - 76.36)
Well water used for livestock	- 1.32	0.58	0.023	0.27 (0.09 - 0.84)
Goats vaccinated against brucellosis	- 1.19	0.46	0.010	0.31 (0.12 - 0.75)
Constant	0.04	0.62		

4.4 Discussion

A range of environmental, management, husbandry, host and agent factors can be directly or indirectly associated with the seroprevalence, transmission and distribution of brucellosis (Al-Majali et al. 2009, McDermott and Arimi 2002). In this study a questionnaire was administered to 146 owners of the 166 sampled flocks to identify putative risk factors for seropositivity to brucellosis in sheep and goat flocks located in the Kurdistan Region of Iraq. The risk factors detected in this study are closely related with a traditional management system for small ruminants in this region.

The final logistic regression model highlighted that flocks which introduced sheep (OR = 4.2, 95%CI 1.0 - 17.3) or goats (OR = 15.2, 95%CI 3.0 - 76.4) in the 12 month period preceding the survey were more likely to be seropositive. Similar findings were reported in Oman (Al-Rawahi 2015) where a significantly higher seroprevalence was detected in imported sheep (0.6%) compared with local sheep (0.1%). Kabagambe (2001) highlighted that the movement of animals between flocks increased the risk of disease transmission and Crawford et al. (1990) reported that purchasing of infected replacement animals was the most important factor responsible for introducing brucellosis into previously free flocks. Boukary et al. (2013) also reported that mixing of newly arrived animals into a herd was highly correlated with brucellosis seropositivity. It is critical that introduced animals originate from a known diseases-free flock and it is also recommended these animals have a one-month period of quarantine on-farm of at prior to mixing with existing livestock to minimise the introduction of false negative animals (Mee et al. 2012) and to maintain a *Brucella*-free flock. The pens for quarantine need to be cleaned and disinfected regularly to prevent the potential exposure of existing livestock to pathogens carried by the newly acquired livestock (Villarroel et al. 2007). Also using birthing pens and regular cleaning of these has been shown to significantly reduced the odds of infection (Musallam et al. 2015). Purchasing animals from sale yards/auctions or livestock traders/dealers is of particular danger for the introduction of disease due to the uncertain origin of the animals or the potential for the animals to be incubating infection prior to introduction (AVA 2016).

In the current study, farmers who had vaccinated their goats in the preceding 12 months (OR: 0.31, 95%CI 0.12 - 0.75) were less likely to have a seropositive flock than those who had not vaccinated their goat flock. Ganter (2015) also highlighted the role of vaccination in preventing brucellosis in sheep and goats and recommended that vaccination should be the main tool for disease control, particularly in low-income countries where the disease is often endemic. As outlined in Chapter Two a range of vaccines have been used to protect animals and flocks against brucellosis, with Rev-1 being the most effective one used in small ruminants (Blasco 2011).

In the current study, farmers who used water sourced from wells were also less likely to have seropositive flocks (OR: 0.27, 95%CI 0.09 - 0.84). This is likely associated with the private ownership of wells, hence sheep and goats from these owner's flocks are less likely to mix with other flocks for watering. The increased risk from contact with other flocks has been highlighted as a reason for disease transmission by others (Godfroid et al. 2011), as well as the potential for environmental contamination (Newell et al. 2010). Although direct contact with infectious material has been identified as a key feature for the horizontal transmission of *Brucella* spp. (Abubakar et al. 2012), and in the current study flocks which grazed with other flocks were more likely to be seropositive, this difference was not significant. Extensive grazing not only increases the likelihood of contact with potentially infected flocks, but also increases the opportunity for direct or indirect contact with wildlife, which can also be a reservoir for *Brucella* spp. (Muma et al. 2006). In Kurdistan sheep and goats can share grazing land with potentially infected wildlife, such as wild boars (*Sus scrofa*) Personal Communication Dr Ali, Kirkuk Hospital, and the role of these in transmission of infection to

domesticated animals cannot be discounted, although in Iraq it is likely that contact with other infected flocks is a more important factor in disease spread.

In this study sheep flocks with a history of abortion were more likely to be seropositive (OR: 4.2, 95%CI 0.92 - 19.0) than those without a history of abortion. This is not surprising as the characteristic clinical sign of brucellosis, particularly in small ruminants, is abortion (Blasco 2011). Renukaradhya (2002) similarly observed that flocks with a history of abortions had a higher seroprevalence of brucellosis. However, in the study reported by Al-Rawahi (2015) in Oman there was no association between a history of abortions in sheep and seropositivity. The differences in results between studies may be due to the sample size or the management of flocks where some flocks are more intensively managed and observed hence increasing the likelihood of detection of abortions, if present.

Incorrect disposal by herders of materials from abortions and potentially contaminated after-birth was also found in this study. This has the potential to result in environmental contamination of grazing pastures and watering points (Nakeel et al. 2016), resulting in the spread of infection. Assenga et al. (2016) reported that a history of retained foetal membranes in cattle herds or goat flocks was a significant risk factor for seropositivity. The aborted, foetal fluids and foetal membranes have been shown to contain a large number of *Brucella* organisms (Assenga et al. 2016, John et al. 2010) and their correct disposal through burning or deep burial is important to reduce environmental contamination and exposure of other animals to the bacteria (Deddefo et al. 2015).

In the current study the livestock owners/managers surveyed did not keep any systematic flock records or use an animal identification system and consequently no reliable data were available regarding the number of births, early mortalities, the birth of weak offspring or the number of abortions or still births occurring each year in the surveyed flocks. There is a need for the Iraqi and Kurdistan Regional Government, through the Ministry of Agriculture, to introduce a formal flock and animal identification system and to support the implementation of vaccination programs against infectious diseases, such as brucellosis and foot and mouth disease (FMD). As part of this extension an educational program should be implemented for livestock owners to improve flock biosecurity, which would also reduce the risk of introduction of other infectious diseases, such as FMD, and reduce the risk of human infection with *Brucella* spp.

As brucellosis can have a significant impact on livestock production, understanding the economic impact of the disease in the Kurdistan Region is key to implementing cost-effective control practices, particularly given the potential for transmission of infection to humans. In the following chapter the results of an economic analysis are presented to enumerate the benefit of disease control in small ruminant flocks.

CHAPTER FIVE

Benefit - cost analysis comparing two different vaccination control strategies against brucellosis in sheep and goats in the Kurdistan Region.

5.1 Introduction

Infection with *Brucella* spp. is responsible for significant economic losses to the small ruminant industry because of abortions, premature births, reduced milk production and decreased reproduction rate (Ganter 2015). Despite the implementation of control programs the prevalence of brucellosis is increasing in many developing countries due to the influences of various sanitary, socioeconomic and political factors (Ganter 2015, Seleem 2010). The difficulties in controlling and eradicating brucellosis are often related to animal husbandry and management practices adopted, such as the coexistence of several livestock species and extensive grazing (Godfroid et al. 2011). Successful elimination programs have always been long, costly and hard to carry through (Godfroid et al. 2011).

In countries where brucellosis is endemic, such as Iraq, control of the disease is by a combination of mass vaccination and prevention of *Brucella* spp. entering farms through biosecurity. These control methods are considered to be the best methods, and frequently the only reasonable strategies, to apply in situations where the disease has a high prevalence and herds/flocks are managed extensively (Corbel et al. 2006).

The basis of any policy to control brucellosis is to limit the exposure of susceptible animals to *Brucella* organisms. The mitigation strategies adopted depend upon the prevalence of the disease, geographical considerations, resource availability (both human and financial) and the desire to either eradicate or control the disease (Banai 2002). Loss of production or productivity is the most significant direct economic impact of brucellosis on the livelihood of farmers (McDermott et al. 2013, McDermott and Arimi 2002). The disease may also affect the local or national economy when a serious outbreak happens through reducing the quantity of animal products, such as meat and milk, available for sale to the general public (Seleem 2010). Substantial and on-going financial support from the government and active involvement of farmers are important for the success of a regional and national brucellosis control program (Corbel et al. 2006). Data on the economic losses from brucellosis and the cost of the control programs proposed are required before selecting and implementing control measures. This study was undertaken to estimate the economic value to the small ruminant industry arising from a mass vaccination programme against brucellosis in sheep and goats in the Kurdistan Region of Iraq and comparing it to the status quo, namely vaccinating only lambs and kids from 3 to 6 months of age.

5.2 Materials and Methods

In the economic analysis reported in this chapter a benefit - cost analysis was undertaken (the total amount lost per year and the average loss per adult female were also calculated) to compare a mass vaccination control program involving vaccination of both young and adult animals for 10 years, with the current vaccination program which involves vaccinating only lambs and kids from 3 to 6 months of age. Based on the results of the questionnaires

administered to farmers (Chapter 4), a total of 57,145 sheep and goats were owned by the 146 surveyed farmers of which 53.3% (30,458) were adult females, 3.7% (2,115) were adult males and 43% (24,572) were lambs or kids under one year of age. Available statistics from the Ministry of Planning for 2010 indicated that the total number of sheep and goats in Kurdistan region and Kirkuk province was 4,497,906 head (3,246,115 sheep and 1,251,791 goats) (KRG 2012). For the purpose of this study only losses in adult female small ruminants (sheep and goats) were included in the model to estimate the costs associated with brucellosis, as the majority of the economic impact of the disease results from abortions and reduction of milk production in these species (Seleem 2010). The planned new vaccination strategy was to vaccinate 100% of the animals (both entire males and females, young and adult animals).

In this study, a discount rate of 4.0% was applied as the average discount rate in the Kurdistan Region in 2014 (KRG 2015). The most likely price of a lamb or kid was estimated at US$50 (range 45 - 55) (data from questionnaire). The overall real prevalence of brucellosis in this study was 4.92% (95%CI 3.7 - 6.4%) among sheep and goats (test results from Chapter 3).

The main economic impact of the disease in sheep and goats is associated with production losses arising from abortions and reduced milk production (McDermott et al. 2013) and these aspects were focused on in this study. Economic analyses of control options for zoonotic diseases are complex and require data which are currently lacking from Kurdistan and Iraq. Consequently for some parameters estimates from other countries, or opinions from the local veterinary services or other experts were used (Tables 5.1 and 5.3). The average abortion rate

attributable to brucellosis in infected pregnant sheep and goats was estimated at 30% (range 19 - 36%) per year (assumption as there is really no good quality study that has calculated the risk of abortion due to brucellosis).

The fertility rate in local goats in Iraq was assumed to be 75.74% (range 72.96 - 78.67%) per season (Raoof et al. 2016). According to Hermiz et al. (1998) the average milk production in local goats in Iraq is 98 kg (range 94.3 - 101.7 kg) spread over 173 days in each of the two lactation periods per year. It is assumed that aborted animals do not produce any milk. A current price of milk of US$ 1 per litre was used in this study (Table 5.1). Due to a lack of available data regarding the average fertility rate and milk production of local sheep in Iraq, the values from local goats in Iraq were used for both sheep and goats in this study.

The direct costs for the new vaccination program (vaccine and its administration) included the salaries of the vaccination team members, transport costs of the vaccine, transportation for the vaccination team members and expenses for holding the sub-district farmers' meetings. To implement a vaccination program in Kurdistan, it is proposed that 90 teams are needed, where each team is comprised of one veterinarian, two veterinary nurses and one driver. These teams work for the Veterinary Medical Centres in the districts and sub-districts for a period of 30 days each year to ensure 100% (all adult and young animals) vaccination coverage is achieved.

Table 5.1: Economic parameters used in this study

Description	Most likely value (min, max)	Reference/source
Abortion rate per year	30% (19 - 36%)	Assumption
*Fertility rate per season	75.7% (73.0 - 78.7%)	Raoof et al. (2016)
Average milk production per adult female per year (kg)	98 (94.3 - 101.7)	Hermiz et al. (1998)
Lambing/kidding seasons per year	2	Questionnaire data (Chapter 4)
Average price of one lamb or kid / USD	$50 (45 - 55)	Questionnaire data (Chapter 4)
Average price of milk per kg	$1USD	Questionnaire data (Chapter 4)
Discount rate	4%	KRG (2015)
Percentage of adult females	53.3%	Questionnaire data (Chapter 4)
Percentage of adult males	3.7%	Questionnaire data (Chapter 4)
Percentage of lambs/kids	43%	Questionnaire data (Chapter 4)
$ (United States dollar)	$1 = 1,190 IQD	https://www.mataf.net/

*Fertility rate per season for local goats in Iraq was used for all adult females (sheep & goats)

The assumptions included in this modelling were:

- Estimated $3,200 salary per team per month = 90 (teams) × 3,200 = $288,000.
- Estimated $20 per day for general consumables (such as ice boxes and ice blocks for packaging vaccines, fuel, syringes, expenses for sub-district meetings and administration) per team = 20 × 30 × 90 = $54,000.
- It was estimated that the *Brucella* vaccine would cost USD $0.10 per dose (Director of the Veterinary services) = 4,500,000 (doses) × $0.10 = $450,000.

The total costs of the mass vaccination control program in the first year = $288,000 (salary) + $54,000 (consumables) + $450,000 (cost of vaccine) = $792,000. It was assumed that the costs of vaccine and general consumables will increase by 4% per year, however the salary was assumed to remain the same for the 10 year period because salaries rise very slowly, if at all, in Kurdistan.

This study assessed the impact of brucellosis on abortions and milk production according to the formulas listed below. Data on the costs of treatment, number of cases of still births, orchitis, epididymitis and mastitis were not included as little data were available regarding these features of the disease in sheep or goats for Iraq and they are of less importance than abortions and reduced milk yield (Lopes et al. 2010, Seleem 2010).

Economic losses = A + M

Where: A is the cost of an abortion [A = number of positive females (sheep and goats) × 30% (abortion rate) × 75.74% (fertility rate) × 2 (lambing/kidding seasons per year) × $50 (average price of one lamb or kid)].

M is the cost of the milk production loss [M = number of positive female (sheep and goats) × 30% (abortion rate) × 75.74% (fertility rate) × 98 kg/year (milk production per adult female kg per year) × $1 (average price of milk per litre is US$)].

Benefit-cost analysis is a method that is commonly used to determine whether the benefits arising from a control program exceed the costs of conducting that program. The median and its 95%CI for the Net Present Value (NPV), Benefit Cost Ratio (BCR), and Internal Rate Return (IRR) were calculated (Marsh 1999). A NPV value greater than zero means that the proposed program is economically profitable, as does a BCR greater than 1 (Marsh 1999).

A sensitivity analysis was carried out to account for uncertainty in values for six input variables (abortion rate, fertility rate, lambing/kidding seasons per year, price of a lamb/kid, milk production per adult female per year and average price of milk per kg) in the mass vaccination control program to identify which input parameters had the greatest effect on the uncertainty of the NPV and BCR through examining the normalised regression coefficients. These variables were expressed as probability distribution functions using @Risk 7.5 student version (Palisade Decision Tools, Palisade Corporation). The model was run for 10,000 iterations using the variables specified as @Risk functions, Pert (minimum, most likely and maximum) distributions (Table 5.2). The protection rate was estimated using a combination of the efficacy of the available vaccine and vaccine coverage. Scenarios of 60% (efficacy of vaccine: 75% and vaccination coverage: 80%), 71.25% (efficacy of vaccine: 75% and vaccination coverage: 95%) and 80.75% (efficacy of vaccine: 85% and vaccination coverage: 95%) in the protection rate were applied.

Table 5.2: List of input variables used as @Risk functions

Name	@ Risk function*
Abortion rate per year (%)	19, 30, 36
Fertility rate per year (%)	72.96, 75.74, 78.67
Average milk production per adult female kg per year	94.3, 98, 101.7
Average price (USD) of one lamb or kid	$45, $50, $55
Average price (USD) of milk per kg	$0.9, $1, $1.1

*Data used in the Pert distribution: minimum, most likely, maximum values

It was assumed that, with the current vaccination programme (vaccinating young animals only), brucellosis would be at an endemic equilibrium, where the number of newly infected animals (sheep or goats) produced by one infectious animal (sheep or goat) during its infectious period would equal 1. In this context, the number of newly infected animals (sheep or goats) each year would be the same as the number of infected animals (sheep or goats) that died or were culled/sold (removed). The calculation of new cases was derived from the following formula (Dohoo et al. 2003):

$$R_e = R_0 \times s$$

Where: R_e is the effective reproduction number, which is the average number of secondary cases that result from an infectious individual in a particular population. R_0 is the basic reproduction number, which is the average number of secondary infections arising from one infectious animal in a totally susceptible population, and s is the proportion of susceptible animals (sheep or goats) in the total population of animals.

Table 5.3: Demographic parameters used to simulate brucellosis transmission dynamics and mass vaccination control program strategy in the Kurdistan Region

Parameter	Description	Value	Reference/source
RP	Real prevalence RP =(TP + Sp -1)÷(Se + Sp -1)	5.8% (4.5 - 7.4%)	Chapter Three
TP	Test prevalence	4.9% (3.6 - 6.3)	Chapter Three
s	Proportion of susceptible animals	0.5008	Calculation (s = 1-RP-pr)
pr	Proportion of protected animals	0.45	Calculation (pr=vc*ve)
vc	Vaccination coverage baseline	60%	Directorate of Dohuk Veterinary
ve	Vaccine efficacy baseline	75%	El Idrissi et al. (2001)
N	Number of adult females (sheep and goats)	2,397,383	Total number of animals × percentage of adult females
Ss	Number of susceptible females	1,200,610	Adult females - infected adult females - protected females
i	Number of infected females	117,951	Adult females × Real prevalence
R_e	Effective reproduction number	1.0	Assumption of endemic equilibrium
R_0	Basic reproduction number $R_0 = R_e/s$	1.9968	Dohoo et al. (2003)
D	Duration of infection	5 years	1/u
u	Replacement sheep per year / Cull rate	20%	Director of the Veterinary services
beta	Transmission coefficient beta = R_e / (N*D*s)	1.66582	Calculated

5.3 Results

Based on the data available, the total production losses due brucellosis in sheep and goats in 2015 (year 1 or baseline) in the Kurdistan Region was estimated to be $6.14 million (95%CI $4.48 - $7.96 million) ($2.56 per adult female) with slightly more losses from abortions than reduced milk production (Table 5.4). By adopting a mass vaccination control program for 10 years the annual production losses in sheep and goats due to brucellosis were estimated to decrease to $1.83 million (95%CI $1.33 - $2.39 million) ($0.76 per adult female) for losses arising from abortions and decreased milk production only (if the total number of adult females remained the same) (Table 5.4).

Table 5.4: Losses (USD) due to brucellosis in baseline and during mass vaccination program

	Number of affected females	**Abortions**	**Reduced milk production**	**Total**
Baseline	140,407	$3,102,727	$3,040,672	**$6,143,399**
Year 2	127,770	$2,823,481	$2,767,012	**$5,590,493**
Year 3	112,520	$2,486,485	$2,436,755	**$4,923,240**
Year 4	98,033	$2,166,334	$2,123,008	**$4,289,342**
Year 5	85,126	$1,881,118	$1,843,495	**$3,724,613**
Year 6	73,851	$1,631,962	$1,599,323	**$3,231,285**
Year 7	64,022	$1,414,773	$1,386,478	**$2,801,251**
Year 8	55,530	$1,227,101	$1,202,559	**$2,429,661**
Year 9	48,178	$1,064,642	$1,043,349	**$2,107,991**
Year 10	41,808	$923,888	$905,410	**$1,829,298**

5.3.1 Benefit - Cost Analysis

The results of the benefit - cost analysis are presented in Tables 5.5 and 5.6 and Figure 5.1. Benefits only included the reduced number of abortions and reduced milk loss in sheep and goats due to the control of brucellosis. For the mass vaccination control program compared to continuing the previous control measure using a 4% discount rate, the median NPV was US$11.22 million (95%CI 6.31 - 16.63 million) and the median BCR was 2.56 (95%CI 1.88 - 3.31). The median cost of the mass vaccination program over the ten-year period was estimated at US$7.18 million (95%CI 7.11 - 7.25 million) and the total median benefit in present day dollars was estimated at US$18.42 million (95%CI 13.43 - 23.83 million).

Table 5.5: Summary of the results of the cost-benefit analysis comparing a mass vaccination control program with continuation of the current control measures of vaccinating young animals only

Years	Benefits (US$)	Costs (US$)	Future Value (US$)	PV of Benefits (US$)	PV of Costs (US$)	NPV (US$)
1	$0.00	$792,000.00	-$792,000.00	$0.00	$761,538.46	-$761,538.46
2	$552,905.89	$812,160.00	-$259,254.11	$511,192.58	$750,887.57	-$239,695.00
3	$1,220,158.31	$833,126.40	$387,031.91	$1,084,716.29	$740,646.34	$344,069.96
4	$1,854,056.89	$854,931.46	$999,125.44	$1,584,855.60	$730,798.99	$854,056.61
5	$2,418,785.44	$877,608.71	$1,541,176.73	$1,988,065.32	$721,330.39	$1,266,734.93
6	$2,912,113.47	$901,193.06	$2,010,920.41	$2,301,485.58	$712,225.97	$1,589,259.61
7	$3,342,147.80	$925,720.79	$2,416,427.01	$2,539,757.65	$703,471.71	$1,836,285.93
8	$3,713,737.94	$951,229.62	$2,762,508.33	$2,713,591.94	$695,054.16	$2,018,537.78
9	$4,035,407.97	$977,758.80	$3,057,649.17	$2,835,224.11	$686,960.36	$2,148,263.75
10	$4,314,100.87	$1,005,349.15	$3,308,751.72	$2,914,451.97	$679,177.87	$2,235,274.11
Total	**$24,363,414.59**	**$8,931,077.99**	**$15,432,336.60**	**$18,473,341.04**	**$7,182,091.83**	**$11,291,249.21**

Table 5.6: Summary of Benefit - Cost analysis of a mass vaccination program conducted over a 10 year period to control brucellosis in sheep and goats in the Kurdistan Region

	Benefits (median and 95%CI)	Costs (median and 95%CI)
PV	US$18,420,624 (13,426,580 - 23,830,207)	US$7,182,070 (7,113,637 - 7,251,452)
NPV	US$11,224,173 (6,309,437 - 16,631,789)	
BCR	2.56 (1.88 - 3.31)	
IRR	67.9% (44.4 - 91.6%)	

The real prevalence of brucellosis in sheep and goats in Kurdistan region was predicted to decrease over 10 years from 5.81% (95%CI 4.5 - 7.4%) to 1.74% (95%CI 1.04 - 2.73%) due to the mass vaccination program. However, the real prevalence, if the current vaccination program was maintained, was predicted to stay at the same current level 5.81% (95%CI 4.5 - 7.4%) (i.e. endemic equilibrium) (Figure 5.1).

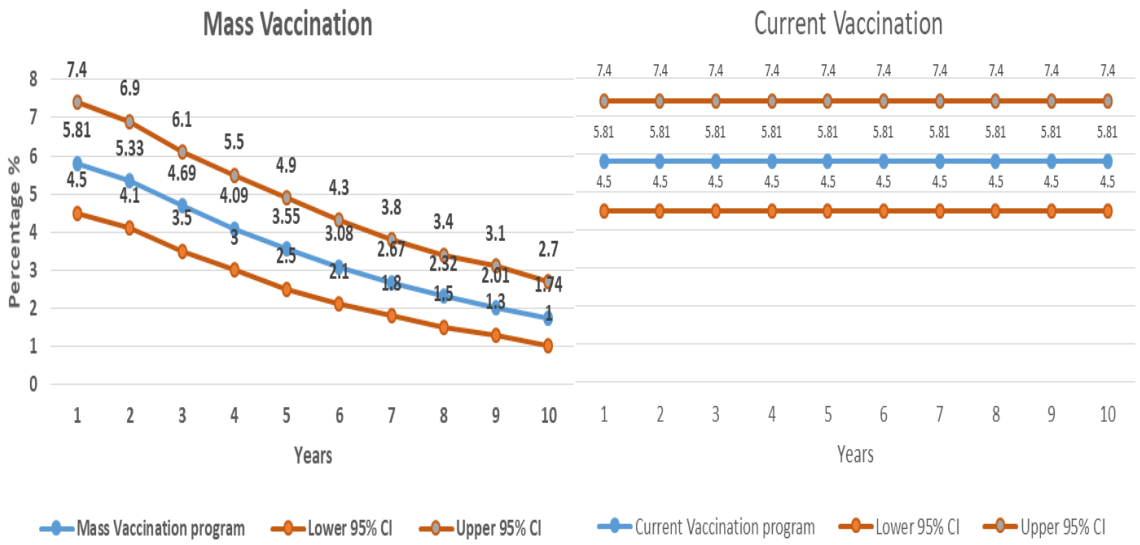

Figure 5.1: Predicted real prevalence of brucellosis in small ruminants over 10 years with a national mass vaccination program compared with the current vaccination program

5.3.2 Sensitivity analysis

In the sensitivity analysis the abortion rate (Regression coefficient = 0.74) had the largest effect on the outcome. The prevalence of the disease (0.63) was the next important factor influencing the outcome. All other factors had minimal impact on the control program (low coefficients values ≤ 0.13) (Figure 5.2).

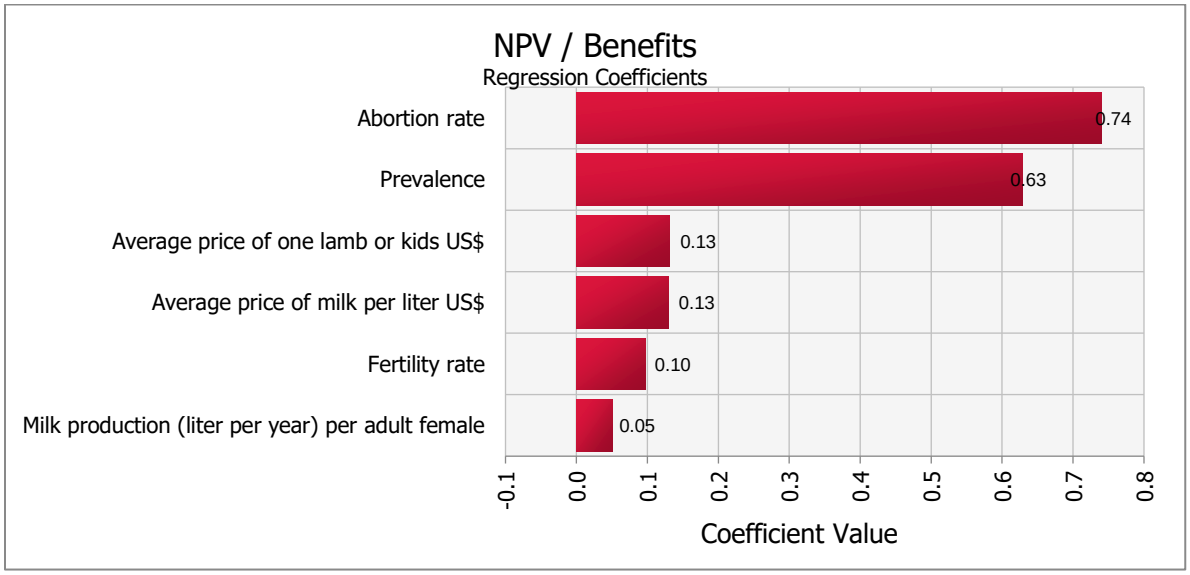

Figure 5.2: Regression coefficients of the sensitivity analysis for the mass vaccination control program

The results of the manual sensitivity analysis (Table 5.7) show that the NPV increased with increasing protection level. Increasing the protection level from 60% to 80.75% resulted in the NPV increasing from US$9,588,653 (95%CI: 5,218,434 - $14,183,401) to US$10,984,762 (95%CI: 6,027,065 - $16,282,818) (Table 5.7).

Table 5.7: Result of manual sensitivity analysis using different protection rates for the vaccinated animals

Protection rate (%)	NPV (US$)	BCR	IRR (%)
60	$9,588,653	2.36	63.99
	(5,218,434 - $14,183,401)	(1.73 - 2.98)	(40.7 - 86.9)
71.25	$10,534,449	2.47	66.29
	(5,827,738 - $15,729,215)	(1.81 - 3.19)	(42.8 - 90.2)
80.75	$10,984,762	2.53	67.39
	(6,027,065 - $16,282,818)	(1.84 - 3.27)	(43.3 - 91.0)

5.4 Discussion

The most effective control program for brucellosis can vary between countries and even regions within a country depending on the relevant risk factors for the disease and the management and husbandry practices adopted, including cultural practices (McDermott et al. 2013). In the current chapter the results of an economic evaluation were presented to assess the net economic value of a mass vaccination control program spanning a 10-year period to control brucellosis in sheep and goats in Kurdistan.

This study found that mass vaccination of animals resulted in a median NPV in the benefit-cost analysis greater than zero and a median BCR ratio greater than one. These results indicate that the proposed vaccination program is financially viable. Benefits in this study only included the reduced number of abortions and reduced milk loss in sheep and goats due to the control of brucellosis. The NPV and BCR would have been even higher if data were available regarding the costs of treatment, number of cases of still births, orchitis,

epididymitis and mastitis as well as the benefits gain from reducing the incidence of infection in humans, however there are currently little data available regarding these features of the disease in sheep, goats or humans for Iraq. Although the proposed program was financially viable, implementing it would face challenges from instability of the veterinary infrastructure through conflicts internal and external to Iraq and uncertainty over vaccine availability. The results of this study are in agreement with the study conducted by Roth et al. (2003) who reported that if the overall costs of vaccinating livestock against brucellosis were allocated to all sectors in proportion to the benefits that would be gained, these interventions could prove profitable and cost effective for both the agricultural and human health sectors.

In this study the proposed small ruminant brucellosis control program in the Kurdistan Region of Iraq was estimated to cost US$7.18 million ($7.11 - $7.25 million) over the 10-year period, with the highest cost associated with the purchase of the vaccine and its delivery. Although vaccination does not eliminate infection, the model predicted a reduction in prevalence to 1.74% (95%CI 1.0 - 2.7) after ten years, at which time it might be possible to evaluate adopting eradication programs, such as test and slaughter programs (Corbel et al. 2006).

In the current study the sensitivity analysis demonstrated that, not surprisingly, improving the protection rate of the vaccine would result in a better economic performance for the vaccination control program. Similar results have been reported in other studies (Alves et al. 2015, Roth et al. 2003). The sensitivity analysis indicated that the abortion rate had the largest effect on the outcome, followed by the prevalence of the disease, with all other factors having

minimal impact on the control program (low coefficients). The results of this study are in agreement with the study adopted by Blasco (2011) who suggested that abortion and infertility are the predominant clinical signs in small ruminants. Although there is a paucity of specific studies, brucellosis is recognised as a source of significant financial loss to livestock industries.

It is critical to not only focus on vaccination, but concurrently establish a strong disease surveillance system involving the monitoring of all abortion cases by the provincial veterinary services to prevent reinfection or spread of disease. In addition, developing a strong quarantine system and preventing the illegal movement of animals from neighbouring countries, especially Syria and Iran, are important for the on-going control of brucellosis (as well as other diseases) in Kurdistan. The benefit of controlling brucellosis in small ruminants is not only important to reduce the economic impact of the disease in sheep and goats but also to reduce the infection in humans, as transmission of the disease between humans is rare and *B. melitensis* is a major cause of *Brucella* in humans (Blasco 2011).

Brucellosis in animals mainly affects reproduction and fertility, and reduces the milk yield and the survival of new-borns lambs/kids, while mortality of adult animals is usually of minor importance. Reduced production or productivity is the most important direct economic impact of brucellosis on the profitability of rearing livestock, with abortions and reduction in milk yield the largest components of these losses (Ducrotoy et al. 2014). Small ruminants represent a major source of meat for Middle Eastern communities (Sherman 2011), therefore improving the livestock industries has been a major focus for many governments in the region

to improve both food safety and food security and the current study highlights the economic benefit in controlling brucellosis through mass vaccination.

Critical to disease control are good management and husbandry measures, particularly associated with the introduction of animals, isolation/quarantine of animals which abort and the presence of wildlife which can act as potential reservoirs for the disease (Godfroid et al. 2005). Therefore, control or elimination of these factors by improving flock biosecurity, disposing of aborted materials properly (for example burning and/or burying aborted materials) and isolating animals which abort from other animals in the flock, as well as purchasing new animals from confirmed brucellosis-free flocks will reduce the disease transmission in this region (Assenga et al. 2016). Reducing the prevalence of brucellosis in livestock, not only improves the productivity of livestock but also reduces the likelihood of disease in the human population as infection of humans nearly always is associated with contact with infected animals or their products (Godfroid et al. 2005). In the following chapter brucellosis in humans in Kurdistan is investigated further.

CHAPTER SIX

A Retrospective Study of Human Brucellosis in Iraq

6.1 Introduction

Brucellosis in humans is widely distributed, with high endemic levels in the Middle East, the Mediterranean region and parts of Asia, Latin America and Africa (Shevtsov et al. 2015, Godfroid et al. 2011, Gwida et al. 2010, Moreno 2002). The most pathogenic and invasive species for humans in descending order are considered to be *B. melitensis, B. abortus* and *B. suis* (Acha and Szyfres 2003). Disease control can be challenging in many regions where the disease is endemic, as these locations/countries are typically characterised by large numbers of poor livestock keepers who adopt either extensive (often pastoral or nomadic) or intensive smallholder livestock systems (Ducrotoy et al. 2014). Animals and their products are the only significant sources of human brucellosis, and transmission predominantly occurs via consumption of unpasteurised dairy products or from direct contact with infectious material, particularly through occupational exposure in livestock keepers, abattoir workers and veterinarians (Corbel et al. 2006). Although attempts have been made to control brucellosis in both people and livestock in Iraq, the incidence of brucellosis in humans remains high (Pappas et al. 2006). It has even been questioned whether control of this disease can be achieved when a country remains plagued by war, famine and poverty (Pappas and Memish 2007).

Although the disease in animals has been eradicated from some countries where it was previously endemic (Pappas et al. 2006), new foci of brucellosis in humans have emerged, especially in central Asia. In addition, the disease is still present in both the USA (126 cases reported in humans in 2015) and in Europe (439 confirmed cases in humans were reported in 2015) (ECDC 2016, CDC 2016). The annual incidence of brucellosis in people residing in endemic countries (such as the Mediterranean, Middle East, Latin America and parts of Asia) is reported to vary from < 0.01 to > 200 per 100,000 population (Dean et al. 2012a, Corbel 1997). Although most humans are infected through occupational exposure to *Brucella* pathogens, with laboratory technicians, abattoir workers, veterinarians and farmers at a greater risk of infection than others (Galinska and Zagórski 2013, Dean et al. 2012a), infection through consumption of unpasteurised dairy products is also common (Kassiri et al. 2013). In 2010 it was estimated that the global burden of foodborne diseases arising from brucellosis alone was 832,633 (95% uncertainty interval (UI) 337,929 - 19,560,440) (arising from eating contaminated products) (Kirk et al. 2015).

Brucellosis in humans is usually a systemic infectious disease, resulting in various clinical manifestations (Corbel et al. 2006). The most common manifestations are fever, intermittent fever, malaise, excessive sweating, fatigue, anorexia, headache, myalgia, chills, weight loss, backache and arthralgia (Mantur 2007, Corbel et al. 2006). Brucellosis in humans may result in hepatomegaly, lymphadenopathy, osteomyelitis, meningitis, splenomegaly, endocarditis and epididymo-orchitis (Rahil et al. 2014). The case fatality rate of untreated brucellosis in humans is around 2% with death usually resulting from heart complications (Vorou et al. 2008).

The first case of human brucellosis in Iraq was confirmed in 1938 (Al Zahawi 1938). In the Kurdistan region there have, however, been very few studies investigating the incidence or prevalence of brucellosis in humans. In 1994, 15.2% of patients with fever presenting at public hospitals in Erbil Province were seropositive on the RBT (Shareef 2006). That same study indicated a higher level of seropositivity among rural residents and females than urban residents and males, respectively, with *B. melitensis* being the predominant species isolated (Shareef 2006). In Erbil Province, Rasul and Mansoor (2012) also found 10.7% of 2,085 patients who presented to the Rizgary and Erbil Teaching Hospitals with fever and signs and symptoms similar to brucellosis, were seropositive on the RBT.

The study reported in this chapter, which examined existing data sourced from the Ministry of Health, Kurdistan Regional Government of Iraq (KRG), was designed to investigate the historical distribution, incidence and cost of brucellosis in humans in Kurdistan.

6.2 Materials and Methods

6.2.1 Analysis of historical data on the incidence of brucellosis in humans in Iraq

This study examined data from 1988 to 2014 sourced from the Ministry of Health, Iraqi Government and the Ministry of Health, KRG. The annual incidence of brucellosis per 100,000 people from 2004 to 2008 in Kurdistan was compared with that for the rest of Iraq

and the incidence of human brucellosis (per 100,000 people) for the period 2009 to 2014 was also compared between the provinces of the Kurdistan region.

Data on the number of cases of human brucellosis from 2009 to 2014 were collected from the Kirkuk Health Directorate and the Ministry of Health, KRG between March and May 2015 and included the number of patients with brucellosis recorded by public hospitals in the different provinces of Kurdistan. In addition, data regarding the number of cases of human brucellosis in Iraq, excluding the Kurdistan region, which had been reviewed by other studies were included in this study to compare the local situation with that of the country.

A survey of health sector experts was also conducted to estimate the total cost of brucellosis in humans. Thirty private health sector experts in Kirkuk province (10 from medical clinics, 10 from diagnostic laboratories and 10 from pharmacies) were selected using a systematic random sample method to estimate the current costs arising from brucellosis. Human ethics approval was obtained from Murdoch University (2014/190) for this research.

The average annual incidence of brucellosis in Iraq (including the Kurdistan region) and relative risks and their 95% confidence intervals were calculated to determine the association between the incidence in different areas and between periods. In addition, a Pearson's correlation coefficient and its P value were calculated to determine the correlation between brucellosis in humans and animals in Kurdistan.

6.2.2 Estimation of the financial burden of brucellosis to humans in Iraq

The cost of brucellosis in humans includes both government health spending and costs incurred by private households containing affected individuals. However, because of the lack of data regarding the cost to the public health sector from human brucellosis in Iraq (including Kurdistan), this study used the current prices (based on a survey of health sector experts) in the private sector to estimate the total cost of brucellosis in humans. The costs included the consultation fee of a doctor, the number of doctor visits, the price of diagnostic tests and the cost of medicines recommended by the World Health Organisation (Ariza et al. 2007), as well as income lost in people suffering from brucellosis (Table 6.1). In this study it was assumed that the average monthly salary in Iraq was $663 ($22.10 per day) (Alghad-Press 2017). For this study the total cost (TC) of brucellosis in humans in the Iraqi Kurdistan region per year was calculated as:

$$TC = (A \times B + C + D + E) \times I$$

Where A is the cost per outpatient visit (doctor's fee); B is the number of outpatient visits per patient; C is the cost of the diagnostic tests; D is the costs of medical treatment per patient (medication costs were assessed on two weeks of streptomycin and six weeks of rifampicin and doxycycline therapy, intravenous fluid therapy, and anti-inflammatory and analgesic medicines); E is the income lost due to the illness per patient and I is the number of human cases in 2014.

Table 6.1: Parameters used to calculate the cost (US$[#]) per brucellosis patient in the private sector in the Iraqi Kurdistan region based on a survey of 30 health sector experts.

Description	Values (minimum, most likely, maximum)
Doctor's fee per visit	$8.40, $25.21, $42.02
Cost of diagnosis (laboratory)	$4.20, $21.01, $33.61
Cost of treatment	$18.70, $26.68, $32.77
Days away from work	7, 10, 14
Number of visits to the doctors	1, 2, 3
Loss of income ($) due to the illness	$154.70, $221.00, $309.40

[#]US$ (United States of America dollar where US$1 = 1,190 IQD) (https://www.mataf.net/)

According to the WHO (2018) the disability-adjusted life years (DALYs) for a disease or health condition are calculated as the sum of the years of life lost (YLL) due to premature mortality in the population and the years lost due to disability (YLD) for people living with the health condition or its consequences:

$$DALY = YLL + YLD$$

The YLL corresponds to the number of deaths multiplied by the standard life expectancy at the age at which death occurs and the basic formula for YLL is:

$$YLL = N \times L$$

Where: N = number of deaths, L = standard life expectancy at age of death in years.

To estimate YLD for a specific disease in a particular time period, the number of incident cases in that period is multiplied by the average duration of the disease and a weighting factor that reflects the severity of the disease on a scale from 0 (perfect health) to 1 (dead) (WHO 2018). The basic formula for YLD is:

$$YLD = I \times DW \times L$$

Where: I = number of incident cases, DW = disability weight, L = average duration of the case until remission or death (years).

DALYs were estimated by assuming that brucellosis was associated with a disability weight of 0.191 (95%CI 0.172 - 0.211) (Dean et al. 2012b) and an average duration of 4.5 years (Singh et al. 2018). As no deaths were reported in two comprehensive surveys carried out on human brucellosis in India (Mantur et al. 2006, Mantur et al. 2004), this study assumed that the disease did not cause death and was only responsible for causing disability in infected persons. Therefore, DALYs were solely estimated from the years lost due to disability (YLD).

To estimate the financial impact of brucellosis in humans in Kurdistan a model was developed using probability distribution functions for the input parameters (Table 6.1) in @Risk 7.5 student version (Palisade Decision Tools, Palisade Corporation) and run for 10,000 iterations. A sensitivity analysis (assessed through examination of the calculated regression coefficients) was performed to account for uncertainty in values, and to determine the economic impact of brucellosis in humans as well as the impact of the number of incidence cases on DALYs occurring due to brucellosis in humans in the Kurdistan region in 2014. This was achieved by using probability distributions for 6 input parameters for the cost of brucellosis in humans, and 3 input parameters for the number of DALYs. The Pert distribution was used for these parameters with data from the experts or literature used to specify the minimum, most likely and maximum values.

6.3 Results

6.3.1 Analysis of historical data on the incidence of brucellosis in humans in Iraq

According to the official records from the Ministry of Health, Iraqi Government, the average annual incidence of brucellosis in Iraq, based on presenting clinical symptoms/signs and laboratory confirmation, for the period 1988 to 2002 was 41.88 cases per 100,000 people (Table 6.2). The annual incidence of brucellosis reported in humans for each year between 1989 and 2002 were significantly higher than that reported for 1988. There were significant differences between years (overall P value < 0.0001) and the lowest annual incidence reported was 10.62 per 100,000 people in 1988 and the highest was 88.2 cases per 100,000 people in 1995 (Table 6.2).

In Table 6.3 the annual incidence of human brucellosis in the Kurdistan Region and other parts of Iraq for the period 2004 to 2008 are summarised. The average annual incidence over this five-year period was 54.11 per 100,000 people in Kurdistan which was significantly higher than the 17.82 per 100,000 people in the rest of Iraq (RR 3.0; 95%CI 1.76 - 5.11). The incidence was also significantly higher in the Kurdistan region compared to other areas of Iraq for every year data were available. The highest incidence in Kurdistan was 66.78 per 100,000 people in 2007. In contrast the highest incidence in the rest of Iraq was 21.82 per 100,000 people in 2005. The lowest incidence in Kurdistan was 37.85 per 100,000 people in 2006, compared with 13.44 per 100,000 people in 2007 for other parts of Iraq.

Table 6.2: The annual incidence (based on clinical and laboratory diagnosis) of brucellosis in humans from 1988 to 2002 in Iraq

Year*	Number of cases *	Human population*	Annual incidence per 100,000 people	Relative Risk (95%CI)
1988	1,892	17,814,801	10.62	1.00
1989	2,464	18,349,311	13.43	1.26 (1.19 - 1.34)
1990	2,819	18,899,860	14.92	1.40 (1.32 - 1.49)
1991	13,106	19,409,189	67.52	6.36 (6.06 - 6.67)
1992	14,546	19,932,244	72.98	6.87 (6.55 - 7.21)
1993	14,989	20,469,395	73.23	6.89 (6.57 - 7.23)
1994	15,476	21,021,022	73.62	6.93 (6.61 - 7.27)
1995	19,040	21,587,514	88.20	8.30 (7.92 - 8.71)
1996	7,531	22,249,812	33.85	3.19 (3.03 - 3.35)
1997	8,911	22,932,429	38.86	3.66 (3.48 - 3.84)
1998	5,305	23,635,988	22.44	2.11 (2.01 - 2.23)
1999	7,297	24,361,133	29.95	2.82 (2.68 - 2.97)
2000	8,030	25,108,525	31.98	3.01 (2.86 - 3.17)
2001	8,166	25,748,669	29.43	2.99 (2.84 - 3.14)
2002	7,189	26,405,133	27.23	2.56 (2.44 - 2.70)

* Salih (2010) who sourced the data from the Ministry of Health, Iraq

Table 6.3: The annual incidence of brucellosis per 100,000 people from 2004 to 2008 in Kurdistan compared with the rest of Iraq (Source: Ministry of Health, Iraqi Government)

Year	Kurdistan region	Iraq excluding the Kurdistan region	Relative Risk* (95%CI)
2004	64.40	20.66	3.05 (1.86 - 4.99)
2005	40.41	21.82	1.82 (1.08 - 3.06)
2006	37.85	16.66	2.24 (1.26 - 3.96)
2007	66.78	13.44	5.15 (2.86 - 9.33)
2008	61.11	16.53	3.50 (2.10 - 6.14)
Average	54.11	17.82	3.00 (1.76 - 5.11)

* Incidence in Kurdistan compared to rest of Iraq

The average annual incidence of brucellosis per 100,000 people for the study period 2009 to 2014 in four different provinces of Kurdistan was 36.74. The highest average incidence was 60.31 cases per 100,000 people in Sulaymani province and the lowest was 16.68 per 100,000 people in Dohuk province (Table 6.4).

Table 6.4: Annual incidence of human brucellosis (per 100,000 people) for the period 2009 to 2014 in four provinces of Kurdistan[#]

Years	**Provinces**				**Average**[*]
	Erbil	**Sulaymani**	**Kirkuk**	**Dohuk**	
2009	50.52	60.34	50.46	27.51	49.26
2010	40.96	74.80	44.18	35.72	51.29
2011	16.18	66.24	41.83	20.38	38.56
2012	12.67	54.78	20.31	11.13	29.37
2013	4.87	53.10	26.86	2.61	24.55
2014	28.68	52.57	20.98	2.71	29.77
Average (2009 to 2014)	25.13	60.03	35.19	16.17	36.74
Relative Risk (95%CI)	1.55 (1.31 - 1.85)	3.71 (3.18 - 4.34)	2.18 (1.84 - 2.58)	1.00	-

[#] Data from the Ministry of Health, KRG and Kirkuk Health Directorate
[*]Average determined using raw data to account for population differences

The annual incidence of human brucellosis in 2014 and the test seroprevalence in sheep and goats in different provinces in Kurdistan are summarised in Table 6.5. The highest incidence in humans in this year was 52.57 (95%CI 49.53 - 55.74) cases per 100,000 population in Sulaymani followed by 28.68 (95%CI 26.19 - 31.32) in Erbil province. Similarly, the highest test seroprevalence of brucellosis in both sheep and goats were in Sulaymani 5.67 (95%CI 3.34 - 8.92) and Erbil 5.11 (95%CI 3.27 - 7.57) respectively. The Pearson's correlation coefficient calculated (0.8) and its P value (0.2004) indicated that there was a strong positive

correlation, but not significant, between the prevalence in sheep and goats and the incidence of brucellosis in humans in Kurdistan in the study year.

Table 6.5: Incidence of human brucellosis (per 100,000 people) in 2014 compared with the seroprevalence to brucellosis in sheep and goats in four provinces of Kurdistan*

Provinces	Annual incidence per 100,000 people (95%CI)	Test seroprevalence in sheep and goats % (95%CI)
Erbil	28.68 (26.19 - 31.32)	5.11 (3.27 - 7.57)
Sulaymani	52.57 (49.53 - 55.74)	5.67 (3.34 - 8.92)
Kirkuk	20.98 (18.74 - 23.42)	3.5 (1.42 - 7.08)
Dohuk	2.71 (1.73 - 3.61)	4.0 (1.1 - 9.93)
Average	29.77 (28.47 - 31.12)	4.9 (3.67 - 6.39)

* Data from Ministry of Health, KRG and Kirkuk Health Directorate

6.3.2 Estimation of the financial burden of brucellosis to humans in Iraq

The median cost per patient diagnosed with brucellosis was estimated to be $321.78 (95%CI $259.53 to $388.72). This equated to a median total cost of brucellosis for all affected humans in the Iraqi Kurdistan region in 2014 of $627,565.41 (95%CI $508,934.35 to $760,727.11). The median annual disability adjusted life years (DALYs) due to human brucellosis in the Iraqi Kurdistan region in 2014 was estimated to be 27.17 (95%CI 15.81 - 42.65) per 100,000 people per year. As outlined in the materials and methods DALYs were estimated only using the number of years compromised due to disability (YLD).

The sensitivity analyses for the cost estimation indicated that the loss of income due to illness (regression coefficient = 0.85) was the most sensitive variable for estimating the total cost of brucellosis followed by the doctor's fee per visit (regression coefficient = 0.37). All other factors had minimal impact on the cost of human brucellosis in Kurdistan (low coefficients) (Figure 6.1). The number of DALYs per 100,000 population was most sensitive to uncertainty in the duration of the disease (regression coefficient = 0.99). Other than for disability weight (regression coefficient = 0.15) all other factors had a coefficient estimate of less than 0.1 (Figure 6.2).

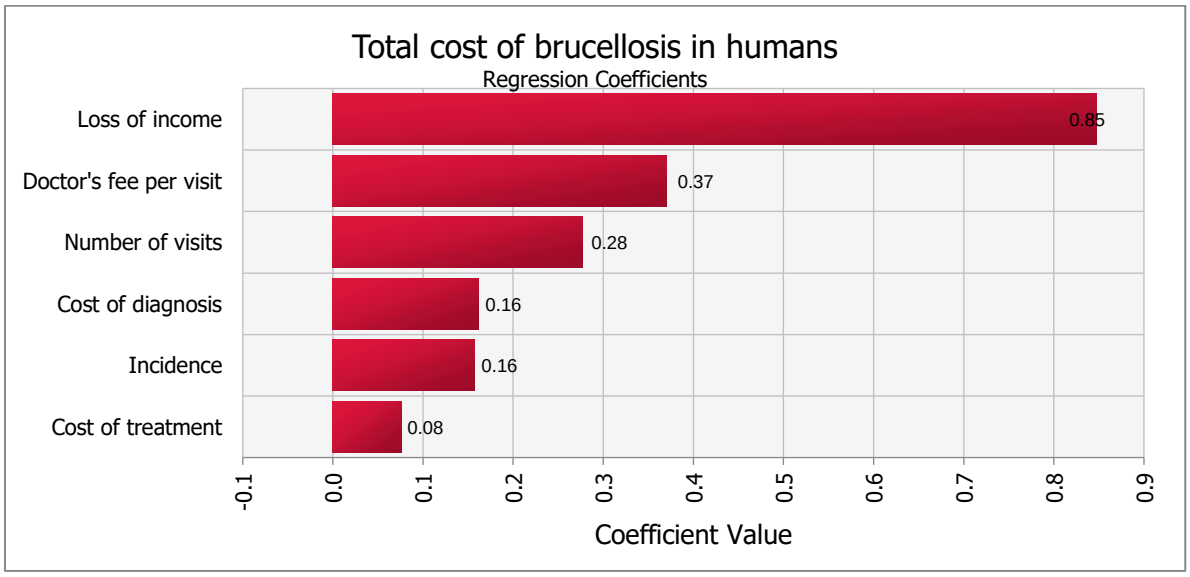

Figure 6.1: Correlation coefficients of the sensitivity analysis for the effect of input parameter values on the total cost of brucellosis in Kurdistan region in 2014

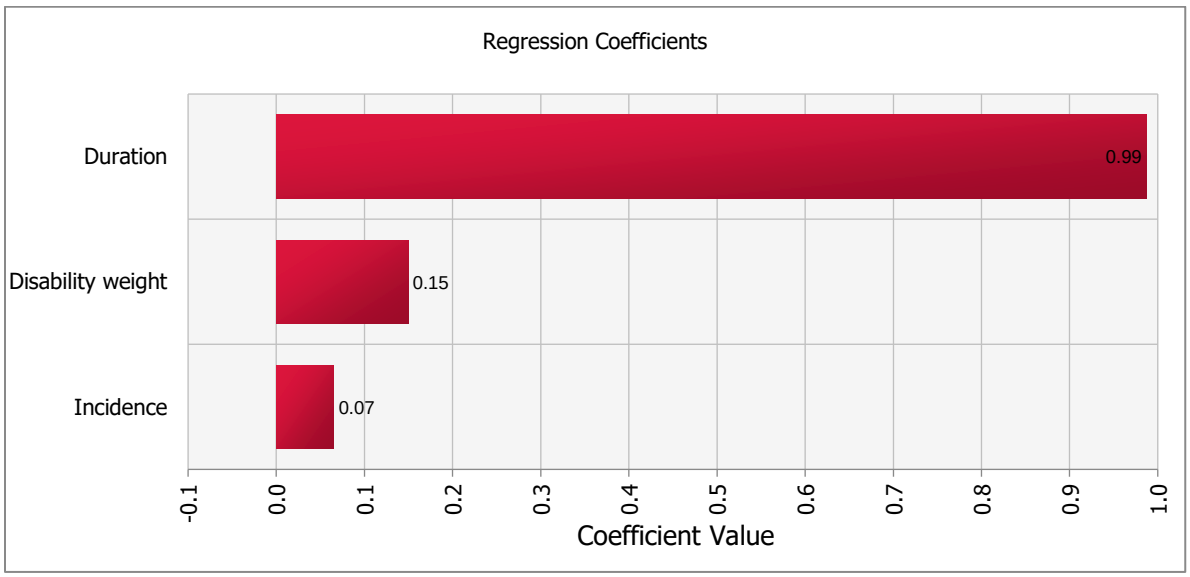

Figure 6.2: Correlation coefficients of the sensitivity analysis for the effect of input parameter values on the number of disability-adjusted life years in Kurdistan region in 2014

6.4 Discussion

In Iraq, brucellosis is a widespread disease of both humans and livestock, and is distributed throughout the country being present in all provinces, including the Iraqi Kurdistan Region (Tables 6.2, 6.3, 6.4, 3.2 and 3.4). The geographical location of Iraq and the Kurdistan Region, where long porous borders are shared with Syria, Iran and Turkey, significant conflicts and wars over the past forty years, the uncontrolled illegal movement of animals from Syria and Iran to Iraq generally and the Kurdistan Region specifically and insufficient preventive and control programs have all contributed to the increased risk and spread of brucellosis within the region (Jaff 2016). The incidence of brucellosis in humans can vary widely, not only between countries but also within countries. This suggests that occupational, demographic and socioeconomic factors play a significant role in infection (Dean et al. 2012a). Identifying these factors and the impact of disease on the population is important

when developing control programs for the disease and prioritizing allocation of scarce resources.

The current study presented data on brucellosis in humans sourced from the Ministry of Health, Iraqi Government for the period from 1988 to 2008. The average annual incidence in Iraq was 41.88 per 100,000 people with the highest incidence of 88.2 cases per 100,000 people in 1995 which was significantly higher than that reported in 1988 (8.30; 95%CI 7.92 - 8.71). The data from the Ministry of Health indicates that the average annual incidence of brucellosis in humans from 2004 to 2008 was on average 3 times (RR 3.0; 95%CI 1.76 - 5.11) higher in the Kurdistan Region than in the middle and southern regions of Iraq. This may be due to a real difference or potentially underreporting due to a weak public health system in the middle and southern regions of Iraq because of the war in 2003 and the subsequent civil war. These hostile activities may have led patients to prefer treatment at private clinics rather than public hospitals. Unfortunately only official records for analysis were available from the public health service and consequently the estimated incidence is likely to be an underestimate of the true situation in Kurdistan and Iraq. Such information is important to be able to allocate sufficient resources to control brucellosis in humans, and future access to data from private health services would help address this issue.

In the present study the highest average annual incidence of brucellosis in humans in the provinces of Kurdistan for the period 2009 to 2014 was in Sulaymani Province (60.03 per 100,000 people). This was not unexpected as the highest seroprevalence of sheep and goats reported in Chapter 3 of this study was also in this province and consequently the risk of

transmission would be expected to be higher here than in other provinces due to the association of contact with infected animals or their products and disease in humans (Kozukeev et al. 2006). This highlights the importance of controlling the disease in livestock to control the disease in humans (Godfroid et al. 2011).

To improve the understanding of the importance and impact of brucellosis in the Kurdistan region, an estimate of disease cost was calculated using data sourced from health sector experts in Iraq. The annual cost of brucellosis in humans in 2014 was estimated to be $627,565.41 (95%CI $508,934.35 - $760,727.11). Not surprisingly the cost of disease was most sensitive to uncertainty in the loss of income due to the illness and the doctor's fee per visit. This highlights the difficulty in making informed disease impact measurements in the absence of empirical data on disease frequency. The current study estimated a DALY of 27.17 (95%CI 15.81 - 42.65) per 100,000 persons per year. The results of this study are higher than the study conducted by Singh et al. (2018) who estimated the disease was responsible overall for 15 DALYs (95%CI 13 - 17) per 100,000 persons per year in India. In that study they assumed that the average duration of disease was six months, there were no mortalities due to the disease and a disability weight of 0.19. The differences in the calculated DALYs are likely to arise from differences in disease within the livestock population, cultural differences between the two populations, and different assumptions made for the average duration of disease in humans and sample size differences.

Unfortunately there were no available data recorded to enable a comparison of the incidence of brucellosis in humans residing in urban regions from those originating from rural areas.

However several previous studies conducted in this region (Jaff 2016, Rasul and Mansoor 2012, Shareef 2006), not surprisingly, have reported a higher incidence in humans from rural areas. This is likely to arise from the greater contact a rural population would have with animals and their products such as aborted placenta, which is recognised as a key risk factor for infection (Rasul and Mansoor 2012). Residents in rural areas are also more likely to be less literate and come from a lower socioeconomic background than urban residents which have been highlighted by Muhammad (2009) and Racloz et al. (2013) as risk factors for infection. Adoption of simple preventive measures, such as boiling milk prior to consumption, can have a significant impact on the exposure of people to the bacteria (Corbel et al. 2006). From the author's experience there also is a need to improve management practices by livestock owners as, anecdotally, some owners sell or slaughter diseased animals for human consumption, including animals which have aborted potentially from brucellosis.

Prevention of brucellosis in humans depends on the eradication or control of the disease in animal hosts (Corbel et al. 2006). To reduce and control the incidence of disease it is very important to apply a One Health Program principle involving aspects targeting the three components of human, animal and environment, to achieve the most effective outcome. This can be attained by veterinarians, public health authorities, farmers and consumers working together to increase awareness and education about the disease - its causes, routes of transmission and methods to avoid exposure to the pathogen. Because the rural health infrastructure is underdeveloped in Kurdistan, as well as in the rest of Iraq, there are insufficient community health workers in the region to control brucellosis with the primary focus of workers being on other, more common and potentially more infectious diseases of humans (Jaff 2016). However brucellosis in humans can be a debilitating disease and awareness of it is important in its control (WHO 2006) and critical to controlling the disease

in humans is effective control and preventive programs in livestock, including vaccination programs (Zinsstag et al. 2007).

The Middle East has traditionally been considered an endemic area for brucellosis with five of the ten countries with the highest incidence of brucellosis in humans in the world being located there (Pappas et al. 2006). This high incidence can be attributed to political instability and the loss of adequate animal and human health services as the region has been plagued by war, famine and poverty for many years. In addition, a predominantly rural population, which is centred on livestock, and the low general literacy level of the population resulting in a lack of adoption of public health interventions would also account for the high incidence of human brucellosis (Pappas and Memish 2007).

In conclusion, this chapter focused on the epidemiology of human brucellosis in Iraq generally and the Kurdistan region specially, from 1988 to 2014. The findings indicated that there was a high positive, although not significant correlation (0.8) between brucellosis in humans and sheep and goats in four provinces in Kurdistan and the disease was responsible for a significant cost to the individual and general public. Therefore adopting a mass vaccination control program in livestock is not only important in the control of brucellosis in animals but also would reduce the risk of infection in humans.

CHAPTER SEVEN

General Discussion

7.1 Introduction

Brucella melitensis, the first species in the genus *Brucella* to be described by Bruce in 1887, causes abortions in pregnant animals and Malta fever in humans. Brucellosis is consistently ranked among the most economically important zoonoses globally. It is a 'multiple burden' disease with economic impacts attributable to disease in livestock, humans and wildlife (McDermott et al. 2013, Seleem 2010, Corbel et al. 2006). The epidemiology and economic impact of brucellosis varies between locations and livestock systems adopted. In the Iraqi Kurdistan Region little was known about the distribution and impact of brucellosis in small ruminants prior to the current study. The study reported in this thesis was designed to further our understanding of the epidemiology of brucellosis in sheep, goats and humans in Kurdistan and involved four main components: undertaking a cross-sectional seroprevalence survey of sheep and goats to determine the distribution of infection within the region; administering a questionnaire to the owners/managers of sampled livestock to identify risk factors for infection at the individual and flock level; evaluating the economic impact of the disease in sheep and goats and the benefit of implementing a vaccination control programme; performing a retrospective study examining existing historical data to determine the distribution and frequency of brucellosis in humans and to estimate the costs arising from the disease in the Kurdistan population.

7.1 Seroprevalence

Brucellosis is included in the list of animal diseases requiring compulsory vaccination in Iraq and the Kurdistan region. The current vaccination program in the Kurdistan region involves vaccinating only lambs and kids from 3 to 6 months of age, and the annual report from Dohuk Veterinary Hospital in 2015 indicated that 60% of lambs and kids in Dohuk Province had been vaccinated. However, even with this level of coverage and government attention to brucellosis, the real prevalence in sheep and goats in 2015 was 5.8% (95%CI 4.5 - 7.4) (Chapter 3). Although this was lower than the 16.4% reported by Salih (2010) in sheep and goat flocks in Kirkuk province, the disease is still likely to be having an economic impact on productivity and the current level of vaccine coverage is unlikely to control an epidemic disease such as brucellosis. The differences between the results of these two surveys may have arisen from differences in: adoption of vaccination control programs across Iraq; management and husbandry practices between provinces; or sampling strategies.

This study found that the test seroprevalence was similar among sheep and goats and between males and females in the sampled provinces. These similarities are expected due to the similar traditional husbandry practices for handling both species and the similar management systems adopted between the sampled provinces. These findings were in agreement with other studies in Sulaymani and Nineveh provinces (Jabary and Al-Samarraee 2015, Al-Hankawe and Rhaymah 2012, Shareef 2006).

7.3 Economic assessment

Brucellosis in sheep and goats is considered to be one of the most economically important zoonotic diseases due to its direct and indirect impact on both livestock and humans (Seleem 2010). Many countries have conducted economic impact evaluations on the benefit of controlling this disease (Santos et al. 2013); however it is difficult to accurately assess the economic losses arising from this disease in the Kurdistan region specifically, and in all of Iraq generally, due to a lack of available data. In this study the economic impact of the disease in sheep and goats was investigated focusing on abortions and reduced milk production to explore the determinants of successful and sustainable control strategies within Kurdistan.

This study (Chapter 5) found that the proposed mass vaccination program of sheep and/or goats over a 10-year period was economically viable (NPV > 0 and BCR > 1). The NPV and BCR would have been even higher if data were available regarding the number of still births and cases of orchitis and epididymitis, and lengthened inter-lambing/kidding interval. The results of this study are in agreement with the study conducted by Roth et al. (2003) regarding a positive return from controlling brucellosis in livestock. The estimated cost in this study for the proposed small ruminant brucellosis control program in the Kurdistan Region of Iraq was US$7.18 million over the 10-year period. The sensitivity analysis in this study indicated that the abortion rate followed by the average price of one lamb or kid had the largest effects on the outcome, and the results agreed with the study undertaken by Seleem (2010) which reported that the most significant economic losses from brucellosis arise from abortions and

consequently the size of the reduction in the abortion rate would be expected to have a major impact on the economic benefit arising from disease control.

The findings from the model used in Chapter 5 predicted that annual whole flock vaccination was a technically effective vaccination strategy and economically viable in small ruminants and the NPV and BCR increased with increasing protection level (vaccine coverage and efficacy of vaccine). However, this control is dependent upon supply of sufficient doses of vaccine to vaccinate approximately 4.5 million sheep and goats each year, which will require support from the national and provincial Governments.

7.2 Risk factors for infection

Identifying and understanding risk factors for a disease are critical for the implementation of effective disease control programs (Porphyre et al. 2010). In general, the risk factors for zoonotic disease transmission are well documented in many parts of the world (John et al. 2010, Busch and Parker 1972). The challenge is to find mitigation strategies that are easy to implement and which simultaneously enable livestock owners to benefit from disease control, whilst ensuring the good health of farmers and their families along with workers in high risk occupations and consumers (Schelling et al. 2007). This study is believed to be the first study to investigate the specific risk factors associated with brucellosis in small ruminants in the Kurdistan region.

The final logistic regression model indicated that sheep and goats flocks which introduced new animals in the 12 month period preceding the survey (OR = 4.2, 95%CI 1.0 - 17.3; OR = 15.2, 95%CI 3.0 - 76.4, respectively) were more likely to be seropositive than those that didn't. Similar findings have been reported in other areas such as Oman, Niger and Uganda (Al-Rawahi 2015, Boukary et al. 2013, Kabagambe 2001, Crawford et al. 1990). It is well known that introducing new animals to flocks/herds, increases the risk of introducing infected animals (Dalrymple 1993), particularly if they are purchased from livestock traders/dealers. Therefore, it is important that all animals are tested with a test or tests of high sensitivity prior to introduction, or introduced animals only originate from a known brucellosis-free flock. Furthermore movement of animals from infected flocks should be restricted to sites such as meat works and sale to other farms/flocks should ideally be prohibited. Furthermore improved hygiene, such as washing hands before smoking or eating, and wearing personal protective equipment, such as gloves, masks and protective eyewear, should be encouraged within all flocks but particularly infected flocks as part of an educational campaign within a control program (Musallam et al. 2015, Robinson 2003). The FAO, OIE and WHO have made specific biosecurity recommendations for reducing the spread of brucellosis within flocks and flocks and to humans in contact with infected flocks. These include isolating individual animals during parturition, appropriate disposal of waste products, disinfecting pens and enclosures, and wearing personal protective equipment that is changed between handling individual animals (Corbel et al. 2006).

The logistic-regression analysis highlighted an association of the source of water with the presence of disease (farmers who sourced water from wells were significantly less likely to have seropositive flocks). Direct contact with infected animals and contact with a

contaminated environment are the main reasons for disease transmission between animal flocks/herds (Shehada and Abu Halaweh 2013, Al-Majali et al. 2009). Using water sourced from private wells would decrease the likelihood of mixing of flocks, as well as potentially decreasing the likelihood of contamination of the water. Privately owned water sources are not only important to decrease the likelihood of a flock acquiring brucellosis but also potentially have economic benefits for farmers by decreasing the presence of other diseases such as rabies and FMD by reducing potential contact with other animals, vectors and wildlife. In areas such as Iraq, where cattle, sheep and goats are often grazed together, disease transmission between livestock species is likely to be one of the key drivers of disease spread and attempts should be made to minimise these contacts between animals of different herds/flocks/disease statuses.

This study found that the farmers who had vaccinated their goats (Rev-1) in the preceding 12 months were significantly less likely to have a seropositive flock (OR: 0.31, 95%CI 0.12 - 0.75). These findings concur with those obtained by Ganter (2015) who reported the role of vaccination in preventing brucellosis in sheep and goats. Accordingly, while vaccination should be the cornerstone of the control effort, it should be accompanied by measures to facilitate and promote the adoption of good hygiene and husbandry practices that minimise the risks of introduction and maintenance of *Brucella* spp. as well as the risk of human infection. However in the 2014 annual report from the Directorate of Dohuk Veterinary DDV (2015) it was documented that only 60% of the lambs and kids between the ages of 3 to 6 months had been vaccinated. It is important that this percentage is increased to at least 80% to allow development of an effective level of herd immunity.

7.4 Brucellosis in humans

The results of the study on human brucellosis indicated that the median cost per patient diagnosed with brucellosis was estimated to be $321.78 (95%CI $259.53 - $388.72) with an annual estimated cost of brucellosis for humans in 2014 of $627,565.41 (95%CI $508,934.35 - $760,727.11) (Chapter 6). Other studies have demonstrated that brucellosis can result in large economic losses which are borne primarily by people involved in the livestock industries, however they do also affect the general community (Singh et al. 2015, Santos et al. 2013).

This study found that there was a high positive correlation (0.8) between brucellosis in humans and animals in the sampled provinces. Zhu (2013) also highlighted that brucellosis in humans is always associated with infected animals or products from those animals, and effective control of the disease in animals will not only reduce the incidence in livestock with benefits to the farming community, but will also result in a lower incidence in humans, benefiting the total community. The current study examined data on the number of cases of human brucellosis from Kirkuk Health Directorate and the Ministry of Health in KRG for the period from 2009 to 2014 and the results indicated that the highest average annual incidence of brucellosis in humans was in Sulaymani Province (60.0 per 100,000 people). This finding was expected as the highest seroprevalence in animals in this study (Chapter 3) was also in Sulaymani Province. Therefore, giving priority or commencing an expanded vaccination programme in this province (or other provinces in Iraq with a high prevalence) will benefit both animals and humans.

In the historical data examined for human brucellosis sourced from the Ministry of Health, Iraqi Government for the period from 1988 to 2002, there were significant differences between years with the highest incidence of 88.2 cases per 100,000 people observed in 1995. This finding is probably associated with the United Nations sanctions on Iraq resulting in insufficient medical support at this time. According to the data from the Ministry of Health, Iraqi Government for the period from 2004 to 2008, the average annual incidence of brucellosis in humans (RR 3.0; 95%CI 1.8 - 5.1) was significantly higher in the Kurdistan Region than in the rest of Iraq. This is likely to be influenced by the fact that official records were only available for the public health services, and as a result of the war in 2003 and the subsequent civil war the public health system in the middle and southern regions of Iraq were weak. Therefore, the estimated incidences of brucellosis in humans in all parts of Iraq are likely to be underestimates of the true situation. Further studies are needed to assess the true incidence (including data from public and the private health sectors) of human cases in Iraq.

Human brucellosis incidence data were only available from officially recorded cases from the Ministry of Health in KRG and the Iraqi Government, which may not reflect the true incidence in Iraq. In Mongolia, Zolzaya et al. (2014) conducted a seroprevalence study in humans and when they compared their results to official data, they found that the level of under-reporting to be 15-fold. Another study conducted in Kyrgyzstan estimated the true incidence to be up to 5.6 times higher than that officially recorded (Bonfoh et al. 2012). Therefore, the estimated DALY and the annual cost of brucellosis in humans in Iraq most likely are significantly under-estimated as well.

7.5 Limitations of the present study

Although the epidemiological study reported in this thesis produced several important findings, there were limitations associated with the study. Restricting the ability to accurately measure the impact of brucellosis is the scarcity of data on the livestock productivity losses attributable to the disease. Consequently for some parameters estimates from other countries or expert opinions were used, these parameters may under or overestimate the local values. These estimates potentially could have impacted upon the economic evaluation of the disease and hence the benefits arising from controlling brucellosis (Chapter 5). Although the questionnaires used in the current study played an important role in the methodology of the epidemiological study and were used for: identification of the risk factors of the disease and the economic evaluation of the disease, several factors, such as the current government policy and reluctant interviewees, also could have impacted upon the results. For example, the authorities have a policy of supporting farmers by supplying animal feed at a discounted price. This may have resulted in farmers concealing the true number (overestimating) of livestock, which could potentially bias the results. With respect to the study on brucellosis in humans, a major limitation with using the retrospective data was a lack of data from private clinics and consequently the estimated incidence is likely to be an underestimate of the true situation in Kurdistan and Iraq. Future access to data from private health services would help address this issue.

Diagnosis of brucellosis is usually made by antigen detection or through serological assays, however no test is 100% accurate (sensitive or specific) (Nielsen and Yu 2010). In the current study two tests have been used. All samples were tested with the RBT as a screening test and only samples positive on the RBT, plus an equal number of negative samples from the same locations, were retested with an ELISA to confirm the animal's positivity. For the RBT, the agglutination intensity is affected by many factors including the amount of antigen, the temperature at which the test is run, the duration between adding the antigen and reading (interpreting the result), the experience and visual acuity of the test interpreter and potential cross-reactions with LPS of other bacteria (Cho et al. 2010). Potential false positive results due to vaccination (Munoz et al. 2005) could also have impacted upon the results. Finally, isolating (culturing) and identifying the infecting *Brucella* species and biotype was not undertaken in the current study because of time, logistical and funding constraints.

7.6 Recommendations

Control measures for diseases, including brucellosis, should be based on sound epidemiological reasoning and it is unlikely that one specific program is suitable for all regions or countries. To plan and implement a program, several considerations for each situation (country or region) have to be undertaken. Firstly it is important to specify whether the goal of the program is the control or eradication of the disease and this is influenced by the financial situation within the country and the impact of the disease. Secondly, it is critical to have accurate information about the factors associated with the disease (Al-Rawahi 2015). These factors include the location and environment where the disease is distributed, the

animal population and the management and husbandry practices adopted, the expected prevalence, the culture of the owners and their willingness to cooperate in a control program. Once the situation with respect to the disease is known, the country may go for a specific strategy or a combination of strategies to control the disease. Ideally, permanent identification of animals and screening of animals with a suitable test, such as the RBT and/or ELISA, is recommended before selecting a specific control program to implement (Chen et al. 2016, Senein and Abdelgadir 2012, Seleem 2010).

Many countries free from *Brucella* have a strong quarantine and border control system, a policy of slaughtering all infected and in-contact susceptible animals, as well as imposing strict restrictions on the movement of animals and vehicles from and around potentially infected premises. After slaughter the carcasses are either burnt or buried on the infected premises, the buildings thoroughly washed and disinfected, movement restrictions applied and the affected farms quarantined. In situations where the prevalence is low, a test and slaughter program could be adopted using the simple, rapid and inexpensive RBT with the positive results confirmed by another more specific test, such as an ELISA. However, the same process may not be ideal in populations with a high prevalence or in a country with a low prevalence but with limited financial resources. Therefore, a confirmatory process using more accurate tests (multiple tests) and different controlling methods has been adopted in many countries (Nielsen and Yu 2010).

Although brucellosis is one of the most common zoonotic diseases (Seleem 2010), many people still lack an awareness of the disease. If farmers had a better awareness of the risks of infection and the impact of the disease on their livestock and families, they would be more likely to take measures both to reduce transmission of the disease in their livestock and to minimise cross-species transmission to themselves and their families. Epidemiological analysis of health, disease and disability in the populations of most developed countries confirms the role of social, economic and environmental factors in determining increased risk of disease and adverse outcomes from disease (Harris et al. 1999). In order to increase awareness and encourage the adoption of better healthy behaviours, the government should conduct annual public education programs on the disease in schools and communities in rural areas. Information should be provided on the clinical signs, transmission routes and preventive and control measures for the disease. The educational message may be provided through various routes including television, radio broadcasts, warning signs, posters and newspapers (Chen et al. 2016).

A policy of test and slaughter of all infected and in-contact suspected infected animals cannot be adopted in Kurdistan at this stage because of the endemic nature of the disease and the lack of suitable compensation for farmers. Consequently vaccination is considered the best way to initially control the disease in the region as the vaccine, Rev-1, is efficacious in adults, as well as animals 3 to 6 months of age, and induces a high and durable immune response (Blasco 1997, Alton and Elberg 1967). Compulsory mass vaccination, free voluntary testing, development of farm and an individual animal identification system and control of the illegal movement of animals from neighbouring countries potentially would reduce brucellosis in both humans and livestock. Despite the limitations of this study, the results of this analysis

suggest that mass vaccination with Rev-1 reduced overall costs and is potentially effective in reducing sheep and goat, as well as human, brucellosis costs. A well-operating surveillance system that incorporates current data collected from the field is required to control brucellosis (Robinson 2003). The main purpose of a surveillance system is to determine the need for immediate or long-term action in response to diseases and to provide information to optimise the use of the available resources through data analysis, determination of priorities, design of alternative actions, and determination of their likely costs and benefits (Corbel et al. 2006). Finally, a stronger border control system is important to prevent illegal movement of animals to Iraq which may impact upon any brucellosis control program. Subsequently a policy of test and slaughter of all infected animals could then be adopted in Kurdistan.

7.7 Future research

In order to obtain a better understanding of the epidemiological patterns of the disease in animals (sheep, goats and cattle) and to determine if sheep and goats are a spill-over host or a true reservoir, isolation and identification of the infecting *Brucella* spp. from sheep, goats and cattle should be further explored in Iraq. Because *Brucella* survives intracellularly in lymph nodes and mammary glands of ruminants after abortion, samples for identification should be taken from the lymph nodes of infected animals or from aborted foetuses (OIE 2009). This research could provide information regarding the species and type of *Brucella* that is circulating in animals within Kurdistan especially and Iraq generally. More importantly, research could help to identify the most effective vaccines, as not all vaccines have the same efficacy against the various *Brucella* spp. (Adone and Pasquali 2013).

Many studies on the economic costs of brucellosis and its control have been carried out in developed (high-income) countries (such as the USA), however there is little information from developing (low-income and middle-income) countries (McDermott et al. 2013) such as Iraq. Due to different feed, animal husbandry and management practices, animal species present and veterinary and medical capacities, the economic impact of brucellosis could vary between species, regions and countries (Godfroid et al. 2013, Corbel et al. 2006, McDermott and Arimi 2002). Therefore, it is important to conduct further economic research into the effect of the disease on livestock in Kurdistan and other parts of Iraq. The results of such studies (amount of milk production in local goats per year, abortion rate due to brucellosis in local goats, number of cases of still births due to brucellosis in sheep and local goats) would allow a more accurate evaluation of the economic impact of the disease to be determined. This would provide direction for design and implementation of more effective control measures of the disease in the long term.

Brucellosis affects a variety of terrestrial animals and wildlife species (Seleem 2010, Godfroid 2002) and occasionally disease can spill-over from wildlife to livestock and vice-versa (Godfroid 2002). In Kurdistan there are thousands of wild deer and it is possible that frequent contact between wildlife species and livestock, in particular sheep and goats, will increase the risks of infection in the livestock. Consequently wildlife control may be required when the prevalence in livestock is low. In addition, infected placental material on pastures can be dispersed by dogs and other carnivorous animals, such as foxes, potentially resulting in exposure of *Brucella* to other susceptible livestock (EC 2001). Therefore, correct disposal of placental material through burning or deep burial is important to reduce environmental contamination and exposure of other animals within the infected flock (Deddefo et al. 2015).

In order to understand if wildlife species have been infected, and if there is a spill-over of infection from domestic animals to wild species or vice-versa, multidisciplinary research is required to study both domestic and wildlife species. If local wildlife are infected, it would increase the risk of the transmission of *Brucella* to domestic livestock and contribute to the persistence of brucellosis within the region. However, controlling brucellosis in wildlife reservoirs is complicated and costly (Olsen 2010). Conversely wildlife species could be exposed to the bacteria shed by infected livestock, so implementing a control program in livestock could minimise transmission to wildlife species and prevent the establishment of a potential wildlife reservoir of infection.

7.8 Conclusions

In conclusion, this study focused on expanding information on the epidemiology of brucellosis in sheep, goats and humans in the Kurdistan region. Based on the findings, it is recommended that in order to effectively control the disease resulting in improved income for local farmers and less disease in the human population, an integrated approach should be implemented including adopting risk-based control measures, mass vaccination and education. These control measures would result in significant benefits to the economy as well as to public health. The evidence gathered and results presented in the thesis highlight how epidemiological, economic, social and political factors can influence the impact of brucellosis within a specific context.

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APPENDIX 1:

Epidemiology of Brucellosis in Sheep, Goats and Humans in Iraqi Kurdistan Region

Questionnaire

Province: District: Sub district:

Village: Record number: 1 Date:

1- How many animals are in your herd? ( )

2- Type and number of your animals?

	Number of males	Number of females
Sheep ≤ 6 months		
Sheep > 6 months		
Goats ≤ 6 months		
Goats > 6 months		

Other animals Yes No Type Number

3- Are your sheep and goats grazed outside your farm?

Yes ( ) No ( )

4- Are your sheep and goats grazed together or do they graze separately?

Together ( ) Separately ( )

5- Are your sheep and goats grazed with other flocks of sheep or goats?

Yes ( ) No ( )

6- If Yes – Approximately how many flocks do your flock graze with? ( )

7- If Yes - How long does your flock graze with other flocks each day? ( ).

8- Approximately how long does your flock graze for, in total, each day? ( ).

9- Approximately how many lambs and kids have been produced over the last 12 months?

Sheep: Male ( ) Female ( ) Unsure: ( ).

Goats: Male ( ) Female ( ) Unsure: ( ).

10- Did you have any abortions in your sheep during the last 12 months?

No: ( )Not Sure: ( )Yes: ( )If yes how many ( ).

11- If you had abortions in your sheep approximately at what stage (month) did the abortions occur?( ) month.

12- Did you have any abortions in your goats during the last 12 months?

No: ( )Not Sure: ( )Yes: ( )If yes how many ( ).

13- If you had abortions in your goats approximately at what stage (month) did the abortions occur?( ) month.

14- Did all the abortions result in the birth of dead foetuses or did some survive for a period of time?

All died ( ) Some survived ( )

15- How did you dispose of the aborted foetuses?

Burnt ( ) Gave to dogs ( ) Threw away ( ) Other please specify ().

16- Have you sold any sheep from your flock during the last year?

No ( ) Yes ( )If yes, how many? ( ).

17- Have you sold any goats from your flock during the last year?

No ( ) Yes ( )If yes, how many? ( ).

18- Have you purchased any sheep for your flock during the last year?

No ( ) Yes ( ) If yes, how many? ( ).

19- If yes where did they come from?

Same village ( ) Different village same sub-district ( )

Different village different sub-district ( )

Different village different district ( ).

20- Have you purchased any goats for your flock during the last year?

No ( ) Yes ( ) If yes, how many? ( ).

21- If yes where did they come from?

Same village ( ) Different village same sub-district ( )

Different village different sub-district ( )

Different village different district ( ).

22- Approximately how much did you spend on food last year for your sheep? ( ).

23- Approximately how much did you spend on food last year for your goats? ( ).

24- Approximately how much did you spend on the treatment of sick sheep in the past year? ( ).

25- Approximately how much did you spend on the treatment of sick goats in the past year? ( )

26- What was the main disease/condition you treated your sheep for?

( ).

27- What was the main disease/condition you treated your goats for?

( ).

28- What is the source of water for your sheep and goats?

River ( ) Well ( ) Spring ( ) Other please specify ( )

29- Do your sheep and goats share these water sources with sheep and goats from other farms? Yes ( ) No ( ) Not sure ( )

30- Do you use electricity on your farm?

Yes ( ) No ( )

31- Approximately how much did you spend on electricity for your sheep over the last 6 months? ( ) Not sure ( )

32- Approximately how much did you spend on electricity for your goats over the last 6 months? ( ) Not sure ( )

33- Are there any paid agricultural workers on your farm?

No ( ) Yes ( ) If yes how many ( )

34- Approximately what is the annual cost of these workers?

Cost ( ) Not sure ( )

35- Were your sheep vaccinated against brucellosis during the last year?

No ( ) Not sure ( ) Yes ( ).

If yes how many times was it vaccinated in the last 12 months? ( )

If yes – In what month were they last vaccinated? ( ).

36- Were your goats vaccinated against brucellosis during the last year?

No ( )Not sure ( ) Yes ( ).

If yes how many times was it vaccinated in the last 12 months? ( )

If yes – In what month were they last vaccinated? ( ).

37- What do you think have been the three most important health problems in your sheep over the last 12 months? ( ).

38- What do you think have been the three most important health problems in your goats over the last 12 months? ( ).

39- Do you know if your sheep are infected with *Brucella*?

Yes ( )No ( ) Don't know ( ).

40- If yes how do you know your sheep are infected? (

).

41- Do you know if your goats are infected with *Brucella*?

Yes ( )No ( ) Don't know ( ).

42- If yes how do you know your goats are infected? (

).

43- Has brucellosis ever been diagnosed in your sheep or goat flock?

Yes ( ) No ( ) Not sure ( ).

44- If yes how many sheep and goats were infected?

Sheep: ( ) Goats: ( ) Don't know ( ).

45- How much did you spend over the past year on your sheep to treat them for brucellosis? ( ).

46- How much did you spend over the past year on your goats to treat them for brucellosis? ( ).

47- How much did you earn from milk, wool & hair sales in the last 12 months from your sheep and goats?

Income ( ) Not sure ( ).

Thank you for taking the time to answer these questions. The results from this survey will help further our understanding of brucellosis in sheep and goats in Kurdistan.