SURVEY OF LIVE BIRD MARKETS FOR *BORELLIA* SPECIES IN CHICKENS AND SOFT TICKS IN KATSINA AND KEBBI STATES, NIGERIA

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DEPARTMENT OF VETERINARY PUBLIC HEALTH AND PREVENTIVE MEDICINE,

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DECEMBER, 2014

DECLARATION

I declare that the work in this thesis titled **"SURVEY OF LIVE BIRD MARKETS FOR *BORELLIA* SPECIES IN CHICKENS AND SOFT TICKS IN KATSINA AND KEBBI STATES, NIGERIA"**was performed by me in the Department ofVeterinary Public Health and Preventive Medicine, Ahmadu Bello University Zaria, under theSupervision of Professor J. K. P. Kwaga and Professor J. Kabir. Theinformation derived from the literature have been duly acknowledged in the text and a list ofreferences provided. No part of this work has been previously presented for another degreeor diploma at any institution.

BABA USMAN

Name of Student	Signature	Date

CERTIFICATION

This thesis, titled **"SURVEY OF LIVE BIRD MARKETS FOR *BORELLIA* SPECIES IN CHICKENS AND SOFT TICKS IN KATSINA AND KEBBI STATES, NIGERIA"**meets the regulations governing the award of the degree of Master of Science of the Ahmadu Bello University, Zaria and has approved for its contribution to knowledge and literary presentation.

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DEDICATION

This work is dedicated to my parents MODIBBO ALHAJI BABA MUHAMMAD AND HAJIYA FATIMA BABA for their love and care. Also to my primary school teacher, MALLUM IYA BANO (May his soul rest in peace) for his patience to remain as primary school teacher throughout his life dedicated to teaching, training and encouraging pupils to be hard working and dedicated.

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ABSTRACT

Borellia is a zoonotic bacterium that is pathogenic to most species of animals and birds which is transmitted mainly through tick bite. The bacterium and its vectors are present in Nigerian live bird markets (LBMs) but the prevalence of *Borellia* and level of LBMs tick colonization have not been established despite the report of human exposure to tick bites and cases of recurrent fever among Nigerians. This research is partly a prevalence study whereby LBMs in Katsina and Kebbi States within the North Western region of Nigeria were sampled. A survey was carried out in the LBMs to search for the presence of ticks,*Borellia* organisms in ticks and local breeds of chicken sold at the markets and risk factors associated with the presence of the bacterium. Eight LBMs were sampled across the two States, 5 in Katsina State and 3 in Kebbi State out of which 75% were colonized by soft ticks (*Argas* species) that were found in crevices of cages and tables. A total of 374 chickens and 54 ticks were examined for *Borellia* species, out of which 38 chickens (10.3%) were positive but none of the ticks sampled was found to harbour the bacteria. Majority of LBMs were located within conventional markets (85%) and there was a history of tick bite to human in 87.5% of the markets. All the markets were found to be operating under similar conditions which include processing of chickens manually without the use of hand gloves. Quarantine services, ante mortem inspection, post mortem inspection and health records were practically absent as well. Absence of tick control programmes and allowing chickens to stay in infested market overnight were found to be important in increasing the level of *Borellia* infection among chickens. Based on the findings authorities should enforce mandatory tick control and biosecurity programmes in all our LBMs for better marketing activities, food security and public health.

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DEFINITIONS AND ABBREVIATIONS

Acaricide; chemicals use for control of tick

AI: Avian Influenza

AICP: Avian Influenza Control Program

Avian: Bird

CDC: Centers for Disease Control and Prevention

FAO: Food and Agriculture Organization of the United Nations

Isolation cage;A cage where birds will be observed for their health status before mixing them with old stock

LBMs: Live Bird Markets

MOA: Ministry of Agriculture

ND: Newcastle Disease

Palm cages: local cages made with raffia tree (palm tree)

Wooden cages: cage made with wood and zinc sheet

CHAPTER ONE

INTRODUCTION

1.1 Background of the Study

Live bird markets (LBMs) are important for selling and buying of poultry in Nigeria and some neighbouring countries. They are preferred places for many people to purchase poultry for consumption throughout the world and are located within village markets or in major markets of towns and cities in Nigeria (Senne*et al*., 1992). The birds are usually bought from multiple producers of free ranging chicken and backyard flocks around the neighbouring communities. Species of birds such as pigeons, guinea fowls, ducks, chickens and a wide range of wild-caught birds are usually mixed together at the markets (Senne*et al*., 1992; Paola*et al*., 2008). They flourish all over the world with increasing number of marketers and buyers with attendant risk to people through the spread of diseases like avian influenza and disease vectors such as lice and ticks (Webster, 2004; Bunza*et al.*, 2008).

Ticks are considered as the most important disease vectors among the arthropods, when it comes to transmitting of diseases to man and animals (Sparagano*et al*., 1999;Parola and Raoult, 2001; Jongejanand Uilenberg, 2004). They are non-permanent ectoparasites of domestic and wild animals, birds, reptiles and man that transmit pathogens such as protozoa, viruses, fungi, rickettsia and bacteria which may cause disease in humans and other animals (Furman and Loomis, 1984; Seifert, 1996). Soft ticks for example, *Argas persicus* transmit fowl cholera, *Salmonella* Pullorum, *Salmonella* Gallinarum and West Nile virus (Stefanov*et al*., 1975; Gyurov, 1983; Abbassy*et al*., 1993; Permin and Hansen, 1998). Buriro (1983) reported that, *Argas persicus* harbours different types of

bacteria including those of genus *Salmonella, Aerobacter, Escherichia, Proteus, Staphylococcus, Flavobacterium, Bacillus, Pseudomonas and Streptococcus.* In another report, Abdu (1987), incriminated *Argas persicus* larvae to be important for simultaneous occurrence of infectious bursal disease and spirochaetosis (borelliosis) in birds.

Borelliosis is a zoonotic disease of birds and mammals including man that is caused by large number of *Borellia* species and transmitted by ticks and lice (Steele, 1979; Barclay and Coulter, 1990; Durden*et al*., 1997). The disease occurs worldwide, most frequently in tropical and subtropical countries with tendency of becoming pandemic and the causative organism has been reported in Nigeria from free ranging indigenous birds and ticks (Steele, 1979; Saidu*et al.,* 1993; Cooper and Bickford, 1993; Anna *et al.,* 2012).

1.2 Statement of Research Problem

Live bird markets in Nigeria and around the world are becoming more popular with an increasing marketing activities, because they satisfy the needs of their customers. However, they present a disease risk to people through the spread of diseases like avian influenza and infestation with disease vectors such as ticks (Webster, 2004; Bunza*et al.,* 2008). Hence, LBMs may act as sources and potential reservoirs of infection for domestic poultry and man.

Human beings are exposed to tick bites in the market, as they make close contact with the free ranging birds that are infested with ecto-parasites and unsold birds may be left in cages for days giving optimum conditions for disease and parasite spread among the

poultry and to the community. Similarly, various bird species mostly indigenous chickens, guinea fowls, ducks, pigeons and wild birds which are drawn from populations that are distant from each other are mixed together in LBMs giving room for parasites and diseases to spread (Paola*et al*., 2008;Usman *et al*., 2012).

The Daily Trust Newspaper of the 8 September, 2008 reported a suspected outbreak of Tick borne relapsing fever (TBRF) at Gubio Community in Borno State following massive infestation with ticks. Subsequent investigation showed persistent infestation of the community with ticks highlighting the need for more detailed study of the occurrence and prevalence of *Borellia* in Nigeria (Yunusa, 2012).

Tick borne relapsing fever is reported to be endemic in sub-Saharan Africa, with *B. crocidurae* being the most prevalent within the west African region and *B. duttonii*in the eastern region, but most often misdiagnosed for malaria, especially as they often occur as co-infections (Miron*et al*., 2004; Cutler, 2006; Nordstrand*et al*.,2007; Vial *et al*., 2006)

There are reports of zoonotic borelliosis in East Africa (Ethiopia) and West Africa (Togo). In addition, zoonotic *Borellia* species were reported from ticks in Nigeria and cattle in Ghana (Bell-Sakyi*et al.,* 2004; Nordstrand*et al.,* 2007; Cutler and Abdissa, 2009; Anna *et al.,* 2012). The presence of the bacteria indicatesa need to establish its prevalence in the soft ticks especially the most predominant, *Argas* species and whether they harbour the zoonotic species since they co-infest birds with

*Onithodorous*specieswhich may give room for the bacterium to extend its hostrange (Bunza*et al.,* 2008).

Borellia live primarily as extracellular pathogen aided in adapting to various host animals by regulating the lipoproteins on their surface and is also established that, they receive virulence plasmid from other pathogenic virulent bacteria (Jauris-Heipke, 1995).

The number of *Borellia* species associated with relapsing fever has been on the increasing, likely due to improvement in diagnostic techniques. These spirochaetes arereported produce undulating febrile disease in humans, with signs and symptoms similar to those of malaria, indicating the need to intensify surveyin such area of research (Cutler, 2006).

Relapsing fever is a neglected disease of public health interest that has potential for causing pandemics. It caused epidemics that affected approximately 50 million people in the past and was associated with death rates of 10 to 40% during the first half of the 20th century (Bryceson*et al*., 1970). Recently, perinatal death ratio of 43.6% due to relapsing fever has been reported from disease-endemic regions of Tanzania (Cutler and Abdissa, 2009).The disease is recognized as the fifth most common killer disease in Ethiopia(McConnel, 2003).

1.3 Justification of the Study

Village chickens are the majority in Nigerian poultry population (NBLS, 2007). They are exposed to parasites and predators while scavenging for food and are mainly sold at live bird markets (Abubakar*et al.,* 2007; NBLS 2007; Usman *et al.,* 2012).Tick

infestation and nuisance of tick bites are likely to constitute a threat to poultry marketing and reduce economic activities in the LBMs. Occurrence of zoonotic *Borellia* species in ticks and birds sold at LBMs could be a source of tick borne relapsing fever to the marketers and the market community.

Live bird markets in Nigeria most often adjoin conventional markets, hence providing intimate contact between human beings, birds and ticks. Thus, there is need for this study in this area to provide information on the occurrence of ticks and *Borellia* species which they may carry and transmit to poultry. Information obtained will be used to sensitize the live bird marketers to embark on control interventions against tick infestation that could translate to better and safer marketing and hence higher profit, food security and better livelihood (AICP, 2008).

This study was also designed to provide information on the relevance of LBMs in the spread of diseases and the prevalence of *Borellia*species in local chickens and in *Argas* species.

1.3 Aim of the Study

This study aimed to investigate the occurrence of soft ticks in LBMs and *Borellia* species in chickens and soft ticks found in LBMs of Katsina and Kebbi States.

1.4 Objectives of the Study are to:

1.determine colonization of LBMs by soft ticks.

2. detect*Borellia* species in the ticks and chickens in the LBMs.

3.determine the risk factors associated with the presence of *Borellia* in LBMs.

1.5 Research Questions

1. Do ticks found at the LBMs harbour *Borellia* species?
2. Are LBMs in Katsina and Kebbi States colonized by *Argas persicus*?
3. Do the chickens sold at the LBMs harbour *Borellia* species?

CHAPTER TWO

LITERATURE REVIEW

2.1 The genus *Borellia*

The genus *Borellia* belongs to the family *Spirochaetaceae* of the order *Spirochaetales*. The order is made up of two families, the free living saprophytes; *Spirochaetaceae* and *Treponemataceae* consist of three genera that are pathogenic for man and animals: they include *Borellia, Leptospira and Treponema*.*Borellia* are highly specialized, spiral-shaped, two-membrane bound Gram – negative bacteria that are easily stained by the ordinary methods such as Giemsa. They differ in length, diameter, tightness of the coils, regularity of the coils, and number of peri plasmic flagella (Goubau*et al.*, 1984; And Stanley, 1986). The length is a function of the age and the nutritional adequateness of the culture medium or experimental animal in which the species is cultured or passaged. It can range from 8µm, in the case of *B. coriaceae* species and up to 30 µm in length in*B. burgdorferi*. The widths of the helices range from that of *B. burgdorferi*, which is the narrowest at about 0.2 to 0.3 µm to those of *B. recurrentis* and *B. persica* which are reported to have diameters of 0.45 to 0.5µm (Karmi and Hovind-Hougen, 1979; Burgdorfer*et al.*, 1982; Hovind-Hougen, 1984; Lane *et al*., 1985).

Borellia live primarily as an extracellular pathogens aided in adapting to various host animals by regulating the various lipoproteins on their surface. They are extremely

difficult to culture *in vitro,* due to their specific nutritional requirements such as long fatty acid chain and an aerobic environment (Perine*et al.,* 1984). The *Borellia* DNA has approximately 30% of G+C mole content and contain both linear chromosomes and numerous plasmids (Jones *et al.,* 1986).*Borellia* species can be differentiated based on their arthropod vector, pathogenicity for laboratory animals and geographic distribution (Goubau*et al.,* 1984)

Several tick species have been associated with the transmission of *Borellia* species in animals, birds, man and reptiles which include *Argas* species, *Ixodes* species and *Onithodoros*species (Saidu *et al.,* 1993; Barbour *et al.,* 1996; Durden*et al*., 1997; Ece*et al.,* 2003).

2.1.1Cultivation of *Borellia* species

Borellia is maintained in nature between the host and the vector that feed on them. They are cultivated through regular passage in susceptible laboratory animals or propagated on chorioallantoid membrane of embryonated chicken embryo (Goubau,1984). Artificial media for cultivation of *Borellia* species have been developed, which include Kelly culture media A, B and C and Fortified Kelly's medium (Barbour and Hayes 1986).

2.2. Borelliosis

Borelliosis is a zoonotic disease of birds and mammals including man that is caused by large number of *Borellia* species and transmitted by ticks and lice (Steele, 1979; Barclay and Coulter, 1990; Durden*et al*., 1997). The disease occurs worldwide, most frequently in tropical and subtropical countries and the causative species have been reported in

Nigeria from free ranging indigenous birds and ticks (Steele, 1979; Saidu*et al.,* 1993; Cooper and Bickford, 1993; Anna *et al.,* 2012).

Borellia anserina is incriminated mainly in avian borelliosis worldwide with *Argas* species as the vector and recent studies have shown that *B. burgdorferi* also affect wild birds and causes mortality similar to that of avian spirochaetosis(Barbour and Hayes 1986; Reed *et al.,* 2003; Abdu, 2014).The avian *Borellia*(*B.anserina*) is motile, stains readily with aniline dyes (in contrast to *Leptospira* and *Treponema*), and is readily soluble in 10 % ox bile and saponin. It has loose coils and can be stained with ease unlike *Treponema* species that have tight coils and in the ease with which it can be stained. It also differs from *Leptospira* in having looser coils and in the absence of a terminal hook as well as in ease of staining. It is easily diagnosed from blood smears but may also be found in sections of almost any tissue. In the tissues it is best demonstrated by some method of silver impregnation techniques and by slow Giemsa staining techniques (Mc Neil *et al*., 1948).

The human *Borellia* is divided into two large genetic groups namely; the relapsing fever *Borellia*and the Lyme disease related *Borellia* species. The relapsing fever group are transmitted by*Ornithodorous* species of ticks and is divided into New World tick-borne relapsing fever that are caused by *B. hermsii, B. turicatae, B. parkeri, B. mazzottii, B. venezuelensis,* and Old World tick-borne relapsing fever that is caused by *B. duttonii, B. crocidurae, B. persica, B. hispanica,* and *B. caucasia*in different parts of the world

(Goubau, 1984; Barbour and Hayes, 1986). The incidence of relapsing fever has been increasing possibly due to improved diagnostics and molecular techniques which have enabled sequence-based characterization of these spirochaetes (Cutler, 2006). Lyme disease is caused by *B. burgdorferi* and is transmitted by *Ixodes* species of ticks in USA and some parts of Europe (Goubau, 1984). *B. recurrentis* causes Louse-borne relapsing fever and is transmitted worldwide by *Pediculushumanus*(Barbour and Hayes, 1986).

2.3. Animal Borelliosis

The most important zoonotic *Borellia*specie is *B.burgdorferi*which causes disease in dogs, horses, cattle and cats, while many wild birds become infected and serve as reservoirs for tick infection (Perker and White, 1992; Gylfe, *et al*., 2000).

Cattle show different clinical signs when infected with different *Borellia*specie for example*B. theileri*is the aetiological agent for bovine borelliosis in Africa, Australia and Mexico and is said to be transmitted by *Boophilusmicroplus*. *B. coriaceae* is associated with epizootic bovine abortion in some parts of the world which include USA and Europe (Barbour and Hayes, 1986).

2.3.1Avian borelliosis

The disease is caused by *B. anserina* which has the following synonyms;*Spirochaeta galinarum*, *Treponemaanserinum*, *Spirochaetaanserina*, and *Spirochaetaanatis*causes avian borelliosis (McNeil *et al*., 1948; Merck Veterinary Manual, 2005). The disease was first described in 1891 by Sakharoff from geese in the Caucasus. Marchoux and Salimbeni(1903) reported the first natural outbreak in fowls

and alsoincriminated*Argas persicus* as the arthropod vectorin Brazil. Since then it has been reported as themajor cause of mortality in fowls in many parts of the world. In Africa, the disease was reported as early as 1906 in Sudan and is now endemic affecting large commercial layer farms in Khartoum State (McNeil *et al*., 1948; Iman*et al.,* 2010). Burroughs (1947) reported the spirochaete fromticks in the United States when he discovered that,specimens of *Argaspersicus*sent to him from Texas were infected with the *Borellia*.

Avian spirochaetosis is becoming more relevant not only because of large losses it causes in large scale layer farms but also because some human *Borellia* species (*B. burgdorferi*) are found in wild and aquatic birds outside their endemic foci(Reed *et al.,* 2003; Iman*et al.,* 2010).

2.3.2 Pathogenesis of avian borelliosis

Avian borelliosis is an acute,febrile, septicaemic disease of birds that is mainly caused by *B. anserina* and transmitted by *A. persicus* (Merck Veterinary Manual, 2005).The disease is worldwide in distribution and natural outbreaks in chickens, geese, turkeys, ducks, grouse, and canaries had been reported (Saidu*et al.,* 1993; Barnes, 1997;Iman*et al.*, 2010). The bacterium is transmitted through saliva or coxal fluid during feeding on birds. Oral, ocular or nasal routes can establish infection and virulent strains can penetrate unbroken skin through contact with infected tissues or blood from recently dead or slaughtered bird (Barnes, 1997). Transmission through accidental inoculation of laboratory workers and the use of syringes and needles for multiple birds has been documented (Favorova*et al*., 1971; Hira and Hussein, 1979). In addition, the bacteria remain infectivefor up to eight days in blood but can be destroyed at low temperature (20 ^{0}F)(McNeil *et al*., 1948).

The clinical signs of the disease depends on the virulence of the strain but chicks are said to be more susceptible to the disease. Avian borelliosis is generally characterized by pyrexia, dullness, weight loss, drop in egg production and anaemia. In addition, there may be greenish diarrhoea, and paralysis of the wings or legs (Iman*etal.,* 2010; Abdu, 2014).

Iman*etal.* (2010) reported that pyrexia occurred on the second day and even apparently healthy chickens showed spirochetaemiain the peripheral blood 48 hours post inoculation in experimentally infected chickens with field isolates of *B. anserina.* The bacterium completely disappeared from the peripheral blood before day fifteen in the birds that recovered within seven days on average (McNeil *et al*., 1948). The characteristic gross lesions include the enlargement and mottling of the spleen which can be detected even in apparently healthy chicks (Iman*etal.,* 2010).

2.3.3 Diagnosis of avian spirochaetosis

Avian borelliosis is easily diagnosed from blood smears but may also be found in sections of almost any tissue. In the tissues it is best demonstrated by methods of silver impregnation, such as that of Levaditi or by the slow method of Giemsa (Mc Neil *et al*., 1948).

History of tick infestation and clinical signs such as fever (45^0C), drop in egg production, paleness of comb and wattle is also diagnostic. Demonstration of the bacteria in Giemsa stained smear is confirmatory. In post mortem examination, the most important lesion is enlargement and mottling of the spleen. Histo-pathologically, spleen

will show haemorrhagic and necrotic foci in the reticular cells with active proliferation of lymphatic cells and thickening of the capillary walls, while in liver changes include accumulation of lymphocytes in the interlobular connective tissue, congestion and thickening of the portal veins (Iman*et al* ., 2010).

Serological diagnosis is questionable as a result of diverse serotypes that exist in some parts of the world. These test are florescence antibody test, complement fixation test and agglutination test (Abdu, 2014). Demonstration of spirochaetal antibody in yolks of eggs laid by infected chicken is also diagnostic for the disease (Merck Veterinary Manual, 2005).

2.3.4 Treatment and prevention of avian Spirochaetosis

The use of antibiotics at the early onset of the disease when the number of bacteria per oil immersion field is low is advisable for better result. The drugs of choice include terramycine, penicillin, bacitracin and streptomycin (Abdu, 2014).

Prevention lies mainly on vector control which include the use of pesticide to eliminate ticks, mosquitoes and mites. Environmental management and sanitation is the helpful in the vector control (Abdu, 2014).Vaccination has been successful in endemic areas and may be produced from phenol or formalin attenuated material from lysates blood, tissue or embryo or egg containing local strain of *Borellia* (Merck Veterinary Manual, 2005). Vaccination of chickens, turkeys and ducks with suspension of *B. anserina* grown in embryonated egg is protective (Abdu, 2014).

2.4.1 Human borelliosis

The history of human borelliosis has been long, but the term relapsing fever was first mentioned by David Craigie in Edinburgh between 1843 and 1848 to describe the clinical sign of the disease (Cuttler, 2009). In Africa, the disease was reported from the outcome of many independent researchers working across central Africa in early 20th century. Ross and Milne discovered the causative agent of the African tick borne relapsing fever in 1904 (Ross and Milne, 1904). The species was identified as a spirochaete, when it was seen in the blood of acutely ill patients. The soft tick *Ornithodorosmoubata* was identified as the vector, but the route of transmission was not well established until when the naturally infected ticks were fed experimentally on cercopithecus monkeys by Dutton and Todd (Cook, 1904; Dutton and Todd, 1905; Wellman, 1905). MacKie in 1907 reported the findings of Dutton and Todd after Dutton himself got infected, in his publication "The role of the human body louse in the transmission of relapsing fever" (Bryceson*et al*., 1970). The genus was later named *Borellia* after AmedeeBoreel, who established that *Borellia* caused relapsing fever and is transmitted by *Ornithodoros* ticks (Cuttler. 2009).

The first spirochaete reported in a wild African mammal was *Spirochaetacrocidurae* (now, *Borelliacrocidurae*), which was found in a shrew *Crocidurastampflii* in Dakar, Senegal (Leger, 1917). The specie is widespread across most of Africa north of the equator and reported to be endemic in West Africa (Bouattour*et al*., 2010; Cutler, 2006; Vial *et al.,* 2006). The *Borellia*species is known to infect a large number of wild and domestic rodents and shrews, and is transmitted by two species of soft ticks, *Ornithodoroerraticus* and *Ornithodorossonrai* (Hoogstraal*et al.,* 1954).

Relapsing fever is an old disease of global interest that has potential for causing pandemics.It caused epidemics that affected approximately 50 million people and was associated with death rates of 10 to 40% during the first half of the 20th century (Brycesone*t al*., 1970). In Africa, tick borne relapsing fever is one of the most important *Borellia* infections that is caused by at least sixteen *Borellia* species of which *Borelliacrocidurae*seem to be of great importance in the western part of Africa (Van Dam *et al*., 1999). The disease has been ranked as the most common bacterial infection in Senegal and is usually listed among the top ten causes of mortality in children of less than five years of age in Tanzania (McConnell, 2003; Vial *et al*., 2006).

In 1930s, approximately one third of the population in Africa was suffering from an epidemic attributed to relapsing fever. Relapsing fever outbreak in Sudan around 1998 / 1999 was estimated to have affected 20,000 members of the Dinka tribe and mortality rate of about 10–14% was recorded (Cutler, 2006). Tick borne relapsing fever due to *B. duttonii* infection is reported to have affected children and pregnant women, and was associated with foetal loss and neonatal deaths in Tanzania. A perinatal death ratio of 436/1,000 (43.6%) in the disease-endemic regions of the country was recorded (McConnel, 2003). In West Africa,*B. crocidurae* is the most prevalent species and rodents serve as reservoirs with up to 18% of rodents in Senegal infected with this spirochaete (Diatta*et al*., 1994; Vial *et al*., 2006).

Tick Borne Relapsing Fever (TBRF) is transmitted by soft ticks, which infests the environment (Busvine, 1975). The bacterium is transmitted through saliva or coxal fluid during feeding on man, animal or bird. Oral, ocular or nasal routes can establish infection and virulent strains can penetrate unbroken skin through contact with infected

tissues or blood from recently dead or slaughtered bird (Barnes, 1997). Transmission through accidental inoculation of laboratory workers and blood transfusion have been documented (Favorova*et al*., 1971; Hira and Hussein, 1979).

Lyme disease is *a*borellialinfection that affect more than one system of the body and is caused by at least three species of tick-borne *Borelia*which include*Borreliaafzelii*,*Borreliagarinii*and*B. burgdorferi* sensulatogenogroup. Theseinclude *B. burgdorferi* sensustricto. The disease is more important in North America, Europe,Northern Asia and Russia (Reed *et al*., 2003). In North America, Lyme disease is maintained by enzootic cycles involving small rodents, such as whitefootedmice (*Peromyscusleucopus*) and is transmitted to man and other animals by hard ticks (*Ixodesscapularis*in the northeast and north central U.S. and *Ixodespacificus*along the pacific coast (Barbour, 1998).

2.4.2 Pathogenesis of human borelliosis

Tick borne relapsing fever is an acute febrile disease caused by spirochaetes of genus *Borellia* that may be transmitted by the bite of an infected vector ticks of the genus *Ornithodoros* (Goubau, 1984). The diseasehas an incubation period of 3 to 18 days in man depending on the infective dose (Bryceson, 1976). The signs begin with bull eye coloration at the site of tick bite and itchy rashes sometimes occurs. This is followed by high fever and severe headache which may be followed by a period of apparent wellness that last for about a week before the next episode. The disease is characterized by recurrent bouts of fever, chills, headache, muscle and joint pains and nausea (Marquam*et al*., 1996). There may be neurological signs, tachycardia, tachypnoea, jaundice, tenderness of spleen and liver with or without splenomegaly(Perine and

Reynolds, 1974). Abnormal bleeding is also reported most commonly, purpura and epitaxis in which the nasal mucosa appear hyperemic with diffused oozing of blood (Perine and Reynolds, 1974).Jarisch-Herxheimer reaction may occur after antibiotic therapy which may be self-limiting. Serious complications or death may occur in poorly managed cases especially in immune compromised and immune deficient patients such as very young, elderly, pregnant and debilitated individuals (Southern and Sandford, 1969).

2.4.3 Diagnosis of human borelliosis

History of recurrent fever and tick bite is suggestive of the disease in endemic areas. Diagnosis of relapsing fever depends mostly on demonstrating the spirochaete in Giemsa-stained blood films (Cutler, 2006). The sensitivity of blood smear is approximately 70 to 80% during the febrile stage of the disease but can be as low as less than 5% during afebrile periods (Schwan*et al*, 1996; Assous and Wilamowski, 2009). Sensitivity can be increased through wet mount preparation and buffy coat smear stain with Giemsa (Butler, 1991; Christer and Sven, 2008) The use of acridine orange–coated quantitative buffy coat tubes, centrifugation, and fluorescence microscopy is effective (van Dam *et al* .,1999; Cobey*et al* .,2001)

Serological methods such as ELISA have limited use due to cross reactivity and frequent false positive reactions. Recombinant GlpQ, a protein found in all relapsing fever due to *Borellia* infection is the most useful especially in retrospective diagnosis, but it cannot differentiate between active and passive disease hence those that recover from the disease will also be test positive (Schwan, 1996; Porcella*et al*., 2000).

Polymerase Chain Reaction is an excellent diagnostic method for relapsing fever (Christer and Sven, 2008). Molecular approaches that allow characterization of relapsing fever *Borellia* species are now in place and can be facilitated through allowing more accurate investigation of the species that are prevalent in different areas and enable characterization of newly discovered spirochaetes (Bunikis and Barbour, 2005). This type of analysis can be performed on frozen blood samples and samples kept for long at 4^0C. The sensitivity of the PCR analysis can be increased with nested PCR protocol (Sin *et al*., 2010). DNA sequences of the 16S rRNA gene, or the 16S rDNA of bacteria contain their respective unique hyper variable regions that is specific for each species species-specific or type-specific signature sequences (Greisen *et al*., 1994; Phillippy*et al*., 2007)

2.4.4 Control, prevention and treatment of human borelliosis

Good environmental management and public education concerning the risk factors associated with TBRF is a key in the disease control and prevention. It is advised to spray shoes, trousers and other protective wares with acaricides and repellants. Infested market areas need to be sprayed regularly for human safety and better marketing activities (Sandford, 1976).

Successful antibiotic treatment is possible only if the individual has an effective immune system. The classical pre-exposure prophylaxis is based on administration of doxycycline which can be used for post exposure treatment as well (Hasin*et al*., 2006). Oral tetracycline or doxycycline for 5 -10 days is generally effective (Butler, 1985). Erythromycin is the effective substitute for children and pregnant women and those allergic to tetracycline (Butler, 1991).

2.5Ticks

Ticks are said to transmit a greater variety of disease causing microorganisms such as protozoa, rickettsiae, viruses and spirochaetes, than any other arthropod vector group, and are among the most important vectors of diseasesaffecting livestock, pets and humans. These are in addition to their parasitism and ability to causeallergy, paralysis and toxicosis (Furman and Loomis, 1984; Seifert, 1996; Jongejan1and Uilenberg, 2004). They belong to the phylum*Arthropoda*, order *Acarina*and are divided into two groups *Argasidae*(soft ticks) and *Ixodidae* hard ticks (Barker and Murrell, 2004). Soft ticks for example, *Argas persicus* transmit fowl cholera, *Salmonella* Pullorum, *Salmonella* Gallinarum and West Nile virus to its hosts (Stefanov*et al*., 1975;Gyurov, 1983; Abbassy*et al*., 1993; Permin and Hansen, 1998).

The world's argasid tickfauna is made up of one hundred and eighty three species (183 species) and is divided into four genera, namely *Argas*, *Carios*, *Ornithodoros*and *Otobius*, but those of medical and veterinary importance belong to three genera namely *Argas*, *Ornithodoros* and *Otobius* (Jongejanand Uilenberg, 2004).

Soft tickslive near their favourite hosts and all the stages of development are parasitic (nymphs, larvae and adults). Adults feed for a short period only on the host and then go back to their hiding place such as crevices, cracks or sand. Larvae of some*Argas* species attach and feed for some days on domestic poultry. Soft ticks often haveseveral nymphal stages (Fig 2.1). Adult *Argas* species mate off the host and the female feeds several timesand produces a small batch of eggs after each meal(Hoogstraal, 1956).

The genus *Argas*is made up of several species that are widely distributed in different parts of the world. They include *A. persicus, A. reflexus, A. monolakensis, Argas brumpti, A. miniatus*and *A. walkerae*(Hoogstraal, 1956). Nigerian LBMs were reported to be colonized by *A. persicus* and*A.walkerae*(Bunza*et al.,* 2008). Buriro (1983) stated that, *Argas persicus* harbours different types of bacteria including those of genus *Salmonella, Aerobacter, Escherichia, Proteus, Staphylococcus, Flavobacterium, Bacillus, Pseudomonas and Streptococcus.* In another report, Abdu (1987) incriminated *Argas persicus* larvae to be important for simultaneous occurrence of infectious bursal disease and spirochaetosis (borelliosis) in birds, butKahl*et al*. (2002) suggested that, for a tick species to be considered as a vector for a particular pathogen, it most fulfill the following conditions;

(1) It should feed on an infectious vertebrate host.

(2) It shouldbe able to acquire the pathogen during the blood meal.

(3) It should be able to maintain thepathogen through one ormore life stages.

(4) It should be able to pass it onto other hosts when feeding again

It is an established fact that, many species of ticks transmit borelliosis to animals and man. They include *A. persicus* which transmits *Borellia anserina, Amblyommaamericanum, A. maculatum, Haemaphysalisleporispalustris, Ixodesbrunneus, I. Minor* and *I. scapularis*transmit *Borellia burgdorferi* (Saidu*et al*., 1993; Durden*et al*., 1997; Slowik and Lane, 2001).

2.5.1*Borellia* in ticks

The tick usually acquires the spirochaete during its larval stage, when it feeds on small animals such as rodents or birds. The ability of these spirochaetes to remain associated with their arthropod vectors throughout their lifespan once they get infected is well

established (Goubau*et al.*, 1984; Kisinza*et al*., 2003). The bacteria is then transmitted among ticks via trans-ovarian transmission in some species: examples are *B. duttonii*, by *Ornithodorosmoubata* and *B. anserina* by *A. persicus* (Aeschlimann, 1958; Zaher*et al.*, 1977). Venereal transmission (male to female) has also been documented especially in *Ornithodoroserraticus* carrying *B. crocidurae* (Geigy,1954).

2.5.2 Tick control

Farmers and researchers have developed various ways of controlling ticks, but every method of tick control has certain shortcomings (Mbati*et al*., 2002; Rajput*et al.*2006). Chemical control with acaricides was considered as the best method, but it was shown recently that ticks have developed resistance against a range of acaricides (Martins *et al*., 1995). In addition, they are toxic and costly resulting in the need for the development of an alternate and absolute control method. Chemicals used for tick control in poultry include acaricides such as arsenical, chlorinated hydrocarbons, organophosphates, carbamates and synthetic pyrethroids (Rajput *et al.*, 2006).

Acaricidal control of soft ticks on poultry and LBMs in general is verydifficult because of the particular hiding behaviour of the ticks in the housing, hence there is need for improved infrastructures that will not permit the parasite to hide, and also cracks and crevices should be filled (Jongejanand Uilenberg, 2004).

Drummond(1983) suggested that, acaricides used in control of ticks on livestock or in the environment should be applied in such a manner that the ticks will be killed, without causing harm to livestock, applicators, the tissues of treated animals and community.

Sanitation and good hygienic practices will reduce colonization of area by ticks alsospray of chemicals such as diazentol to poultry and pens will help back yard famers and live bird marketers to control ticks.

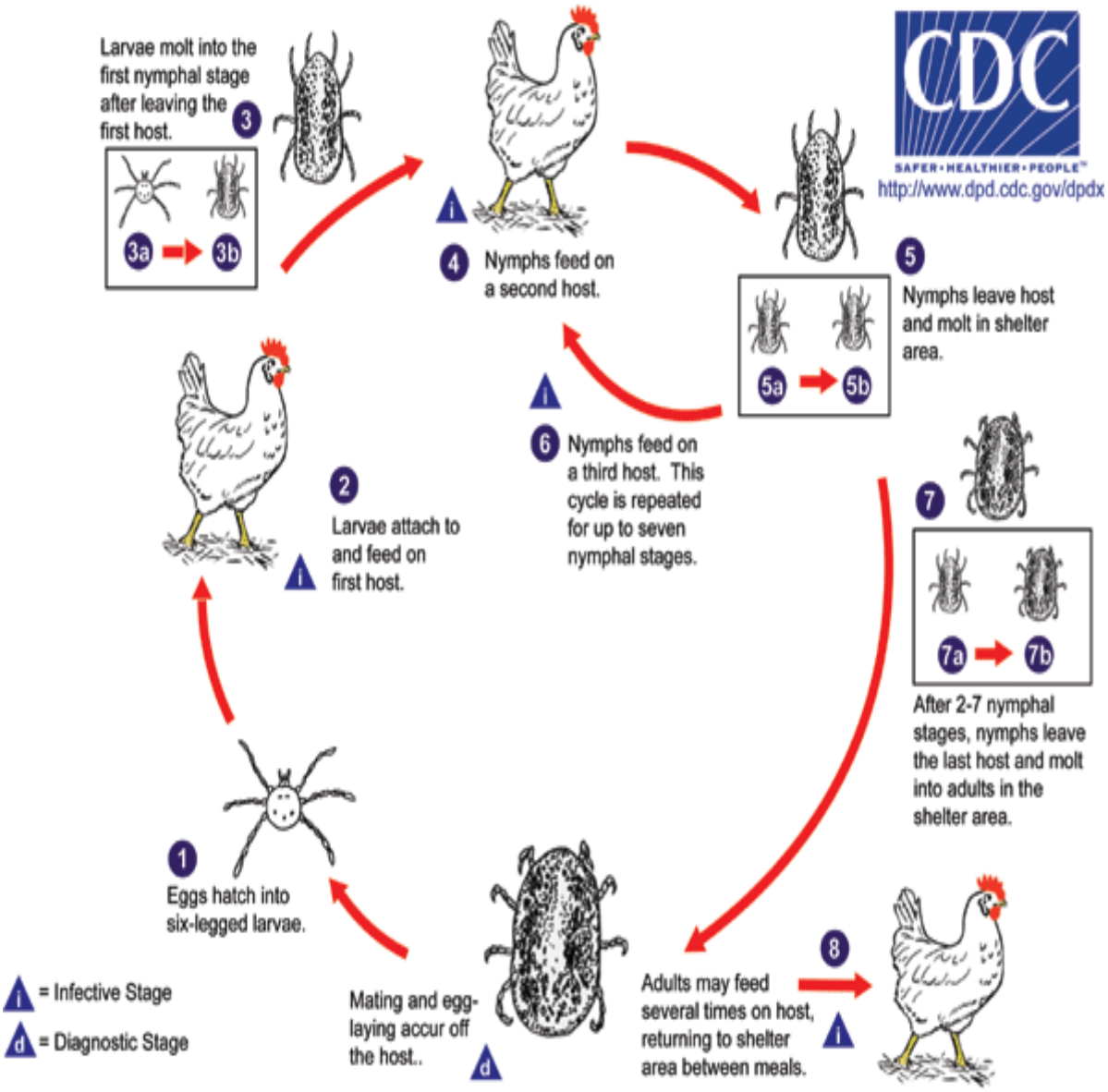


Fig. 2. I Life cycle of soft ticks adapted from http/www.dpd.cdc.gov/dpdx

2.6. Live Bird Markets (LBMs)

Poultry is said to be the most numerous species of farm animal according to African Livestock Population Statistics for 1995 and more than 80% of the Nigerian poultry are free roaming village poultry under extensive system, consisting of over 143 million birds (Adene and Oguntade, 2006.; NBLS, 2007). Village chicken production is an extensive production system where birds survive by scavenging for food around their environment with little input from the owners who are mostly women, children and aged people (Abubakar *et al.,* 2007). They contribute substantially to annual egg and meat production and are mainly sold at live bird markets *(*(Akinwumi*et al.,* 1979; Sonaiya, 1997; Paola *et al.,* 2008)

The Nigerian LBMs are of traditional structure in which poultry and poultry products are displayed in an open system for buyers and sellers (AICP, 2008). They are preferred places for many people to purchase poultry for consumption throughout the world and are located within village markets or in major markets of towns and cities in Nigeria (Senne*et al*., 2003; AICP, 2008). The birds are usually bought from multiple producers of free roaming chicken and back yard flocks around the neighbouring communities. Species such as pigeons, guinea fowls, ducks, chickens and a wide range of wild-caught birds are usually mixed together at the markets (AICP, 2008; Paola *et al.,* 2008).Village poultry may be transported to market in locally made cages, tied by the legs, held in the hand, by foot or on a lorry (Paola *et al.,* 2008).

There are two types of LBMs in Nigeria according to the AICP (2008) , namely daily and weekly LBMs. Weekly markets are the LBMs that operate once or twice a week

and are mostly located in rural or pre-urban areas of local governments. They drain village poultry and serve as source of birds to daily market. Villagers and some final consumers also buy birds for their consumption, home rearing and/or for sacrifices (AICP, 2008; Paola *et al*, 2008). Daily LBMs are mostly located at the pre-urban and cities of the country. Direct interaction between producers and final consumers is rare as the birds are moved to cities from distant and nearby villages. Processing of poultry take place in most of the markets under poor hygienic conditions and they serve as last destination (AICP, 2008). Some of the markets are colonized with lice and ticks which are parasites of the birds and vectors of various diseases (Buriro, 1983; Bunza*et al.*, 2008).

The suggested biosecurity plan for LBMs in order to have a safer and better poultry business in Nigeria as reported by AICP (2008) are;

1. The LBMs must be isolated and confined within a controlled area; distant from human dwellings and farms.
2. There should be a mandatory routine disinfection and cleaning up of the market every forth night.
3. LBMs are to be sited in well-drained locations that are far away from wild birds' concourse.
4. LBM operators must avoid inter-market visitation and contact with dead birds. They should use mobile phones for contacts and business. .
5. All visitors and customers must be restricted to the Reception/Bird view and car park areas only.
6. Trucks bringing in birds must be thoroughly disinfected after every delivery.

7. Drivers, Repairmen, Delivery Personnel and all Operators are to wear protective clothing, clean foot coverings and overalls (plastics) which they must not share with others.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Area

The study areas were Katsina and Kebbi States of Nigeria. The States were randomly selected from the North Western part of the country which is made up of seven states. These include Jigaa, Kaduna, Kano, Katsina, Kebbi, Sokoto and Zamfara states. Both of the States share boarders with Niger republic, are semi-arid and have large poultry markets.

3.1.1 Katsina State

Katsina State is located in the North-Western region of Nigeria, bordering Niger republic, Kaduna, Kano and Jigawa States. The State is located on longitude7°30′E and latitude 12°15′N with a land area of approximately 23,938 Km². It has 34 local government areas and a population size of 5,792,578 people as at the 2006 census (www.katsinastategov.org). There are twenty registered LBMs in the state, located at Sabuwar Kofa, Yankutungu, Kankia, Kita, Dankame, Border (Jibiya), Kankara, Bakori, Batsari, Sheme, Mai'adua, Kafur, DanMusa, Yantumaki, Dutsinma, Jikamshi, Dandume, Mashi and Funtua. The LBMs sampled were Sabuwar Kofa, Border (Jibiya), Kafur, Dandume and Funtua LBMs(Fig. 3.1).

3.1.2 Kebbi State

The state is located in North Western Nigeria and occupies a land mass of 36,800 km^2. It shares boundaries with Sokoto state on the North Eastern axis, Zamfara state on the Eastern part, Niger state on the Southern part and Republic of the Niger on the western part. The state has twenty-one Local Government Areas and a human population of about 3,238,628. Kebbi

state lies between Longitute $11^{0}30'$E and Latitude 11.500^{0} N (2006 census; www.kebbistate.gov.ng). There are nine registered LBMs that are located atBirnin Kebbi, Argungu, Yauri, Zuru, Jega, Dadinkowa, Bagudo, Bunza,andKamba. Three LBMswere sampled for the study;Birnin Kebbi, Argungu and Jega LBMs (Fig. 3.2).

3.2. Study Design

This was a cross sectional study. LBMs from Katsina and Kebbi States were considered for the study. Approximately 25% of the registered LBMs were sampled in each of the states using Avian Influenza Control Programme (AICP) data on LBMs (2006). Markets were randomly selected using simple random sampling and examined for presence of ticks, bio-security practices and risk factors for tick colonization and spread of borelliosis.

Simple random sampling method was used for market selection and convenience sampling method was adapted in selecting birds at the markets for blood collection. Ticks were searched for by beating the cages and tables lightly but repeatedly with hand to dislodge them from their hide outs.

3.3 Blood Collection and Staining Techniques

Blood samples were collected from the wing vein of the chickens using a 2ml syringe and 21 gauge sterile hypodermic needle. The skin was cleaned with alcohol to disinfect the area and to make the vein visible. The blood was transferred into labeled test tubes containing anticoagulant (Heparin) and transported in Coleman boxes with ice, to the

Veterinary Public Health Laboratory, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria.

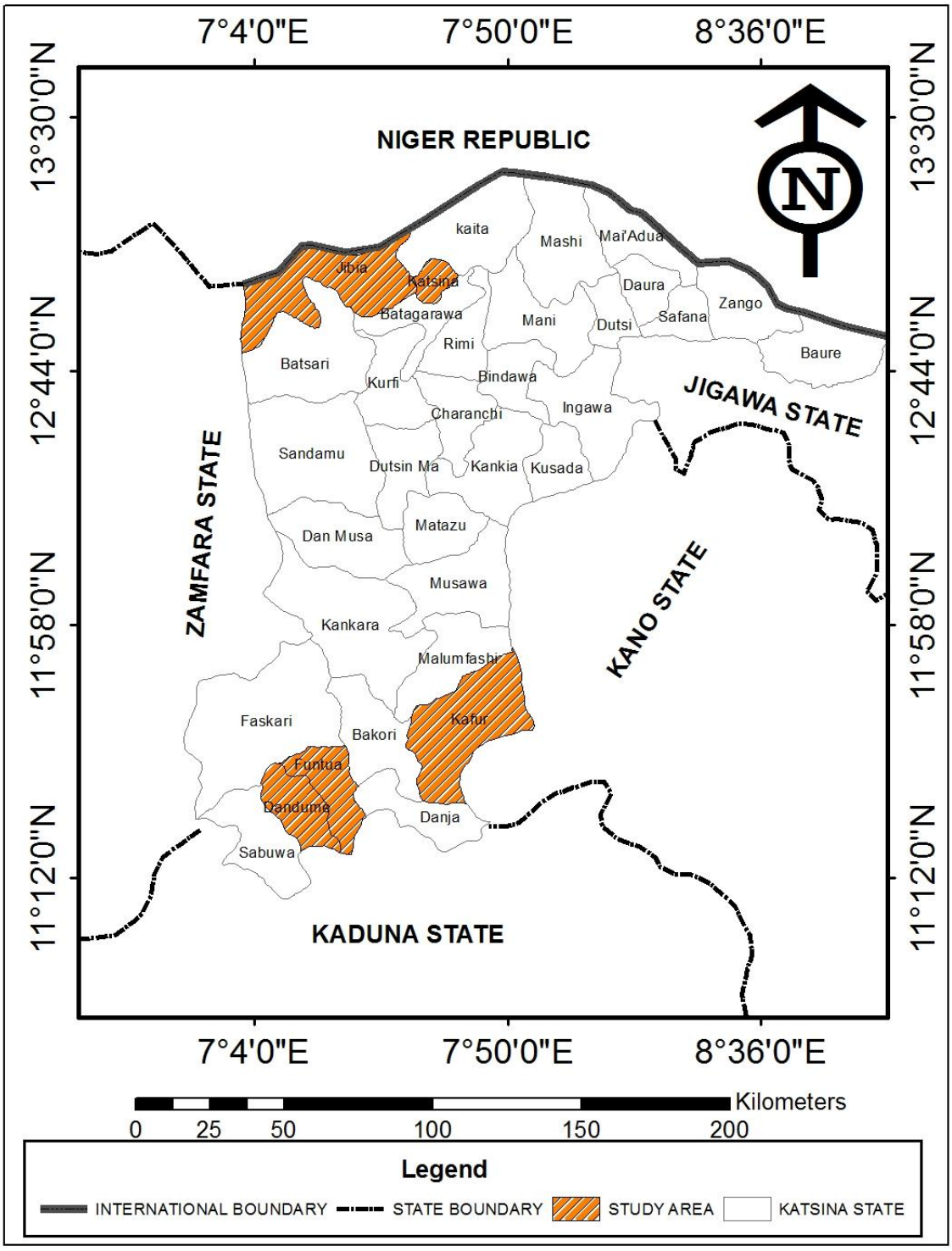


Fig. 3.1: Map of Katsina State Modified From Administrative Map of Katsina State

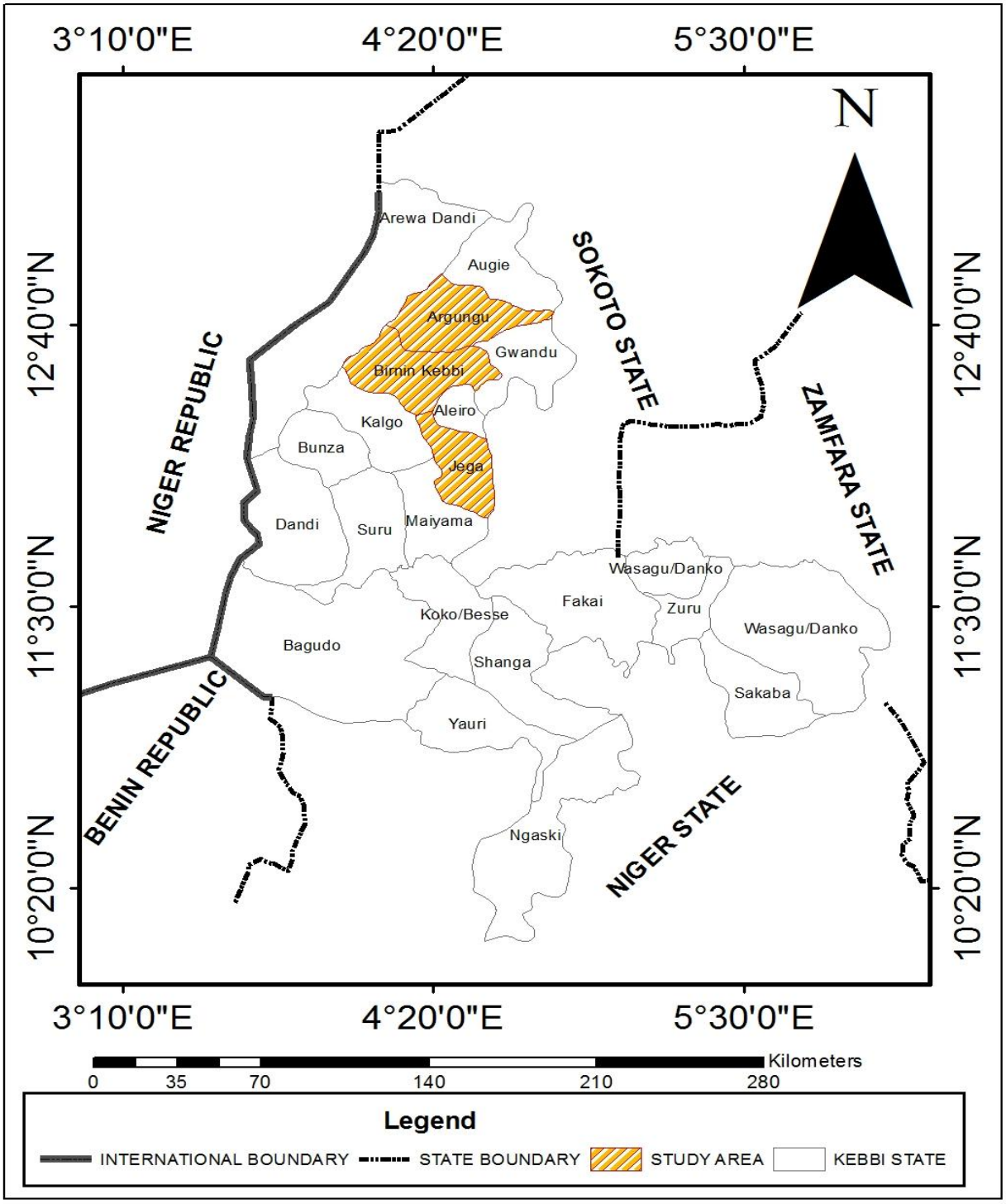


Fig 3.2: Map of Kebbi State Modified From Administrative Map of Kebbi State.

Blood samples were examined using thin blood smear technique to detect *Borellia* species (Christer and Sven, 2008). One drop of blood was placed on a clean grease free glass slide and thin smear was made and allowed to dry, fixed in methanol for five minutes, air dried, stained with Giemsa stain for 45 minutes and then washed under running water. The slide was examined under x1000 magnification using oil immersion. The film with blue to purple spiral thread-like bacteria was considered as positive (Garcia, 1999; Christer and Sven, 2008).

3.4 Tick Collection and Identification

Ticks were searched for and dislodged from crevices by beating the cages and tables several times continuously with hand. The ticks were then collected into a labeled screw-capped plastic containers containing moist cotton and transported to Veterinary Parasitology Laboratory, Ahmadu Bello University Zaria.

The ticks were disinfected using 70% alcohol and examined under the stereo microscope using x10 magnification power. This was done by placing each specimen on to a clean grease free slide with the aid of a pair of forceps. Confirmation of the tick species was achieved through observation of their morphological structures using tick identification keys provided by Hoogstraal (1956) and Walker (2003). Soft ticks have leather like skin that is devoid of cuticle but peripheral striations on its dorsal part, with lateral suture and ventral visible capitum with long legs (Appendix I).

3.4.1 Collection and examination of heamolymph for *Borellia* species

Newly collected ticks were disinfected with 70% alcohol and air dried. One leg of a tick was nipped just above the first coxa and the resulting haemolymph was smeared on grease free and disinfected microscopic glass slide. One smear was made per slide and

air dried, fixed in methanol for five minutes, allowed to air dry, stained with Giemsa stain for forty five minutes and then washed under running water. The blood film was examined under x1000 magnification power. The film with blue to purple spiral like bacteria was considered as positive (Christer and Sven, 2008; Kamani*et al*., 2011).

3.5 PCR Assay for Detection of *Borellia* Species

3.5.1 DNA extraction

DNA was extracted from chicken blood samples that were positive for *Borellia* species in order to obtain the *Borellia* genomic DNA using the DNeasy Tissue Kit (QIAGEN, Germany) as described by the manufacturer with modifications.

The protocol involves putting of 200 µl of the blood samples into Eppendorf tube (38 samples). Then 500 µl of lysis buffer was added to each tube and incubated at 65^0c for 30 minutes in water bath, cooled to room temperature and transferred to spin tubes. The spin tubes were centrifuged at 10,000 rpm for 3 minutes using sigma refrigerated centrifuge (Appendix II) and the flow through was discarded. Five hundred (500) µl of washing buffer I (W1) was added to each spin tube, spun for 5 minutes at 10,000 rpm and the flow through was discarded. The procedure was repeated with 500 µl of washing buffer II (W2) as in W1 and finally, the tube were spun empty. The filters were then transferred to new Eppendorf tubes and 50 µl of elution buffer was added to each and centrifuged at 10,000 rpm for 3 minutes. The Eppendorf tubes containing the DNA filtrate were labelled and stored at -4^0c prior to use for PCR.

3.5.2 PCR Procedures for identification of *Borelli*a species in blood samples

Hemi-nested Polymerase Chain Reaction (PCR) as described by Sin *et al*. (2010) was optimized and carried out to increase the PCR sensitivity of the specie - specific amplification for the 16S rDNA gene.

The genomic DNA preparation was first separated on agarose gel to establish presence or absence of the DNA. Amplirum^{R} MBC076 Purified DNA of *Borellia burgdorferi* was purchased from Vircell^{R} microbiologist and used as positive control in the test.

The extracted DNA was used as template for the primary PCR and its amplicon was used as template for the nested PCR using Gene Amp ^{R} PCR system 9700. QIAGEN long range PCR kit was used, seven microliters of the DNA template was mixed with the components in accordance to the manufacturer's instruction to produce 25 µl reaction volume. This involves the use of 4µl of DNAse free water, 5µl Q solution, 5µl Enzyme buffer, 1µl each of Primer1, Primer 2, dNTPs and Taq polymerase. The mixture was spun to collect all the components before setting on the PCR machine.

The primers used were LD1 (5'-ATGCACACTTGGTGTTAACTA) and LD2 (5'-GACTTATCACCGGCAGTCTTA) for the primary PCR and thermocycling steps were programmed to 30 cycles at 85°C for 30 seconds, 52°C for 30 seconds, and 65°C for 1 minute after an initial heating of the cover, this was followed with final extension at 72°C for 7 minutes. The nested PCR primer was TEC1 (5'CTGGGGAGTATGCTCGCAAGA) and LD2 and the thermocycling steps were programmed to initial heating at 94 °C for 5 minutes, 85 °C for 10 minutes and 65 °C for 10 minutes in a Gene Amp ^{R} PCR system 9700 (Marconi and Garon, 1992; Cyr *et*

al., 2005). Agarose gel of 2% concentration containing 5 µg/ml of ethidium bromide was prepared and used for separating the PCR amplicon.

3.6 Determination of Risk Factors Associated with *Borellia* Infection in the Live Birds Markets

A structured questionnaire was prepared and administered to marketers at the sampled LBMs within the study areas. The questionnaire was validated by pretesting to poultry marketers in LBMs in Samaru and Tudunwada LBMs in Zaria.

The questionnaire (Appendix II) was designed to obtain information on the market, attitudes and practices predisposing to *Borellia* infection. The questionnaire was administered through on spot interview by the researcher while interviewing a marketer in Hausa language.

3.7 Statistical Analysis

Results obtained from this study was analyzed using Statistical Package for Social Sciences (SPSS) version 20.0 to carry descriptive analysis. Chi – squared test and Odds ratio were used to test the strength of the association between the categorical variables and $P< 0.05$ was considered significant. Tables and graphs were used to show level of tick infestation, history of tick bite and tick control program in different markets. *Borellia* prevalence was calculated using the formula;

$$\text{Prevalence} = \frac{\text{No of birds with } Borellia}{\text{Total number of birds sampled}} \text{ X } 100$$

3.8 Study Instruments and Materials

1. Structured LBMs questionnaire.
2. Sample bottles with EDTA anticoagulant for blood collection.

3. Coleman box with ice pack for blood transportation.
4. Syringes and needle.
5. 70% ethanol.
6. Cotton wool.
7. Plastic containers with screwed caps for tick collection and transportation.
8. Laboratory record book
9. Examination hand gloves
10. Glass slides
11. Stereo microscope
12. Bi ocular light microscope
13. Copling jar
14. Giemsa reagents; methanol, Giemsa powder, glycerol, water
15. QiagenDNeasy Tissue Extraction Kit
16. Agarose tank, Power pack and combs
17. Agarose powder
18. Running buffer (TAE)
19. Ethidiumbromide
20. Thermo cycler Gene Amp ^{R} PCR system 9700
21. Bio Rad documentation machine
22. Refrigerated centrifuge
23. PCR Pipette
24. Eppendorff tubes
25. PCR tubes
26. Computer
27. Digital camera

CHAPTER FOUR

RESULTS

4.1. Colonization of Live Bird Markets

A total of eight LBMs were sampled, five in Katsina State and three in Kebbi State. Soft ticks were found in crevice of cages and tables ofBorder market, Sabuwar Kofa Market, Funtua market, Dandume market and Kafur marketduring the survey period, giving 60% tick colonization (n = 5) of the markets sampled in Katsina State. All the markets sampled in Kebbi State were found to be infested with soft ticks (100% colonization). The market were Birnin Kebbi market, Jega market and Argungu market. A total of 6 (75%) out of the 8 LBMs sampled were colonized by soft ticks for the two states. Soft ticks collected from crevice of cages and tables in LBMs of both the States were identified as *Argas* species.

Birnin Kebbi, Jega, Funtua and Border LBMs reported having experienced tick infestation always but Argungu, Sabuwar Kofa, Dandume and Kafur markets reported having tick infestation occasionally especially during wet season when tick experience is at peak (Figure 4.1).

There were reports of having occasional tick bite experience in all the markets except in Sabuwar Kofa market where there was no history of tick bite at all. Only marketers at Funtua market practiced regular tick control program, while five markets namely, Argungu, Dandume, Kafur, Jega and Jibiya markets did not practicecontrol program at all, but Sabuwar Kofa and Birnin Kebbi markets occasionally sprayed the market with acaricides (figure 4.2).

All the 8 markets do not carry out ante mortem and post mortem examination and did not keep records for diseased or dead birds. Processing of chicken took place in all the markets manually, without the use of protective clothing, indicating very low bio security practice.

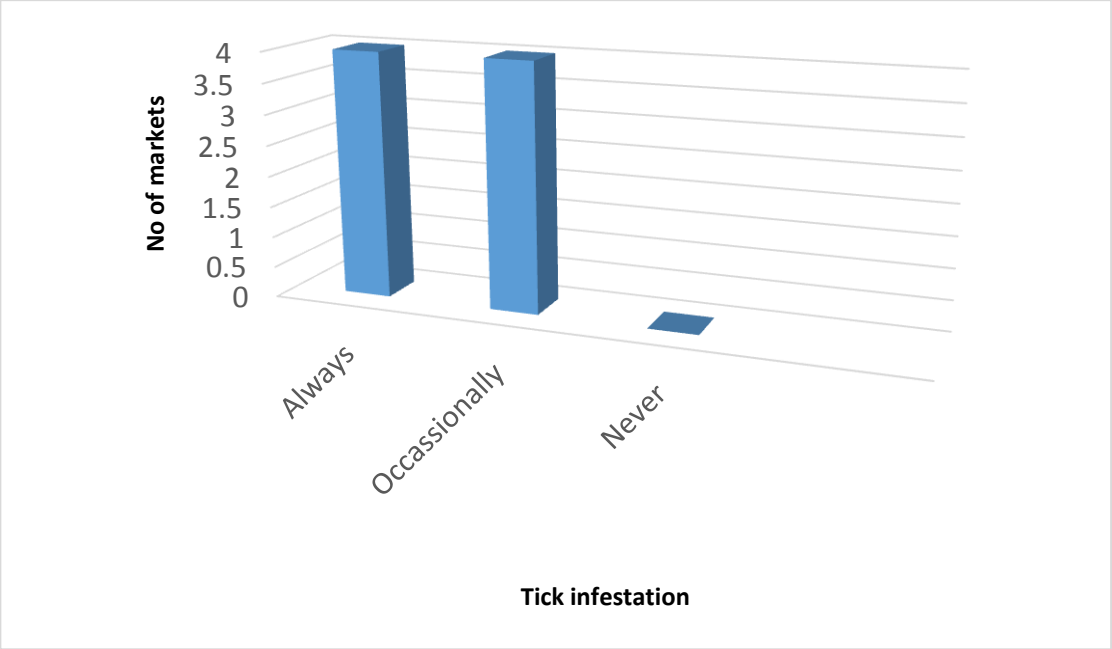


Fig. 4.1 Distribution of LBMs based on the history of tick infestation at the market

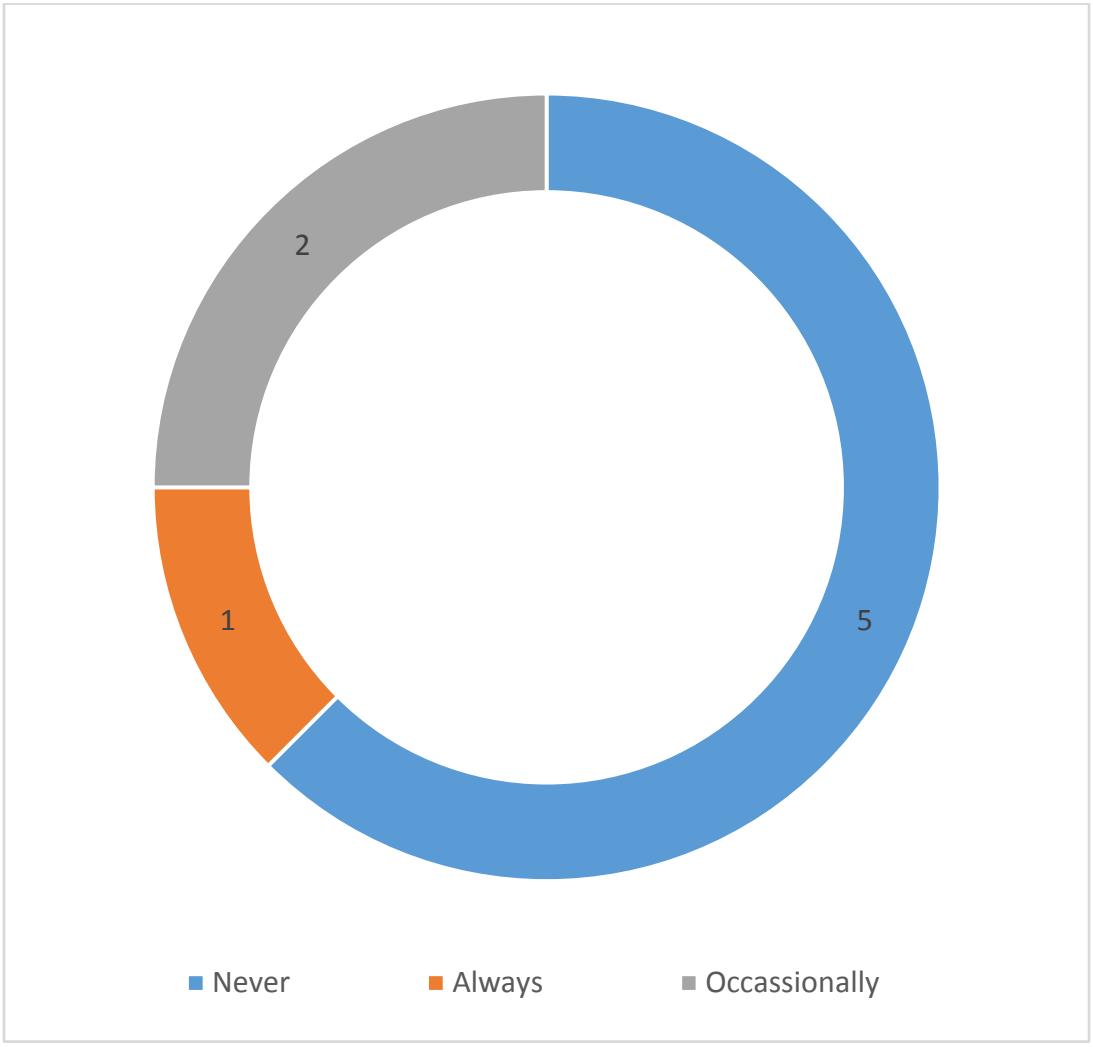


Fig 4.2 Distribution of LBMs based on tick control program

4.2. Detection of *Borellia* Species from Soft Ticks

Out of the54 smears made fromtick's haemolymphs,no*Borellia* specie was detected by microscopy.

4.3 Presence of *Borellia* Species in Chicken

Out of the 374 chickens examined, 38 (10.3%) were positive for *Borellia* species. There were 14 positive samples in Katsina state (n = 232) and 24 positive samples in Kebbi state (n = 142) giving the prevalence rate of *Borellia* species in chickens sold at the LBMs of the Katsina and Kebbi states of 16.9% and 6.0% respectively (plate 4.1) .

4.4 Detection of Zoonotic *Borellia* Species

The *Borellia* positive samples were further subjected to PCR technique. DNA was successfully extracted from 35 out of 38 samples. Hemi nested PCR technique was carried out as described using species - specific primers for the detection of *Borelliaburgdorferi*species. The resulting gel image shows no band at the expected band size of 293 bp, but it had band size at 180 bp after several optimization process (Appendix II)

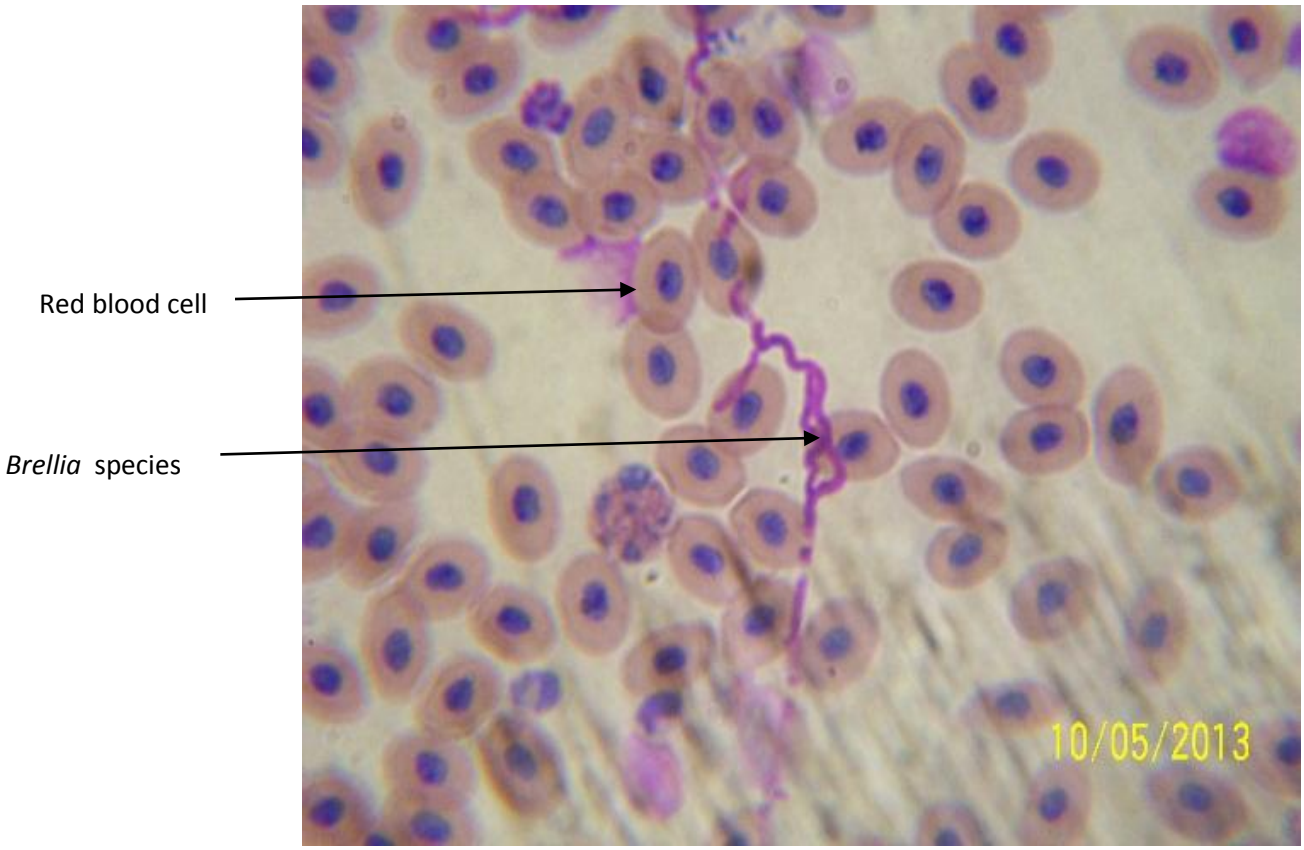


Plate 4.1*Borellia* speciesin Giemsa stained thin blood smear (Bk14)

4.5 Distribution of *Borellia* Based on Various Risk Factors

Prevalence of *Borellia* species was classified based on the state where the markets are located. Out of 232 chicken blood samples examined in Katsina state 24 (10.3) were positive for the *Borellia* species, while *Borellia* were detected from 14 (9.9%) out of 142 chicken tested in Kebbi State.There was no significant difference in the occurrence of *Borellia* species in chickens in LBMs in the two states ($P > 0.05$) and Odds Ratio of 1.05 (Table 4.1).

The LBMs were grouped based on marketing days, either daily or weekly. A total of 299 samples were derived from weekly LBMs and 75 from daily LBMs. *Borellia* species were found to be more frequentin chickens sampled from daily market (16%) compared to those sampled from weekly markets (8.7%).There was no statistical significant difference between the occurrence of *Borellia* species in markets that operate daily and weekly ($P > 0.05$).

Location of the LBM in respect to the conventional market was studied to find out the difference in the prevalence of *Borellia* species from chickens sold at the markets.Markets located far away from the conventional marketshad higher prevalence of 28 % while LBMs located within the main markets had 9.7 % prevalence of *Borellia*. The least prevalence was found in LBMs segregated from the conventional market with a *Borellia* prevalence of 2.0%. These differences were found to be statistically significant ($P < 0.05$).

Distribution of *Borellia* species based on the type of housing at the market was studied to assess effect of living birds in markets on the prevalence of *Borellia*. Some markets kept all their birds in cages (All in cages) while in other markets some birds were in cages (some in cages) while others were not. *Borellia* species prevalence of 14.3 % was found in markets where all the birds were in cages while a prevalence of 5.9 % was recorded in markets where some birds were kept in cages (Table 4.1). This difference based on the housing type was found to be significant ($P < 0.05$) and Odd ratio is 0.4 was observed.

Wooden cages with zinc sheet cover and palm cages were the two cages found at the LBMs studied (Appendix III). Palm cages are also used for transportation of chickens from market to market. Effect of these cages on the occurrence of*Borellia*among birds in the market was determined. There was no significant difference in the occurrence of *Borellia* between the two cage types ($P > 0.05$) but the prevalence was found to be higher in wooden cages (13.2%) than in palm cages (8.9%) (Table 4.1).

Isolation cages for keeping sick birds was used as criterion to find out the distribution of the *Borellia* species. Birds examined were differentiated basedon the presence of isolation cages in which we categorized the markets as ‘Few marketers’ having isolation cages, ‘some marketers’ having isolation cages and‘none of the marketers’ having isolation cages. Prevalence of 7.6%, was recorded for few marketers, 13.0 some marketers and 6.9 for none marketer having isolation cages. No significant differencewas observed ($P < 0.05$).

Borellia species prevalence from different markets based on the method of disposing the birds was observed. All the markets sold part of their stock alive. Some markets sold just ‘few alive’ while others sold ‘some’ of their birds depending on festivity seasons. The result was analyzed and presented on the Table 4.1. No significant difference (P>0.05) was observed. The prevalence of 18.80% and 5.08% were recorded for ‘Few’ birds are sold alive and ‘Some’ birds are sold alive respectively

End of market day activities was used to study the prevalence of the *Borellia* species. There was no market that slaughtered the remaining birds at the end of the day. In most of the markets (75%) birds are left to stay in their cages at the end of the day while others (25%) moved them to the neighbouring market. Prevalence of *Borellia* species differed in these markets. The study recorded a prevalence of 11.5% in birds that are left to remain in the market overnight and 7.4% in those that took the birds to the next market, but this was not statistically significant (P >0.05) (Table 4.1).

Table 4.1: Risk Factors Associated with the Spread of *Borellia* Species in LBMs of Katsina and Kebbi States

Risk factors	Variable	No positive (%)	Total	OR (95%)	P value
State	Katsina	24 (10.3%)	232	0.9(0.4 -1.9)	>0.05
	Kebbi	14 (9.9%)	142		
Type of market	Daily	12 (16.0)	75	0.5 (0.24 – 1.89)	>0.05
	Weekly	26 (8.7)	299		
Proximity to main market	Far from market	14(21.9)	64	5.7 (3.0 – 6.3)	>0.05
	Segregated from market	23(8.8)	260		
	In market	1 (2)	50	1	
Type of cages	Palm	23(8.8)	260	1.6 (0.9-3.1)	>0.05
	Wooden	15 (3.2)	114		
Type of housing	All in cages	27 (14.3)	189	0.4 (0.2 -0.8)	< 0.05*
	Some in cages	11 (5.9)	185		
Birds sold alive	Few birds	13 (12.4)	105	0.7(0.4 – 1.5)	> 0.05
	Some birds	25 (9.3)	269		
End of day	Left at the market	29 (11.5)	252	0.6 (0.3 – 1.3)	> 0.05
	Taken to the next market	9 (7.4)	122		
History of tick bite in market	Occasionally	24 (7.7)	310	0.3 (0.15– 0.6)	< 0.05*
	Never	14 (21.9)	64		
Isolation cages	Some marketers	(13.0)	115	0.5 (0.3 – 1.2)	>0.05
	Few marketers	99 (7.6)	99		
	None of marketers	160 (6.9)	160	1	
Tick control program	Always	14 (21.9)	64	0.4 (0.2 – 0.5)	<0.05*
	Occasionally	13 (10.4)	125		
	Never	11 (5.9)	185	1	

- Shows factors that are statistically significant

CHAPTER 5

5.0 DISCUSSION

Live bird markets sampled in Katsina and Kebbi States of the North Western Nigeria were colonized by soft ticks and the ticks were found in the crevices of cages and tables. Thefinding is in agreement with the report of Bunza*et al*. (2008), who reported that Sokoto LBM was colonized by ticks and that of Abdu (2014) who reported that, adult soft ticks hide in crevices during the day time. The infestation of the market is likely due to the fact that majority of the birds sold are village chickens that carry various parasites as reported by Biu*et al.* (2007) and Usman *et al*. (2012). The presence of ticks in the markets may affect business activities through nuisance caused by tick bites, transmission of diseases such as TBRF and Q fever to the marketers, and transmission of pathogens to the poultry (Buriro, 1985; Seifert, 1996). This may contribute to national food insecurity, increase in number of hospital visits and poverty.

The Soft ticks found were *Argas* speciesas reported by early researchers that *Argas*ticks are the most predominant chicken ectoparasites and the most prevailing in LBMs as reported from Sokoto LBM (Biu*et al*., 2007; Bunza*et al*., 2008; Usman *et al*., 2012). Abdu (1987), reported that, *Argas persicus* larvae were involved in the concurrent transmission of infectious bursal disease and avian spirochaetosis. Therefore, there is

risk of transmitting such diseases to healthy chickens sold at the markets which may cause poultry mortality especially in communities that get their initial or replacement stock from LBMs.

A history of tick biteswere reported in all the markets (always 42.0% and occasionally, 58.0%) The nuisance caused by tick bite at the markets and possible transmission of tick borne diseases such as relapsing fever to the marketers and the market community may interfere with marketing activities and community wellbeing. This study did not categorically determine the impact of the disease, however occurrence of the disease in Northern Nigeria may contribute to rural poverty level through loss of man power, increase expenses on medical bills and poor productivity.

Tick control program was absent in 37.2 % of the LBMs, but 19% reported the spraying withacaricides always while 43.8% sprayedacaricides occasionally in efforts to control ticks. The finding indicated low awareness level on problems of ticks by respondents which in turn may contribute to increase in tick bite nuisance and tick borne diseases to the marketers and the market community.

Haemolymph smears examined from 54 engorged *Argas*species were negative for *Borellia*species. This may be due to the low prevalence of the bacteria in the ticks as reported by Anna *et al.* (2012) where a prevalence of 0.4% was reported from questing hard ticks.

Borellia species was detected in 38 out of 374 chickens tested. The outcome showed that, *Borellia* species is circulating in the study area among the village chickens, which is in agreement with the report of Saidu*et al.* (2003). Poultry infection with the bacteria can lead to destruction of heamatopoitic organs resulting in anaemia, poor production and death (Iman*et al*., 2010).

The outcome of PCR using species - specific primers (Marcon and Garon, 1992) did not yield the expected amplicon (293 bp) indicating the likelihood that, the species detected microscopically were not members of *Borellia burgdorferi*, which are zoonotic. However, there were some non – specific amplicons.

Risk factors associated with spread of *Borellia* species in LBMs were assessed through the use of questionnaires. The results of the study showed that health records, quarantine, ante mortem and post mortem inspection of birds were practically absent in all the markets sampled, which is in agreement with the report of AICP (2008). This meansthat early detection of any disease problem or signals may not be possible and the market community is at risk of disease outbreak. In addition, trace back activities of any disease to LBMs will be difficult as the marketers move from one market to another without keeping any record of number of sick birds, death, source of the birds and the transactions carried out at the end of the day.

Katsina state recorded higher prevalence of *Borellia*species (10.4%) than Kebbi State (9.9%), though thiswas notstatistically significant. Both states are in the Sahel savannah and share borders with Niger Republic. This is likely the pioneer report on prevalence of *Borellia*species in these States.

Daily LBMs had higher prevalence of *Borellia*species (19%) compared to weekly LBMs (9.5%). Higher prevalence of *Borellia*speciesin daily markets may be associated with keeping the birds in the market overnight which increases the exposure to tick bite, unlike in weekly markets where birds from different places are brought together with low chances of transmission.Hoogstraal (1956) reported that soft ticks hide in crevices during day time around their host and are more active at night when probably they feed on their hosts, coupled with less disturbance of the ticks at night. The risk of tick bite is not to the birds only but also to the security personnel who guard the markets during night (See appendix IV).

Prevalence of *Borellia*specieswas found to be lower in markets that were segregated from the conventional market (2%) and higher (28%) in markets located far from them. Research findings to support the outcome is not readily available, but may be due to level of awareness that some poultry diseases are zoonotic and control measures are in place.

The prevalence of *Borellia*specieswas higher in markets where all the birds were kept in cages (14.3%) than where only some are in cages (5.9%). This may be associated with the fact that markets where all birds were kept in cages, are left overnight at the market. Published literature on effects of cages on disease transmission are limited. This finding may be a compounding factor in the spread of disease since it is not a good practice to leave birds outside cages.

End of day activities shows that, birds are left at the market in about75% of the LBMssampled permitting the spread of parasites and diseases especially since TBRF is transmitted mainly by soft ticks which are more active at night (Busvine, 1975; Abdu, 2014). The finding is in agreement with earlier report by (Paola*et al*., (2008) that birds are kept in markets over night in most daily.

Use of protective clothing was absent among marketers in all the markets and they processed chickensmanually; AICP (2008) reported that ‘‘the dependents of the marketers manually process the birds’’ which may permit *Borellia* infection since a very virulent strain has been reported to penetrate intact skin (Barnes, 1997).

All the cages used in the markets were made of palm or wooden materials, which by construction have many crevices which soft ticks are known to inhabit. The studydemonstrates that the type of cages may also help in vector stability and disease spread.

CHAPTER SIX

6.1 CONCLUSION

Live bird markets in Katsina and Kebbi States were found to be mostly located within or around an existing market and are very important in poultry business especially when it involves selling and buying of village chickens. The research shows that, LBMs in the study areas were colonized by soft ticks (*Argas*) and most of the birds allowed to remain in the market at the end of the daywhich gives room for parasites and diseases to spread.*Argas* species were found in crevice of cages and tables during the survey and there was a low commitment towards tick control by marketers despite the history of tick bites in 87.5% of the markets. The marketers at LBMs studied processed birds manually without the use of any protective wares. Quarantine of newly introduced chickens, ante mortem examination, post mortem examination, and health records were absent in all the markets, but few marketers had isolation cages.Thus, application of biosecurity is minimal in all the markets.

Village chickens sold at the LBMs were foundto harbour *Borellia* species. *Borellia* prevalence of 10.2% was established for the two states, but their zoonotic potential was not established by the PCR technique used. In addition, *Borellia*was not detected by microscopy from haemolymph smears of ticks collected during the survey.

Risk factors associated with the bacterial transmission were studied. All the factors were analyzed, those found mostly in daily LBMs contribute to the rate of the bacterial transmission in chickens than those found in weekly LBMs. The most important factors found to result in increased prevalence of *Borellia*were presence of ticks and overnight stay of birds in tick infested markets.

Live bird markets may serve as sources of soft ticks and village chicken may promote the transmission of *Borellia* to the marketers and neighbouring communities because they harbours soft ticks.

6.2 RECOMMENDATIONS

Based on the findings of the present study, the following recommendations are made;

1. Authorities should enforce mandatory tick control programs in all LBMs for better marketing activities, food security and public health.
2. Marketers should be encouraged to use protective clothing during handling and processing of birds.
3. Birds should not be kept overnight in tick infested areas.

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APPENDECES

Appendix I Questionnaire

Live Birds Markets Assessment Questionnaire

The purpose of this form is to determine conditions present in Live Bird Markets that may favour ticks infestation and or spread of tick transmitted diseases.

Please fill or tick where applicable.

Section: A

1. Serial No……
2. Market name: ……....
3. Location: …….
4. LGA: ……
5. State: ……
6. Type of LBM: ……..

Section B: Infrastructure

Market location: in market ( ) Segregated from market ( ) Far from market ( )

Loading area: Present not functional ( ) Present functional ( ) Absent ( )

Nature of birds housing: Some in cages ( ) All in cages ( ) None in cages ( )

Type of cages: palm ( ) Wooden ( ) Metal ( ) Plastic ( )

Isolation cages: All marketers ( ) some marketers ( ) few marketers ( )

Section C: Practices

Sellers having protective cloths: Some ( ) all ( ) None ( )

Availability of AM inspection: Some times ( ) all times ( ) Absent ( )

Availability of PM inspection: Some times ( ) all times ( ) Absent ( )

Preventive services: Some times ( ) all times ( ) Absent ( )

Availability of quarantine services Some times ( ) all times ( ) Absent ( )

Records available: Mortality ( ) Morbidity ( ) Both ( ) None ( )

What happen to birds at the end of the day: Taking to the next market ( ) left to sleep in market ( ) Slaughter ( )

Birds are sold alive All ( ) some ( ) few ( )

Processing of birds: Some times ( ) all times ( ) Not at all ( )

Section D: Ticks

Experience of ticks in the market: Occasionally ( ) Always ( ) Never ( )

If present, where are they mostly notice? Everywhere ( ) on the birds ( ) on the Table ( ) Crevices ( ) other ( )

Experience of tick bite in the market: Occasionally ( ) Always ( ) Never ( )

Presence of tick control program: Occasionally ( ) Always ( ) Never ( )

What season of the year do you experience highest infestation?

Hot dry ( ) cold dry ( ) wet ( )

Appendix II

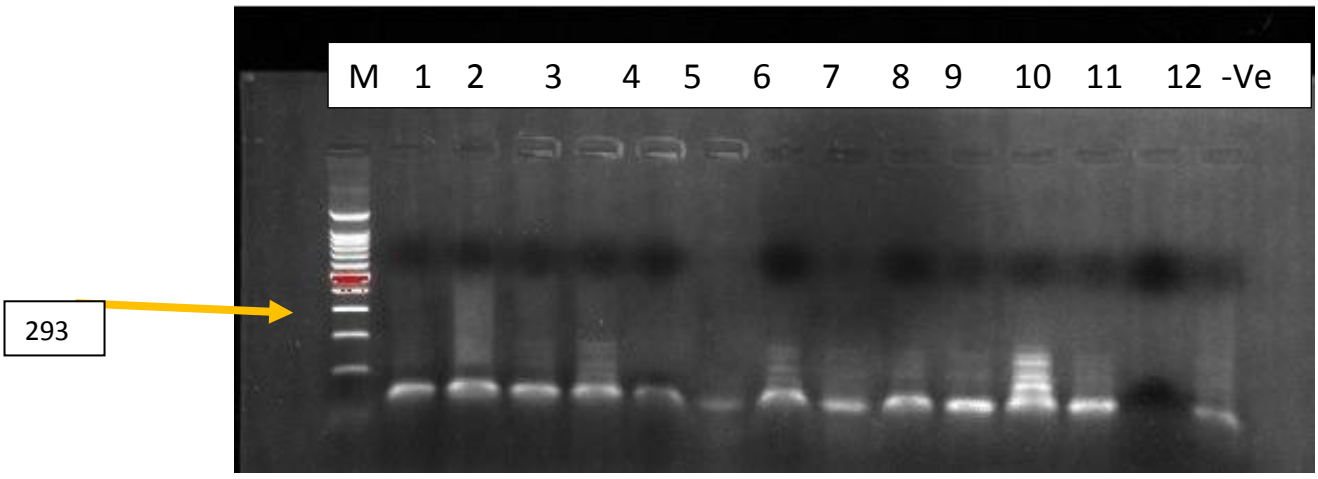


Plate II. Gel image of unknown *Borellia* species

Lane 1 Molecular size markers (lane M) in based pairs are indicated on the left

Lane 2: KT44 Lane 3: JBY24 Lane 4: KT43

Lane 5:KT03Lane 6:JBY21Lane 7: JBY29Lane 8: JBY13Lane 9: BK47Lane 10: BK74Lane 11: BK73Lane 12: KT45Lane 13: KT35Lane 14: Negative control

Lane 15: Positive control

Appendix III



A security personnel at LBM

Appendix IV

(A)



(B)



Type of cages

(a) Palm cages from Jega LBM
(b) **Wooden cage from Sabuwar kofa LBM**