INFECTIOUS ABORTION AND ASSOCIATED RISK FACTORS IN DAIRY CATTLE FARMS IN NAKURU DISTRICT, KENYA

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other university

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DEDICATION

To

My wife Damaris and my family, The Okumu's

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LIST OF ABBREVIATIONS

AEZ - Agroecological zone

BA – *Brucella abortus*

BPAT – Buffered plate agglutination test

BDV – Border disease virus

BVD – Bovine viral diarrhoea

BVDV – Bovine viral diarrhoea virus

CSFV – Classical swine fever virus

EAAPP –East African agricultural productivity project

EED – Early embryonic death

ELISA – Enzyme linked immunosorbent assay

FPA – Fluorescence polymerisation assay

IFAT – Indirect fluorescence antibody test

IFS – International foundation for science

NC – *Neospora caninum*

NDDP – Nakuru District development plan

NVIL – Nakuru veterinary investigations laboratory

OIE – World organization for animal health

OR – Odds ratio

RBT – Rose Bengal plate test

SAT – Serum agglutination test

TMB - Tetramethylbenzidine

US – United States

VN – Virus neutralization

ABSTRACT

Agriculture plays a significant role in the Kenyan economy. According to estimates, 15% of the total farm revenue in Kenya is generated from livestock products, of which 3.5% is from the dairy sector. Several factors, however, hamper productivity of the sector. These include feeds, husbandry, marketing and animal diseases among others. Reproductive diseases limit the dairy industry from achieving optimum efficiency. Some of the diseases that cause major reproductive wastage, including abortion, in the dairy industry in many parts of the world include leptospirosis, campylobacteriosis, bovine viral diarrhoea, brucellosis and neosporosis.

Typically, the diagnostic rate for cases of abortion in the livestock sector is low. In Kenya, Leptospirosis, Campylobacteriosis and Brucellosis have been confirmed as causes of abortion in dairy cattle. However, there is a paucity of information on other pathogens such as Bovine viral diarrhoea virus (BVDV) and *Neospora caninum* (NC), and their interrelationships as causes of abortion, yet these have a major role on reproductive wastage globally. Additionally, there is no comprehensive study that has been carried out on the causes of abortion in Kenyan dairy cattle herds.

This study was therefore designed to investigate the infectious causes of abortion and associated risk factors in dairy cattle in Nakuru District, a major dairying area in Kenya. Across-sectional study was carried out to determine: 1) management practices in dairy cattle farms in Nakuru; 2) prevalence of antibodies against Bovine Viral diarrhoea Virus (BVDV), *Brucella abortus* (BA) and *Neospora caninum* (NC) in cattle; and 3)

prevalence of NC in farm dogs in the selected farms. A prospective epidemiological study was then undertaken to investigate the effects of BVDV, BA and NC on the occurrence of bovine abortion in the dairy cattle herds. A questionnaire was also administered to animal health service providers in Nakuru District to determine their knowledge, attitude and practices on bovine abortion.

In the 398 tested cattle on the 64 dairy herds, the prevalence of antibodies to BVDV, NC and BA was 79.1, 25.6 and 16.8%, respectively. Of the cattle seropositive to NC, 83.3% were also seropositive to BVDV (OR =1.4) and 13.7% to BA (OR = 0.7). Of the cattle seropositive to BVDV, 17.1% were also seropositive to BA (OR = 1.1). The seroprevalence of NC in 84 tested farm dogs on 53 dairy farms was 17.9%, and lack of confinement was associated with its occurrence (OR =4.5, $P<0.05$).

The prospective study revealed reproductive wastage in 260 monitored pregnant dairy cattle on the 64 dairy farms, with an incidence of 11.1% (28/260) for abortion, while the incidence of other foetal losses was 1.1% (3/260) The incidence rates of the NC, BVD and BA in this study was 1.1, 0.06 and 0.5 new infections/100 cow months at risk respectively. The foetal losses were observed in animals less than 96 months old, and occurred in mid-gestation. *Neospora caninum* was associated with most cases (29.0%) of foetal losses, followed by mixed infections of NC and BVDV (12.9%), BVDV (9.9%) and co-infections of BA and NC (6.5%). Age of the dam was the only factor significantly associated (negatively) with foetal loss in the present study ($p<0.05$).

The factors that animal health service providers considered associated with the occurrence of dairy cattle abortion were: infections (77.2%), malnutrition (40.9%), inclement weather (37.8%) and intoxication (4.5%). The infectious diseases that they considered associated with the occurrence of bovine abortion were: brucellosis (92.4%), Rift Valley fever (43.9%), leptospirosis (27.3%), toxoplasmosis (9.1%), infectious bovine rhinotracheitis (6.0%), trichomoniasis (4.5%), bovine viral diarrhoea virus (3.0 %), fungal and yeast infections (3.0%) and neosporosis (1.5%).

The present study confirmed the occurrence of all three abortifacient pathogens in the study area, and it is the first study to document the presence of NC in dairy cattle in Kenya. Although animal health providers perceived BA to be the pathogen most likely to be the cause of bovine abortion, BVDV was the abortifacient agent found to have the highest antibody prevalence, and NC was the pathogen most associated with cases of abortion in the present study. The high prevalence of BVDV and the high frequency of abortions associated with NC seropositivity may have been due to the lack of adequate control measures such as vaccination, screening and isolation of new introductions and improved biosecurity for these two diseases; this was probably due to lack of awareness of the presence of the conditions in dairy cattle in Kenya and consequently low levels of knowledge by the animal health industry players on the impact of these diseases.

The knowledge of the animal health providers needs to be updated through training to make them aware of the disease trends in their areas of practice since they are in the frontline of disease control measures. In addition, a policy on the control of

abortifacient pathogens should be developed in order to reduce the losses associated with these infections.

CHAPTER ONE

1. GENERAL INTRODUCTION

The Kenyan economy, like in many other developing countries, is based on agriculture which supports approximately 80% of the rural labour force and is practiced mainly at a small-scale level. Small-scale farmers mainly produce for subsistence but in the event of surpluses, the excess sold thus contributing to wealth and thus improving the farmer's quality of life. Large-scale farms on the other hand are run commercially and almost all the produce is sold for profit (Wilson *et al*., 2005).

Estimates have shown that 15% of the total farm revenue in Kenya is generated from livestock products, of which 3.5% is contributed by the dairy industry. Kenya is also considered as having one of the most developed dairy sectors in Sub-Saharan Africa (EAAPP, 2010). The main products are milk, milk products (butter, cheese and yoghurt among others), replacement heifers, meat and hides, among others. Through the years, there has been improvement of the dairy breeds by the upgrading of local Zebu cattle breeds with exotic dairy cattle breeds through artificial insemination, importation of exotic bulls and the importation of high-producing dairy cattle. These genetic improvements have led to the country having over 5 million improved dairy cattle, the largest population in the Eastern and Southern Africa region, making the country self-sufficient in dairy products. Smallholder dairy farms contribute 60% to 90% of the total milk produced, even though individual cow productivity is low (Goldson and Ndeda, 1985; Walshe, 1987; Mbogoh, 1985; Omore *et al*., 1994).

Several factors have hampered the productivity, growth and development of the dairy industry namely feeds and feeding, husbandry, marketing and animal diseases (Muriuki, 2004). Among the reproductive disorders, problems such as abortion, metritis, stillbirths and infertility have continued to impact negatively on the dairy sector (Agumbah, 1977; Abuom, 2006). Many infectious diseases have been associated with abortion in dairy cattle. These include bovine viral diarrhoea, brucellosis and neosporosis, among others (Radostits *et al*., 1994; Carpenter *et al*., 2006; Murray, 2006; Stahl *et al*., 2006; Nuotio *et al*., 2007). Factors reported to increase the exposure of dairy cattle to abortifacient pathogens include the geographical location, environmental factors such as exposure to farm dogs, management factors such as crowding, and use of natural mating, among others (Radostits *et al*., 1994; Faye *et al*., 2005; Konnai *et al*., 2008; Mekonen *et al*., 2010). However, such information is limited in Kenyan dairy herds. Elsewhere, each case of abortion in dairy cattle has been estimated to lead to losses of around US $500 to $900 (Thurmond *et al*., 1990; Carpenter *et al.*, 2006). Bovine viral diarrhoea, brucellosis and neosporosis are the diseases that play a major role in reproductive wastage in dairy herds (Carpenter *et al.*, 2006*;* Konnai *et al.*, 2008; Mekonen *et al.*, 2010; Yang *et al*., 2012). Epidemiologic and economic data are therefore required to provide evidence on the importance of these reproductive diseases to the dairy industry in Kenya. The evidence can inform policy and interventions aimed at reducing the impacts of the diseases. The present study was designed to investigate the infectious causes of abortion and associated risk factors in dairy cattle farms in Nakuru District, a primary dairy production area in Kenya.

CHAPTER TWO

2. LITERATURE REVIEW

Abortion is the termination of pregnancy at a stage where the expelled foetus is of recognizable size ranging from 45 to 260 days of gestation (in this study) and not viable (Roberts, 1986; Hafez and Hafez, 2000). Abortion is important to the dairy industry because it disrupts the scheduled lactation and replacement of heifers and cows. It also leads to culling, thus adversely affecting herd genetics, all of which lead to reduced farm revenue. In addition, abortion storms can be psychologically catastrophic to the farmer. Some diseases that cause abortion in cattle, such as brucellosis, are also zoonotic (Carpenter *et al*., 2006; De Vries, 2006).

The aetiology of abortion is diverse and several risk factors have been reported including genetic, environmental (nutrition, temperature extremes and toxins, among others), management (crowding and use of natural mating), geographical factors and infectious factors, with infections contributing up to 90% of the abortions (Radostits *et al.*, 1994; Faye *et al*., 2005; Konnai *et al.* 2008; Mekonen *et al*., 2010). The important infectious agents that have been reported to cause abortion in cattle can be viral (bovine viral diarrhoea virus (BVDV), infectious bovine rhinotracheitis virus (IBRV) and Rift Valley fever virus), bacterial (*Brucella abortus*, *Campylobacter fetus*, *Salmonella* spp., *Escherichia coli*, *Leptospira* spp.), Chlamydiae (*Chlamydophila abortus*), protozoa (*Neospora caninum* and *Tritrichomonas foetus*) as well as several fungal species (*Absidia* spp *and Aspergillus* spp.) among others (Radostits *et al.*, 1994; Carpenter *et al*., 2006; Murray, 2006; Stahl *et al*., 2006; Nuotio *et al.*, 2007). General principles in the

diagnosis of bovine abortion include the collection of a complete history of the case and relevant epidemiological data, such as recent introductions into the farm, determination of the number of animals affected, examination of the breeding, health and feeding records, careful examination of the affected dam(s), and collection of the expelled foetus and placenta for pathological and microbial examination. Furthermore, samples such as paired serum samples, urine, milk and vaginal swabs can also be collected for analysis. The results are then collated and analyzed to reach a diagnosis (Miller, 1986). However, the diagnostic rate in bovine abortions is very low due to the diverse range of pathogens involved, as well as the fact that factors affecting the dam, foetus and placenta may be involved (Radostits *et al*., 1994). Abortion also often follows an initial infection which may have gone on for several weeks or months; the aetiology often is not detectable by the time the abortion occurs. The high cost of laboratory work to aid in the diagnosis of bovine abortion also compounds the problem. Positive diagnostic rates of 17 % and 43 % have been reported in British and United States of America dairy cattle herds, respectively (Carpenter *et al.*,2006; Murray, 2006).

Scanty reports on abortion and pathogens that can cause abortion in Kenya are available, where *Leptospira* spp. and *Campylobacter* spp. have been confirmed to occur (Agumbah, 1977; De Souza, 1982; Macharia, 1989; Odima, 1994). Also, a review of the records at Nakuru regional veterinary investigation laboratory (NVIL) revealed that between January 1997 and October 2007, 1,182 cases of abortion were reported. Only 124 (10.4 %) were positively identified as due to brucellosis, while the rest (89.6 %) had no definitive diagnosis and therefore interventions were difficult to institute to reduce the

problems. The Rose-Bengal test for brucellosis was the only test carried out on cases of abortion in this area. Other tests were not performed due to lack of reagents and equipment to run the tests. Therefore, there is an urgent need for research to identify the causes of bovine abortion in Kenya and their associated risk factors.

Bolstering the argument for research on abortion in cattle, over the past 20 years, a newly diagnosed pathogen, *Neospora caninum* has also been identified as a cause of both endemic (abortions occurring at a constant rate in a population) and epidemic abortion (abortions occurring at increased rates in a population) in cattle. It is currently the most commonly diagnosed pathogen causing bovine abortion in many countries (Frossling *et al.*, 2003; Dubey and Schares, 2006; Fernandez *et al*., 2006; Murray, 2006; Silva *et al*., 2007). *Neospora caninum* is known to be harboured by canids, and livestock farms can succumb to abortion storms when cattle consume *N. caninum* oocysts fecally shed by canids into their feed or water sources (Dubey and Schares, 2006). Recent studies in the Philippines have also indicated that concurrent infection with bovine viral diarrhoea virus (BVDV) could be a contributory factor to *N. caninum* induced abortion in cattle (Konnai *et al.*, 2008). Neosporosis has never been reported in Kenyan dairy cattle, and since 1996, the status of BVDV in Kenya has not been ascertained. Therefore, it is important to investigate whether *Neospora caninum* and BVDV play a role in abortion in Kenyan dairy cattle herds, and to understand the risk factors of bovine abortion in order to assist in development of control programs for bovine abortion.

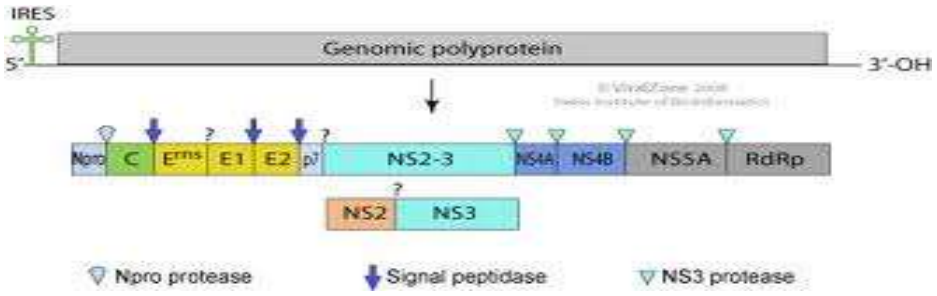
Each case of abortion in dairy cattle has been estimated to lead to losses of around US $500 to $900 (Thurmond *et al.*, 1990; Carpenter *et al.*, 2006). These losses

can be attributed to loss of replacement calves, reduced milk production, and premature culling of productive cows and heifers. In addition, some of the causes of abortion, such as *Brucella abortus,* Rift Valley fever virus, *Toxoplasma* and *Leptospira,* are zoonotic, thus posing a risk to human health (Carpenter *et al.*, 2006; Murray, 2006). However, no reports are available that estimate the economic impact of bovine abortion in Kenya.

2.1 Bovine viral diarhoea (BVD)

Bovine viral diarrhoea is a disease caused by bovine viral diarrhoea virus (BVDV). Bovine viral diarrhoea is one of the most important diseases of cattle worldwide (Lopez *et al.,* 1993; Almeida *et al.,* 2010). It is an important cause of diarrhoea, reproductive problems and reduced milk yield in affected herds (Lindberg and Houe, 2005). This is a Pestivirus in the family Flaviviridae that is closely related to border disease virus of sheep and classical swine fever virus of pigs (OIE, 2004). The disease occurs worldwide and infections may be subclinical in some animals (Lindberg and Houe, 2005). The genomic structure of BVDV has been deciphered and from which constituting antigens may be visualised (see figure 2.1below).

Figure 2.1. Genomic structure and constitutive antigens of BVDV



Source: Swiss institute of bioinformatics (2008). Retrieved 4th November 2014.

Bovine viral diarrhoea virus is transmitted by direct contact with saliva, faeces, semen, urine, tears and milk of infected cattle, or by inutero infection of foetuses (Radostits *et al*., 1994). Infection of naive pregnant cows and heifers may lead to abortion and other reproductive disorders, such as early embryonic death (the death of a conceptus within the first 2 months after conception) in the first 45 days, foetal death and mummification. The other effects of BVDV are birth of calves with congenital defects, calves with poor growth rates, and increased average age at first calving in affected herds (Kabongo and Van Vuuren, 2004; Heuer *et al.*, 2007). The virus has also been shown to depress ovarian function in infected heifers by disrupting gonadal steroidogenesis, and impairing the quality of oocytes produced (Fray *et al.*, 2000; Altamarand *et al.*, 2013). Infection from day -9 to 45 of gestation results in reduced conception rates and infertility, early embryonic death and infertility. From day 45-75 of gestation, infection with BVDV will result in abortions, intrauterine growth retardation, and calves with congenital defects especially of the nervous system. Infection in late gestation (125-285) results in birth of normal calves with neutralising antibodies (Grooms, 2004). This virus has a high affinity for leucocytes and reduces their numbers in infected animals. This immunosuppression potentiates the effects of other pathogens, including abortifacient ones, such as *Neospora caninum* (Bjorkman *et al.*, 2000; Lopez *et al.*, 1993; Konnai *et al.*, 2008).

Among the risk factors for BVDV infection in cattle are increased age and the origin of the animal (Mainar-Jaime *et al.*, 2001); pasturing and increased herd sizes

(Houe *et al*., 1995); and dam factors, such as high BVDV titres at calving and increased parity (Muñoz-Zanzi *et al.,* 2003).

Several methods have been developed to detect BVDV infection in cattle (OIE manual, 2008). These include virus isolation in bovine tissue culture (kidney, lung, testis and turbinate cells), immunohistochemistry to detect virus antigen in tissue, nucleic acid detection by polymerase chain reaction, and serological tests, such as virus neutralisation and enzyme-linked immunosorbent assay (ELISA). Samples collected for analysis include: bulk milk to determine the herd status, individual milk, serum, and plasma samples to determine individual animal sero-status, as well as tissue samples for immunohistochemistry. Serological tests, such as ELISA, are commonly employed in explorative studies since they can be used to determine the sero-status of large numbers of animals sampled in a population (OIE Manual, 2008).

Bovine viral diarrhoea virus seropositivity rates of up to 70%-81% have been reported in South American dairy cattle herds (Lertora, 2003 cited in Gogorza *et al.*, 2005; Stahl *et al*., 2006). Prevalence rates in European and American herds have been reported to be between 21-98% in unvaccinated cattle herds (Waldner, 2005; Ahmad *et al.,* 2011). The disease in Africa has been detected in a variety of domestic and wild ruminants (Kabongo and Van Vuuren, 2004). A study in extensively reared Small East African Zebu cattle in coastal Kenya reported a prevalence of 48.5% (Kenyanjui *et al.*, 1994). In a Namibian study, 58% of cattle were found to be positive to ruminant Pestiviruses (Depner *et al.*, 1991). Bovine viral diarrhoea virus has also been detected by immunohistochemistry in 22.2% of aborted bovine foetuses in South Africa (Njiro and

Nkosi, 2009). In Tanzania, antibody prevalence rates of 12% have been reported in the cattle population (Msolla *et al.*, 1988). Therefore, there are reports of BVDV infection in various cattle herds in Africa; however, few reports are available on the occurrence of this disease in the rest of Eastern Africa and Kenya.

2.2 Brucellosis

Brucellosis is an important disease of humans, and domestic and wild animals worldwide and is also a serious zoonosis (Mekonen *et al*., 2010). In female cattle, the disease is characterised by abortions from the 5th month of gestation, infertility, mastitis, retained placentae and arthritis, while in males, the condition is characterised by orchitis and epididimytis (Radostits *et al*., 1994). All these manifestations lead to losses in the production system. Several species of the bacterium *Brucella* can cause brucellosis in cattle; however, *Brucella abortus* is the primary bovine pathogen (Godfroid *et al.*, 2011). Brucellosis in cattle is spread by ingestion of contaminated pasture, feed and water, licking aborted foetuses or genital exudates from recently aborted cows or carrier cattle that have calved normally (Arthur *et al*., 1999).

While vaccination of cattle with strains S19 and RB51 has been the cornerstone of Brucellosis control programmes in the developed world, adequate information on its occurrence in the developing world is lacking and the adoption of control programmes is still low (Godfroid *et al.,* 2011).

Several risk factors for bovine brucellosis have been reported. Among these are increased herd sizes, increased age, sex of the animal, husbandry practices such as animal confinement, contact with wildlife, geographical area, keeping different

breeds in a herd (Faye *et al.,* 2005; Muma *et al*., 2007; Matope *et al.,* 2010; Mekonen *et al.,* 2010).

Various techniques have been used to diagnose bovine brucellosis. These include the use of staining techniques, such as modified acid fast staining, culture, and molecular techniques, such as polymerase chain reaction. However, in most epidemiological studies, serological tests, such as Serum agglutination test (SAT), Rose-Bengal test (RBT), Buffered plate agglutination test (BPAT), Fluorescence polarisation assay (FPA) and ELISA, are often used. The limitations to the use of serological tests are false positives from vaccinated animals, cross-reactivity with other gram-negative bacteria, and low sensitivity from tests such as SAT and RBT (OIE manual, 2009).

Individual animal seroprevalences of brucellosis of between 4.9%and 19% have been reported in Africa (Muma *et al.*, 2007; Mekonen *et al.*, 2010). However, most of these studies have determined the occurrence of antibodies against *Brucella* spp., as opposed to *Brucella abortus,* which is the primary bovine pathogen. Thus, there have been no studies conducted to specifically determine the prevalence of *Brucella abortus* and its impact on bovine abortion.

2.3 Neosporosis

Neosporosis is a disease caused by *Neospora caninum*. This is a protozoan coccidian parasite that structurally resembles, and is genetically related to, *Toxoplasma gondii* (Silva *et al*., 2007). There are two species of *Neospora* currently recognised; *Neospora caninum* which causes clinical disease in dogs, cattle, sheep, equines and many wild animal species, and *Neospora hughesi,* which has been associated with causing

reproductive losses and myoencephalitis in horses (Marsh *et al* , 1999; Peters *et al.*, 2001; Hall *et al.,* 2006; Fernandez *et al*., 2006; Villabolos *et al*., 2006). Dogs are the definitive hosts of *Neospora caninum* and cattle are among the intermediate hosts.

Cattle become infected by ingestion of feed and water contaminated by oocysts shed in dog faeces, or by congenital infection (Jenkins *et al.*, 2002; Wouda, 2000; Pan *et al.*, 2004). This parasite has been reported to be the most important cause of abortion and neonatal mortality in beef and dairy cattle populations worldwide (Romero *et al.*, 2002; Boger and Hattel, 2003; Frossling *et al.*, 2003; Waldner, 2005; Dubey and Schares, 2006; Fernandez *et al.*, 2006; Murray, 2006; Paradies *et al*., 2007; Silva *et al.*, 2007).

Abortions in cattle due to *Neospora caninum* occur from 3 months of gestation but are most common from 5-6 months of pregnancy. Other signs presented by infected cattle are foetal resorption, mummification, autolysis, and stillbirth, and some calves are born alive with neuromuscular defects, while other calves are apparently healthy but persistently infected (Dubey and Schares, 2006). The incidence of abortion is often repeated in subsequent pregnancies, and congenital/vertical transmission from seropositive dams to their offspring is important in the epidemiology of neosporosis (Dubey *et al.*, 2007). Reported risk factors for bovine abortions due to *Neospora caninum* include geographical location, exposure to dogs, and being pregnant heifers (Pare *et al.*, 1997; Wouda *et al.*, 1998; Koiwai *et al.*, 2006; Dubey and Schares, 2006).

Various methods have been used to diagnose neosporosis in animals (Dubey, 1999). These include histopathology of tissues from aborted foetuses and still-births, parasite isolation from sacrificed animals, inoculation in mice, molecular techniques such

as polymerase chain reaction, and oocyst recovery from dog faeces. However, serology (ELISA and immuno-fluorescent antibody test [IFAT]) has been the most common technique used to diagnose neosporosis since it can be done ante-mortem and post-mortem. Serology is useful in epidemiological studies since it can be used to reliably test exposure and infection in large animal populations (Dubey and Schares, 2006; Silva *et al.,* 2007). The antigens commonly used in the serodiagnosis of neosporosis would include the major antigens of *Neospora caninum* e.g. surface antigen-1 (SAG-1) and surface antigen-1 related sequence 2 (SRS -2) which differentiates this organisn fron related ampicomplexa group protozoa (*Neospora hughesi* and *Toxoplasma gondii*) (Marsh *et al*, 1999) and could be the ones used in the coated wells which could not be revealed for commercial reasons (Soderlund, 2014).

The seropositivity for *Neospora caninum* in dairy cattle has been reported in South America (Garcia-Vasqueza *et al.*, 2002; Romero *et al.*, 2002; Moore, 2005; Lista-Alves *et al.*, 2006; Stahl *et al.*, 2006), North America (Chi *et al.*, 2002; McDole and Gay, 2002; Boger and Hattel, 2003; Tiwari *et al.*, 2007), Europe (Otranto *et al*., 2003; Václavek *et al.*, 2003; Canada *et al.*, 2002; Canada *et al.*, 2004), Asia (Koiwai *et al*., 2005; Koiwai *et al.*, 2006; Konnai *et al.*, 2008), and Africa (Njiro *et al.*, 2011; Ayinmode and Akanbi, 2013). The general seroprevalence of this disease globally ranges from 1.9% to 39.7%.

Barber *et al.* (1997) reported the occurrence of *Neospora caninum* antibodies in dogs in Tanzania (22%); however, in the same study, no *Neospora caninum* antibodies were detected in dogs in Kenya. *Neospora caninum* antibodies have also been detected in

wildlife in Kenya (Ferroglio *et al.,* 2003). However, there are no studies on neosporosis prevalence in Kenyan dairy cattle, its risk factors, and its effect on their reproduction.

2.4 Hypothesis

Bovine viral diarrhoea virus, *Brucella abortus* and *Neospora caninum* are important causes of dairy cattle abortion in Nakuru District, Kenya and their occurrence is influenced by environmental, management and agro-ecological factors, with these agents working synergistically as causes of bovine abortion in this area.

2.5 Overall objective

To improve the detection, prevention and control of bovine abortions caused by bovine viral diarrhoea virus, *Brucella abortus* and *Neospora caninum* through an assessment of their seroprevalence in dairy cattle herds, and an epidemiological study of their associated risk factors.

2.6 Specific objectives

1. To estimate the seroprevalence of BVDV, BA and NC infections in dairy cattle in Nakuru District, Kenya.
2. To determine the prevalence and risk factors of *Neospora caninum* in farm dogs in Nakuru District, Kenya.
3. To determine the pattern of occurrence and associated risk factors of abortion/foetal loss in dairy cattle due to the three agents in Nakuru District, Kenya.
4. To determine the knowledge, attitudes and practices (KAP) of animal health service providers on bovine abortion in Nakuru District, Kenya.

2.7 Justification

There are reports on bovine abortion in Kenya (Odima, 1994). Abortion risks in a dairy herd should not exceed 5% (Radostits *et al.*, 1994); in Kenya, incidence risks of abortion of 9% have been reported (Odima, 1994). In addition, between January-August 2007, cases of abortion accounted for 9.7% of the samples submitted to the Nakuru regional veterinary investigation laboratory (unpublished data). Previous studies in Kenya have neither determined the specific causes nor the risk factors for bovine abortion.

Neospora caninum and bovine viral diarrhoea virus have been reported as major causes of bovine abortion worldwide (Dubey and Lindsay, 1996; Almeida *et al*., 2013). There are no reports on the impact of these two pathogens on abortion in dairy cattle in Kenya. Synergy between the two in causing abortion has been postulated but no data is available to confirm whether this exists or not (Konnai *et al.*, 2008).

An epidemiological study into abortion in dairy cattle herds in Kenya will therefore lead to an increased understanding of the extent of the problem and aid in the identification of agroecological and management risk factors. This will aid in laying down appropriate control strategies. Successful detection and reporting will also provide an estimate of the abortion incidence that is occurring.

CHAPTER THREE

3 MATERIALS AND METHODS

3.1 Study area

This study was carried out in the former greater Nakuru District which has an area of 7,242.3 km^2 and lies at an altitude of 1,520-3,098 m above sea level. It is one of the main dairy farming zones in Kenya and is also the main catchment area for dairy cattle breeding stock in Kenya. Though Nakuru is a large-scale farming district in terms of land area, with many large-scale farms averaging 1,100 acres, many small-scale farms with average sizes of between 0.3-10 acres do exist (NDDP, 2001; Economic survey, 2007; Statistical abstract, 2007).

Figure 3.1 Map of Nakuru District and its location in Kenya (shaded green)

Nakuru district has two distinct agroecological zones (AEZ), namely lower highland 3 (at an altitude between 2,100-2300 m above sea level) and upper highland 2 (at an altitude of between 2,400-2,550 m above sea level). Rainfall is bimodal and reliable, with the long rains experienced from March-May while the short rains are

experienced from July-August. Average rainfall is 800mm/annum and 1024mm/annum in the lower highland 3 and upper highland 2 agroecological zones, respectively. The dairy cattle population in this area ranges from 100,000-120,000, most of which are large herds on large-scale farms.

3.2 Selection of study farms and animals

A list of more than 300 dairy cattle farms was collected from the local animal production offices and dairy societies and divided into small- and large-scale farms, with large-scale farms having ≥30 dairy cattle and small-scale ≤ 29 cattle. From the list provided, 70 farms were selected based on their willingness to participate in the study following a face to face interview with each farmer as well as logistical and budgetary considerations. A stratified random sampling procedure was used; 50 farms having ≤ 29 dairy cattle and 20 farms having ≥ 30 dairy cattle were randomly selected into the study. For the prevalence survey, serum samples were collected from 398 randomly selected dairy cattle (pregnant and non-pregnant; over 6 months old) of the estimated 200,000 dairy cattle on the farms, based on the following formula:

$$n = \frac{4\mathrm{xP}(1-\mathrm{P})}{L^2}$$

where; n = sample size, L=Precision (0.15) and P= incidence risk estimate (25%), using 95% confidence levels (Dohoo *et al.*, 2009).

Based on the formula above (Dohoo *et al.*, 2009), 398 serum samples were collected for the prevalence survey. Subsequently, 279 dairy cattle confirmed pregnant

by rectal palpation (40 - 60 days post service) were selected for the prospective study. However, 19 dairy cattle were lost to follow-up due to sales.

3.3 Study design

Questionnaires (Appendix 2) were administered to animal health providers in Nakuru district to get information on the potential risk factors of bovine abortion (area of practice, level of education [degree, diploma, certificate, informal], years of experience in animal health service provision, perceived importance of abortion, risk factors of abortion, specific infections associated with abortion, gestation stage of abortion [early, midterm and late], management of abortion and handling of samples in cases of abortion.

3.3.1 Prevalence survey

In the first phase of the study, a prevalence survey was initiated to screen 398 dairy cattle in 64 dairy cattle farms randomly selected cattle for exposure to BVDV, *Brucella abortus* and *Neospora caninum* through serology. Information from individual animals such as age, breed, sex, production system, herd size, medical and reproductive history, vaccination status. Blood samples were collected from the coccygeal veins of individual animals and serum extracted for serology. The average herd size for small scale farms was 8.3 $\pm$ 6.7 while the average herd size in the large scale farms was 91.7 $\pm$ 124.6. This high standard deviation for large scale farms was because of 2 farms which had 592 and 153 animals each.

3.3.2 Prospective study

For the prospective study, blood samples were collected from the coccygeal veins of cows and heifers restrained in a crush. The blood was allowed to clot at room

temperature and the serum harvested manually into labelled Eppendorf tubes before being frozen at -40^{o}C. This was done at monthly intervals beginning from the time pregnancy was diagnosed by rectal palpation between 40 - 60 days post-service. This was done monthly until the animal calved at term or the pregnancy was lost. Animals that died or were sold from the farms during this period were considered as withdrawals.

Blood testing was done monthly to monitor changes in antibody titres to BVDV, *Brucella abortus* and *Neospora caninum*. Rectal palpation was also performed monthly to test for continued pregnancy in the animals. Detection, diagnoses and subsequent abortion recordings were made by the veterinarian and farmer if he/she saw conceptus expulsion, and the date of an abortion was estimated retrospectively. In addition, detailed data on management practices, such as breeding methods, were collected at this time. Detailed information and herd history was collected using a questionnaire (Appendix 1). Individual animal data such as the age, breed, medical and reproductive history, serological status and outcome (e.g. abortions and malformations) of the rectally detected pregnancy were recorded in an individual animal record sheet (Appendix 3). The data was collected by observation, examination of records where available, and face to face interviews with the farmers.

An animal that had a gestation that lasted >260 days was considered to constitute a parity, and animals were classified as 0 parity if they had not been served or had a gestation (got pregnant) less than 260 days. For animals that had gestation lengths more than 260 days and aborted at the current reproductive outcome, the parity was considered to be 0, 1st, 2nd, 3rd, 4th or $\geq$ 5th. Abortion was any pregnancy that was lost between initial

pregnancy diagnosis by rectal palpation (around 60 days) and day 260 of gestation. Early abortion was defined as those occurring between initial day of pregnancy diagnosis and 100 days of gestation, mid-term abortions were between 100 days and 210 days while late abortions were defined as those occurring after day 210 (Stahl *et al*., 2006). Cattle were manually restrained in a crush, 3ml of blood was obtained from the coccygeal vein into plain 5 ml vacutainer tubes labelled with the date of collection, farm identity and type (small/large scale), and animal identity. The blood was allowed to clot at room temperature in standing racks. Serum was harvested by decanting into corresponding labelled eppendorf tubes and stored at -40^{o}C awaiting transport and analysis in the laboratory.

Single serum samples were also collected from dogs in the randomly selected farms to test for presence of antibodies against *Neospora caninum*. The dogs were restrained manually and 3 ml. of whole blood was collected from the cephalic vein into plain vacutainer tubes. The blood was allowed to clot at room temperature and the serum harvested manually into labelled Eppendorf tubes before being frozen at -40^{o}C.

3.3.3 Sample analysis

Serum samples were screened for the presence of antibodies to bovine viral diarrhoea virus (BVDV), *Brucella abortus* and *Neospora caninum (NC)* and according to the tests and standards set in the OIE manual (www.oie.int, search for BVDV, NC and BA).

3.3.3.1 Bovine viral diarrhoea virus (BVDV)

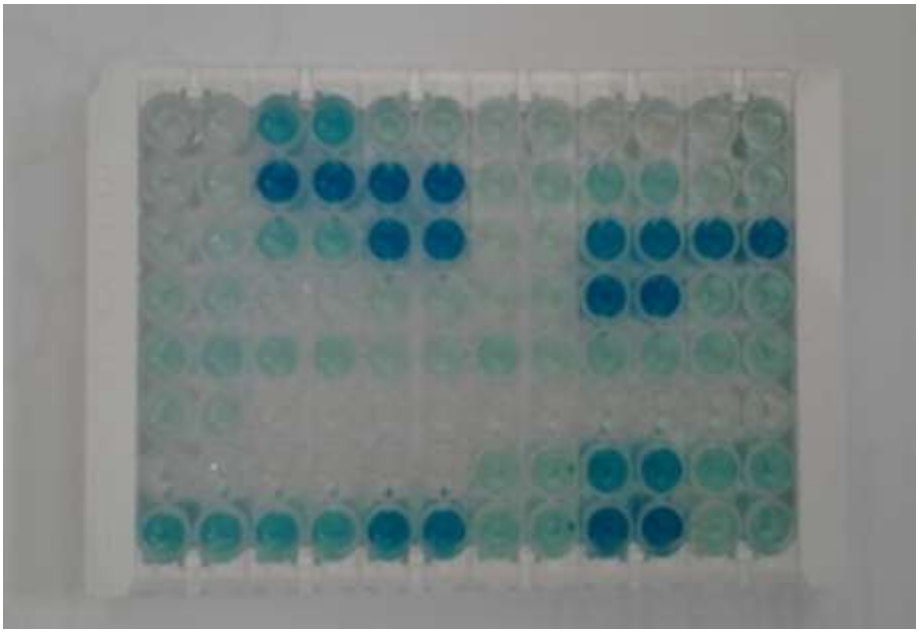
Serum samples were tested for antibodies to BVDV using a commercial BVDV test kit (Herdcheck BVDV Ab, IDEXX Laboratories, Switzerland AG). This kit has a sensitivity of 100% and specificity of 95%. It contains a cocktail of antigens that are able to detect the majority of BVDV 1 and BVDV 2 antigens likely to be found in most parts of the world (Soderlund, 2014. personal communication). The cocktail of antigens was most likely derived from the structure in Figure 2.1 and could not be revealed for industrial reasons. The large original protein coded by the BVDV genome is cleaved into several antigens some of which were in this kit.

The reagents were allowed to come to room temperature. Antigen coated plates were obtained and the sample position recorded on a worksheet. One hundred microlitres of sample diluent was added into each sample well and 25 µl of the negative and positive controls was added into wells A1-A2 and A3-A4 respectively; 25 µl of the test samples were then added in duplicate into each sample well from well A5 according to their respective positions on the worksheet. The contents of the wells were gently mixed taking care to avoid mixing. The plate was incubated for 90 minutes at room temperature (18-25 ^{o}C) in a humid chamber to avoid evaporation.

The liquid contents were then aspirated into a waste reservoir and each well was washed with 300µl of wash solution provided by the manufacturer and washed 5 times. The residual wash solution in the plates was gently tapped onto absorbent material. One hundred microlitres of anti-bovine horse radish peroxidase conjugate was then dispensed into each well, and the plate was incubated at room temperature for 30 minutes. The

liquid contents were again aspirated into a waste reservoir, and each well was washed with 300µl of wash solution 5 times. One hundred microlitres of (Tetramethylbenzidine) TMB substrate solution was added into each well, and the plate was incubated for 10 minutes in darkness at room temperature. One hundred microliters of stop solution was then dispensed into each well in the same order as the substrate solution was dispensed. Positive wells developed a hue of blue and negative wells were colourless.

Figure 3.2 A ninety six well Idexx ELISA plate for screening antibodies to BVDV. Note the hue of blue in wells A1 –A2 (positive control wells)



The spectrophotometer (MR-96A, Shenzen mindray biomedical electronics company limited. Shanzen China) was set at 450 nm and blanked on air. The ELISA plate placed on the receptacle and the absorbance/optical density (O.D.) measured. The mean optical densities for the negative and positive controls in wells A1-A2 and A3-A4 respectively were calculated using the formulae below given by the manufacturer

The negative control (N.C.) mean O.D. was calculated as $= \frac{N.C.1+N.C.2}{2}$

The positive control (P.C.) mean O.D. was calculated as $= \frac{P.C.1+P.C.2}{2}$

The sample /positive ratio (S/P) was calculated using the formula provided by the manufacturer

$$S/P = \frac{\text{Sample O.D. mean} - \text{N.C. mean O.D.}}{\text{P.C. mean O.D.} - \text{N.C. mean O.D.}}$$

The results were interpreted as

S/P values <0.2 = Negative

S/P values ≥ 0.2 but < 0.3 = Suspect

S/P values ≥ 0.3 = Positive

Samples that tested suspect, were rerun confirm the ambiguity of the results. Samples that remained suspect in the second run, their O.D.s were compared to the negative and positive control means and were assigned according to the nearest O.D. category.

3.3.3.*2 Brucella abortus*

A commercial ELISA antibody test kit (Chekit Brucellose Serum Antibody Test Kit, IDEXX Laboratories,Switzerland AG) was used to test serum samples for exposure to *Brucella abortus*. This kit has a sensitivity and specificity 99% and 95% respectively. The antigens coated to coat the wells were not revealed for commercial resons but are

recommended for specific serodiagnosis of *Brucella abortus* under European Directive (CEE 64/432, modifications of Annex C dated on 21/03/2002) as in www.idexx.com (retrieved 4th November 2014)

Ninety microlitres of wash solution was dispensed into each well of the microtitre plate. Ten µl of the negative control and positive control samples were dispensed into wells A1-A2 and A3-A4, respectively. Ten µl of the undiluted test samples were then dispensed in duplicate into appropriate wells of the microtitre plate based on their positions on the worksheet. The plate was then carefully and gently shaken while avoiding spillage and then incubated in a humid chamber at 37 ^{o}C for 60 minutes. After this period, each well was washed with 300µl of Chekit wash solution thrice. The residual wash fluid was firmly tapped on absorbent material and 100 µl of Chekit-Brucellose anti-ruminant horse radish peroxidase conjugate dispensed into each well.The plate went through a second wash cycle after which 100µl of Chekit substrate was dispensed into each well.The plate was then incubated for 15 minutes at room temperature 18^{o}C-25^{o}C. After which the reaction was stopped by adding 100µl of Chekit stop solution into each well. Ths colour change was similar to the one described in 3.3.3.1 above. The results were read using a spectrophotometer at a wavelength of 450nm within 2hours of stopping the reaction.

For valid results, the mean O.D. of the positive control did not exceed 2.0 and the mean O.D. of the negative control did not exceed 0.5.

$$\frac{S}{P}\ Value = \frac{\text{O. D. sample} - \text{O. D. mean N. C. x 100}}{\text{O. D. mean P. C.} - \text{O. D. mean N. C.}}$$

Samples with values lower than 80 % were classified as negative while samples with values greater than or equal to 80%were classified as positive as recommended by the test kit manufacturer

3.3.3.3 *Neospora caninum*

An antibody ELISA test was carried out to test for exposure to *Neospora caninum* (CHEKIT *Neospora* ELISA Test Kit, IDEXX Laboratories, Switzerland AG) (Wu *et al.*, 2002). Serum samples were prepared by diluting them 100-fold with sample diluents (5 µl of sample mixed with 500 µl of sample diluents). One hundred microlitres of undiluted negative and positive control sera were dispensed into well A1-A2 and A3-A4, respectively. One hundred microlitres of the diluted test samples were then dispensed in duplicate into appropriate wells of the microtitre plate based on their positions on the worksheet. The plate was then incubated at room temperature for 30 minutes, after which the liquid contents were aspirated. Each well was then washed with 300 µl of phosphate buffered wash solution four times. One hundred microlitres of anti-bovine horse radish peroxidase was dispensed into each well, and the plate was incubated for 30 minutes at room temperature. The wells were again washed with 300 µl of the wash solution four times.

One hundred microlitres of substrate was then added into each well and the plate was incubated for 15 minutes at room temperature. After this period, 100 µl of stop solution was dispensed into each well to stop the reaction. The positive wells appeared a hue of yellow in colour.

The ELISA spectrophotometer reader was blanked on air, and the absorbance/optical density was measured and recorded at a wavelength of 650nm.

For the assay to be valid, the P.C. mean O.D. – N.C. mean O.D. had to be $\geq$ 0.150 and the N.C. mean O.D. had to be $\leq$0.20.

$$\text{S/P ratio} = \frac{\text{O.D. Sample} - \text{N.C.mean O.D.}}{\text{P.C. mean O.D.} - \text{N.C.mean O.D.}}$$

The results were interpreted as

Samples with S/P ratios < 0.5 = negative

Samples with S/P ratios $\geq$ 0.5 = positive.

3.3.3.4 Sampling of dogs for *Neospora caninum*

An antibody ELISA test was carried out on dog serum samples to test for exposure to *Neospora caninum* (CHEKIT Neospora ELISA Test Kit, IDEXX Laboratories, Switzerland AG) (Wu *et al.*, 2002). The protocol was as for Neosporosis in the bovine samples.

3.4 Data management

Data from the survey (age, breed, sex, production system, herd size, medical and reproductive history and vaccination status) and serological status were entered for each animal and each pathogen and stored in Microsoft Office Excel 2007 (Microsoft Corporation, 2007). The data were screened for any entry errors. The data were imported into Genstat® 13th edition, service pack two, for analysis (VSN international).

3.4.1 Statistical analysis

Descriptive statistics, including prevalence and incidence risk, were computed for abortion parameters and management variables. Correlation analyses were performed to test and quantify associations between variables while Chi-square tests were used for differences in dichotomous variables between groups.

To determine if an abortion was caused by one of the 3 test pathogens, the monthly antibody titres to *Brucella abortus*, *Neospora caninum* and BVDV were examined around the times of the reported abortions. A four-fold increase in a titre for a specific pathogen before and after the abortion was used to indicate the likely aetiology of the abortion by that pathogen (Graham *et al.,* 1998). Monthly titres for both aborted and non-aborted cows and heifers were used to calculate cause-specific incidence risks of abortion.

Multiple logistic regression procedures were used to model the effects of potential risk factors (age, breed, parity, herd size, production system, breeding method, reproductive history, location) on abortion incidence (outcome variable). The backward elimination procedure was used to eliminate the factors that were not significant at $P<0.05$ in the overall model. Factors that were significant ($P<0.05$) were retained in the final model. Cause-specific models were explored for pathogens that caused at least 10 abortions. Potential clustering of animals within farms was controlled for by including farm as a random effect in the models.

Infertility was defined as the diminished or absent capacity to produce viable offspring. Early embryonic death was the period between when pregnancy could be

detected and 2 months of gestation. Repeat breeder syndrome was defined as a cow/heifer that was served more than three times without conception.

CHAPTER FOUR

4 SEROPREVALENCE AND RISK FACTORS OF BOVINE VIRAL DIARRHOEA VIRUS, *Brucella abortus* AND *Neospora caninum* ANTIBODIES IN DAIRY CATTLE HERDS IN NAKURU DISTRICT, KENYA

4.1 Introduction

Abortion is important to the dairy industry because it disrupts the scheduled lactation and replacement of heifers (Carpenter *et al*., 2006; De Vries, 2006). The aetiology of abortion is diverse (see Chapter 2 of this thesis) with several risk factors reported (Radostits *et al*., 1994).

General principles in the diagnosis of bovine abortion include the collection of a complete history of the case, epidemiological data and collection of relevant samples. These are then collated and analyzed to reach a diagnosis (Miller, 1986).

Reports on abortion and abortifacient pathogens in Kenya are available. Pathogens such as *Leptospira spp., Brucella abortus,* BVDV and *Campylobacter fetus* among others have been confirmed to occur (Agumbah, 1977; De Souza, 1982; Macharia, 1989; Odima, 1994). However, there are few reports on the occurrence of other important abortifacient pathogens in this country. This study was therefore designed to determine the prevalence and risk factors of three important bovine abortifacient pathogens namely BVDV, BA and NC in dairy cattle herds in Nakuru District, which is a major dairying zone in Kenya (See chapter 3 of this thesis for location of study site).

4.1.1 Bovine Viral Diarrhoea

Bovine viral diarrhoea virus is a Pestivirus in the family Flaviviridae that is closely related to border disease virus (BDV) of sheep and classical swine fever virus (CSFV) of pigs (OIE manual, 2004) and is one of the most important diseases of cattle worldwide (Lopez *et al.,* 1993; Lindberg and Houe, 2005; Almeida *et al.*, 2010).

Seropositivity rates of between 70%-81% have been reported in South American dairy cattle herds (Lertora, 2003 cited in Gogorza *et al.*, 2005; Stahl *et al*., 2006) while prevalence rates in European and American herds have been reported to be between 21-98% in unvaccinated cattle herds (Mainar-Jaime *et al*., 2001; Waldner, 2005; Ahmad *et al.*, 2011). Several reports on its occurrence in some parts of Africa are available. In Southern Africa, it has been detected in a variety of domestic and wild ruminants in South Africa (Kabongo and Van Vuuren, 2004) and in Namibia, 58% of cattle were found to be positive to ruminant Pestiviruses (Depner *et al.*, 1991). In addition, bovine viral diarrhoea virus has also been detected by immunohistochemistry in 6 out of 27 (22.22%) aborted bovine foetuses in South Africa (Njiro and Nkosi, 2009). In Eastern Africa, a study in Tanzania reported antibody prevalence rates of 12% in the cattle population (Msolla *et al.*, 1988) while a seroprevalence of 48.5% was reported in extensively reared Small East African Zebu cattle in coastal Kenya (Kenyanjui *et al.*, 1994).

Several methods are recommended and have been used to detect BVDV infection in cattle (OIE manual, 2008). Serological tests have been preferred for ease of performance, their ability to detect occurrence of disease in large populations, as well as

their cost effectiveness (Carpenter *et al*., 2006). Such tests include virus neutralisation and ELISA among others (OIE manual, 2008). Samples collected for analysis include bulk milk (bulk and individual cow), serum and plasma samples to determine individual animal sero-status, and tissue samples for serology and immunohistochemistry. Serological tests, such as ELISA, are commonly employed in explorative studies since they can be used to determine the sero-status of large numbers of animals sampled in a population (OIE manual, 2008).

Several risk factors for BVDV infection in cattle have been reported worldwide. Houe *et al*., (1995) reported that increased herd sizes and the purchase of replacement animals increased the risk of BVDV spread to susceptible herds. In a Spanish study, Mainar-Jaime *et al.,* (2001) reported that increased age, as well as the geographical origin of the animal, were important risk factors to BVDV seropositivity, with a four-fold increase in seroprevalence found between animals less than 2 years old and those more than 5 years old. Other reported risk factors include pasturing (Houe *et al*., 1995), high anti-BVDV antibody titres at calving (increases of in-utero calf infection and birth of persistently infected calves), and increased parity (Muñoz-Zanzi *et al.,* 2003). In addition, the use of artificial insemination breeding technique without the institution of biosecurity measures on the farm has been shown to increase the risk of BVDV spread by 2.8 times, most likely due to contamination of the herd through contaminated insemination equipment and personnel (Almeida *et al.* 2013).

4.1.2 Brucellosis

This is one of the most important diseases of domestic animals, wild animals and humans including Africa where its seroprevalence has been estimated at between 4.9 and 15.8% (Faye *et al*., 2005, Muma *et al*., 2007; Matope *et al.*, 2007; Mekonen *et al.*, 2010). In cattle, the disease is characterised by abortions, infertility, mastitis, and retained placentae (OIE manual, 2009). Several species of the bacterium *Brucella* can cause brucellosis in cattle; however, *Brucella abortus* is the primary bovine pathogen (Godfroid *et al.*, 2011).

Various techniques used to diagnose bovine brucellosis. Serological tests such as serum agglutination test (SAT), Rose-Bengal test (RBT), buffered plate agglutination test (BPAT), fluorescence polarisation assay (FPA) and ELISA, are often used most epidemiological surveys. The limitations to the use of serological tests are false positives from vaccinated animals, cross reactivity from other gram-negative bacteria, and low sensitivity from tests such as SAT and RBT (OIE manual, 2009). A serodiagnostic technique with high specificity and sensitivity would be most preferred diagnostic technique as this would reduce genetic resource wastage occasioned by slaughter and disposal of false positive animals in an eradication campaign.

Risk factors for bovine brucellosis include increased herd sizes and age, sex of the animal, husbandry practices such as animal confinement, contact with wildlife, geographical area, keeping different breeds in a herd, (Faye *et al.*, 2005; Muma *et al*., 2007; Matope *et al.*, 2010; Mekonen *et al.*, 2010). Thus there have been no studies conducted to specifically determine the prevalence of *Brucella abortus* and its impact on

bovine abortion in this country. This phase of the study was therefore conducted to determine the prevalence of *Brucella abortus* in Kenyan dairy cattle herds.

4.1.3 Neosporosis

Neosporosis is a disease caused by *Neospora caninum* which is a protozoan coccidian parasite that structurally resembles and is genetically related to *Toxoplasma gondii* (Silva *et al*., 2007). Dogs are the definitive hosts of *Neospora caninum*, and cattle are among the intermediate hosts (Dubey and Schares, 2006). This pathogen is currently reported as the most important cause of bovine abortion worldwide.

Serology (ELISA and Immuno-Fluorescent Antibody Test [IFAT]) has been the most common technique used to diagnose neosporosis since it can be done ante-mortem and post-mortem. Serology is also useful in epidemiological studies since it can be used to reliably test exposure and infection in large animal populations (Dubey and Schares, 2006; Silva *et al*., 2007).

Risk factors for bovine abortions due to *Neospora caninum* include geographical location, exposure to dogs, and whether they are heifers (Pare *et al.*, 1997; Wouda *et al.*, 1998; Dubey and Schares, 2006; Koiwai *et al.*, 2006). Cattle become infected congenitally or by ingestion of oocysts shed in dogs feaces in feed and water (Jenkins *et al*, 2002; Pan *et al*., 2004).

The occurrence of *Neospora caninum* in dairy cattle has been reported in South America (Garcia-Vasqueza *et al.*, 2002; Romero *et al.*, 2002; Moore, 2005; Lista-Alves *et al.*, 2006; Stahl *et al.*, 2006), North America (Chi *et al.*, 2002; McDole and Gay, 2002;

Boger and Hattel, 2003; Tiwari *et al.*, 2007), Europe (Otranto *et al.*, 2003; Václavek *et al.*, 2003; Canada *et al.*, 2002; Canada *et al.*, 2004), Asia (Koiwai *et al*., 2005; Koiwai *et al.*, 2006; Konnai *et al.*, 2008) and Africa (Njiro *et al.*, 2011; Ayinmode and Akanbi, 2013) with infection prevalence risks of between1.9% to 39.7 % being reported. A recent systematic review reports the median estimate of the global economic impact of *N. caninum* infections/abortions to be US $1.3 billion per annum (Reichel *et al*., 2013).

Barber *et al.* (1997) reported the occurrence of *Neospora caninum* antibodies in dogs in Tanzania; however, in the same study, no *Neospora caninum* antibodies were detected in dogs in Kenya. *Neospora caninum* antibodies have also been detected in wildlife in Kenya (Ferroglio *et al.*, 2003).

Therefore, there are reports of BVDV infection in various cattle herds in Africa; however, few recent reports are available on the occurrence of this virus in dairy cattle in Eastern Africa and Kenya and its impact on bovine abortion. Similarly, though there are reports on the occurrence of Brucellosis in Kenya, none of the studies were specific to the identification of *Brucella abortus* this being the primary bovine *Brucella* pathogen. As for, there are no reports of its occurrence as an abortifacient pathogen of cattle exists in Kenya. A serological survey using antigens specific for these three pathogens would be most desirable. The choice of the ELISA antibody test for these three pathogens using Idexx test kits with known antigens (Refer to chapter 3.3.3) that would bind antibodies to these three pathogens was chosen

4.2 Materials and Methods

4.2.1 Study area

The study was carried out in 2010 in the former greater Nakuru District, a major dairy farming zone which has an area of 7,242.3 km^2 and lies at an altitude of 1,520 to 3,098m above sea level (NDDP, 2001).

4.2.2 Selection of study farms and animals

A list of farms was collected from the local animal production offices and dairy societies and categorised into small and large-scale farms, with large-scale farms (> 30 dairy cattle) and small-scale (≤ 29 cattle). A stratified random sampling procedure was used; they were stratified as large or small-scale farms. Sixty-four farms of the total 70 farms were included into the study. Two hundred and forty two animals (60.8%) were from small scale farms while 156 (39.2%) were from large scale farms.

4.2.3 Data and sample collection.

Serum samples were collected from randomly selected dairy cattle (pregnant and non-pregnant). Using a data collection sheet (Appendix 3), information on the age, breed, sex, production system (free-range/zero-grazed), breeding method, herd size, parity, medical history, reproductive history and vaccination status was collected by interviewing farmers and by observation in the selected farms.

4.3 Sample handling and laboratory analysis

The samples were handled and analysed as described in section 3.3.3.

4.4 Data management and analysis

4.4.1 Data management

Data from the survey and serological status were entered and stored in Microsoft Office Excel 2007 (Microsoft Corporation, 2007). The data were screened for any entry errors. The data were imported into Genstat® 13th edition, service pack two, for analysis (VSN international).

4.4.2 Statistical analysis

Descriptive statistics were computed for seroprevalences and management variables. Chi-square tests were used for differences in dichotomous variables between groups.

Multiple logistic regression procedures were used to model the effects of potential risk factors (independent variables) on seropositivity (outcome variable). The backward elimination procedure was used to eliminate the factors that were not significant at $P<0.05$ in the overall model. Factors that were significant ($P<0.05$) were retained in the final model. Potential clustering of animals within farms was controlled for by including farm as a random effect in the models.

4.5 Results

4.5.1 Descriptive statistics

4.5.1.1 Response rate and number of animals selected into the study

A total of 87.1% (64/70) of the farmers interviewed were willing to participate in the study. Each farm also constituted a household.

4.5.1.2 Animal variables

Friesians were the most common breed encountered comprising 68.1% (271) of the selected animals. The rest of the breeds were Ayrshire at 18.1% (72/398), Guernsey at 6.0% (24/398), Jersey at 5.5% (22/398), Sahiwal at 2.0% (8/398), and 1 cow (0.25%) was a dairy cross-breed.

With regard to the age of the animals selected into the study, of the 398 animals sampled, 178 (44.7%) were 49-96 months old, 132 (33.2%) were 13-48 months old, 84 (21.1%) were more than 98 months old and 4 (1.0%) were between 6-12 months old.

The parity of the selected animals ranged from 0-9. The mean was 3.1 with a standard deviation of 2.1, median of 3. Fifty-seven (14.3%) animals were heifers.

4.5.1.3 Feeding system

Among the large-scale farmers, free-range grazing was more common than stall feeding at 86.5% and 13.5% respectively. However among the small-scale farmers, stall feeding was more common than free-range grazing, at 52% and 48%, respectively.

4.5.1.4 Breeding methods

Among the dairy cattle selected for this study, 280 (70.4%) were bred by artificial insemination using imported semen, 85 (21.4%) were bred by artificial insemination using local semen, and only 1 (0.4%) had been bred by embryo transfer. Of the 398 animals sampled, 32 (8.0%) had not been bred.

4.5.1.5 Vaccination

The level of vaccination against reproductive diseases in dairy cattle selected for this study was low. Only 7 (1.8%) and 4 (1.0%) had been vaccinated against brucellosis and BVD, respectively. All these vaccinations had been done in animals at one farm.

4.5.1.6 Reproductive history

With regard to the reproductive history, 160 (40.2%) of the selected animals were reported to have developed at least one reproductive disease. The frequency of these reproductive disorders were abortion and infertility at 14.3% (57/398) each, repeat breeder syndrome (i.e. bred at least 3 times for a conception) at 6.8% (27/398), retained placenta at 2.8% (11/398) and deformed foetuses at 2.0% (8/398). However, these percentages could be underestimates given the poor record-keeping of the farmers.

4.5.1.7 Seroprevalence results

Of the 3 abortifacient pathogens under investigation, BVDV had the highest seroprevalence at 79.1% (95% CI=75.2%-83.0%). The seroprevalence of *Neospora caninum* was 25.6% (95% CI=21.6%-29.6%), while that of *Brucella abortus* was 16.8% (95% CI=13.2%-20.4%).

4.5.2 Disease variables

4.5.2.1 BVDV

BVDV was the most common abortifacient pathogen in this study with a seroprevalence of 79.1% (315/398). The frequency of antibodies to BVDV was high in all the age groups though it was marginally higher in dairy cattle 49-96 months old at 83.7% (149/178). With regard to parity, the frequency of BVDV antibodies was higher in

dairy cattle with parity 2 at 88.8% (56/63), parity 3 at 82.1% (60/73), parity 4 at 79.2% (42/53) and parity ≥5 at 76.7% (79/103).

The frequency of BVDV antibodies was higher in the Friesians at 81.5% (221/271), Ayrshires at 76.4% (55/72) and Jerseys at 81.8% relative to Guernseys at 16/24 (66.7) and Sahiwals at 62.5% (5/8).

The frequency of BVDV antibodies among free-ranging dairy cattle at 81.6% (205/251) was marginally higher than in stall-fed dairy cattle at 74.8% (110/147). The frequency was also somewhat higher in large-scale farms 80.7% (126/156) than small-scale farms 78% (129/242).

Of the dairy cattle with a history of reproductive disorders, some were also seropositive for BVDV: abortion at 84.2% (48/57), infertility at 82.5% (47/57), repeat breeder syndrome at 88.9% (24/27), deformed foetuses at 100% (8/8) and retained placenta at 72.7% (8/11). The odds ratios for cattle having a positive BVDV titre and the reproductive disorders were: abortion at 1.5; infertility at1.3; repeat breeder at 2.2; and retained placenta at 0.7. The details are shown in Table 4.1.

4.5.2.2 *Neospora caninum*

Neospora caninum at 25.6% (102/398) was the second most common abortifacient pathogen encountered in this study. The frequency of antibodies to NC was 25% (1/4) in dairy cattle 6-12 months old, 29.5% (39/132) in dairy cattle 13-48 months old, 27.5% (49/178) in dairy cattle 49-96 months old and 21.4% (18/84) in dairy cattle more than 98 months.

The frequency of NC antibodies was higher in the Guernseys at 29.2% (7/24). The prevalence in the other breeds was Ayrshires at 27.7% (20/72), Jerseys at 27.3% (6/22) and Friesians at 25.4% (69/271). With regard to parities, the frequency of NC antibodies was higher in dairy cattle with parity 0 at 31.5% (18/57), parity 4 at 26.4% (14/53), parity ≥5 at 27.2% (28/103).

In addition, the frequency of NC antibodies among free-ranging dairy cattle, at 26.7% (67/251), was marginally higher than in stall-fed dairy cattle at 23.8% (35/147) though this difference was not statistically significant (P>0.05). The frequency was also marginally higher in large-scale farms at 26.9% (42/156) than in small-scale farms at 24.8% (60/242).

Of the dairy cattle with a history of reproductive disorders, some were also seropositive to NC: abortion at 22.8% (13/57), infertility at 21.1% (12/57), repeat breeder syndrome at 33.3% (9/27), deformed foetuses at 12.5% (1/8), and retained placenta at 18.2% (2/11).The odds ratios for cattle having a positive *Neospora caninum* titre and the reproductive disorders were: abortion at 1.2; infertility at 1.3; repeat breeder syndrome at 1.5; deformed foetuses at 2.5; and retained placenta at 1.6.The details of these results are shown in Table 4.1.

4.5.2.3 *Brucella abortus*

In this study, the prevalence of *Brucella abortus* antibodies, at 16.8% (67/398), made it the third most common abortifacient pathogen. The frequency of antibodies to *Brucella abortus* was 14.3% (19/132) in dairy cattle 13-48 months old, 19.1% (34/178) in dairy cattle 49-96 months old, and 16.7% (14/84) in dairy cattle more than 96 months old.

None of the dairy cattle 6-12 months old tested positive for antibodies to *Brucella abortus*.

The frequency of *Brucella abortus* antibodies was higher in the Sahiwal breed at 37.5% (3/8), Jersey breed at 13.6% (3/22) and the Ayrshire breed at 19.4% (14/72) relative to Friesians at 16.2% (44/271) and Guernseys at 12.5% (3/24). With regard to parity, the frequency of *Brucella abortus* antibodies was higher in dairy cattle with parity 0 at 19.3% (11/57) and parity 2 at 22.2% (14/63). The prevalence in the other parities is shown in Table 4.1.

The frequency of *Brucella abortus* antibodies among free-ranging dairy cattle, at 17.1% (43/251), was marginally higher than in stall-fed dairy cattle at 16.3% (24/147), though this difference was not statistically significant ($P>0.05$). The frequency was also higher in large-scale farms at 17.3% (27/156) than in small-scale farms at 16.5% (40/242).

Of the dairy cattle with a history of reproductive disorders, some were also seropositive to *Brucella abortus*: abortion at 15.8% (9/57), infertility at 15.8% (9/57), repeat breeder syndrome at 11.1% (3/27), retained placentae at 36.4% (4/11) and deformed foetuses at 25% (2/8). The odds ratios for cattle with a positive *Brucella abortus* titre and the reproductive disorders were: abortion at 1.1, infertility at 1.1, repeat breeder syndrome at 1.6 and deformed foetuses at 1.7. In this study, none of the dairy cattle reported to be bred by natural mating were seropositive to BA antibodies. The details of these results are shown in Table 4.1.

Table 4.1 Descriptive statistics and univariable associations between seropositivity to BVDV, NC and BA their risk factors in among dairy cattle in Nakuru District, Kenya, 2010

Epidemiological data	Abortifacient pathogen			
	Positive (%) to BVDV (315)	Positive (%) to NC	Positive (%) to BA	p-value
Age				
• 6-12 months	3/4 (75)	1/4 (25)	0/25 (0)	
• 13-48 months	101/132 (76.5)	39/132 (29.5)	19/132 (14.3)	>0.05
• 49-96 months	149/178 (76.5)	49/178 (27.5)	34/178 (19.1)	
• ≥96 months	62/84 (73.8)	18/84 (21.4)	14/84 (16.7)	
Parity				
• 0	43/57 (75.4)	18/57 (31.5)	11/57 (19.3)	
• 1	35/49 (71.4)	12/49 (20.3)	4/49 (8.2)	
• 2	56/63 (88.8)	16/63 (25.4)	14/63 (22.2)	>0.05
• 3	60/73 (82.1)	14/73 (19.2)	11/73 (15.1)	
• 4	42/53 (79.2)	14/53 (26.4)	9/53 (17)	
• ≥ 5	79/103 (76.7)	28/103 (27.2)	18/103 (17.5)	
Feeding system				
• Free ranging	205/251 (81.6)	67/251 (26.7)	43/251 (17.1)	>0.05
• Stall feeding	110/147 (74.8)	35/147 (23.8)	24/157 (16.7)	
History of reproductive disorders				
• Abortion	48/57 (84.2)	13/57 (22.8)	9/57 (15.8)	>0.05
• Infertility	47/57 (82.5)	12/57 (21.1)	9/57 (15.8)	
• Repeat breeder syndrome	24/27 (88.9)	9/27 (33.3)	3/27 (11.1)	
• Deformed foetuses	8/8 (100)	1/8 (12.5)	2/8 (25)	
• Retained placenta	8/11 (72.7)	2/11 (18.2)	4/11(36.4)	

4.5.2.4 Interrelationship between diseases

Of the 102 dairy cattle seropositive to *Neospora caninum*, 85 (83.3%) were also seropositive to BVDV (OR =1.4) and 14 (13.7%) were also seropositive to *Brucella abortus* (OR = 0.7). Of the 315 dairy cattle seropositive to BVDV, 54 (17.1%) were also seropositive to *Brucella abortus* (OR =1.1).

4.5.2.5 Regression analysis of risk factors

In the final models, being an animal older than 49-96 months was marginally associated with BVDV seropositivity (P=0.065). Therefore, no other predictor variables

remained significant in this final model. No variables were significant in the final models for NC seropositivity or BA seropositivity.

4.6 Discussion

Bovine viral diarrhoea has been reported to be one of the most common reproductive diseases of cattle populations worldwide (Lopez *et al*., 1993; Almeida *et al*., 2010). In this study, BVDV was the most commonly encountered abortifacient pathogen in the study population, with a prevalence of 79.1% (315/398). This was comparable to other studies on this disease in dairy cattle populations (Lertora, 2003 cited in Gogorza *et al*., 2005; Stahl *et al*., 2006). A previous study on extensively reared Small East African Zebu cattle at the Coast Province, Kenya, had reported a prevalence of 45.8% (Kenyanjui *et al*., 1994*)*. However that study used the virus neutralization (VN) test, which is a less sensitive test (Graham *et al.* 1998), which may partly explain the differences in results. The high prevalence in the current study may have also been due to the lack of an active BVDV surveillance in this part of Kenya. Indeed, most animal health providers in this area reported little knowledge on the importance of this pathogen as a cause of bovine abortion, as discussed in section 7.3. The exclusion of BVD in most of the farms' herd health plans may have also led to the high prevalence, as has been reported in previous studies (Humphry *et al*., 2012).

The prevalence of BVDV infection has been reported to be reduced by employing herd biosecurity measures such as disinfection, double fencing, as well as keeping a closed herd (Kampa *et al*., 2004: Gates *et al*., 2013). In this study, the frequency of antibodies to BVDV was slightly higher in free-ranging cattle (81.6%) than stall-fed cattle (74.8%). This difference may have been brought about by the increased chance of free-ranging cattle encountering potential sources of infection, such as cattle from other

herds or carrier domestic and wild ruminants. Large-scale farms had a significantly higher frequency of BVDV seropositive dairy cattle relative to small-scale farms. Previous studies had reported similar findings (Presi *et al.*, 2011). This may have been due to the increasing chances of having persistently infected animals among large populations of animals that would act as sources of infection to the rest of the herd. Indeed, the large-scale farms also had a higher proportion of animals free-ranging than the small-scale farms, and therefore farm size likely is confounding any relationship between free-range management and BVDV seropositivity.

Bovine viral diarrhoea infection of naive pregnant cows and heifers has been reported to lead to abortion and other reproductive disorders such as early embryonic death, foetal death and mummification, birth of calves with congenital defects, calves with poor growth rates, increased age at first calving and depressed ovarian function in affected herds (Kabongo and Van Vuuren, 2004; Heuer *et. al*., 2007; Fray *et al*., 2000; Altamarand *et al.*, 2013). Bovine viral diarrhoea virus has also been reported to be fetopathogenic in cattle, thus leading to early embryonic death, repeat breeder syndrome, and abortion in this species (Rufenacht *et al.*, 2001; Yang *et al*., 2012). Indeed, in this study, a high percentage (84.2%) of dairy cattle with clinical history of abortion and 88.9% of dairy cattle with clinical history of repeat breeder syndrome (possibly as a sequel to early embryonic death) were seropositive for BVDV, whereas 78.2% and 78.4% of dairy cattle without a clinical history of abortion or repeat breeding were seropositive for BVDV, for ORs of 1.5 and 2.2 respectively; the fetopathogenic effects of the virus may have been responsible for these histories. Bovine viral diarrhoea virus has

also been reported to suppress ovarian function in affected animals due to disruption of gonadal steroidogenesis, thus leading to infertility (Fray *et al*., 2000; Altamarand *et al.*, 2013). Similar findings were reported in this study, where 82.5% of the dairy cattle with a clinical history of infertility were seropositive for BVDV, whereas 78.6% of dairy cattle without a clinical history of infertility were seropositive for BVDV, for an O.R. of 1.3, although these differences were not statistically significant.

Neospora caninum has been reported as the most important cause of bovine abortion in dairy cattle populations worldwide (Romero *et al.*, 2002; Boger and Hattel, 2003; Frossling *et al.*, 2003; Waldner, 2005; Dubey and Schares, 2006; Fernandez *et al.*, 2006; Murray, 2006; Paradies *et. al*., 2007; Silva *et. al*., 2007; Asmare *et al*., 2013). The prevalence of antibodies to *Neospora caninum* in this study was 25.6% (102/398). This prevalence was high compared to other studies that had reported prevalences of between 1.9%-39.7% (Chi *et al*., 2002; McDole and Gay, 2002; Boger and Hattel, 2003; Tiwari *et al*., 2007, Otranto *et al*., 2003; Václavek *et al*., 2003; Canada *et al*., 2002; Canada *et al*., 2004; Koiwai *et al*., 2005; Koiwai *et al.*, 2006; Konnai *et al*., 2008; Asmare *et al*., 2013; Ayinmode and Akanbi, 2013).

The low level of knowledge by farmers and animal health providers has been reported to hinder disease control programmes (Sarrazin *et al.,* 2013). In this study, there was a low level of knowledge on abortion causes by animal health providers, as reported in Chapter 7. Thus, the high prevalence of NC in this study may be associated with the low level of knowledge on this disease and the subsequent lack of a control programme.

The prevalence of antibodies to most infectious diseases is normally higher in older animals relative to the younger ones due to their greater chance of having encountered the pathogen(s) (Nazir *et al*., 2013; Talafha and Al-Majali, 2013). However, the age of the animal did not seem to have an effect on the prevalence, with only marginal differences being experienced between the different age groups. With vertical transmission of *Neospora caninum* being common (Schares *et al*., 1998), differences between age groups would be less likely.

Asmare *et al*., (2013) had reported that the odds of NC seropositivity were increased by 1.8 times in large relative to small-scale farms. Similar findings were reported in this study, where the frequency of antibodies to NC was marginally higher in large-scale farms relative to the small-scale farms. This may have been caused by the fact that most of the large-scale farms had more land available for grazing, thereby increasing the chances of exposure of their cattle to pasture and water contaminated by oocysts in faeces of domestic and wild canids. In addition, 22.8% of the seropositivity to NC was associated with clinical histories of abortion (OR=1.2), infertility (OR= 1.3) and repeat breeder syndrome (OR=1.5). Note that none of these differences were statistically significant.

Brucellosis is among the most important diseases in livestock in developing countries such as Kenya, leading to losses associated with abortion in livestock, infertility, and mastitis, and it is also a serious zoonosis (Mekonen *et al*., 2010). Individual animal prevalences of between 4.9-16.9% have previously been reported in Africa (Kadohira *et al.,* 1997; Muma *et al*., 2007; Matope *et al*; 2011[a;] Matope *et al*;

2011[b]). In this study, the seroprevalence of *Brucella abortus* was high at 16.8%. The high prevalence of dairy cattle seropositive to *Brucella abortus* in this study may have been due to the lack of a coordinated surveillance and control programmes for this disease in the study area and indeed Kenya as a whole.

Kadohira *et al.* (1997) reported that the seroprevalence of *Brucella abortus* was higher in older cattle. This study reported similar findings, where the prevalence was somewhat higher in cattle more than a year old relative to the younger ones. This may have been due to their increased chances of exposure to the infection due to their older age, with heifers less than a year old having less chances of exposure to potential sources of infection. The prevalence was high in all the parities and older age groups, with only marginal differences.

Kadohira *et al.* (1997) had also reported that free-ranging was a risk factor of Brucellosis in cattle. This study reported similar findings, with the prevalence of antibodies to *Brucella abortus* being marginally higher in free-ranging cattle relative to stall-fed dairy cattle. This higher prevalence may have been due to the fact that free-ranging increases the chances of the disease spreading by contact between animals in the herd. The prevalence was also somewhat higher in large-scale farms relative to small-scale farms, since they practiced more free-range grazing than the small-scale farms.

Reproductive disorders, such as abortion, and retained placenta due to placentitis; infertility due to chronic endometritis and ovariobursal adhesions, and repeat breeder syndrome have been associated with *Brucella abortus* infection in cattle (Arthur *et al,*.1999*;* OIE, 2009). Similar findings were found in this study where dairy cattle

seropositivity to *Brucella abortus* was somewhat associated with reproductive histories of abortion (OR=1.1), infertility (OR=1.1), repeat breeder syndrome (OR=1.7) and deformed foetuses (OR=1.7).

As discussed earlier, BVDV and NC are important abortifacient pathogens on their own (Dubey and Schares, 2006; Yang *et al*., 2012). However, the effects of concurrent infections by NC and BVDV in causing abortion in cattle has been reported in previous studies (Bjorkman *et al*., 2000; Weston *et al.*, 2012). The concurrent infections had been thought to be due to the immunosuppressive effects of BVDV, increasing the chances of foetal infection by NC in pregnant cattle, thus increasing the chances of abortion. Indeed, in this study, of the 102 dairy cattle seropositive to *Neospora caninum*, 85 (83.3%) were also seropositive to BVDV, and 14 (13.7%) were also seropositive to *Brucella abortus.* Of the 315 dairy cattle seropositive to BVDV, 54 (17.1%) were also seropositive to *Brucella abortus*. Konnai *et al*. (2008) had reported similar findings in cattle herds with abortion in Philippines; however, the biological impact of such co-infections requires further investigation.

4.7 Conclusion and recommendations

The findings of this study show a consequential prevalence of antibodies to all three abortifacient pathogens under investigation. A coordinated surveillance and control programme on abortifacient pathogens should be developed and implemented, which would reduce the losses associated with these diseases in dairy cattle.

CHAPTER FIVE

5 SEROEPIDEMIOLOGICAL SURVEY OF *Neospora caninum* AND ITS RISK FACTORS IN FARM DOGS IN NAKURU DISTRICT, KENYA

5.1 Introduction

Neosporosis in dogs is caused by *Neospora caninum*, a coccidian parasite that was first described in 1984 (Dubey, 1999). The parasite is related structurally, genetically and immunologically to *Toxoplasma gondii* (Silva *et al.,* 2007). Dogs act as the definitive and intermediate hosts of this parasite (Dubey, 1999), although many other mammalian hosts have been described as intermediate hosts of *Neospora caninum*. Among these are cattle, sheep, goats, deer, moose, water buffalo, and camels (Gondim *et al.,* 2004). Wild canids, such as wolves, coyotes and foxes, can also act as definitive hosts for *N. caninum* (Dubey, 1999; Gondim *et al.*, 2004; Gondim, 2006; Steinman *et al.,* 2006). Experimental infection has been induced in cats, monkeys, mice, pigs, and gerbils (Dubey, 1999). The rate of vertical transmission in canine neosporosis has been reported to be low; therefore horizontal transmission through direct ingestion of tachyzoites in placentae, aborted foetuses, or improperly cooked meat with tissue cysts is thought to be the main route of spread (Dubey, 1999; Lopes-Sicupira *et al*., 2012)

The impacts of canine neosporosis are two-fold. Canine neosporosis is important to the dog population because congenital infection can lead to neuromuscular defects and mortality (Paradies *et al,* 2007). *Neospora caninum* oocysts shed in canid faeces can also lead to horizontal transmission to ruminants, especially cattle, leading to reproductive losses, such as abortion, from the third month of gestation, since the parasite is

fetopathogenic (Dubey and Lindsay, 1996; Dubey, 1999; Dubey, 2005; Gondim, 2006; Yang *et al.,* 2012). A recent systematic review reports the median estimate of the global economic impact of *N. caninum* infections/abortions to be US$1.3 billion per annum (Reichel *et al*., 2013).

There are reports on the occurrence of canine neosporosis in various parts of the world, with seroprevalence levels ranging from 0% to 32% being reported (Barber *et al.,* 1997; Dubey, 1999; Hornok *et al*., 2006; Paradies *et al.,* 2007; Silva *et al.,* 2007; Cruz-Vázquez *et al*., 2008). A previous Kenyan study reported that out of 140 dogs screened, none was seropositive to *Neospora caninum* (Barber *et al*., 1997). However, this Kenyan population of dogs was a non-farm dog population, and therefore not necessarily representative of the farm dog population or population of dogs intentionally fed abattoir foetuses.

Paradies *et al*., (2007) had reported that the prevalence of neosporosis in rural and urban dogs in Italy was 26% and 14.6%, respectively. The same study also reported that the prevalence in free-roaming dogs (35.8%) was double compared to confined dogs (17.3%), and that the 13.9% prevalence in young dogs less than 1.5 years old was less than in older dogs more than 5 years old, at 37.8%. Lopez-Sicupira *et al.* (2012) had reported similar findings, and in addition, reported higher prevalences in older dogs, dogs not fed cooked meat, and non-pure-bred dogs. However, further research is needed to verify which of these risk factors are valid in the African and/or Kenyan context, and to quantify the amount of risk the factors represent.

The purpose of this study was to determine the seroprevalence of *Neospora caninum* and associated risk factors in farm dogs in Nakuru District, Kenya. This was part of a wider longitudinal study on infectious causes of abortion in dairy cattle in Nakuru District, a primary dairy cattle rearing area.

5.2 Materials and methods

5.2.1 Study area

The study was carried out in 2010 in the former greater Nakuru District which has an area of 7,242.3 km^2 and lies at an altitude of 1,520m to 3,098m above sea level (NDDP, 2001).

5.2.2 Farm and animal selection

A list of dairy cattle farms was collected from the Nakuru District animal production office. Using a random number table, 64 farms were selected and agreed to participate in the study. Sixty-one of the 64 participating farms had dogs. Farmers' consent to participate in the study was sought if they were eligible (had at least one dog resident on the farm). Eighty four dogs were therefore included in the study from these participating farmers.

5.2.3 Sample collection, handling, and laboratory analysis

Serum samples were collected from the cephalic veins of 84 farm dogs. After blood collection into plain vacutainer tubes, the blood was allowed to clot, and then put in a cooler with an ice pack (4^oC) until it was transported back to the laboratory for processing within 4 hours. After centrifuging the blood, serum was harvested into

labelled Eppendorf tubes, and stored at -20^{o}C awaiting screening for antibodies to *Neospora caninum,* after all samples were collected.

The samples were assayed in a commercial ELISA antibody test kit for cattle (CHEKIT *Neospora* ELISA Test Kit, IDEXX Laboratories, Switzerland AG) to test the dog serum for exposure to *Neospora caninum*, with some modifications, as recommended by Wu *et al.* (2002).

The methods and result interpretation are as described in chapter 3.3.3 for cattle.

5.3 Data collection, management and analysis

A survey was conducted to collect data on each dog's age (categorized as < 3 years old or $\geq$ 3 years), breed (purebred or crossbred), sex (male or female), diet (allowed to eat abattoir and aborted bovine foetuses and placentae or not), periurban (urban farms around Naivasha [an urban area in Nakuru District] or not), and confinement (confined or free-roaming).

Data from the survey and serological results status were entered and stored in Microsoft Office Excel 2007 (Microsoft Corporation, 2007). The data were screened for any entry errors. The data were imported into Genstat® 13th edition, service pack two, for analysis (VSN international).

Descriptive statistics, including prevalence and correlations, were determined for the outcome and risk factor variables of interest from the survey.

5.3.1 Analytical statistics

In Genstat®, the Pearson's Chi-squared test was used to determine unconditional associations between predictor variables and *Neospora caninum* seropositivity, with the significance set at $P<0.05$.

Multiple variable logistic regression was carried out to model the effects of potential risk factors on the seropositivity of *Neospora caninum* in farm dogs in Nakuru District, while controlling for the effects of confounding of other variables in the model. Interaction terms were explored as a cross-product variable between the variables that were significant in the model or for which interaction was hypothesized. A backward elimination procedure was used to build the model of main effects and interaction effects on the seropositivity outcome. Factors that were found significant ($P\leq0.05$) were retained in the final model. Odds ratios, as a measure of strength of association between the significant variables ($P<0.05$) and the outcome, were calculated.

5.4 Results

5.4.1 Descriptive statistics

Of the 61 farms selected to participate in the study, samples were collected from dogs in 53 farms. Dogs from 8 farms could not be traced during the scheduled visits due to roaming. A total of 84 dogs were sampled in the farms. The number of dogs in the farms ranged from 1-3.

Overall, 15 of the 84 (17.9%) dogs sampled were positive for antibodies to *Neospora caninum.* At least one seropositive dog was found in 12 of 53 farms (22.6%). No cases of clinical neosporosis were encountered in the dog population.

5.4.2 Chi-squared analyses of risk factors

The seroprevalence in relation to other variables is shown in Table 5.1. The seroprevalence of *Neospora caninum* in free-roaming dogs (36.4%) was significantly higher than in confined dogs (11.3 %) ($P < 0.05$). The seroprevalence of *Neospora caninum* was also higher in dogs not fed foetal parts (33.3%) versus those fed foetal parts (16.4%) though this difference was not statistically significant. There were no significant differences in seroprevalence ($P>0.05$) for the remaining variables.

Table 5.1 Descriptive statistics and univariable association between seropositivity for *Neospora caninum* and epidemiological data for 84 dogs on 53 dairy farms in Nakuru District, Kenya in 2010

Epidemiological data	Examined animals	Number Positive (%) to *Neospora*	Odds Ratio	p-value
Free-roaming				
• No	62	7 (11.3)		
• Yes	22	8 (36.4)	4.48	<0.05
Foetal feeding				
• No	12	4 (33.3)		
• Yes	61	10 (16.4)		
• Unknown	11	1 (9.1)	0.39	>0.05
Sex				
• Female	19	2 (10.5)		
• Male	65	13 (20)	2.12	>0.05
Breed				
• Cross-breed	79	14 (17.7)		
• Pure-breed	5	1 (20.0)	1.16	>0.05
Age				
• <3YRS	54	9(16.7)		
• ≥3 YRS	30	6 (20.0)	0.8	>0.05
Location				
• Peri-urban dogs (Naivasha)	11	1 (9.1)		
• Rural dogs (Rongai, Molo and Njoro)	73	14 (19.2)	2.84	>0.05

5.4.3 Regression analysis of risk factors

In the final model, lack of confinement was significantly associated with seropositivity for *Neospora caninum* in dogs on dairy cattle farms in Nakuru District (OR=4.48, P=0.047). No other predictor variables remained significant in the final model (Table 5.1).

5.5 Discussion

This is the first positive report of *N. caninum* in farm dogs in Kenya, with a seroprevalence of 17.9%. A study by Barber *et al.,* (1997) had found no seropositive dogs in Kenya. This was probably due to differences in the dog populations under study; the dogs in the Barber study were feral dogs and therefore not specifically associated with farms, while the dogs in the current study were all farm dogs. This agricultural source increased their chances of interacting with cattle which are the main intermediate hosts of this canine parasite. However, the seroprevalence in the current study was within the range reported in other studies (Dubey, 1999[0-31%]; Hornok *et al.,* 2006 [2.9%]; Paradies *et al.,* 2007 [20.9%]).

Congenital infection through transplacental infection and horizontal transmission through direct ingestion of oocysts in placentae, tissue cysts or improperly cooked meat are the routes of spread of NC in dogs (Lopez-Sicupira *et al*., 2012). Lack of confinement was the only factor significantly associated with seropositivity to *Neospora caninum*. Indeed the prevalence in free-roaming farm dogs was more than three times higher than in confined farm dogs, likely due to increased chances of exposure to potential sources of infection such as aborted bovine foetuses and placentae. This finding was in agreement

with a Brazilian study which found an odds ratio of 2.2 for lack of confinement and canine neosporosis (Lopez-Sicupira *et al.,* 2012), while controlling for whether the dog came from a rural, urban or peri-urban setting. Our study also controlled for residence because it only sampled farm dogs, although some of the dogs did come from peri-urban farms around Naivasha. Other studies found higher seroprevalences for *N. caninum* in rural/farm dogs compared to urban dogs (Hornok *et al.,* 2006; Paradies *et al.*, 2007; Lopez-Sicupira *et al.,* 2012). However, the Brazilian study by Lopez-Sicupira (2012) did not investigate if there was an interaction between setting and confinement, so it remains unclear whether free-roaming farm dogs are even more at risk of *N. caninum* exposure than confined urban dogs.

The prevalence of *N. caninum* was slightly higher in the rural areas of the study (Molo, Njoro and Rongai) at 19.2%, as opposed to Naivasha at 9.1%, which is a more urban setting, although this difference was not statistically significant (P>0.05). Others (Lopez-Sicupira *et al.,* 2012; Hornok *et al.,* 2006) have reported similar differences between rural and urban dog populations, possibly due to their greater likelihood to encounter cattle offal.

In this study, there was a trend for seroprevalence of *Neospora caninum* to increase with age, as reported by others (Paradies *et al.,* 2007; Cruz-Vázquez *et al*., 2008; Lopes-Sicupira *et al.,* 2012), although this difference was not statistically significant. The increased prevalence in older dogs may be due to their cumulative risk of exposure throughout their life relative to the younger dogs. Gondim *et al*. (2004) reported that some infected young animals may fail to seroconvert or may have a slow rate of sero-

conversion when infected by *Neospora caninum*. This delayed seroconversion may also have led to this trend of lower prevalence rates in the younger dogs.

Despite many dogs being fed foetal parts from the abattoirs, their seroprevalence was marginally lower than those not fed foetal parts, though the association was not statistically significant. This difference may be attributed to the fact that these foetal parts may have been cooked prior to being fed to the dogs (as mentioned by some farmers), thus reducing their infectivity. Cooking of foetal parts was not asked among the initial farmers, making it impossible to check for interactions between cooking and feeding foetal parts. The owners mentioned that the dogs not fed foetal parts were more likely to scavenge on carcasses, as well as hunt birds and rodents that are potential sources of infection (Costa *et al*., 2008).

Hornok *et al.* (2006) and Lopez-Sicupira *et al*. (2012) both reported a higher NC seroprevalence in dogs relative to bitches. In this study, the prevalence of antibodies to NC was somewhat higher in males than females, though the association was not statistically significant. This difference may be due to the fact that more people were likely to confine females in order to avoid unwanted breeding, thus reducing their chances of getting infected with NC.

Most studies on canine neosporosis have been based on the serological status of sampled dogs due to the low and intermittent shedding of oocysts by the host, making oocysts difficult to detect in field conditions (Barber *et al*., 1997; Paradies *et al.*, 2007; Silva *et al.,* 2007). Risk factors of infection prevalence are usually less informative for understanding factors of transmission than infection incidence studies (Dohoo *et al.*,

2009). Therefore, a risk factor incidence study of sero-conversion of dogs to *N. caninum* or a study of oocyst-shedding dogs (recently infected) would be a useful addition to the literature.

This study suggests that canine neosporosis is common in Kenyan dairy cattle farms and that lack of confinement does predispose dogs to infection. Measures should be taken to reduce exposure of dogs to potential sources of infection, such as through placentae and foetuses from abattoirs. Faecal contamination of water and feed sources by potential definitive hosts should also be avoided in order to break its life cycle. In addition, studies should be carried out to determine the reproductive effects of *Neospora caninum* on cattle in Kenya.

CHAPTER SIX

6 THE EFFECT OF BOVINE VIRAL DIARRHOEA VIRUS, *Brucella abortus* AND *Neospora caninum* ON THE INCIDENCE OF ABORTION/FOETAL LOSS IN DAIRYCATTLE HERDS IN NAKURU DISTRICT, KENYA

6.1 Introduction

The success of most animal production systems is dependent on successful reproduction that leads to survival of a conceptus to term (Vanroose *et al.*, 2000). While high fertilization rates of up to 90% have been reported, up to 65% of embryos are estimated to be lost before term (Prenatal losses) leading to significant economic losses and biological waste to the animal industry (Arthur *et al.*, 1999). Some of the losses include disruption of scheduled lactations and loss of replacement stock. They also lead to culling, which adversely affects herd genetics, and some of these losses can be psychologically catastrophic to the farmer (Carpenter *et al*., 2006; De Vries, 2006).

Reproductive diseases often cause prenatal losses by creating a hostile uterine environment, such as in endometritis, or by having a direct cytolytic effect on the foetus, as is seen in BVDV infections or placentitis (Vanroose *et al.*, 2000). Systemic diseases that lead to fever can also cause prenatal losses by three main methods; denaturing embryonic proteins; elevated prostaglandins from the infection leading to luteolysis; and elevated steroids leading to direct foetal loss. These steroids may also cause immunosuppression, making the foetus and dam vulnerable to infection (Vanroose *et al.*, 2000).

Some of the important infectious agents that have been reported to cause prenatal losses in cattle are bovine viral diarrhoea virus (BVDV), *Brucella abortus*, *Campylobacter foetus*, *Chlamydophila abortus*, *Escherichia coli*, infectious bovine rhinotracheitis virus, *Leptospira* spp, *Salmonella* spp., *Neospora caninum*, Rift Valley fever virus, *Toxoplasma gondii*, as well as several fungal species, such as *Absidia* spp and *Aspergillus* spp. (Radostits *et al*., 1994; Vanroose *et al*., 2000; Carpenter *et al*., 2006; Murray, 2006; Stahl *et al*., 2006; Nuotio *et al*., 2007).

Prenatal losses can be classified into two types, embryonic mortality/early embryonic death and foetal death (Vanroose *et al.,* 2000). Early embryonic death is thought to be more common than foetal death, accounting for up to 95% of the prenatal losses. However, it is less commonly reported since most of the losses occur early in gestation in the pre-implantation stage and are therefore more difficult to detect (Vanroose *et al*., 2000; Diskin and Morris; 2008). Early embryonic death in cattle manifests as return to oestrous or an extension of the inter-oestrus interval; foetal death on the other hand can result in either foetal mummification or abortion (Arthur *et al.*, 1999).

The general principles in the diagnosis of bovine prenatal loss involve the collection of a complete history of the case and relevant epidemiological data, such as recent introductions into the farm, determination of the number of animals affected, examination of the breeding, health and feeding records, careful examination of the affected dam, and collection of the expelled foetus and placenta for pathological and microbial examination. Furthermore, samples such as paired serum samples, urine, milk

and vaginal swabs can also be collected for analysis. The results are then collated and analyzed to reach a diagnosis (Miller, 1986).

The diagnostic rate in bovine abortions is very low. This is due to the diverse range of pathogens involved, as well as the fact that factors affecting the dam, foetus and placenta may also be involved (Radostits *et al*., 1994). Prenatal abortion also often follows an initial infection which may have gone on for several weeks or months; the aetiology often is not detectable by the time the abortion occurs. The high cost of laboratory work also compounds the problem. Positive diagnostic rates of 17 % and 43 % have been reported in British and American dairy cattle herds, respectively (Carpenter *et al.*, 2006; Murray, 2006). Other methods, such as the use of ultrasound scans, have helped to improve the diagnosis of early embryonic death (Arthur *et al*., 1999).

Reports on prenatal loss and pathogens that can cause abortion in Kenya are available; *Leptospira* and *Campylobacter* have been confirmed to occur (Agumbah, 1977; De Souza, 1982; Macharia, 1989; Odima, 1994). A review of the records at Nakuru regional Veterinary Investigation Laboratory (NVIL) revealed that between January 1997 and October 2007, 1,182 cases of abortion were reported. Only 124 (10.4 %) were positively identified as brucellosis while the rest (89.6 %) had no definitive diagnosis. The other causes remained unknown and therefore interventions were difficult to institute to reduce the problem. Therefore, there is an urgent need for research to address causes of bovine abortion in Kenya and their associated risk factors.

Factors that have been reported to increase the risk of abortion in dairy cattle herds include: being a heifer; being a cow more than 10 years old; feeding on communal

pastures; lack of vaccination against abortifacient diseases; and reproductive problems such as retained placentae, dystocia, uterine prolapse and stillbirth in the previous pregnancies (Waldner and Garcia, 2013; Waldner, 2014).

This phase of the study was designed to determine the effect of Bovine Viral Diarrhoea Virus, *Brucella abortus* and *Neospora caninum* on the incidence of abortion/foetal loss in dairy cattle herds in Nakuru District,

6.2 Materials and methods

This phase of the study was carried out between January 2010 and May 2011 in the former greater Nakuru District.

6.2.1 Selection of study farms and animals

Farms were randomly selected as described in Section 3.3.2 and a pregnancy diagnosis was performed by rectal palpation. Animals found to be 40 - 60 days pregnant were selected into the study. Information on the age, breed, sex, production system (free-range/zero-grazed), breeding method, herd category (large/small scale), parity, medical history, reproductive history and vaccination status was also collected by interviewing farmers and by observation on the selected farms.

Rectal palpation was performed monthly to test for continued pregnancy in cows, and serum samples were collected monthly until the cow calved or the pregnancy was lost (abortion/EED/mummification). The time of pregnancy loss was considered to be when the foetal loss was detected. Blood testing was done monthly to monitor rise in antibody titres (to BVDV, *Brucella abortus* and *Neospora caninum*). Detection, diagnoses and subsequent abortion recordings were made by the veterinarian and/or farmer and the date of an abortion was estimated retrospectively. Animals that died or were sold from the farms during this period were considered as withdrawals.

6.2.2 Sample handling and laboratory analysis

The monthly serum samples collected were handled and analysed as described in Section 3.3.3.

6.3 Statistical analysis

6.3.1 Descriptive statistics

Descriptive statistics were computed for abortion incidence risk (overall and cause-specific) and frequency of management variables, as well as incidence ratesfor new infections and/or recrudescence/reinfection of existing infections, regardless of abortion incidence. Assuming cows would only have one four-fold increase in titre during the pregnancy monitoring period, the incidence rate of new infections and/or recrudescence/reinfection of existing infections (regardless of abortions) was calculated as:

$$\frac{\text{number of cows with 4X titre increases}}{\text{number of cow months at risk}}$$

where the number of cow-months at risk was determined by:-

$$(\text{No. of cows} - \text{half No. of cows with 4X titre increases} - \text{half No. withdrawals}) * 7 \text{ months.}$$

The incidence risk of abortion was calculated as:

$$\frac{\text{Number of cows aborting X } 100}{\text{Number of cows} - 0.5 \text{ withdrawals}}$$

To calculate cause-specific incidence risks of abortion, monthly antibody levels to BVDV, *Brucella abortus* and *Neospora caninum* were examined around the times of the reported abortions, with a four-fold increase in a titre for a specific pathogen indicating the likely aetiology of the abortion by that pathogen (Graham *et al.,* 1998).

6.3.2 Analytical statistics

In Genstat®, correlation analyses were performed to test and quantify univariate associations between continuous variables. Pearson's Chi-square tests were used for differences in dichotomous variables between groups (e.g. those that aborted versus those that didn't abort).

Multivariable logistic regression was carried out to model the effects of potential risk factors on the incidence of abortion in dairy cattle in Nakuru District, while controlling for the effects of confounding of other variables in the model. Interaction terms were explored as a cross-product variable between the variables that were significant in the model or for which interaction was hypothesized. A backward elimination procedure was used to build the model of significant main effects and interaction effects on abortion. Factors that were found significant ($P < 0.05$) were retained in the final model.

Odds ratios, as a measure of strength of association between the significant model variables ($P < 0.05$) and the outcome, were calculated. Overall models and cause-specific models were explored for pathogens that caused at least 10 abortions. Potential clustering of animals within farms was controlled for by including farm as a random effect in the models.

6.4 Results

6.4.1 Descriptive statistics

6.4.1.1 Animals selected and monitored in the study

Of the 279 cattle selected into the study, 260 were followed up to the termination of their pregnancies. There were 19 cattle (6.9%) that were lost to follow-up due to sales from the selected farms.

6.4.1.2 Animal variables

Friesians were the most common breed encountered, comprising 53.8% (140) of the selected cattle. The rest of the breeds were Ayrshire 25.8% (67), Guernsey and Sahiwal at 6.9% (18) each, Jersey 5.8% (15) and 2 (0.77%) were dairy cross-breeds.

With regard to the age of the selected animals, 48.8% (127/260) were 49-96 months old, 40.8% (106) were 13-48 months old and 10.4% (27) were more than 96 months old.

With regard to the parity of the selected animals, the mean was 2.7 with a standard deviation of 2.0, median 3, minimum 0 and maximum 9.

6.4.1.3 Location

In this study, 42.3% (110) of the selected animals were from Rongai, 24.6% (64) were from Molo, 13.1% (34) were from Naivasha, and 20% (52) were from Njoro.

6.4.1.4 Feeding system

In this phase of the study, zero-grazing/stall-feeding at 51.5% (134) was marginally higher than free-range grazing at 48.5% (126) as a method of rearing dairy

cattle. Of the 260 animals monitored, 72.3% (188) were from small-scale farms while 27.7% (72) were from large-scale farms. Among the large-scale farmers, free-range grazing was more common than stall feeding, at 58.3% and 41.7%, respectively. However, among the small-scale farmers, stall-feeding was marginally higher than free-range grazing, at 51.5% and 49.5%, respectively.

6.4.1.5 Breeding methods

Among the dairy cattle selected for this phase of the study, for the current pregnancies, 66.9% (174/260) were bred by artificial insemination using imported semen, 30.8% (80/260) were bred by artificial insemination using local semen, and 2.3% (6/260) were bred by natural mating.

6.4.1.6 Reproductive history

With regard to the reproductive history, 14.2% (37/260) of the selected animals were reported to have developed at least one reproductive disorder. The frequency of these disorders were abortion at 6.9% (18/260), infertility at 6.1% (16/260) and repeat breeder syndrome 1.2% (3/260).

6.4.2 Abortion incidence

Of the 260 animals monitored in the study, 11.9% experienced reproductive wastage; the incidence of abortion was 11.1% (28) while the incidence of early embryonic death, deformed foetus at term and foetal mummification was 0.4% (1) each.

6.4.2.1 Univariable associations with abortion

With regard to the age of the monitored animals, cases of abortion were more common in the young and middle aged. No cattle more than 96 months old aborted. The frequency of abortion was highest in dairy cattle 13-48 months 14.1% (15/106) while the frequency in cattle 48-96 months old was 10.2% (13/127) and this difference was statistically significant ($P<0.05$, OR=1.4). Cases of early embryonic death, deformed foetuses and mummified foetuses were only reported in cattle 13-48 months old occurring at a frequency of 0.4% each.

With regard to the breeds, the frequency of abortions was highest in Friesians, occurring at 13.5% (19/140), and this difference was statistically significant ($P<0.05$, O.R. =1.93). The frequency in the other breeds was Sahiwal 11.1% (2/18), Ayrshire 8.9% (6/67) and Jersey 6.6% (1/15). No cases of abortion occurred in the Guernsey (0/18) and Dairy crosses (0/18). Deformed foetuses and mummified foetuses only occurred in Friesian cattle, both with a frequency of 0.7% (1/140). In addition, the only breed that had early embryonic death was a Friesian at 0.7% (1/140). This animal was found pregnant at less than 60 days and returned to cycling 7-10 day later indicating foetal loss without observation which is suggestive of early embryonic death.

Most of the foetal losses occurred in dairy cattle with low parity. The frequency of abortion was higher ($P < 0.05$, OR = 2.9) in dairy cattle with parity ≤ 3 (18.6 %) relative to cattle with parity > 3 (6%). Animals with parity more than 6 did not develop any foetal loss. The details are shown in Table 6.1.

Table 6.1 Distribution of foetal loss (n=31) by parity in dairy cattle farms in Nakuru District, Kenya in 2010-2011

Reproductive disorders	Parity	Examined animals	Number (%) that had foetal loss
abortion	0	39	2 (5.1)
	1	38	8 (21)
	2	50	5 (10)
	3	46	5 (10.8)
	4	34	4 (11.7)
	≥ 5	53	4 (7.5)
Deformed foetus at term	2	50	1 (2.0)
Mummified foetus	1	38	1 (2.6)
Early embryonic death	3	46	1 (2.1)

The frequency of abortions was highest in dairy cattle from Njoro at 17.3% (9/52). The frequency in the other areas was Naivasha at 11.7% (4/34), Molo at 10.9% (7/64) and Rongai at 7.2% (8/110). When comparing the frequency of abortion between the Naivasha (an urban area) and the rural areas (Molo, Rongai and Njoro), the differences were marginal, occurring at 11.7% and 11.8%, respectively. This difference was not statistically significant ($P>0.05$, OR = 1.1). The single cases of foetal mummification and deformity were both reported in Rongai, while the single case of EED was reported in Molo (1.5%).

The frequency of abortion in cattle bred by artificial insemination (A.I.) using imported semen was 16.3% (13/80), higher than in cattle bred by artificial insemination using local semen at 8.6% (15/174). This difference was statistically significant ($P<0.05$, OR=2.1). Cases of foetal deformity and mummification were also only reported in cattle bred by artificial insemination with imported semen, at 1.3% (1/80) each. The single dairy cow that had early embryonic death was bred by A.I. using local semen.

With regard to the herd sizes, the frequency of abortion in large-scale farms, at 12.5% (9/72), was marginally higher than in small-scale farms at 10.1% (19/188). However, this difference was not statistically significant ($P>0.05$, OR = 1.3). Cases of foetal deformities (0.53%), mummification (0.53%) and early embryonic death (0.53%) were only reported in the small-scale farms.

With regard to the medical histories of the selected dairy cattle, of the 28 animals that aborted in this study, 2 (7.1%) had histories of abortions in previous pregnancies

while 1 (3.5%) had a history of infertility. The animals that had foetal deformity and mummification as well as EED did not have histories of previous reproductive problems.

6.4.2.2 Stage of foetal loss

Eighty percent of the foetal losses occurred between 4-7 months of gestation. The details are as shown in Table 6.2 and the Kaplan – Meier survival analysis in Table 6.3.

Table 6.2 Stage of gestation of foetal loss (n=31) in 260 dairy cattle in Nakuru District, Kenya in 2010-2011

Month	Number	Percentage
< 2	1 (E.E.D.)	3.2
3	2	6.5
4	7	22.6
5	5	16.1
6	8	25.8
7	5	16.1
8	2	6.5
Term	1 foetus with deformities	3.2

Table 6.3 Kaplan-Meier output on stage/time of foetal loss (n=31) in 260 dairy cattle herds in Nakuru District, Kenya

Time of foetal loss (Months)	Survival function	95% confidence limits	
2	0.9962	0.989	1.000
3	0.9846	0.970	1.000
4	0.9577	0.933	0.982
5	0.9385	0.909	0.968
6	0.9077	0.873	0.943
7	0.8923	0.855	0.930
8	0.8846	0.846	0.923
9	0.8808	0.841	0.920

6.4.2.3 Incidence rates of NC, BVD and BA

The incidence rates of the NC, BVD and BA pathogens in this study population were 1.1 new infections/100 cow-months at risk, 0.06 new infections/100 cow-months at risk and 0.5new infections/100 cow-months at risk respectively.

6.4.2.4 Infections vs foetal loss

Of the three infections under investigation as causes of abortion/foetal loss, *Neospora caninum* (NC) was associated with the most losses at 29.0% (9/31), followed by mixed infections with NC and BVDV at 12.9% (4/31). BA infection led to 6.5% of the foetal losses (2/31). Ten (32.3%) of the cases of foetal loss were not associated with any of the three infections under investigation. The animal that had foetal mummification had a four-fold increase in antibody titres to BVDV, while the animal that had a deformed foetus had a four-fold increase in antibody titres to NC and BVDV. The single case of EED was not associated with any infection. The details are as shown in Table 6.4.

Table 6.4 Distribution of infections associated with foetal loss (n=31) in 260 dairy cattle in Nakuru District, Kenya, 2010-2011

Abortifacient pathogen	Number	Percentage
NC	9	29.0
NC[1] and BVDV	4	12.9
BVDV	3	9.7
BA	2	6.5
NC[1] and BA	2	6.5
NC[1], BA and BVDV	1	3.2
No NC, BA or BVDV infection	10	32.3
Total	31	100

[1]Indicates animals that had co-infections (i.e four-fold rise in antibody titres to more than one pathogen with subsequent foetal loss)

6.4.3 Regression analysis

The results of the multivariable regression analysis indicated that the only factor associated (negatively) with the occurrence of bovine abortion in the study was the age of the animal ($P<0.05$). The breeding system was only marginally significant. The cause-specific models had no significant independent variables left in the models.

Table 6.5 Multivariable regression analysis of risk factors for foetal loss (n=31) in 260 dairy cattle in Nakuru District, Kenya

Factor	Coef.	Std. Err.	Z	P value	95% Confidence Interval	
[1]Age	-.6298745	.2561126	-1.93	0.013	-1.421137	-.075376
Breeding system	-.5788346	.3647293	-1.42	0.102	-.1360217	.1241272

[1]The only factor significant in the final model

6.5 Discussion

In this study, all three pathogens were present in the population and were associated with reproductive wastage. While BVDV was the most common abortifacient pathogen in this study, NC was associated with the most foetal losses (29.0 % by itself). This was consistent with other studies (Romero *et al*., 2002; Boger and Hattel, 2003; Frossling *et al*., 2003; Garcia –Vazqueza *et al*., 2002; Vaclavek *et al*., 2003; Canada *et al*., 2004; Waldner, 2005; Dubey and Schares, 2006; Fernandez *et al*., 2006; Murray, 2006; Paradies *et al*., 2007; Silva *et al*., 2007; Yang *et al*., 2012), who found that NC was associated with 3.9% - 69% of all diagnosed abortions in dairy cattle herds. Although BVDV was the most prevalent of the three abortifacient agents in the present study, it was not the one most associated with foetal loss. This may have been due to the fact that most BVDV abortions often occur when previously unexposed dams are infected during gestation since they are not immune to the virus (Grooms, 2004; Lanyon *et al*., 2014).Thus, with the high prevalence rates of BVDV in this study, most animals may have been previously exposed and recovered, and therefore the low rates of abortion/foetal loss attributable to this pathogen. Previous studies have tried to investigate interrelationship between BVDV and NC as causes of abortion/foetal loss. In this study, the pair of pathogens led to 12.9% of the foetal losses, though the association was not statistically significant ($P>0.05$).

In this study, positive diagnostic rates for foetal loss of 67.7% were achieved. This was much higher than rates of between 17% and 56.3% that have been reported in previous studies (Carpenter *et al*., 2006; Murray, 2006; Yang *et al*., 2012). This high rate

of positive diagnosis may have been due to the fact that the animals selected into the study were closely monitored until the foetal loss occurred. Samples had therefore been collected prior to the foetal loss and after, thus increasing the chances of diagnosis. All the other reports were based on samples collected after the foetal loss. By this time, it is not easy to detect the aetiology since infection normally precedes foetal loss by weeks or months (Carpenter *et al.*, 2006; Murray, 2006).

Several infections, such as BA and BVDV, have been reported to be transmitted through the use of artificial insemination (Eaglesome and Garcia, 1997). In this study, the frequency of abortions was significantly higher in dairy cattle bred by artificial insemination using imported semen relative to those AI bred using local semen. This difference may be due to the potential of imported semen having strains of abortifacient pathogens that our local cattle population are not immune to. In addition, the poor biosecurity measures seen in most farms in this study may lead to increased chances of transmission of some of these pathogens, such as BVDV, by animal health providers, as has been reported in previous studies (Presi *et al*., 2011).

Neospora caninum was the pathogen most associated with abortion in this study. Abortions in cattle due to NC have been reported to occur commonly from 5-6 months of gestation (Stahl *et al*., 2006); this may have been the reason why 80% of the abortions/foetal loss in this study were recorded from 4-7 months of gestation.

In this study, cases of foetal loss were more common in young and middle-aged dairy cattle. Indeed, no dairy cattle more than 96 months old had abortion or any foetal loss. This may have been due to younger animals being naive to most abortifacient

pathogens, thus making them more likely to contract these infections and subsequently abort. A similar trend was seen in dairy cattle with lower parity having more foetal loss than the ones with higher parity (Schares *et al*., 1998). In fact, the age of the dam was the only statistically significant risk factor ($P<0.05$) to the occurrence of bovine abortion in this study.

The main limitations of this study were the failure to recover aborted foetuses as well as placental tissue, which would have helped to enhance the diagnosis of the actual causes of abortion. Farmers and animal health providers should be informed on the importance of submitting these samples in cases of abortion. Further research should also be carried out to determine the effects on production, as well as economic impact of abortifacient pathogens in the dairy cattle industry in this country.

CHAPTER SEVEN

7 AN ASSESSMENT OF THE KNOWLEDGE, ATTITUDES AND PRACTICES RELATED TO BOVINE ABORTION AND FOETAL LOSS AMONG ANIMAL HEALTH SERVICE PROVIDERS IN NAKURU DISTRICT, KENYA

7.1 Introduction

Kenya's economy is mainly based on agriculture, and estimates have shown that 15% of the total farm revenue in Kenya is generated from livestock products, 3.5% is contributed by the dairy industry (EAAPP, 2010). Kenya is also considered as having the most developed dairy sector in sub-Saharan Africa with over 5 million improved dairy cattle, the largest population in the Eastern and Southern Africa region, which has made the country self-sufficient in dairy products. Smallholder dairy farms contribute 60% to 90% of the total milk produced (Goldson and Ndeda, 1985; Walshe, 1987; Mbogoh, 1985; Omore *et al*., 1994). The main products are milk and processed milk products, replacement heifers, and meat from dairy bulls and culled cows (EAAPP, 2010).

Despite the progress that the Kenyan dairy industry has undergone over the past 100 years, several factors have hampered its productivity, growth and development. Among these are feeds and feeding, husbandry, high costs of inputs, poor marketing, low adoption of new technologies, lack of extension services, low milk value addition, and animal diseases (Muriuki, 2004; EAAPP, 2010).

Among the problems affecting the animal industry, reproductive problems such as abortion, metritis, stillbirths and infertility have continued to impact negatively on the

Kenyan dairy sector (Agumbah, 1977; Abuom, 2006). Some of these diseases, such as bovine viral diarrhoea, brucellosis and neosporosis, have been reported elsewhere as important reproductive diseases (Radostits *et al*., 1994; Carpenter *et al*., 2006; Murray, 2006; Stahl *et al*., 2006; Nuotio *et al*., 2007). However, in Kenya, very few studies have been carried out to determine the occurrence of these diseases or their impact on dairy cattle abortion.

In chapters 4 and 5 and 6 of this thesis, a high prevalence of BVDV, NC and BA in dairy cattle was found in Nakuru District, as well as their impact on reproduction and abortion. Nakuru District is one of the main dairy farming zones in Kenya, and is also the main catchment area for dairy cattle breeding stock in Kenya (NDDP, 2001; Economic survey, 2007; Statistical abstract, 2007). An assessment of knowledge, attitudes and practices of animal health service providers are important in determining some risk factors to disease occurrence in populations, and in influencing policy on their control (Holt *et al*., 2011). Therefore, this phase of the thesis was designed to determine the awareness, knowledge and practices of the different groups of animal health providers in Nakuru District in relation to bovine abortion. The intention was that the information generated would be used to formulate continuing professional development courses for these people so they can disseminate correct information and best practices to farmers in the study area, and elsewhere.

7.2 Materials and methods

7.2.1 Study area and population

The study was carried out in 2010 in the former greater Nakuru District, which has an area of 7,242.3 km^2 and lies at an altitude of 1,520m to 3,098m above sea level (NDDP, 2001). It is a dairy farming zone.

Farmers who had been selected earlier (section 3.2) were asked for telephone contacts of animal health service providers in the region. These animal health service providers were contacted by phone to determine their willingness to participate in the study. The animal health service providers included veterinarians, animal health technologists, animal health technicians, and traditional animal health practitioners.

7.2.2 Data collection

Face-to-face, semi-structured questionnaires (Appendix 2) were administered to those who were willing to participate in the study (66). Open ended questions were administered to 66 animal health service providers in Nakuru District and the answers were quantified with respect to a give response. From these were generated a frequency table of answers. Data on their level of education (Degree, Diploma, Certificate, and Others), years of experience (less than 5 years, 5-10 years, and more than 10 years), perception on occurrence of dairy cattle abortion (e.g. common, not common, and unsure), factors associated with occurrence of abortion (malnutrition, intoxication, inclement weather, and infections) and infections suspected to be causing abortion (brucellosis, leptospirosis, toxoplasmosis, neosporosis, BVD, Rift Valley fever, infectious bovine rhinotracheitis, and fungal and yeast infections) were collected. Information on

whether they attempted to establish the stage of gestation when abortion occurred, sample collection and handling, as well as the fate of aborted foetuses and placentae, was also determined.

7.2.3 Data management and analysis

7.2.3.1 Data management

Data on the responses from the open ended questions in the questionnaires were entered and stored in Microsoft Office Excel 2007 (Microsoft Corporation, 2007). The data were screened for any entry errors.

The data were imported into Genstat® 13th edition, service pack two, for analysis (VSN international).

7.2.3.2 Descriptive statistics

Descriptive statistics (percentages, means, standard deviations, ranges) were determined for the variables of interest.

7.2.3.3 Analytical statistics

Simple logistic regression was carried out to determine associations among the level of training/years and experience of the animal health providers (predictor) and their response/perception on bovine abortion (importance, causes, risk factors and management).

7.3 Results

7.3.1 Descriptive statistics

Of the 66 animal health service providers interviewed, 11 (16.7%), 24 (36.4%), 27 (40.9%) and 4 (6.1%) were veterinarians, diploma-holders, certificate-holders and traditional practitioners, respectively. In terms of experience, 24.3% (16), 34.8% (23) and 27 (40.9%) had experience of less than 5 years, 5-10 years, and more than 10 years respectively.

Forty-three (65.2%) of the respondents reported that cases of abortion were common in their practice, 13 (19.7%) reported that abortion was not common in their practice, while 10 (15.2%) were not sure.

The factors considered associated with the occurrence of dairy cattle abortion were infections 51 (77.2%), malnutrition 27 (40.9%), inclement weather 25 (37.8%), and intoxication 3 (4.5%). Three respondents (4.5%) were not sure what factors lead to abortion in cattle.

The infectious diseases considered to be associated with bovine abortion were brucellosis 61 (92.4%), Rift valley fever 29 (43.9%), leptospirosis 18 (27.3%), toxoplasmosis 6 (9.1%), infectious bovine rhinotracheitis 4 (6.0%), trichomoniasis 3/66 (4.5%), bovine viral diarrhoea 2 (3.0 %), fungal and yeast infections 2 (3.0%) and neosporosis 1 (1.5%).

Of the 66 respondents who participated in the study, 39 (59%) reported that they made an attempt to find out the stage of gestation the abortion occurred when they

encountered such cases. The gestational stage of occurrence of the abortions they had seen was reported to be early (10.2%), mid-term (30.7%), and late (7.6%), and combinations of mid-term and late abortions (15.3%).

In managing cases of abortion, 55 (83.3%) gave systemic antibiotics, 44(66.6%) collected samples for analysis, 26 (39.3%) removed retained placentae, 17 (25.7%) recommended skipping of heats before the next service, 10 (15.1%) recommended culling, and 1 (1.1%) inserted intrauterine pessaries to treat the infection. Of the 44 animal health service providers who collected samples for analysis, 44 (100%) collected serum samples, 11 (25%) collected foetal fluids, 9 (20.4%) collected vaginal secretions, 8 (18.1%) submitted the foetus to the laboratory, 4 (9%) submitted placental tissue and 1 (2.2%) collected bacteriological samples using tampons.

7.3.2 Comparison of the level of training with knowledge and practices on bovine abortion

Veterinarians reported that abortion was perceived to be common in the areas where they practiced, while traditional practitioners reported abortion was not common in their practice. This difference was statistically significant ($P<0.05$).

Most respondents, regardless of the level of education, associated the occurrence of abortion with infections. Other common factors were inclement weather (increased rainfall) and malnutrition. There was a statistically significant difference in the perception of infections, weather and malnutrition as risk factors for abortion between the veterinarians/diploma-holders and the certificate-holders/traditional practitioners ($P<0.05$).

Veterinarians recognized the most infectious diseases associated with abortion (brucellosis, leptospirosis, toxoplasmosis, neosporosis, BVDV, RVF, IBR, fungi/yeasts and trichomoniasis). The diseases thought to be most associated with the occurrence of abortion were brucellosis and Rift Valley fever. Neosporosis and bovine viral diarrhoea were the diseases least commonly associated with abortion by animal health service providers in Nakuru District. More veterinarians listed *Toxoplasma gondii*, BVDV and *Leptospira* spp. as major causes of bovine abortion compared to the providers with other levels of training, and these differences were statistically significant ($P<0.05$).

Fifty-nine percent (59%) of the animal health service providers in Nakuru district tried to determine the stage of gestation at which an abortion occured. One hundred percent (100%) of the veterinarians interviewed did try to establish the stage of abortion, whenever possible. The frequency of determination of the stage of abortion in the rest of the groups was: diploma - 83.3 %; certificate - 25.9 %; and traditional practitioners - 25%. This difference was statistically significant ($P < 0.05$). Most of the abortions were perceived to occur in the mid-term and late gestation.

Most of the animal health service providers in Nakuru District managed cases of abortion by administration of systemic antibiotics and collection of samples. All the veterinarians interviewed collected samples for analysis and gave systemic antibiotics. A large percentage of the veterinarians also recommended skipping heats before the next service (91%), and 63.6 % recommended culling. These differences in management were statistically significant ($P<0.05$) when compared to the other training levels. None of the veterinarians performed invasive intrauterine procedures, such as removal of retained

placentae and administration of intrauterine pessaries, unlike the other training levels, but this difference was not statistically significant ($P>0.05$).

Sixty-six percent (66%) of the animal health service providers in Nakuru District collected samples in cases of abortion for analysis. Most samples were collected by veterinarians and the most common sample collected was serum. There were statistically significant differences ($P<0.05$) in the types of samples collected (serum, foetuses, foetal fluids, vaginal secretions, placentae and tampons) between veterinarians and the other training levels. The details of these results are shown in Table 7.1

Table 7.1 Distribution of knowledge, attitudes and practices on bovine abortion by level of training among animal health service providers in Nakuru District, 2010.

Knowledge[1], attitudes[2] and practices[3]	Level of training			
	Degree (n=11)	Diploma (n=24)	Certificate (n=27)	Traditional practitioners (n=4)
Occurrence of abortion[1,2]				
• Common[a]	100	83.3	44.5	0
• Uncommon	0	16.7	22.2	75
• Not sure	0	0	33.3	25
Risk factors associated with abortion[1]				
• Malnurition[a]	63.6	47.7	37	0
• Intoxication[a]	9.1	4.2	0	25
• Inclement weather	91.0	54.2	7.4	50
• Infections[a]	100.0	95.8	55.6	20
• Not sure	0	0	7.4	0
Infections associated with bovine abortion[1]				
• Brucellosis	100	95.8	85.2	100
• Leptospirosis[a]	81.8	33.3	4.2	0
• Toxoplasmosis[a]	54.5	0	0	0
• Neosporosis	9.1	0	0	0
• BVDV[a]	18.2	0	0	0
• RVF	100	45.8	25.9	0
• IBR	36.4	0	0	0
• Fungi and yeasts	18.2	0	0	0
• Trichomoniasis	27.3	0	0	0
Perceived gestation stage of abortion[1,3]				
• Early (<3 mons.)	0	0	57.1	0
• Midterm (3-6 mons)	18.2	45.0	42.9	100
• Late (>6 mons.)	27.3	55.0	0	0
• Midterm and late	54.5	0	0	0
Management of abortion cases[1,3]				
• Collect samples[a]	100	91.7	33.3	50
• Systemic antibiotics[a]	100	87.5	77.8	50
• Intrauterine pessaries	0	4.2	0	0
• Remove retained placentae	0	58.3	37.0	50
• Recommend culling[a]	63.6	4.2	7.4	0
• Skip heats before next service[a]	91.0	12.5	14.8	0
Samples collected in abortion cases[1,3]				
• Serum	100	91.6	33.3	50
• Foetus	72.7	0	0	0
• Foetal fluids	54.5	1.8	0	0
• Vaginal secretions	54.5	2.7	0	0
• Placentae	36.3	0	0	0
• Tampons	9	0	0	0

[a]Statistically significant difference (P<0.05) between the groups' responses

7.3.3 Comparison of the years of experience against perceptions and management of bovine abortion

An analysis of the years of experience in animal health service provision was carried out. Most of the respondents (40.9%) had experience of more than 10 years, while 34.9% had experience of between 5-10 years and 24.2% had experience of less than 5 years. Those with less than five years of experience reported that cases of abortion were common in their practice more than the other groups. However, this difference was not statistically significant ($P>0.05$).

An analysis of the years of experience in animal health provision against the importance of abortion revealed that those with <10 years of experience reported abortion to be an important condition relative to those with >10 years of experience. However, this difference was not statistically significant ($P>0.05$).

An analysis of the factors considered associated with bovine abortion revealed that animal health providers with > 10 years experience associated infectious agents with the occurrence of abortion more than those with < 10 years. On the other hand, animal health service providers with < 5 years experience associated malnutrition with abortion more than those > 5 years experience however none of these differences were statistically significant ($P > 0.05$).

Brucellosis (88.8%-95.6%) was the most common disease considered associated with abortion in dairy cattle in Nakuru District by all the groups, regardless of the years of experience. Conditions such as neosporosis, BVD and fungal and yeast infections were only reported by animal health providers with less than 10 years of experience. There

were differences in the responses between the groups, however, none of them were statistically significant (P>0.05).

Thirty-nine animal health service providers (36/66; 59%) in Nakuru District reported that they determined the stage of gestation in cases of abortion. The group with the highest level of response in determination of the stage of abortion/gestation were those with 5-10 years of experience (73.9%). Those with < 5years and > 10 years of experience determined the stage of abortion less often, at 68.8% and 40.7%, respectively. This difference was statistically significant (P<0.05).

There were differences in the management of cases of abortion between the animal health service providers based on their years of experience. Those with less than 5 years of experience were more likely to recommend culling, and this difference was statistically significant (P < 0.05).

In addition, most of the samples were collected by those with experience between 5-10 years, while those with more than 10 years of experience collected samples least frequently. However, these differences were not statistically significant (P>0.05). There were differences in the handling of samples collected from cases of abortion. Animal health service providers with more than 10 years of experience were more likely to keep samples chilled prior to submission to the laboratory, as well as use transport media. This difference was statistically significant (P<0.05). The details are of these results are shown in Table 7.2.

Table 7.2 Distribution of knowledge, attitudes and practices on bovine abortion by years of practicing experience among animal health service providers in Nakuru District, 2010.

Knowledge attitudes and practices		Years of practicing experience		
		< 5 years (n=16)	5-10 years (n=23)	>10 years (n=27)
Occurrence of abortion	• Common	68.8	65.2	62.9
	• Uncommon	18.7	22.7	18.5
	• Not sure	12.5	13.0	18.5
Importance of abortion	• Very important	50.0	52.2	44.4
	• Moderately important	43.8	30.4	33.3
	• Not important	6.2	13.0	22.2
	• Not sure	0.0	4.3	0.0
Factors associated with abortion	• Malnutrition	56.2	39.1	40.7
	• Intoxication	0.0	4.3	7.4
	• Inclement weather	43.8	47.8	33.3
	• Infections	62.5	86.7	92.6
	• Not sure	0.0	4.3	7.4
Infections associated with bovine abortion	• Brucellosis	93.75	95.6	88.8
	• Leptospirosis	25.0	34.7	22.2
	• Toxoplasmosis	18.7	4.3	7.4
	• Neosporosis	0.0	4.3	0.0
	• BVDV	0.0	8.6	0.0
	• RVF	50.0	39.1	37.0
	• IBR	12.5	0.0	3.7
	• Fungi and yeasts	6.2	4.3	0.0
	• Trichomoniasis	12.5	0.0	3.7
Perceived gestation stage of abortion	• Early (<3 months)	18.1	11.8	0
	• Midterm (3-6 months)	54.5	23.5	45.6
	• Late (>6 months.)[a]	18.1	52.9	54.4
	• Midterm and late	9.3	11.0	0
Management of abortion cases	• Collect samples	68.8	60.9	63.0
	• Systemic antibiotics	93.8	78.3	81.5
	• Intrauterine pessaries	6.3	4.3	0.0
	• Remove retained placentae	18.8	26.1	40.7
	• Recommend culling[a]	31.3	13.0	7.4
	• Skip heats before next service	31.3	30.4	18.5
Samples collected in abortion cases	• Serum	68.8	78.3	66.6
	• Foetus	6.3	13.0	0
	• Foetal fluids	25.0	17.4	0
	• Vaginal secretions	18.8	21.7	7.4
	• Placentae	12.5	8.7	0
Sample handling	• Keep serum and fluids chilled in a cool box [a]	31.3	39.1	66.6
	• Store in transport media[a]	0	8.7	74.1
	• Keep foetuses and placentae chilled[a]	0	8.7	29.6

[a]Statistically significant difference (P<0.05) between the group's responses

7.4 Discussion

The findings of this study revealed that a large percentage (65.15%) of the animal health service providers in Nakuru District reported abortion as a common occurrence in their practice. In addition, there were significant differences ($P<0.05$) in the responses based on the level of training, with more veterinarians and diploma-holders reporting that abortion was a common occurrence in their practice relative to the other animal health workers with less training. These differences were not apparent when analysis was done comparing the years of experience with how common abortion was ($P>0.05$). This means that years of experience in provision of animal health provision did not change the perception on the occurrence of abortion. This may be due the records of the occurrence of abortion were not kept and most of the respondents relied on memory.

There was also limited awareness on the causes and risk factors of bovine abortion among animal health providers with less training in Nakuru District. The factors considered most commonly associated with the occurrence of bovine abortion were infections (77.2 %), malnutrition (40.9%) and inclement weather (increased rainfall) (37.8%). Veterinarians and diploma-holders did correctly associate more factors with the occurrence of abortion than certificate-holders and traditional practitioners (Table 7.1), and this difference was statistically significant ($P < 0.05$). These differences likely were due to their better training.

The important infectious agents that have been reported to cause abortion in cattle worldwide are bovine viral diarrhoea virus (BVDV), *Brucella abortus, Campylobacter foetus, Chlamydophila abortus, Escherichia coli,* infectious bovine rhinotracheitis virus,

Leptospira spp, *Salmonella* spp., *Neospora caninum*, Rift Valley fever virus, *Toxoplasma gondii* and several fungal species, such as *Absidia* spp and *Aspergillus* spp. (Radostits *et al*., 1994; Carpenter *et al*., 2006; Murray, 2006; Stahl *et al*., 2006; Nuotio *et al*., 2007). In this study, most animal health providers associated *Brucella abortus* and Rift Valley fever virus with the occurrence of bovine abortion. This is most likely due to the wide publicity received by these two abortifacient pathogens not only in the study area, but also in the whole country due to the recent year 2007 Rift-Valley fever outbreak. Worldwide, there have been reports of *Neospora caninum* and BVDV being among the most common abortifacient pathogens in the bovine species (Dubey, 1999; Bjorkman *et al.*, 2000). However, in this study, the level of awareness on these two diseases was very low among all cadres of animal health providers in Nakuru District, being reported as causes of bovine abortion at 1.5% and 3% of the time, respectively. This may have been due to lack of research on the status of these two diseases in Kenyan cattle or lack of fora for continuing professional development courses for animal health providers to enable them to update their knowledge on current trends in infectious causes of abortion. In addition, NC may have only recently been introduced into the Kenyan veterinary medicine curriculum and students who graduated earlier may not have this education.

Veterinarians associated more infectious diseases with the occurrence of abortion as opposed to the other cadres animal health service providers. These differences were however not apparent when the years of experience were compared with the range of pathogens associated with abortion. These differences were probably due to their exposure to a wider range of pathogens during their training relative to the other cadres.

Most respondents (59%) tried to determine the stage of abortion. Mid- and late-gestation abortions were reported to be the most common. This may have been due to the fact that foetal loss at this stage is more noticeable clinically as opposed to early embryonic death and abortion prior to three months. Veterinarians and diploma-holders were also more likely to determine the stage of abortion, probably due to the fact that they better understood the clinical significance of this finding, since different abortifacient pathogens cause abortion at different stages of the gestation period; therefore an animal health provider who makes an effort to determine this parameter is more likely to narrow down the causes of abortion. Animal health providers with less than 10 years of experience were more likely to determine the stage of abortion ($P<0.05$), perhaps due to improved recent training on the diagnosis and management of abortion cases.

Sample collection and administration of systemic antibiotics were the two most common methods of managing cases of abortion in this study. It was interesting to note that none of the veterinarians in this study performed invasive intrauterine procedures such as removal of retained placentae and administration of intrauterine pessaries whenever they encountered abortion cases. This difference may have been due to the fear of contracting zoonotic abortifacient diseases such as brucellosis and Rift Valley fever. Furthermore, the reasons for the other cadres performing these procedures may have been economical due to the perception that failure to be seen to treat the animals may lead to reduced/lack of payment by the farmers, especially if they are less knowledgeable on taking samples and/or treating with systemic antibiotics.

Abortion has been shown to increase the risk of culling of cattle in many herds, regardless of knowledge on the cause of abortion in an animal (Thurmond and Hietala, 1996). Culling is often recommended for diseases such as brucellosis and bovine viral diarrhoea due to the carrier status of the persistently infected animals and risk of spread in the herd. In this study, animal health providers with more than 5 years of experience were less likely to recommend culling. This may have been due to lack of awareness of the carrier state of BVD or brucellosis, or due to loyalty to the farmers they had served for many years, which probably made it more difficult for them to recommend management practices that would be perceived to lead to losses (Holt *et al.,* 2011).

Standard samples collected in cases of abortion include serum, milk, placentae, foetuses, vaginal secretions and urine. These samples often need to be preserved either by chilling or being kept in transport media in order to prevent deterioration and improve the diagnostic rates (Miller, 1986). The most common sample collected among respondents in this study was serum samples. Though it is recommended that paired serum samples should be collected to improve diagnostic rates in cases of abortion, in this study, none of the respondents routinely did this, which may be part of the reason for the limited collection of samples, at least among the non-veterinarians.

Veterinarians collected the widest range of samples among the different cadres of training, and this also may have been due to their broader training. A lower percentage of animal health providers with more than 10 years' experience collected samples for analysis in cases of abortion. This was probably due to the fact that due to their long years of experience, they would have noticed that there was limited diagnostic capacity in

most of the laboratories in the region and nationally. However, this same group with more than 10 years of experience was more likely to keep the samples they collected chilled, as well as use transport media, compared to the other groups.

The findings of this phase of the study indicate low levels of knowledge among animal health providers on important bovine abortifacient pathogens such as BVDV and NC as well as their risk factors. This would therefore make their control difficult. Therefore, there is a need to enhance their knowledge and skills by conducting professional development courses as well as review of curricula for training animal health service providers in this country.

CHAPTER EIGHT

8 CONCLUSIONS AND RECOMMENDATIONS

The following conclusions were drawn from this study

a) Bovine viral diarrhoea was the most common abortifacient pathogen when checking for serum antibody exposures in 398 dairy cattle in Nakuru District. *Neospora caninum* was the second most common, followed by *Brucella abortus*. Increased age was significantly associated with increased prevalence of BVDV exposure. Open grazing and large herd sizes were marginally associated with increased prevalence of all three pathogens.

b) The seroprevalence of NC in 84 farm dogs in Nakuru District was high at 17.9%. Lack of confinement was significantly associated with increased risk of infection.

c) The incidence risk of abortion among the 260 monitored pregnant cattle was high at 11.1%. There was also 1 early embryonic death, 1 deformed foetus, and 1 foetal mummification. Of the three pathogens examined as causes of abortion/foetal loss, the majority of the abortions were associated with infection by *Neospora caninum* (29.0%) and co-infections by *Neospora caninum* and bovine viral diarrhoea (12.9%).

d) There were very low levels of knowledge by all cadres of animal health providers on the variety of abortifacient pathogens possible. Most associated the occurrence of abortion with *Brucella abortus* or Rift Valley fever virus infection with some knowing about leptospirosis, while the findings of this study revealed that BVDV was the most prevalent pathogen, and NC was the pathogen associated with the most cases of abortion.

To reduce the losses attributed to these infections, the following recommendations should be implemented.

1. A comprehensive policy (including best management practices) on the diagnosis, control and prevention of abortifacient pathogens, including BVDV, *N. caninum* and *B. abortus* in dairy cattle should be developed by the animal health industry and Department of veterinary services.
2. The capacity of the veterinary laboratories to diagnose abortion in cattle needs to be enhanced, including diagnosis of neosporosis and BVD.
3. The knowledge levels of the animal health providers on the causes of abortion in cattle needs to be enhanced, by review of curricula for students, as well as through continuing professional education programmes for existing animal health workers. This knowledge would help them to offer better clinical services, as well as extension information to the farmers.
4. Research institutions such as the University of Nairobi should offer regular and relevant seminars on current trends on bovine abortion should to animal health service providers in Nakuru District to change their attitudes on bovine abortion.
5. The skills of animal health service providers in Nakuru District and indeed the whole of Kenya, should be should be enhanced on through practicums on how to manage cases of abortion.

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APPENDICES

APPENDIX 1 FARMERS' SURVEY QUESTIONNAIRE

PART 1: BACKGROUND INFORMATION.

1) Location of farm_____ Farm size_____

2) Owners details – Name_____

Age_____

Level of education_____

Profession (if any)_____

Occupation_____

3) Do you belong to any dairy society? Yes_____ No_____

4) If yes, which one?_____

HERD STRUCTURE

Mature cows_____ Heifers_____ Mature bulls_____

Bull calves_____ Heifer calves_____

Other animals in the farm_____

_____

_____

GENERAL MANAGEMENT PRACTICES.

1) Deworming_____

2)Vaccination_____Which ones_____

3) Dipping/spraying_____

4) Steaming up._____

5) Dry cow therapy. _____

6) Confirmation of pregnancy.Lack of heat.___Veterinarian.___Inseminator.____

Abdominal distension___Others (specify) _____

METHOD OF SERVICE

What type of service is used?

-Own bull

-Communal bull.

-AI Local_____ Imported_____

-Embryo transfer.

DISEASE RANKING

Is abortion an important disease on your farm? Yes___ No____

Which diseases would you consider as being the most important affecting cattle on your farm?

_____
_____
_____
_____

APPENDIX 2 VETERINARIANS AND PARAVETERINARIANS SURVEY QUESTIONNAIRE

Date_____

Name_____

Location_____

Level of training_____

Years of practice_____

Is abortion/fetal loss commonly encountered in your area of practice? Yes_____No_____

How important is it? (on a scale of 1-10)_____

What do you think is the cause of these abortions?_____
_____
_____

What other diseases are commonly encountered in your area of practice?_____
_____
_____
_____

How do you handle cases of abortion?

Submit samples for analysis_____

Give antibiotic cover_____

Recommend culling_____

Skip heats before service_____

Others_____
_____

APPENDIX 3 INDIVIDUAL ANIMAL FOLLOW-UP RECORD

Farmer's name_____Farm ID. _____.

Location _____ Cow's ID. (Name / number)_____

Age. _____

Visit date	Health status	Pregnancy status	Sample collected and identity	Therapeutic interventions