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Epidemiological studies of brucellosis, campylobacteriosis and trichomonosis, and other factors affecting calving rate in cattle herds in northern Nigeria

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Declaration

The experimental work and results described in this thesis is my original work (except where the input of others is acknowledged), conducted in the Department of Production Animal Studies, Faculty of Veterinary Science, University of Pretoria, South Africa, in the Faculty of Veterinary Madicine, Ahmadu Bello University, Zaria, Kaduna state, Nigeria and in the National Veterinary Research Institute Zonal Office, Yola, Adamawa state, Nigeria. This work has not been submitted in any other form to any other University or academic institution. I declare the above statement to be true.

Hassan M. Mai

December 2012

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Dedication

To: Alhaji Muhammadu Mai (Bappa Manga),

Hajja Fadimatu Aiya Mai,

Hajja Aishatu Indo Mai,

Barr. Nasiru Muhammadu Mai

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List of abbreviations

AFLP- Amplified fragment length polymorphism

AI- Artificial insemination

ANOVA- Analysis of variance

AP- Apparent prevalence

ATCC- American type culture collection

BCS- Body condition score

c-ELISA- Competitive enzyme-linked immunosorbent assay

Cff- *Campylobacter fetus fetus*

CFT- Complement fixation test

Cfv- *Campylobacter fetus venerealis*

CI- Confidence interval

CR- Calving rate

DNA- Deoxy ribonucleic acid

FP- False positive

FPA- Florescent polarization assay

FPSR- False positive serological reactions

H_2S- Hydrogen sulphide

i-ELISA- Indirect enzyme-linked immunosorbent assay

LGA- Local government area

MLVA- Multiple locus variable number tandem repeats analysis

MRT- Milk ring test

OIE- *Office International des Epizooties* (The World Organization for Animal Health)

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PCR- Polymerase chain reaction

PFGE- Pulsed-field gel electrophoresis

RBPT- Rose-Bengal plate-agglutination test

RFLP- Restricted-fragment-length-polymorphism

rpm- Revolutions per minute

roh- Rate of homogeneity

SAT- Serum agglutination test

Se- Sensitivity

Sp- Specificity

subsp.- Subspecies

TP- True prevalence

TSI- Triple sugar iron agar

VMAT- Vaginal mucus agglutination test

ZIP- Zero-inflated Poisson model

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Abstract

EPIDEMIOLOGICAL STUDIES OF BRUCELLOSIS, CAMPYLOBACTERIOSIS AND TRICHOMONOSIS, AND OTHER FACTORS AFFECTING CALVING RATE IN CATTLE HERDS IN NORTHERN NIGERIA

H.M. Mai

Livestock production, of which cattle production is a major component, plays a key role in the socio-economic development of Nigeria, with 70-80% of the nation's population of over 150 million engaged in agriculture and the livestock industry as their major occupation. Cattle production provides essential food products – meat, milk, and other dairy products, animal power, fuel, transport and organic manure for arable farming. However, the productivity and reproductive performance of cattle in Nigeria is generally low due to many factors, including a number of infectious reproductive diseases resulting in decreased calving percentage, infertility, abortion and decreased milk production. Brucellosis is one of the most important reproductive diseases and widespread zoonosis in the world and previous studies have indicated an increase in its occurrence in cattle in Nigeria. In addition, bovine campylobacteriosis and trichomonosis are widespread diseases associated with bovine infertility worldwide.

However, there is little recent or reliable information on the prevalence of these important diseases in Nigeria and their effect on reproductive performance. Most studies have used non-representative samples, small sample sizes and relatively non-specific diagnostic tests. Few studies have been conducted on bovine campylobacteriosis and trichomonosis in Nigeria and none on the concurrence of brucellosis and campylobacteriosis. Therefore, a large cross-sectional study covering Adamawa, Kaduna and Kano states of northern Nigeria was designed. A multistage random cluster sampling strategy was used to sample 4,745 cattle from 271 herds, including diverse production systems. The objectives of the study were to estimate, at the animal and herd level, the seroprevalence of brucellosis in adult cattle, the prevalence of bovine genital campylobacteriosis and trichomonosis in bulls and the association between the three diseases. In addition, the study aimed to identify herd-level managemental and environmental risk factors for each of the diseases, as well as risk factors

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for within-herd seroprevalence of brucellosis. Further objectives were to determine the reproductive efficiency and occurrence of reproductive disorders in the herds, and to estimate the effect of the three infectious diseases, as well as other factors, on calving rate.

Serum samples were tested for antibodies to *Brucella* using the Rose-Bengal plate-agglutination test (RBPT) and positives were confirmed using a competitive enzyme-linked immunosorbent assay (c-ELISA). Thirty-seven percent of all animals were RBPT positive, and after confirmation with c-ELISA the overall true animal-level prevalence, adjusted for test sensitivity and specificity and for sampling weights and clustering in the complex survey design, was 26.3% (95% CI, 22.1%-31.0%). Of the herds sampled, 210 (77.5%; 95% CI, 68.6%-84.5%) had at least one animal positive to both tests; this did not differ significantly between states ($P = 0.538$). A significantly higher seroprevalence of brucellosis was found in males than in females ($P < 0.001$), in non-pregnant than in pregnant females ($P < 0.001$), and in cattle >7 years than in cattle <4 years of age ($P < 0.001$). Seroprevalence was highest in the pastoral management system (45.1%) while the commercial system had the lowest seroprevalence with 15.9% ($P < 0.001$).

Preputial samples of 602 bulls from 250 herds were tested for *Campylobacter fetus* and *Tritrichomonas foetus* using culture and identification. The estimated true animal-level prevalence of *C. fetus* infection in bulls was 16.4% (95% CI: 13.0%-20.7%), of which 18.5% was *C. f. fetus* and 81.5% was *C. f. venerealis*. Of the latter, 92% were *C. f. venerealis* biovar intermedius, the major aetiology of bovine genital campylobacteriosis. A higher prevalence was found in bulls >7 years old (33.4%) than in bulls 4-5 years old (13.6%) ($P = 0.018$). Prevalence was highest in the Gudali breed (28.8%) and in pastoral herds (43.5%). There was a strong positive association between the presence of campylobacteriosis and brucellosis, both within bulls (OR = 8.3, 95% CI: 5.2-13.4) and within herds (OR = 16, 95% CI: 3.8-68) ($P < 0.0001$). All the samples tested for trichomonosis using different isolation methods were negative.

Multilevel logistic regression models were used to identify herd-level risk factors for brucellosis and campylobacteriosis. The odds of both *Brucella* seropositivity and *C. fetus* infection increased significantly with the presence of small ruminants (sheep and/or goats) on the same farm and with the introduction of animals to the farm without quarantine. In addition, *Brucella* seropositivity was positively associated with larger herd size, with the pastoral management system and with the presence of a crush or improvised chute on the

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farm, while regular or occasional gynaecological examination was associated with reduced odds of seropositivity. Initial purchase of stock from a market, regular or occasional gynaecological examination, failure to practice regular or occasional herd prophylactic measures and high rainfall were associated with increased odds of *C. fetus* infection. A zero-inflated Poisson model showed that the presence of small ruminants, the introduction of animals without quarantine, and borrowing or sharing of breeding bulls were associated with a higher within-herd seroprevalence of brucellosis within infected herds, while routine provision of mineral supplementation was associated with a lower within-herd seroprevalence.

Of all the cows, only 6.5% were lactating and pregnant, 37% were lactating but non-pregnant, 26% were non-lactating but pregnant and 30% were non-lactating and non-pregnant. The calculated annual calving rate was 51.4%. There was a positive association at the herd level between brucellosis infection and the occurrence of abortion, retained afterbirth, stillbirth and weak calves or calf mortality. Multilevel linear regression analysis showed that *Brucella* and *C. fetus* infection of herds were each independently associated with an absolute reduction in calving rate of 14.9% and 8.4%, respectively, and that there was a strong inverse relationship between within-herd *Brucella* seroprevalence and calving rate. In addition, the presence of small ruminants, introduction of animals without quarantine and the presence of crush or improvised chute were associated with lower calving rates, whereas larger herd size, supplementary feeding, routine mineral supplementation, isolation and observation of the cow during parturition and removal of afterbirth were associated with higher calving rates.

The prevalences of brucellosis and campylobacteriosis were higher than previous reports, suggesting an increase in the prevalence of both diseases in northern Nigeria. The pastoral management systems of the traditional Fulanis may be encouraging the dissemination of the diseases, which are having an adverse effect on reproductive performance. Public enlightenment of the farmers about the diseases, vaccination and appropriate potential national control measures are recommended in order to improve the productivity of the cattle herds.

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Chapter 1

Introduction and literature review

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Background

Reproductive performance is vital to the continued existence of cattle farming, since it determines the production efficiency of an animal or herd and maintenance of a desirable level of production (Zemjanis, 1974). Cattle production is the major component of livestock production in most developing countries and is mainly in the hands of numerous small farmers. Nigeria has a cattle population of 18.4 million and 95% of the population is in the hands of the traditional Fulanis who are mostly in the cattle belt of northern Nigeria (Rikin, 1988; Anon, 1994). Prior to independence, agriculture was the most important sector of the economy, accounting for more than 50% of gross domestic product (GDP) and more than 75% of export earnings. With the discovery of petroleum, the agricultural sector was neglected and declined. Agriculture has suffered from years of mismanagement, inconsistent and poorly conceived government policies, and lack of basic infrastructure. Still, the sector accounts for over 26.8% of GDP and two-thirds of employment (Anon., 2012a). Cattle production provides a continuous source of essential food products – meat, milk, and other dairy products. It also generates employment opportunities and income to millions of rural dwellers, animal power, fuel, transport, hides and organic manure for arable farming mainly in the Sudano-Sahelian ecological zones. Cattle are also a source of government revenue through taxes ("Jangali") and serve as an index of social prestige among the nomadic Fulanis and Shuwa Arab pastoralists (Lamorde, 1998). However, the productivity and reproductive performance of cattle in Nigeria are generally low due to many factors including poor management systems, inadequate nutrition, poor genetic potential, inadequate veterinary services, unfavourable environmental factors and widespread infectious and parasitic diseases (Mukasa-Mugerwa, 1989). The low productivity is reported as one of several adaptive features helping to keep these animals in equilibrium with their stressful environment (Tizikara, 1985). In an attempt to improve on the germplasms of cattle, cross-breeding programmes using artificial insemination (AI), as well as exotic breeds of cattle, were introduced over 65 years ago to upgrade the genetic potential of the indigenous Nigerian cattle (Anon., 2012b).

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Productivity and reproductive performance of cattle

The productivity of cattle is largely dependent on reproductive performance. The reproductive performance of a herd has been shown to be one of the most important starting points in any animal improvement package (Mukasa-Mugerwa *et al*., 1992). For instance, the success of any dairy enterprise depends largely on the reproductive efficiency of the herd. It is therefore important that reproductive problems be identified and remedied as rapidly as possible (De Kruif, 1978). In the livestock industry, the optimal reproductive rate is that which gives maximum economic profit per breeding female per year, and in the case of cattle, producing a calf per cow per year (Pelissier, 1976). To achieve this, pregnancy must be accomplished within 90 days postpartum. However, calving intervals in Nigeria are much longer than 365 days (Oyedipe *et al*., 1981a). Yet, there is no clear-cut management strategy towards improving the reproductive efficiency of cattle. Indigenous cattle are well adapted to the harsh environmental conditions of the tropics and have the ability to utilize high-fibre forages such as crop residue which would have gone to waste. However, the output from *Bos indicus* cattle in terms of milk and meat is low. *Bos indicus* cattle attain puberty late, experience long calving intervals, decreased in calving rate, decreased milk production, low number of calves per cow lifetime and have short productive life (Zemjanis, 1974; Mukasa-Mugerwa, 1989; Voh Jr and Otchere, 1989).

Cattle reproductive performance in Nigeria

The annual growth in population and demand for food in Nigeria is over 3%, while growth rate in food production is 1.0-1.5%, leaving a shortfall of 1.5-2.0% in annual food supplies (Ikhatua, 2002). There is therefore, the need to intensify livestock production, particularly cattle, commensurate with the ever increasing human population to avoid a food security crisis. However, there is generally a substandard development of the livestock industry in Nigeria (Lamorde, 1998).

To improve on reproductive performance and economic benefit of cattle, there should be proper understanding of reproductive parameters, reproductive disorders and factors influencing reproductive efficiency of cattle. Several reproductive parameters have been assessed and, although perhaps not valid estimates due to small and biased studies, most have been found to be suboptimum in Nigerian cattle, such as an average age at puberty of 40.2 months (Knudson and Sohael, 1970), age at first calving of 60 months (Zemjanis 1974;

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Pullan, 1979), calving to first ovulation of 4.6 months (Dawuda *et al.*, 1988), calving to first conception of 7.8 months (Eduvie and Dawuda, 1986), number of services per conception of 1.9 (Zakari, 1981), and conception rate of 50% and first service conception rate of 46.7% (Voh Jr *et al*., 1987). Other reproductive parameters considered include calving interval of 24 months (Voh Jr and Otchere, 1989), annual calf crop of 27-55% (Lamurde and Weinman, 1972), calving rate of 34-55% (Nuru and Dennis, 1976; Zakari, 1981), number of calves per cow lifetime of 2.5 (Zemjanis, 1974), productive life of cattle of 4 to 10 years (Voh Jr and Otchere, 1989) and weak oestrus activity and poor cyclicity (Zakari, 1981).

Reproductive performance is the single most important factor determining the production efficiency of individual animals as well as the entire livestock industry of a country (Zemjanis, 1974). Reproductive indices in Nigeria are affected by several factors such as genetics (Pullan, 1979), adverse environmental conditions (Mai, 1997), age and parity of the dam (Voh Jr and Otchere, 1989), nutrition and body condition score of the dam (Zakari, 1981; Oyedipe *et al.,* 1982b), suckling (Eduvie and Dawuda, 1986), infectious diseases (Ocholi *et al*., 2004a; Mshelia *et al.*, 2010b) and inadequate oestrus detection (Mai *et al*., 2002). Calving rate, calving interval, age at first calving and cow productive life are important factors in cattle breeding herds (Voh Jr and Otchere, 1989; Arthur *et al*., 1996; Youngquist, 1997). The continuous movement of cattle as a result of trade and for grazing, sharing of bulls, and communal grazing by farmers are common practices in Nigeria, putting herds at risk of venereal infections. These husbandry practices also affect the reproductive performance of cattle.

Measures of reproductive performance

There is need to continuously monitor the reproductive performance of a herd for good cattle management. Several measures of reproductive performance have been used, which determine the total number of viable offspring produced by all adult females over a particular time period. The fertility measures used most commonly include: calving interval, days open, age at first calving, calving percentage, calving rate and number of services per conception (McDowell *et al*., 1973; Mukasa-Mugerwa, 1989; Mokantla *et al.*, 2004). These parameters except calving rate and calving percentage do not consider proportion of females that failed to conceive nor provide information on calf viability or survival. Calving interval only takes into account females that have successive parturitions and excludes all of the heifers and any cows that failed to conceive.

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The calving rate is defined as the calves due in one year (including pregnancies) divided by the total number of cows and postpubertal heifers in the herd (Stonaker *et al*., 1976; Voh Jr and Otchere, 1989). This can be determined from one day's examination of a herd. The calving percentage on the other hand considers only the number of calves born per number of female cattle exposed to a bull expressed as a percentage (Mokantla *et al*., 2004). Although the calving percentage accounts for abortions, the farm will have to be visited monthly for a period of one year, particularly the fact that calving occurred year-round in communal herds, which requires monitoring of pregnancies and recording of calvings as they occur. This approach is susceptible to poor, inadequate records of the herds. There are no written records of events and livestock owners rely on what they can recall from memory. The solution to this requires trans-rectal palpation of cattle for pregnancy and cyclicity to determine the actual status of the herds. Furthermore, calving percentage is prone to selection bias due to selective culling of non-conceiving or late conceiving cows before subsequent calving. In this case, the calving percentage will be higher than if the herd was assessed by pregnancy diagnosis before culling had occurred.

The total annual calving rate is an estimate of all the calving activity within a 12 month period; it is a good measure of fertility giving an overview of the reproductive performance of a herd in the previous and the current year (Youngquist, 1997). The calving rate considers the number and estimated ages of calves in the herd as well as the number of pregnant animals and estimated ages of foetuses which give a true picture of breeding efficiency. The calving rate is easily measured and more reliable reproductive parameter than the other indices which can be determined from a single visit. However, single-day examination of a herd for calving rate is prone to some errors: it does not take into account cases of abortion and embryo mortality, and secondly there may be errors in the aging of pregnancies. While some of these errors result in overestimation of annual calving rate, others will result in underestimation. Calving rate is crucial for herd replacement, but is influenced by nutrition, bull fertility, pregnancy loss, disease, environmental factors, management factors and breed (Morrow, 1986; Mickelson, 1990). In traditional agropastoral production systems in Nigeria, calving rate has been reported as 55% (Voh Jr and Otchere, 1989).

Infectious reproductive diseases of cattle

Infectious reproductive diseases remain a major constraint to cattle productivity across all agro-ecological zones and production systems in Africa. Such infectious reproductive

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diseases result in huge economic losses by causing abortion, embryo mortality, repeat breeding, irregular oestrus, retained afterbirth, stillbirth, birth of weak calves and calf mortality and pyometra (Godfroid *et al*., 2004; McFadden *et al*., 2004; BonDurant, 2005). The conditions consequently reduce milk production, prolong calving interval and can cause a large reduction in calf crop (Rae, 1989; Jimenez *et al*., 2011; Mekonnen *et al*., 2011). It was estimated that bovine brucellosis causes a 20% to 25% loss in milk production (OIE, 2011a). Losses in meat and milk production as a result of brucellosis is estimated at $800 million annually in the USA (Richey and Harrell, 2008), $224 million in Nigeria (Esuruoso, 1979) and $37.5 million in South Africa (Anon, 1990). Estimates of annual losses to the USA beef industry due to trichomonosis vary greatly and have been as high as $650 million (Speer and White, 1991; Daly, 2005). Such estimates are not available for Nigeria.

Over the years both governmental and non-governmental interventions in the Nigerian livestock sector have imported semen for AI programmes towards upgrading the genotype of local cattle population. However, there is no information on the screening of AI semen for *Campylobacter fetus, Tritrichomonas foetus* or *Brucella abortus* in Nigeria, even though it is mandatory to test semen and embryos intended for international trade for these pathogens (OIE, 2011a,b), since semen of chronic carrier bulls creates the risk of spread of the diseases within AI programmes or across national borders.

Brucellosis

Aetiology, microbiology and transmission

Brucellosis is one of the most important reproductive diseases and widespread zoonoses in the world (Poester *et al*., 2002). Previous studies have indicated an increase in the occurrence of bovine brucellosis in Nigeria (Ocholi *et al*., 2004a). Bovine brucellosis is caused by *Brucella abortus*, a facultative non-sporulating, non-encapsulated intracellular coccus, coccobacillus or short Gram-negative rod approximately 0.5-0.7 µm in diameter and 0.6-1.5 µm long and the colonies are 0.5-1.0 mm in diameter (Alton *et al*., 1988; OIE, 2011a). Bovine brucellosis is transmitted by contact, ingestion, inhalation and even venereally (Nicoletti, 1980; Bercovitz, 1998), while calves become infected *in utero* or by ingesting infected colostrum or milk (Nicoletti, 1980; Bale and Nuru, 2001). Mammary gland macrophages may provide the intracellular environment for the persistence and permanent localization of *B. abortus* in the mammary gland of chronically infected cattle (Nicoletti,

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1980), and the bacteria may be excreted intermittently in milk throughout the lactation period (Godfroid *et al*., 2004). The disease is also important in other domestic animals, wildlife and humans. Some animals may remain infected for life and spread the disease at parturition or to their calves *in utero* or via their milk (Nicoletti, 1980).

Other factors that may contribute to the spread of brucellosis include indiscriminate buying-in of animals without quarantine (Bale and Kumi-Diaka, 1981), mixed herds of cattle with flocks of sheep or herds of goats (Kabagambe *et al*., 2001; Megersa *et al*. 2011a) and certain breeds of cattle (Karimuribo *et al.,* 2007; Junaidu *et al*., 2008). A significantly higher prevalence has been reported in older cattle (Kadohira *et al.*, 1997; Kubuafor *et al*., 2000), females (Jegerfa *et al.,* 2009; Mekonnen *et al.* 2010; Junaidu *et al*., 2011), non-pregnant cows (Ibrahim *et al*., 2010) and lactating cows (Nicoletti, 1980; Junaidu *et al*., 2011). Calving pens and wet, muddy lush pastures also play a significant role in the spread of the disease (Crawford *et al*., 1990). However, risk factors observed in one particular agro-ecological region do not necessarily apply to another area with different ecological settings and husbandry practices (Matope *et al*., 2010; Mekonnen *et al*., 2010).

More than 500,000 new human cases of brucellosis have been reported annually worldwide, and it is considered a pathogen that has potential for development as a bio-weapon (Seleem *et al*., 2010). In places where brucellosis is endemic, humans can be infected via contact with infected animals or consumption of their products, mostly milk and milk products (Kubuafor *et al*., 2000). Many of the farmers in Nigeria take no measures to protect themselves against brucellosis and are quite willing to drink unpasteurized milk. In northern Nigeria, milk is usually preserved by souring, which does not destroy brucellae as they are preserved in the milk fat (Eze, 1978). Chronic brucellosis can mimic any chronic disease, including tuberculosis, human immunodeficiency virus, rheumatologic disorders, and other conditions including enteric fever, malaria, cholecystitis, thrombophlebitis, fungal infection, autoimmune disease and tumors (Mantur *et al.*, 2007). Initial symptoms are easily confused with those of influenza (Chain *et al*., 2005). Unfortunately, farmers who have symptoms of undulating fever and joint pain very rarely seek medical help. If they do, the fever is usually ascribed to malaria or typhoid. Human brucellosis is often misdiagnosed and thus under-reported in the medical literature (Mantur *et al*., 2006). Symptoms can be very diverse depending on the site of infection and include encephalitis, meningitis, spondylitis, arthritis, endocarditis, orchitis, and prostatitis (Acha and Szyfres, 2003). Spontaneous abortions,

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mostly in the first and second trimesters of pregnancy, are seen in pregnant women infected with *Brucella* (Khan *et al*., 2001). Specific high-risk occupational groups include farm workers, veterinarians, ranchers, herdsmen and meat inspectors (Tabak *et al*., 2008). Making such people aware of a high risk of human brucellosis could encourage them to seek medical assistance and proper diagnosis of their symptoms, with collaboration between veterinary and medical disciplines.

Pathogenesis

Depending on the route of infection, *B. abortus* penetrates the mucous membrane of the pharynx or alimentary tract and move to the reticulo-endothelial system, where the bacteria are phagocytised by neutrophils and macrophages which carry them to the regional lymph nodes where they multiply and cause lymphadenitis which may last for some months (Enright, 1990; Godfroid *et al.*, 2004). The resulting bacteraemia may resolve, persist for several months or be recurrent for at least two years particularly during pregnancy in 5-10% of cases. The organisms then localize in various organs, especially the gravid uterus, udder, supramammary lymph nodes, the spleen and other lymph nodes (Godfroid *et al.*, 2004), while in the bulls, the organisms are localized in the testes and male accessory sex glands (Nicoletti, 1980; Enright, 1990).

The allantoic fluid factor erythritol that is elevated from about the fifth month of pregnancy stimulates the localization, growth and multiplication of the organism in the endometrium of the gravid uterus and in foetal membranes of cattle resulting in endometritis and placentitis (Cunningham, 1977; Thoen and Enright, 1986). Following these abnormalities and depending on the severity of the infection, abortion, stillbirth, premature birth, birth of a viable or non-viable calf may occur (Nicoletti, 1980; Enright, 1990). Although the mechanism of abortion in the second trimester is not clear, trophoblastic cells in which the brucellae multiply secrete steroids such as cortisol. There is also increase in oestrogen and prostaglandin synthesis and decreased production of progesterone that normally maintains pregnancy, thereby inducing parturition (Enright, 1990). Some cows are resistant to infection with *B. abortus* because their macrophages have the ability to destroy *B. abortus*. The reduction of this ability in susceptible cows results in the establishment of chronic infection (Harmon *et al.*, 1989). This macrophage activity is greater in cows that are genetically resistant to infection (Qureshi *et al*., 1996). Furthermore, it has been reported that phagocytes develop antimicrobial defence mechanisms, such as oxidative burst, acidification of phagosomes, or fusion of phagosomes

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with lysosomes to eliminate pathogens (Godfroid *et al*., 2004). Of all the cytokines, IFN-α is the most important and activates the macrophages inducing the intracellular killing of brucellae (Baldwin and Parent, 2002).

Clinical signs

There is a large variation in the incubation period of brucellosis. The length of the incubation period is affected by the size of the infective dose, and the age, sex, stage of gestation, and immunity of the infected animal (Crawford *et al*., 1990). It has been reported that cows infected at service abort after an average interval of 225 days, those infected at seven months' gestation abort about 50 days later (Thomson, 1950). Congenitally infected calves manifested clinical signs 18 months later; and the longest incubation period ever recorded in a cow was nine years (La Praik *et al.*, 1975). Several factors contribute to the abortion in herds such as management practices, susceptibility of the pregnant animals, severity of challenge, duration of infection in herds, and environmental factors such as the quality of pastures, cattle density, climate and topography (Cunningham, 1977; Nicoletti, 1980; Crawford *et al*., 1990).

In infected herds, the clinical signs include decreased calving percentage, delayed calving, infertility, decreased milk production, abortions, stillbirth, birth of weak calves and uni- or bilateral hygromas (McDermott and Arimi, 2002; Ocholi *et al*., 2004a). *Brucella abortus* in bulls also causes acute or chronic uni- or bilateral orchitis, epididymitis, seminal vesiculitis and hygroma (Kumi-Diaka *et al*., 1980a; Godfroid *et al*., 2004). In fully susceptible herds with brucellosis, abortion rates vary from 30 to 70% (Godfroid *et al.,* 2004). Abortion typically occurs during the first pregnancy following infection, and although there is invasion of the gravid uterus during subsequent pregnancies, abortion rarely recurs (Pappas *et al*., 2005). Since the reproductive performance of these carrier animals is unaffected, they are left in the herds in Nigeria, making effective control programmes extremely difficult. In camels, brucellosis represents the major cause of infectious abortion in Africa and the Middle East (Tibary *et al*., 2006).

Diagnosis

There is no single test to identify *B. abortus* with absolute certainty. However, a combination of growth characteristics, colonial and cellular morphology, staining properties, agglutinating antisera and biochemical reactions will allow an accurate identification (Alton *et al.*, 1988).

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Antibody response of cattle against *B. abortus* has been extensively used for the serological diagnosis of bovine brucellosis (OIE, 2011a). Because of the variable incubation period and the often subclinical nature of the disease in most animals, a definitive diagnosis should be based on the isolation and identification of *B. abortus* and on positive serological results based on the detection of antibodies in blood, milk, whey, vaginal mucus, or seminal plasma (Anon, 1986; Alton *et al.*, 1988; OIE, 2011a). The diagnostic tests for bovine brucellosis are subdivided into three groups as follows: 1) for demonstration of *B. abortus*, 2) for detection of immunoglobulins, and 3) those dependent on allergic reactions to *B. abortus* (Anon, 1986; Alton *et al.*, 1988). The ideal diagnostic test should detect infection early during the long and variable incubation period, detect carriers, not be influenced by the presence of 'non-specific' antibodies, and differentiate between responses to vaccination and those due to field infection (Brinley Morgan, 1977). There is no single test that has all these qualities (Nielsen, 2002).

The tests for the demonstration of *B. abortus* include microscopic examination using Ziehl-Neelsen stain in which the *Brucella* spp. stains red against a blue background (Stamp *et al.*, 1950), although other organisms such as *Coxiella burnetti* and *Nocardia* spp. are weakly acid-fast and present the same colour reaction hence making it difficult to differentiate from *Brucella*. Culture, identification and typing require the supramammary lymph nodes if dealing with carcasses; retropharyngeal, parotid, mandibular or iliac lymph nodes can also be collected. Other specimens include udder tissue, uterus, milk, hygroma, testes and accessory sex glands (Nicoletti, 1980; Corner *et al.*, 1987). Recovery of *B. abortus* from supramammary, parotid and mandibular lymph nodes is almost 100% (Corner *et al.*, 1987). In cases of abortion, foetal membranes, lungs, abomasal content, liver, spleen of aborted fetuses and from live cows uterine discharge, colostrum or milk (Alton *et al.*, 1988) are to be collected. The organism should be grown on a selective media such as *Brucella* or Columbia agar with appropriate supplements and culture conditions. Procedures for identification are described (OIE, 2011a). The isolated brucellae have to be typed to determine the species and biovar as described by Bale and Kumi-Diaka (1981) and Ocholi *et al.* (2004a) in Nigeria, and Anon (1986) and Alton *et al.* (1988) elsewhere. Although culture and identification of aborted material is the method of choice for diagnosing early infections (Erasmus, 1986), the procedure is laborious, time consuming, costly, and cannot routinely be used as a diagnostic procedure. Furthermore, the probability of successful recovery of *B. abortus* is strongly reduced when the material is heavily contaminated or when only a few organisms are present. Hence, negative culture results do not exclude infection (Bercovich, 1998).

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Molecular methods for the differentiation of several *Brucella* species include real time polymerase chain reaction (PCR) (Hinic *et al.*, 2008), restricted-fragment-length-polymorphism (RFLP) (Bricker, 2002), and pulsed-field gel electrophoresis (PFGE) (Jensen *et al*., 1999). As with other molecular methods, these techniques are time consuming and expensive. In addition, there is no reproducible and robust technique that allows for the differentiation between strains belonging to the same biovar (Bricker, 2002). Also, removal of polymerase indicators is needed from field samples and food products before employing such diagnostic techniques (Bricker, 2002).

The detection of specific immunoglobulins involves using whole-cell suspensions as antigen, such as the serum agglutination test (SAT), the Rose Bengal plate test (RBPT), the complement fixation test (CFT), enzyme-linked immunosorbent assays (ELISA) and the milk ring test (MRT) to detect antibodies directed against the oligo-polysaccharide moiety of the smooth lipopolysaccharide (Anon, 1986; Alton *et al.*, 1988; OIE, 2011a). These tests are suitable for large surveys, large-scale campaigns, control and eradication programmes and trading purposes (Anon, 1986; OIE, 2011a). The ideal tests should be inexpensive, rapid and simple screening tests with high sensitivity followed by more specific confirmatory test on the positives from the screening test. The screening and confirmatory tests can be interpreted either in series or parallel depending on epidemiological context and objective of testing. In South Africa, serum samples are screened with RBPT, while CFT is used as a confirmatory test (Anon, 1986). The CFT shows the best correlation with *B. abortus* isolation in naturally or experimentally infected animals (Nielsen, 1995). It has been reported that vaccine antibodies interfere less with CFT than with agglutination tests (OIE, 2011a). However, the test is cumbersome and requires specialized labour and strict quality control of the reagents. In addition, in rare situations, when sera show excess or predominance of IgG2 antibodies, reading may be similar to that of the prozone phenomenon (OIE, 2011a), leading to false-negative results. This is because the IgG2 reacts with the antigens, preventing IgG1 from binding and fixing the complement (Chappel, 1989). In such rare cases ELISAs or florescent polarization assay (FPA) with higher sensitivity and specificity may be required to confirm the diagnosis. Complement fixation test is regarded as confirmatory test for detection of infected animals worldwide. Unlike SAT, the titres do not wane as the disease becomes chronic (Anon, 1986). However, vaccination with strain 19 vaccine stimulates serological titres giving false positives. Consequently, experience, expertise and accurate records of vaccination and birth dates are required to be able to accurately interpret the results.

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The antigen for RBPT has been stained with Rose-Bengal dye. The most abundant antibody in the serum of infected animals, IgG1, agglutinates more strongly with the antigen than does IgM and IgG2 (Levienx, 1974; Brinley Morgan, 1977; Anon, 1986). The test is cheap and easy to perform with few false negative results which are usually obtained in chronic cases of the disease. However, false positives occur because of its relatively low specificity, usually due to the presence of IgM as a result of strain 19 vaccination (OIE, 2011a). Bacteria such as *Yersinia enterolitica* serovar IX, *Vibrio cholera* and *Escherichia coli* O:157 have also been reported to produce cross reacting antibodies to brucellosis (Nielsen *et al.*, 2004; Munoz *et al.*, 2005), with *Y. enterolitica* the most common cause of false positives. In one study, for every three positive RBPT animals, only one tested positive on c-ELISA (Muma *et al*., 2012). This is a recommended test by OIE for the international cattle trade (OIE, 2011a).

The SAT has been the major serological test used for the diagnosis of brucellosis. Although SAT is not the most sensitive, it contributed to the international harmonization of brucellosis control and eradication programmes (Bercovich, 1998). The test can be used for the detection of acute infections because it measures principally IgM rather than IgG1, IgG2 and IgA antibodies (Alan *et al.,* 1976; MacMillan, 1990). The SAT is also used to diagnose brucellosis only on a herd basis because it yields false-negative and false positive results (Nielsen *et al*., 1981; Corbel *et al.,* 1984). The SAT is still used in some countries as a screening test despite its low sensitivity. The non-specific agglutination is reduced by the incorporation of EDTA, with no effect on *B. abortus* agglutination titres of sera from infected cattle (MacMillan and Cockrem, 1985). This preparation is a very specific test and useful in identifying new infections as early as two weeks (Godfroid and Kasbohrer, 2002). However, in chronic cases, it gives false negative results (Saegerman *et al*., 1999).

The ELISAs have higher sensitivity and specificity, do not show the prozone phenomenon and can be automated (OIE, 2011a). The ELISA can be used for screening and confirmation of brucellosis in both milk and serum, and can differentiate between vaccinated cattle and cattle with naturally occurring brucellosis (Stemshorn *et al.,* 1985; Sutherland, 1985; Bercovich and Taaijke, 1990). The procedure of ELISA is simpler than CFT (Sutherland *et al*., 1986). The antigens, conjugates, and substrates that have been used for the assay have been extensively reviewed by Nielsen *et al*. (1988). Indirect ELISA (i-ELISA) is more sensitive in detecting antibodies to *B. abortus* than RBPT, SAT and CFT but false positives are obtained from animals vaccinated with strain 19 vaccine (Nielsen, 2002). The test is

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intended to replace RBPT, SAT and CFT (Paweska *et al*., 2002). Competitive enzyme-linked immunosorbent assay (c-ELISA) uses monoclonal antibody (MAb) that has the ability to compete with low affinity antibody, therefore, it has a higher specificity than i-ELISA (Sutherland, 1985; Nielsen *et al.*, 1995). The c-ELISA partially reacts in animals vaccinated with strain 19 vaccine, with cross reactive bacteria such as *Yersinia enterolitica* O:9 infection (Aguirre *et al.*, 2002; Godfroid *et al*., 2002) and with lacteal immunoglobulin present in colostrum (Sutherland *et al*.,1986; Kerkhofs *et al*., 1990). Aguirre *et al.* (2002) further reported that the reaction of the MAb antibody of c-ELISA with that of strain 19 vaccine or cross reactive infection was less than the other tests. The c-ELISA test, with a higher specificity than RBPT, CFT and FPA, is therefore an ideal confirmatory test (Muma *et al*., 2007a). However, it is expensive and rarely been used in Nigeria in naturally infected cattle, and never in a large study including different production systems. The FPA is a simple and rapid technique for measuring antigen/antibody interaction and may be performed in the laboratory or in the field (Nielsen and Gall, 2001). The mechanism of the assay is based on random rotation of molecules between the soluble antigen and the complex antigen-antibody in solution. Molecular size is the main factor influencing the rate of the rotation, which are inversely related (OIE, 2011a). The test is rapid, easy to use, reagents do not have to be rinsed, and the equipment is portable (Nielsen *et al.*, 2001). The FPA has shown promising results and the ability to differentiate between S19 vaccinated and unvaccinated animals (Nielsen and Gall, 2001; Nielsen *et al*., 2006). However, initial acquisition of equipment is costly.

The MRT is used in lactating animals as a herd screening test for brucellosis based on fat globules, agglutinins and stained *Brucella* cells (antigens) that agglutinate forming a coloured cream layer on top (Anon, 1986; Alton *et al.*, 1988). The MRT is cheap, easy, quick to perform, sensitive screening test and reliably specific used on bulk milk samples, although its sensitivity decreases with large herds and few positive animals (Bercovich, 1998; Godfroid *et al.,* 2004). Milk can be obtained cheaply and more frequently than blood samples and is often available centrally at dairies. When a positive test result is obtained, all cows contributing milk should be blood tested. The milk i-ELISA is a sensitive and specific test, and is particularly valuable for testing large herds. The MRT is a suitable alternative if the ELISA is not available (OIE, 2011a). Factors that may cause false positive results include high prevalence of mastitis, high proportion of cows in early or late gestation, recent (within three to four months) vaccination with strain 19 vaccines, hormonal disorder, colostrum and milk

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at the end of lactation period, and souring of milk (Alton *et al.*, 1988; Kerkhofs *et al.*, 1990). Excessive heating of milk or storing for longer than five minutes at 45°C may cause false negative results and pasteurized milk cannot be effectively tested by the MRT (Alton *et al.*, 1988).

The brucellin skin test is an alternative immunological test, which can be used for screening unvaccinated infected herds by eliciting an allergic response, provided that a purified (free of smooth lipopolysaccharide) and standardized antigen preparation (e.g. brucellin INRA; Brucellergen-Rhone-Merieux, France) is used. The efficacy of these antigens to detect brucellosis, their characteristics and limitations has been reviewed (Olitzki, 1970). The brucellin skin test has a very high specificity of up to 99%, such that serologically negative unvaccinated animals that are positive reactors to the brucellin test should be regarded as infected animals (Pouillot *et al*., 1997). Also, results of this test may aid the interpretation of serological reactions thought to be false positive serological reactions (FPSR) due to infection with cross reacting bacteria, especially in brucellosis-free areas (Pouillot *et al*., 1997). Not all infected animals react, therefore this test alone cannot be recommended as the sole diagnostic test or for the purposes of international trade (OIE, 2011a). It has been reported that the brucellin skin test is the only test that can distinguish between *Yersinia enterolitica* O:9 and *B. abortus* (Saegerman *et al*., 1999; Godfroid *et al.,* 2002). Calfhood vaccination with strain 19 may interfere with the interpretation of skin test due to brucellae allergens (Saegerman *et al*., 1999).

Other tests not commonly used for the diagnosis of bovine brucellosis are 2-mercaptoethanol and rivanol tests. They are used to detect specific IgG and also to differentiate between infected and vaccinated cattle (Stemshorn *et al*., 1985).

Campylobacteriosis

Aetiology, microbiology and transmission

Bovine genital campylobacteriosis is a widespread disease associated with bovine infertility worldwide (Jimenez *et al*., 2011). Bovine genital campylobacteriosis is caused mainly by *Campylobacter fetus venerealis*, a thin Gram-negative curved rod that may form S-shapes, slender, seagull-shapes and spirals, and can be cultured at 37°C for at least 3 days in a microaerobic atmosphere, although it can also grow at 25°C (OIE, 2011b). *Campylobacter*

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fetus is 0.01-0.08 µm in width and 0.5-0.8 µm in length. In old cultures or when exposed to oxygen, the cells appear coccoid in shape (Ng *et al*., 1985). They are motile having a single polar flagellum at one or both ends of the cells with a characteristics corkscrew motion (Irons *et al*., 2004a). The colonies are slightly grey-pink, round, convex, smooth and shiny, with a regular edge and a diameter of 1-3 mm (OIE, 2011b). *Campylobacter fetus* can be differentiated from each other by using standard biochemical parameters but this may give a wrong identification for some specific species (Vargas *et al.*, 2003).

The disease is transmitted by venereal contact, through AI using contaminated semen or contact with contaminated bulls (Garcia *et al*., 1983a; Cobo *et al*., 2004; OIE, 2011b). *Campylobacter fetus venerealis* is a major cause of infectious infertility, they survive in the genital tract of cow and bull (Corbeil *et al*., 1981; Penner *et al*., 1988; McFadden *et al*., 2004). Bulls with bovine genital campylobacteriosis carry the bacterium subclinically in their prepuce; older bulls above three years may remain permanently infected and transmit the disease sexually or serve as source of infection through the use of semen from infected bulls (Garcia *et al*., 1983a; Bryner, 1990; Irons *et al.*, 2004a). Sub-speciation of *C. fetus venerealis* indicated an unusually high prevalence of *C. fetus venerealis* biovar intermedius in South Africa (Schmidt *et al*., 2010). Similar *C. fetus* subspecies genomic analysis assay specific for *C. fetus* subsp. *venerealis* biovar intermedius in Australia (Moolhuijzen *et al*., 2009) and Italy (Nigrelli *et al.,* 1984) were reported. The occurrence of this isolate has not been studied in Nigeria. *Campylobacter fetus fetus* is found in the genital and intestinal tracts of cattle and sheep and is transmitted by ingestion of contaminated material (Dufty and Vaughan, 1993) or occasionally by venereal contact (Hum 1987; McLaren *et al*., 1988) causing lowered fertility and abortion mostly in sheep and sporadic abortion in cattle (McLaren *et al*., 1988). *Campylobacter fetus fetus* is reported to cause a wide variety of invasive diseases in humans such as cellulitis (Briedis *et al*., 2002), bacteraemia (Zonjos *et al.*, 2005), perinatal sepsis (Fujihara *et al*., 2006) and infection of the thyroid gland (Goegebuer *et al*., 2007).

Bovine genital campylobacterios dissemination is also encouraged by newly introduced bulls, cows and heifers from endemic herds (Woldehiwet *et al*., 1989), importation of bulls for cross breeding purposes (Nuru, 1974) and lack of effective control of mass cattle movements across international borders (Mshelia *et al*., 2010b). Other factors associated with spread of bovine genital campylobacteriosis include use of communal bulls or having more than one bull in a herd (Mukasa-Mugerwa, 1989), communal grazing (Pefanis *et al*., 1988), lack of

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vaccination (Hoffer, 1981), genetic differences in susceptibility between different cattle lines (Dufty *et al*., 1975), contact with contaminated bedding (Hjerpe, 1990), and transmission via fomites and between bulls (Hoffer, 1981; Mukasa-Mugerwa, 1989).

Pathogenesis

Following invasion of the genitalia of naïve cows and heifers by *C. fetus*, the organism only establishes itself during the luteal phase of the oestrus cycle, localizing in the vagina (Schurig *et al*., 1974; Mshelia *et al.*, 2007). The numerous neutrophils in the uterus during oestrus help to protect the susceptible host by destroying many of the organisms (Corbeil *et al*., 1975) and infection during the luteal phase does not spread any further and has no effect on fertility in 10 to 20% of cases (Dufty and Vaughan, 1993). In the remainder, the organisms migrate through the cervix into the uterine body after five to seven days and then may continue to spread during the following weeks. Colonisation of the uterus stimulates a local immune response in which IgG, IgM and IgA are produced (Van Aert *et al*., 1977). The duration of protective immunity acquired after natural infection is two to four years (Dufty and Vaughan, 1993). Bovine genital campylobacteriosis interferes with placentation causing lowered fertility, early embryo mortality and abortion in females (Hum *et al*., 1994a; Campero *et al*., 2005). Female cattle experience transient infertility associated with inflammation of the reproductive tract (Irons *et al*., 2004a). The infection is generally self-limiting, recovery taking three to four months (De Keyser, 1986; Dufty and Vaughan, 1993). In the bull, *C. f. venerealis* is confined to the fornix of the prepuce and the penis (Samuelson and Winter, 1966), with a weak and localized antibody response due to insufficient antigenic stimulation as a result of antigenic variation and superficial nature of the infection leading to prolonged survival of the organism (Van Aert *et al*., 1976; Bier *et al*., 1977).

The spread of the organism from the intestine or gall bladder into the system results in bacteraemia. Osborne and Smibert (1964) reported that the *C. fetus* endotoxins may cause abortion due to hypersensitivity reaction or a drop in the maternal blood progesterone levels. The surface of *C. fetus* is protected with S-layer protein antigens called anti-phagocytic macrocapsule composed of high-molecular-weight proteins and can change the size and crystalline structure of the predominant protein expressed. This property enhances the virulence of pathogenic *C. fetus* (Blaser and Gotschlich, 1990; Nitta *et al.,* 1997; Casademont *et al.,* 1998). The S-layer proteins found in pathogenic *Campylobacter* have pathogenic significance due to antigenic variation by shifts in expression of the S-layer proteins in

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chronic venereal infections (Blaser and Gotschlich, 1990; Brooks *et al*., 1996; Thompson, 2002).

Clinical signs

Bovine genital campylobacteriosis is subclinical in most cases. However, there are two forms of the disease, the acute and the chronic forms (Mshelia *et al*., 2007). The acute form is characterized by catarrhal vaginitis, cervicitis and mucopurulent discharge. In cases of ascending infection, endometritis ensued, creating an unfavourable environment which is responsible for deprivation of oxygen to the embryo resulting in early embryonic death (Firehammer, 1979; Ware, 1980; McFadden *et al*., 2004; Mshelia *et al*., 2007). The chronic form of the disease is seen in endemic areas and older cows where the pregnancy rate of the herd is usually not affected and the fertility of the herd may be fluctuating (Hoerlien *et al.,* 1964). The abortion rate in such herds is between 3-10% occurring between 4-6 months of gestation (De Keyser, 1986; Hum, 1987; Dufty and Vaughan, 1993; Irsik and Shearer, 2007). Other clinical signs include irregular oestrous cycles, repeat breeding, decrease in conception or pregnancy rates and long calving intervals (Hum, 1987; Bawa *et al*., 1991; Van Bergen *et al*., 2006). Pregnancy rate can be as low as 20%, with sterility in up to 11% of infected heifers (McCool *et al*., 1988) and up to 10% of animals may become permanently infected (Cipolla *et al*., 1994). Major clinical sign of campylobacteriosis in a herd is large numbers of cows found open during routine pregnancy diagnosis (Irons *et al*., 2004a). No signs of infection are evident in bulls (Vandeplasse *et al*., 1963). The microorganism does not interfere with semen quality or breeding ability (Bier *et al*., 1977).

Diagnosis

The diagnosis of bovine genital campylobacteriosis is based on history, clinical signs, laboratory diagnosis such as serology, culture and identification, and molecular techniques. Bulls are the most important when making a diagnosis of campylobacteriosis on a herd basis, as the probability of a positive diagnosis in them is far greater than in cows (Irons *et al*., 2004a). Serology includes antigen detection which involves fluorescence antibody techniques. This is commonly complemented by cultural methods using preputial samples (Garcia *et al*., 1983b; Dawson, 1986). The test sensitivity and specificity of fluorescence antibody techniques are 93% and 88.9% respectively (Figueiredo *et al.*, 2002). It is important that the preputial material be collected correctly to ensure accuracy of the diagnosis. If

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sample is to exceed 6-8 hours before culture, a transport medium must be used to ensure viability of *C. fetus* (Dufty and Vaughan, 1993; Hum *et al*., 1994a). Several methods for the collection of preputial smegma have been investigated in order to improve the reliability of selective culture-based diagnostic procedures (McMillen *et al*., 2006). Tissues from aborted foetus should be homogenized and filtered through a membrane filter with a 0.65 µm pore size (Irons *et al.*, 2004a). The sample is inoculated on a commonly used selective medium such as Columbia agar base containing *Campylobacter* Skirrow's supplement, antimicrobials and incubation at appropriate conditions as described by OIE (2011b). A high percentage of *C. f. venerealis* strains are susceptible to polymyxin B, an antibiotic used in all *Campylobacter* selective media and transport enrichment media (Jones *et al.*, 1985).

One method of antibody detection is the vaginal mucus agglutination test (VMAT) which is used to detect antibodies from vaginal mucus, foetal fluid and preputial washings (Lander, 1990). It is a convenient herd screening test for *C. fetus* detecting about 50% of all infected cows, but it has the limitation of large number of false positives and false negatives due to dilution of agglutinins by mucous from oestrus cow, contamination of samples with blood, inflammatory products or antibodies to *C. f. fetus* (Clark, 1967; Dufty and Vaughan, 1993; OIE, 2011b). The ELISA has also been used for the detection of antibodies in vaginal mucus (Hewson *et al.*, 1985; Mshelia *et al*., 2010b). The ELISA test was reported to be more sensitive than the VMAT with a specificity of 98.5% (Hum *et al*., 1994b). Other serological tests employed for *C. f. venerealis* include ELISA to detect antibodies (Hum *et al*., 1991; Akhtar *et al*., 1993a) and monoclonal antibody-based ELISA (Brooks *et al.,* 2004; Devenish *et al*., 2005).

Molecular techniques have used PCR analysis of the 16s rRNA gene of *Campylobacter* spp. (Hum *et al*., 1997), amplified fragment length polymorphism (AFLP) (Wagenaar *et al.*, 2001) and PFGE (Salama *et al.,* 1992; On and Harrington, 2001) for the detection of *C. fetus* and distinguishing between the two *C. fetus* subspecies. In comparison to conventional PCR techniques, 5' *Taq* nuclease assays are highly sensitive and specific and the amount of target DNA in the assay can also be accurately quantified (Mackay, 2004). Thus, this test is highly suited for routine diagnostic applications. In addition, 5' *Taq* nuclease demonstrates significant improvements for the detection of *C. f. venerealis*-infected animals from crude extracts following prolonged transport of samples (McMillen *et al*., 2006). However, molecular methods are time consuming and require expensive apparatus, reagents and

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knowledge. Schmidt *et al*. (2010) demonstrated the incorrect classification based on subspecies-specific PCR assays, making phenotypic characterization following bacterial culture superior to molecular characterization of *C. fetus* subspecies. Therefore, field studies continued to rely upon either selective culture (Hum *et al.*, 1994a; Schmidt *et al*., 2010), ELISA (Hum *et al*., 1991) or direct immuno-fluorescence test (Martinez *et al.*, 1986). Although, Wagenaar *et al*. (2001) reported that culture and identification are not reliable diagnostic methods in distinguishing *C. f. venerealis* from *C. f. fetus*.

Trichomonosis

Aetiology, microbiology and transmission

Trichomonosis is also a widespread disease associated with bovine infertility worldwide (BonDurant, 2005). *Tritrichomonas foetus* can be identified by the presence of three anterior flagellae and an undulating membrane because of its wave-like motion on one side of the organism when viewed under phase-contrast or dark-field microscopy (Irons *et al.,* 2004b; BonDurant, 2005). It measures about 9-25 x 3-12 µm (Van Someren, 1946). The protozoan is pyriform and elongated from fresh samples of infected animals, while on culture it shows pleomorphism and a tendency to become spherical (Mundt, 1954). *Tritrichomonas foetus* is transmitted venereally, but can also be transmitted mechanically by insemination instruments, by a susceptible bull mounting a recently contaminated bull by homosexual mounting or by gynaecological examination through vaginal examination (Yule *et al.*, 1989; Mardones *et al.*, 2008). Bulls older than 4 years rarely recover spontaneously; they therefore become life-long carriers, whereas cows clear the infection spontaneously (Kimberling *et al*., 1988; BonDurant, 1997; Irons *et al*., 2004b). In addition, the organism can survive the standard processing methods for semen used in AI (Monke, 1998). Prevalence of the disease is high in areas where natural breeding is practiced (Cobo *et al*., 2004). Economic losses associated with the disease can be ascribed to the increased number of days that cows are open, decreased calving percentage, decreased growing time of calves born later in the season, cost of culling and replacing animals, and the cost of treatment (McCool *et al*., 1988; Rae, 1989; Rae *et al.,* 1999). Trichomonosis caused by *T. foetus* is not zoonotic; the zoonotic species is *T. vaginalis* which is the most common pathogenic protozoan infection of humans (Soper, 2004). A prevalence of 18.7% trichomonosis has been reported in women attending antenatal clinic in Nigeria, characterized by vaginitis and lower abdominal pains (Jatau *et al.,* 2006).

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Pathogenesis

In older bulls, *T. foetus* readily colonises the preputial and penile mucosa, confining itself to the preputial cavity without invading the mucosa (Parsonson *et al*., 1974). The organism was also isolated from the seminal vesicles (Parsonson *et al*., 1974; Roberts, 1986). Older bulls remain chronically infected although spontaneous recovery has been recorded (Kimberling *et al*., 1988). The maintenance of infection in older bulls may be as a result of increase in number and size of the irregular crypts in the epithelium of the penis of mature bulls and may be the reason for increased susceptibility (Samuelson and Winter, 1966; Cobo *et al.*, 2011).

The organism migrates from the vagina through the cervix to the uterus and fallopian tubes producing cervicitis, endometritis and salpingitis (BonDurant, 1985; Rae and Crews, 2006). The development of salpingitis may result in fertilization failure (Clark *et al.*, 1983) but generally conception is not affected (Parsonson *et al*., 1976). However, endometritis may cause embryo or foetal death due to interference with nutrition. Foetuses that die in the early stage of gestation are retained and such cows develop pyometra which may not resolve for many months (BonDurant, 1985; Mickelsen *et al*., 1985; Rae and Crews, 2006). With the exception of cows that develop pyometra or abort, most of the infections resolve spontaneously within 2-4 months (Parsonson *et al*., 1976; BonDurant, 1997). On rare occasions cows might carry the infection throughout pregnancy and calve at term (Godger and Skirrow, 1986). Repeated exposure in some animals leads to transient infection which is normally cleared within three weeks (Skirrow and BonDurant, 1990a). This partial immunity is sufficient to carry over from one breeding season to another, thereby reducing the pregnancy loss in the subsequent season (Clark *et al*., 1983). However, cows become fully susceptible after 15 months (Clark *et al*., 1983). Normally fertility is restored 2-6 months after the initial infection (Abbitt, 1980). Only IgG and complement-mediated defences are known to kill trichomonads (Corbeil, 1994). Bulls show local processing of antigens in preputial secretions following challenge (Soto and Parma, 1989), while heifers exhibit significant systemic antibody response by 11 weeks after infection (Soto and Parma, 1989; Skirrow and BonDurant, 1990a). It has been reported that *T. foetus* exists in the intestine, oesophagus and abomasum of the foetus (Rae and Crews, 2006).

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Clinical signs

Uncertainty regarding the gestational ages of pregnancies following the breeding season are often the first indication of trichomonosis in a herd (BonDurant, 1997). Ayoade *et al*. (1990) reported that repeat breeding suggests a diagnosis of trichomonosis in a herd. The calving percentages in chronically infected herds can be up to 85%, while in newly infected herds lower percentages are expected (Clark *et al*., 1983; Ball *et al*., 1987). Following infection in the bull, trichomonosis is symptomatic (BonDurant, 1985). In the early stage of infection in the bulls, balanoposthitis, difficulty in micturition and lack of interest to mate may be noticed (Jubb *et al*., 1993). In some cases swelling of the prepuce with accumulation of mucopurulent exudates in the preputial cavity may occur (Riedmuller, 1978).

In females, trichomonosis is characterized by aberrant oestrous cycles, infertility, a low percentage of early abortions, and pyometra (Irons *et al*., 2004b). Pyometra is not a common occurrence; however, it has been reported in 5 to 10% of cows and 21.3% of exposed animals in one herd (Mickelsen *et al*., 1985; BonDurant, 1997). Embryonic and foetal deaths from 14-18 days and up to 7 months of gestation have been reported, resulting in irregular estrous cycles (BonDurant, 1985; Clark and Dufty, 1986). Infections affect neither semen quality nor sexual behaviour (Martin-Gomez *et al*., 1998; Adeyeye *et al*., 2012). Cervicitis, endometritis, salpingitis, and mucopurulent vaginal discharge may be noticed in cows and heifers (BonDurant, 1997).The first observable physical signs are pyometra and abortion (Rae and Crews, 2006).

Diagnosis

An infected herd usually shows moderate calving percentages, long calving intervals, failure of heifers to conceive, sporadic abortions, aberrant oestrus cycles, repeat breeding, and post coital pyometra in some cases (BonDurant, 1985; Skirrow and BonDurant, 1988). However, a positive diagnosis of trichomonosis depends on the demonstration of live *T. foetus* in samples from preputial scraping of bulls, genital tract of females, or aborted foetal and placental tissues. Bulls are the best animals to select for testing, but because of the fluctuation in the number of organisms and moderate sensitivity of the screening tests used, testing samples at least three times a week is recommended before certifying a bull free of the infection (Clark *et al.,* 1983; Parker *et al.*, 1999). Direct field examination of the samples using dark field, phase contrast, differential contrast, or normal bright field microscopy can be used (Irons *et*

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al., 2004b). Direct examination of different samples shows different test sensitivity: sheath wash 20% (Ribiero, 1999), vaginal secretions 36% (Skirrow and BonDurant, 1990b), cervical secretions 40% (Skirrow and BonDurant, 1990b) and vaginal mucus 73.9% (Ribiero, 1999). The low sensitivity may be as a result of non-detection of the disease in animals with low numbers of the organisms (Ribiero, 1999). Direct examination of uterine secretions from clinically normal animals is less sensitive, and the cervix tends to be the best area for the isolation of the organisms (Ribiero, 1999). *T. foetus* may be isolated from exudates collected from cervix, uterus and fallopian tubes of the infected animals depending on the stage of infection (Roberts, 1986).

Culture is the gold standard for the diagnosis of bovine trichomonosis (OIE, 2011c). A commercially available culture kit (In-Pouch TF, Biomed Diagnostics) facilitates transport of the sample, incubation and examination in situations where sophisticated laboratory facilities are not available (Thomas *et al.*, 1991; Borchardt *et al.*, 1992). However, the kit is expensive, recovery of other trichomonads is possible (BonDurant *et al.*, 1999) and sensitivity reduces by 10% after transportation of samples overnight (Kittel, 1998). The sensitivity of In-Pouch and Diamonds culture media is between 70-95%, with In-Pouch having the advantage of rapid identification of positive samples (Kittel, 1998; Thomas *et al.*, 1991; Borchardt *et al.*, 1992). Serological tests include vaginal mucus agglutination test with mucus from the cervical region of the vagina. This test lacks sufficient specificity (Riedmuller, 1978), although it is useful in the diagnosis of existing infection and cleared infection (OIE, 2011c). ELISA on serum of heifers and cows showed a sensitivity and specificity of 85% and 95% respectively, but it does not identify infected bulls (BonDurant 1997; Irons *et al.*, 2004b). A haemolytic test on blood samples has also been used (Campero *et al*., 1998), while intradermal test is used as a screening test for females (Soto and Parma, 1989). Histologically, haematoxylin and eosin-stained tissues do not differentiate trichomonads from leukocytes (Rhyan *et al.*, 1995), however, immunohistochemistry is the most ideal staining technique using poly- and monoclonal antibodies (Campero *et al*., 1985; Rhyan *et al.*, 1995).

Polymerase chain reaction has shown promising results in identifying *T. foetus* (Parker *at el.*, 2001; Campero *et. al*., 2003). Its advantages include increased analytical sensitivity, faster diagnostic turnaround time, the fact that the organisms in the collected sample are not required to be viable, and ability to analyse large numbers of samples. Furthermore, it has been observed that primers can be used to differentiate between *T. foetus* and non-*T. foetus*

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trichomonads sometimes found in preputial samples (BonDurant *et al.*, 1999; Campero *et al*., 2003). Campero *et al.* (2003) further reported that the diagnostic sensitivity of PCR tests is similar to that of the In-Pouch TF kit (Campero *et al*., 2003). Polymerase chain reaction techniques are a good substitute for microscopy in that they have a faster diagnostic turnaround time, and they also allow the detection of dead organisms. However, despite the advantages of PCR, the test also has limitations such as: the presence of contaminants in the vagina and preputial cavity during sample collection, blood and urine from preputial scraping and preputial washing reduce the sensitivity of PCR (Irons, 2002).

Infectious reproductive diseases of cattle in Nigeria and other African countries

Brucellosis

Investigators have reported serological evidence of *Brucella* infection across Nigeria, particularly in cattle, sheep and goats. In institutional farms and other ranches or dairy farms, prevalence in cattle ranged between 3.7% and 48.8% (Esuruoso and Van Blake, 1972; Esuruoso, 1974; Junaidu *et al* 2008), while in the traditional nomadic Fulani cattle herds, the prevalence was between 0.4% and 26% (Nuru and Dennis, 1975; Eze, 1978; Ocholi *et al*., 1996). Many of the studies in Nigeria are from the southern part and are mostly from abattoir investigations. In the northern part, locations of the studies are restricted, with small sample sizes and using a non-specific diagnostic test. In South Africa, the prevalence decreased on a national scale from 10.5% in 1976 to 1.4% in 1988/89 (Anon., 1989). Herd prevalences of 8.4% and 15% were reported in Cameroon (Bayemi *et al.,* 2009) and Ethiopia (Ibrahim *et al*., 2009), respectively.

Campylobacteriosis

Bovine genital campylobacteriosis was first reported in Nigeria in 1974 in a herd that imported Devon cattle (Nuru, 1974) and Bawa *et al.* (1987) reported bovine genital campylobacteriosis in an AI herd. In 1991, *C. f. venerealis* was isolated from 12 bulls and one cow while *C. f. fetus* was isolated from three bulls and four cows in the genital tract. The overall animal- and herd-level prevalence of campylobacteriosis in the study covering three states of Nigeria was 2.9% (20/689) and 20% (16/79), respectively (Bawa *et al.*, 1991). Mshelia *et al.* (2010b) reported a 3.7% prevalence of *C. fetus* in culture from preputial washings of bulls and 7/66 cows (11%) had specific IgA antibodies to *C. f. venerealis* in

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vaginal mucus, while 6/40 herds (15%) with reproductive failure were *C. f. venerealis* positive. Very recently, animal- and herd-level prevalences of 3.7% and 22.2% respectively have been reported (Mshelia *et al.*, 2012). A survey in a communal grazing area in South Africa showed a high prevalence of 28.7% (Pefanis *et al*., 1988), whereas another study showed a prevalence of 1.9% in southern Africa (Madoroba *et al*., 2011).

Trichomonosis

Studies conducted on *T. foetus* in Ibadan, southern Nigeria reported a prevalence of 14.9% (Akinboade, 1980). The disease has been reported in several breeds such as Bunaji, Bokoloji, Red Bororo, Keteku (Akinboade, 1980), and in Ndama and their crosses (Ayoade *et al*., 1990). Infection has been reported in younger bulls 1-2 years old (Ayoade *et al*., 1990) and the prevalence in bulls is higher than in the cows (Akinboade, 1980). Very recently, there was failure to detect trichomonosis in Sokoto, northern Nigeria (Adeyeye *et al*., 2011). In addition, failure to detect trichomonosis has also been reported in other parts of Africa, Malawi (Klastrup and Halliwell, 1977) and Tanzania (Swai *et al.,* 2005). However, prevalence of 4.6 % among Holstein bulls in Egypt (Gawade *et al.*, 1981); 7.1% (Pefanis *et al.,* 1988), 26.4% (Erasmus *et al.,* 1989), 1.8% (Kitching, 1999) and 25% (Ribiero, 1999) in South Africa; and 4.1% (Madoroba *et al.*, 2011) in southern Africa have been reported.

Concurrent infections

It has been reported that cattle could simultaneously harbour *C. fetus* and *T. foetus* (Pefanis *et al*., 1988), and *B. abortus* and *C. fetus* (Zhoa *et al*., 2011). Concurrent herd infection of trichomonosis with *C. fetus venerealis* is found in communal farming systems in South Africa (Mickelsen *et al.*, 1985; Pefanis *et al*., 1988; Perez *et al.*, 1992). However, there is no evidence to suggest that infection with one causative agent predisposes to infection with the other agent (Campero *et al*., 1987; McCool *et al.*, 1988). *Tritrichomonas foetus* and *C. fetus venerealis* infections present similar clinical signs, hence the need to do laboratory tests to differentiate them.

Problem statement

Brucellosis, campylobacteriosis and trichomonosis cause serious economic losses in the livestock industry. Despite the apparent increase in the prevalence of bovine brucellosis and bovine genital campylobacteriosis in Nigeria, there is a dearth of information on the

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prevalence of brucellosis covering a wide geographical area and diverse management systems from studies using an accurate diagnostic test. There is also paucity of information on risk factors for brucellosis or campylobacteriosis using well-structured epidemiological studies, with appropriate multivariable methods to control for confounding. Knowing the risk factors and other epidemiological features of the diseases is essential for the development of cost-effective and efficient control programmes. The concurrent occurrence of these reproductive diseases in cattle in Nigeria is also not known. The situation of the infectious reproductive diseases in Nigeria is disturbing. The pastoral management system and handling of cases of abortion and hygroma by the traditional Fulanis who are the main livestock farmers as well as porosity of the international borders that may predispose livestock to cross infection may be responsible for the occurrence of these diseases. Furthermore, the influence of brucellosis, campylobacteriosis and trichomonosis on reproductive performance has not been well studied in Nigeria. Understanding the key infectious causes of infertility will facilitate the improvement of poor reproductive performance of cattle in Nigeria.

Study objectives and outline of the thesis

This study aimed to determine the animal- and herd-level seroprevalence of brucellosis in northern Nigeria, in private and government-owned cattle herds with different production systems, including some settled and pastoral Fulani herds that usually resist attempts to investigate their herds. This was addressed in Chapter 2, in which a multistage random sampling approach was used to select farms in three states of northern Nigeria. A total of 4,745 cattle from 271 herds were tested for brucellosis using a simple and rapid screening test, the Rose-Bengal plate-agglutination test (RBPT) and positives were confirmed using a highly specific confirmatory test, the competitive enzyme-linked immunosorbent assay (c-ELISA). In addition, seroprevalence was compared between states, management systems and different categories of animals defined by breed, sex, age, pregnancy status and lactation status. In order to obtain accurate prevalence estimates, results were adjusted for test sensitivity and specificity, as well as for sampling weights and clustering in the multistage survey.

A further objective was to determine the animal- and herd-level prevalence of campylobacteriosis and trichomonosis in mature bulls in cattle herds in northern Nigeria; and the concurrent occurrence of brucellosis and campylobacteriosis in such herds. This objective was addressed in Chapter 3 in which preputial smegma samples of 602 bulls from 250 herds

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were tested for campylobacteriosis and trichomonosis using culture and identification. Accurate techniques were used for both diseases, with a sensitivity and specificity of over 95% for each disease. As for brucellosis, appropriate adjustments were made for test performance and survey design.

Another objective was to identify herd-level risk factors for *C. fetus* infection, and for *Brucella* seropositivity as well as within-herd seroprevalence of brucellosis in cattle in northern Nigeria. This was addressed in Chapter 4 in which hierarchical mixed-effects logistic regression models were used to model risk factors for herd-level infection with each of the two diseases. In addition, a zero-inflated Poisson model was developed to identify factors associated with within-herd seroprevalence of brucellosis within infected herds.

The last objective of this study was to quantify the reproductive performance and occurrence of reproductive disorders in cattle herds in three states of northern Nigeria. This was addressed in Chapter 5, in which structured questionnaires, farm records, and reproductive examinations by trans-rectal palpation were used and calving rates were estimated for each herd. This information was then combined with the results of the diagnostic tests reported in Chapters 2 and 3 to model the effect of the infectious reproductive diseases and other environmental and management factors on calving rate using a multilevel linear regression model.

Finally, an overview of the results obtained, and the degree to which the aims and objectives of the study were attained are discussed in Chapter 6. The robustness and shortcomings of the data and analyses that were done in the study and recommendations for further investigations are also discussed. Recommendations on the prevention and control of the reproductive diseases, implementation of better management systems and other measures by farmers to improve on the productivity and reproductive performance of cattle in Nigeria are addressed in Chapter 7.

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Chapter 2

A large seroprevalence survey of brucellosis in cattle herds under diverse production systems in northern Nigeria

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Abstract

This study was carried out to investigate the status of brucellosis in cattle under various management systems in Adamawa, Kaduna and Kano states, northern Nigeria. Using multi-stage sampling, serum samples of 4,745 cattle from 271 herds were tested using the Rose-Bengal plate-agglutination test (RBPT) and positives were confirmed using a competitive enzyme-linked immunosorbent assay (c-ELISA). Prevalence estimates were calculated by adjusting for sampling weights and where possible for test sensitivity and specificity. Thirty-seven percent of all animals were RBPT positive, and after confirmation with c-ELISA the overall animal-level prevalence, adjusted for sampling weights, was 26.3% (95% CI, 22.1%-31.0%). Of the herds sampled, 210 (77.5%; 95% CI, 68.6%-84.5%) had at least one animal positive to both tests; this did not differ significantly between states ($P = 0.538$). Mean within-herd seroprevalence in positive herds was 30.2% (95% CI, 25.3%-35.1%) and ranged from 3.1% to 85.7%. Overall animal-level seroprevalences of 29.2% (95% CI, 22.5%-36.9%) (n = 1,827), 23.3% (95% CI, 18.9%-28.3%) (n = 1,870) and 26.7% (95% CI, 18.8%-36.7%) (n = 1,048) were observed in Adamawa, Kaduna and Kano states, respectively ($P = 0.496$). A significantly higher seroprevalence was found in males (38.2%; 95% CI, 31.7%-45.2%) than in females (24.7%; 95% CI, 20.4%-29.5%) ($P < 0.001$) and in non-pregnant females (27.8%; 95% CI, 22.9%-33.5%) than in pregnant females (17.2%; 95% CI, 13.6%-21.5%) ($P < 0.001$). Seroprevalence increased with increasing age ($P < 0.001$), from 13.5% (95% CI, 8.9%-19.9%) in cattle <4 years to 35.0% (95% CI, 28.5%-42.3%) in cattle >7 years. Seroprevalence also varied between management systems ($P < 0.001$): pastoral systems 45.1% (95% CI, 38.6%-51.9%), zero-grazing systems 23.8% (95% CI, 6.8%-59.2%), agro-pastoral systems 22.0% (95% CI, 17.3%-27.8%), and commercial farms 15.9% (95% CI, 9.5%-25.5%). Seroprevalence did not differ significantly between breeds or lactation status. This is the first large study to assess the prevalence of bovine brucellosis over a wide geographic area of northern Nigeria, in a variety of management systems and using accurate tests. The seroprevalence of brucellosis was high, and higher than results of previous studies in northern Nigeria. The pastoral management systems of the traditional Fulanis may be encouraging the dissemination of the disease. Public enlightenment of the farmers about the disease, vaccination and appropriate national control measures are recommended.

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Introduction

Brucellosis is one of the most important and widespread zoonoses in the world (Poester *et al*., 2000). *Brucella abortus* infection in cattle is endemic in Nigeria, resulting in huge economic losses due to decreased calving percentage, delayed calving, culling for infertility, cost of treatment, decreased milk production, abortions, stillbirth, birth of weak calves and loss of man-hours in infected people (McDermott and Arimi, 2002; Ocholi *et al.*, 2004a; Adamu, 2009). Infection in bulls also causes orchitis, epididymitis, seminal vesiculitis and hygroma (Kumi-Diaka *et al.*, 1980a; Godfroid *et al*., 2004). In Nigeria and some countries where cattle are kept in close association with sheep and goats, infection can also be caused by *B. melitensis* (Bale *et al.*, 1982; Ocholi *et al.*, 2004a).

Infection occurs via contaminated feed or water, by inhalation, through the conjunctiva, or by contact with infected aborted materials; while calves become infected *in utero* or via infected colostrum or milk (Nicoletti, 1980). Venereal transmission has also been reported (Bercovich, 1998). In fully susceptible herds, abortion rates vary from 30 to 70% (Godfroid *et al*., 2004). Infection may be lifelong, and during subsequent pregnancies there is invasion of the gravid uterus and allantochorion; abortion rarely recurs, but uterine and mammary infection recurs (Pappas *et al*., 2005). Since the reproductive performance of these carrier animals is unaffected, they are retained in herds in Nigeria despite the presence of pathognomonic clinical signs in some cases, making effective control programmes extremely difficult.

Prevalence of bovine brucellosis varies widely across Nigeria, and between herds in the same area (Nuru and Dennis, 1975; Ocholi *et al.*, 1996), with reported seroprevalences of 0.2% to 80.0% (Pam, 1995; Bertu *et al*., 2012). In institutional farms, abattoir surveys and other ranches or dairy farms in southern Nigeria, prevalence in cattle ranged between 3.7% and 48.8% (Esuruoso and Van Blake, 1972; Esuruoso, 1974; Esuruoso, 1979; Cadmus *et al.*, 2008), while in the traditional nomadic Fulani cattle herds, the prevalence was between 0.4% and 26% (Nuru and Dennis, 1975; Eze, 1978; Ocholi *et al.*, 1996). Recently, a within-herd prevalence of 32.2% on a prison cattle farm (Junaidu *et al.*, 2008); and 19.5% seropositive and 25.3% positive milk samples (Junaidu *et al.*, 2011) were reported in northern Nigeria. Prevalence studies in other surrounding countries indicated 8.4% in Cameroon (Bayemi *et al*., 2009), 7% in Chad (Scheling *et al*., 2003), 41% in Togo (Akapo, 1987) and 6.6% in Ghana (Kubuafor *et al.*, 2000). Brucellosis has also been reported in many other parts of Africa (Anon., 1989; Musa *et al.*, 1990; Bernard *et al*., 2005; Berhe *et al.*, 2007; Karimuribo

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et al., 2007; Matope *et al.*, 2011; Muma *et al.,* 2012), although detailed information on its prevalence is still lacking for most countries (McDermott and Arimi, 2002).

Although prevalence is high and variable in many countries, surveillance for the disease is generally poor (McDermott and Arimi, 2002; Ocholi *et al.*, 2004a; OIE, 2011a; Bertu *et al.*, 2012). Factors assumed to be responsible for variation in prevalence include purchase of infected cattle from the market for replacement or upgrading, nature of animal production, demographic factors, regulatory issues, climate, deforestation and wildlife interaction (Bale and Kumi-Diaka, 1981; Musa *et al.*, 1990; Avong, 2000; Muma *et al.,* 2007b; OIE, 2011a). Furthermore, one major factor contributing to the spread of the disease is the free movement of animals practiced by the nomadic Fulani herdsmen, who own about 95% of all food animal populations in Nigeria (Bale and Kumi-Diaka, 1981; Rikin, 1988; Ocholi *et al.*, 2004a). Other factors that may influence the prevalence of brucellosis in Nigeria include management system (Nuru and Dennis, 1976; Atsanda and Agbede, 2001), the herding of different species together (Bale *et al*., 1982; Ocholi *et al.*, 2004a,b; Junaidu *et al.*, 2008), use of common pastures and water sources (Bertu *et al.*, 2012), age (Ocholi *et al.*, 1996; Junaidu *et al.*, 2011), breed (Esuruoso, 1974; Cadmus *et al*., 2008), sex (Atsanda and Agbede, 2001; Junaidu *et al.*, 2011), lactation status (Junaidu *et al.*, 2011) and season (Nuru and Dennis, 1976; Bertu *et al.*, 2012). However, other variables such as pregnancy status and state have not been assessed. All these risk factors need to be taken into consideration in the design and execution of effective control programmes in Nigeria.

In the development of a livestock industry, disease eradication and control are paramount. The continuous movement of cattle as a result of trade and for grazing is a common practice in Nigeria, putting many herds at risk of brucellosis infection. So too do the sharing of bulls and use of open-range grazing. Reports have indicated that trade cattle in and from the northern states, and also those from across the northern borders of Chad and Niger showed evidence of infection (Esuruoso, 1974; Cadmus *et al*., 2008; Bertu *et al*., 2012). More than 20% of the trade cattle came from outside Nigeria (Esuruoso, 1974). The nature of the communal grazing systems used by the nomadic Fulanis, as well as porosity of the borders, predisposes livestock in the study area to infection, with a serious risk to human health (Esuruoso, 1974; Nuru and Dennis, 1976; Rikin, 1988). Even in developed countries, despite the preventive and control measures that exist, there is still a high potential for transmission and spread of *Brucella* organisms via animals and their products (Acha and Szyfres, 2003).

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Recent estimates of losses in meat and milk production as a result of brucellosis are $800 million annually in the USA (Richey and Harrell, 2008), in excess of $224 million in Nigeria (Esuruoso, 1974) and $37.5 million in South Africa (Anon., 1990).

Many of the studies conducted on brucellosis in Nigeria have been from the humid southern part (Esuruoso and Van Blake, 1972; Esuruoso, 1974; Cadmus *et al*., 2008) and were mainly abattoir surveys which were not representative of the general population. Abortions in Adamawa province in the late 1940's caused so much concern that *B. abortus* strain 19 vaccination was implemented in 1949, 1953 and 1956 (Nuru and Schnurrenberger, 1975). Since then no surveys to our knowledge have been done on brucellosis in Adamawa province, except that of Atsanda and Agbede (2001) who used the RBPT and SAT, and very recently Bertu *et al.* (2012) who analysed some samples collected from sick animals using RBPT and a rapid field test. Most studies in northern Nigeria were based on small sample sizes or suspect samples submitted to laboratories, or were done in restricted locations or in abattoirs (Bale and Kumi-Diaka, 1981; Pam, 1995; Ocholi *et al.*, 1996; Ocholi *et al.*, 2004a; Junaidu *et al.*, 2008; Adamu, 2009; Junaidu *et al.*, 2011; Bertu *et al.*, 2012). In addition, most studies in Nigeria have relied on the relatively non-specific RBPT and few have used a more specific confirmatory test such as ELISA (Ocholi *et al.*, 1996). Reported sensitivity and specificity of RBPT in Zambian cattle were 90.0% and 75.0% (Muma *et al*., 2007a) and for c-ELISA they were 98.0% and 99.0%, respectively (Nielsen *et al.,* 1996).

This study was prompted by an apparent increase in the occurrence of bovine brucellosis in Nigeria (Ocholi *et al.*, 2004a; Junaidu *et al.*, 2011), and therefore the need to obtain an accurate estimate of its prevalence and examine the role of the commonly practiced traditional management system of pastoral Fulanis. There is a dearth of studies using a specific diagnostic test and covering wider geographical areas and different management systems. The objective of this study therefore was to use a structured, multistage sampling strategy, combined with a sensitive and specific diagnostic test, to estimate the animal- and herd-level seroprevalence of bovine brucellosis in three states of northern Nigeria. A secondary objective was to estimate and compare seroprevalence between different management systems and to assess the effect of certain animal-level risk factors on seropositivity in both private and government herds, including some settled and pastoral Fulani herds that usually resist attempts to evaluate their herds.

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Materials and Methods

The research protocol for this study was approved by the Animal Use and Care Committee of the University of Pretoria (Protocol no. V073-08).

Selection of study states

Three states out of the nineteen were sampled from the northern region of Nigeria. The states selected were Kaduna, Kano and Adamawa (Fig. 2.1). Their selection was based on their location, proximity to a reliable laboratory, farming systems, human and cattle populations, cooperation from farmers, sharing of international borders and variety of cattle breeds.

Adamawa state

The state has a total land area of 42,159 km^2 and a cattle population of 3.8 million, lying between latitudes 8°N and 11°N and longitudes 11.5°E and 13.5°E (Bourn *et al.*, 1992). There are two main vegetation zones in the state: the sub-Sudan characterized by short grasses and short trees commonly found in the northern parts and the Guinea savannah zones where the vegetation is thick with tall grasses and trees in the southern parts. Average daily temperature between 15.2°C and 42°C, and relative humidity ranging from 27-79% were recorded. The rainy season commences from May and ends in October, with average annual rainfall of 1600 mm in the southern parts and 759 mm in the northern parts (Adebayo and Tukur, 1999).

Kaduna state

Kaduna state has a land area of 48,473 km^2 and a cattle population of 3.1 million, and is located between latitudes 9°N and 11.3°N and longitudes 10.3°E and 9.6°E (Bourn *et al.*, 1992). The state extends from the Sudan savannah in the north to the tropical grassland of the Guinea savannah in the south. Daily temperatures range from 14-30°C with a relative humidity of 12-72%. The rainy season is usually from April through November, with greater variation in the northern part. The annual rainfall varies, decreasing from an average of about 1530 mm in the southeast to about 1,015 mm in the northeast (Oyedipe *et al.*, 1982a; Mai, 1997).

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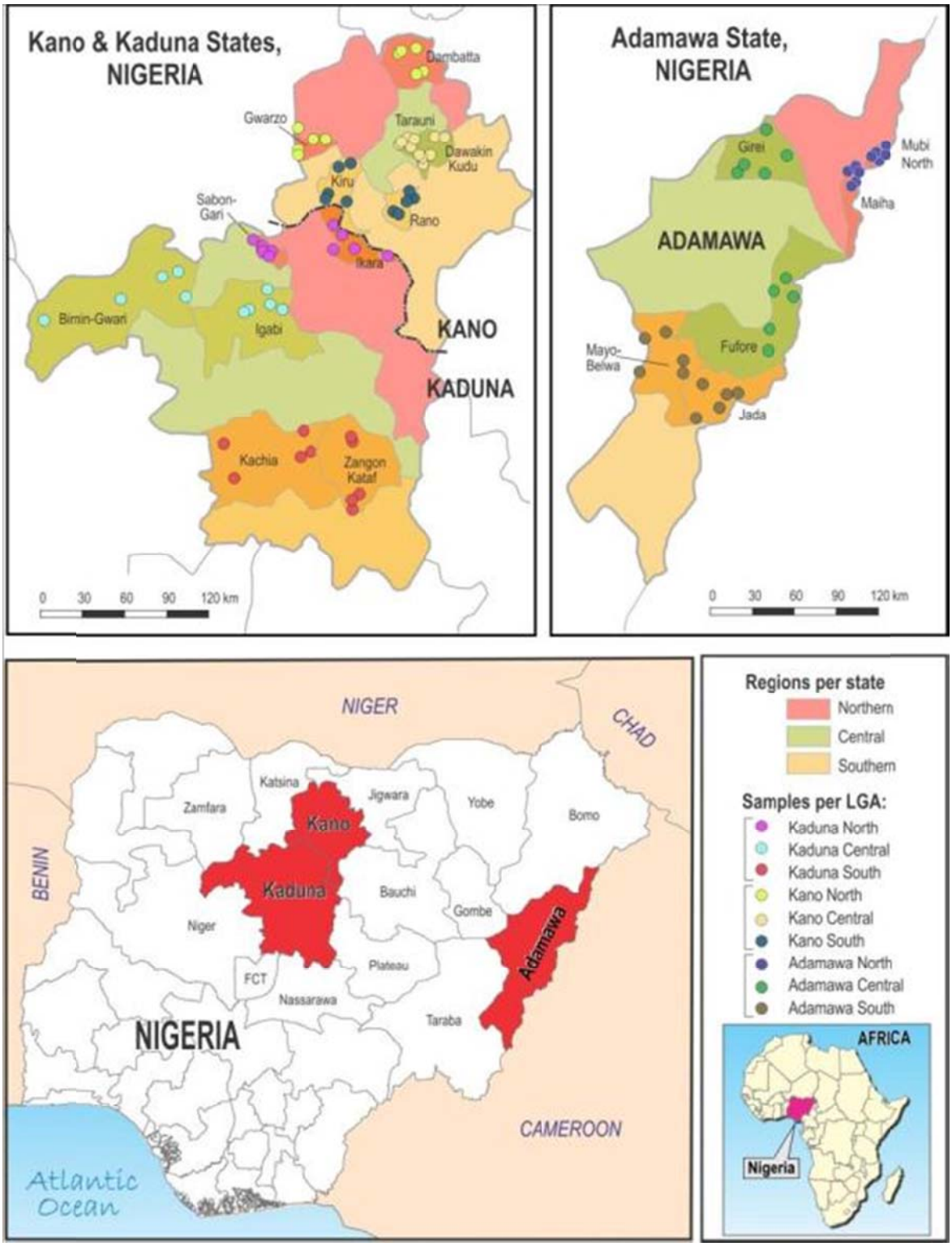

Figure 2.1. Map of Nigeria showing the three states, 18 local government areas and 89 wards sampled in northern Nigeria

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Kano state

This state has a land area of 42,593 km^2 and cattle population of 3.2 million. It is situated at latitudes 12°N and longitudes 9°E (Bourn *et al.*, 1992). The location is within the Sudan savannah in the north and the Guinea savannah vegetations in the south which provides ample natural fodder for cattle to graze. The temperatures range from 26-40°C with a relative humidity of 11-68%. The rainfall with a duration of about 3–5 months between May and September, ranges from over 1,000 mm in the extreme south to a little less than 800 mm in the extreme north (Anon., 2012c,d).

Sample size

To calculate the required number of farms to be sampled in order to estimate the prevalence of *B. abortus*-infected herds, an expected herd prevalence (P_{exp}) of 40%, desired absolute precision (d) of 10% and a confidence level of 95% were applied using the formula $n = 1.96^2 P_{\text{exp}} (1 - P_{\text{exp}})/\text{d}^2$ (Thrusfield, 2005), resulting in a required sample size of 93 farms. However, multistage cluster sampling was used because of its practical advantages and flexibility. Therefore, the design effect (D) of the survey was calculated using the formula $D = 1 + (b - 1)\ roh$ (Bennett *et al.*, 1991), where b is the average number of samples per cluster and *roh* is the rate of homogeneity, equivalent to the intra-cluster correlation coefficient (ρ) in single-stage cluster sampling. It was decided to sample approximately 12 to 13 farms per local government area ($b = 13$). An intra-cluster correlation coefficient of $\rho = 0.09$ was reported for *B. abortus* in cattle (Otte and Gumm, 1997); in order to account for the multistage design, a higher value of 0.15 was used for *roh*. The design effect was therefore calculated to be $D = 2.8$ which, multiplied by the original calculated sample size, gave a required sample size of 261 farms. Ultimately, a total of 271 herds was sampled.

Survey design

Each of the three selected states was divided into three geographic zones: northern, central and southern (Fig. 2.1). Six local government areas (LGA's) were randomly selected from each state (two LGA's per geographic zone), using as sampling frame a list of all LGA's in each zone. Similarly, within each selected LGA's, approximately 50% of the wards (4 to 6 per LGA's) were randomly selected (Fig 2.1). Within each selected ward, available herds were used. Since no sampling frames were available for selection of herds within wards, herds were selected by visiting the farms and taking the first few that agreed. An average of

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three herds was selected per ward, giving an average of 15 herds selected per LGA. Animals sampled in each selected herd included all the breeding bulls and other mature bulls, first calf heifers that had calved at least six weeks previously, and all the mature heifers and cows.

The following four management systems were encountered: 1) a pastoral system which is practiced by the Fulanis that move for long distances from location to location in search of pasture during the dry season, while in the rainy season their animals graze close by since pasture is available; 2) an agro-pastoral system in which animals do not travel long distances, but graze communally and return in the evening, and are given supplementary feeds, including crop residues, particularly during the critical period of the dry season; 3) commercial farms that are intensively managed, usually fenced and in some cases paddocked, and where the cattle are well supplemented with feeds in addition to sown and natural pastures; and 4) zero-grazing herds that are also intensively managed but the cattle are more restricted or tethered in one location and are provided with feeds where they are confined.

Demographic data and sample collection

Blood was collected from the jugular, coccygeal or saphenous veins into Vacutainer® tubes, which were immediately placed into an ice bath and transported to the laboratory within a maximum of 7 hours. When the outside ambient temperature was cool, the clot was allowed to form in the vacutainer tube in the field before transportation. The samples were centrifuged at 3,000 rpm for 15 minutes and the serum was removed and stored at −20°C until analyzed.

Pre-tested and structured questionnaires (Appendix I) were administered during the sample collection to determine the profile of the animal, including the presence of hygroma, orchitis or epididymitis, as well as information about the farm and herd. Pregnancy diagnosis was determined by trans-rectal palpation. Age was estimated using farm records, dentition and, in some cases, cornual rings.

Data obtained from the serological test and questionnaires were stored in a spreadsheet programme (Excel 2007; Microsoft Corp., Redmond, WA, U.S.A.).

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Screening using Rose-Bengal plate-agglutination test

The RBPT (VLA, Weybridge, UK) was done on all samples in accordance with the manufacturer's instructions. All samples testing positive or which were inconclusive using the RBPT were further subjected to c-ELISA.

Confirmation using competitive enzyme-linked immunosorbent assay

This test was performed using a competitive ELISA (c-ELISA) kit (COMPELISA, VLA, Weybridge, UK) according to the manufacturer's instructions, in order to confirm the RBPT positive and inconclusive samples. The optical density (OD) was measured at 450 nm using a microplate ELISA reader (SIGMA DIAGNOSTICS EIA Multi-well Reader II). A positive/negative cut-off was calculated as 60% of the mean OD of the four conjugate control wells. Any test sample giving an OD equal to or below this value was regarded as positive.

Data analysis

A positive herd was defined as any herd that had at least one animal positive to both RBPT and c-ELISA. For each ward the sampling fraction was calculated as the product of the proportion of wards sampled within the LGA and the proportion of LGAs sampled within the state. The sampling weight was then calculated as the inverse of the sampling fraction. Because it was not possible to calculate the proportion of farms sampled within each ward, and because all eligible animals on each farm were tested, the same sampling weight was assigned to every animal within a ward. Estimates of the animal- and herd-level seroprevalences were calculated by state, management system, age, sex and breed using the '*svy*' command in STATA 12, which accounts for sampling weights, stratification and clustering in the multistage survey design to produce adjusted prevalence estimates and standard errors. Seroprevalence estimates were then compared using the Chi-square test, corrected for the survey design using the second-order correction of Rao and Scott (1984). Animal-level prevalences were also calculated and were adjusted for the sensitivity and specificity of the serial testing system. The sensitivity of the test series was calculated as: $Se = Se_{RBPT} \times Se_{ELISA} = 90.0\% \times 98.0\% = 87.9\%$ and the specificity was calculated as $Sp = 1 - (1 - Sp_{RBPT}) \times (1 - Sp_{ELISA}) = 1 - (1 - 75.0\%) \times (1 - 99.0\%) = 99.8\%$. True prevalence was then calculated using the formula of Rogan and Gladen (1978): $TP = (AP + Sp - 1) / (Se + Sp - 1)$, where AP = apparent prevalence, Se = sensitivity of the test series, Sp = specificity of the test series.

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To adjust for confounding amongst animal-level factors (age, sex and breed), as well as state and management system, their association with brucellosis seropositivity was assessed using a hierarchical mixed-effects logistic regression model. Age, sex, breed, state and management system were included as categorical fixed effects. Local government area, ward and herd were included as nested random effects, with ward nested within LGA and herd nested within ward. In addition, data were restricted to females only and pregnancy and lactation status were added to the model in order to estimate their association with brucellosis seropositivity. No variable selection or elimination was done. Fit of the models excluding the random effects was assessed using the Hosmer-Lemeshow goodness-of-fit test. All analyses were done using STATA 12 (Stata Corporation, College Station, TX, USA) and a significance level of 5% was used.

Results

Data from 4,745 samples from 271 herds were available for analysis. Of the 271 herds included in the study, there were 225 herds (84.9%) with at least one animal testing positive based on RBPT and 210 herds (77.5%) based on c-ELISA. The herd-level seroprevalence of brucellosis in the three states combined, adjusted for the sampling weights, was estimated to be 77.5% (95% CI, 68.6%-84.5%). The herd-level seroprevalences for both tests in the individual states adjusted for the sampling weights are shown in Table 2.1. Twenty three per cent of all herds sampled and 30% of the infected herds had animals with hygromas (Table 2.1). Of the 63 herds in which hygromas were present, 54 (64.3%) were from Adamawa, 7 (13.7%) from Kano and 2 (2.7%) from Kaduna states (Table 2.1). Only one herd with animals with hygromas (1.6%) was negative for brucellosis.

A total of 4,745 samples was tested with RBPT, of which 1,735 (36.6%) were positive. Of these, 1,137 (65.5%) were confirmed to be seropositive for brucellosis upon further testing by c-ELISA, giving 34.5% overall false positives (42.8%, 24.7% and 27.1% in Adamawa, Kaduna and Kano states, respectively) (Table 2.2). Based on c-ELISA, the estimated overall survey adjusted true animal-level seroprevalence was 26.3% (95% CI, 22.1%-31.0%) (Table 2.3). The prevalence for Adamawa state was 29.2% (95% CI, 22.5%-36.9%), for Kaduna state 23.3% (95% CI, 18.9%-28.3%) and for Kano state 26.7% (95% CI, 18.8%-36.7%) (Table 2.3).

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Table 2.1. Herd-level seroprevalence of bovine brucellosis based on RBPT and c-ELISA, adjusted for sampling weights, and presence of hygroma, orchitis or epididymitis in three states of northern Nigeria

State	n	RBPT pos. (%)	c-ELISA pos. (%)	95% CI	Hygroma (%[#])	Orchitis/ epididymitis (%[#])
Adamawa	100	89 (88.0)	84 (82.3)	66.8 - 91.5	54 (64.3)	12 (14.3)
Kaduna	105	78 (74.8)	75 (72.0)	58.6 - 82.4	2 (2.7)	12 (16.0)
Kano	66	58 (89.6)	51 (78.5)	61.2 - 89.4	7 (13.7)	7 (13.7)
Overall	271	225 (84.9)	210 (77.5)	68.6 - 84.5	63 (30.0)	31 (14.8)

Key:
n: Sample size
c-ELISA: Competitive enzyme-linked immunosorbent assay
RBPT: Rose-Bengal plate-agglutination test
CI: Confidence Interval
[#] Percentage of c-ELISA-positive herds showing hygroma, orchitis or epididymitis

Table 2.2. Apparent animal-level seroprevalence of bovine brucellosis in three states of northern Nigeria based on RBPT and percent (%) of samples positive by c-ELISA tests

State	n	RBPT pos. (%)	c-ELISA pos. (%)	FP (%)
Adamawa	1827	892 (48.8)	510 (27.9)	382 (42.8)
Kaduna	1870	511 (27.3)	385 (20.6)	126 (24.7)
Kano	1048	332 (31.7)	242 (23.1)	90 (27.1)
Overall	4745	1735 (36.6)	1137 (24.0)	598 (34.5)

Key:
n: Sample size
RBPT: Rose-Bengal plate-agglutination test
c-ELISA: Competitive enzyme-linked immunosorbent assay
FP (%): False positives (proportion of RBPT positive animals that were c-ELISA negative)

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Table 2.3. Animal-level seroprevalence of brucellosis in cattle in three states of northern Nigeria, by breed, sex, age, management system, pregnancy status and lactation status, adjusted for sampling weights and test sensitivity and specificity

Variable and level	n	Adjusted prev. (%)	95% CI (%)	*P*-value*
All Animals				
State				0.496
Adamawa	1827	29.2	22.5 - 36.9	
Kaduna	1870	23.3	18.9 - 28.3	
Kano	1048	26.7	18.8 - 36.7	
Management system				<0.001
Zero-grazing	101	23.8^{ab}	6.8 - 59.2	
Commercial	642	15.9^{a}	9.5 - 25.5	
Agro-pastoral	2758	22.0^{a}	17.3 - 27.8	
Pastoral	1244	45.1^{b}	38.6 - 51.9	
Breed				0.392
Bunaji	3052	27.5	22.5 - 33.2	
Gudali	863	26.3	22.1 - 31.1	
Bos taurus	118	15.1	6.6 - 31.0	
B. taurus x B. indicus	267	21.8	11.7 - 37.0	
Other *B. indicus*	445	24.7	17.8 - 33.5	
Sex				<0.001
Males	596	38.2^{a}	31.7 - 45.2	
Females	4149	24.7^{b}	20.4 - 29.5	
Age				<0.001
< 4 years	559	13.5^{a}	8.9 - 19.9	
4 - 5 years	2038	23.8^{b}	19.9 - 28.1	
5 - 7 years	1312	29.8^{c}	22.6 - 38.2	
> 7 years	804	35.0^{c}	28.5 - 42.3	
Females Only				
Pregnancy status				<0.001
Pregnant	1367	17.2^{a}	13.6 - 21.5	
Non-pregnant	2835	27.8^{b}	22.9 - 33.5	
Lactation status				0.635
Lactating	1819	25.3	20.7 - 30.5	
Non-lactating	2386	23.2	19.3 - 29.9	
Total	4745	26.3	22.1 - 31.0	

Figures with different superscripts within the same variable differ significantly ($P < 0.05$).
* *P*-value determined using the Chi-square test, corrected for the survey design using the second-order correction of Rao and Scott (1984).

Overall mean within-herd seroprevalence of brucellosis was 24.6%. Within positive herds, the mean prevalence of seropositive animals, adjusted for survey design, was 30.2% (95% CI,

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25.3%-35.1%) and ranged from 3.1% to 85.7%. The distribution of within-herd seroprevalence in infected herds for the various management systems is shown in Fig. 2.2. Mean within herd seroprevalences in the different management systems, from lowest to highest, were: commercial 18.5% (95% CI, 10.2%-26.8%), agro-pastoral 25.2% (95% CI, 19.0%-31.4%), pastoral 43.0% (95% CI, 38.6%-47.5%) and zero-grazing 51.1% (95% CI, 46.9%-55.3%).

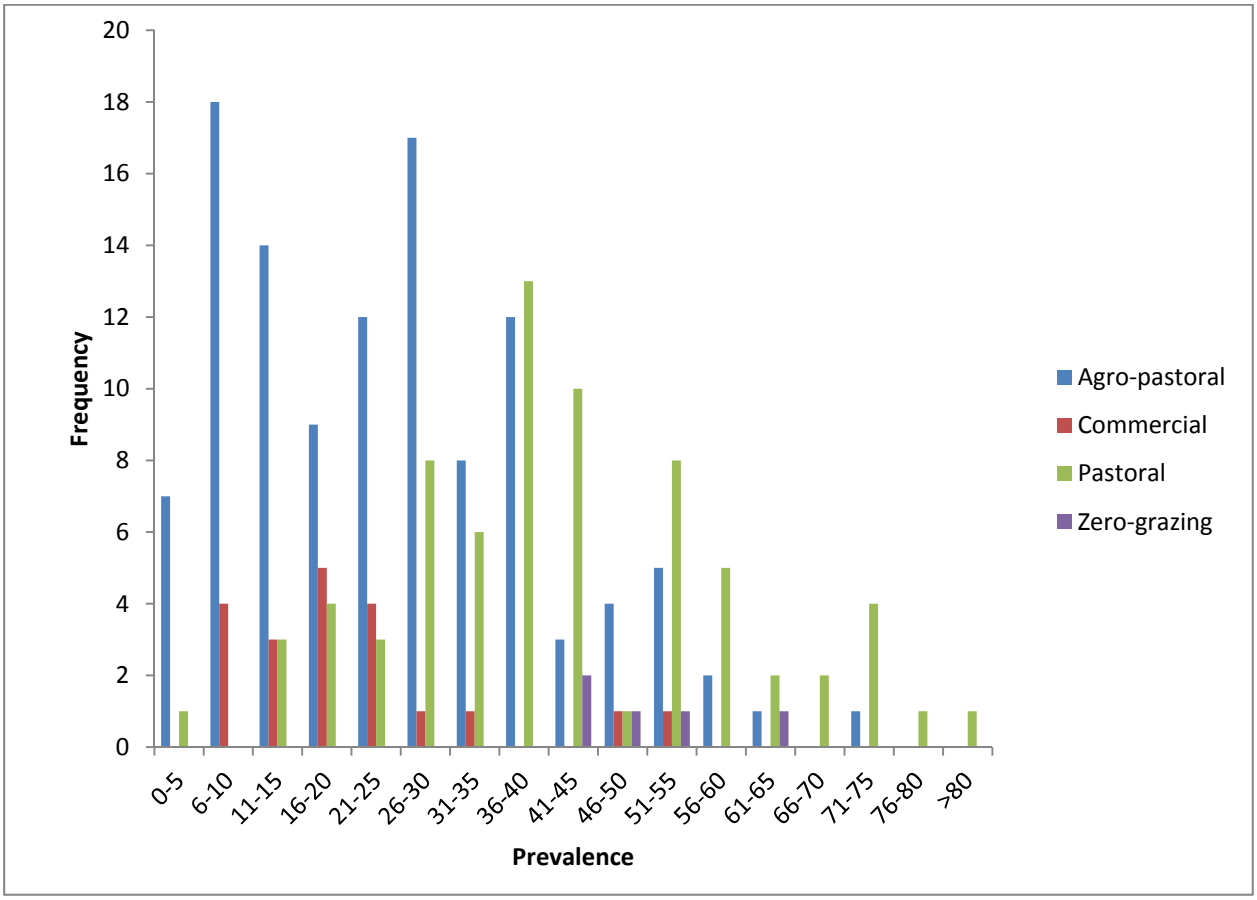

Figure 2.2. Distribution of within-herd prevalence of seropositive animals in brucellosis-positive herds in the different management systems

The seroprevalence differed significantly between males (38.2%; 95% CI, 31.7%-45.2%) and females (24.7%; 95% CI, 20.4%-29.5%) ($P < 0.001$), between cattle <4 years (13.5%; 95% CI, 8.9%-19.9%) and >7 years of age (35.0%; 95% CI, 28.5%-42.3%) ($P < 0.001$), between commercial farms (15.9%; 95% CI, 9.5%-25.5%) and pastoral farms (45.1%; 95% CI, 38.6%-51.9%) ($P < 0.001$), and between non-pregnant (27.8%; 95% CI, 22.9%-33.5%) and pregnant females (17.2%; 95% CI, 13.6%-21.5%) ($P < 0.001$). There was no significant difference in seroprevalence between lactating (25.3%; 95% CI, 20.7%-30.5%) and non-lactating (23.2%; 95% CI, 19.3%-29.9%) animals ($P = 0.635$) or between breeds ($P = 0.392$),

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although *Bos taurus* had the lowest prevalence of 15.1% (95% CI, 6.6%-31.0%) and Bunaji had the highest prevalence of 27.5% (95% CI, 22.5%-33.2%) (Table 2.3).

The associations between animal-level factors and brucellosis seropositivity, adjusted in the multivariable model for confounding both by the other animal-level factors and by state and management system, are shown in Table 2.4. The greater odds of seropositivity in males (OR = 1.98; 95% CI, 1.54-2.54; $P < 0.001$) remained, as did the monotonic increase with increasing age (e.g., for >7 y vs. < 4 y: OR = 3.82; 95% CI, 2.72-5.36; $P < 0.001$) and the increased odds in non-pregnant compared to pregnant cows (OR = 1.84; 95% CI, 1.49-2.27; $P < 0.001$). Neither state nor management system acted as confounders of the effects of the above variables, as their exclusion resulted in <10% change to coefficients. Although there was ultimately no significant effect of breed, there was some confounding both by state and by management system, with their exclusion from the model resulting in up to 66% and 180% change in coefficients, respectively. Similarly, there was some confounding of the effect of lactation status on seropositivity by both state and management system. The odds of seropositivity were significantly higher in the pastoral management system than in the other management systems (e.g., vs. agro-pastoral: OR = 3.52; 95% CI, 2.50-4.95; $P < 0.001$). The random effects in the hierarchical model showed that there was significant variation in brucellosis seropositivity between herds within ward and between wards within LGA, but not between LGAs within state.

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Table 2.4. Animal-level risk factors for brucellosis seropositivity, adjusted for state and management system: results from hierarchical mixed-effects logistic regression models

Risk factor and level	OR	95% CI (OR)	*P*-value[#]
All Animals			
Breed			
Bos taurus	1*	–	–
Gudali	1.52	0.65 - 3.54	0.334
Bunaji	1.52	0.66 - 3.50	0.327
Other *B. indicus*	1.59	0.67 - 3.75	0.293
B. taurus x *B. indicus*	1.71	0.71 - 4.11	0.230
Sex			
Females	1	–	–
Males	1.98	1.54 - 2.54	<0.001
Age			
< 4 years	1	–	–
4 - 5 years	1.70	1.26 - 2.31	0.001
5 - 7 years	2.50	1.82 - 3.44	<0.001
> 7 years	3.82	2.72 - 5.36	<0.001
Females Only			
Pregnancy status			
Pregnant	1	–	–
Non-pregnant	1.84	1.49 - 2.27	<0.001
Lactation status			
Lactating	1	–	–
Non-lactating	1.17	0.96 - 1.43	0.112
Confounders			
State			
Kaduna	1	–	–
Kano	1.09	0.66 - 1.78	0.737
Adamawa	1.54	0.96 - 2.45	0.073
Management system			
Agro-pastoral	1	–	–
Commercial	1.06	0.55 - 2.04	0.860
Zero-grazing	1.32	0.53 - 3.31	0.555
Pastoral	3.52	2.50 - 4.95	<0.001
Random effects			
LGA	–	–	1.000
Ward	–	–	<0.001
Herd	–	–	<0.001

[#] Wald *P*-value
* Reference level

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Discussion

The study revealed that bovine brucellosis is still prevalent in the three states of northern Nigeria covered, with a herd-level prevalence of 77.5%, higher than the 40% reported in Zimbabwe (Matope *et al.*, 2011), 42% in Ethiopia (Berhe *et al.*, 2007), 56% in Uganda (Bernard *et al.*, 2005) and 63% in Brazil (Aguair *et al.*, 2007). Interestingly, a very similar herd prevalence of 77.8% was reported 40 years ago in southern Nigeria (Esuruoso and Van Blake, 1972). The dissemination of Ndama cattle, reportedly the most heavily infected breed (Esuruoso, 1974), to various parts of the country as foundation stocks because of their good beef conformation and resistance to trypanosomosis and dermatophilosis infection may have contributed to the high prevalence in other parts of the country. Other interstate movement and trade in cattle across the country, as well as the nomadic nature of the pastoral Fulanis may also have contributed to high infection rates (Esuruoso, 1974; Rikin, 1988; Cadmus *et al*., 2008; Bertu *et al*., 2012).

Livestock production in Nigeria was dominated by nomadic pastoralism long before the advent of the colonial era. In the1930s the government established stock farms for dairy herds by selective breeding. In the same period, mixed farming policy such as agro-pastoral production system as well as range management was introduced for livestock improvement in Nigeria (Anon., 2012b). In the 1940s to 1950s, government investigation and breeding centres in settled herds all over the country and AI were established. It was also within this period that exotic breeds of cattle were introduced to upgrade the local stock (Anon., 2012b). Brucellosis infection rate of over 30% was reported during the 1940s at various livestock centres in Nigeria characterized by abortion storms (Esuruoso and Van Blake, 1972; Esuruoso, 1974). Attempts were made to vaccinate cattle against brucellosis but it was limited and irregular (Esuruoso, 1974; Cadmus *et al.*, 2008). Between the 1970s and 1990s, about 96% of the cattle were zebu-type cattle, most of which were tended by traditional Fulani pastoralists (Anon., 2012e). In addition, in the 1970s, 30 to 40% of the beef consumed in Nigeria was imported from Niger, Chad, and other neighboring countries (Anon., 2012e). These factors may have influenced the increase in prevalence of bovine brucellosis in Nigeria (Acha and Szyfres, 2003; Ocholi *et al*., 2004a).

An overall adjusted animal-level true prevalence of 26.3% (95% CI, 22.1-31.0%) was obtained in this study (Table 2.3). Of the three states sampled, Adamawa state had the highest apparent animal-level seroprevalence of 29.2%, although this was not significantly higher

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than in Kano (26.7%) or Kaduna (23.3%). However, after adjustment for confounding, the difference between Adamawa and Kaduna approached significance ($P = 0.07$). In addition, Adamawa state showed the highest number of cattle exhibiting hygroma, seen in 54 of 84 positive herds (Table 2.1). Adamawa state borders on Cameroon, and constant trans-border movement of cattle has been reported to result in transmission of contagious bovine pleuropneumonia (Chima *et al*., 2001). Cross-border movement has been implicated in the transmission of brucellosis by previous investigators in Nigeria (Esuruoso, 1974; Bale and Kumi-Diaka, 1981) and elsewhere (Kubuafor *et al*., 2000; Menachem, 2002), and although not directly implicated by this study, it is possible that it may be a risk factor for brucellosis in Adamawa state.

The variations in the results of the two tests showed that many of the RBPT results were falsely positive because of its relatively low specificity, with Adamawa state showing the greatest discrepancy between the two tests (Table 2.2). The c-ELISA of all the samples was done in the same laboratory at the same time, while screening using RBPT was done in two different laboratories at different times but by the same investigator, thereby reducing the possibility of laboratory error or subjective interpretation. Since none of the herds sampled had been vaccinated against brucellosis, the antibodies responsible for the false positives were likely from other sources. Some bacterial pathogens such as *Yersinia enterolitica* serovar IX, *Vibrio cholera*, *Escherichia coli* O:157, *Salmonella* spp. and *Sternotrophomonas maltophilia* have been reported to produce cross reacting antibodies to brucellosis (Nielsen *et al*., 2004; Munoz *et al*., 2005), with *Y. enterolitica* being the most significant cause of false positives. It is possible that the prevalence of one or more sources of cross-reacting antibodies was higher in Adamawa than in the other two states. In a Zambian study, for every three positive RBPT animals, only one tested positive on c-ELISA, except among animals which had aborted, where the ratio was close to one (Muma *et al*., 2012). The c-ELISA test, with a higher specificity than RBPT, CFT and FPA, and therefore an ideal confirmatory test (Muma *et al*., 2007a), has however rarely been used in Nigeria in naturally infected cattle, and never in a large study including different production systems.

The animal-level prevalence reported in this study (26.3%) is higher than recent reports from northern Nigeria (Junaidu *et al*., 2011). Furthermore, the prevalence is much higher than the 9.8% and 18.6% using RBPT and MRT respectively in indigenous cattle in abattoirs in western Nigeria (Cadmus *et al*., 2008), 20% in government farms in the north using SAT

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(Eze, 1978), and 6.6% in cattle herds in northern Nigeria using ELISA (Ocholi *et al.*, 1996), but lower than a recent report of 45% from samples of sick animals in Adamawa state, Nigeria, using RBPT and rapid field test (Bertu *et al.*, 2012). However, the result is consistent with the 32% within-herd prevalence reported in one prison farm in northern Nigeria using serum agglutination test (SAT) and MRT (Junaidu *et al*., 2008); and the 38.0% using RBPT and SAT in cattle in government Livestock Investigation and Breeding Centers in Kano (Bale and Kumi-Diaka, 1981). Studies elsewhere showed prevalences between 49% and 60% among breeding cows and heifers, dairy farms and abattoir surveys in the southern and western states of Nigeria on the basis of SAT, with CFT on doubtful results (Esuruoso and Van Blake, 1972; Esuruoso, 1974). The animal-level prevalence obtained in this study was also much higher than those reported in South Africa (Anon., 1989), North Africa (Refai, 2000) and East Africa (Berhe *et al.*, 2007; Ibrahim *et al.*, 2010). However, a higher prevalence of 41% was reported in Togo, West Africa (Akapo, 1987). McDermott and Arimi (2002) also reported a higher prevalence in sub-Saharan Africa. Although some of the variation in results between studies may be due to the use of different diagnostic techniques, considering only those studies that used the same serological test as the present study, the prevalence of bovine brucellosis appears to be increasing in northern Nigeria. Several reports have previously indicated that brucellosis is on the increase in Nigeria (Bale and Kumi-Diaka, 1981; Ocholi *et al.*, 2004a; Junaidu *et al*., 2011) and other developing countries (McDermott and Arimi, 2002; OIE, 2011a). Lack of proper surveillance and control measures in most parts of Africa may be contributing to this increase, as may the importation of animals and their products from more developed countries despite the preventive and control measures in such countries (McDermott and Arimi, 2002; Acha and Szyfres, 2003; OIE, 2011a). Nevertheless, despite reports showing the extent of brucellosis in Nigeria, there is no record of a proper brucellosis control programme in the country (Ocholi *et al.*, 2004a; Bertu *et al.,* 2012).

Traditional Fulani herds practicing nomadism or pastoralism showed the highest prevalence followed by the zero-grazing and agro-pastoral systems, with the lowest prevalence being recorded in commercial herds. The findings by Nuru and Dennis (1975), Eze (1978), and Ocholi *et al*. (1996) who reported prevalences of between 0.4% and 26% in traditional nomadic Fulani herds; and Atsanda and Agbede (2001) who reported a slightly higher infection rate of 5.1% among nomadic cattle herds than among settled cattle herds (4.4%) in Adamawa state, are consistent with the findings of our study. Furthermore, Rikin (1988) and

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Ocholi *et al*. (2004a) reported the prevalence of brucellosis to be rising among pastoral and semi-pastoral herds which comprise about 95% of the cattle population in Nigeria (Nuru and Dennis, 1976); Bale and Kumi-Diaka (1981) indicated free movement of the pastoral Fulani herdsmen and interaction of cattle with those of other Fulani herdsmen as major factors in spreading brucellosis. These observations agree with our findings, as do reports by McDermott and Arimi (2000) and Matope *et al*. (2011) of highest occurrence in pastoral production systems in arid and semi-arid areas in Zimbabwe and other parts of Africa, and Bernard *et al*. (2005) in Uganda and Berhe *et al.* (2007) in Ethiopia who also reported a higher seroprevalence in the transhumance system than in sedentary cattle.

The odds of brucellosis seropositivity were 3.5 times greater amongst pastoral herds than agro-pastoral herds in this study. The high prevalence of brucellosis in a pastoral management system may partly be attributed to long distance movement of cattle in search of pasture and water and comingling in communal grazing areas and at watering points, particularly during the dry season. Musa *et al*. (1990) observed in Sudan that clinical manifestation of brucellosis often began during adverse weather conditions and famine. During such times animals become concentrated on scarce pastures and around watering points, which may become contaminated with aborted foetal materials or fluids from infected normal calvings. Many pastoralists do not isolate cows during parturition or dispose of the afterbirth following calving, resulting in contamination of the environment and transmission of brucellosis within and between herds. Other possible risk factors for brucellosis associated with the pastoral management system in Nigeria include bull sharing which may result in venereal transmission of brucellosis (Bercovich, 1998), purchasing of livestock from markets without quarantine (Bale and Kumi-Diaka, 1981) and interaction of cattle with wildlife (Avong, 2000; Muma *et al*., 2007b).

The prevalence of brucellosis in zero-grazing systems in this study was also high. This is contrary to the report by McDermott and Arimi (2002) of low prevalence due to very low level of between-herd contacts. However, Bayemi *et al.* (2009) and Karimuribo *et al.* (2007) observed a high prevalence in intensively managed herds. Cattle in zero-grazing systems in Nigeria are generally bought from the open market for a fattening programme, which may explain the high prevalence in such systems.

Reports indicate that about 20% of infected pregnant animals do not abort, while 80% of animals that abort as a result of *B. abortus* infection, do so only once (Anon., 1986) and

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thereafter will usually carry the pregnancy to full-term and appear healthy. In herds that have chronic brucellosis and do not introduce new animals, very few or no abortions occur and the disease is almost impossible to recognise clinically (Crawford *et al*., 1990). The emphasis in livestock production in Nigeria is on the ability of the females to produce calves; as long as cows produce, farmers tend to keep them, even if they have a history of abortion. In bulls, brucellosis causes no impairment of libido or breeding capacity (Kumi-Diaka *et al*., 1980a) and the disease is subclinical in most animals (Godfroid *et al*., 2004). For these reasons, farmers seldom cull infected animals from their herds, contributing to the high prevalence observed in this study. These apparently healthy cattle that are reproducing normally serve as permanent carriers of brucellosis. Some cattle may get rid of the infection within a few months, while others may remain infected for life, thereby transmitting the disease at subsequent parturitions (Nicoletti, 1980; Anon., 1986). This scenario could make control of the disease in Nigeria an extremely difficult task, requiring a well-designed and coordinated eradication policy and good cooperation of all sectors of the industry. Strategies such as immunization and the identification of and selection for genetic resistance factors may be required to make significant progress in control of the disease.

Cows with visible hygromas, but reproducing normally, are also left in the herds. All forms of hygromas were encountered in this study including fluid accumulation in some infected animals on the cervical region, between the nuchal ligament, shoulder, flank, primary thoracic spines and most commonly the carpal and stifle joints (Fig. 2.3). Over 23% of all herds sampled and 30% of the infected herds had hygroma (Table 2.1). This is the first report of this manifestation of the disease in Nigeria. Similar clinical signs have been reported elsewhere (Musa *et al.*, 1990; OIE, 2011a); these authors also used the term hygroma for fluid accumulation in locations other than the joints. The hygromas are localized in carpal and other bursae and contain large numbers of the organisms (Faul and Bosman, 1987; Jubb *et al*., 1993). The traditional Fulani cattle rearers practice ‘firing’ of the hygroma lesions, by using a hot knife to incise the swelling through the capsules, when large numbers of the localized brucellae are discharged from the hygroma and contaminate the environment, further encouraging the spread of the disease (Fig. 2.3). The herd that had hygroma and was serologically negative is consistent with a previous report that 13% of brucellosis positive animals were serologically negative (O’Hara and Christiansen, 1978).

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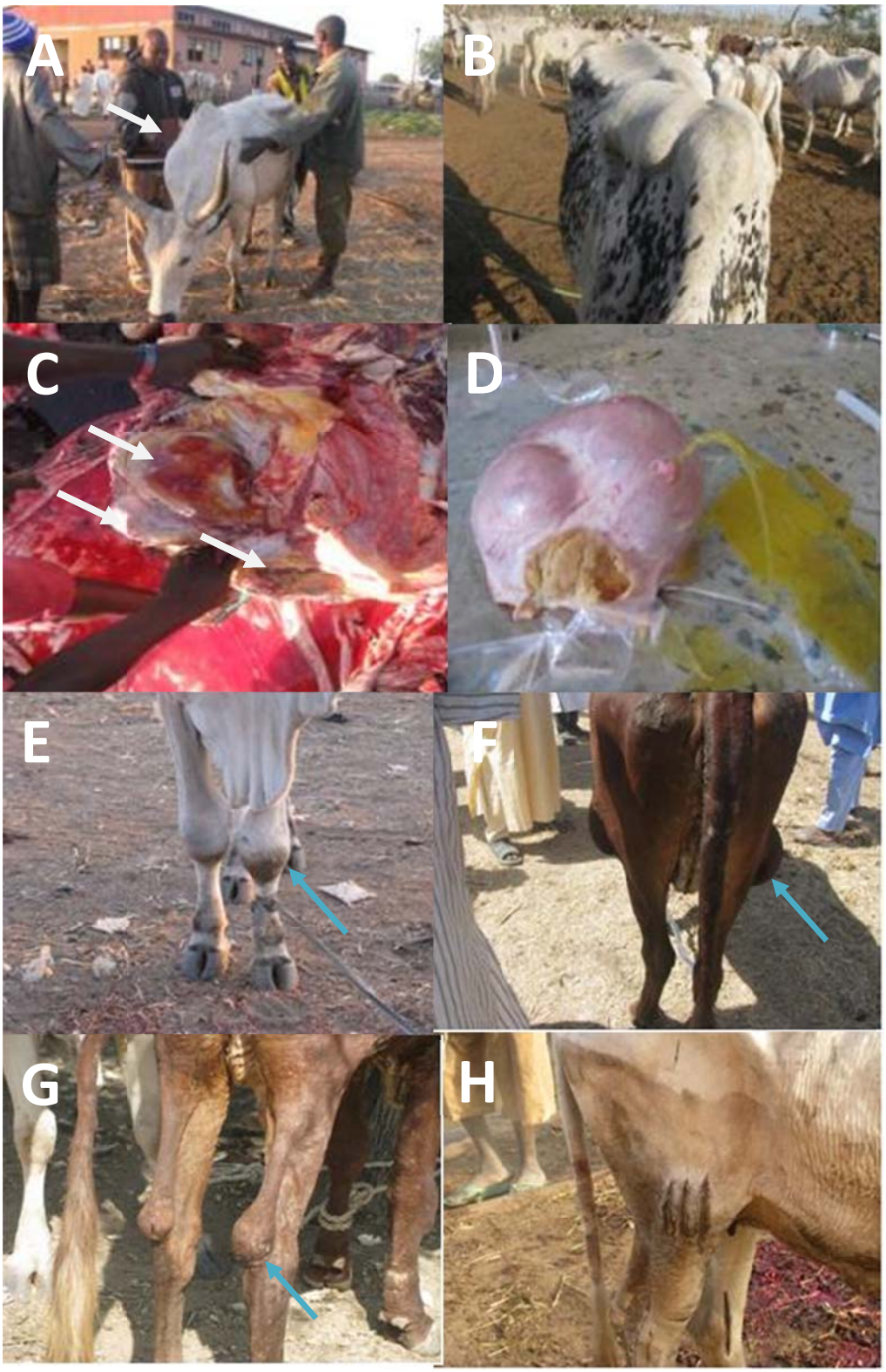

Figure 2.3. Various hygroma lesions encountered in study herds, capsules at *post mortem* examination and firing of hygroma lesions practiced by the pastoral Fulanis. A: Hygroma on right cervical region; B: Hygroma on withers or thoracic spine; C: Capsules from hygroma at post mortem; D: Capsule punctured with fluid post mortem; E: Bilateral carpal hygroma; F: Bilateral hygroma of stifle; G: Bilateral hygroma of hock joint; H: Firing of hygroma by pastoral Fulanis

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It is also possible that some of the hygromas observed in this herd may have been due to another aetiology, such as intermittent mild trauma to the precarpal area caused by lack of bedding or a poorly designed feed bunk (Kenyon, 2012). This may partly explain the very high prevalence of hygromas seen amongst the seropositive animals in Adamawa state. Nevertheless, the presence of hygroma in one or more animals in a herd appeared to be a fairly specific predictor of herd seropositivity, with estimated specificity of 98.4%. It could therefore be used in participatory disease surveillance, although its estimated sensitivity of only 29.5% would mean that other signs of herd infection would also have to be considered.

Although not statistically significant, the prevalence of brucellosis was somewhat lower in *Bos taurus* breeds than amongst indigenous breeds. This finding is consistent with reports by Kubuafor *et al.* (2000) in Ghana. Karimuribo *et al.* (2007) in Tanzania stated that the proportion of seropositive animals was significantly higher in indigenous than in crossbred cattle. However, Muma *et al*. (2007a) reported no association between *Brucella* seropositivity and cattle breed. The better management in the exotic herds, stall or intensive feeding that minimizes contact between animals and herds may be responsible for the low prevalence. The distribution of breeds between management systems in this study varied, with highest number of Bunaji found in agro-pastoral followed by pastoral systems; Gudali in agro-pastoral and commercial systems; and *Bos taurus* were mainly in commercial farms. However, adjustment for management system did not change the result. Esuruoso (1974) reported that the Ndama breed was the most affected breed in western Nigeria, while Cadmus *et al.* (2008) found the Red Bororo breed to have the most positive reactors followed by Bunaji. Junaidu *et al*. (2011) reported the Sokoto Gudali breed to have the highest prevalence, followed by Azuwarq, with Bunaji having the least prevalence. Genetic variation is an important factor in conferring resistance or tolerance of cattle breeds to a wide range of diseases, and the antibody response of animals classified as resistant to infection by *B. abortus* differed significantly from that of susceptible animals (Martinez *et al*., 2010). Significant genetic variability in resistance/susceptibility to brucellosis has been detected in cattle and associated with a 3' untranslated polymorphism in the *Slc 1a1* gene (Barthel *et al.*, 2001). This aspect needs further studies in Nigeria.

The prevalence of brucellosis was significantly higher in males than females, and this did not change after adjusting for age, management system, state or breed. This difference between sexes is consistent with reports by Chimana *et al.* (2010) who recorded more seropositive

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cases in bulls (12.5%) compared to females (8.1%). However, our findings are contrary to other reports that showed significantly higher prevalence in females than males (Junaidu *et al.*, 2008; Junaidu *et al*., 2010) or no difference between sexes (Ocholi *et al.*, 1996; Kubuafor *et al*., 2000; Bayemi *et al*., 2009). Fifteen percent of the infected herds had animals with clinical evidence of orchitis and/or epididymitis (Table 2.1). Reports in Nigeria and elsewhere indicated that testes, epididymis and other accessory sex organs may be affected (Kumi-Diaka *et al*., 1980a; Godfroid *et al.*, 2004).

In the present study, the prevalence of brucellosis increased with age, with the odds of having brucellosis 3.8 times greater amongst cattle >7 years than those <4 years old. This is consistent with previous reports (Kubuafor *et al.*, 2000; Bandyopadhyay *et al.*, 2009; Junaidu *et al.*, 2011). The higher prevalence of brucellosis in older cattle can be attributed to constant exposure of the cattle over time to the infectious agent. However, Cadmus *et al*. (2008) observed no difference between cattle >3 years and 1–3 years old, whereas Matope *et al*. (2011) reported decreased frequency of brucellosis with increasing age, with 2–4 years old having higher odds of being seropositive compared to those >7 years. They concluded that some older cows may not exhibit detectable antibody titres possibly due to latency, which is common in chronic brucellosis.

The significantly higher prevalence in non-pregnant compared to pregnant animals in this study did not change after adjusting for age, breed, state and management system. This finding is consistent with the observation in Ethiopia by Ibrahim *et al*. (2010) but contrary to reports by Mekonnen *et al.* (2011). Pregnant cattle above five months of gestation are more susceptible to *Brucella* infection due to the preferential localization of *Brucella* in the uterus in which allantoic fluid factors such as erythritol stimulate the growth of *Brucella* (Godfroid *et al.*, 2004). However, the greater probability of abortion in infected animals could explain the higher seroprevalence in non-pregnant animals.

The difference in prevalence between non-lactating and lactating cows was not significant, consistent with reports by Mekonnen *et al.* (2011) and Medeiros *et al*. (2011) but inconsistent with findings by Soomro (2006) and Ibrahim *et al*. (2009). A prevalence of 25% in lactating cows was recently reported in Nigeria by Junaidu *et al*. (2011) and 80.7% in Pakistan by Soomro (2006). This is of public health importance particularly in those Fulanis observed to be drinking raw milk directly from the udder of the cow, since *B. abortus* has been isolated from raw and sour milk of Fulani cattle in Nigeria (Eze, 1978; Bale and Kumi-Diaka, 1981).

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Brucellosis remains one of the most common zoonotic diseases worldwide with more than 500,000 human cases reported annually (Seleem *et al.*, 2010); many of the farmers take no measures to protect themselves against brucellosis and are quite willing to drink unpasteurized milk. In this area, milk is usually preserved by souring, which does not destroy brucellae as they are preserved in the milk fat (Eze, 1978). Unfortunately, infected farmers with symptoms of undulating fever and joint pain very rarely seek medical help, and if they do, the fever is usually ascribed to malaria or typhoid, therefore human brucellosis is likely to be greatly under-diagnosed.

Conclusion

It is evident from this study that bovine brucellosis is endemic in northern Nigeria at a high prevalence, with the majority of herds in all management systems being infected and the traditional management systems, particularly of the pastoral Fulanis, and lack of control measures are encouraging the spread of the disease. The first priority should be increased education and farmer extension, particularly amongst the nomadic Fulanis, regarding the zoonotic risk associated with milk consumption and contact with aborted materials. Food derived from animal sources must be properly cooked, and protective clothing must be provided for people dealing with infected cattle, blood and meat. Further surveys in other locations and improved management systems are necessary. If possible, the government should be involved in isolation and slaughtering of seropositive reactors for brucellosis, and compensation of farmers, identification of resistant breeds, and implementation of appropriate control measures. Integrating vaccination against brucellosis into the annual vaccination programme of livestock is highly recommended. Cooperation between neighbouring countries and intensifying border patrols in order to restrict movement of cattle across borders are also suggested.

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Chapter 3

Animal- and Herd-level Prevalence of Bovine Genital Campylobacteriosis and Trichomonosis in Cattle Herds in Northern Nigeria

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Abstract

A survey of 602 bulls in 250 herds was conducted to determine the prevalence of campylobacteriosis and trichomonosis, and their concurrence with brucellosis, in cattle in three states of northern Nigeria. The estimated true animal-level prevalence of *C. fetus* infection was 16.4% (95% CI: 13.0-20.7), of which 18.5% was *C. f. fetus* and 81.5% was *C. f. venerealis*. Of the latter, 92% were *C. f. venerealis* biovar intermedius strains. Animal-level prevalences in Adamawa, Kano and Kaduna states were 31.8%, 11.6% and 8.3% respectively. Of the 250 herds, 78 (25.5%, 95% CI: 19.4-32.7) had at least one infected bull. Herd-level prevalence was 51.3% in Adamawa state (n = 94), 20.7% in Kaduna state (n = 93), and 14.3% in Kano state (n = 63). *Campylobacter fetus venerealis* occurred in 67 herds (21.7%), *C. f. fetus* in 18 herds (6.3%), and 7 herds (2.5%) had a combination of both subspecies. Significantly higher prevalence was found in bulls >7 years 33.4% (95% CI: 16.6-56.3) than in bulls 4-5 years 13.6% (95% CI:10.4-17.7) (P = 0.018); in Gudali 28.8% (95% CI: 19.8-39.9) compared to Bunaji breed 11.5% (95% CI: 8.1-16.1) (P= 0.002); and in pastoral 43.5% (95% CI: 24.7-64.4) compared to agro-pastoral herds 17.4% (95% CI: 10.8-26.8) (P= 0.01). Prevalence was lowest in Bunaji cattle and in agro-pastoral management systems. All the samples tested for trichomonosis using different isolation methods were negative. There was a strong positive association between the presence of campylobacteriosis and brucellosis (P< 0.0001), both within bulls (OR = 8.3, 95% CI: 5.2-13.4) and within herds (OR = 16.0, 95% CI: 3.8-67.7). It was concluded that campylobacteriosis is highly prevalent in northern Nigeria with *C. fetus venerealis* biovar intermedius as the major aetiology of bovine genital campylobacteriosis, which may contribute to the poor reproductive performance of Nigerian indigenous cattle. Potential control measures are proposed.

Introduction

A number of infectious agents have been implicated in infertility and abortions in cattle (Bale and Kumi-Diaka, 1981). Bovine genital campylobacteriosis and trichomonosis are economically important venereal diseases that occur worldwide and are characterized by infertility, abortion, irregular oestrous cycles and long calving intervals (Bawa *et al*., 1991; Kimsey, 1997). The symptoms of campylobacteriosis are very similar to those of trichomonosis and these diseases tend to occur in areas with extensive cattle management and

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natural breeding (Cobo *et al.*, 2004). Bovine genital campylobacteriosis also causes repeat breeding and embryo mortality due to cervico-vaginitis, salpingitis and endometritis (Firehammer, 1979; Bawa *et al*., 1987; McFadden *et al*., 2004) and decrease in conception or pregnancy rates (Hum, 1987; Van Bergen *et al*., 2006). Pregnancy rate can be as low as 20% and abortion rate as high as 10% (Hum, 1987). Sterility may occur in up to 11% of infected heifers (McCool *et al*., 1988). Trichomonosis is also associated with reproductive tract invasion, mild to severe pyometra, low birth weight and decreased calf crop of up to 50% (Rae, 1989; BonDurant, 2005). Embryonic and foetal deaths from 14-18 days have been reported (Clark and Dufty, 1986). Campylobacteriosis and trichomonosis may not be detected in a herd until the time routine pregnancy diagnosis is carried out when large numbers of cows are found open (Irons *et al*., 2004a).

Campylobacter fetus fetus and *C.f. venerealis* are among the causes of infectious infertility. Bovine genital campylobacteriosis is commonly caused by *C. f. venerealis* (Mshelia *et al*., 2010a). Bulls carry *C. f. venerealis* subclinically in their prepuce, and older bulls above three years may remain permanently infected and transmit the disease sexually or serve as sources of infection through the use of semen from infected bulls in AI programmes (Irons *et al*., 2004a; OIE, 2011b). Female cattle experience transient infertility associated with inflammation of the reproductive tract (Irons *et al.*, 2004a). Up to 10% of animals may become permanently infected and act as sources of infection (Cipolla *et al*., 1994). Sub-speciation of *C. f. venerealis* has been an area of some interest; there is an unusually high prevalence of *C. f. venerealis* biovar intermedius in South Africa (Schmidt *et al*., 2010). The occurrence or prevalence of this isolate has not been studied in Nigeria. *Campylobacter fetus fetus* is found in the genital and intestinal tracts of cattle and sheep and is also reported to affect humans (Fujihara *et al*., 2006).

The prevalence of *C. fetus* infection varies according to location. A survey in a communal grazing area in South Africa showed a high prevalence of 28.7% (Pefanis *et al*., 1988), whereas 40-47% of dairy cows were found to be seropositive in one study in the USA (Akhtar *et al*., 1993a). The only published field studies on the occurrence of bovine genital campylobacteriosis in Nigeria were by Nuru (1974), Bawa *et al.* (1987), Bawa *et al.* (1991) Mshelia *et al*. (2010b) and very recently, Mshelia *et al*. (2012). However, these studies were done on few herds and in limited locations, and in some cases targeted animals with a history of reproductive failure. Bawa *et al.* (1991), in a study of 79 herds in 3 states, isolated *C. f.*

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venerealis from 12 bulls and one cow while *C. f. fetus* was isolated from three bulls and four cows in the genital tract, with an overall animal-level prevalence of 2.9% and a herd prevalence of 20%. In the Lake Chad basin of northern Nigeria, 3.7% of preputial washings of bulls yielded growth of *C. fetus* in culture and 11% of cows had specific IgA antibodies to *C. f. venerealis* in vaginal mucous from a sample of 150 cows in 6 herds (Mshelia *et al*., 2010b). The occurrence of bovine genital campylobacteriosis is grossly under-estimated and under-reported in Africa (Mshelia *et al*., 2010a; OIE, 2011b).

Trichomonosis is caused by *Tritrichomonas foetus*. Bulls are the long-term carriers and primary reservoirs of *T. foetus* whereas cows clear the infection spontaneously. Strong evidence exists that bulls older than 4 years rarely recover spontaneously; they therefore become a permanent source of infection (BonDurant, 1997). However, infections affect neither semen quality nor sexual behaviour (Martin-Gomez *et al*., 1998). In addition to the venereal route, mechanical transmission can also occur under certain circumstances (Kimsey, 1997). Also, semen used in AI is another source of transmission, as the organism can survive the standard AI processing methods (Monke, 1998). Prevalence of the disease is high in areas where natural breeding is practiced (Cobo *et al.*, 2004). Bull infection rates ranged from relatively low (2-8%) in some herds to high (15-44%) in others (Rae *et al.*, 1999; Fitzgerald, 1986; Madoroba *et al*., 2011). In a large study of bulls in Colorado and Nebraska, only 0.17% were positive for trichomonosis (Grotelueschen *et al.*, 1994).

The estimates of the annual losses to the USA beef industry as a result of trichomonosis vary greatly and have been as high as US$ 650 million (Speer and White, 1991). However, estimates are not available for Nigeria. Despite the huge economic losses due to infertility, cost of culling and replacing animals, and cost of treatment reported elsewhere (Rae *et al*., 1999), the magnitude and extent of this disease in Nigeria is largely unknown. The only studies conducted on *T. foetus* in Nigeria were by Adeyeye *et al*. (2011), Akinboade (1980) and Ayoade *et al.* (1991) who reported prevalences of 0%, 14.9% and 100% respectively.

There have been no studies on the concurrence of campylobacteriosis, trichomonosis and brucellosis in Nigeria, nor any recent studies covering different states and production systems. Concurrent herd infection with trichomonosis and *C. f. venerealis* in bulls was shown to be common in South Africa (Pefanis *et al*., 1988) and northern Australia (McCool *et al*., 1988). Concurrent infection with *C. fetus* and *T. foetus* has been reported to cause reproductive failure in camels (Wernery *et al.*, 1992). However, there is no evidence to

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suggest that infection with one agent predisposes to infection with the other agent (Campero *et al*., 1987). Determining the extent of concurrence of diseases is a useful tool in devising control strategies likely to impact on both diseases. It is therefore a means of optimizing the use of scarce resources, and is particularly valuable in under-resourced areas such as Nigeria.

Diagnostic methods for these conditions are not ideal. The sensitivity and specificity of culture for campylobacteriosis were 94.0% (Hum *et al*., 1994b) and 100.0% (Andrew and Frank, 1974) respectively, and for trichomonosis 76.0% and 98.5% (Cobo *et al.,* 2007) respectively. Different sampling, transport and laboratory processing conditions can cause variability in the results obtained in specific circumstances.

Farming practices and government policy in Nigeria are conducive to the continued occurrence of these diseases. Reports indicate that venereally transmitted diseases like campylobacteriosis and trichomonosis are easily transmitted by communal bulls in management systems commonly found all over Africa (Tekleye *et al*., 1988). The continuous movement of cattle as a result of trade and for open-range grazing, and sharing of bulls is practiced in Nigeria, putting many herds at risk of venereal infections. The semen of chronic carrier bulls may be a source of infection in AI programmes and even across international borders (Van Bergen *et al*., 2006; OIE, 2011b). Over the years both governmental and non-governmental interventions in the livestock sector have imported semen for AI programmes. There is no information on the screening of AI semen for *C. fetus, T. foetus* or *B. abortus* in Nigeria, even though it is mandatory to test semen and embryos intended for international trade for these three organisms (OIE, 2011a,b,c).

The study of infectious reproductive diseases, especially campylobacteriosis and trichomonosis in Nigeria has not received as much attention compared to other non-reproductive diseases such as contagious bovine pleuropneumonia, rinderpest and trypanosomosis. The desire of the Nigerian government to promote dairy production and the upsurge of commercial dairy production through the use of AI programmes to upgrade Nigerian indigenous cattle, without proper screening of semen for infectious reproductive diseases, are of concern to the livestock industry. Therefore, this study was designed in order to determine the animal- and herd-level prevalence of campylobacteriosis and trichomonosis in mature bulls in cattle herds in three important cattle producing states of northern Nigeria, as well as the concurrence of the two infections with each other and with *B. abortus* infection.

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Materials and Methods

The research protocol for this study was approved by the Animal Use and Care Committee and the Research Committee of the University of Pretoria (Protocol no. V073-08).

Sampling area and sample size

Three states (Kaduna, Kano and Adamawa) out of the nineteen northern states of Nigeria were sampled (Fig. 2.1, Chapter 2). The selection was based on their location, proximity to a reliable laboratory, farming systems, human and animal populations, cooperation from farmers, sharing of international borders and variety of animal breeds. Adamawa state is located on the border with Cameroon, between latitudes 8°N and 11°N and longitudes 11.5°E and 13.5°E, with a combination of sub-Sudan and Guinea savanna vegetation. Kaduna state lies between latitudes 9°N and 11.3°N and longitudes 10.3°E and 9.6°E and extends from the tropical grassland of Guinea savanna to the Sudan savanna. Kano state is at latitude 9°E and longitude 12°N and is situated within the Sudan and Guinea savanna vegetation zone.

In order to estimate the herd-level prevalence of campylobacteriosis and trichomonosis, a multistage cluster sampling strategy was used in which local government areas (LGA) were sampled within states and wards were sampled within LGA. Assuming an estimated herd prevalence (P_{exp}) of 20% for both diseases, an absolute allowable error (d) of 10% and a confidence level of 95%, the formula $n = 1.96^2 * P_{exp}(1 - P_{exp})/d^2$ (Thrusfield, 2005) was used to calculate a required sample size of 62 herds using simple random sampling. An intra-cluster correlation coefficient of $\rho = 0.25$ was used to account for the multistage cluster sampling strategy, since it is unlikely to exceed this value in the majority of infectious diseases (Otte and Gumm, 1997). This was used to calculate a design effect (D) of 4.0 using the formula $D = 1 + (b - 1)\ roh$ (Bennett *et al.*, 1991), where b is the average number of samples per cluster, in this case approximately 13 farms per LGA, and *roh* is the rate of homogeneity, equivalent to the intra-cluster correlation coefficient (ρ) in single-stage cluster sampling. This resulted in a required sample size of 248 herds. To ensure even distribution across states, each state was divided into 3 geographical zones based on established administrative divisions. In each zone, two LGA's were randomly selected, giving a total of six LGA's per state and eighteen LGA's from the three states (Fig. 2.1, Chapter 2). On average in each LGA, 5 wards were randomly selected, and in each ward 3 farms were sampled, giving 271 herds in total, of which 250 contained bulls eligible for sampling. Every

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eligible bull in each of the selected herds was sampled. An infected herd was defined as one in which one or more *C. fetus* or *T. foetus* infected bulls were detected.

Sampling for *Campylobacter fetus* and *Tritrichomonas foetus* from bulls

All post-pubertal bulls present in each selected herd were sampled. Each animal was sampled once only. The same samples were used for both *C. fetus* and *T. foetus*. Samples were collected by a preputial scraping technique as follows: The prepuce was cleaned with paper towel and the hair around the preputial orifice clipped where necessary. Preputial smegma samples were collected from bulls by insertion of a 53-cm rigid AI pipette attached to a sterile 20 ml disposable hypodermic syringe, through the preputial orifice to the fornix of the prepuce. The preputial lining and *glans penis* were scraped vigorously for about 45 seconds while applying vacuum with the plunger of the syringe as described by Irons *et al*. (2004a). The pipette was withdrawn gradually while still applying negative pressure to the plunger. For *C. fetus*, the sample was directly inoculated in Mueller Hinton broth (Oxoid, CM0405B) for transportation to the laboratory and culture. For *T. foetus*, the same sample was inoculated directly in both a combined commercial transport and culture medium (In-Pouch™ TF system; Biomed Diagnostics, San Jose, California, USA) and *Trichomonas* medium (Oxoid, CM0161B) enriched with foetal calf serum and chlorampenicol. In addition, a drop of the fresh sample was put on a clean glass slide with cover slip; and viewed directly under normal bright field microscope with correct condenser settings in the field for *T. foetus*. These were done under the shade. The media were used for the detection of *C. fetus* and *T. foetus* as detailed below.

Sampling for *Campylobacter fetus* and *Tritrichomonas foetus* from aborted foetuses

Where tissues from recent abortions were available in any herd during a visit, the abomasal contents, liver and the lungs of the foetus were removed using sterile instruments, put in sterile polythene bags and sent to the laboratory on ice (at 4-8°C).

Isolation of Campylobacter fetus and Tritrichomonas foetus

Isolation of *Campylobacter fetus* from bulls

Preputial smegma samples were streaked out using a 25 µl loop onto the surface of Columbia agar base (Oxoid, CM0331B) containing *Campylobacter* Skirrow's supplement (Oxoid

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SR0069E), 7% citrated/defibrinated blood, polymixin B, trimethoprim, vancomycin and amphotericin B and blood agar without antibiotics. Plates were incubated in a hydrogen enriched microaerophilic atmosphere consisting of 10% CO_2, 10% H_2, and 80% N_2 using *Campylobacter* system Gas Generating Kit (Oxoid, BR0060A) with palladium catalyst at 37°C for 72 hours, shielded from light. At 72 h, a representative of a dewdrop colony which was smooth, shiny and grey to pink in colour with organisms that were Gram-negative, vibroid in shape, oxidase- and catalase-positive was transferred to blood agar base (Oxoid, CM0055), streaked for purity and incubated under the same conditions as the sample described above. Each culture and incubation run was verified by using control strains of *C. f. fetus* and *C. f. venerealis* (ATCC 33247 and 19438 respectively). The identification of each colony obtained was confirmed using the Dry spot latex agglutination test (Oxoid, DR150M), according to manufacturer's instructions.

The isolates were subjected to biochemical testing for H_2S production using TSI agar (Oxoid, CM0277B), for aerobic growth, for growth at 25°C and 42°C and in the presence of 1% glycine and 3.5% NaCl, and for sensitivity to cephalothin and nalidixic acid. *Campylobacter fetus* grew at 25°C, did not produce H_2S using TSI agar, was sensitive to cephalothin but resistant to nalidixic acid, and was oxidase and catalase positive. Two phenotyping tests, i.e. tolerance to 1% glycine and H_2S production using lead acetate paper (OIE, 2011b), were used to differentiate the subspecies. *Campylobacter fetus fetus* grew on 1% glycine medium and produced H_2S. *Campylobacter fetus venerealis* did not grow on 1% glycine and did not produce H_2S. *Campylobacter fetus venerealis* biovar intermedius did not grow on 1% glycine but produced H_2S in L-cysteine supplemented "sensitive medium" (Véron and Chatelain, 1973).

Isolation of *Tritrichomonas foetus* from bulls

The first 300 preputial samples collected above were each directly inoculated into a commercial transport and culture medium (In-Pouch™ TF system; Biomed Diagnostics, San Jose, California, USA) that allows for growth of the trichomonads and direct microscopic examination without aspiration of the inoculums; and the last 302 samples in a Whirl-Pak bag (99100007 lot 10257, U11657, USA) containing 10 ml of *Trichomonas* medium enriched with heat-inactivated foetal calf serum (CN 3332 Highveld Biological, Lyndhurst 2106, RSA) (80ml/l medium) and 2ml chloramphenicol/l (CAPS Pharmaceuticals, RSA) (Irons *et al.*, 2002). The medium was examined directly through the plastic pouch in the field under a

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standard light microscope using a magnification of 100 or more for the presence of motile protozoa with three flagella. The pouch was incubated at 37°C and examined every 24 h for 7 days. In addition, the prepared enriched *Trichomonas* medium was dispensed aseptically into sterile McCartney bottles in 10 ml aliquots and used as both transport and culture medium for isolation of *T. foetus* with microscopic examination of the medium at intervals from day 1 to day 7 after inoculation, taken from the bottom of the bottle. For wet preparation or direct microscopy, a drop of inoculated sample was put on a clean glass slide with a cover slip for immediate observation under the microscope at x10 and x40 magnification. The result was recorded as positive when Trichomonad organisms displaying unique morphological characteristics were present, or negative if there was no growth of Trichomonads.

Isolation of *Campylobacter fetus* and *Tritrichomonas foetus* from aborted foetuses

One case of foetal abomasal content was inoculated directly onto Colombia agar (with Skirrow's supplement, antibiotics and amphotericin B) and enriched *Trichomonas* medium for *C. fetus* and *T. foetus* respectively. Foetal lung was homogenized and filtered through a membrane cellulose acetate filter with a 0.65 µm pore size (Sartorius Biotech GmbH 37070 Goettingen, Germany) and inoculated on to the culture media.

Other data collected

Herds were categorized into one of the four main management systems described as follows: Pastoral herds are those in which the cattle graze communally during the rainy season and pre-dry season, but may cover large distances in search of pasture and water during the critical period of the dry season. Natural pasture is the major source of feed for livestock in the pastoral system. Agro-pastoral herds are herds that go out for communal grazing in the morning and return in the evening without covering large distances but in addition supplementary feeds are provided. The farmers are involved in agricultural activities and use crop residues for their livestock. Commercial farms are usually organized and fenced farms that are well managed with paddocked, improved pasture and a regular supply of supplementary feeds. Zero-grazing cattle are tethered and feeds are constantly supplied to them. The breed of each animal was recorded in the following categories: Bunaji, Gudali (Adamawa Gudali and Sokoto Gudali), other *Bos indicus* (Rahaji, Wadara, Ndama and Brahman), *Bos taurus* (Friesian, Simmental, Jersey and Brown Swiss) and *Bos indicus* x *Bos*

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taurus crosses. Age was estimated using farm records, dentition and, in some cases, cornual rings.

As part of a separate study (Mai *et al.*, 2012), all the breeding bulls and other mature bulls, first calf heifers that had calved at least six weeks previously, and all the mature heifers and cows in each herd were tested for brucellosis using the RBPT, with confirmation using a c-ELISA kit (COMPELISA, VLA, Weybridge, UK).

Data analysis

The prevalences of campylobacteriosis and trichomonosis, overall and within states, age, breed and management systems were estimated both at animal and herd-levels, taking into account sampling weights in the multistage survey design. Prevalence estimates were then compared using the Chi-square test, corrected for the survey design using the second-order correction of Rao and Scott (1984). At the animal-level, true prevalence (TP) was then calculated by correcting the apparent prevalence point estimates and confidence limits using the formula described by Rogan and Gladen (1978): $TP = (AP+Sp - 1)/ (Se+Sp - 1)$, where AP = apparent prevalence, Se = sensitivity, Sp = specificity. The values used for sensitivity and specificity of the culture system for detection of *C. fetus* were 94.0% (Hum *et al.*, 1994b) and 100% (Andrew and Frank, 1974) respectively, and for *T. foetus* detection they were 76.0% and 98.5% respectively (Cobo *et al.,* 2007). Potential confounding between breed, state, age and management systems was assessed using Mantel-Haenszel odds ratios. The concurrent presence of brucellosis was assessed at both the animal- and herd-level using the chi-square test and the strength of association estimated using the odds ratio. All statistical analyses were done using STATA 12 (Stata Corporation, College Station, TX, USA).

Results

Herd-level prevalence of campylobacteriosis

A total of 250 herds comprising 602 bulls aged between 4 and ≥ 7 years, with between 1 and 24 bulls per herd (median: 1; inter quartile range: 1, 2) were sampled. Of the 250 herds sampled, 78 herds contained at least one bull positive for *C. fetus*, giving a herd-level prevalence, adjusted for sampling weights, of 25.5% (95% CI: 19.4-32.7). There were significant differences in the herd-level prevalence of campylobacteriosis between the three states (P = 0.0006), with the prevalence in Adamawa state (51.3%) being higher than in both

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Kaduna (20.7%) (P = 0.003), and Kano (14.8%) (P = 0.002) states (Table 3.1). Similarly, the herd-level prevalence varied between management systems (P = 0.03), being higher in pastoral (43.5%, 95% CI: 24.7-64.4) than in agro-pastoral (17.4%, 95% CI: 10.8-26.8) systems (P = 0.01).

Table 3.1. Prevalence of herds with *Campylobacter fetus*-positive bulls in three states of northern Nigeria, adjusted for sampling weights

State	Herds sampled	Negative	Cff	Cfv	Herds with Cff&Cfv	Total positive	95% CI
Adamawa	94	44	10	45	5	50(51.3%)[a]	37.0, 65.4
Kaduna	93	74	6	14	1	19(20.7%)[b]	14.5, 28.7
Kano	63	54	2	8	1	9(14.3%)[b]	6.8, 29.2
Total	250	172 (74.5%)	18 (6.3%)	67 (21.7%)	7 (2.5%)	78 (25.5%)	19.4, 32.7

[a,b]Values with different superscripts are significantly different (P< 0.01)

Key:
Cff: *Campylobacter fetus fetus*
Cfv: *Campylobacter fetus venerealis*

Animal-level prevalence of campylobacteriosis

Of the 602 bulls tested, 108 were positive for *C. fetus*; the animal-level prevalence, adjusted for the survey design and test sensitivity and specificity, was 16.4% (95% CI: 13.0-20.7). Adamawa state showed the highest animal-level prevalence of 31.8%, significantly greater than that of Kano (11.6%) (P = 0.001) and Kaduna (8.3%) (P = 0.0001) (Table 3.2). The fact that Adamawa state showed the highest prevalence did not change with adjustment for management system using the Mantel-Haenszel technique.

Table 3.2. Prevalence of *Campylobacter* spp. in individual bulls from three states in northern Nigeria, adjusted for sampling weights and for test sensitivity and specificity

State	No. sampled	No. of positive bulls		Total positive *Campylobacter* spp.	95% CI
		C. fetus fetus	*C. fetus venerealis*		
Adamawa	235	12	63	75 (31.8%)[a]	23.3, 41.9
Kaduna	257	6	15	21 (8.3%)[b]	5.9, 12.4
Kano	110	2	10	12 (11.6%)[b]	7.6, 17.2
Overall	602	20 (3.2%)	88 (13.3%)	108 (16.4%)	13.0, 20.7

[a,b]Values with different superscripts are significantly different (P< 0.01).

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There was a significant increase in prevalence of *C. fetus* infection with increasing age, with bulls ≥7 years having the highest prevalence (33.4%) (Table 3.3). Infection was also more prevalent in the Gudali (28.8%) than in the Bunaji (11.5%) breeds (P = 0.002) (Table 3.3).This crude association between breed and *C. fetus* prevalence did not change materially with adjustment for either management system or age group. However, after adjustment for state, the association was reversed; the odds of being seropositive was significantly higher for *Bos taurus* bulls than for both Gudali bulls ($OR_{M\text{-}H}$ = 4.26; P = 0.046) and other *Bos indicus* bulls ($OR_{M\text{-}H}$ = 7.11; P = 0.013).

Animal-level prevalence of *C. fetus* did not differ between the management systems. However, after adjustment for state, the odds of being positive were significantly higher for pastoral systems than for both agro-pastoral ($OR_{M\text{-}H}$ = 3.69; P = 0.0001) and zero-grazing ($OR_{M\text{-}H}$ = 4.16; P = 0.0003) systems, but did not differ between pastoral and commercial farms.

Table 3.3. Animal-level prevalence of *Campylobacter fetus* in cattle in three northern states of Nigeria, adjusted for sampling weights and test sensitivity and specificity

Variable	n	% positive	95% CI
Management system			
Zero-grazing	68	17.6	12.6- 23.9
Commercial	31	24.7	9.9- 50.1
Agro-pastoral	347	13.5	8.4- 21.0
Pastoral	125	22.2	14.8- 32.0
Age			
<4 years	23	8.8	1.6- 36.2
4-5 years	339	13.6[a]	10.4- 17.7
5-7 years	200	18.7	12.1- 27.9
≥7years	30	33.4[b]	16.6- 56.3
Breed			
Bunaji	344	11.5[c]	8.1- 16.1
Gudali	149	28.8[d]	19.8- 39.9
Bos taurus	28	17.8	5.2- 46.7
B.taurus x *B.indicus*	31	18.1	3.5- 58.6
Other *B.indicus*	50	16.4	7.3- 32.9

[ab, cd]Values with different superscripts differ significantly (ab: P = 0.018 and cd: P = 0.0016).

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Distribution of *C. f. venerealis*, *C. f. fetus* and *C. f. venerealis* biovar intermedius strains

In all states, *C. f. venerealis* was isolated more frequently than *C. f. fetus*. The distribution of *C. f. fetus*, *C. f. venerealis* and both subspecies in herds in the various states is shown in Table 3.1. Of the positive herds, 76.9% had *C. f. venerealis* alone, 14.1% had *C. f. fetus* alone and 9.0% had both *C. f. venerealis* and *C. f. fetus*. Of the positive cases, 20 (3.2%) were identified to have *C. f. fetus* infection and 88 (13.3%) had *C. f venerealis* infection (Table 3.2). Of the 88 *C. f. venerealis* isolates, 81 (92%) were *C. f. venerealis* biovar intermedius strains. Both *C. f. fetus* and *C. f. venerealis* were never found together in the same bull. Both *C. f. fetus* and *C. f. venerealis* were found more in herds with bulls >7 years (7.5% and 27.5%) than bulls <4 years (0% and 8.3%) respectively. The prevalence of *C. f. fetus* and *C. f. venerealis* were higher in zero-grazing herds (25.0% and 62.5%), followed by pastoral herds (10.5% and 38.2 %), commercial herds (13.0% and 17.4%) and agro-pastoral herds (3.5% and 20.3%) respectively.

Association between campylobacteriosis and brucellosis

There was a significant positive association between the occurrence of campylobacteriosis and brucellosis both at animal-level (*OR*= 8.3; $P < 0.0001$) and at herd-level (*OR* = 16.0; $P< 0.0001$) (Table 3.4). Culture for *C. fetus* from the aborted material was negative.

Table 3.4. Concurrent occurrence of campylobacteriosis and brucellosis at animal and herd-level in cattle herds from three states in northern Nigeria

		Campylobacteriosis		
		+	–	Total
Animal-level (bulls)				
	Brucellosis +	81	131	212
	Brucellosis –	27	363	390
	Total	108	494	602
Odds ratio = 8.3 (95% CI: 5.2, 13.4), $\chi^2 = 91.3$, $P< 0.0001$				
Herd-level				
	Brucellosis +	76	121	197
	Brucellosis –	2	51	53
	Total	78	172	250
Odds ratio = 16.0 (95% CI: 3.8, 67.7), $\chi^2 = 23.6$, $P< 0.0001$				

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Animal- level prevalence of trichomonosis

Trichomonosis was not isolated from any bulls using the methods of isolation carried out in this study. The upper 95% confidence limit for the prevalence of positive test results in the population was 0.50%. The absence of any positive tests suggested that the specificity of the test was greater than the 98.5% reported by Cobo *et al.*(2007); therefore, adjusting for a test sensitivity of 76% and specificity of 100%, an upper 95% confidence limit of 0.65% was calculated for the true animal-level prevalence of trichomonosis in the population.

Discussion

In the recent past, most emphasis, has been placed on brucellosis as the most prominent reproductive disease of cattle in Nigeria. It is evident from the result of this study that *C. fetus* is common in northern Nigeria with an estimated overall animal-level prevalence in bulls of 16.4% and herd prevalence of 25.5%. A similar study performed in Nigeria 20 years ago reported a lower prevalence of 2.9% of cattle and 20% of herds (Bawa *et al*., 1991). Although the authors sampled both bulls and cows, the herd-level prevalence is comparable to our study, however, the animal-level prevalence has increased. They reported prevalences of 2.3% in bulls and 5.0% in cows in Kaduna state and 0% in bulls and 7.7% in cows in Kano state. This finding is also higher than animal- and herd-level prevalences of 3.7% and 22.2% respectively (Mshelia *et al.*, 2012). Our data therefore suggest that there has been an increase in the prevalence of this disease.

An animal-level *C. fetus* prevalence of 10-15% with a herd-level prevalence of 53.8% was reported in Malawi (Klastrup and Halliwell, 1977). Animal-level prevalences of between 22% and 47% have been reported in other countries (Villar and Spina, 1982; Akhtar *et al.,* 1993a). In the three states sampled, the animal-level prevalence varied from 8.3% in Kaduna to 31.8% in Adamawa state. The proximity of Adamawa state to Cameroon border may contribute to the high prevalence in the state. Mshelia *et al*. (2010a,b) reported high incidence of bovine venereal campylobacteriosis in developing countries, where natural breeding of cattle is widely practiced, and implicated mass cattle movement across the borders of Nigeria as a major risk factor.

In the association between breed and *C. fetus* prevalence, adjustment for management system and age did not change the association; therefore, the breed differences were not due to differences in management system or age. However, after adjustment for state, the odds of

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being *C. fetus* positive was significantly higher for *Bos taurus* bulls than for both Gudali bulls and other *Bos indicus* bulls. This may be because Gudali bulls were encountered predominantly in Adamawa state which has a higher prevalence. Based on this finding, *Bos taurus* may be more susceptible than other breeds to campylobacteriosis, whereas Bunaji and Gudali, which are the most predominant breeds in northern Nigeria, may be less susceptible. Klastrup and Halliwell (1977) also found more of the disease in exotic than indigenous cattle. However, further studies including adjustment for other possible confounders are required to be able to determine breed susceptibility.

The association between management system and *C. fetus* prevalence, adjusted for state, showed that bulls in pastoral herds are more likely to be infected with *C. fetus* than bulls found in either agro-pastoral or zero-grazing farms. However, more of the pastoral herds were found in the lower prevalence Kaduna state than in Adamawa state, which initially masked the fact that pastoral herds had a higher odds of infection. Comingling with other cattle, sharing of grazing land and common watering points, which are common in northern Nigeria, contribute to the spread of genital campylobacteriosis. Evidence of this is the very high prevalence (43.5%) amongst herds with a pastoral management system. A prevalence of 28.7% was reported in communal grazing area in South Africa (Pefanis *et al*., 1988). Trespassing bulls from neighboring herds doubled the risk of a herd being positive to bovine genital campylobacteriosis (Jimenez *et al*., 2011). Furthermore, transmission in cattle is known to be associated with sharing, renting or mixing of bulls and comingling cattle on common grazing land or extensive cattle management (Tekleye *et al*., 1988; Cobo *et al*., 2004; Jimenez *et al*., 2011). However, zero-grazing too showed a high herd-level prevalence of 42.3%. Although there is restricted movement in zero-grazing thereby reducing contact and prevalence of *C. fetus* between animals, 62.5% of the herds belonging to zero-grazing system bought bulls from the open market, and 62.5% of the herds also borrow or share bulls for breeding, which may contribute to the high prevalence recorded in this study. The agro-pastoral system showed the lowest prevalence. The nutritional and husbandry practices, regular herd prophylactic measures and grazing system of the agro-pastoral herds may reduce the prevalence of *C. fetus*.

The prevalence of genital campylobacteriosis was higher in older bulls, with 33.4% of bulls over 7 years of age identified as being infected. It has been reported that bulls older than 3 years may remain permanently infected, which may be asymptomatic and a source of re-

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infection of the herd (Bawa *et al*., 1991; Irons *et al*., 2004a). The retention of infection in older bulls than the younger bulls may be due to the increase in number and size of the irregular crypts in the epithelium of the penis and persistent colonization of the lower reproductive tract of mature bulls by *C. fetus* (Samuelson and Winter, 1966; Cobo *et al.*, 2011). Antigenic variation may also enhance persistence of the carrier state in bulls (Samuelson and Winter, 1966). However, McCool *et al.* (1988) and Swai *et al*. (2005) observed that both younger and older bulls could remain carriers after infection. This requires further investigation.

Of the two subspecies of *C. fetus*, *C. f. venerealis* was more common, comprising 81.5% of the isolates. This is in agreement with other studies (Hum, 1987; Bawa *et al*., 1991; Irons *et al.*, 2004a). It is noteworthy that *C. f. venerealis* is more pathogenic than *C. f. fetus* (Zhoa *et al.*, 2010; OIE, 2011b). This is the first report of *C. f. venerealis* biovar intermedius in Nigeria. This isolate constituted the great majority of all isolates. Similar results were obtained in South Africa (Schmidt *et al*., 2010), who also demonstrated the incorrect classification based on subspecies-specific PCR assays, making phenotypic characterization following bacterial culture superior to molecular characterization of *C. fetus* subsp. In a genomic analysis of *C. fetus* subspecies, two assays were specific for *C. f.* subsp. *venerealis* AZUL-94 strain, with a further single assay specific for the AZUL-94 strain and *C. f.* subsp. *venerealis* biovar intermedius in Australia (Moolhuijzen *et al*., 2009). In a study of the prevalence of genital campylobacteriosis infection in cattle in the Mantova region of Italy, out of the eight *C. f. venerealis* isolates, five were *C. f. venerealis* biovar intermedius (Nigrelli *et al.,* 1984). The assays developed in the genomic analysis of *C. fetus* subspecies highlight the complexity of targeting strain specific virulence genes for field studies for the molecular identification and epidemiology of *C. fetus* (Moolhuijzen *et al*., 2009).

The odds of having campylobacteriosis were 8.3 times greater amongst bulls with brucellosis than those without brucellosis, and herds positive for brucellosis were 16 times more likely to have bulls positive for campylobacteriosis. Both conditions were more common in older bulls, and half of all the bulls that had hygroma, a common manifestation of brucellosis, also had genital campylobacteriosis. This is consistent with the findings of Zhao *et al*. (2010), who reported some degree of mixed infections of campylobacteriosis and bovine brucellosis. This strong association between the occurrences of the two infections suggests that, although their major mode of transmission is different, they share common risk factors. Further

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investigation of risk factors for the two diseases would be useful in order to inform control measures for both diseases. Another possible explanation may be that one infection predisposes an animal to the other infection, possibly due to an effect on the immune system. Neta *et al*. (2010) reported that *B. abortus* invades phagocytic and non-phagocytic host cells of cattle, inhibiting phagosome-lysosome fusion thereby affecting the innate and adaptive immunity against brucellosis. Such cattle may also be susceptible to campylobacteriosis.

It is likely that the frequent infertility, embryo mortality, abortion, irregular oestrous cycles with subsequent long calving intervals, low pregnancy rates, and low milk yield experienced by cows and heifers in some herds in northern Nigeria may be as a result of genital campylobacteriosis. The isolation of *C. fetus* in indigenous cattle herds in northern Nigeria demonstrates the potentially important role of the organisms in infectious infertility, with *C. f. venerealis* being the most prevalent. Mshelia *et al*. (2010b) showed an association between detection of IgA antibodies against *C. fetus venerealis* in the vaginal mucous and poor herd fertility including abortion and stillbirth; while Bawa *et al.* (1987) and Nuru (1974) isolated *C. f. venerealis* from cases of abortion in cattle herds in Nigeria. Further study is required to investigate the association between infertility experienced in Nigerian herds and *C. f. venerealis* infection.

Although trichomonosis may go undetected in a herd because of its insidious nature (Rae and Crews, 2006), *T. foetus* was not isolated from any of the bulls sampled in this study despite using the most ideal and efficient diagnostic protocol, including a gold standard test (Rae and Crews, 2006) and a commercial transport and field culture kit (In-Pouch™ TF system) which has a high sensitivity of up to 95%-100% (Thomas *et al*., 1991; Appell *et al.*, 1993). Even assuming a test sensitivity of only 76%, we could be 95% certain that the true prevalence of trichomonosis in the area was below 0.65%. Considering the extent of bull sharing and comingling of cattle in our study area, it would be expected that, were the infection present, the true prevalence would be far greater than 0.65%, therefore it is probable that the disease is absent in our study area. This finding is in agreement with surveys conducted in 384 bulls in Malawi (Klastrup and Halliwell, 1977); in 58 bulls in Tanzania (Swai *et al.,*2005) and most recently in 299 males, 101 female cattle and 3 abomasal and foetal fluids in northern Nigeria (Adeyeye *et al*., 2011). It therefore suggests that trichomonosis may not be a significant problem in northern Nigeria despite reports of its occurrence in southern Nigeria (Akinboade, 1980; Ayoade *et al.*, 1991). Breed and ecological differences may be responsible for the

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variation in disease occurrence in northern and southern Nigeria as shown for trichomonosis by Erasmus *et al*. (1989) in South Africa. Most of the breeds in the south are the short and humpless Ndama, Muturu and Keteku; while the climate is relatively hot with high humidity as opposed to the relatively cool and dry northern parts. Very recently it was shown that *T. foetus* may transform into pseudocysts or endoflagellar form because of adverse environmental conditions or presence of drugs (Pereira-Neves *et al*., 2012) and may therefore not be detected. A more extensive survey, including the other states in northern Nigeria, would be necessary to determine whether or not the disease is present; further efforts to isolate *T. foetus* and to investigate its epidemiology in northern Nigeria are suggested.

Conclusion

Bovine genital campylobacteriosis should be considered in all investigations of herd infertility problems in northern Nigeria, since more than 50% of herds are infected in some areas. Specific feasible protective factors associated with the agro-pastoral management system could be identified and applied. Proper implementation of adequate biosecurity or preventive measures to reduce the risk of reinfection in affected herds is also recommended. Furthermore, vaccination of cattle against genital campylobacteriosis is very effective and should therefore be recommended for Nigerian cattle. Where possible, culling or treating of exposed or known infected bulls and females should be considered, with testing of all replacement and virgin breeding bulls and heifers during quarantine prior to introduction into the herd. Also, porosity of international borders likely predisposes livestock to infection and should be monitored as a policy requirement. Further studies on breed and age susceptibility, correlation of infection with infertility, and the biological significance of the different *C. fetus* subspecies are indicated. Further molecular characterization of the respective isolates to identify unique genomic regions for sub-speciation would also be of interest.

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Chapter 4

Herd-level risk factors for *Campylobacter fetus* infection, *Brucella* seropositivity and within-herd seroprevalence of brucellosis in cattle in northern Nigeria

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Abstract

Brucellosis and campylobacteriosis are economically important diseases affecting bovine reproductive efficiency in Nigeria. A survey was conducted in 271 cattle herds in Adamawa, Kaduna and Kano states of northern Nigeria using multistage cluster sampling. Serum from 4,745 mature animals was tested for *Brucella* antibodies using the Rose-Bengal plate test and positives were confirmed in a series-testing protocol using the competitive enzyme-linked immunosorbent assay. Preputial scrapings from 602 bulls were tested using culture and identification for *Campylobacter fetus*. For each disease, a herd was classified as positive if one or more animals tested positive. For each herd, information on potential managemental and environmental risk factors was collected through a questionnaire administered during an interview with the manager, owner or herdsman. Multilevel logistic regression models were used to model the odds of herd infection for each disease. A zero-inflated Poisson model was used to model the count of *Brucella*-positive animals within herds, with the number tested as an exposure variable. The presence of small ruminants (sheep and/or goats) on the same farm, and buying-in of >3 new animals in the previous year or failure to practice quarantine were associated with increased odds of herd-level campylobacteriosis and brucellosis, as well as increased within-herd counts of *Brucella*-positive animals. In addition, high rainfall, initial acquisition of animals from markets, practice of gynaecological examination and failure to practice herd prophylactic measures were positively associated with the odds of *C. fetus* infection in the herd. Herd size of >15, pastoral management system and the presence of a handling facility on the farm were associated with increased odds, and gynaecological examination with reduced odds of herd-level *Brucella* seropositivity. Furthermore, the zero-inflated Poisson model showed that borrowing or sharing of bulls was associated with higher counts, and provision of mineral supplement with lower counts of *Brucella*-positive cattle within herds. Identification of risk factors for bovine campylobacteriosis and brucellosis can help to identify appropriate control measures, and the use of zero-inflated count model can provide more specific information on these risk factors.

Introduction

Brucellosis and campylobacteriosis are economically important bacterial diseases of cattle that are endemic in Nigeria (Ocholi *et al*., 2004a; Mshelia *et al*., 2010a). Brucellosis is a

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zoonotic disease that is caused by *Brucella abortus* and *B. melitensis*, and is transmitted from animals to man through ingestion of raw milk or unpasteurized cheese or direct contact with infected materials via abraded skin (Solorio-Rivera *et al.*, 2007; Godfroid *et al*., 2011; Junaidu *et al*., 2011). Transmission among animals is mainly through mucous membranes following contact with contaminated materials, by inhalation and *in utero* (Queipo-Ortuno *et al*., 1997; Solorio-Rivera *et al.*, 2007). Infected animals spread brucellosis horizontally at parturition or to their calves *in utero* or via their milk (Nicoletti, 1980; Bale and Nuru, 2001). The disease is endemic in sub-Saharan Africa and the prevalence varies according to agro-ecological region (McDermott and Arimi, 2002; Muma *et al*., 2007a). Bovine genital campylobacteriosis is a venereally transmitted disease that is caused by *Campylobacter fetus venerealis* (Devenish *et al.*, 2005). It is also transmitted through AI using frozen infected semen, even with the use of antibacterial agents (Garcia *et al*., 1983a), and via contaminated semen collection equipment (OIE, 2011b). Artificial insemination using uninfected semen remains the single most effective means of preventing spread of campylobacteriosis (Irons *et al.*, 2004a).

In Nigeria, free movement and intermingling of the nomadic and extensively managed Fulani herds encourages the spread of brucellosis (Bale and Kumi-Diaka, 1981; Ocholi *et al.,* 2004a). Similar observations have been made in other parts of Africa (Berhe *et al.,* 2007; Matope *et al.,* 2010), where brucellosis often began during adverse weather conditions and famine (Musa *et al*. 1990). However, prevalence may also be high in intensively managed herds (Karimuribo *et al.*, 2007; Jergefa *et al.*, 2009), whereas extensive management in smallholder farms may limit the spread of infection (Madsen, 1989). Trade cattle, especially across Nigeria's northern borders with Chad and Niger, show evidence of infection (Esuruoso, 1974). This is in agreement with reports by Cadmus *et al*. (2008) in Nigeria and Kubuafor *et al*. (2000) in Ghana. Possibilities also exist of the transmission of *Brucella* spp. from wildlife to domestic cattle (Avong, 2000; Muma *et al*., 2007b). Indiscriminate buying-in of animals without quarantine (Bale and Kumi-Diaka, 1981) and mixing of cattle with sheep and goats and sometimes horses (Ocholi *et al*., 2004b) may disseminate brucellosis. Increased susceptibility of some breeds of cattle to brucellosis has been reported (Karimuribo *et al.,* 2007; Junaidu *et al*., 2011). A higher prevalence in older cattle (Kubuafor *et al*., 2000; Samaha *et al*., 2009; Mai *et al*., 2012), females (Mekonnen *et al.* 2010), males (Mai *et al*., 2012), non-pregnant cows (Ibrahim *et al*., 2010; Mai *et al*., 2012) and lactating cows (Nicoletti, 1980) has been reported in Africa.

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The prevalence of brucellosis is therefore influenced by husbandry and management system, herd size, population density, age, sex, type of animal, hygiene, socio-economic factors, herd immunity and adequacy of veterinary services (Crawford *et al*., 1990; Omer *et al*., 2000; Ocholi *et al.,* 2004a; Mekonnen *et al*., 2010), as well as intensity of contact with infected herds and with contaminated environmental sources (Madsen, 1989; Megersa *et al*., 2011b). However, risk factors observed in one particular agro-ecological region do not necessarily apply to other areas with different ecological settings and husbandry practices (Matope *et al*., 2010; Mekonnen *et al*., 2010).

The following factors have been associated with the introduction or spread of bovine genital campylobacteriosis: introduction of cows and heifers from endemically infected herds (Woldehiwet *et al*., 1989), importation of bulls for cross-breeding purposes (Nuru, 1974) and lack of effective control of mass cattle movements across international borders (Mshelia *et al*., 2010b). Other factors associated with campylobacteriosis include the use of communal bulls or having more than one bull in a herd (Mukasa-Mugerwa, 1989), communal grazing (Pefanis *et al*., 1988), lack of vaccination (Hoffer, 1981), genetic differences in susceptibility between different cattle lines (Dufty *et al*., 1975), contact with contaminated bedding, fomites or mechanical transmission (Hjerpe, 1990), and contact between infected and non-infected bulls (Hoffer, 1981; Mukasa-Mugerwa, 1989). Transmission of infection between cows probably does not occur naturally (Clark, 1971). Young bulls under five years of age are difficult to infect (Samuelson and Winter, 1966); however, both younger and older bulls could remain carriers for up to 18 weeks post-infection (Bier *et al*., 1977) and bulls older than three years may remain permanently infected. Heifers and cows of all ages are susceptible (Dufty *et al*., 1975; Irons *et al.*, 2004a); however, the infection is more persistent in heifers than in cows (Dufty and Vaughan, 1993).

Campylobacteriosis and brucellosis cause heavy economic losses resulting from abortions, herd infertility, embryo mortality, irregular oestrus, reduced pregnancy rate, increased calving intervals, birth of weak calves, increased culling rates, decreased milk production and veterinary costs (Bawa *et al*., 1991; Ariza *et al*., 1995; Devenish *et al.*, 2005; Mekonnen *et al*., 2010). Despite reports on the distribution of campylobacteriosis and brucellosis in cattle in Nigeria and their increase in prevalence (Ocholi *et al*., 2004a; Mshelia *et al*., 2010a; Mai *et al*., 2012; Mai *et al.*, unpublished data), little information on risk factors for brucellosis or campylobacteriosis has been generated from representative, well designed epidemiological

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studies, using appropriate multivariable methods to control for confounding. Knowing the risk factors and other epidemiological picture of the diseases is essential for the development of cost-effective and efficient control programmes. The aim of this study was to identify herd-level risk factors associated with campylobacteriosis and brucellosis, as well as factors associated with the within-herd seroprevalence of brucellosis, in cattle herds in three states of northern Nigeria.

Materials and Methods

The research protocol for this study was approved by the Animal Use and Care Committee and the Research Committee of the University of Pretoria (Protocol no. V073-08).

Study areas

Adamawa, Kaduna and Kano states were selected from the nineteen northern states of Nigeria (Fig. 2.1, Chapter 2) based on animal population, location, willingness of farmers, and types of farms and animals. Adamawa state is located on the border with Cameroon with a land area of 42,159 km^2 and cattle population of 3.8 million, located between latitudes 8°N and 11°N and longitudes 11.5°E and 13.5°E, with a combination of sub-Sudan and Guinea savanna vegetation, with temperatures ranging between 15.2°C and 42°C, and relative humidity of 27-79%. Kaduna state, with a land area of 48,473 km^2 and cattle population of 3.1 million, lies between latitudes 9°N and 11.3°N and longitudes 10.3°E and 9.6°E and extends from the tropical grassland of Guinea savanna to the Sudan savanna, having a temperature range of 14-30°C with a relative humidity of 12-72%. Kano state, with a land area of 42,593 km^2 and cattle population of 3.2 million, is at latitude 12°N and longitude 9°E and is situated within the Sudan and Guinea savanna vegetation zone.

Sample size and sampling strategy

The herds used for this study were selected using random, multistage cluster sampling for concurrent studies on the prevalence of the two conditions and their effect on calving rate. The initial sample size to estimate the prevalence of *B. abortus*-infected herds was determined by using the formula $n = 1.96^2 * P_{exp}(1 - P_{exp})/d^2$ (Thrusfield, 2005), with expected herd prevalence (P_{exp}) of 40%, desired absolute precision (d) of 10% and confidence level of 95%, resulting in a required sample size of 93 farms. Because of the multistage cluster sampling strategy, the design effect (D) was calculated using the formula $D = 1 + (b - 1)\ roh$

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(Bennett *et al*., 1991), where *b* is the average number of samples per cluster and *roh* is the rate of homogeneity, equivalent to the intra-cluster correlation coefficient (ρ) in single-stage cluster sampling. Approximately 12 to 13 herds per local government area (b = 13) were sampled. An intra-cluster correlation coefficient of ρ = 0.09 was reported for *B. abortus* in cattle (Otte and Gumm, 1997); in order to account for the multistage design, a higher value of 0.15 was used for *roh*. The design effect was therefore calculated to be D = 2.8 which, multiplied by the original calculated sample size, gave a required sample size of 261 farms. Ultimately, a total of 271 herds was sampled.

For campylobacteriosis an estimated herd prevalence (P_{exp}) of 20%, precision (d) of 10% and a confidence level of 95% was used, using the formula described above (Thrusfield, 2005). The required sample size was 62 herds using simple random sampling. An intra-cluster correlation coefficient of ρ = 0.25 was used to account for the multistage cluster sampling strategy, since it is unlikely to exceed this value in the majority of infectious diseases (Otte and Gumm, 1997). This resulted in a design effect of D = 4 and therefore a required sample size of 248 herds. Ultimately, 250 eligible herds were sampled.

Each state was divided into 3 geographic zones based on established administrative divisions. In each zone, two local government areas (LGA's) were randomly selected from a list of all LGA's in each zone, giving a total of six LGA's per state and eighteen LGA's from the three states (Fig. 2.1, Chapter 2). On average in each LGA, 5 wards were randomly selected from a list of all wards in the LGA, and in each ward, 3 farms were sampled from a locally-obtained list of farms following earlier visits and consent from the farmers, giving 271 herds in total. Of these, 250 contained bulls eligible for sampling for campylobacteriosis.

Animal sampling

For brucellosis, first calf heifers that had calved at least six weeks previously, cows and mature bulls and heifers in each of the selected herds were bled by collecting about 10 ml of blood, which was placed into an ice bath and transported to the laboratory for centrifugation, serum separation and storage at -20°C until ready for analysis. For campylobacteriosis, all breeding bulls and other mature bulls in the herds were sampled using a preputial scraping for *C. fetus* as described by Irons *et al*. (2004a).

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Serological testing for *Brucella abortus*

As part of a concurrent study (Mai *et al.*, 2012), all the animals sampled above were tested for brucellosis using the RBPT (Veterinary Laboratories Agency (VLA), Weybridge, UK), with confirmation of positive reactors using a c-ELISA kit (COMPELISA, VLA, Weybridge, UK). An infected herd was defined as one in which one or more animals was positive to both RBPT and c-ELISA (series-testing protocol).

Isolation of *Campylobacter fetus* from bulls

Preputial smegma samples were used to isolate *C. fetus*. After culture for 72 h, a representative of a dewdrop colony that was Gram-negative, vibroid in shape, and oxidase- and catalase-positive was transferred to blood agar base (Oxoid, CM0055), streaked for purity and incubated under microaerophilic conditions for 72 h. Each culture and incubation was verified by using control strains of *C. f. fetus* and *C. f. venerealis* (ATCC 33247 and 19438 respectively). The isolates obtained were subjected to biochemical testing for H_2S production using TSI agar (Oxoid, CM0277B), aerobic growth, growth at 25°C and 42°C and in the presence of 1% glycine and 3.5% NaCl, and sensitivity to cephalothin and nalidixic acid. The details of the isolation procedure for *C. fetus* were as described by OIE (2011b). Each bull was categorized as positive or negative depending on the isolation of *C. fetus*, and any herd with at least one positive bull was considered a positive herd.

Questionnaire

An interview-based, pre-tested, structured questionnaire (Appendix I) was administered on each farm to gather information on potential risk factors for herd-level *Brucella* seropositivity and *C. fetus* infection at the same time that blood samples and preputial scrapings were collected. The questions related to environmental conditions and management practices on the farm over the previous 12-24 months. Detailed definitions for each variable are listed in Table 4.1. As far as possible, the herdsmen were interviewed in the presence of the owner or farm manager, in a face-to-face interview lasting 30 to 45 min, in one of the two major local languages (Fulani and Hausa), conducted by the principal investigator or his trained assistant. Incentives were also given to the farmers where necessary by providing free curative and prophylactic treatment of their animals. Mean annual rainfall figures for each farm were obtained from the nearest meteorological station.

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Table 4.1. Levels and definitions of categorical risk factors evaluated

Variable and level	Definition
Type of breeding	
AI and natural mating	Herd uses both artificial insemination (AI) and natural mating
AI only	Herd that practices only AI
Natural mating (plough/trade/feedlot)	Plough, trade or feedlot cattle herd that practices natural mating only
Natural mating only	Conventional herd that practices natural mating only
Management system	
Intensive	Paddocked or fenced commercial farms that are always supplemented with concentrate or sown pastures
Agro-pastoral	Grazing cattle cover short distances and return to be confined; supplementary feeds given during critical periods
Pastoral	Cattle graze freely on fallow land close to the place of settlement of the owners during rainy season and cover long distances moving from place to place in search of feed during dry season
Supplementary feeding	
None	No supplementary feeding given
Fodder/Bran	Fodder or bran provided in addition to natural pasture
Concentrate	Concentrates given
Mineral supplementation	
No	No mineral supplementation given
Yes	Mineral/salt lick or potash provided
Pasture environment	
Enclosed	Pasture fenced or paddocked
Unenclosed	Farms with unfenced pasture and animals graze close to settlement
Free range	Farms in which animals have free access to natural pasture and may wander long distances
Pasture establishment	
No	Farms where pasture was not developed, cattle depend only on natural grazing
Yes	Farms where pasture was established or planted for the cattle
Water source	
Piped	Water provided from taps or boreholes
Flowing	Water obtained from river or stream
Stagnant	Water obtained from earth dams, lakes, ponds or open wells

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Housing	
Open barbed wire	Cattle confined using wooden stakes and barbed wire
Open half way and roofed	Half solid wall and half open with roofing sheets
Open solid enclosure	Solid wall with no roofing
Hygiene/floor type	
Floored	Housing is provided with a hard solid floor
Unfloored/muddy	Housing is not floored, only bare earth surface
Care during parturition	
No	Cows calve together with herdmates without special attention
Yes	Isolation and observation of the cow during parturition, including removal of afterbirth
Herd prophylactic measures	
No	No preventive measures such as deworming, tick control, haemoparasite control or vaccination
Yes	Regular preventive measures such as deworming, tick control, haemoparasite control or vaccination
Borrow or share bulls	
No	Herd in which the breeding bull is not borrowed or shared
Yes	Herd in which breeding bull is borrowed from or shared with other herds
Presence of small ruminants	
No	No sheep or goats grazing with the cattle herd
Yes	Sheep and/or goats grazing with the cattle herd
Presence of dogs	
No	No dogs associated with the herd
Yes	Dogs accompany the grazing cattle herd
Presence of horses	
No	No horses kept with the cattle
Yes	Horses present on the farm
Presence of camels	
No	No camels kept with the cattle
Yes	Camels present on the farm
Presence of chickens	
No	No chickens kept or scavenging around the cattle herd
Yes	Chickens kept or scavenging around the cattle herd
Multiple herds	
No	Cattle owner has just one herd
Yes	Cattle owner has more than one herd

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Initial animal acquisition	
Inherited	Owner inherited cattle from onset
Other farms	Owner bought cattle from other commercial farms
Market	Owner initially bought cattle from the market
Buying-in of new animals	
Closed herd	No new cattle introduced during the past 12 months
Bought <3, quarantine	Fewer than 3 animals acquired over the past 12 months, and quarantined before introducing them into the herd
Bought >3 or no quarantine	Owner bought more than 3 animals or did not quarantine them before introduction into the herd
Socio-economic status of farmer	
Full-time	Farmer engages only in livestock and/or arable farming
Part-time	Farmer has other business besides farming
Gynaecological examination	
No	Herds where no trans-rectal palpation or other gynaecological or obstetrical examination was done
Yes	Herds where regular or occasional trans-rectal palpation or other gynaecological/obstetrical examination was done
Specialist attending to animals	
No	No veterinarian or livestock assistant attending to the cattle
Yes	A veterinarian or livestock assistant attending to the animals
Handling facility	
No	No handling facility on the farm
Yes	The presence of a crush, locally made chute or other handling facility used to restrain animals
Annual rainfall	
<700 mm	Mean annual rainfall in the area is less than 700 mm
700-1000 mm	Annual rainfall in the area is between 700-1000 mm
>1000 mm	Annual rainfall in the area is greater than 1000 mm
Herd size	
≤15	Herd contains 15 or fewer mature cattle
>15	Herd contains more than 15 mature cattle

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Statistical analysis

All independent variables used in the analysis were categorical; herd size (number of mature animals) was dichotomized using the median into ≤15 and >15 animals and annual rainfall was categorized into <700 mm, 700-1000 mm and >1000 mm. The independent variables were tested for bivariable associations with each of the outcome variables (*C. fetus* infection and *Brucella* seropositivity) using the two-tailed Fisher's exact test. For selection of independent variables for inclusion into the initial multiple logistic regression models, the entry criterion was $P < 0.20$. Each model (one for *C. fetus* infection and one for *Brucella* seropositivity) was developed by backward elimination, dropping the least significant independent variable (with the exception of state, which was kept in the model) until all the remaining predictor variables were significant ($P_{Wald} < 0.05$). Each independent variable not in the model was then re-entered into the model and retained if significant. All biologically plausible two-way interactions between variables remaining in the model were tested and retained if significant. The fit of the logistic regression models was assessed using the Hosmer-Lemeshow goodness-of-fit test. Multilevel (hierarchical) logistic regression models were then constructed in order to account for the multistage sampling design by including LGA and ward as nested random effects.

Possible confounding by *C. fetus* infection status in the model of *Brucella* infection in the herd was then investigated, firstly by including herd *C. fetus* status as an additional predictor and secondly by using a conditional logistic regression model with the same predictors, stratified on herd *C. fetus* status. Changes in the coefficients for the other predictors were observed. The same was done, vice-versa, to the model of *C. fetus* infection by including herd brucellosis status as a predictor and by conditional logistic regression stratifying on herd brucellosis status.

A multivariable zero-inflated Poisson (ZIP) model was then used to identify factors associated with the count of *Brucella*-positive animals within a herd. This was not done for campylobacteriosis, since only bulls were tested and the majority of herds (200/250) contained only one or two bulls. The Poisson component modeled the count of *Brucella*-positive animals in the herd, while the inflation component accounted for the excess zero counts, i.e. negative herds, by simultaneously using logistic regression to model the odds of the herd being *Brucella*-negative (Dohoo *et al.*, 2009). The number of animals tested in each herd (n) was included in the Poisson model as an exposure variable to account for the size of

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the population at risk, i.e. the coefficient for *ln* (*n*) was constrained to be 1. To account for clustering of observations, state was included as a fixed effect in both parts of the model and Huber-White sandwich (robust) variance estimates were used. Initially, the variables significant in the first logistic regression model were included in each part of the ZIP model and backwards elimination, followed by re-testing of each independent variable, were performed as above until all variables remaining in the models were significant ($P < 0.05$). Interactions were not assessed in this model. The fit of the ZIP model vs. the standard Poisson model was assessed using the Vuong statistic, large positive values (>1.96) of which favour the ZIP model (Dohoo *et al.*, 2009). To test for overdispersion, the fit of the corresponding zero-inflated negative binomial model vs. the ZIP model was assessed using a likelihood-ratio test. All analyses were done using STATA 12 (Stata Corporation, College Station, TX, U.S.A.) and a significance level of 5% was used.

Results

Brucellosis serology was carried out on 4,745 animals from 271 farms and *C. fetus* isolation was done on 602 bulls from 250 farms, since 21 farms contained no bulls. Questionnaires were administered on all 271 farms. Herd size (number of animals tested) ranged between 4 and 41 (median: 16; interquartile range (IQR): 12, 21) and in those herds with bulls there were between 1 and 24 bulls per herd (median: 1; IQR: 1, 2). Of the 271 herds sampled for brucellosis, 210 (77.5%) were classified as seropositive, while 78 (31.2%) of the 250 herds sampled for campylobacteriosis were positive. Of the 250 herds tested for both diseases, 76 (30.4%) were positive for both, 121 (48.4%) were positive for *Brucella* only and 2 (0.8%) were positive for *Campylobacter* only. It was established that none of the herds had been vaccinated against brucellosis or campylobacteriosis.

In the bivariable analysis (Table 4.2), several variables were associated ($P < 0.2$) with herd-level *Brucella* and/or *C. fetus* infection (indicated by superscripts in the table) and were selected for the multivariable analyses. The Hosmer-Lemeshow goodness-of-fit test showed adequate fit for both the *Brucella* ($P = 0.930$) and the *Campylobacter* ($P = 0.922$) models. The final multilevel logistic regression models are shown in Table 4.3 for campylobacteriosis and in Table 4.4 for brucellosis.

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Table 4.2. Bivariable analysis of categorical risk factors for herd-level campylobacteriosis and brucellosis in cattle in three states of northern Nigeria

Variable and level	Campylobacteriosis			Brucellosis		
	No. of herds	No. positive (%)	*P*	No. of herds	No. positive (%)	*P*
State[a,b]			<0.001			0.096
Adamawa	94	50 (53.2)		100	84 (84.0)	
Kaduna	93	19 (20.4)		105	75 (71.4)	
Kano	63	9 (14.3)		66	51 (77.3)	
Type of breeding[a,b]			<0.001			0.165
AI and natural mating	45	15 (33.3)		46	37 (80.4)	
AI only	1	0 (0.00)		14	10 (71.4)	
Natural mating (plough/trade/feedlot)	13	12 (92.5)		13	13 (100.0)	
Natural mating only	191	51 (26.7)		198	150 (75.8)	
Management system[a,b]			<0.001			<0.001
Intensive	31	12 (38.7)		36	25 (64.9)	
Agro-pastoral	143	30 (30.0)		158	113 (71.5)	
Pastoral	76	36 (47.4)		77	72 (93.5)	
Supplementary feeding[a,b]			0.005			<0.001
None	25	15 (60.0)		25	25 (100.0)	
Fodder/bran	107	33 (30.8)		110	94 (85.5)	
Concentrate	118	30 (25.4)		136	91 (66.9)	
Mineral supplementation[a,b]			<0.001			<0.001
No	77	36 (46.8)		78	74 (94.9)	
Yes	173	42 (24.3)		193	136 (70.5)	
Pasture environment			0.916			0.204
Enclosed	52	15 (28.9)		63	52 (82.5)	
Unenclosed	7	2 (28.6)		7	7 (100.0)	
Free range	191	61 (31.9)		201	151 (75.1)	
Pasture establishment[b]			0.398			0.076
No	191	61 (31.9)		201	151 (75.1)	
Yes	59	17 (28.8)		70	59 (84.3)	
Water source[a]			0.002			0.328
Piped	65	12 (18.5)		75	54 (72.0)	
Flowing	114	33 (29.0)		118	92 (77.9)	
Stagnant	71	33 (46.5)		78	64 (82.1)	
Housing[a,b]			0.007			0.076
Open barbed wire	155	54 (34.8)		163	131 (80.4)	
Open half way and roofed	62	10 (16.1)		71	48 (67.6)	
Open solid enclosure	33	14 (42.4)		37	31 (83.4)	

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Hygiene/floor type[a,b]			0.021			0.125
Floored	60	12 (20.0)		67	48 (71.6)	
Unfloored/muddy	190	66 (34.7)		204	162 (79.4)	
Care during parturition[a,b]			<0.001			<0.001
No	93	44 (47.3)		94	85 (90.4)	
Yes	136	20 (14.7)		154	105 (68.2)	
Herd prophylactic measures[a,b]			<0.001			0.067
No	107	48 (44.9)		109	90 (82.6)	
Yes	143	30 (21.0)		162	120 (74.1)	
Borrow/share bull[a,b]			<0.001			<0.001
No	156	27 (17.3)		172	118 (68.6)	
Yes	94	51 (54.4)		99	92 (92.9)	
Presence of small ruminants[a,b]			<0.001			<0.001
No	96	12 (12.5)		106	62 (58.5)	
Yes	154	66 (42.9)		165	148 (89.7)	
Presence of dogs[b]			0.261			0.014
No	227	69 (30.4)		247	187 (75.7)	
Yes	23	9 (39.1)		24	23 (95.8)	
Presence of horses			0.466			0.408
No	239	74 (31.0)		258	199 (77.1)	
Yes	11	4 (36.4)		13	11 (84.6)	
Presence of camels[a,b]			0.151			0.097
No	245	78 (31.8)		262	201 (76.7)	
Yes	5	0 (0.00)		9	9 (100.0)	
Presence of chickens[b]			0.257			0.002
No	169	50 (29.6)		179	129 (72.1)	
Yes	81	28 (34.6)		92	81 (88.0)	
Multiple herds[a,b]			<0.001			<0.001
No	163	33 (20.3)		168	117 (69.6)	
Yes	87	45 (51.7)		103	93 (90.3)	
Initial animal acquisition			0.212			<0.001
Inherited	114	30 (26.3)		125	87 (69.6)	
Other farms	13	6 (46.5)		14	8 (57.1)	
Market	123	42 (34.2)		13 2	115 (87.1)	
Buying-in of new animals and quarantine[a,b]			<0.001			<0.001
Closed herd	67	7 (10.4)		78	44 (56.4)	
Bought<3, quarantine	29	2 (6.90)		31	15 (48.4)	
Bought>3 or no quarantine	154	69 (44.8)		162	151 (93.2)	
Socio-economic status of farmer[a,b]			0.005			0.104
Full-time	174	45 (25.9)		189	142 (75.1)	
Part-time	76	33 (43.4)		82	68 (82.9)	

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Gyneacological examination[a]			<0.001			0.393
No	163	32 (19.6)		170	131 (77.1)	
Yes	73	34 (46.6)		87	65 (74.1)	
Specialist attending to animals[a,b]			0.040			<0.001
No	52	22 (42.3)		52	49 (94.3)	
Yes	198	56 (28.3)		219	161 (73.5)	
Handling facility[b]			0.516			0.124
No	191	60 (31.4)		200	151 (75.5)	
Yes	59	18 (30.5)		71	59 (83.1)	
Annual rainfall [a]			0.012			0.779
<700 mm	40	5 (12.5)		40	33 (82.5)	
700-1000 mm	60	19 (31.7)		65	50 (76.9)	
>1000 mm	150	54 (36.0)		166	127 (76.5)	
Herd size[a,b]			0.042			0.001
≤15	118	30 (25.4)		124	85 (68.6)	
>15	132	48 (36.4)		147	125 (85.0)	

[a]Variable significant (P< 0.20) for campylobacteriosis and therefore considered in the multivariable model.
[b]Variable significant (P< 0.20) for brucellosis and therefore considered in the multivariable model.

Factors positively associated with herd-level *C. fetus* infection in the multivariable analysis were the presence of small ruminants (sheep and/or goats) on the same farm, buying-in of >3 new animals or no quarantine, gynaecological examination, initial acquisition of animals from markets and high annual rainfall (Table 4.3). The practice of herd prophylactic measures against diseases was protective. After adjustment for the other predictors, large differences in the odds of herd-level *C. fetus* infection were seen between states; however, the random effects parameters indicated that there was no significant variation between LGA's or wards. Neither inclusion of herd *Brucella* status in the model nor conditional logistic regression stratifying on herd *Brucella* status (models not shown) resulted in any major changes in the coefficients for the other predictors. However, herd *Brucella* status was a significant predictor of herd *Campylobacter* status in the multilevel logistic regression model (OR = 13.7; P = 0.008).

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Table 4.3. Risk factors for herd-level *Campylobacter fetus* infection in 250 cattle herds in three states of northern Nigeria: results of a multilevel logistic regression model

Variable and level	*OR*	95% CI (*OR*)	*P*
State			
Kaduna	1	–	–
Adamawa	11.4	3.72, 34.9	<0.001
Kano	63.5	5.98, 674	0.001
Small ruminants present			
No	1	–	–
Yes	4.72	1.40, 15.9	0.012
Herd prophylactic measures			
No	1	–	–
Yes	0.15	0.05, 0.44	0.001
Buying-in of new animals and quarantine			
Closed herd	1	–	–
Bought ≤3, quarantine	0.48	0.06, 4.09	0.499
Bought >3 or no quarantine	10.9	2.46, 47.9	0.002
Gynaecological examination			
No	1	–	–
Yes	5.26	1.93, 14.4	0.001
Initial animal acquisition			
Inherited	1	–	–
Other farms	1.27	0.54, 3.02	0.582
Market	14.8	1.95, 112	0.009
Annual rainfall			
<700 mm	1	–	–
700-1000 mm	14.4	1.73, 120	0.014
>1000 mm	100.6	7.26, 1394	0.001
Random effects: Variance (SE)			
LGA	1.33×10^{-17} (2.25×10^{-9})		
Ward	3.09×10^{-20} (1.60×10^{-10})		
Likelihood ratio test vs. standard logistic regression model: $P = 1.000$			

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The odds of herd-level *Brucella* seropositivity were positively associated with the presence of small ruminants on the same farm, buying of >3 new animals or no quarantine, herd size >15 animals, the pastoral management system and the presence of handling facilities on the farm (Table 4.4). The practice of gynaecological examination in the herds was associated with lower odds of seropositivity. After adjustment for the other predictors in the model, odds of brucellosis varied between states, but did not vary significantly between LGA's or wards. Neither inclusion of herd *Campylobacter* status in the model nor conditional logistic regression stratifying on herd *Campylobacter* status (models not shown) resulted in any major changes in the coefficients for the other predictors.

The distribution of the count of *Brucella*-positive cattle in herds is shown in Fig. 4.1. The mean count of positive animals was 4.2 and the variance was 12.2, suggesting overdispersion, and zero counts were recorded in 22.5% of herds. A zero-inflated model therefore seemed appropriate. The Vuong statistic for the final ZIP model was 4.01 ($P < 0.0001$), indicating substantially better fit than the standard Poisson model. The likelihood-ratio test for the corresponding zero-inflated negative binomial model was not significant ($P = 0.499$), therefore the ZIP model was used and is shown in Table 4.5.

The logistic component of the model produced results consistent with those of the multivariable logistic regression model in Table 4.4, except that herd size was no longer significant. Note that the logistic component of the zero-inflated model (Table 4.5) models the odds of a herd being *Brucella*-negative, rather than positive, therefore the coefficients have the opposite sign and the odds ratios are inverted. In the Poisson component, the presence of small ruminants, buying-in of >3 new animals or no quarantine and borrowing or sharing of bulls were positively associated with the count of *Brucella*-positive cattle in herds. Herds in which mineral supplementation was given had significantly fewer *Brucella*-positive animals.

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Table 4.4. Risk factors for herd-level *Brucella* infection in 271 cattle herds in three states of northern Nigeria: results of a multilevel logistic regression model

Variable and level	*OR*	95% CI (*OR*)	*P*
State			
Kaduna	1	–	–
Adamawa	1.62	0.55, 4.77	0.381
Kano	4.47	1.33, 15.0	0.015
Small ruminants present			
No	1	–	–
Yes	6.36	2.24, 18.1	0.001
Buying-in of new animals			
Closed herd	1	–	–
Bought <3, quarantine	0.72	0.23, 2.25	0.570
Bought >3 or no quarantine	10.7	3.12, 36.8	<0.001
Herd size			
≤15	1	–	–
>15	2.81	1.11, 7.16	0.030
Management system			
Intensive	1	–	–
Agro-pastoral	2.92	0.70, 12.2	0.141
Pastoral	24.3	3.41, 174	0.001
Gynaecological examination			
No	1	–	–
Yes	0.12	0.03, 0.49	0.003
Handling facility			
No	1	–	–
Yes	21.2	4.33, 104	<0.001
Random effects: Variance (SE)			
LGA	6.19×10^{-21} (7.45×10^{-11})		
Ward	0.364 (0.766)		
Likelihood ratio test vs. standard logistic regression model: $P = 0.867$			

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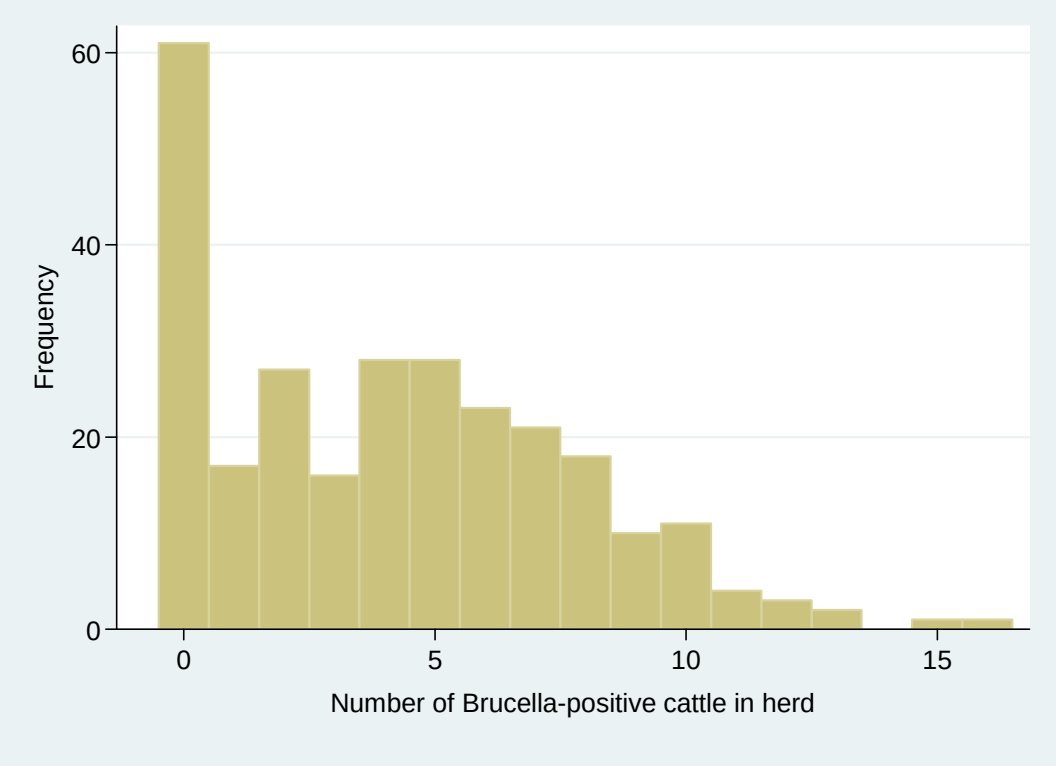

Figure 4.1. Distribution of number of *Brucella*-positive cattle in 271 herds in northern Nigeria

Table 4.5. Factors associated with the number of *Brucella*-positive cattle in 271 cattle herds in three states of northern Nigeria: results of a zero-inflated Poisson regression model with robust standard errors

Variable and level	Count ratio[a] or odds ratio[b]	95% CI	*P*
Poisson model			
State			
Kaduna	1	–	–
Adamawa	1.06	0.91, 1.22	0.451
Kano	1.17	1.01, 1.36	0.035
Small ruminants present			
No	1	–	–
Yes	1.45	1.16, 1.81	0.001
Buying-in of new animals			
Closed herd	1	–	–
Bought ≤3, quarantine	0.94	0.65, 1.37	0.764
Bought >3 or no quarantine	1.87	1.52, 2.30	<0.001

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Mineral supplementation			
No	1	–	–
Yes	0.76	0.67, 0.86	<0.001
Borrow or share bulls			
No	1	–	–
Yes	1.28	1.08, 1.51	0.004
ln(number tested)	(exposure)	–	–
Logistic (inflation) model			
State			
Kaduna	1	–	–
Adamawa	0.51	0.13, 2.10	0.354
Kano	0.27	0.07, 1.13	0.074
Small ruminants present			
No	1	–	–
Yes	0.16	0.05, 0.57	0.004
Buying in of new animals			
Closed herd	1	–	–
Bought<3, quarantine	1.98	0.34, 11.6	0.450
Bought>3 or no quarantine	0.11	0.02, 0.58	0.010
Management system			
Intensive	1	–	–
Agro-pastoral	0.14	0.03, 0.80	0.027
Pastoral	0.02	0.00, 0.18	0.001
Gynaecological examination			
No	1	–	–
Yes	21.5	3.67, 126	0.001
Handling facility			
No	1	–	–
Yes	0.01	0.00, 0.07	<0.001

[a]Count ratio for Poisson model
[b]Odds ratio for logistic model, with outcome=1 as reference

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Discussion

In this study the variation in herd-level prevalence of campylobacteriosis and brucellosis between states in northern Nigeria was due to a variety of measured and unmeasured factors. Although Adamawa state had the highest prevalence of *C. fetus* infection, after adjustment for the other significant risk factors in the multivariable model, cattle herds in Kano state showed the highest odds of infection (Table 4.3). This may partly be explained by factors such as the practice of buying-in larger numbers of animals without quarantine, which was much more prevalent in Adamawa than in Kano ($P = 0.002$), and by rainfall, which was much lower in Kano than in Adamawa and Kaduna ($P < 0.001$). However, it is clear that other important factors, unmeasured in this study, also influenced the risk of herd-level *C. fetus* infection. Variation between states was not as marked for *Brucella* seropositivity; nevertheless, the higher odds in Kano state in the multivariable model indicate that other unmeasured risk factors were present.

The observed variation in brucellosis seroprevalence between states is consistent with reports by McDermott and Arimi (2002), Jergefa *et al.* (2009) and Megersa *et al.* (2011b) who showed that cattle management practices and other agro-ecological factors in different locations that promoted or restricted contact between herds could influence brucellosis. In addition, the environmental survival of *Brucella* spp. depends partly on climatic conditions, with the pathogen likely to survive longer in wet and cold compared to dry and hot areas (Nicoletti, 1980; Lepeuple *et al.,* 2004). *Brucella* spp. are very susceptible to sunlight and heat; they survive for a few hours in hot and dry months, although in summer they can survive in wet soil for up to 7 days (Nicoletti, 1980). The relatively drier climate in Kano state may have helped prevent the seroprevalence of brucellosis from being higher than in the other states despite the presence of other unmeasured risk factors.

Another reason for the relatively high prevalence of both diseases in Adamawa may be the fact that the state borders Cameroon and there is unrestricted movement of cattle across the border. Similar observations were made for campylobacteriosis in Nigeria (Mshelia *et al*., 2010b) and for brucellosis in Nigeria (Esuruoso, 1974; Cadmus *et al*., 2008), in Ghana (Kubuafor *et al*., 2000) and in Ethiopia (Mekonnen *et al.*, 2010). Mixing of animals from different areas can facilitate spread of brucellosis between herds (Kubuafor *et al*., 2000) and contact with bulls from different herds as in communal grazing increases the risk of venereal transmission of *C. fetus* infection (Pefanis *et al*., 1988).

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There was a significant positive association between the presence of small ruminants and the odds of campylobacteriosis in this study. *Campylobacter fetus* is found in the genital and intestinal tracts of sheep (Kimberling, 1988), which can transmit the disease to cattle. Likewise, the presence of small ruminants in the herds was positively associated with the odds of brucellosis. This is in agreement with results of serological surveys in Uganda and Ethiopia in which the odds of testing *Brucella* seropositive were higher in sheep and goat flocks co-grazing with cattle, suggesting the possibility of cross-species transmission of *Brucella* infection (Kabagambe *et al*., 2001; Megersa *et al*., 2011a). A similar observation has been made with respect to keeping small ruminants together with camels (Al-Majali *et al*., 2008). In addition, horses can be infected by contact with infected cattle, but infection of cattle by horses is not likely to occur (Ocholi *et al*., 2004b).

There was a positive association between conducting gynaecological examinations and the odds of *C. fetus* infection. It is possible that during regular or occasional trans-rectal palpation in a herd health fertility programme for pregnancy diagnosis and assessment of cyclicity, contamination of the female reproductive tract could occur. Therefore, gynaecological examination may have been responsible for the mechanical spread of this organism within and possibly also between herds, particularly where vaginal examination was also done and basic sanitary practices were not applied. Mechanical transmission has been reported (Mukersa-Mugerwa, 1989; Hjerpe, 1990) and contaminated bedding and fomites are known to transmit campylobacteriosis (Hoffer, 1981; Hjerpe, 1990). In addition, where semen collection and AI are practiced, the instruments may be contaminated and play a role in the dissemination of the bacteria (Garcia *et al*., 1983a; OIE, 2011b). On the other hand, the negative association between gynaecological examination and the risk of herd brucellosis seropositivity observed in this study suggests that *Brucella* is unlikely to be transmitted in this way, and may be due to the fact that, in general, the herds in which gynaecological examination was conducted were better managed herds with better sanitary conditions and therefore lower risk of infection. Indeed, although already accounted for in the multivariable model, herds that conducted gynaecological examination were more likely to be maintained as closed herds (OR = 2.4; P = 0.002). It is likely that the farmers had some knowledge of brucellosis and were therefore cautious of introducing the disease or had some form of *Brucella* disease control measures on their farms. Although not assessed in this study, farmer's knowledge of brucellosis was reported to be associated with lower odds of herd seropositivity in Zambia (Matope *et al.*, 2010).

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Herds that had bought in >3 new animals or did not practice quarantine were far more likely to be seropositive for brucellosis, suggesting that buying-in of infected animals is an important mechanism of introduction of the disease. Very similar results were obtained in our study for campylobacteriosis. Although it was reported from Argentina that buying bulls was associated with a 35% decrease in the risk of campylobacteriosis infection, likely due to a decrease in disease transmission associated with introduction of virgin, uninfected bulls (Jimenez *et al*., 2011), it is unlikely that the replacement stock in Nigeria is free from the disease. Neither brucellosis nor campylobacteriosis is likely to be detected during the period of quarantine, since most infections are subclinical (Godfroid *et al*., 2004; Irons *et al*., 2004a), although in rare occasions, depending on the stage of infection, hygroma may manifest in the case of brucellosis. Therefore, this risk factor is likely to be an effect mainly of the number of animals introduced rather than the practice of quarantine. In general terms, for both disease conditions, the more often cattle are introduced into the herd, the greater the risk of introducing infected cattle (Mukasa-Mugerwa, 1989; Crawford *et al*., 1990; Omer *et al*., 2000).

Initial acquisition of animals from market was associated with significantly higher odds of *Campylobacter* infection than buying from other farms or inheriting animals. This suggests that farmers that acquire their initial stock from market stand the risk of buying infected animals. Cattle acquired from the market are likely to come from a mixture of farms, localities and management systems, and are therefore likely to include one or more infected animals. The practice of carefully acquiring healthy animals from other farms that are better managed as initial stock reduces the risk of infection and is therefore recommended to farmers. The majority of the agro-pastoral herds, which had significantly lower prevalence of *C. fetus* infection in this study, were inherited.

The practice of herd prophylactic measures against diseases was associated with reduced odds of campylobacteriosis. These included farm management practices that help to ensure a healthy status of the herd, such as deworming, tick control, haemoparasite control or vaccination, although specific vaccination against campylobacteriosis was not practiced by any herds in the study. Such practices may help to ensure healthy immune status of the herds and may reduce the risk of infection with *C. fetus*, and are likely also an indication of overall better herd management. Active engagement of the producer to keep a high sanitary performance of the herd, as well as proactive policies of identification and removal of

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animals infected with diseases such as campylobacteriosis will reduce the risk of herd infection in such organized farms (Jimenez *et al.*, 2011).

High rainfall was positively associated with the odds of herd *C. fetus* infection. It has been reported that the occurrence of enteric campylobacteriosis increased with rainfall (Taema *et al*., 2008). *Campylobacter fetus fetus* is a commensal of the gastrointestinal tract of cattle and sheep and is spread by ingestion of contaminated material (Dufty and Vaughan, 1993); therefore wet conditions may be more favourable for its spread. However, since *C. f. venerealis*, the predominant subspecies in this study, occurs in the genital tract and is transmitted venereally, the reason for this association is unclear, and it may also be an effect of other management factors associated with rainfall.

The higher brucellosis seropositivity observed in the large herds (>15 animals) in this study is consistent with previous findings (Berhe *et al*., 2007; Muma *et al*., 2007b; Mekonnen *et al*., 2010), although contrary to others (Karimuribo *et al*., 2007; Jergefa *et al.,* 2009). In Ethiopia, animals from smaller herds were reportedly at greater risk of acquiring brucellosis, a finding attributed to the fact that extensive farms held larger numbers of animals than intensive farms, which showed higher risk of brucellosis seropositivity (Jergefa *et al.,* 2009). However, in our study areas, herd size was smaller in pastoral than in intensive systems (P = 0.005), and yet large herd size and pastoral management system were both independently associated with increased odds of brucellosis. In most of the herds in our study, there was the potential for contact with animals from other herds, particularly in the pastoral and to a lesser extent the agro-pastoral system. It is likely that the larger the herd the more frequent the contacts with other herds, which may explain the higher risk of herd infection in larger herds in our study.

The higher seroprevalence of brucellosis in pastoral systems is consistent with results of other studies (McDermott and Arimi, 2002; Ocholi *et al*., 2004a). As in Ethiopia (Megersa *et al*., 2011b), the pastoral Fulanis in Nigeria settle in clusters of households with their herds in close proximity. Their nomadic nature, covering long distances in search of pasture particularly during the dry season, is likely to result in stress to the animals and exposure to infected animals and contaminated environments, resulting in increased risk of brucellosis. Musa *et al*. (1990) reported that brucellosis often began during adverse weather conditions and famine. Furthermore, nomadism may also increase interactions with wild life, which can increase the risk of acquiring brucellosis (Muma *et al*., 2007b). In addition, most of the

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pastoral Fulani herds do not isolate pregnant cows during parturition in order to remove and appropriately dispose of the afterbirth following calving. Cattle from farmers, who disposed of foetal membranes in the fields or gave them to dogs, as well as those in communal grazing systems, were found to have a high proportion of seroreactors (Mekonnen *et al.,* 2010).

The fact that management system was no longer a significant risk factor for *C. fetus* infection in the multivariable model shows that the observed difference in prevalence between management systems was at least partially accounted for by the other variables in the multivariable model. Indeed, the two variables accounting for the majority of this confounding were herd prophylactic measures and buying-in of animals from the market; with these two variables excluded from the model the odds of *C. fetus* infection was far greater in pastoral than in intensive (OR = 13.2; P = 0.001) or in agro-pastoral (OR = 7.9; $P <$ 0.001) systems.

The strong positive association between the presence of a handling facility and herd brucellosis seropositivity is likely due to the fact that such facilities may be shared by nearby farmers and even used by more than one herd at the same time. This would therefore increase contact with neighbouring herds and increase risk of transmission of the disease.

The zero-inflated Poisson model (Table 4.5) showed that risk factors may differ somewhat for the two biological processes in herd infection, namely the herd becoming *Brucella*-positive and the extent of infection within the herd. Although conventional count models have previously been used in bovine brucellosis (Muma *et al*., 2007b; Matope *et al*., 2010), zero-inflated count models such as the ZIP model used in our study may be more appropriate to use all the information in the data to provide more insight into the two biological processes. This was recently demonstrated in a study of risk factors for developmental orthopaedic disease in young horses (Lepeule *et al.,* 2011).

The logistic (inflation) component of the ZIP model identified the same risk factors for herd brucellosis infection as did the logistic regression model (Table 4.4), with the exception of herd size which was not significant in the former. The Poisson component showed that the presence of small ruminants and buying of >3 new animals or no quarantine were associated not only with the odds of herd seropositivity, but also with the extent of the within-herd infection. In addition, the Poisson component identified two further factors associated with the count of *Brucella*-positive animals within infected herds, i.e. the within-herd

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seroprevalence. Firstly, the borrowing or sharing of bulls was associated with higher counts, which suggests venereal transmission of brucellosis. The brucellae localize in the testicles and in the non-gravid uterus and venereal transmission is a significant route of spread of infection (Bercovich, 1998). It has also been reported that leakage from the penis of an infected bull may contaminate feed and water of susceptible pregnant cows (Anon., 1977). Secondly, herds that received mineral supplementation had significantly lower counts of seropositive animals. This is consistent with a report by Grunert (1984) that mineral and nutritional deficiencies increased the occurrence of *Brucella* infected herds, although mineral supplementation may also have been an indicator of other good management practices that reduced the within-herd spread of infection.

Conclusion

This study revealed that both campylobacteriosis and brucellosis were endemic in northern Nigeria. The presence of small ruminants on the farm and buying-in of new animals without quarantine were positively associated with campylobacteriosis, brucellosis and within-herd proportion of *Brucella*-positive animals. Lack of herd prophylactic measures, initial animal acquisition from markets and high rainfall were associated with increased odds of *C. fetus* infection, while the pastoral management system, larger herd size and presence of a handling facility were associated with higher odds of *Brucella* seropositivity. In addition, borrowing or sharing of bulls was associated with higher counts, and mineral supplementation with lower counts of *Brucella* seropositive cattle within herds. Management systems practiced in northern Nigeria, particularly by the traditional Fulani pastoralists, likely facilitate the spread of both campylobacteriosis and brucellosis. Attention to risk factors identified in this study will help to inform effective control programmes.

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Chapter 5

Reproductive diseases, reproductive disorders and factors affecting calving rate of cattle in three states of Northern Nigeria

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Submitted

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Abstract

The presence of brucellosis and genital campylobacteriosis, reproductive disorders and demographic and management characteristics were determined and related to calving rate of cattle herds in Adamawa, Kaduna and Kano states. Serum samples, preputial scrapings, questionnaire survey, trans-rectal palpation and farm records were used from 271 herds. The Rose-Bengal plate test (RBPT) and competitive enzyme-linked immunosorbent assay (c-ELISA) were used for brucellosis serology while culture and identification from preputial samples were used for campylobacteriosis. A herd was classified as positive if one or more animals tested positive. A multilevel linear regression model was used to determine the effect of herd-level *Brucella abortus* seropositivity, *Campylobacter fetus* infection and other factors on calculated calving rate. The reproductive performance of the cattle herds was generally poor: Only 6.5% of the nursing cows were pregnant and 51.1% were non-pregnant and acyclic; the mean annual calving rate was 51.4%; and 1.6% of females were found to have anatomical malformations of the genitalia or foetuses. There was a positive association at the herd level between brucellosis infection and the occurrence of abortion, retained afterbirth, stillbirth, pyometra and weak calves or calf mortality. *Brucella abortus* and *C. fetus* infection of herds were each independently associated with an absolute reduction in calving rate of 14.9% and 8.4%, respectively (both $P < 0.001$). There was also a strong negative association between within-herd *Brucella* seroprevalence and calving rate ($P < 0.001$). In addition, the presence of small ruminants, introduction of animals without quarantine and the presence of a handling facility were associated with lower calving rates, whereas larger herd size, supplementary feeding, routine mineral supplementation, isolation and observation of the cow during parturition and removal of afterbirth were associated with higher calving rates. Farmer education and measures to improve the fertility of cattle herds are suggested.

Introduction

The reproductive efficiency of indigenous Nigerian cattle is comparatively low (Pullan, 1979). Failure to conceive and delayed return to oestrus with subsequent long calving intervals have frustrated AI programmes (Oyedipe *et al*., 1982a; Eduvie and Oyedipe, 1991). A previous estimate of the prevalence of postpartum anoestrous indicates a worrisome level of this problem in Nigerian cows (Eduvie and Dawuda, 1986; Dawuda *et al*., 1989). Abortion

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has a severe economic impact due to loss of the offspring, loss of milk, culling and replacing affected animals and increased calving interval. Other sources of economic loss include culling for infertility, delayed calving, decrease in calving rate and decrease milk production due to subclinical mastitis (Esuruoso, 1979; Akhtar *et al*., 1993b).

The reasons for poor productivity are multi-factorial, including poor management systems, inadequate nutrition, poor genetic material, inadequate veterinary services and widespread infectious and parasitic diseases (Mukasa-Mugerwa, 1989). About 95% of all food animal populations in Nigeria are in the hands of nomadic and semi-nomadic traditional farmers (Rikin, 1988) who utilise relatively inefficient production systems resulting in low productivity (Nuru, 1981). It is therefore unlikely that the output from the livestock industry will match the population growth unless the causes of poor productivity are identified and addressed.

Reproductive indices reported in nomadic herds in Nigeria include age at first calving of 60 months, calving interval of 17-24 months, annual calf crop of 40% and total number of calves produced by a cow of 2.5 (Zemjanis 1974). Other indices include age at puberty of 40.2 months (Knudson and Sohael, 1970), calving to first conception of 7.8 months (Eduvie and Dawuda, 1986) and first service conception rate of 46.7% (Voh Jr *et al*., 1987). These indices are affected by several factors such as genetics (Pullan, 1979; Mukasa-Mugerwa, 1989), adverse environmental factors (Mai, 1997), age and parity of the dam (Voh Jr and Otchere, 1989), nutrition (Oyedipe *et al.,* 1982b), suckling (Eduvie and Dawuda, 1986; Dawuda *et al*., 1989), infectious diseases (Ocholi *et al*., 2004a; Mshelia *et al.*, 2010b) and inadequate oestrus detection (Mai *et al*., 2002).

Reproductive disorders are a major reason for low reproductive efficiency in cattle and subsequent lifetime productivity (De-Vecchio *et al*., 1992). Prevalence of genital tract malformations of 2.1% (Abdullahi and Chaudhari, 1996) and 13.6% (Chaudhari and Paul-Bokko, 2000) were reported in indigenous Nigerian cows. Reports elsewhere indicated 21% in native Egyptian cows (Wahid *et al*., 1991) and 36.8% in Ethiopian Zebu cattle (Albati *et al*., 2006). In addition, a 35% abortion rate and 17-59% rate of repeat breeders were reported in Nigerian Fulani herds (Nuru and Dennis, 1976**)**. In a study to determine calving ease in indigenous cattle, 46% of herds reported dystocia (Akpa *et al*., 2011). Reports from the field indicate that abortion is a common feature among livestock in Nigeria but cases are not always subjected to detailed laboratory investigations (Okoh, 1980). Most of these studies in

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Nigeria did not associate the conditions with infectious diseases. Elsewhere, Degefa *et al.* (2011) reported an incidence of abortion of 8.7% and retained afterbirth 18.3% in Oromia, Ethiopia, while Megersa *et al.* (2011a) reported 13.8% abortion in Borana, Ethiopia. In Rwanda, a 13% abortion rate, 33% retained afterbirth and 37% dystocia were reported (Chatikobo *et al.*, 2009).

Amongst the infectious diseases, brucellosis and bovine genital campylobacteriosis have been known to be prevalent in Nigeria and implicated in infertility and abortions (Ocholi *et al.*, 2004a; Mshelia *et al.*, 2010a) resulting in huge economic losses. These venereal diseases are transmitted by communal bulls in management systems commonly found all over Africa (Tekleye *et al.*, 1988). *Brucella abortus* is a major zoonosis and exist worldwide (Godfroid *et al.,* 2005). In certain circumstances *C. f. fetus* can be zoonotic (Fujihara *et al.,* 2006). Brucellosis is characterized by abortion, stillbirth, retained afterbirth and birth of weak calves in females (Aguair *et al.*, 2007; Degefa *et al.*, 2011), and epididymitis and orchitis in males (OIE, 2011a). *Brucella abortus* was isolated from two aborted foetuses of cattle in Nigeria (Ocholi *et al.*, 2004a). It was concluded that most abortions were associated with *Brucella* seropositivity in cattle (Degefa *et al.*, 2011; Megersa *et al* 2011a). The abortion is as a result of both hypoxia from placental damage and by fetal invasion (Jones *et al.*, 1997) which will result in early embryonic death or spontaneous abortions in the first trimester of gestation, although occasionally sporadic abortions between 4 and 7 months of gestation have been reported (Anderson, 2007). In addition, retained afterbirths, stillbirths, birth of weak calves and repeat breeders were also positively associated with brucellosis (Aguair *et al.*, 2007; Degefa *et al.*, 2011), although Ibrahim *et al.* (2010) found no association between retained afterbirth and brucellosis. Reports elsewhere indicated that brucellosis increased calving interval (Mekonnen *et al.*, 2010; Mekonnen *et al.*, 2011). A 10% decrease in the number of calves was observed in RBPT-positive cows (McDermott *et al.*, 1987; Megersa *et al.*, 2011a).

Bovine genital campylobacteriosis, commonly caused by *C. f. venerealis*, is characterized by infertility, repetition of oestrus, embryonic mortality and late abortions (Bawa *et al.*, 1991; Campero *et al.,* 2005; Mshelia *et al.*, 2010a). The consequences of these are prolonged calving interval and low pregnancy rate (Roberts, 1986; McFadden *et al.*, 2004). In Nigeria, *C. fetus* was isolated in an artificially inseminated herd with history of abortion and repeat breeding (Bawa *et al.*, 1987), and from two cases of abortion encountered during a survey (Bawa *et al.*, 1991). Recently, Mshelia *et al.* (2010b) showed a 15% infection rate in herds

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with history of abortion and stillbirth, and 4% in those without any history of reproductive failure. Abortion at a mean foetal age of 6 months occurs in 5 to 10% of animals (Dufty and Vaughan, 1993). Jimenez *et al*. (2011) reported abortion rate of 27.1% in beef herds in Argentina. McCool *et al*. (1988) showed that permanent infertility may result in up to 11% of infected heifers. A study of communal farms in South Africa showed high prevalence of campylobacteriosis and trichomonosis (Pefanis *et al*., 1988). Campylobacteriosis is more problematic in heifers, while no signs of infection are evident in bulls (Vandeplassche *et al*., 1963). The semen quality and breeding ability of bulls are unaffected (Irons *et al*., 2004a). The persistent nature of the disease in bulls makes control difficult, since asymptomatic animals may transmit the infection at coitus or during AI (Schurig *et al*., 1975).

Annual calving rate is a robust indicator of breeding performance and herd fertility, taking into account all pregnancies and calves in the herds over a one year period (Stonaker *et al*., 1976; Voh Jr and Otchere, 1989). The total annual calving rate is an aggregate of all the calving activity within one year period. It is a good measure of fertility giving a broad overview of the reproductive performance of a herd in the previous and the current year. It considers the number and estimated ages of calves in the herd as well as the number of pregnant animals and estimated ages of foetuses. It can be readily calculated using a consistent method in herds with different calving patterns. It is also independent of the season in which the data is collected, which can be a confounder with other indices in herds with seasonal calving patterns. The calving rate is therefore easily measured and a reliable reproductive parameter. The calving rate was therefore used as the variable which best represented the herd reproductive performance. Single-day examination of a herd and prediction of calving rate from findings of gynaecological examinations is prone to some errors in that it cannot account for cases of abortion and errors in the aging of pregnancies. Some of these errors result in overestimation of annual calving rate while others will result in underestimation. The impact of these omissions is also likely to be affected by the general prevalence of pregnancy loss after pregnancy diagnosis. There is no available information regarding pregnancy loss following pregnancy diagnosis in cattle herds infected with either brucellosis or campylobacteriosis in Nigeria. However, a report in Holstein dairy cows in North America estimated the loss to be 5% (Warnick *et al*., 1995).

Calving percentage is a closely similar reproductive parameter to the calving rate and accounts for abortions. However, computations of calving percentage involves visiting the

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farm at least monthly for a period of one year to monitor and record calving as they occur; since bulls are with the breeding females all the time allowing breeding, conception and calving occurring throughout the year. The calving percentage therefore has limitation because there are no written records of events and the parameter will have to depend on poor, inadequate records of the herds or what the farmers or herdsmen can recall in the last one year. To deal with this situation requires trans-rectal palpation of cattle for pregnancy and cyclicity to determine the actual status of the animals in the herds in addition to what the farmers can recall. Calving percentage is also susceptible to selection bias as a result of selective culling of non-conceiving or late conceiving cows before subsequent calving, thereby overestimating the calving percentage as opposed to when the herds were assessed by pregnancy diagnosis before culling ensued as reported by Fahey *et al.* (2002).

The influence of brucellosis, campylobacteriosis and trichomonosis on calving rate has not been well studied on a herd basis in the communal farming system (Pouilly *et al*., 1994; Mokantla *et al*., 2004). It is well acknowledged that infertility in livestock production is a serious reproductive problem worldwide. Studies of the prevalence of infectious causes of infertility such as bovine brucellosis, bovine genital campylobacteriosis and trichomonosis have been conducted in Nigeria (Ocholi *et al.*, 2004a; Mshelia *et al*., 2010b; Mai *et al*., 2012; Mai *et al*., unpublished data). However, the actual impact of infectious diseases and other factors on calving rate of cattle in Nigeria has not been adequately documented. The purpose of this study was firstly to determine the reproductive efficiency and prevalence of reproductive disorders of Nigerian cattle based on physical examination for pregnancy and cyclicity, and secondly to investigate the effect of infectious reproductive diseases, as well as managemental and environmental factors, on calving rate in cattle herds in northern Nigeria.

Materials and Methods

The research protocol for this study was approved by the Animal Use and Care Committee and the Research Committee of the University of Pretoria (Protocol no. V073-08).

Study areas and study design

Three states, namely Adamawa, Kaduna and Kano were selected from the nineteen northern states of Nigeria. Adamawa state is situated on latitudes 8-11°N and longitudes 11.5-13.5°E, with sub-Sudan vegetation in the northern parts and Guinea savannah vegetation in the southern parts. Kaduna state is on latitudes 9-11.3°N and longitudes 10.3-9.6°E extending

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from the Sudan savannah in the north and tropical grassland of Guinea savannah in the south. Kano state is situated at latitudes 12°N and longitudes 9°E, located within the Sudan savannah in the north and the Guinea savannah vegetation in the south.

A cross sectional study was conducted using multistage cluster sampling. Each of the three selected states was divided into three administrative geographical zones, and two local government areas (LGA's) were randomly selected from each zone, giving a total of six LGA's from each state, using as sampling frame a list of all LGA's in each zone. Approximately 50% of wards were randomly selected from a list of all wards in each selected LGA (Fig. 2.1, Chapter 2). An average of three herds was selected per ward, giving an average of 15 herds selected per LGA. Therefore, a total of 271 herds were sampled from the three states. Since no sampling frames were available for selection of herds within wards, herds were selected by visiting the farms and enrolling them as they consented to participation.

Animal and herd classification

Selected herds were visited once each between July, 2008 and June, 2009. Herd and individual animal data collection, and animal sampling were done during this visit.

All the postpubertal bulls, and postpubertal heifers and cows were sampled in each selected herd. A postpubertal bull was defined as a bull that had been successfully mounting other cows or heifers by achieving intromission. They weighed approximately 200 kg or above. A postpubertal heifer was a female that had been observed exhibiting oestrus or standing to be mounted by a bull and on trans-rectal examination, they typically have either of the functional structures i.e corpus luteum or follicle on their ovaries. They were weighing about 200 kg.

Four management systems were encountered during the study. The pastoral management system was characterized by cattle grazing on fallow land close to the place of settlement of the owners during the rainy season but covering long distances, some even migrating, during the critical period of the dry season in search of natural pasture. Agro-pastoral management was characterized by cattle grazing locally and supplementation with mostly crop residues particularly during the dry and pre-rainy seasons. Commercial management systems were organized farms that were usually fenced with paddocked, improved pastures and concentrate

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provided as supplementary feeds. Zero-grazing systems were farms in which the cattle were confined or even tethered with restricted movement and feed was provided.

Sample collection and testing for *Brucella abortus*

Animals selected for blood sampling for brucellosis were first calf heifers which had calved at least six weeks previously, cows and postpubertal heifers and bulls. About 10 ml of blood was collected from the jugular, coccygeal or saphenous veins into Vacutainer® tubes, and placed into an ice bath and transported to the laboratory for centrifugation, serum separation and storage at -20°C until ready for analysis. The Rose-Bengal plate agglutination test for brucellosis using RBP antigen (VLA, Weybridge, UK) and confirmation of RBPT positive samples with c-ELISA (VLA, Weybridge, UK) were carried out as recommended by OIE (2011a). Sampling and testing methods are discussed in detail in Mai *et al* (2012).

Sample collection and isolation of *Campylobacter fetus* from bulls

Preputial scrapings were collected from all postpubertal bulls in the herds as described by Irons *et al*. (2004a) and used to isolate *C. fetus* as described by OIE (2011b). At 72 h, a representative of a dewdrop colony that was Gram-negative, vibroid in shape and oxidase- and catalase-positive was transferred to a blood agar base (Oxoid, CM0055), streaked for purity and incubated under microaerophilic conditions for 72 h. Each culture and incubation was verified by using control strains of *C. f. fetus* and *C. f. venerealis* (ATCC 33247 and 19438 respectively). These isolates obtained were subjected to biochemical testing for H_2S production using TSI agar (Oxoid, CM0277B), aerobic growth, growth at 25°C and 42°C and in the presence of 1% glycine, 3.5% NaCl and sensitivity to cephalothin and nalidixic acid.

Additional data collection

Interview-based, structured questionnaires (Appendix I) were administered to the livestock owners on each farm at the time of sample collection, in order to gather information on potential animal-level and herd-level factors affecting calving rate. As far as possible, the herdsmen were interviewed in the presence of the owner or farm manager for about 30-45 minutes. Interview questions were focused on events on the farm over the past 12-24 months. Management, herd structure, location and environmental variables with a potential impact on calving rates were recorded.

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Age was estimated using farm records, dentition and, in some cases, cornual rings. Body condition score (BCS) was obtained as described by Pullan (1978) and assigned by the same veterinarian for all animals. History of abortion, retained afterbirth, stillbirth, irregular oestrus, repeat breeders, dystocia, weak calf and calf mortality were recorded from the questionnaires. Pregnancy diagnosis and cyclicity were determined using trans-rectal palpation as described by Arthur *et al*. (1996). Some reproductive disorders were also observed via trans-rectal palpation, including ovarian disorders such as inactive or acyclic ovaries, ovarian cysts and agenesis of ovaries, and uterine disorders such as pyometra, unicornuate uterus and freemartinism. All data were stored in Microsoft Excel spreadsheet (Microsoft Corp., Redmond, WA, U.S.A.).

Determination of annual calving rate

For the calculation of annual calving rate in each herd, the formula of Stonaker *et al.* (1976) and Voh Jr and Otchere (1989) was used to determine the number of animals likely to calve in the twelve months period, as follows:

Calving rate = number of calvings in one year / number of postpubertal heifers and cows

$$= (b + e + g + 2h + i) / (a + b + c + d + e + f + g + h + i)$$

where: a is the number of open, dry cows

b is the number of open cows nursing a calf under 6 months of age

c is the number of open cows nursing a calf 6 months of age and over

d is the number of pregnant dry cows under 2 months of gestation

e is the number of pregnant cows under 2 months of gestation and nursing a calf under 6 months of age

f is the number of pregnant cows under 2 months of gestation and nursing a calf 6 months of age and over

g is the number of pregnant dry cows at 2 months of gestation and over

h is the number of pregnant cows at 2 months of gestation and over and nursing a calf under 6 months of age

i is the number of pregnant cows at 2 months of gestation and over and nursing a calf 6 months of age and over.

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Statistical analysis

The unit of analysis was the herd and the outcome variable was the calving rate. Each independent variable (brucellosis, campylobacteriosis and the management and environmental variables) were tested for bivariable association with the outcome using Student's *t*-test or ANOVA. Variables associated with the outcome at $P < 0.2$ were selected for the multivariable model. A multilevel, mixed-effects linear regression model with state as a fixed effect and nested random effects for LGA and ward was then constructed. Backward elimination was applied until all remaining variables were significant ($P < 0.05$), after which all other predictor variables were tested by adding them back into the model and retained if significant. Significance of the random effects for LGA and ward was assessed by comparing models with and without random effects using a likelihood ratio test. Fit of the final model was evaluated using a plot of residuals versus fitted values and a normal probability plot of residuals.

Herd-level associations of *Brucella* seropositivity and *C. fetus* infection with abortion, retained afterbirth, stillbirth, repeat breeder, dystocia, pyometra, uterine prolapse, birth of weak calves or calf mortality, and hygroma were analyzed using Fisher's exact test. An association between within-herd *Brucella* seroprevalence and calving rate was also determined.

All statistical analyses were done using STATA 12 (Stata Corporation, College Station, TX, USA) and a significance level of $\alpha = 0.05$ was used.

Results

Herd structure

The herd structure studied is shown in Table 5.1. The average bull: female ratio was one mature male to eight mature females. The herd size ranged between 7 and 119 animals (median: 34; interquartile range (IQR) 25, 43).

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Table 5.1. Herd structure and breed, management system and reproductive status of cattle sampled from three states of northern Nigeria.

Variables	Total	Proportion of group (%)
Herd structure		
Bulls	602	6.0
Heifers	1,137	11.3
Cows	3,068	30.4
Bull calves and growers	1,285	12.8
Young bulls	1,038	10.3
Heifer calves and growers	1,276	12.7
Young heifers	1,663	16.5
Total	10,069	
Breed		
Bunaji	3,097	64.4
Gudali	870	18.1
Other *Bos indicus*	448	9.3
Bos taurus	120	2.5
B. taurus x *B. indicus*	272	5.7
Total[a]	4,807	
Management system		
Pastoral	1,263	26.3
Agro-pastoral	2,793	58.1
Commercial	650	13.5
Zero-grazing	101	2.1
Total[a]	4,807	
Reproductive status		
Suckling	1,818	43.3
Non-pregnant	1,545	36.8
Pregnant	273	6.5
Non-Suckling	2,384	56.7
Non-pregnant	1,290	30.7
Pregnant	1,094	26.0
Total[a]	4,807	

[a] [These totals were number of mature animals from the herds that were sampled]

Reproductive parameters

The mean annual calving rate was 51.4%, ranging between 0% and 100%, while the pregnancy rate, defined as the proportion of cows and postpubertal heifers which were found to be pregnant, was 32.5%.

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Reproductive status and body condition score

A total of 4,202 females consisting of 1,137 heifers and 3,065 cows were studied. Of these, 2,384 (56.7%) were non-suckling and 1,818 (43.3%) were suckling, and 1,367 (32.5%) were pregnant and 2,835 were non-pregnant. Of the suckling cows, 273 (15%) were pregnant, 609 (33.5%) were non-pregnant but cyclic and 936 (51.5%) were non-pregnant and acyclic. Body condition score ranged from 2 to 5 (median: 3; IQR 3, 4). Using two categories of BCS (≤3 and ≥3.5) as described by Pullan (1978), there was a significant difference in BCS between cyclic and non-cyclic cows ($P < 0.0001$) and between suckling and non-suckling cows ($P < 0.0001$).

Reproductive status of heifers and parity of cows and heifers

The reproductive performance records of heifers in this study indicated that at <2 years, two heifers were cyclic while five were acyclic (data not shown). Of 99 heifers at 2 years of age, 13% were pregnant, 21% were non-pregnant and cycling, and 66% were acyclic. At 3 years however, 22% of 381 heifers were pregnant, 55% were non-pregnant and cycling, and 23% were acyclic. Out of 480 heifers at 4 years, 45% were pregnant, 44% were cycling, and only 11% were acyclic. At 5 years of age, 57% of the heifers were pregnant (data not shown). These suggest that the general age at puberty of heifers in the study population is between 2 and 3 years while the age at first calving is between 3 and 5 years. The ages at puberty and first calving were projected from the estimated age and parity of dams derived from Table 5.2.

In addition, Table 5.2 shows the distribution of parity by age. Most (76.4%) of the heifers were between the ages of 3 and 4 years. Between 4 and 5 years of age, 68% of the heifers were uniparous and over 70% had calved. At the age of 6 and 7 years, most of the cows were in second or third parity, although 18 animals had not yet calved.

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Table 5.2. Age and parity of dams

Age (years)	Parity 0	1	2	3	4	5	6	7	8	9	10	Total
<2	7	0	0	0	0	0	0	0	0	0	0	7
2	99	4	0	0	0	0	0	0	0	0	0	103
3	381	46	0	0	0	0	0	0	0	0	0	427
4	480	241	21	0	0	0	0	0	0	0	0	742
5	149	581	131	40	2	0	0	0	0	0	0	903
6	13	284	285	65	21	0	0	0	0	0	0	668
7	5	50	204	107	25	6	0	0	0	0	0	397
8	0	6	91	143	57	10	2	0	0	0	0	309
9	0	2	6	78	50	22	5	1	0	0	0	164
10	0	0	6	33	50	36	7	1	0	0	0	133
11	0	0	1	8	14	13	8	6	1	0	0	51
12	0	0	0	6	7	15	9	10	6	1	0	54
13	0	0	0	0	0	2	3	3	1	1	0	10
14	0	0	0	0	1	0	0	0	0	0	1	2
15	0	0	0	0	0	0	0	0	0	1	3	4
Total	1134	1214	745	480	227	104	34	21	8	3	4	3974
% of Total	28.5	30.5	18.7	12.1	5.7	2.6	0.9	0.5	0.2	0.1	0.1	

Number of calves per cow lifetime in the herd and productive life of the cows

It was calculated from Table 5.2 that 2,840 cows were examined and had complete information about their ages. The cows had given birth and accounted for a total production of 6,054 calves. The average number of calves per cow in this study therefore was 2.1, i.e: total number of calves produced / total cows examined. This is an underestimation of true lifetime production in that it includes animals which are still in the productive state. Furthermore, Table 5.2 showed that very few animals were kept beyond 10 years; we can therefore conclude from this finding that the productive life of cattle in this study area is up to 10 years.

Factors associated with calving rate

The distribution of the various environmental and managemental factors and their bivariable association with calving rate at the herd level is shown in Table 5.3. The crude absolute difference in calving rate between *Brucella* positive and *Brucella* negative herds was 33.2%, while that of *C. fetus* positive and *C. fetus* negative herds was 24.2%. All the herds that were

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Brucella negative had a calving rate of over 50%, while 55 of the *Brucella* positive herds had a calving rate of <50% (Fig. 5.1). The mean calving rates for *Brucella* positive, *Brucella* negative, *C. fetus* positive and *C. fetus* negative herds were 43.6%, 76.8%, 33.1% and 57.3% respectively. In addition, there was a strong negative association between within-herd *Brucella* seroprevalence and calving rate ($P < 0.001$) (Fig. 5.3).

Table 5.3. Bivariable analysis of categorical predictors for calving rate in herds in three states of northern Nigeria

Predictor and level	No tested	Calving rate (%)		*P*-value
		Mean	SD	
Brucella infection[a]				<0.001
No	59	76.8	9.2	
Yes	192	43.6	21.8	
Campylobacter fetus infection[a]				<0.001
No	166	57.3	22.2	
Yes	66	33.1	18.0	
State[a]				0.033
Adamawa	87	46.1	23.5	
Kaduna	98	55.2	22.8	
Kano	66	52.7	25.8	
Method of breeding[a]				0.026
AI and natural mating	44	52.5	24.0	
AI only	11	70.1	25.0	
Natural mating only	196	50.1	23.8	
Use of AI[a]				0.11
No	196	50.1	23.8	
Yes	55	56.0	25.0	
Management system[a]				<0.001
Zero-grazing	3	76.2	12.3	
Commercial	26	66.2	25.4	
Agro-pastoral	146	58.1	21.3	
Pastoral	76	32.6	17.3	
Supplementary feeding[a]				<0.001
None	25	21.9	7.6	
Fodder/bran	105	46.3	22.1	
Concentrate	121	62.0	21.5	
Mineral supplementation[a]				<0.001
No	69	32.2	17.6	
Yes	182	58.7	22.2	

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Fencing pasture				0.257
Enclosed	57	56.1	25.4	
Unenclosed	7	50.5	21.7	
Free range	187	50.0	23.8	
Pasture establishment[a]				0.122
No	187	50.0	23.8	
Yes	64	55.5	24.9	
Water source[a]				<0.001
Piped	69	63.2	21.2	
Natural flowing	112	46.6	22.5	
Natural static	70	47.6	25.8	
Housing[a]				<0.001
Open barbed wire	153	46.4	23.3	
Open half way and roofed	66	63.1	24.6	
Open solid enclosure	32	51.5	18.9	
Hygiene/floor type[a]				<0.001
Floored	63	63.1	23.0	
Unfloored/natural bear earth	188	47.5	23.3	
Isolation and observation of cow during parturition and removal of afterbirth[a]				<0.001
No	94	35.1	19.1	
Yes	154	61.4	21.4	
Routine prophylactic measures[a]				<0.001
No	97	40.0	22.1	
Yes	154	58.6	22.5	
Borrow/share bull[a]				<0.001
No	166	60.4	21.8	
Yes	85	33.9	18.2	
Presence of small ruminants[a]				<0.001
No	97	65.6	19.5	
Yes	154	42.5	22.5	
Presence of dogs[a]				0.036
No	227	52.9	24.5	
Yes	24	37.5	13.7	
Presence of horses				0.684
No	240	51.3	24.0	
Yes	11	54.3	27.2	

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Presence of camels				0.729
No	243	51.3	24.2	
Yes	8	54.3	24.3	
Presence of chickens[a]				0.0002
No	161	55.7	23.3	
Yes	90	43.8	23.9	
Multiple herds[a]				0.013
No	166	54.1	23.6	
Yes	85	46.1	25.3	
Purpose of keeping animals[a]				0.0002
Small scale local dairy	187	52.5	24.1	
Dairy and Beef	29	61.3	23.2	
Beef	35	37.5	18.9	
Initial purchase of stock from a market[a]				<0.001
Inherited	118	55.1	24.0	
Other farms	14	71.5	21.2	
Market	119	45.4	27.7	
Buying-in new animals and quarantine[a]				<0.001
Buy <3 + quarantine	30	68.4	11.8	
Buy >3 or no quarantine	147	38.3	20.2	
Close herd	74	70.6	15.9	
Socio-economic status of farmer[a]				0.031
Full-time	176	53.6	23.7	
Part-time	75	46.4	24.9	
Routine veterinary examination				0.351
No	163	50.4	24.0	
Yes	83	53.4	14.3	
Specialist attending to animals[a]				<0.001
No	48	32.6	17.9	
Yes	203	55.9	23.3	
Presence of crush or local chute at the farm[a]				0.061
No	187	49.8	23.9	
Yes	64	56.3	24.9	
Rainfall				0.409
<700 mm	40	47.2	26.6	
700-1000 mm	56	53.8	24.5	
>1000 mm	155	51.7	23.1	

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Herd size				0.834
<=15 cattle	114	51.8	23.9	
>15 cattle	137	51.1	24.3	

[a]Variable significant (P< 0.20) for calving rate and therefore considered in the multivariable model.

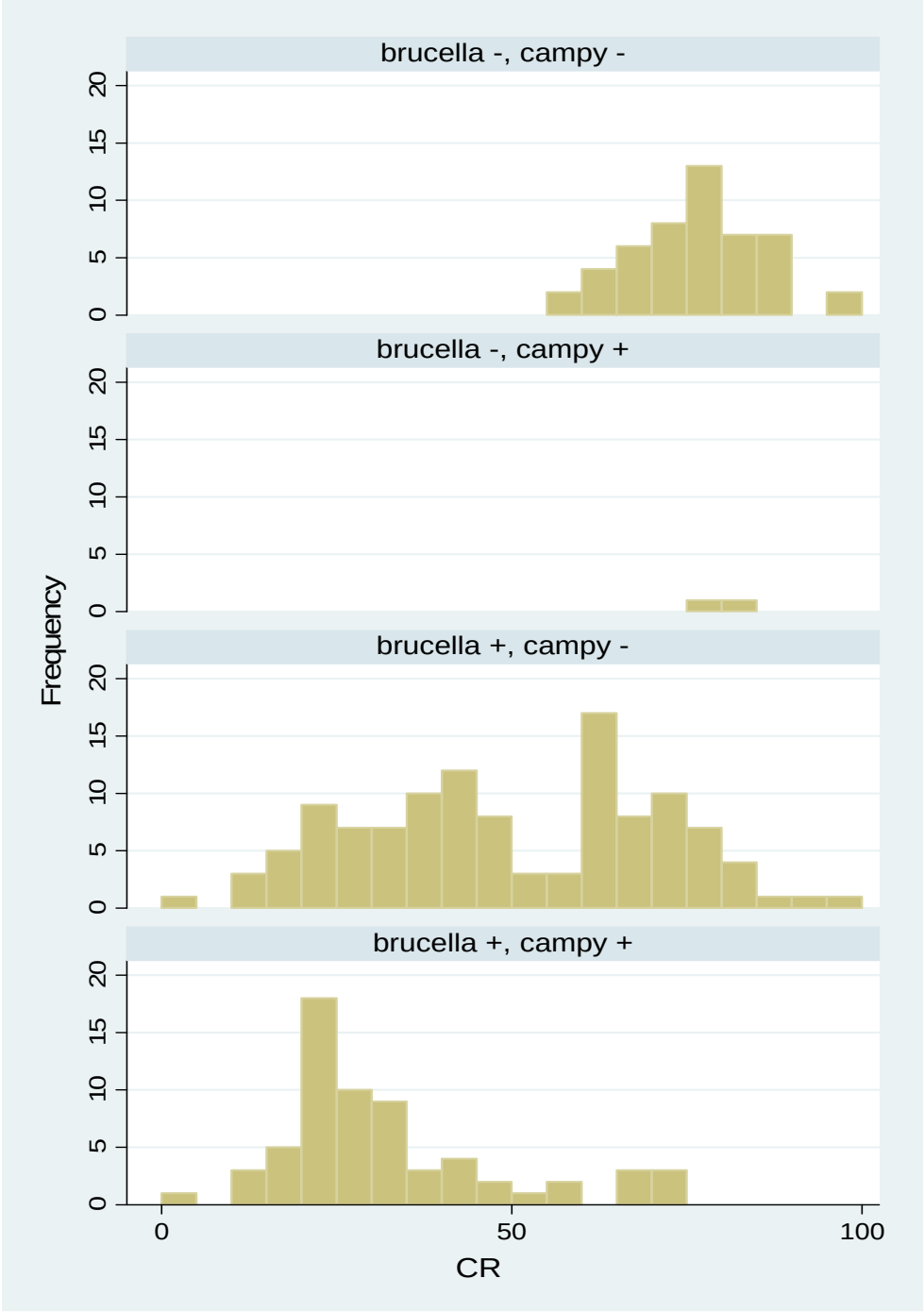

Figure 5.1. Calving rate in *Brucella abortus* positive and negative herds, and *Campylobacter fetus* positive and negative herds

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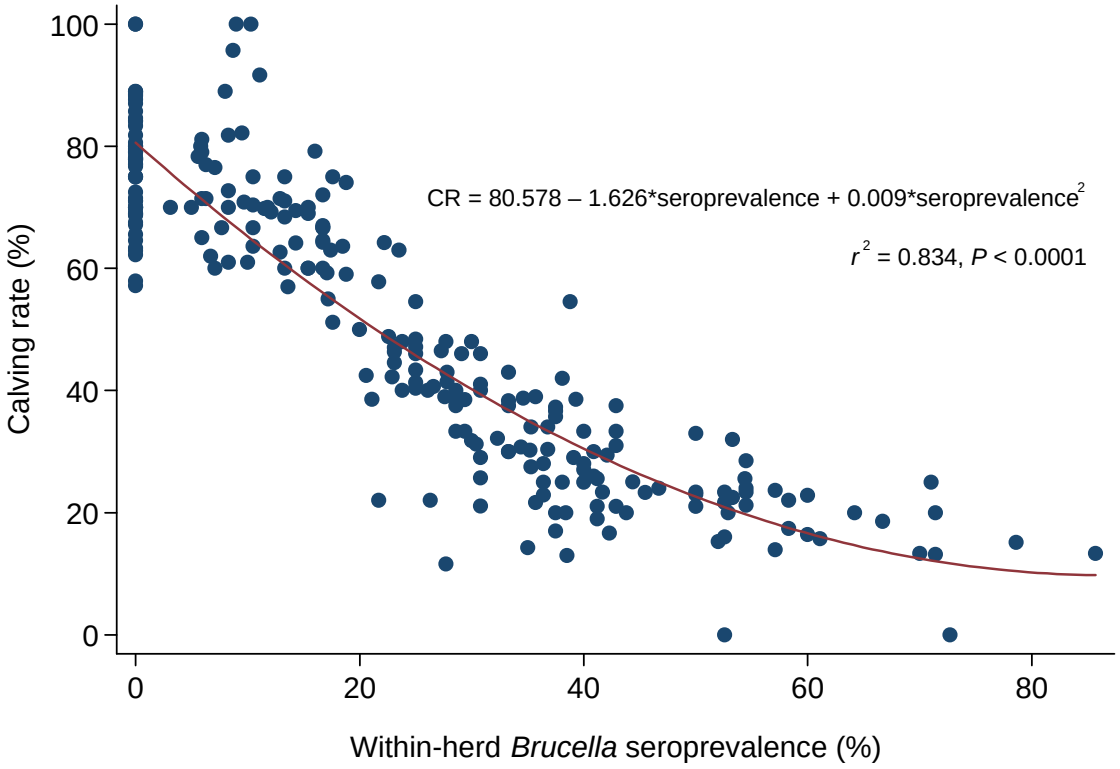

Figure 5.2. Scatter plot of calving rate (CR) vs. within-herd *Brucella* seroprevalence, with least squares quadratic fit.

The final multilevel regression model of factors associated with calving rate is shown in Table 5.4. After adjustment for confounding by the other variables in the model, *Brucella* herd infection was associated with an absolute reduction in calving rate of 14.9%. In addition to this, *C. fetus* herd infection was associated with a further reduction in calving rate of 8.4%.

Herds that received fodder and bran were associated with 6.5% higher calving rate (P = 0.044) and herds that received concentrate with 7.9% higher calving rate (P = 0.037). In addition, mineral supplementation and isolation and observation of cows during parturition and removal of afterbirth were associated with higher calving rate. Furthermore, the presence of small ruminants, the presence of a handling facility and the introduction of new animals, particularly the introduction of >3 animals without quarantine were significantly associated with lower calving rates in such herds (Table 5.4). Herd size was initially not significant in the bivariable analysis but after adding to the final model and adjusting for other variables the association with calving rate was significant.

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Table 5.4. Factors associated with calving rate in cattle herds in northern Nigeria: Results of a multiple linear regression model

Risk factor and level	Coefficient	95% CI	*P*- value
Brucella infection			
No	1	-	-
Yes	-14.9	-20.01, -9.62	<0.001
Campylobacter fetus infection			
No			
Yes	-8.41	-12.93, -3.88	<0.001
State			
Adamawa	1	-	-
Kaduna	1.76	-2.84, 6.35	0.452
Kano	-0.24	-5.40, 4.92	0.928
Supplementary feeding			
None	1	-	-
Fodder and bran	6.54	0.46, 12.63	0.044
Concentrate	7.86	0.46, 15.30	0.037
Mineral supplementation			
No	1		
Yes	6.45	1.71, 11.20	0.008
Isolation and observation of cow during parturition and removal of afterbirth			
No	1		
Yes	7.54	3.09, 11.98	0.001
Small ruminants			
No	1	-	-
Yes	-7.81	-12.41, -3.22	0.001
Buy-in new animals			
Close herd	1	-	-
Buy <3 + quarantine	-6.44	-12.53, -0.38	0.038
Buy >3 or no quarantine	-15.23	-20.31, -10.16	<0.001
Presence of crush or local chute at the farm			
No	1	-	-
Yes	-9.97	-16.08, -3.76	0.002
Herd size			
< or = 15	1	-	
> 15	4.98	1.17, 8.80	0.011

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Random effects: Variance (SE)	
LGA	0.408 (5.055)
Ward	7.82×10^{-9} (3.51×10^{-8})
Likelihood ratio test vs. standard linear regression model: $P = 0.9966$	

Reproductive disorders and hygroma

Two classes of reproductive disorders were determined in this study. The first consisted of reproductive disorders observed through trans-rectal examination, with a prevalence of 1.6%. A total of 69 animals were diagnosed with anatomical malformations of the genitalia and foetuses as shown in Table 5.5. The second was the occurrence of reproductive disorders at the herd level from reports or records of owners. Prevalence of the latter ranged from repeat breeder with the highest prevalence of 53.1%, to orchitis/epididymitis with a prevalence of 12.1% (Table 5.5). Hygroma occurred in 23.2% of herds. Abortion, retained afterbirth, stillbirth, repeat breeder, orchitis/epididymitis and hygroma were significantly positively associated with the occurrence of both brucellosis and campylobacteriosis ($P < 0.01$). The birth of weak calves or calf mortality, and pyometra were significantly associated with brucellosis only ($P < 0.01$).

Discussion

Reproductive indices are vital in the determination and management of herd fertility. It is obvious from this study that several factors are responsible for poor reproductive efficiency of cattle. Previous extensive studies on the reproductive performance of cattle in traditional herds in northern Nigeria are more than two decades old (Voh Jr and Otchere, 1989) and there is a lack of data quantifying the impact of infectious causes of infertility (Ocholi *et al.*, 2004a; Mshelia *et al.*, 2010a). This report provides current information on reproductive performance and factors affecting calving rates in cattle in Nigeria. It is the only report that considers various management systems in one study.

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Table 5.5. Herd-level prevalence of reproductive disorders and hygroma; and occurrence of genital abnormalities detected during gynaecological examinations

Disorder reported by farmers	n	No. pos	Prev. %
Abortion	257	123	47.9[a,b]
Retained afterbirth	257	108	42.0[a,b]
Stillbirth	257	70	27.2[a,b]
Repeat breeder	258	137	53.1[a,b]
Pyometra	257	45	17.5[a]
Weak calf/calf mortality	257	92	35.8[a]
Irregular oestrus	258	97	37.6[a,b]
Dystocia	257	54	21.0
Mastitis	257	91	35.4
Uterine prolapse	257	38	14.8
Ochitis/Epididymitis	250	31	12.4[a,b]
Hygroma	271	63	23.2[a,b]
Occurrence of genital abnormalities during gynaecological examinations			
Agenesis of ovaries and atrophy of tubular genitalia		16	
Uterine unicornis		7	
Ovarian cyst		24	
Hypoplasia of ovaries and tubular genitalia		4	
Freemartin		11	
Foetal maceration		2	
Foetal mummification		3	
Double cervix		1	
Total abnormalities		69	
Total females (n)		4,205	
Prev. %		1.6	

[a]Variable significantly associated with brucellosis ($P< 0.01$)
[b]Variable significantly associated with campylobacteriosis ($P< 0.01$)
n – Herd sample size (denominators)

The average herd size of 37.0 in agro-pastoral production systems obtained in this study (data not shown) is similar to 38.3 reported by Voh Jr and Otchere (1989) in agro-pastoral herds; but the herd size of 34.1 in pastoral herds (data not shown) is lower than 45.9 reported by Otchere (1986) in the same management system.

From the global perspective, the previous two decades have witnessed a steady rise in the incidence of bovine infertility (Murray, 1959; Lopez-Gatius, 2003). The overall calving rate obtained in this study is similar to the 52-55% calving rate reported in Colombia (Stonaker *et*

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al., 1976) and the 55% observed by Voh Jr and Otchere (1989) in traditional agro-pastoral system in Nigeria. The pregnancy rate in this study is lower than 42% reported by Voh Jr and Otchere (1989). However, our study does not provide conclusive evidence to support a decline in fertility of the study population based on reports from northern Nigeria. It is also evident from the age and parity data (Table 5.2) that strict culling based on poor reproductive performance is not practiced.

It is apparent from this study that brucellosis and campylobacteriosis have a significant impact on calving rate, and that there is a clear negative relationship between within-herd *Brucella* seroprevalence and calving rate. The outcome of brucellosis such as abortion, retained afterbirth, stillbirth and birth of weak calves or calf mortality affect the overall calving rate of infected herds. This tends to agree with reports by Aguair *et al*. (2007) and Degefa *et al.* (2011). It is also consistent with the report that a 10% decrease in the number of calves was observed in *Brucella* positive cows (McDermott *et al.*, 1987; Megersa *et al*., 2011a). Campylobacteriosis causes similar clinical signs and therefore may be associated with infertility thereby lowering calving rate and other reproductive indices (McCool *et al*., 1988; Campero *et al*., 2005; Jimenez *et al*., 2011). Due to the fact that almost all *C. fetus* positive herds were also positive for *Brucella*, it was not possible to accurately quantify the impact of campylobacteriosis alone. However, a combination of brucellosis and campylobacteriosis was associated with poorer calving rate in this study than brucellosis alone (Fig. 5.1), which would suggest that campylobacteriosis has an additional negative effect. Despite this, our data confirms that it is possible to maintain good calving rate with only brucellosis or campylobacteriosis infections, and even with a combination, a calving rate in excess of 70% is possible, provided that the within-herd seroprevalence of brucellosis is below about 20% (Fig. 5.2). The fact that females often abort once and following that they reproduce normally in the case of brucellosis, and the acquired immunity conferred by *C. fetus* challenge, may explain the acceptable calving rates observed in some infected herds.

Although management system was not significant in the multivariable analysis for calving rate, the multivariable model shows that the observed difference in calving rate between the management systems was partially accounted for by the other variables in the multivariable model. In the bivariable analysis, the calving rate differed significantly between the various management systems ($P< 0.001$). The crude calving rate being lowest in the pastoral system may be as a result of the movement of the pastoral Fulani herdsmen and interaction of their

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cattle with other Fulani herdsmen particularly at watering points during the dry season which may expose them to infection thereby lowering the calving rate. It was recently observed that the presence of brucellosis was positively associated with pastoral management (Mai *et al*., 2012).

It was observed from this study that providing supplementary feeding and mineral supplementation were associated with higher calving rate, as were the isolation and observation of cows during parturition and removal of the afterbirth, and the presence of a handling facility associated with lower calving rates. Such effects may be by proxy, in that the education level of the herd owner, availability of other sources of income, focus on other activities may all have impact on the general level of management, condition and health of the herd. Likewise, larger herd size is likely to be associated with increased animal movements, with the associated increased risk of contact with infectious agents. Indeed, farmers that introduce >3 animals without quarantine were found to have 15% lower calving rate than farmers that introduce <3 animals plus quarantine. In the current study, the association with herd size was obscured due to confounding, in multivariable analysis calving rate was significantly associated with herd size with larger herds having higher calving rate. The reason for this is not clear. The commercial and zero-grazing herds showed higher calving rates but have smaller herd sizes. It is also likely that confounding by other variables in the multiple linear regression model accounted for this variation.

It was observed that over 61% of the multiple herd owners engaged in introducing >3 animals without quarantine in their herds. This is a risky practice due to the potential for introducing infections that may lower the calving rate. Reports indicate that ownership of multiple herds potentially increases the risk of a herd being infected with brucellosis (Richey and Harrell, 1997), which may also affect the calving rate.

Herds that had small ruminants had significantly lower calving rates. The reason for this association is not known. Cross infection of infectious reproductive diseases may be possible between species thereby lowering the calving rate. This tends to agree with findings by Megersa *et al*. (2011a).

Herds with handling facility had significantly lower calving rate due probably to sharing with other herds thereby increasing risk of disease transmission. In commercial herds presence of a

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crush would be expected to improve general management therefore reducing infection and improving calving rate.

The estimated age at puberty observed in this study is lower than those of Mukasa-Mugerwa (1989) who showed average age at puberty of *Bos indicus* as 40 months. Furthermore, the estimated age at first calving is also lower than 4 to 5 years reported by Voh Jr and Otchere (1989) and 5 years by Zemjanis (1974). In addition, the age at first calving in indigenous tropical cattle of between 3 and 5 years, between 4 and 7 years for the second time and between 5 and 8 years for the third (Wilson, 1985) tend to agree with our findings (Table 5.2). Furthermore, the average age at first calving in Nigerian Zebu cattle of about 5 years under traditional agro-pastoral system reported by Pullan (1979) and Otchere (1986) is consistent with our study in which the highest number of animals showed age at first calving of five years (Table 5.2). This study also revealed that age at first calving in cattle in northern Nigeria can also be as low as 2 to 3 years, meaning the animals attained puberty and conceived at about 1 to 2 years old. Oyedipe *et al*. (1982b) indicated that under improved management where seasonal nutritional stress is reduced, it is possible to achieve average age at first calving a little over 3 years.

The important age of reproduction of up to 10 years (Voh Jr and Otchere, 1989) is consistent with our findings. Almost all of the cows had been culled by the age of 10 years. The low number of calves per cow may be attributed to late age at first calving, long calving intervals and early culling age. Suckling and nutrition are in a large part responsible for this reproductive inefficiency (Voh Jr and Otchere, 1989). The lifetime production was projected from the estimated age and parity of dams derived from Table 5.2.

Zemjanis (1974) stated that a within-herd individual abortion rate of less than 5% is within normal limits, such abortions being sporadic and usually non-infectious. The abortion rate associated with infection by *C. fetus* reported by Jimenez *et al*. (2011) in Argentina is in agreement with our study but much higher than reports by Dufty and Vaughan (1993). Studies elsewhere incriminated brucellosis in causing abortion, retained afterbirths, stillbirths, birth of weak calves and repeat breeders (Aguair *et al*., 2007; Degefa *et al*., 2011 Megersa *et al* 2011a) which is in agreement with our findings. The variation in the prevalence of abortion and retained afterbirth in the various studies may be as a result of difference in production system, husbandry practices, cattle breed, environmental factors and infection.

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Brucella seropositive cows have a higher abortion rate, with the risk increasing with parity, and longer calving intervals than seronegative ones (Mekonnen *et al*., 2011), suggesting active *Brucella* spp. infection (Matope *et al.*, 2011). Since most infected cows usually abort only once (OIE 2011a), this may have been forgotten and could distort the association between history of abortion and seropositivity (Matope *et al*., 2011). This also encourages farmers to keep such infected animals in their herds since they will continue calving, thereby serving as foci of infection in disseminating brucellosis. The positive association between herds with abortion and those seropositive to brucellosis in this study implies that brucellosis is a significant cause of the abortions observed in Nigerian herds. This confirms other findings in Nigeria (Ocholi *et al*., 2004a), Ethiopia (Ibrahim *et al*., 2010; Degefa *et al*., 2011; Megersa *et al*., 2011a) and Zambia (Matope *et al.*, 2011).

The finding of stillbirth or weak calves or calf mortality associated with brucellosis in this study was in line with Megid *et al*. (2010) who stated that late abortion and premature or full-term birth of dead or weak calves predominated in pregnant animals with brucellosis. However, there was no association in this study between calf mortality or birth of weak calves and campylobacteriosis. The prevalence of dystocia reported by Akpa *et al.* (2011) in Nigeria and Chatikobo *et al*. (2009) in Rwanda are higher than our findings. The high incidence of dystocia and other related conditions may be as a result of poor feeding, twinning and management as reported by Arthur *et al*. (1996).

Although orchitis/epididymitis and hygroma were observed to be associated with campylobacteriosis in this study, they are not clinical features of campylobacteriosis. It is likely that the signs are as a result of other conditions such as brucellosis.

The isolated cases of double cervix, foetal mummification and maceration are consistent with the general pattern of these diseases (Ajala *et al*., 2000; Lopez-Gatius *et al*., 2002). A prevalence of 1.6% reproductive disorders diagnosed by trans-rectal palpation agrees with Abdullahi and Chaudhari (1996). The prevalence is lower than reports from slaughter surveys of 13.6% gross genital abnormalities in Nigeria (Chaudhari and Paul-Bokko, 2000), 21% in Egypt (Wahid *et al.*, 1991) and 38.6% in Ethiopia (Albati *et al*., 2006). This is to be expected because slaughtered cows are not representative of the entire population. Sample size, breed, management, diagnostic technique and criteria, and location may be additional reasons for the variations in prevalence. Most of the reproductive disorders observed in this study were also reported in abattoir studies in Nigeria (Kumi-Diaka *et al*., 1980b) and elsewhere (Abalti *et*

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al., 2006). Trans-rectal palpation is a real-time herd management tool enabling managemental decisions based on early diagnosis of conditions and reproductive status. Abattoir surveys are suitable for surveys but are not suitable for early diagnostic purposes.

Body condition score is a management tool that has proved useful in the assessment of the nutritional status of dairy and beef cows (Hady *et al*., 1994, Montiel and Ahuja, 2005). Poor BCS of cows, mainly caused by poor management, was also considered to play a major role in reducing pregnancy rates (Lopez-Gatius *et al*., 2002). Their results further suggest that an abrupt loss of nutritional status postpartum can impair uterine involution, and cause pregnancy failure in the early foetal development period when the placentomes develop. In addition, a one unit reduction in BCS from previous partum to 30 days postpartum resulted in a 2.4-fold increase in pregnancy loss. Highly significant associations between BCS and pregnancy status ($P < 0.0001$) and BCS and cyclicity status ($P < 0.0001$) were observed in this study.

Most of the animals in this study and other studies considered were anestrous which corroborates with the findings that anestrous is a major problem in the tropics and subtropics due to inadequate nutrition, high ambient temperature and high parasite burdens and disease (Mukasa-Mugerwa, 1989). Furthermore, it was reported that declining reproductive efficiency was the main disorder due to inactive ovaries related to seasonal anoestrus, resulting in profound economic losses, since it increases the length of the calving interval (Voh Jr and Otchere, 1989). In a study of genital tract abnormalities, Chaudhari and Paul-Bokko (2000) in Nigeria reported that 55.5% of cows were cyclic while 44.5% were acyclic while Albati *et al*. (2006) in Ethiopia reported 46.0% and 53.6% cycling and non-cycling cows respectively. In our study, 54.4% of the cows were cyclic as against 45.6% acyclic. This is in agreement with reports by Voh Jr and Otchere (1989) in traditional agro-pastoral management systems in northern Nigeria. This highlights the inhibitory effect of suckling and negative energy balance on resumption of ovarian activity postpartum, as noted by Eduvie and Dawuda (1986), Dawuda *et al*. (1989) and Reksen *et al*. (2002).

Conclusion

In conclusion, the reproductive performance of the cattle herds in this study was generally poor. *Brucella abortus* and *C. fetus* infections reduced the calving rate. In addition, presence of small ruminants, the introduction of animals without quarantine and presence of handling

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facility were also associated with lower calving rates. Supplementary feeding, mineral supplementation, isolation and observation of the cow during parturition and removal of the afterbirth and larger herd size were associated with improved annual calving rates. Suckling and nutrition also contributed to the high prevalence of anoestrus. Herd health management programmes to control infectious reproductive diseases as well as addressing the effect of suckling and malnutrition are advocated. In particular, supplementary feeding, isolation and observation of cows during parturition and removal of afterbirth should be encouraged while introduction of animals without quarantine, sharing handling facilities and mixing herds with small ruminants should be avoided.

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Chapter 6

General Discussion

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Infectious reproductive diseases have a serious impact on the productivity of cattle worldwide and are a major cause of reproductive failure in cattle in Nigeria (Ocholi *et al*., 2004a; Mshelia *et al*., 2010a). This study focused on the potentially important infectious reproductive diseases namely brucellosis, bovine genital campylobacteriosis and trichomonosis, in three cattle-producing states in Nigeria. The animal- and herd-level prevalences of brucellosis and bovine genital campylobacteriosis, concurrence of infections, the risk factors associated with the diseases and with the within-herd seroprevalence of brucellosis, and reproductive disorders and factors affecting calving rates were investigated.

This study was conducted in Adamawa, Kaduna and Kano states in northern Nigeria which is the cattle belt with over 90% of the cattle population of the country. A total of 4,807 cattle consisting of 4,205 females and 602 males, in 271 herds, were studied. Both RBPT and c-ELISA were done in series on serum samples for the diagnosis of brucellosis, and isolation and identification from preputial scrapings for the diagnosis of campylobacteriosis and trichomonosis. The RBPT was used as a screening test for brucellosis because it is a simple, rapid test with high sensitivity, therefore few false negative results due to chronic cases will be obtained (OIE, 2011a). The c-ELISA was used as a confirmatory test because of its high sensitivity and specificity, and lack of the prozone phenomenon (OIE, 2011a). The c-ELISA has not previously been used on large samples from different production systems in Nigeria. Culture and identification were the gold standard tests used in this study for the diagnosis of both campylobacteriosis and trichomonosis (Irons *et al*., 2004a,b; OIE, 2011b).

Of the diseases, brucellosis showed the highest animal- and herd-level prevalence estimates of 26.3% (95% CI, 22.1%-31.0%) and 77.5% respectively, while for bovine genital campylobacteriosis the estimates were 16.4% and 25.5% respectively. A variety of prevalences of bovine brucellosis have been reported in Nigeria. However, many studies have not distinguished between animal-level, herd-level or within-herd prevalence, resulting in confusion and difficulty in comparing results between studies. This study addressed all the three types of prevalences and attempted to relate the findings to other studies reporting similar types of prevalences. No studies in Nigeria considered within-herd prevalence of brucellosis in various herds with different management systems as well as within-herd prevalence of brucellosis and calving rate. The situation is better with bovine genital campylobacteriosis; the few studies that were conducted in Nigeria distinguished between animal- and herd- level prevalences.

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To compound the lack of clarity in the literature on different prevalence estimates, most previous studies used samples not representative of the target population or did not use appropriate adjustments for sampling weights and clustering in calculation of prevalence estimates and standard errors. This study used a formal, multistage, probability sampling strategy and prevalence estimates and their standard errors were adjusted for the survey design. This resulted in reliable estimates which can be used as a basis for assessing future changes.

The animal- and herd- level prevalences of brucellosis obtained in this study were higher than prevalences previously reported in other parts of northern Nigeria. This may suggest an increase in the prevalence of brucellosis, likely due to several managemental and environmental factors. The pastoral management system showed the highest prevalence of brucellosis and campylobacteriosis which corroborates with the findings of Nuru and Dennis (1975) and Eze (1978) in traditional nomadic Fulani herds in Nigeria, and Cobo *et al*. (2004) and Jimenez *et al*. (2011) in extensive farms elsewhere. Of the three states sampled, Adamawa state showed the highest animal- and herd-level prevalences. It is the only state that shares an international border, and it is likely that there is cross infection of the disease across the border as reported by Esuruoso (1974) and Bale and Kumi-Diaka (1981). Similarly, a higher prevalence of bovine genital campylobacteriosis than previously reported was obtained in this study.

The pastoral management system and the attitude of the pastoralists towards brucellosis increased the risk of transmission of the disease among cattle herds. The pastoralists in this study have been involved in borrowing and or sharing of bulls and indiscriminate buying-in of cattle into the herds without quarantine (Bale and Kumi-Diaka, 1981). These factors further contributed to the spread of brucellosis and campylobacteriosis since both diseases can be transmitted venereally (Pefanis *et al*., 1988; Bercovich, 1998). The management of parturition and afterbirth, 'firing' of hygromas, and retention of cows with chronic brucellosis because of their productivity, and of infected bulls because of their normal libido and breeding capacity by the pastoralists likely has also enhanced the transmission of brucellosis.

Bovine trichomonosis is likely to be absent in northern Nigeria, at least in the states included in this study. This study did not detect the organism from any of the bulls sampled despite using appropriate diagnostic techniques. Even after adjusting for imperfect test sensitivity, the calculated true prevalence of trichomonosis in the study area was below 0.65%, meaning

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that the disease is likely to be absent. In previous studies the reported prevalence was also 0% in northern Nigeria (Adeyeye *et al*., 2011) and some other parts of Africa (Klastrup and Halliwell, 1977; Swai *et al*., 2005). However, bovine trichomonosis has been diagnosed in southern Nigeria, albeit more than 20 years ago (Akinboade, 1980; Ayoade *et al.*, 1991). It is possible that the differences in intrinsic and environmental factors and management systems between the northern and southern parts of Nigeria are responsible for this variation in occurrence.

Although there have been several studies on prevalence and some on risk factors of brucellosis in Nigeria, none to the best of our knowledge have used appropriate epidemiological methods to control for confounding. This study determined both animal-level and herd-level risk factors associated with both brucellosis and campylobacteriosis, using multilevel, multivariable models to control for both confounding and hierarchical data structure. Cattle herds in contact with small ruminants, and herds that bought in more than three cattle or practiced no quarantine, had the highest risk of being *Brucella* seropositive and *C. fetus* positive. Similar findings have been reported (Kimberling, 1988; Megersa *et al*., 2011a). In addition, Mukasa-Mugerwa (1989) and Crawford *et al*. (1990) reported that the number of new animals introduced in a herd increases the risk of introducing infected animals for both campylobacteriosis and brucellosis. However, one study indicated that buying bulls was associated with a 35% decrease in the risk of campylobacteriosis infection in Argentina, which was likely due to a decrease in disease transmission associated with the introduction of virgin or uninfected bulls (Jimenez *et al*., 2011). In the Nigerian situation, the animals from the open market are more likely to be infected since they are from different sources, some from poor management systems.

A zero-inflated Poisson model was used to identify factors associated with the within-herd seroprevalence of brucellosis in this study. The value of using within-herd seroprevalence as an outcome, rather than only herd-level brucellosis status, became clear in the final study, when a very clear negative association was found between seroprevalence and calving rate within herds. The presence of small ruminants and buying >3 animals or no quarantine were again both associated with the within-herd seroprevalence of brucellosis. Borrowing or sharing of bulls and mineral supplementation were associated with higher and lower within-herd counts of brucella positive cattle, respectively. This is consistent with venereal

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transmission and spread of brucellosis, while the effect of mineral supplementation is consistent with a previous study (Grunert, 1984).

The outcomes investigated in the initial chapters of the thesis, namely herd-level brucellosis and campylobacteriosis, as well as within-herd *Brucella* seroprevalence, affected the overall calving rate and other reproductive parameters of cattle herds. This is consistent with previous reports (McDermott *et al.*, 1987; Jimenez *et al*., 2011). However, our study further demonstrated a clear negative association between within-herd *Brucella* seroprevalence and calving rate. Furthermore, the positive association between abortion and *Brucella* seropositivity in this study suggests that brucellosis is an important cause of abortion in Nigerian cattle herds. The same observation was made regarding stillbirth, birth of weak calves or calf mortality in this study which is consistent with previous reports (e.g. Megid *et al.*, 2010). The combination of brucellosis and campylobacteriosis resulted in poorer calving rates than a single infection. It has been reported that *C. f. venerealis* is more pathogenic than *C. f. fetus* (Zhoa *et al*., 2010). This study also demonstrated *C. f. venerealis* as the commoner of the two subspecies.

The findings of this study may therefore largely explain the poor reproductive performance of cattle in northern Nigeria. Hitherto, non-infectious causes have largely been implicated in infertility, but it is now obvious that infectious diseases particularly bovine brucellosis and bovine genital campylobacteriosis are also strongly associated with reproductive inefficiency of cattle. It is quite worrisome that despite the high prevalence of brucellosis in Nigeria and its zoonotic implications, there is currently no control programme in place.

To the best of our knowledge, this is the largest survey on the prevalence of brucellosis conducted in Nigeria. The study included data even from the conservative, nomadic, Fulani herdsmen who usually resist attempts to study their animals. Such extensive data on Fulani pastoralists are usually not available. The data also considered extensive risk factors associated with brucellosis and campylobacteriosis using structured epidemiological techniques that controlled for confounding. This approach has not previously been used in the study of brucellosis or bovine genital campylobacteriosis in Nigeria. In addition, this study also fills the gap regarding the scanty literature on bovine genital campylobacteriosis. Except for a study by Mshelia *et al.* (2010b) and very recently Mshelia *et al.* (2012), the last study on the disease was over two decades ago (Bawa *et al.,* 1991). Our finding of *C. f. venerealis* biovar intermedius is also the first report in Nigeria. Furthermore, hygromas were observed in

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unconventional sites such as the cervical region, thoracic spine and the withers in Nigerain cattle. The study also observed for the first time the concurrence of bovine genital campylobacteriosis and brucellosis in Nigerian cattle herds.

This study covered only three states out of the nineteen states of northern Nigeria due to logistical reasons. However, since environmental and management factors are similar in many of the other states, there is good reason to believe that the findings may be applicable to a large part of northern Nigeria. There was difficulty in convincing some of the farmers to consent to the programme, nevertheless it was largely possible to apply the planned random selection of farms in order to ensure collection of a representative sample. A limitation of the study was the failure, due to logistical and regulatory constraints, to export samples from Nigeria for further studies, hence the inability to isolate and characterize the *B. abortus* isolates or to further characterize the *C. fetus* isolates.

The vast majority of the *Campylobacter* isolates in this study were *C. f. venerealis* biovar intermedius, consistent with the findings of Schmidt *et al.* (2010) in South Africa. Studies in future could include molecular characterization of the respective Nigerian isolates of *C. fetus* to confirm and distinguish between *C. f. fetus*, *C. f. venerealis* and *C. f. venerealis* biovar intermedius using AFLP analysis as described by Wagenaar *et al.* (2001), although Schmidt *et al.* (2010) concluded that bacterial culture is superior to PCR in identifying *C. fetus* subspecies. Further studies to evaluate the virulence properties of Nigerian isolates of *C. fetus* by characterization of S-layer protein genes as described by Nitta *et al. (*1997) are suggested.

A limitation of using serology alone in studying the epidemiology of brucellosis is that it cannot identify the *Brucella* spp. that induced antibodies in the host (Godfroid *et al*., 2012). Since our study identified the presence of small ruminants in cattle herds as a risk factor associated with brucellosis, other *Brucella* spp. besides *B. abortus* may have been present. Further studies to characterize Nigerian *B. abortus* isolates by full multiple locus variable number tandem repeats analysis (MLVA) and RFLP for both genotyping and subspecies identification as described by Alton *et al.* (1988) and Bricker (2002) may therefore be indicated.

Further surveys of brucellosis in other states or locations in Nigeria are advocated. It is important, however, that such studies are well designed in order to obtain representative samples from the respective target populations, and that appropriate statistical procedures are

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used to account for the study design and to adjust for confounding. The aspect of breed susceptibility may merit further investigation, to identify breeds that may be relatively resistant to brucellosis or campylobacteriosis. Exploring more Fulani herds and identification of more factors associated with both disease conditions, and with reproductive performance are indicated.

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Chapter 7

Conclusions and Recommendations

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This is the first large study that clearly demonstrates a strong association between infection status for brucellosis and campylobacteriosis with poor reproductive performance in Nigerian cattle herds. It also identified managemental, environmental and other risk factors for the diseases, as well as risk factors for poor reproductive performance in the form of low calving rates. This study revealed that both bovine brucellosis and bovine genital campylobacteriosis are endemic in northern Nigeria, particularly in the pastoral Fulani herds. Of the three states sampled, Adamawa state showed the highest prevalence of both disease conditions. In addition, male animals, non-pregnant females, older cattle and herds under a pastoral management system also showed significantly higher brucellosis seroprevalence. Regarding genital campylobacteriosis, older bulls, the Gudali breed and herds in the pastoral management system showed higher prevalence. Prevalence was lowest in Bunaji cattle and in agro-pastoral management systems. There was a very high prevalence of *C. fetus venerealis* biovar intermedius amongst the *C. fetus* isolates, accounting for over 90% of isolates. *Campylobacter f. venerealis* biovar intermedius is therefore the major aetiology of bovine genital campylobacteriosis in Nigeria, contributing to the poor reproductive performance of indigenous cattle. All the bulls tested for trichomonosis using different isolation methods were negative. Variables associated with the risk of brucellosis seropositivity and *C. fetus* positive herds included presence of small ruminants and buying-in of animals. Risk factors for brucellosis seropositivity were pastoral management system, larger herd size and presence of a handling facility, while risk factors for *C. fetus* positive herds were failure to apply herd prophylactic measures, initial acquisition of animals from markets and high rainfall.

The following recommendations are given in order to control or prevent infectious reproductive diseases as well as to improve the calving rate:

- Compulsory vaccination of calves and adults against brucellosis and campylobacteriosis, and provision of vaccination certificates
- Public enlightenment, particularly amongst the pastoral Fulanis, about the diseases and modern methods of management, the zoonotic nature of the diseases, implications of raw consumption of milk and contact with aborted material. This can be done along their migratory routes through posters, video films and leaflets in local Fulani and Hausa languages.
- Protecting the abattoir workers, health inspectors, veterinarians, etc. during occupational activity by providing protective clothing

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- Proper cooking of meat and meat products of animal origin
- Avoid mixing cattle with other species of livestock such as small ruminants in one herd
- Take precautionary measures while conducting trans-rectal palpation and gynaecological/obstetrical examination to reduce the risk of transmitting infection between animals
- Farmers should avoid using same facilities, instruments and materials for different herds.
- Farmers should avoid borrowing and sharing of bulls
- Supplementary feeding, mineral supplementation, and care during parturition should also be encouraged.
- Farmers should practice routine herd health prophylactic measures in their herds such as vaccination, deworming, tick control and haemoparasite control

Although perhaps difficult or even impossible to implement, the following measures may also be considered:

- Government should as far as possible provide incentives such as free basic treatments and distribution of supplementary feeds at subsidized rates, practice a "test and slaughter" policy for positive reactors, including compensation for the farmers, and ensure strict control of movement of cattle across the local and international borders.
- Farmers should opt for agro-pastoral management system of rearing cattle as far as possible. Although this measure may be difficult particularly for the nomadic pastoralists, farmers should be encouraged to practice arable farming and conserve the crop residues as well as to buy supplementary feeds during harvest when they are very cheap. These can be supplemented during the critical period rather than migrating.
- Considering the prevention and management of infectious reproductive diseases in all herd health fertility programmes of cattle herds
- Culling of infected bulls and females
- Avoid indiscriminate buying-in of new animals from the market and if replacement is necessary, restrict to a few number and practice quarantine and screening for infectious reproductive diseases during the quarantine. Replacement and virgin breeding bulls must be quarantined and tested.

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- Farmers should ensure that their initial stock of cattle are acquired from better managed farms rather than from the open market.
- Take steps to overcome the stressful effect of suckling and malnutrition so as to improve the productivity of Nigerian cattle. This can be achieved by providing supplementary feeding, and grouping suckling animals and giving them proper feeding commensurate with their requirements.

Measures to further investigate brucellosis include further surveys in other locations/states using RBPT as a screening test, because it is simple, rapid and cheap. However, the seropositive animals should be confirmed using a more specific test. Campylobacteriosis should also be further surveyed in other locations in Nigeria using culture and identification. Further studies on molecular characterization of Nigerian isolates of *B. abortus* and *C. fetus* using molecular techniques such as PCR, AFLP and PFGE are suggested. In addition, an extensive survey of trichomonosis to cover more northern states is suggested in order to determine if the disease exists in the region.

Generally, the results of this study will contribute to the implementation of prevention and control measures aimed at reducing the prevalences of the two disease conditions and improve the productivity and reproductive performance of cattle in Nigeria.

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Appendix I. Questionnaire

SECTION I

GENERAL FARMER/HERD INFORMATION

1. PROFILE OF OWNER

Name of Farmer/Farm_____

Address/Location/State_____

Longitudes and Latitudes_____Land area of farm_____

Date_____Sex_____Rainfall_____

Purpose of keeping Animals: Small scale local dairy; Dairy and beef; Beef; Others (specify) (Tick appropriate)

Main occupation: Civil servant/Businessman/Politician/Fulltime farmer (Tick appropriate)

2. HERD STRUCTURE

a. Cattle

BREED	Bunaji	Sokoto Gudali	Adamawa Gudali	Rahaji	Wadara	Local cross	Exotic cross	Pure exotic	Others	**Total**
MALES										
< 6 months										
6-12 months										
1-2 years										
2-3 years										
3-4years										
>4 or mature										
FEMALES										
< 6 months										
6-12 months										
1-2 years										
2-3 years										
3-4 years										
>4 years or mature										
Open and dry cows										
Open & nursing <6 months										

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Open & nursing >6 months										
Pregnant <2 months & dry										
Pregnant <2 months & nursing <6 months										
Pregnant <2 months & nursing >6 months										
Pregnant >2 months & dry										
Pregnant >2 months & nursing <6 months										
Pregnant >2 months & nursing >6 months										
Total										

b. Other species kept at the farm

Species	**Total**
Sheep	
Goat	
Horses	
Camels	
Pigs	
Donkeys	
Dogs	
Poultry	
Others (Please specify)	

c. Ownership of multiple herds Yes_____ No_____ If yes, No. of herds_____

3. MORTALITY PATTERN AND CULLING

Number of calves aged 0-12 months that were born weak or died shortly in the last 12-24 months

	Total no. of viable calves born normal	Total no. of calves born weak or died shortly	Suspected cause of death

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Number of young (1-2 years) that died within the last 12-24 months

	Total no. of young animals	No. of young died	Suspected cause of death

Number of adults that died within the last 12-24 months

	Total no. of adult animals	No. of adults Died	Suspected cause of death

Culling within the last 12-24 months

	Males	Females	Total	Reasons for culling

4. REPLACEMENT STOCK OVER THE PERIOD 12-24 MONTHS

	No. of animals				Reasons for replacement
Replacement stock	Males	Females	None	Total	

a) Source(s) of replacement stock
i) Market
ii) Other Farms
iii) Imported
iv) Others (specify)

5. QUARANTINE

	No. of animals				Activity or herd health during quarantine
Quarantine	Males	Females	None	Total	

6. INITIAL METHOD OF ANIMAL ACQUISITION

i. Inherited_____
ii. Other farms_____
iii. Market_____
iv. Others (specify)_____

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7. MANAGEMENT

A. Type of management system
i. Commercial (Intensive)_____
ii. Agro-pastoral_____
iii. Pastoral_____
iv. Zero-grazing (Intensive)_____

B. Housing:
i. Open barbed wire
ii. Open half way and roofed
iii. Open solid enclosure

C. Hygiene/floor type:
i. Floored
ii. Unfloored
iii. Muddy

D. Feeding and water source:
1. Supplementary feeding
i. Conserved forage e.g silage
ii. Hay
iii. Crop residue
iv. Bran
v. Concentrate
vi. None

2. Mineral supplementation (potash, salt or mineral lick; replenish after exhaustion or available at time of visit)
i. Yes
ii. No

3. Pasture development
i. Yes
ii. No

4. Pasture environment
i. Fenced or paddocked pasture
ii. Pasture available but not fenced
iii. Free access to all kinds of natural pasture

5. Water source (Tick as applicable if more than one)
i. Well
ii. Tap
iii. Bore hole
iv. Earth dams
v. Stream
vi. River
vii. Ponds
viii. Others (specify)

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E. Medical

i. Herd prophylactic measures

Tick most appropriate	Regularly/Common	Occasionally/Uncommon	Not done
Deworming			
Ectoparasite control			
Heamoparasite control			
Screening for some diseases?			

ii. Gynaecological examination

Tick most appropriate	Regularly/Common	Occasionally/Uncommon	Not done
Trans-rectal palpation/Gynae./ Obst. exam.			

iii. Vaccination

Tick most appropriate	Regularly/Common	Occasionally/Uncommon	Not done
Contagious Bovine Pleuropneumonia (CBPP)			
Haemorrhagic septicaemia			
Anthrax			
Black quarter			
Brucellosis			
Foot and mouth disease			
Others (specify)			

iv. History of diseases

Tick most appropriate	Yes	No	
History of outbreak of diseases (Name)			If yes, what outbreak?_____
Hygroma			If yes, common site_____
Any form of traditional treatment?			If yes, for what is it used?_____

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F. Observation during parturition and removal of placenta?

i. Yes
ii. No

G. Specialist (veterinarian or livestock assistant) attending to animals?

i. Yes
ii. No

H. Provision of handling facilities in form of crush, locally made chute or others?

i. Yes
ii. No

8. BREEDING

A. Breeding methods:
i. Any AI?_____ Source of semen_____
ii. Any natural mating?_____
iii. Natural mating and AI_____
iv. Any natural mating with plough/feedlot or trade bull?_____

B. Do you share, loan or borrow bulls?

i. Yes If yes give reasons_____
ii. No

SECTION II

GENERAL REPRODUCTION

To be determined by the veterinarian executing the research or the veterinarian based at the farm by using records, interviews and trans-rectal palpation.

1. FERTILITY INDICES:

Parameter	Average value
Age at puberty	
Age at first calving	
Post partum anestrus	
Calving rate	
Pregnancy rate	
Number of calves/cow/life time	
Cows/bulls life span	
Cyclicity (%)	

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2. OBSERVED REPRODUCTIVE DISORDERS IN THE HERDS IN THE PREVIOUS 12-24 MONTHS:

A. Previous abortion
   i. Yes
   ii. No

B. Stillbirth
   i. Yes
   ii. No

C. Retained afterbirth
   i. Yes
   ii. No

D. Uterine prolapse
   i. Yes
   ii. No

E. Pyometra
   i. Yes
   ii. No

F. Repeat breeders
   i. Yes
   ii. No

G. Irregular oestrus
   i. Yes
   ii. No

H. Dystocia
   i. Yes
   ii. No

I. Mastitis
   i. Yes
   ii. No

J. Ochitis/Epididymitis
   i. Yes
   ii. No

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SECTION III

GENERAL COMMENTS

What in your opinion are the greatest impediments to dairy or beef cattle production in Nigeria?

1) In the past?_____

2) In the present?_____

Personal appraisal of the general conditions of the herd

Any significant observations on the environment and of factors that may affect the cattle_____

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Appendix II. Different management systems

Friesian/Jersey commercial farm in Adamawa state

Friesian commercial herd in Kaduna state

Agro-pastoral system with crosses in Kano state

Agro-pastoral system with crosses in Kano state

Zero-grazing Rahaji herd in Adamawa state

Zero-grazing Bunaji/Rahaji herd in Adamawa state

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Typical pastoral herd confined with wooden stakes and barbed wire

Watering point during dry season in Adamawa state

Congregation of herds at watering point during the dry season in Adamawa state

Pastoral Fulanis on the move with Sokoto Gudali herd and flock of sheep

Herd of Sahelian goats being grazed with cattle

Bunaji herd with flock of Yankasa sheep

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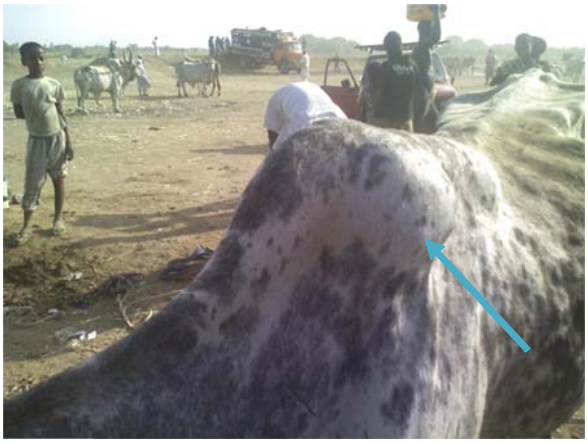
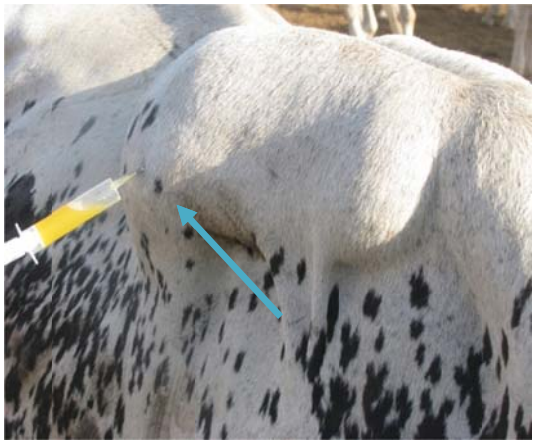
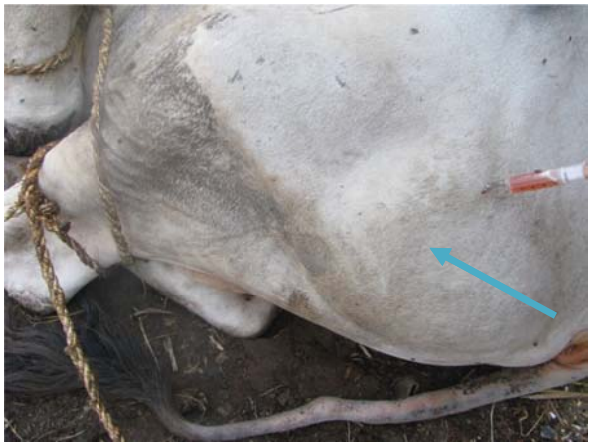
Appendix III. Hygroma lesions and traditional method of firing hygroma for treatment of brucellosis



Hygroma on withers of 8- year old Bunaji cow

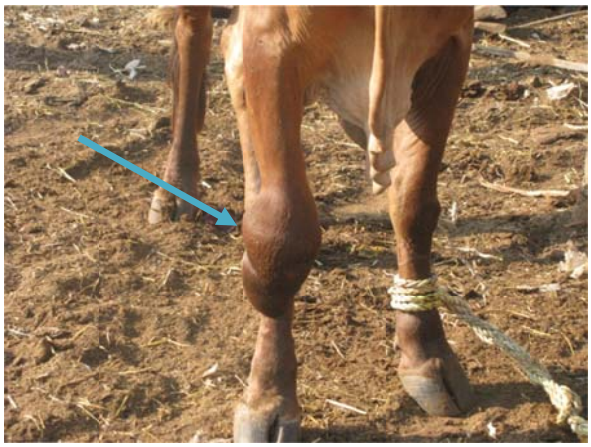


Hygroma (with yellowish fluid) on withers of 8- year old Bunaji cow

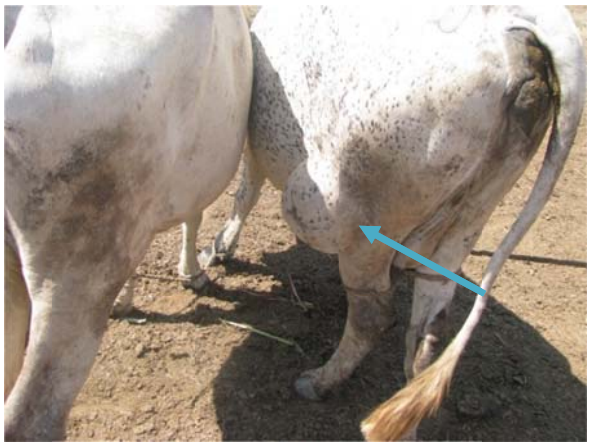


Hygroma on gluteal region of 6-year old Gudali bull

Bilateral carpal hygroma in an old Bunaji bull



Unilateral carpal hygroma in a 7-year old Gudali bull



Unilateral stifle hygroma in a 6-year old Bunaji cow

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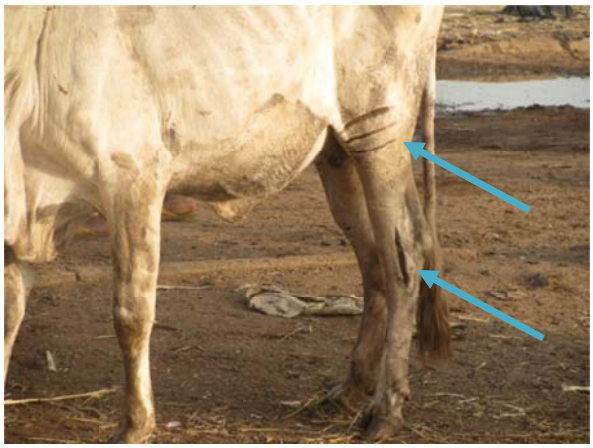
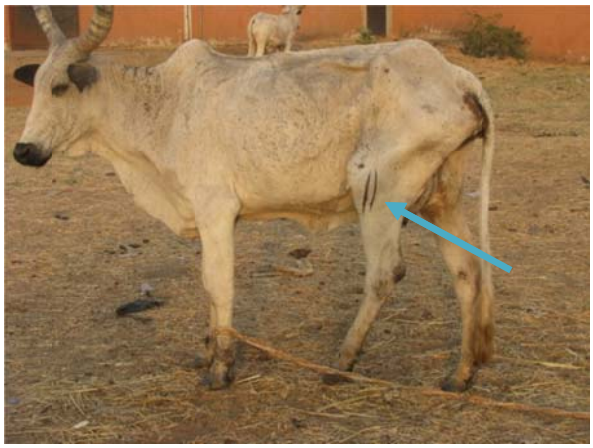
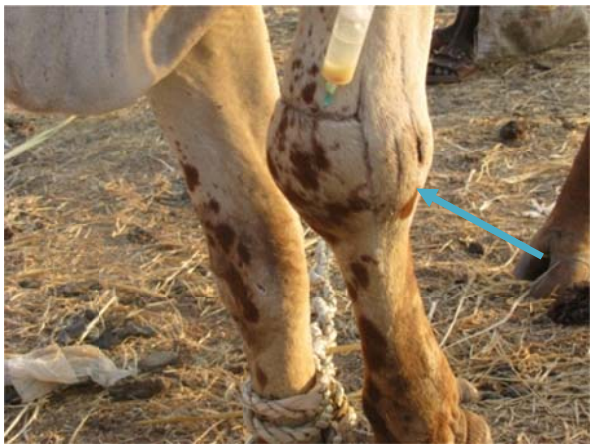


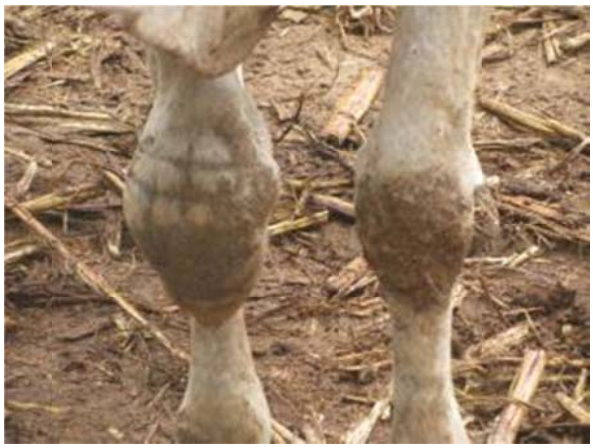
Unilateral firing of hock and stifle joints as traditional method of treatment of brucellosis



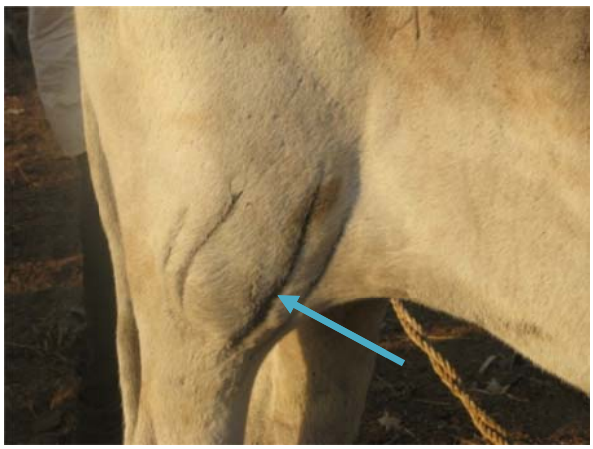
Unilateral firing of stifle joint



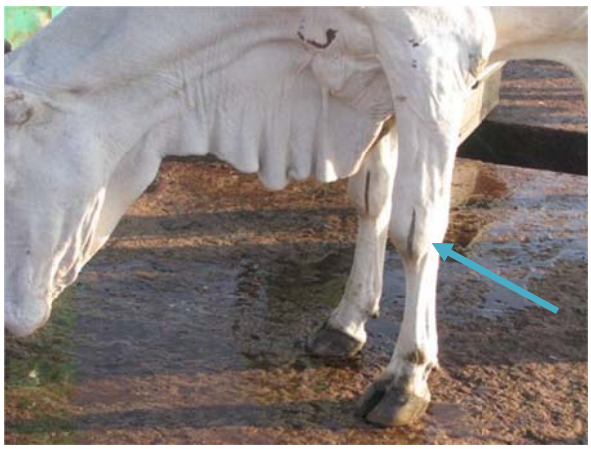
Unilateral firing of carpal joint



Unilateral firing of carpal joint



Unilateral firing of stifle joint



Bilateral firing of carpal joint

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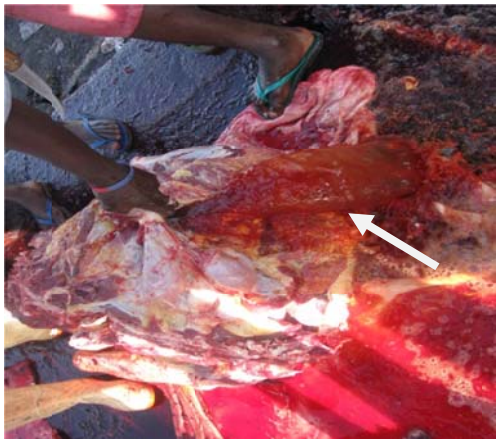
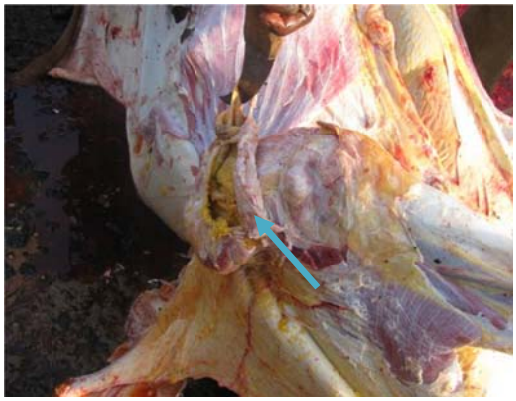
Appendix IV. Different hygromas and hygroma fluids at *post mortem*

Cervical hygroma

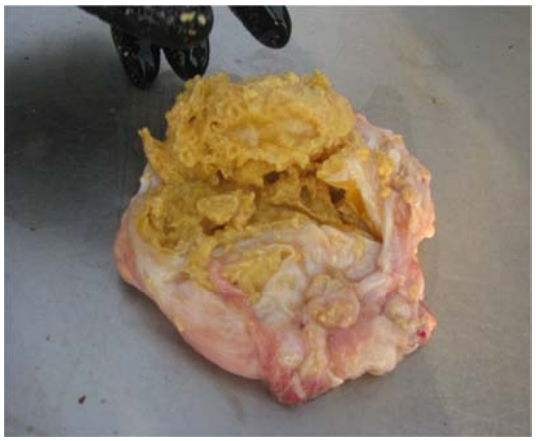


Punctured cervical hygroma with thick yellowish fluid

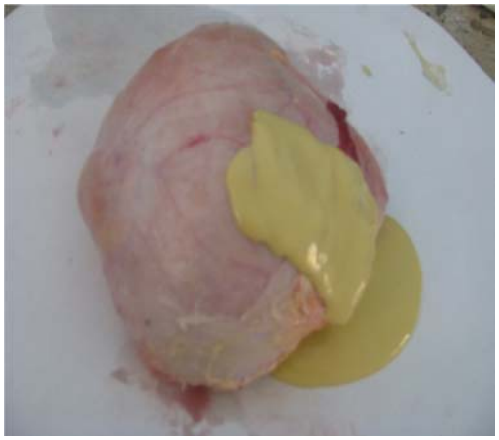
Carpal hygroma excised



Stifle hygroma excised

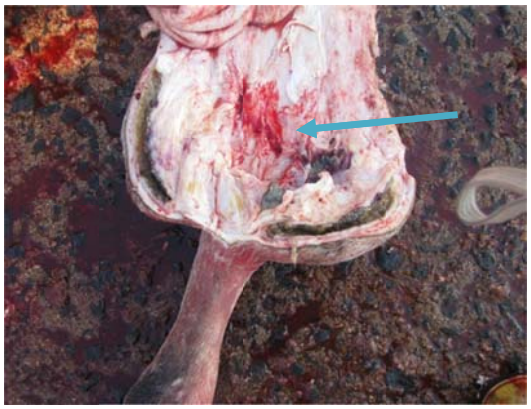


Exteriorised hygroma with flaky exudate



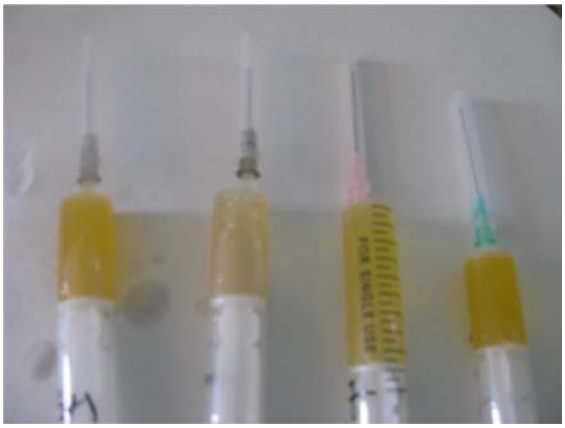
Exteriorised hygroma with thick exudate

© University of Pretoria



Empty carpal hygroma

Carpal hygroma with capsules intact



Different hygroma fluids depending on the stage of infection

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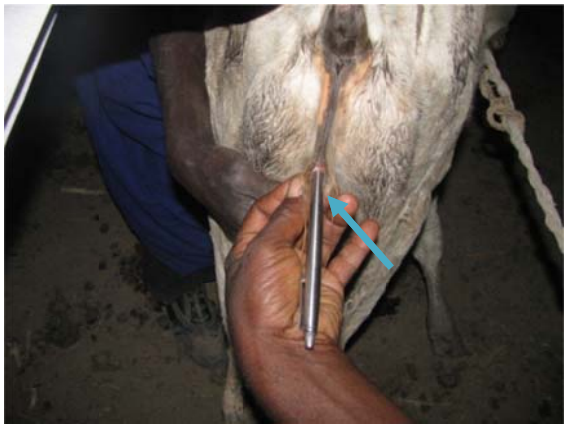
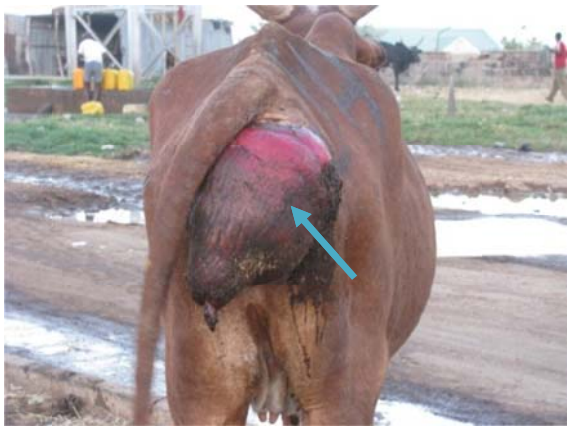
Appendix V. Reproductive abnormalities encountered and some laboratory results



Freemartin in a yearling Bunaji heifer (pencil test)

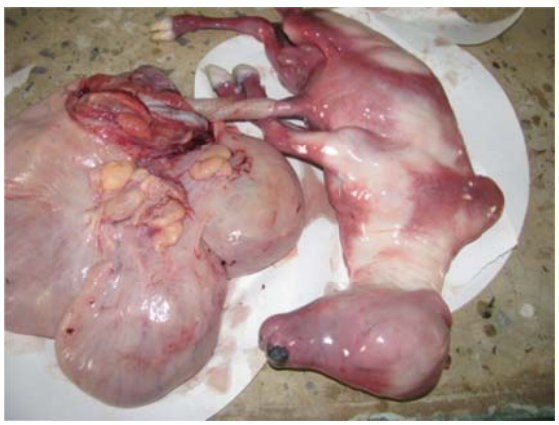
Freemartin in a 5-year old Rahaji heifer (pencil test)

Freemartin in a 6-year old Bunaji heifer (showing narrow vulva)



Uterine prolapse in a Rahaji cow

Both rectal and anal prolapse in a 7-year old Sokoto Gudali



Foetus removed for sample collection following slaughter of a *Brucella* positive cow with cervical hygroma

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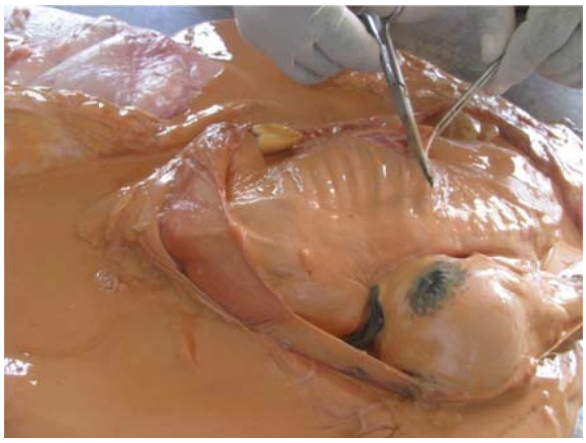
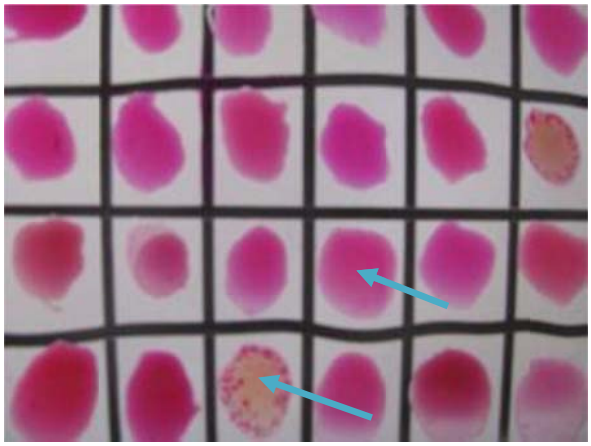


Mummified foetus inside the uterus

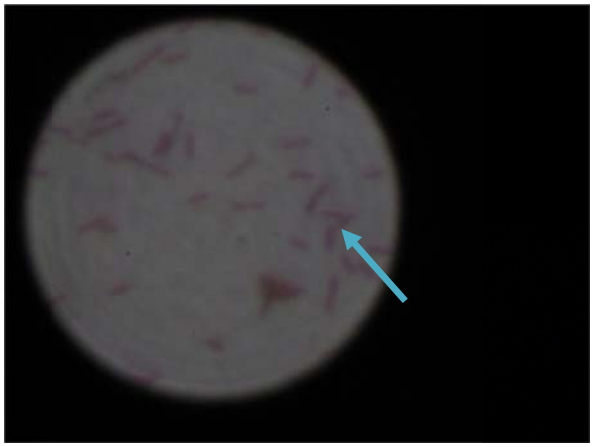
Exteriorised mummified foetus

Orchitis in a 3-year old Wadara bull

5- year old Friesian cow and her aborted foetus



RBPT + (agglutination) and RBPT – (clear, no agglutination)



Campylobacter fetus under the microscope

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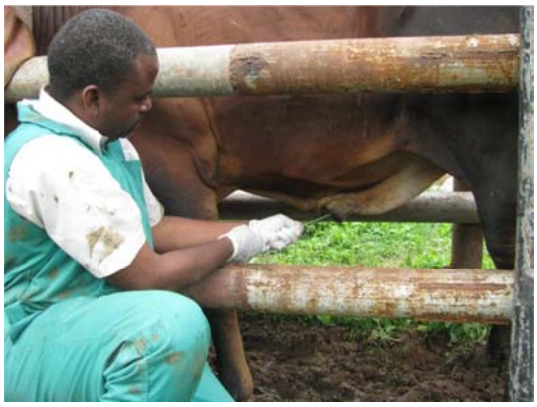
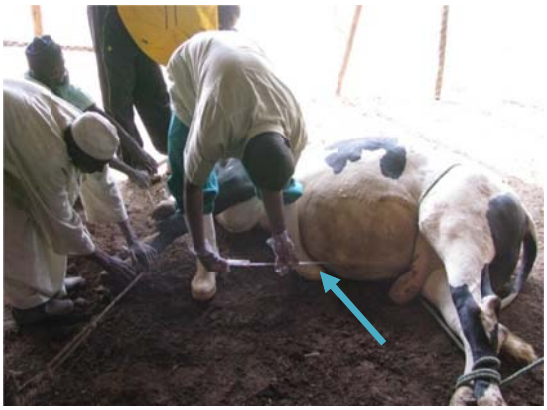
Appendix VI. Sample collection, trans-rectal palpation and aging by dentition

Bleeding via jugular vein and trans-rectal exam of Brahman cow using a crush

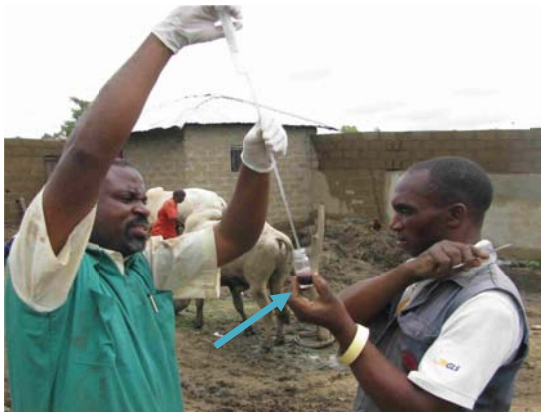
Bleeding via jugular vein & rectal exam of Bunaji cow using local restraint



Preputial scraping of Brahman bull in a crush



Preputial scraping of Friesian bull using local restraint



Gently flushing preputial material into transport medium

Car battery-powered refrigerator for transportation of samples from the field

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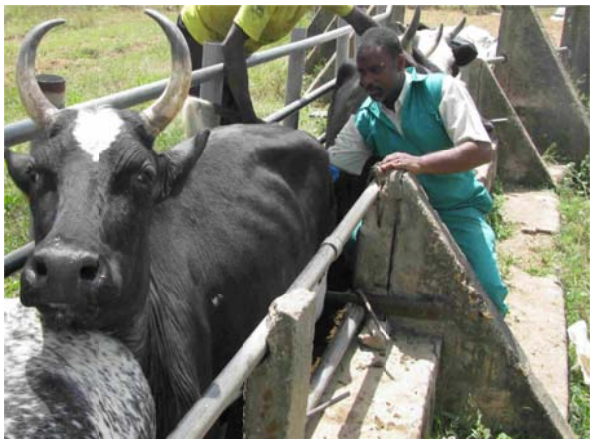
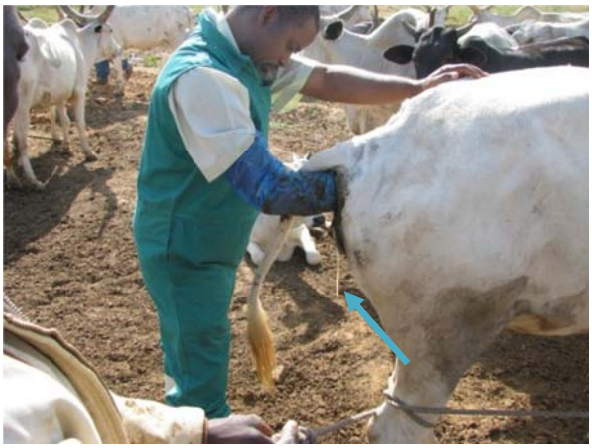
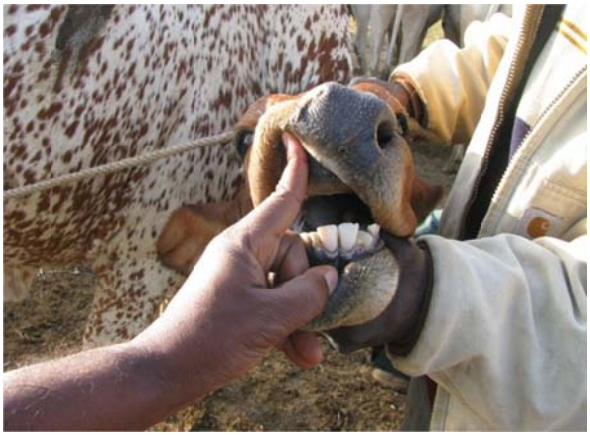


Trans-rectal palpation of a Gudali x Friesian cross in a crush



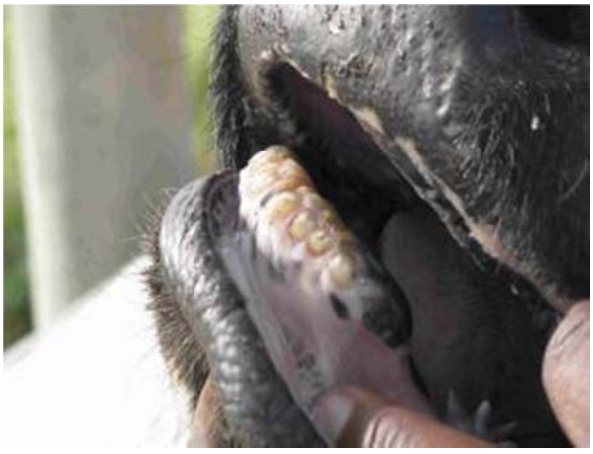
Trans-rectal palpation using local restraint of a Bunaji cow in oestrus (mucous discharge)

A 3-year old Bunaji heifer pushed out of a muddy herd for examination/sampling



A 2-year old (1 pair of permanent incisors) Gudali heifer pregnant

A 3-year old (2 pairs of permanent incisors) Bunaji heifer pregnant



A 15-year old Bunaji cow with all teeth worn (“gummer”)

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Appendix VII. Administering questionnaire, herd prophylactic measures, milking, mixed herds and feeds

Administering questionnaire in an agro-pastoral herd housed in barbed wire/wooden stakes

Regular prophylactic measures in an agro-pastoral herd – dewormers, antibiotics and antiprotozoans used

Reconstituting acaricide for tick control using knapsack sprayer

Administering questionnaire and advising farmer following herd prophylactic measures

Hand milking by a Fulani woman

A herdsman preparing to drink milk directly from the udder of a cow

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A 3-year old Wadara bull still suckling

Double-half-hitch restraint of Bunaji bull for sampling

Mixed Bunaji cattle herd with camels in the background

Mixed Bunaji cattle in the background herding with horses

Stacked crop residues against the dry season

Lopping green leaves of trees during the critical period of the dry season

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Appendix VIII. Some bulls sampled

Sokoto Gudali

Adamawa Gudali

Wadara

Sokoto Gudali

Sokoto Gudali

Bunaji/Adamawa Gudali cross

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Friesian

Jersey

Bunaji

Rahaji

Adamawa Gudali

Brahman

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