CHARACTERIZATION OF *CAMPYLOBACTER* SPECIES ISOLATED FROM SHEEP AND EFFECTS OF CRUDE EXTRACTS *OF ACACIA NILOTCA* AND VI*TEXDONIANA* ON THE ISOLATES

By

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A thesis submitted to the Postgraduate School,
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Award of Degree of Master of Science.

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DECLARATION

I hereby certify that the work presented in this thesis is original and was carried out by me under the supervisions of Prof. J.O. Adekeye, Dr J.K.P. Kwaga and Dr J.O.O. Bale of Ahmadu Bello University, Zaria.

The work of other investigators are duely acknowledged and referred to accordingly. I declare that no part of this thesis has been submitted elsewhere for the award of a Degree or Diploma.

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CERTIFICATION

This thesis entitled "Characterization of *Campylobacter* Species Isolated from Sheep and Effects of Crude Extract of *Acacia nilotica* and *Vitex doniana* on the Isolates" by Mashood Abiola Raji meets the regulations governing the Award of the Degree of Master of Science of Ahmadu Bello University, Zaria and is approved for its contribution to scientific knowledge and literary presentation.

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DEDICATION

I dedicate this project to Almighty Allah for his kindness, mercy and love for keeping me alive till today for the purpose best known to Him

and

To my late mother and grandmother, I love you all.

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ABSTRACT

Campylobacter species isolated from Kaduna State were characterized into their species and bioserogrouped. Their susceptibilites to the extract of the leaves of *Acacia nilotica* bark of *Vitex doniana were* studied. A total of 1100 sheep samples were collected from Kaduna abattoir and National Animal Production Reach Institute (NAPRI), Shika. The samples comprised 250 gallbladder, 250 intestinal contents, 100 fetal stomach contents from Kaduna abattoir while 250 rectal swabs and 250 vaginal swabs were obtained from sheep at the NAPRI Small Ruminant Programme flocks.

Of the total of 1100 samples, 39(3.54%) yielded *Campylobacter* organisms. The highest isolation rate (6.8%) was from samples of intestinal contents followed by those from gall bladdcrs(4.0%). Samples from the vaginal swabs and fetuses had the lowest isolation rates of 2.80% and 0% respectively.

Of all the 39 *Campylobacter* isolates, 79% were characterized as C. *fetus* subsp *jejuni*, 13% as *c. coli, and* 8.0% as C. *laridis- Campylohacter coli and C. laridis* were isolated from gall bladder and intestinal contents only but not from vaginal and rectal swabs. *Campylobacter jejuni* biotype 1 accounted for 40.3% of the total isolates, C laridis biotypes 1 and 11 were also isolated and accounted for 5 % and 3 % of the isolates respectively and 5% of the isolates were non-typcable

Serogroups 36 and 4 were the commonest serogroups repesenting 10.3% and 7.7%of all serogroups respectively. Other serogroups recorded in sheep in Kaduna State were 29 (5.1%),20(7.69%) and 55 (2.6%). They all belonged to *C. jejuni Campylobacter coli were* represented by serogroups 4 (7.7%) 6 and 84 repesenting 2.56% each, C .*laridis* isolates were not

serotyped. There was no significant difference in the recovery of the different serogroups from the different specimens investigated in sheep at two locations surveyed.

The minimum inhibitory concentration of A nilotica ethanol extract was 80 mg/ ml while that of the water extract for *Acacia nolitica was* 250 mg/ ml. Both the water and ethanol extracts of the *V doniana had no* inhibitory effect on the *Campylobacter* isolates at concentrations up to 500 mg/ml.

The isolation of *Campylobacter* organisms from rectum, vagina, gallbladder, and intestinal contents is a clear indication that sheep may serve as a reservior of these organisms in Nigeria.Similarities between documented human *Campylobacter* isolates in Nigeria and those in the present study raise the possibility of cross-transmission between sheep and man. It is concluded that biotyping and serogrouping can be used for epidemiological study of campylobacteriosis due to *Campylobacter jejuni in* sheep in Kaduna State of Nigeria.

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CHAPTER ONE

INTRODUCTION

1.1 Small Ruminant Production In Nigeria

Small ruminants (sheep and goat) which are very important towards meeting the protein requirement of the Nigerian populace continue to be neglected resources in developing countries. For instance, about 50% of the sheep and over 80% of the goats of the world are found in the third world countries (David-West, 1985). By 1982, the world population of sheep was 1130.8 million while that of goats was 468.8 million (F.A.O,1982) . In Africa, sheep and goat constitutes 16.3% and 31.7% of the ruminant population respectively. It has also been estimated that goats contribute 16.0% and sheep 5% of the total estimated 813,000 metric tonnes of meat produced per year domestically in Nigeria (F.A.O., 1992).

The livestock population of Nigeria has been estimated at 11 million cattle, 30 million goats and sheep, 0.9 million pigs and about 150 million poultry (Ademosun, 1985). The livestock industry in Nigeria has a capital value of about N2.5 to N3 billion and contributes between 5 - 7% of the Gross Domestic Product (GDP). It is a matter of grave concern to policy makers, researchers, and even producers that this enormous magnitude of livestock resources does not show the capacity to meet the animal protein needs of Nigerians (David-West, 1985).

The major constraints to the production systems are inefficient use of the goat and sheep genetic potentials, poor management system, health problems and inadequate marketing outlets (Hussain *et al.*, 1983). This trend, if left uncorrected, is likely to continue with severe inadequate animal protein inbalance among Nigerian populace.

World wide, reproductive disease and wastage which include abortion and preweaning mortality have been identified as one of the major economic losses in livestock production

(Dennis, 1974). There have been few reports on the causes of kid and lamb mortality in Nigeria (Adu *et al.*, 1979 ; Osuagwuh and Akpokodje, 1981). In addition, information on cases of abortions in sheep and goats are very scanty (Falade *et al.*, 1975; Okoh, 1980). Among disease causing agent in small ruminants is *Campylobacter* organisms.

Campylobacter species are now recognized as major enteric pathogens in man and animals, causing diarrhoea, enteritis and occasionally septicemia and have been reported to equal or exceed *Salmonella* species and *Shigella* species in prevalence (Prescott and Manroe, 1982). Until recently *Campylobacter fetus* subsp *fetus* was primarily regarded as an animal pathogen that caused bovine and ovine abortion and sterility. However, since 1980, sporadic and epidemic food borne disease attributed to *C. fetus* subsp *fetus* have been reported in man (CDC, 1981). *Campylobacter fetus* subsp *jejuni* and other related species have long been established as causative agents of human enterocolitis (Ketley, 1997).

Vibrionic abortion in sheep was first recognized in 1913 by Mcfadyean and Stockman. In sheep, the disease is characterized by abortion, still birth and birth of weak lambs during the late pregnancy which may or may not survive, and gastroenteritis in lambs (Kimberling, 1988). The species of *Campylobacter* reported by several workers in cattle and sheep all over the world include *Campylobacter fetus* subsp *fetus* and *Campylobacter fetus* subsp *jejuni* (Firehammer *et al.* 1962; Clark, 1971; Coker and Dosumun-Ogunbi 1986). In Nigeria, attention has been focused on *Brucella* organisms as the cause of infectious abortions and coliforms as agents of enteritis in sheep and goats (Bale, 1980; Njoku, 1972; Otesile and Oduye, 1983). It is evident however, that the isolation rates of these organism and even serological evidence of brucellosis in aborting ewes have left no one in doubt that other organisms particularly the more fastidious *Campylobacter* organisms may also be involved

(Bawa *et al.*, 1991). The insufficient knowledge of these organisms and the difficulty in their isolation and identification may be responsible for the poor attention they have received until now .

Many epidemiological studies in different parts of the world have indicated the role of domesticated animals as a natural reservoir for *Campylobacter* organisms (Blaser *et al.*, 1980; Grant *et al.*, 1980). In Nigeria, Adetosoye and Adeniran, (1987), reported the isolation of *Campylobacter fetus* subsp jejuni biotype I and II from different breeds of ovine, caprine, bovine, canine, porcine and poultry, at Ile-Ife located in the western part of Nigeria.

1.2 Justification for the Study

Epizootic abortion or vibrionic abortion commonly referred to as ovine campylobacteriosis is a devastating disease in sheep and goats. It renders the time, effort and money used on raising these animals wasted during pregnancy. This is because up to 50% of the flock can abort when the disease outbreak occurs (Collins and Ross, 1984; Fujita *et al.*, 1995). Furthermore, infected ewes are usually unthrifty and emaciated resulting in very poor market values. Lambs born alive by affected dams are very weak and may not survive. The country cannot afford to continue with such colossal losses of small ruminants at this particular time of economic depression and high cost of beef in the market.

While not minimising the importance of nutrition and other management practices, one of the keys to improving ruminant production in Nigeria is through a systematic prophylactic and curative animal health programme. The greatest impact of research on ruminant production in Nigeria has been in the area of animal health, yet poor health still remains one of the greatest constraints to intensive and improved ruminant production (Voh *et al.*, 1993). This

contradiction may be due partly to lack of adequate knowledge of field disease situation or failure to direct animal health programme to the producer that matter.

One would therefore not adequately talk about ruminant production and its improvement without critically looking at the disease situation in the field of sheep and goats especially for important disease agent such as *Campylobacter* for its effective control and prevention in Nigeria. With a view to improving small ruminant production in Nigeria and attempt to elucidate the role of domestic sheep as a natural reservoir of these important agents of abortion and enteritis in the northern part of Nigeria, this study was designed with the following objectives:

(a) To determine the occurrence of ovine *Campylobacter* in Kaduna State of Nigeria.

(b) To characterize the isolates by biotyping and serotyping methods.

(c) To study the antimicrobial effects of extracts of the two medicinal plants against the *Campylobacter* isolates.

CHAPTER TWO

LITERATURE REVIEW

2.1. **Significance and Historical Context of** *Campylobacter*

Campylobacter jejuni and related species are causative agents of human enterocolitis and are the most common bacterial cause of diarrhoea in many industrialized countries. *Campylobacter jejuni* infection is consequently responsible for major public health and economic burden (Tauxe, 1992; ACMSF, 1993). It has been estimated that about 3000 human campylobacteriosis cases are reported per month in the United Kingdom (Skirrow, 1987; Healing *et al.*, 1992). It is also estimated to be responsible for 7% of all cases of gastroenteritis in Great Britain (Porter and Duguid, 1989). Nevertheless, the public awareness on campylobacteriosis remains limited. After nearly two decades of scientific investigation, much of the biology of *Campylobacter spp* and mechanisms by which they cause disease are still relatively poorly understood. More recently, scientific and governmental interest has been rekindled on the public health problem posed by *Campylobacter* (Tauxe, 1992; ACMSF, 1993). The incidence of *Campylobacter* infection has also clearly risen dramatically and this probably reflects a change in eating habits. For example an increase in the popularity of chicken and pork. The peak incidence rate is in young adults and young children (Tauxe, 1992). However, when considered the proportion of fecal samples within each group, the number of stool containing *Campylobacter* is higher in young adults (Butzler and Skirrow, 1979). Below the age of 45, there is yet an unexplained, but consistently higher incidence of infection in male compared to females (Ketly, 1997). Most cases appear to be sporadic and show a consistent seasonality (Tauxe, 1992).

The incidence of infection derived from laboratory isolation is almost certainly a significant underestimate of the true rate of the disease in the general population(Tauxe,1992).A limited study in a general population (Kendall and Tanner, 1982), calculated an annual incidence of 1.1% and is close to an estimate of 1.0% made for the US population (Tauxe, 1992).

Campylobacter infection in developing countries appears to have different clinical and epidemiological characteristics to that described above for industrialized nation (Taylor, 1992).The clinical spectrum of enteric disease due to *C. jejunni* and *C. coli* ranges from a severe inflammatory diarrhoea to a generally mild non-inflammatory watery diarrhoea. The former is the most common clinical presentation of patients from industrialized nations whilst the latter is the usual pattern seen in developing nations. Disease is usually restricted to children with no apparent peak in adults, no strong pattern of seasonality and a higher incidence of infection complicated by a higher rate of asymptomatic carriage. Many of these differences are probably due to higher rate of exposure and infection early in life, resulting in a different pattern of immunity (Taylor, 1992).

Although strain differences have been correlated with clinical symptom (Ruiz-Palacios *et al.*, 1992; Everest *et al.*, 1992), there is little evidence for the presence of different types of strains in developing countries compared to those found in industrialized countries (Taylor 1992). Indeed, the importance of *Campylobacter jejuni* as a cause of travellers diarrhoea (Taylor 1992), has led to the observation that the spectrum of illness in travellers is similar to that described in the initial disease (Ketley, 1997).

King (1957), first reported the isolation of *Campylobacter fetus* subsp *fetus* in human being. The major symptoms of disease in man include: abortion, premature birth, nausea,

headaches, diarrhoea, vomiting and undulating fever similar to those produced by brucellosis and malaria (Buxton and Frazer, 1979). It is responsible for infertility, abortion and sterility encountered in some of the breeding animal flocks. The reported incidence and prevalence of campylobacteriosis in humans and animals vary widely from country to country. In New Zealand, ovine abortion occurs sporadically, and is still an important disease in livestock farms. The annual national loss from abortion in 1980 was estimated to be Z$ 0.9 and 1 million and caused approximately 60 - 70% of all abortion storms in sheep and cattle in that country (Jopp, 1982).

The economic losses that occur as a result of campylobacteriosis in many countries has not been fully evaluated. This may be due to poor disease surveillance systems coupled with inadequate diagnostic facilities (Raji *et al.*, 1997).

2.2. **Classification and Nomenclature of *Campylobacter***

The most distinctive feature of *Campylobacter* is their spiral morphology. Taxonomically, they are most related to the spiral, flagellated, saprophytic bacteria, and they are currently included in the family *Spirillaceae*. They are short, curved rods or spiral in shape and it was this particular feature that allowed them to be described and classified as *Vibrios*, when they were first reported (Buxton and Frazer, 1979). It was not until 1963 that their proper taxonomic status was recognized and the genus *Campylobacter* (Greek curved rod) was established (Sebald and Veron, 1963).

Smith and Taylor (1919), following their first description, initially assigned *Campylobacter* to the genus *Vibrio* by Mcfadyean and Stockman in 1913. The species was

named *V. fetus* subsp *intestinalis*. *Vibrio fetus venerealis* was the cause of vibrionic abortion in cattle. When the culture of the organism is subjected to biochemical tests it did not produce hydrogen sulphide (using lead acetate strip) and did not grow in 1% glycine or in 3.5% sodium chloride.

Vibrio fetus intestinalis cause sporadic abortion in cattle. On biochemical testing the culture of the organism was either hydrogen suphide negative or weakly positive and did not grow in 3.5% sodium chloride but grew in 1% glycine.

Bryner *et al.* (1962) divided *V. fetus* into biotypes. Type 1 was H_2S positive and grew well in 1% bile and 1% Glycine but not in 3.5% sodium chloride.

The genus *Campylobacter* is divided into two groups according to the ability of the organism to produce catalase. Catalase negative *Campylobacter* (*C. sputorum*, *C. bubulous*) are not known to be pathogenic for man and animals. The catalase positive group consists of *C. fetus* and a closely related set of organism's first recognized by King (1957), and referred to as "Related *Campylobacter*". Veron and Chatelain (1973), reclassified *C. fetus* subsp *fetus*. *C. fetus* subsp *fetus* was the same as *V. fetus* subsp *intestinalis* (Florents, 1960). Likewise *C. fetus* subsp *fetus* was the same as florents. *V. fetus* subsp *venerealis*. *C. fetus* subsp *venerealis* biotype *intermedius* is similar to subtype I of Bryner *et al.* (1962). *C. coli* is similar to *V. coli* described by Doyle (1944) and *C. jejuni* similar to *V. fetus* of Jones *et al.*(1931).

Smibert (1974), classified the genus *Campylobacter* as follows; *C. fetus* subsp *fetus* which is the same as *V. fetus* subsp *venerealis* of Florent. *C. fetus* subsp *intestinalis*, the same as Florent's *V. fetus* subsp *intestinalis*. *C. fetus* subsp *jejuni* represent the *C. coli* and *C. jejuni* of Veron and Chatelain (1973), related to *Vibrio* of King (1957).

2.3 The Genus *Campylobacter*

Members of the genus *Campylobacter* are curved, spiral rods 1.5 - 3.5μm long by 0.2 - 0.4μm wide. They are Gram negative, non-spore forming, microaerophilic, motile with single polar flagellum at one or both ends (Smibert, 1978). They may also be S-shaped or gull shaped. The ends of the cells are usually pointed. *Campylobacter fetus* may appear ribbon-shaped, being composed of a chain of single curved cells. In old cultures' cells may become coccoid (Ogg, 1969). These appear to be a degenerative form because cultures composed mainly of coccoid forms are non-viable. In this form they resemble some of the chemoheterophilic spirilla (Karmali *et al.*, 1981).

Motility is by means of a long single polar flagellum which is two to three times the length of the cells. A few cells may have more than one flagellum at both ends. *Campylobacter* have a unique corkscrew type of motility. Cells quickly move across a microscopic field twirling rotating in a spiral-like motion.

2.4 Morphology of *Campylobacter fetus* subsp *fetus*

Campylobacter fetus subsp. *fetus* was formerly known as *Campyobacter fetus* subsp *intestinalis*. Several types of colonial forms are found on agar on primary isolation (Bryner *et al.*, 1962). The smooth colonies are about 1mm in diameter, colourless to slightly cream coloured. Rough colonies are small, round, finely granular, opaque and white to cream or tan coloured. They are 1 - 2 mm in diameter. The cut glass appearance of colonies does not develop in primary culture, but the colonies are low flat, greyish to tan-coloured and translucent with an irregular edge. Colonies on blood agar are non-hemolytic, round, 1 - 2mm in

diameter, smooth, convex, and greyish white or light tan coloured.

When the colonies are subjected to biochemical testing, hydrogen-sulphite is produced in a medium containing cysteine with lead acetate impregnated paper strip. Culture of the organism grow on slant with 0.1% sodium selenite and semisolid medium contain 1% glycine. A few strains have been reported to grow at 25°C.

2..4 .1 Morphology of *Campylobacter fetus* subsp *jejuni*:

Two types of colonies are found on primary isolation (Smibert, 1965). One is low, flat, greyish, finely granular and translucent with an irregular edge. It spreads along the direction of the streak and tends to swarm and coalesce. The other is round, 1 - 2mm in diameter, raised, convex, entire, smooth and glistening. It has a translucent edge with a darker, dirty brownish, slightly opaque centre.

Colonies on blood agar are non-hemolytic. The culture grows in broth and usually produce a butyrous sediment. When the colonies are subjected to biochemical tests, hydrogen-sulphide is produced in a medium containing cysteine with lead acetate-impregnated paper strips as the method of detection. It also grows on slants with 0.1% sodium selenite and slightly on Mac Conkey agar and in a medium with 1% glycine. The organism grows at 42°C but not at 25°C.

2.5 Genetics of *Campylobacter*

Campylobacter species have a small genome of approximately 1.6 - 1.7 Mbp of AT rich DNA, the ratio of G:C is approximately 30% (Owen and Leaper, 1981; Chang and Taylor, 1990; Nuijten *et al.*, 1990; Taylor *et al.*, 1992). An exception appears to be the *C. upsaliensis* genome which has a size of 2Mbp. It's size difference may arise from a large duplication of chromosomal sequences (Bourke *et al.*, 1995). The small size of the genome in *Campylobacter* is perhaps reflected in their requirement for complex media for growth and their inability to ferment carbohydrates and to degrade complex substances (Griffiths and Park, 1990).

Extrachromosomal elements in the form of both conjugative plasmids and bacteriophages have been reported in *Campylobacter* sp. (Taylor, 1992).

The number of cloned genes from *Campylobacter* sp is rather low in comparison to many other enteric pathogens (Taylor, 1992). This is illustrated by the number of gene sequences deposited in Gene Bank (approximately 50 different entries, excluding rRNA and uncharacterized sequences), perhaps a surprising situation considering the importance of this pathogen (Ketly,1997). It is generally accepted that genes from *Campylobacter* spp are often difficult to clone and subsequently analyse; this is thought to be due to several possible factors, including the high AT content resulting in promoter-like sequences, lack of expression or both in *Escherichia coli* and different patterns of methylation or codon usage.A series of shuttle vectors have been constructed that contain both *E. coli* and *Campylobacter coli* origins of replication and *Campylobacter* derived antibiotics resistance genes (Labigne-Roussel *et al.*,1987;Yao *et al.*,1993; Wang and Taylor 1990;Purdy and Park 1993). The identification of genes and particularly of those unique to the *Campylobacter* is even more difficult due to the

fact that transposons of either Gram-negative or Gram-positive origin or recombinant transposons (Ketley, 1995) have not been found to transpose in *Campylobacter*.

2.6 Epidemiology of *Campylobacter* in Man and Animals.

Most of the information on the epidemiology of vibriosis has been obtained during the past thirty-five years and particularly during the past two decades. The disease is reported to be widespread among cattle and sheep (Vandeplassche *et al.*, *1963)*. Its detection in England in 1913, the United States of America in 1918, Denmark in 1920, and Germany in 1922, was followed by reports from Australia in 1943, Sweden in 1946, South Africa in 1948, and Holland in 1949. Positive blood tests in cattle for *Campylobacter* have been reported from most states including Hawaii and from Canada. Undoubtedly the disease was introduced into the United States with the importation of cattle from Europe (WHO, 1994). The incidence of the disease is unknown, but present day clinical observations, together with the results of agglutination tests and the isolation of *V. fetus* from animals indicate that the infection is far more extensive than was suspected and, furthermore, is a very important cause of abortion and sterility in cows and sheep. In England, the first record by Mcfadyean and Stockman (1913), reported that in sheep the average abortion rate from vibrionic infection was 23.2% and that 10% of the sheep in infected flocks suffered from sterility. In the United States, some data have been obtained by Carpenter in 1919. He observed that approximately 50% of the ewes in the flocks he examined either aborted or gave birth to premature lambs. Plastridge *et al.*(1964), noted that the annual abortion rate in twelve herds comprising 363 cows varied from 4.0 to 20%. The average percentage of animals aborting was 12%. In another herd, sixteen or 30%

of fifty-four cows and heifers aborted. Among 450 bovine abortions in Connecticut, Plastridge *et al.* (1964), observed 44% of them to be vibrionic abortion. Twenty-three per cent were diagnosed by the isolation of *V. fetus* and 21% by means of agglutination test. In New York, Robertson *et al.*, (1959) isolated *V. fetus* from sixteen (11.5%) of 139 bovine fetuses. Leaver and Hart (1960) reported that *C. fetus* subsp *fetus* was diagnosed in 40% of the infertility problem herds and was found to be present in all the major dairying districts of Victoria, Australia. Data on the number of cases of bovine and ovine vibriosis in the United States reported by the Agricultural Research Service, U.S. Department of Agriculture have been available since, July, 1958. Abortion in goats due to *V. fetus* has been reported by Dobbs and McIntyre (1951). Truelblood and Post (1958), isolated the organism from aborted fetuses of antelope,(*Antilocapra americana)* in Wyoming. Diliello *et al.* (1950), postulated swine to be a reservoir due to the similarity observed in *Vibrio* strains isolated from swine to those from cattle and sheep. Results of several studies on occurrence of human campylobacteriosis have shown marked variations between countries. Population studies in the Netherlands suggest that 1800 - 2300 cases per 100,000 individuals occur annually (W.H.O., 1994). Comparable studies in the U.K. and U.S. A. indicate 1,000 - 1100 cases per 10,000. In Sweden and Netherlands, Cam*pylob*acter has been isolated from 11 and 12% respectively of patients with diarrhoea, in comparison with 7% and 5% yielding *Salmonella*. Little is known about the economic impact of the disease in different countries, but the costs are likely to be significant. Results of studies conducted in Africa (Nigeria), in Asia (Bangladesh, China, India, Thailand) and Central America (Mexico) suggest that *Campylobacter* is a significant cause of diarrhea in children (WHO, 1994). Epidemiological studies suggest that *Campylobac*ter infections occur as a result

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of repeated exposure, for example in the environment of poultry processing plants. This appears to prevent illness but not colonization, a finding which has been confirmed by epidemiological studies in developing countries where asymptomatic infections are frequent (Humphrey, 1994). Evidence suggests that protection is immunologically mediated and indicates that vaccine development is a possibility. This is supported by the passive immunization of infants that are breast-fed by apparently immune mothers.

2.7 *Campylobacter* infections in Nigeria

Organisms belonging to the genus *Campylobacter* are now recognized as important agents of gastroenteritis in human and various species of animals throughout the world (Butzler and Skirrow, 1979; Blaser *et al.*, 1980; Aaris *et al.*, 1995). They are also established as animal pathogens of importance that cause bovine and ovine abortion and sterility (Collins and Ross, 1984; Blom *et al.*, 1995). The species *Campylobacter fetus* subsp *jejuni* causes enteritis in man and animals (Prescott and Manroe, 1982; Alabi *et al.*, 1986), and abortion in sheep (Varga *et al.*, 1990). *C. fetus* subsp *fetus* is associated with abortion and other reproductive disorders in cattle and sheep (Smibert, 1978), and also occasionally with human infections (Righter *et al.*, 1983).

Although, the annual incidence of abortion associated with *C. fetus* subsp *fetus* varies every year some farms experience major outbreaks, sometimes affecting large number of the animals in a flock (Collins and Ross, 1984). *Campylobacter* infections cause serious economic losses to the livestock industry, and severe morbidity in man thus constituting an important public health problem recognized world wide (De'mole and Bosman 1978; Gurgan and Diker,

1994; Coker and Adefeso, 1994).

In Nigeria, *Campylobacter* infections is endemic and has been reported sporadically from various parts of the country (Ibeziako *et al.*, 1987; Bawa *et al.*, 1991; Coker and Adefeso, 1994). However many cases of enteritis and abortion in livestock and humans in Nigeria are still attributed to diseases like colibacillosis, salmonellosis, brucellosis, trypanosomiasis (Njoku, 1972; Ikede *et al.*, 1988). This situation may be due to the long existing records of these diseases reported in Nigeria (Raji *et al.*, 1997).

The true picture of the incidence and prevalence of the disease in Nigeria is not known. In addition, the distribution of the disease in Nigeria is not properly documented as the system of disease reporting is not very efficient. However, evidence of *Campylobacter* infections has been reported in humans, cattle, sheep goats, pigs, chickens, ducks, cats dogs as well as contamination of well water and meat (Adekeye *et al.*, 1987, Flegbe *et al.*, 1987).

2.7.1 *Campylobacter* infection in Humans

The major form of *Campylobacter* infections in humans in Nigeria is enteritis (Coker and Dosumun-Ogunbi, 1986; Alabi *et al.*, 1986). Gastroenteritis due to *C fetus subsp jejuni* was reported in northern Nigeria in 1981 (Lawande *et al.*, 1981) and Southern part of Nigeria in Ile-Ife, in 1983 (Coker and Dosumun-Ogunbi, 1983). *Campylobacter* enteritis affects all age groups but with highest incidence in children under the age of 5 years (Coker and Adefeso, 1994; Adegbola *et al.* 1991). The incidence reported in Lagos by Coker and Dosumun-Ogunbi (1983; 1984) were 5% and 11% respectively and 16.5% by Coker and Adefeso (1994). While Olubunmi and Adeniran (1986) reported an incidence of 7% in Ile-Ife.

The incidence of diarrhoea caused by *C. jejuni/coli* is common in male, and the ratio of male to female is 2:1 (Coker and Adefeso, 1994). *Campylobacter* organisms were isolated in almost equal proportion from patients with diarrhoea as well as from asymptomatic carriers in Ile- Ife (Adegbola *et al.*, 1991).

2.7.2 Bovine Campylobacteriosis.

Campylobacteriosis in the cow is characterized by infertility, abortion, anestrous and irregular return to heat (Bawa *et al.*, 1991). The prevalence of campylobacteriosis in Borno, Kaduna, and Kano State of Nigeria was found to be 2.9%. Infections were found to be caused by *C. fetus* subsp *fetus* and *C. fetus* subsp *venerealis* (Bawa, 1987). The major pathological lesions associated with the infections were confined to the vaginal and cervical mucosae. *C. fetus* subsp *venerealis* was isolated more frequently than subsp *fetus* in bulls.

Elegbe *et al.* (1987), conducted an epidemiological survey of *Campylobacter* spp on farms in Ile-Ife Nigeria. The prevalence of *Campylobacter* infection found in their study was 28% and they isolated, *C. jejuni*, *C. coli* and *C. fetus*. Ibeziako (1987), conducted a survey on the occurrence of *Campylobacter* spp in cattle in Zaria. In that study, she isolated *C. jejuni* from 1.1% of rectal swabs from cattle. There was no isolation of the organism from local milk samples collected.

2.7.3 Ovine and Caprine Campylobacteriosis

Enteritis is the major clinical sign of ovine and caprine campylobacteriosis. Olubunmi and Adeniran (1986), isolated *C. jejuni* biotype 1, *C. coli* and *C. fecalis* from fecal samples of sheep and goats. They established a prevalence of 12.5% and 6.25% in sheep and goats respectively. Adegbola *et al.* (1991) and Adetosoye and Adeniran (1987) were unable to isolate the organism from sheep and goats.

2.7.4 Avian Campylobacteriosis

It has been established that poultry in particular are frequently colonized by a large number of *Campylobacter* organisms. This has been demonstrated by Adegbola ***et al.*** (1991), who reported 85% isolation rate from fecal samples of chickens. Adekeye (1986), in Zaria examined feces of 368 ducks usually congregating in groups around eleven different wells for *Campylobacter fetus* subsp *jejuni*. Water samples from the wells were similarly cultured. The prevalence rates of the organism were 54.6% in duck feces and 36.4% in the well water. He found that well water which may be contaminated by duck feces can serve as a potential source of *Campylobacter* for human infection. In another study by Adekeye *et al.* (1987), a survey for *C. fetus* subsp *jejuni* was carried out among 487 live birds in 36 flocks and poultry carcasses. The organism was isolated from 12.3% of the birds in 19 flocks and from 5 of 70 poultry carcasses.

Elegbe *et al.* (1987), carried out an epidemiological survey for *Campylobacter spp* on poultry farms in Ile-Ife, and isolated the organisms from 17 (25.8%) of 66 chickens and 16 (20.5%) of 78 turkeys. Olubunmi and Adeniran (1986), in the same area isolated the

organism from 24 (15.4%) of 156 poultry fecal sampled.

2.7.5 Porcine Campylobacteriosis

Initially there was doubt as to the presence of porcine campylobacteriosis in Nigeria, until Olubunmi (1982), demonstrated enteric lesions associated with porcine campylobacteriosis. Adegbola *et al.* (1991), reported a prevalence of 32.4% in pigs, while Olubunmi and Adeniran (1986) found a prevalence of 27.5%. The species isolated were *C. jejuni* and *C. coli*. Taylor and Olubunmi (1981), demonstrated that *C. coli* are particularly associated with pigs and this species of *Campylobacter* is often found in the intestine of pigs.

2.7.6 Canine and feline Campylobacteriosis

There is little information on canine and feline *Campylobacter* infections in Nigeria. The few reports indicated the prevalence to be 20% in dogs and no isolation in cats (Olubunmi and Adeniran, 1986), whereas Elegbe *et al.* (1987), reported a prevalence of 17.6% in dogs.

2.7.7 Equine and Camel Campylobacteriosis

To our knowledge there is no documented report on the occurrence of *Campylobacter* infections in horses and camels in Nigeria. This is an area that requires closer investigation.

2.7.8 Serotypes of *Campylobacter* isolated in Nigeria

The serotyping scheme of Lior *et al.*, (1982) has been widely adopted and used. It is based on a rapid slide agglutination technique with five bacteria and absorbed antisera for the

detection of heat labile antigenic factors. Alabi *et al.* (1986), have shown that serogroups 2,4, 29,36 and 53 represented more than 60% of all human isolates that were isolated from cases of *Campylobacter enteritis* in Nigeria. The serogroups 2,4,29 and 36 constituted the majority (61.7%) of serogroups in animals. The pattern of distribution strongly suggests that these serogroups are responsible for most cases of enteritis in humans and animals in Nigeria.

2.7.9. Control Measures in Nigeria

To date, the need for the control of campylobacteriosis in Nigeria has received little attention. It is not even listed among the reportable diseases. In developed nations, vaccination of cattle against *Campylobacter* prior to mating have shown to give good protection. A commercially prepared vaccine has been used successfully in cattle herds as soon as a diagnosis of *Campylobacter* infection is made.

Control measures in man will primarily depend on control of the disease in animals. Safe handling of infected animal's fecal material (Adegbola *et al.*, 1990), and treatment of infected animals and man with erythromycin, kanamycin, and tetracycline has also been recommended (Coker and Adefeso, 1994).

In animals, bulls that are infected should not be used for either breeding or for artificial insemination. When abortion occurs, fetal membranes and fetuses should be disposed and premises in which abortion occurred should be properly disinfected. It is essential that contamination of food and water supply should be avoided.

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2.8. **Sheep as a Reservoir of *Campylobacter* Organisms**

A number of studies have shown that sheep may be inapparent carriers, harbouring *V. fetus* var *intestinalis* in the intestine (Bryans and Shepherd, 1960; Smibert, 1965). The occurrence of the organisms in the intestinal tracts of other animals and birds may serve as secondary reservoirs of infection for sheep (Meinershagen *et al.* 1965; Dennis, 1967; Watson, *et al.* 1967). Dennis(1967), isolated *Campylobacter spp* from carrion crows which were found to be highly pathogenic in pregnant ewes. Watson *et al.* (1967) were unable to induce abortion by oral administration of a serotype I strain from a similar source.

In experimental studies, Meinershagen *et al.* (1965), showed that the carrion eating American Magpie (*Pica pica*) could harbour highly pathogenic serotype I organisms in the intestinal tract for seven months after oral administration. Recently, Casanovas *et al.* (1995) reported campylobacteriosis among children in a nursery school as a result of consumption of contaminated milk pecked on by Magpie bird. Dennis (1967), suggested that the raven (*Corvus coronoids*) may act as a carrier of ovine vibriosis. This is of particular interest, because it would help to explain the so-called "explosive" outbreaks which occurred in closed flocks.

Concern over possible transmission of *Campylobacter* from sheep to man and other animals have been justified by detection of the organism in this particular host (Wyffel, 1988; Olubunmin and Adeniran, 1986; Bird *et al.* 1984).There is evidence to suggest that infections in sheep may originate from chickens, turkeys, ravens, magpies, sparrows, startling and other species of birds. The fact that *C. fetus* has been detected in the feces of clinically normal sheep, in the vaginas of pregnant ewes which have not aborted and also in the vaginas of virgin yearling illustrates the resistance which normal sheep can develop against this type of infection

(Buxton and Fraser, 1979).

The gall bladder of sheep appears to be an important organ which the organism colonizes (Firehammer *et al.*, 1962; Firehammer and Hawkins, 1964; Watson *et al.*, 1967; Bryner *et al.*, 1971; 1972). Most of the strains isolated from the gall bladder by Bryner *et al.* (1972), were either serotype I or V. Firehammer and Hawkins (1964), showed that strains isolated from this organ could induce abortion when injected into the rumen of pregnant sheep.

The bile of ewes was examined for *Campylobacter spp* and not all *Vibrio* like organism isolated from the bile samples were pathogenic for pregnant ewes (Bryans and Smith, 1960). Cultures of *V. fetus* inoculated into the gall bladder revealed that infection persisted for thirty to thirty-eight days. Gall bladder infection, determined by culturing of bile sample, persisted in one experimental ewe for 1 year. This particular ewe died while giving birth to a full-term dead vibrio-infected lamb and may have served as a source of infection for other ewes in the same pen which later aborted from vibriosis (Firehammer *et al.*, 1962).

2.9 Pathogenesis of *Campylobacter jejuni* Infections

The pathogenesis of *Campylobacter* infection is not clearly understood (Arimi, 1987). Various studies have been carried out on human volunteers and in animal models, and in human and animal cell lines *in-vitro*, all aimed at understanding the mechanisms involved in the production of disease, but so far no suitable animal model has been found (Arimi, 1987).

Several pathogenic mechanisms have been proposed, mainly for the enteric disease with special reference to *C. fetus* subsp *jejuni* and *C. fetus* subsp *coli* (Walker *et al.*, 1986). The report on the infective dose indicates that infection with *C. fetus* subsp *jejuni* is dose

related since a sizeable number must pass the stomach acid barrier and reach the intestines. *In vitro* studies have shown rapid destruction of *C. fetus* subsp *jejuni* by hydrochloric acid (Blaser *et al.*, 1980), indicating that gastric acid is an effective barrier against infection. Black *et al.* (1988), studied the relationship between ingested dose of *C. fetus* subsp *jejuni* and the rate of infection and illness in human volunteers and found an increase in the rate of infection from 50% to 100% as the inoculum was raised from 8×10^2 to 10^8CFU. Ingestion of as few as 500 or 800 cells of *C. jejuni* in milk (Black *et al.*, 1988; Robinson, 1981), have been found to cause infection and symptoms of disease in volunteers. The strong buffering capacity of milk may protect *Campylobacters* from the stomach acid. After passing the stomach acid barrier, *Campylobacter* must colonize the gut mucosa in order to establish infection and produce disease. Morooka *et al.* (1985), demonstrated colonization of the intestinal tract with motile flagellated strains of *C. jejuni* and not with non motile mutant strains, whether flagellated or non-flagellated. Newell *et al.* (1985), further reported successful colonization of the gastrointestinal tract of 5-day old BALb/c mice with motile and non motile of *C. jejuni*. Lee *et al.* (1986), investigated mucus colonization in the mouse as a determinant of pathogenicity in intestinal infection by *C. jejuni*. They found that organisms do survive in the mucous, and are motile in this environment, sometimes penetrating deep into the crypts where large numbers were observed.

The invasive nature of shigellosis is characterized by bloody diarrhoea (Levine *et al.*, 1983) and bloody diarrhoea is also seen in *Campylobacter* enteritis(Butzler *et al.*, 1974; Mandal *et al.*, 1984). All these suggest invasiveness of the species of *Campylobacter*. However, controlled sereny test for invasiveness using guinea pigs have proved negative

(Manninen *et al.*, 1982). It is not clear whether the sereny test method is suitable for testing *Campylobacter* for invasiveness or not. It is possible that the strains used were not invasive. However, experiments which have been carried out using chicken embryos (Butzler *et al.*, 1979; Field *et al.*,1986), oral infection of 3-8 days old chickens (Butzler *et al.*, 1974 and Newell *et al.*, 1985), and *in vitro* tissue culture systems (Newell *et al.*, 1985), have clearly demonstrated that a large number of *C. jejuni* strains are invasive.

C. jejuni is known to produce at least two exotoxins: a cytotoxin (Walker *et al.*, 1986,) and a heat labile (LT) cholera like enterotoxin (McCardell *et al.*, 1984). *Campylobacter* isolates from patients with secretory diarrhoea produce LT enterotoxin which stimulates cyclic adenine monophospate. The toxin is structurally and immunologically similar to cholera toxin (CT) and the heat labile (LT) enterotoxin of *E. coli* (Walker *et al.*, 1986; McCardell *et al.*, 1984; Klipstein *et al.*, 1985). It appears that bloody diarrhoea is mainly produced by invasive enterotoxin producing strains. The role of LT enterotoxin in the colonization of the gut mucosa has been demonstrated with *V. cholerae* 01 in a rabbit model (Pierce *et al.*, 1985). It now remains to be demonstrated whether *Campylobacter* enterotoxin has a similar role in the colonization of the intestinal mucosa since CT and *Campylobacter* LT enterotoxins are similar.

C. jejuni was found to survive within the macrophages for 6 - 7 days, despite the observation that by 96 hrs conversion to coccal forms appeared to be completed. However, as no antibiotics were present, the possibility that the surviving bacteria were in fact extracellular cannot be ruled out. One strain of *C. jejuni* was phagocytozed by guinea pig resident peritoneal macrophages, while cells of a *C. coli* strain were readily ingested by the macrophages (Black *et al.*, 1988, Duffy *et al.*, 1980).

C. coli cells were killed more than they were phagocytozed, suggesting an extra cellular killing mechanism. It is difficult to make generalizations about possible differences between C. *jejuni* and C. *coli*, because the published reports only present data from one strain or species (Konkel *et al.*, 1992).

Two studies suggest that macrophages, but not complement, are important in the clearance of C. *jejuni*. The relevance of these studies with a murine intra-peritoneal model to natural enteric infections, however, is questionable. It appears that some strains of C. *jejuni* and C. *coli* are efficiently phagocytozed by polymononuclear cells and neither specific immunoglobulin nor complement seem to be required for efficient phagocytosis of C. *jejuni* by macrophages.

2..10. The Pathogenesis of Vibrionic Abortion

The pathogenesis of the vibrionic abortion caused by *Campylobacter fetus* subsps *fetus* has been poorly defined. Speculations and hypotheses have been proposed for the mechanism of *Campylobacter* abortions (Firehammer *et al.*,1962). But with the advent of molecular biology and molecular techniques, detailed studies are now being carried on the pathogenesis of *Campylobacter* abortion. Reports from various workers indicate that ingested organisms gain access to the blood circulation from the small intestine via portal circulation or lymphatic system and carried to the various part of the body (Turker and Robertstad, 1959; Laing, 1979; Clark *et al.*, 1969).

To cause abortion, C. *fetus* must reach the placenta and fetus via the maternal blood stream from the gastrointestinal tract following ingestion (Jensen *et al.*., 1961). The bacteria

penetrate the mucosae at unknown points and establish a bacteraemia which persist for one to two weeks. The blood transports the organisms to the gravid uterus where they induce placental infection (Jensen, 1974). At the hyalinized tips of the uterine septa, the bacteria pass through the walls of maternal capillaries, invade the extravasated blood in the lacunas, and enter the tissue of gravid uterus. At the chorionic epithelial cells of the uterus the infection then move into the fetal blood. Most severely infected uteruses abort the damaged, dead or weakened fetuses, but the precise mechanism which initiates the abortion is not known. Some ewes die from retained dead fetuses and peritonitis. During and following abortion, some organisms localize in the gall bladder and produce a carrier status in the affected sheep. Recovered animals are positive for specific agglutinins and are immune to new attacks for at least 2 years (Gilmour *et al.*, 1975).

The rate of clearance of *Campylobacter* from the blood is the same in pregnant and non pregnant ewes, but is dependent on the bacterial morphology, with coccoid forms being eliminated more slowly. When inoculated intravenously, *Campylobacter* may result in a fatal shock-like syndrome with a generalized thrombosis and death (Bryans and Smith, 1960).

2.11 Prevalence and General Distribution of *Campylobacter Spp*

Bovine campylobacteriosis is characterized by infertility and sporadic abortion. There is no evidence of infertility in sheep although abortions occur. Sjollema *et al.*, demonstrated in 1949 that bovine campylobacteriosis is a venereal disease Firehammer *et al.* (1964) also found that the ram was not a significant source of ewe infection and that the disease was not venereally transmitted.

It has been found for bovine and ovine campylobacteriosis that the bovine strains of *Campylobacter fetus* are somehow more fastidious in their nutritional requirement than cultures of ovine origin (Firehammer *et al.*, 1964). Serologic differences have also been demonstrated between isolates from the two species of animals.

Firehammer and Lovelace (1962) examined cultures which were isolated during an outbreak of ovine abortions and concluded that they were probably *Campylobacter bubulous* rather than *Campylobacter fetus*. That was probably the first recorded outbreak of abortion in ovine attributed to this organism. Whereas vaginal infection with *C. bubulous* may be found in both aborting and non aborting ewes, such infection might account for the isolation which were made after the outbreak of abortion.

Outbreaks of diarrhoea associated with *C. fetus* subsp *jejuni* are known to occur at rates equivalent to or greater than *Salmonella* infections in man (Skirrow, 1989). Outbreaks of *C. fetus* subsp *fetus* diarrhoea are rare. Klein *et al.* (1986), reported a mixed outbreak of *C. fetus* subsp *fetus/ jejuni* diarrhoea following consumption of raw milk at a common meal. However, since 1980 *C. fetus* subsp *fetus* has been implicated in four reported outbreaks of human disease in North America, three of which were suspected to be caused by contaminated foods, raw milk, raw calf liver, and cottage cheese (CDC, 1981; Klein, *et al.*, 1986). *C. fetus* subsp *venerealis* has rarely, if ever, been implicated in human illness (Penner, 1991). Several foodborne outbreaks of *Campylobacter* enteritis have been associated with ingestion of raw milk, undercooked chicken and pork (Robinson *et al., 1979*).

Campylobacter upsaliensis, also known as catalase negative or weak reacting thermotolerant *Campylobacter* species, was first isolated from the feces of healthy and

diarrhoeic dogs in 1983 (Sandstedt *et al.*, 1983). *C. upsaliensis* has been isolated from feces of children and adults with diarrhoea (Goossen *et al.*, 1990; Patton *et al.* 1989; Taylor *et al.*, 1989), and from blood of paediatric patients and adults with septicemia (Lastovica *et al.*, 1980; Taylor *et al.*, 1989). Gurgan and Diker (1994), reported the isolation of *C. upsaliensis* from the blood and fetal placental material of a woman who aborted after contact with an asymptomatic cat. Similarly, Steinkraus and Wright (1994), reported a case of septic abortion caused by *C. fetus* subsp *fetus* at last trimester of pregnancy.

The distribution of ovine campylobacteriosis varies throughout the world. Sporadic cases occur in some countries, and as high as 40 per cent prevalence has been reported in Turkey and 45 per cent in New Zealand by Erdogan *et al.* (1993) and Orr, (1990), respectively. In Turkey, Karaman *et al.* (1993), reported an isolation rate of 0.6 per cent in ewes and serological evidence were demonstrated in 497 (11%) of the total samples. In Italy, samples from 21 aborted fetuses and placental, 106 uterine swabs, 211 rectal swabs from adult sheep and 278 rectal swabs from lambs were cultured. Fourty five strains of *C. jejuni* were isolated from rectal swabs, 31 of which were from adults and 14 from lambs, 5 of the later were from 63 lambs with diarrhoea. Of the 18 *Campylobacter coli* isolates, 16 were from rectal swabs (5 from adults and 11 from lambs and 2 from uterine swabs) (Valente *et al.*, 1991). Varga *et al.*, (1990), in Hungary reported the isolation of *Campylobacter spp* from 38 aborted ovine fetuses in 23 sheep flocks examined, 29 of which were *C. fetus* subsp *fetus*, and 22 were *C. jejuni*. The isolates were examined biochemically and serologically for heat stable antigens. In that study, abortion was caused in 18 (78.3%) flocks by *C. fetus* subsp *fetus*, and while in 5(21.7%) of the remaining flocks by *C. jejuni*. It was suggested that *C. jejuni* is involved in

ovine abortion unlike earlier reports which suggested that only *C. fetus* subsp *fetus* was responsible for ovine abortion.

In Germany, the prevelence of abortion due to *Campylobacter* was found to have a low prevalence of 0.4 per cent (Plagemann, 1989). Other agents such as *Chlamydia psittaci* var ovis, *Salmonella abortus* var ovis, *Coxiella burnetii*, and *Listeria monocytogenes* were recorded as main agents of ovine abortion in that study. Kenar and Erganis (1994), isolated *Campylobacter* from 18 of the 20 vaginal swabs obtained from ewes, of which 12 were *C. fetus* subsp *fetus* and 6 *C. jejuni*.

A survey of the prevalence of *C. fetus* from the gall bladder of sheep slaughtered in Iowa, United Stated of America showed that 17(9.1%) of 186 sheep were infected (Bryner *et al.*, 1972). Smibert (1965), isolated *C. fetus* from the feces of 26 (16.8%) of 214 sheep, the prevalence being higher in animals aged 3 to 20 months (23.1%) than those aged 2 to 10 years (4.2%). In another survey conducted in 1976 on the prevalence of *C. fetus* in the gall bladder of sheep sent to slaughter from farms in Victoria and South-Wales in Australia, *C. fetus* was isolated from 33(3.3%) of 1015 sheep. In that study, the rate was slightly higher in lambs (4%) than in adult sheep which consisted predominantly of ewes (2.7%). Thirty of the 33 isolates were identified as *C. fetus* subsp *jejuni* and the remaining 3 were found to be *C. fetus* subsp *intestinalis* (Marsh and Firehammer, 1953).

2.12 Antimicrobial Susceptibility of *Campylobacter spp*

The range of therapeutic agents suitable for use in treating *Campylobacter* infections is limited by the frequent occurrence of resistance to the more readily available antibiotics

(Butzler *et al.*, 1979; Vanhoof et *al.*, 1978). The antibiotics generally recommended for therapy of infections caused by thermophilic *Campylobacter* species is erythromycin (Butzler and Skirrow 1979; Ricketts and Hopes, 1984). Shutte (1969), has also recommended erythromycin for treatment of *C. fetus* subsp *venerealis* infection in cattle, although a combination of streptomycin and penicillin is more widely used. Erythromycin is not active against all *Campylobacter* isolates (Brunton *et al*, 1978; Karmali *et al.*, 1981; Seeker, 1983).

The susceptibility of *Campylobacter fetus* to various antibiotics has been studied using dilutions of antibiotic on Muller-Hinton agar. Butzler *et al.*, (1974), determined the sensitivity of 114 human strains of *C. fetus* (presumably subsp *intestinalis).* Gentamycin and erythromycin were the most active although some strains were resistant to these agents. Plastridge *et al.*, (1964), studied 22 strains of *C. fetus* isolated from chicken, cattle, sheep and man. They were found to be highly susceptible to tetracycline and penicillin G and resistant to bacitracin and polymyxin B. In another study, the range of sensitivity of *C. fetus* in ug /ml was 16-640 for bacitracin 0.8-6.4 for chloramphenicol, 1-4 for dihydrostreptomycin 0.8-3.2 for erythromycin, 4-10 for neomycin, 16-64 for novobiocin, 2-16 for oxytetracycline, 0.5 - 16 for penicillin and 5-320 for polymyxin B (Plastridge *et al.*, 1962).

Bokkenheuser (1970), reported eight different strains of *C. fetus* from human infections based on the result of sensitivity using antibiotic disks. All eight were sensitive to erythromycin (14µg) chloramphenicol (30µg), and streptomycin (10µg). Six of the eight strains were sensitive to penicillin (10µg) and seven were sensitive to tetracycline (30µg). Resistance of some strains of *C. fetus* to penicillin and tetracycline means that antibiotic sensitivities of clinical isolates must be determined. Treatment of patients with antibiotics for about four weeks

was recommended. Unfortunately there was no report on the susceptibility of the three subspecies of *C. fetus* to antibiotics or their zone of inhibition. Sizes of inhibition of different strains of each of subspecies to different concentration of antibiotic disk should be noted before treatment.

Baffone *et al.*(1992), studied the in vitro sensitivity of 85 strains of *C. jejuni* biotype1 from various sources to 14 antimicrobial agents. Of the strains tested, 98.8% were sensitive to kanamycin and nitrofurantoin, 96.4% to chloraphenicol and gentamicin, 92.9% to cephalothin and erythromycin and 90.5% to ampicillin and tetracycline. The effectiveness of nalidixic acid, (75.2%), trimethoprin-sulfamethoxozole,(69.4%), sulfamethoxazole alone (72.9%), cefsulodin (22.3%) and metronidazole (9.4%)were lower, whereas trimethoprin was completely ineffective. The effectiveness of erythromycine in the treatment of human *C. jejuni* infection was confirmed. Gentamycin and cephalothin were considered as alternative to erythromycin drugs in case of *Campylobacter* infection.

In Trinidad and Tobago, Adesiyun *et al.*, (1993), tested the susceptibility of *C jejuni* isolated from faecal samples of domestic animals and found that, they exhibited resistance to one or more antibiotics and the highest susceptibility was to streptomycin (75.5%), Kanamycin (28.6%) and neomycin (26.7%).

Antibiotic resistance among *Campylobacter* is a world wide problem. Organisms acquire resistance due to indiscriminate use of antibiotics. In Nigeria, erythromycin which is the drug of choice in the treatment of *Campylobacter* infection is now known to have developed resistance to this antibiotic (Coker and Adefeso, 1994).

2.13 Transportation of Specimens to the Laboratory

Campylobacter are typically microaerophilic, that is they are sensitive to level of oxygen as high as those present in air. The need was recognized early, to develop methods to keep samples under microaerophilic conditions (e.g. 3.5% O_2, 10% CO_2 and 86.5% N_2 for *C. fetus).* Generally, these conditions can be obtained using either single or commercially mixed gases. In certain situations, particularly in developing countries, innovative equipment for producing gas-mixtures such as those described by Terzolo, (1987), can be followed. A significant advancement in culture techniques was the development of transport enrichment media (TEM), adapted for field use (Clark and Duffy, 1978). The distinct advantage of TEM is its ability to maintain survival or even growth of *C. fetus* during transport at 18-37°C, thus obviating the need to have the samples arrive in the laboratory on the same day (Garcia *et al.* 1983;1984). It was also observed that a delay of 2-5 days in receiving samples in TEM do sigaificantly affect the recovery of *C. fetus.* Furthermore, this methodology has been found quite suitable for use under tropical conditions and no refrigeration of samples is required. Concerns about the existence of polymxin B sensitive strains (Jones *et al.*, 1985) led to the suggestion of excluding this antibiotic in transport media (Howard, 1986). However, polymyxin B inhibits the growth of *C. sputorum* subsp *bubulous,* a non-pathogenic saprophyte that is commonly encountered in the reproductive tract. The inclusion of polymyxin B in TEM, therefore reduces subsequent complications in the identification of *Campylobacter* isolates. Other transport media have also been found to be suitable for *C. fetus* isolation (Lisle *et al.*, 1982). Recently Lander (1990) described the development and application of a transport

enrichment medium in order to improve the accuracy of diagnosis. The submission of samples of both unfiltered and filtered preputial washing in the medium has been recommended. *C. jejuni* can also be preserved in the TEM broth but they are less sensitive to the atmospheric oxygen than the *C. fetus*. Fecal samples from cases of suspected gastroenteritis suspected to be due to *C. jejuni* can be transported in the TEM for 24-72 hours before subsequent culture.

2.14. Laboratory Procedures

When samples arrive in the laboratory, the TEM vials are incubated at 37°C for 4 days after which isolation of the organism in suitable media is performed. Cysteine-heart agar supplemented with sheep blood and antibiotics is satisfactory for initial isolation (Plastridge *et al.*, 1961). A blood-free selective medium containing cefoperazone which has been recommended for recovering thermophilic *Campylobacter* can also be used to isolate *C. fetus* (Bolton *et al.*, 1983). Either fresh samples or those maintained in transport media are streaked onto duplicate plates containing the appropriate antibiotics or they are passed through 0.65µm filters and then streaked onto blood agar with or without antibiotics (Shepler *et al.*, 1963; Clark and Dufty, 1978). For propagation in broth, the medium described by Dennis and Jones (1959), has been confirmed to be quite satisfactory for bulk growth (Walt and Vander, 1987).

The commercial selective media mostly used for the isolation of *Campylobacters* from human and animals have been recognized all over the world (Adekeye *et al.* 1987; Lander, 1990; Denton and Clark,1992). They are Skirrow's medium (Skirrow, 1989), Butzler's medium (Butzler and Skirrow, 1979) and Blaser's medium (Blaser *et al.*, 1978). A modification of Blaser's medium called campy-BAP (BBL) is also available and widely used. Their

compositions vary; for instance, Skirrow's medium contains Blood Agar Base, Blaser's Medium Brucella medium Base (Oxoid), Lander and Gills selective and enrichment medium contains Veal Infusion Broth (Difco) and Preston's medium Nutrient broth NO. 1 (Oxoid), solidified with New Zealand agar.

The use of bood from different animal species have also been reported for the different commercial media mentioned. For instance Skirrow medium use 5-7% lysed horse blood. It is essential that lysed horse blood should be used in Skirrow's medium in order to ensure neutralization of trimethoprin antagonists otherwise *Proteus spp* will grow (Butzler and Skirrow, 1979). Butzler's medium contains 10-15% defibrinated whole sheep blood. Blaser's medium use 10% defibrinated whole sheep blood, Lander and Gills medium, 7% lysed horse blood. There are many commercial antibioties for different *Campylobacter* media in order to inhibit the growth of contaminants and to ensure selectivity of the different media. They are prepared as freeze dried antibioties. Butzler medium selective supplement SR (85 Oxoid) contains bacitracin (2500 units/L), Cycloheximide (50mg/L), colistin sulphate (10000 units/L), cephazolin sodium (15mg/L) and novobiocin (5mg/L). Skirrows medium contains vancomycin (10mg/l) polymyxin (2500IU/l) and trimethoprim (5mg/l). This supplement is referred to as Oxoid SR 69. It is different from SR 85 by its selective action growing at 43°C for the thermophilic *Campylobacters* while the SR 69 Oxoid is for *Campylobacter* strains that grows at 37°C.

The Blaser-Wang medium contains selective supplement Oxoid SR 98 which is based on the formulation of Skirrow but with the addition of Amphotericin B (2mg/l) and Cephalothin (15mg/L). The antibiotic supplement reduces the normal enteric flora without loss

of *C. fetus*. Amphotericin B inhibit the growth of *Candida albicans* and cephalothin improves the selectivity of the supplement. Bottom and Coates, (1983) and Karmali *et al.,* (1981), reported blood free charcoal based (CCDA) on selective agar in anaerobic condition as a useful medium for the isolation of *Campylobacter* species, while Botton and Robertson (1982), recommended that Prestons medium to be more selective than Skirrow medium and suitable for any kind of specimen for the isolation of *C. jejuni* and *C. coli*.

2.15 Biochemical Identification

Suspected colonies on blood agar are confirmed by various biochemical tests as well as by the fluorescent antibody (FA) test (Clark *et al.,* 1969; Clark, 1971).

Morphologically, *C. fetus* cells are small, Gram negative curved to spiral rods and have a rather corkscrew type of motility. Most have a single, polar flagellum, however strains of subsp *venerealis* may have several flagella located on one end appearing like a tuft (Bryner *et al.*, 1962). Since *Campylobacter* do not ferment sugars, routine fermentation tests have limited value. The differentiation of the subspecies of *C. fetus* also requires experience, as the glycine-tolerance and H_2S tests in various laboratories may show quite variable results. The use of 0.1% sodium selenite in broth may be a useful test for *C. fetus* subsp *fetus* identification (Neil *et al.*, 1985).

2.16. Biotyping and Serotyping Techniques

Campylobacter species do not utilize carbohydrates as a source of energy and is thus relatively of little merit upon routine biochemical testing (Kiggens and Plastridge, 1958). The

biotype II (33.0%). All *C. laridis* isolates belonged to biotype I. The combination of the biotyping scheme with the serogroups of *Campylobacter* provided additional epidemiological markers by further differentiating the serogroups by species and biotypes.

Magee *et al.* (1996), reported that resistotyping with or without additional biotyping proved to be a convenient, simple and inexpensive method well suited to use in local laboratories. They mentioned that it is a strong candidate for first line national and local surveillance of *Campylobacter* infections, fulfilling a need for monitoring of the cause of enteric disease due to *Campylobacter* infections. In Nigeria, Olubunmi and Adeniran (1986), classified *Campylobacter* isolates based on method previously described by Chaletain and Vero(1973).

The early attempts to determine the serological groups or identity of these organisms were made in 1957 by King (1957), who showed that related *"Vibrio"* organisms and *Vibrio fetus* isolated from human disease were antigenically different. In 1971, Berg *et al.*, divided the *Campylobacter* group (*Vibrio fetus)* into three serotypes based on heat-stable antigens. *Campylobacter jejuni* and *C. coli* possess several surface antigens and it is clear that there are a great many serotypes. Several serotyping systems have been developed but two principal ones have emerged, each detecting a different class of antigen. In the one system, heat stable antigens are detected by passive hemagglutination (Penner and Henessy, 1980). In the other heat labile antigens are detected by slide agglutination of live bacteria (Lior *et al.*, 1982). To date, the Penner system has recognized 60 serotypes. The production of antisera and interpretation of the test results is straight forward with that system, even though the test is time consuming to perform. On the other hand, the Lior system has recognized some 68 serotypes.

In this method, raising of antisera is more difficult (high titer sera and many cross absorption are required), but the actual typing technique is simple. *Campylobacter* serotyping is not yet generally available.

2.17. Treatment

Ovine strains of *C. fetus* like bovine strains have been shown to be sensitive to many antibiotics in vitro. In orally inoculated ewes a highly significant reduction in the incidence of abortion resulted from the use of intramuscular penicillin and dihydrostreptomycin combination with oral chlortetracycline but in field trails there was no benefit. There is some risk in the feeding of chlortetracyline to sheep (Gilmour *et al.*, 1975).

Furazolidone by mouth following experimental infection has not proved to be effective in preventing abortion. Chlortetracycline at 80mg per ewe daily fed continuously for several days before lambing has proved effective in the prevention of abortions. The cost of this, its possible dangers and the erratic pattern of disease outbreaks does not justify its use in this way (Laing, 1979).

2.18. Control and Prevention of Ovine Campylobacteriosis

Vaccination against ovine and bovine campylobacteriosis prior to mating has been shown to give good protection. Initially it was shown that a vaccine incorporating a single serotype give maximum immunity to *Campylobacter* infection but now a combined type I and V formalin killed adjuvant is used. *C. fetus* vaccine is generally employed. This will produce an immunity for up to 4 months, but annual inoculation of replacement ewes is advisable

(Plastridge, 1955).

A commercially prepared vaccine has also been used successfully in flocks as soon as a diagnosis of campylobacterosis was made. At the same time two daily injections of ($8x10^5$ i.u procaine penicillin G and dihydrostreptomycin sulphate were given and this combination was effective in preventing further abortions(Laing,1979).

Different methods of vaccination have been recommended by different workers. Luckkin *et al.*, (1987), used an inactivated emulsified vaccine (in use since 1980) for controlling abortion caused by *Campylobacter fetus*. A single dose of 1ml was injected into "parental tissue" during 3 weeks before or after artificial insemination as a preventive measure. Nikanorov *et al* (1984), recommended the use of formaldehyde inactivated vaccine containing mineral oil adjuvants prepared from *C. fetus subsp intestinalis* for immunization in sheep.

Miller and Jensen, (1963), reported a high degree of protection by an oil adjuvant formalin killed bacitracin against challenge by oral administration of a culture. All of 63 vaccinated ewe lambed normally while 22 of 25 unvaccinated controls aborted. Of 69 ewes vaccinated with an aluminium potassium sulphate adjuvant bacitracin, three aborted. There is available a commercially prepared aluminum hydroxide adsorbed bacitracin vaccine in U.S.A., although their efficacy is doubtful (Nikanorov *et al* (1984).

Vaccination of female breeding stock may prove to be the practical method. A significant degree of protection against bovine vibriosis can be conferred on heifers and cows by subcutaneous injections of living *Vibrio fetus (venerealis)* (Osborne, 1952). Most cattle herds are normally grazed under range and semi-range conditions. In situations like this, effective control of bovine vibriosis is difficult. Artificial insemination (AI) could now be used

in suitable areas to control the disease and it is probable that, with the extension of AI services, bovine vibriosis will become relatively uncommon in cattle (Clark *et al.*, 1967).

Studies on natural epizootic of ovine vibriosis indicate that immunity follows abortion. Ewes aborting from infection with *Vibrio fetus* were reported to be immune to vibrionic abortion for at least one year and later investigations increased the time to three years. Reports also indicated that some ewes may become carriers and serve as a reservoir of infection for pregnant ewes during the subsequent lambing season (Jensen *et al.*, 1957). However, the disease is self-limiting in that it is unusual for abortions to occur in a self-contained flock the year after an outbreak of abortion. The flocks which are in contact with aborting animals but which do not themselves abort and are non-pregnant females, do not abort at subsequent pregnancies. This development of immunity to abortion can be used to ensure that an outbreak is confined to one lambing season (Gilmour *et al.*, 1975).

Whatever control measures are attempted, general sanitary precaution must not be neglected. Aborting ewes should be isolated until all discharges have ceased preferably for a period of three to four weeks. Fetuses and fetal membranes should be submitted for laboratory diagnosis or destroyed and the area in which abortion occurred disinfected. It is essential that placental and dead lambs should be incinerated or buried in unslaked lime. Contamination of feed and water supply should be avoided.

2.19. Antimicrobial Activities of Medicinal Plants in Nigeria.

In recent times, there has been an increased awareness of the importance of traditional medicine in the health care of human populations in developing countries. Efforts are being

made in different countries to carry out research into traditional medicine and to integrate it into modern orthodox medicine (Nwude and Ibrahim, 1980).

Herbal medicine has been practiced for over 2000 years before the advent of western medicine and that even the health care of the western world started with traditional methods. But even with these facts it is said that western scientists are just beginning to appreciate the use of *medicinal plants in curing diseases* (Kossowon, 1991). Kossowon (1991), also cited that scientists have tested the medicinal properties of only two percent of the 250,000 plant species of the flowering plants known to man and about 120 plant based drugs used in western medicine are derived from only 95 species of the plants.

Plants have traditionally served as man's most important weapon against pathogens, and in fact, it seems that even Neanderthals knew and made use of medicinal plant (Solecki, 1975). As early as 2000, BC the Chinese were using moulds to treat festering ulcers and the ancient Egyptians are known to have applied moulds to an open wound (Oldfield, 1984).

It is only relatively recently, however, with the advent of modern technology and synthetic chemistry that we have been able to reduce our total dependence on the plant kingdom as a source of medicines. We nonetheless continue to rely on plants to a much greater degree than is commonly realized. Almost half of all prescriptions dispensed in the U.S.A contain substances of natural origin and over 50% of these medications contain a plant derived active principle (Farnsworth and Biorgel, 1977). In 1974 alone, the U.S. imported 24.4 million dollars worth of medicinal plants for drug development (Caufield, 1982).

Awosika (1986), stated that in view of the present unbearable increase in the cost of drugs, Nigeria has no choice but to start to develop drugs from the over 500 plants indigenous

to the nation. Medicinal plants are safe, unfake, simple, available, affordable, effective, economical and with uncomplicated method of therapy when compared with emergence of resistant strains of microorganisms. The abuse of antibiotics and genetic versatility of microbes have rendered some hitherto effective antimicrobial agents useless (Lannette *et al.*, 1985).

In spite of the innovation in the area of drug development, infectious diseases still account for seven per cent of all deaths in United State (Mitscher and Raghav-Rao, 1984). Due to the problems of drug resistance and high cost of drugs, scientists especially in Africa and other developing countries are conducting research into local plants which are used in traditional medicine (Akpulu, 1991).

In Nigeria, it is estimated that sixty per cent of the rural population of the country depend on plant based drugs (Ghani *et al.*, 1989). The use of plant based drugs is equally increasing in Nigeria. This implies that plant based drugs which for centuries had remained man's prime therapeutic agents are still preferred for treating a large number of ailments (Farnsworth and Morris, 1976).

2.20.1 Biology of *Vitex doniana*

Vitex doniana belong to the family *Verbenaceae*. The plant is widely distributed throughout the tropics and with rare exceptions the leaves are opposite and digitately compound. In Nigeria this combination distinguishes *Vitex* from all other genera(Keay,1989). There are about a dozen species of vitex in Nigeria. Some of them grew as shrubs.

Vitex domina Sweet FW TA ed. 2.

This is the most abundant and widespread vitex in Savannah regions. It is readily

recognised by its long stalked glaborous leaves which are usually rounded at the apex,although they may be indented or occasionally have a very short triangular tip. The fruits are edible. The local Nigerian names of this plant are the following:

Hause: Dinya, Fulani: Galbihi, Yoruba: Oori-nla, Igbo: Ucha Kor

The tree is about 15m high and 3m in girth, with a dense rounded crown and dark green foliage.The bark is gray to pale brown, finely fissured longitudinally and fibrous, yellowish when slashed, darkening on exposure. Leaves with a stout common stalk 7 - 15 cm long with 5 very broad elliptical leaflets mostly rounded at the apex and tapering at the base, with about 10 pairs of prominent lateral nerves. Flowers appears around January to April and they are pinkish white about 7 - 12 mm long. Fruits also appears around March and they are broadly ellipsoid, about 2.5 cm in diameter.

In Sierra Leone, they are regarded as good remedy for conditions due to avitaminosis associated with sores of the corners of the mouth and eyes, and sometimes paralytic symptoms in advanced cases (Keay, *et al.*,1964) .

In Eastern Sudan, the fruits when roasted are said to be a substitute for tea. Ink is made from it by boiling the black fruit and young leaves or from the bark.

The fruit and in some cases the bark and leaves are used for the treatment of diarrhoea and dysentery (Keay,1989). In France an infusion of the leaves is used for colds and the crude extract of plant roots is also used for stomach troubles. In Northern Nigeria, *Loran* growing on this tree is a remedy for leprosy.

Phytochemistry of the plant revealed that the bark is made up of flavonones, sterol and leucoanthocyanidins. The leaves contain iridoid glycosides e.g. nishindaside and negudoside

and flavones. The seed and oil (vanillin) contain a mixture of alkaloids and pthalic acid derivative and terpines (Kirtikar and Basu, 1975). The leaves are useful in gonococcal epididymitis.

A compound oil prepared from the juice of the plant and eleven other substances in different proportion is thought to be specific for syphilis and other venereal disease (CCRAS, 1990).

2.20.2. Biology of *Acacia nilotica*

Acacia belong to the *leguminosae* which comprise 650 genera and 18,000 species and the largest family of flowering plants after the compositae and orchidaceae. Compared with those families and many others, the leguminosae are notably generalist ranging from forest giant to tiny ephemerals, with great diversity in the methods of acquiring the essentials of growth and mode of reproduction and defence.

Minosoideae is one of the three widely recognized subfamilies of leguminosae. It contains approximately 50-60 genera distributed throughout tropical, subtropical and warm temperate regions of the world. The distribution of the species among the 56 genera recognized by Hutchinson(1964), is very uneven.

Almost two thirds of the known species fall into three genera; *Acacia* with 1200 species, *Mimosa* with 400-500 species, *Inga* with 350 - 400 species (Elias, 1974). Many of the genera are small nearly one-half of those recognized by Hutchinson, (1964) are monotypic and most of these are African. The tribe *Acacieae* contains a single genus *Acacia* of about 1200 species in tropical and subtropical regions. More than half of these occur in Australia, many in Africa and

the Americas, the fewest in Asia. This tribe are traditionally defined by valvate calyx lobe and indefinitely numerous stamens either free or very infrequently united only at the base (Hutchinson and Dalziel, 1958). *Acacia nilotica* (Linn.) Willdl ex Del (Syn A. arabaca) the species with several varieties in Semi-arid parts of Africa and India. It is a deciduous tree of 15m and 35cm diameters. In West Africa the pods are the main source of tanning material. They yield good firewood, charcoal and timber for agricultural implements. Their leaves are used as fodder. It grows in an area with sea level of about 1200m and with annual rainfall 300 - 900mm and temperature of 15 - 40^{0}C.

The plant has been examined in Senegal for its antidiarrhoeal effects. Two types of tannins were identified as gallotannins and catechins. It was postulated that high concentration of tannin could explain both the use of this plant for tanning hide and skin for shoe-making in Senegal and its therapeutic use in treating diarrhoea (Mashn, 1975).

Extensive studies of *Acacia* heartwoods and barks, carried out in recent years by South African and Australian chemists,lead to the discovery and widespread use of these plants as sources of commercial tannins (Guinet and Vassal, 1978).

CHAPTER THREE

MATERIALS AND METHODS

3.1. The Survey for Campylobacter in Zaria and Kaduna.

Rectal and vaginal swabs from sheep were collected for isolation of *Campylobacter* species.Two hundred and fifty samples of vagina and rectal swabs each were collected from the sheep farm of the National Animal Production Research Institute, Shika, Zaria. The slaughtered sheep were randomly sampled for *Campylobacter spp* from the slaughter house at Kaduna main abattoir at Kasuwan shanu. The samples included two hundred and fifty samples of intestinal content, gall bladder swabs each and hundred samples of the stomach contents of fetuses derived from slaughtered pregnant ewes. The samples obtained were inoculated into sterile Ferrous Bisulphate Pyruvate Broth (FBP) containing GIBCO nutrient broth which served as transport and enrichment medium (TEM). The TEM was brought to the Laboratory within 4 hours of collection. It was then inoculated and streaked onto Butzler's media immediately.

3.2. Media Preparation

Butzler's selective medium was prepared by adding 20 gm of Oxoid Blood Agar Base No. 2 to 500 ml of sterile distilled water and dissolved by bioling over bunsen burner with occassional shaking, then autoclaved at 121°C for 15 lb/sq in pressure for 15 minutes. The autoclaved medium was then placed in water bath to cool the temperature to 50-55°C. At this temperature (50-55°C), the selective antibiotic supplemnet (SR 85 Oxoid) was then added aseptically to the medium after reconstituting the vial with 3.0 ml of 50/50 v/v

ethanol/water . The growth supplement (F.B.P), supplement SR 84 Oxoid was also reconstituted with 2.0 ml of sterile distilled water added aseptically to the 500 ml of the medium at the same temperature. The temperature of the water bath with the medium was then adjusted to 40°C and then 10-15% defibrinated sheep blood was added aseptically. The medium was then poured into sterile petri-dishes and allowed to settle. The plates were stored at 4°C for not longer than 1- 2 days prior to use.

3.3. Culture Procedure

The enriched samples (TEM) were inoculated onto Butzler selective medium(SR 85) using a Pasteur loop. The selective agar plates were streaked for the isolation of *Campylobacter* organisms. A reference strain of Weybridge *C. fetus* subsp *fetus* obtained from Dr E.K. Bawa,(No. 84/793), was inoculated onto selective medium to test for the ability of the selective media to support the growth of *Campylobacter.* The plates were then incubated at 37°C for 48 hours under microaerophilic condition. Microaerophilic condition was generated using candle extinction method (Coker and Dosumun-Ogunbi, 1986).

3.4. Detection of *Campylobacter*

The plates were examined for *Campylobacter colonies* after 72 hours of incubation. If growth was not observed, the plates were further incubated for 3 more days, after which they were discarded as negative.

3.5. Colonial Characteristics

Any smooth, butyrous, fine, pinpoint colonies which was slightly butter-like coloured or water droplike, non- haemolytic on agar was Gram stained and identified under microscope using oil immersion 100 objective lens magnification. *Campylobacter* appear as small curved or long spiral Gram negative rod,and motile organisms. They were further subjected to biochemical tests for confirmation within 2-4 days.

3.6. Biochemical Characterization

All the organisms preliminarily identified as *Campylobacter* were subjected to the following biochemical tests for confirmation according to standard procedures as described by Smibert,(1979); Cowan and Steel,(1974); Skirrow and Benjamin,(1980).

(a)Catalase test:- This test was done on the pure culture by adding 3% Hydrogen peroxide solution to a loopful of colonies placed on clean glass slide and observed for the effervescence.

(b) Motility test:- A few drops of sterile distilled water was placed on a glass slide to which a few colonies of the test *Campylobacter* organism was added. The smear was covered with a cover slide and observed microscopically,under oil immersion using high power magnification(x100). The result was then recorded as motile or non-motile.

(c)Oxidase test:- A piece of filter paper was smeared with a small loopfull of the test *Campylobacter* organism and 2 to 3 drops of 1% tetramethyl-P-phenylenediamine dihydrochloride was added. A positive reaction was shown by the development of a dark purple colour within 10 seconds.

(d)**Nitrate reduction test**:- Nitrate broth was inoculated with suspected *Campylobacter* organism and incubated for 24 hours followed by addition of 1 ml of nitrate reagent A and 1 ml of nitrate B. A brick red colour showed the presence of nitrite and indicated that nitrate has been reduced.

(e)**Urease test**:- Urea agar slant was inoculated with suspected *Campylobacter* organism and incubated at 37°C for 24 hours. Red colour indicates positive reaction while negative slant remain unchanged. Most *Campylobacter spp* are urease negative.

(f)**Glycine tolerance test**:- This was done using thioglycolate broth containing 1% glycine in a tube.The tube was inoculated with a test organism and incubated at 37°C for 24hours. The tube was then examined for growth after 48 hours.The growth of *C. fetus subsp intestinalis* is inhibited in the presence of 1% glycine whereas *C fetus subsp jejuni* is not (Karmali *et al.*, 1981).

(g)**Salt tolerance test**:- Test *Campylobacter* organisms were inoculated into tubes (18 x 150 mm with metal cap) containing thioglycollate broth with 3.5% sodium chloride. The tubes were then incubated at 37°C for 24 hours. *C sputorum* and some *Campylobacter* from fetal samples grow well in the 3.5% sodium chloride concentration (Karmali *et al.*, 1981).

(h)**Heat tolerance test**:- Test organisms were streaked on freshly prepared duplicate blood agar plates and incubated seperately at 37°C and,42°C for 24 hours. *C fetus* grow well at 37°C whereas *C jejuni* grow well at 37-42°C (Karmali *et al.*, 1981).

(i)**Hydrogen sulphite production**:- The test organisms were inoculated into nutrient broth in a test tube into which was inserted a strip of 1% lead acetate impregnated filter paper. The

H_2S production was then recorded as negative or positive depending on the dark colour production on the strip after incubation at 37°C for 48 hours.

(j)**Citrate test**:- Christensen's citrate slant was incoculated with test *Campylobacter* isolates and were incubated at 37°C for 48 hours. The presence of growth and citrate utilization changed the colour of the slant from light green to blue colour.

(k)**Selenite broth test**:- 1% selenite broth was used for the suspected *Campylobacter* organisms. The broth was inoculated and incubated at 37°C for 48 hours. The presence of growth is indicated by the turbidity of the broth whereas unchanged broth was recorded as negative.

(l) **Deoxyribonuclase test (DNA) hydrolysis**:- DNA(Bovine pancreatic DNase-Grade II Cat No. 104 159 (EC 3.1.21.1) was purchased from Boehringer, Mannheim, Germany. DNA was dissolved in sterile distilled water at a concentration of 2 mg/ml. DNA solution was added to the Oxoid nutrient agar base immediately before autoclaving and poured into the plates as soon as the medium cooled to about 50-55°C. The suspected *Campylobacter* was inoculated and incubated at 37°C for 48 hours. It was then flooded with N-HCl. A positive result was shown as a clear zone around the growths and a negative result as opaque.

(m) **Acid production and H_2S production in tripple sugar iron agar**:- The Tripple sugar iron medium was prepared and inoculated with the suspected *Campylobacter* organisms. It was then incubated at 37°C for 48 hours. Acid production was indicated by a change in colour of the slant and butt to yellow while H_2S production resulted in dark colouration and unchanged result recorded as negative.

(n) **Hippurate salt hydrolysis**:- For the determination of hippurate hydrolysis, one loopful of a young culture of each strain under investigation was suspended in 2 ml of physiological saline solution. After that, 0.5 ml of 5% sodium hippurate was added to the physiological saline solution and the suspension incubated at 37°C for 15 minutes in a water bath. After incubation, 1 ml of 3.5% ninhydrin solution was added without mixing and the reaction was recorded as positive if a purple colour appeared within 10 minutes and negative if the solution remained unchanged.

3.7. Serotyping

Deoxyribonuclase (Bovine pancreatic DNase-Grade II Cat No 104 159 (EC 3.1.21.1) was purchased from Boehringer, Mannheim, Germany.A 0.1% stock solution of the DNase was prepared in PBS (0.01 M, PH 7.2) and stored in an aliquots at -20°C. The working solution of the DNase was prepared by diluting 1 ml stock solution with 4 ml of PBS and 0.5 ml of the aliquots were then dispensed into a screw cap tubes and stored frozen at -20°C when not used immediately. PBS-merthiolate (1:10,000) was prepared by dissolving 1 gm of merthiolate in 99 ml of PBS to make 100 folds dilution. The 100 fold diluted merthiolate was further diluted using 99 ml of PBS to obtain 10,000 folds. A drop of merthiolate was added to the diluted antisera as preservative against bacterial agents. A 2% NaCl solution used for determining autoagglutination was also prepared by dissolving 2 gm of NaCl in 100 ml of distilled water. Clean glass slides were used for agglutination.

The antisera were used according to the Laboratory Centre for Enteric Pathogens, National Reference Service for *Campylobacter*, Ottawa, Canada (NRSCC),which

recommended by NRSCC. If the strain showed less than 70% motility, it was subcultured to Mueller-Hinton Broth and incubated for 24 hours at 37°C before repeating the agglutination test.

3.9. Screening of Plant Extracts for Antimicrobial Activities against *Campylobacter* Isolates.

The plants used in this study were collected from Samaru village. They were all identified by Mr.U.S Gallah at the Herbarium Section of the Department of Biological Science, Ahmadu Bello University, Zaria. The plants were sun-dried and then separately ground in a mortar. A suspension of each dried powder in water or ethanol was made in the ratio of one to five (1:5). The suspensions were left overnight for adequate suspension.

The materials were then filtered using wire guaze filter and the filtrates were dried using the hot plate to evaporate the solvent used. They were weighed to determine the quantity of the powdered extract of each suspension. The powdered extract was resuspended in sterile distilled water using 2gm in 5ml. The pH of the powdered extract was determined using pH meter Model 7020 manufactured by Electronic Instrument Limited, Kent, U.K and the temperature was adjusted to 37°C. The bacterial species that were used as test organisms were *Campylobacter* isolates from sheep in this study. *Bacillus subtilis* ATCC strain obtained from Dr. J. Kabir of the Departement of Veterinary Public Health and Preventive Medicine, Ahmadu Bello University, Zaria was used as a sensitive control test organism.

3.10 Antimicrobial Activities of *Vitex doniana* and *Acacia nilotica*

Paper discs of 1 cm diameter were punched out with a puncher from Whatman filter paper No. 1. The discs were put into a covered Petri-dish and sterilized by autoclaving. Fifty pieces of the disc were then soaked singly into different dilutions of the compound (2gm/ 10ml) to get concentration of 200 mg until they saturated. Other paper disc concentrations of the extracts powder prepared and used were 200mg, 20mg and 2mg. They were then labelled as VDE (*Vitex doniana* ethanol)extract, VDW (*Vitex doniana* water)extract, and ANE (*Acacia nilotica* ethanol)extract, ANW (*Acacia nilotica* water)extract respectively. The excess dilution of the extract was then evaporated using the hot plate and the discs were dried up.

McFarland turbidity standared suspension No 1 was made by adding 0.1ml of 1% of Barium chloride to 9.9ml of 1% of Tetraoxosulphate (VI) acid. This turbidity approximates a bacteria density of about 3×10^8 organisms per ml. 0.2 ml of the standardized suspensions of the bacterial test organisms were each pipetted onto Mueller Hinton Agar plate and spread evenly with the aid of sterile bent glass rods (Odama *et al*,. 1986; Akpulu, 1991) on the agar. Sterile forceps was used to placed the discs onto agar plates. The plates were then allowed to stand for 30 minutes for proper diffusion of the active components in the disc into the agar, after which the plates were incubated at 37^{O}C for 24 hours.Following incubation, the zones of inhibitions were then measured to the nearest millimeter using a ruler (Erickson and Sherris, 1971).

3.11. Determination of Minimum Inhibitory Concentration (MIC)

The culture of *Campylobacter* organisms (in nutrient broth) was suitably diluted with sterile physiological buffer saline, such that 1 ml of the broth contained approximately 3×10^8

organisms. The dilution was done by comparing the density with a suspension of barium sulphate as earlier described. Serial dilution of the extract of the plants were made by adding 10 gm of the extract to the 20 ml of the molten Mueller Hinton agar and autoclaved to achieve concentration of 500 mg/ml. Other concentrations of the extracts used were 500 mg, 250 mg, 125 mg, 65 mg as described earlier. Concentration such as 200 mg and 80 mg were also used to determined the exact inhibition value especially after other concentrations have been used (Apkulu, 1991). One drop of 0.02 ml of the standardized culture was added onto the surface of Mueller Hinton agar containing various concentration of the extracts and streaked in three different directions and incubated at 37°C for 24 hours. The minimum inhibitory concentration was the least concentration of the extract showing no visible growth of the organism(Williams and Popoola, 1983).

CHAPTER FOUR

RESULTS

4.1. Isolation of *Campylobacter* Species from sheep in Zaria and Kaduna.

The isolates that were motile Gram negative curved to spiral rods suspected to be *Campylobacter* were catalase positive, oxidase positive, nitrate positive, did not produce acid and H_2S in Triple sugar iron agar, but grew well at 37°C and 42°C but not at 25°C. They also grew in 1% glycine but not in 3.5% Sodium chloride. Those of them that were hippurate positive but H_2S and Deoxyribonuclease(DNase) negative were categorized as *C jejuni* while *C. coli* were hippurate , H_2S, and Dnase negative. *C. laridis* were H_2S positive, but hippurate and Dnase negative. *Campylobacter fetus* was not identified among the isolates.

Table 4.1 shows the species and biotypes of *Campylobacter* isolates obtained from different anatomical sites of sheep examined.

Of a total of 1100 samples examined, 39(3.54%) yielded *Campylobacter* organisms. The highest isolation rate (6.8%) was from samples of intestinal contents followed by those from gall bladders (4.0%). Samples from the vagina and fetuses had the lowest isolation rates of 2.0% and 0% respectively.

Of the 39 *Campylobacter* isolates from all the sources, 79% were identified as *C. jejuni*, 13% as *C. coli* and 8.0% as *C. laridis*. *C. coli* and *C. laridis* were isolated from gall bladder and intestinal content only.

Table 4.1. Biotypes of *Campylobacter* species from Different Anatomical Sites of Sheep in Zaria and Kaduna .

Anatomical site/location	Number of samples	Number Positive	Isolation Rate(%)	*Campylobacter species*	Biotypes of *Campylobacter* isolated	Percentage of biotypes
Gall bladder Kaduna	250	10	4	*C. jejuni*(8)	biotype I(5)	50
					biotype II(1)	10
					biotype III(2)	20
				C. coli(1)	biotype I(1)	10
				C. laridis(1)	boitype II(1)	10
Intestinal contents Kaduna	250	17	6.8	*C. jejuni*(11)	biotype I(6)	34
					biotype II(2)	12
					biotype III(1)	6
					biotyep IV(2)	12
				C. coli(4)	biotype I(2)	12
					biotype II(2)	12
				C. laridis(2)	biotype I(2)	12
Rectum Zaria	250	7	2.8	*C. jejuni*(7)	biotype I(2)	28
					biotype II(4)	57
					biotypeIV(1)	15
Vagina Zaria	250	5	2.0	*C. jejuni*(5)	biotype I(3)	60
					biotype II(1)	20
					biotype III(1)	20
Fetuses Kaduna	100	-	-	-	-	-
Total	1100	39	3.54	-	-	-

4.2 Biotypes of Campylobacter Species Isolated Ffom Sheep in Zaria and Kaduna

C. jejuni isolates from the vagina, gall bladder and intestinal contents belonged to biotype I mainly, whereas, those of rectal swabs were mostly bioytpe II. There was no isolation of *C. jejuni* biotype IV from the gall bladder, rectal swabs and intestinal contents. Although biotype I was the most predominant of all the *C. jejuni* isolates, both biotype I and II were isolated from all the anatomical sites of slaughtered sheep sampled from sheep in kaduna state.

Table 4.2 shows that *Campylobacter jejuni* biotype 1 was the most prevalent biotype, which accounted for 40.3% of the total isolates, while biotype II represented (20.5%) and Biotypes III and IV representing 10.2% and 8.0% respectively. *C. coli* isolates were of biotypes I and II which occur with frequencies of 8%and 5 % respectively. *C. laridis* were also placed into biotypes 1 and II representing (5%)and (3%) respectively from gall bladder and intestinal content only.

Table 4.2.Percentage of different biotypes of *Campylobacter* Species Isolated from Sheep.

Campylobacter Species	Different Biotypes	Number of isolates	Percentage of total isolates
C. jejuni	Biotype I	16	40.3
	Biotype II	8	20.5
	BiotypeIII	4	10.2
	Biotype IV	3	8.0
C. coli	Biotype I	3	8
	Biotype II	2	5
C. laridis	Biotype I	2	5
	Biotype II	1	3

4.3. Serotypes of *Campylobacter* Isolated from Sheep in Zaria and Kaduna.

Table 4.3. shows the distribution of different serogroups of *Campylobacter* species isolated from various anatomical sites of sheep in Zaria and Kaduna . The commonest serogroups were 36(10.3%),20(7.7%), 4(7.7%), and 9(7.7%). Other serogroups recorded in sheep in Kaduna State were 21(5.8%) and 29, 84, 8 representing (5.1%) each They all belonged to *C. jejuni* . *Campylobacter coli* were represented by serogroups 4 (7.7%), 6 and 84, representing 2.6% each. *C .laridis* isolates were not serotyped because the packed antisera sent from NRSCC-Canada did not contained *C. laridis* . There was no significant difference in the recovery of the different serogroups from the different specimens investigated, although serogroup 36 was found to occur in all anatomical sites except in the gall bladder. Serogroups 4 and 9 were the commonest serogroups found in the gall bladder representing 20% each. Other serogroups found in the gall bladder were 53,20,24, and 84 occurred in equal proportional of 10% each. Serogroups from the intestinal contents were 20,,9,36,and 4, repesenting 11.77% each. The serogroups from rectal swabs were 4,18,36,22,45 and 5 representing 14.29 % each. Vaginal serogroups were 8(40%) and serogroups 55,36,29, which occurred in equal proportional of 20% each.

Table 4.3. Distribution of Different Serogroups of *Campylobacter* Species Isolated from Various Anatomical Sites in Sheep in Zaria and Kaduna .

Type Campylobacter	Serogroup	As % of Anatomical Site Isolates				As % of Total Isolates
		Gall bladder	Intestine	Rectum	Vagina	
Campylobacter jejuni	4	2(20)	-	1(14.29)	-	7.69
	36	-	2(11.77)	1(14.29)	1(20)	10.26
	53	1(10)	-	-	-	2.56
	20	1(10)	2(11.77)	-	-	7.69
	9	2(20)	2(11.77)	-	-	7.69
	84	1(10)	1(5.88)	-	-	5.13
	6	-	1(5.88)	-	-	2.56
	29	-	1(5.88)	-	1(20)	5.13
	8	-	-	-	2(40)	5.13
	24	1(10)	-	-	-	2.56
	21	-	1(5.88)	-	-	5.88
	45	-	-	1(14.29)	-	2.56
	5	-	-	1(14.29)	-	2.56
	18	-	-	1(14.29)	-	2.56
	55	-	-	-	1(20)	2.56
	1	-	1(5.88)	-	-	2.56
	22	-	-	1(14.29)	-	2.56
Campylobacter coli	4	1(10)	2(11.77)	-	-	7.69
	6	-	1(5.88)	-	-	2.56
	84	-	1(5.88)	-	-	2.56

Table 4.4 : Distribution of different serogroups found in the gall bladder, vaginal swabs, intestinal content swabs, and rectal swabs of sheep in Kaduna State

Campylobacter strains	Serogroup	No.	Percentage
	4	5	13
	53	1	3
Thirty nine different	36	4	10
strains of	20	3	8
Campylobacter species	9	4	10
isolated	84	3	8
	6	2	5
	29	2	5
	8	2	5
	24	1	3
	21	1	3
	1	1	3
	22	1	3
	45	1	3
	5	1	3
	18	1	3
	55	1	3
	NT	2	5
	NIP	3	8
TOTAL	17	39	100

NT=Not typeable isolates

NIP=Not included in the pack

4.4 Antimicrobial activites of the Extracts of the Leaves/Bark *Vitex doniana* and *Acacial nilotica* on *Campylobacter* Isolates from Sheep.

Table 4.5 shows the pH of ethanol and water extracts of *Acacia nilotica* and *Vitex doniana* . The pHs of both the ethanol and the water extracts of *Acacial nilotica* were lower than those of *Vitex doniana*. The pH values of ethanol and water extracts of *Acacial nilotica* were 4.40 and 4.60 respectively, while those of *Vitex doniana* was 5.35 for ethanol and 5.30 for the water extract

Appendix 1. shows the effect of the extracts on *Campylobacter* isolates using paper disc diffusion method. The ethanol extracts of *Acacial nilotica* had profound inhibitory effect at the lower concentration on the isolates while the ethanol and water extracts of *Vitex doniana* had no effect on them.

Appendix 2. shows the minimum inhibitory concentrations (MICS) produced by the extracts. The MIC for the ethanol extracts of *Acacial nilotica* was 80 mg/ml while the water extract of the same plant was 250mg/ml. The *Vitex doniana* (MIC) was above 500mg/ml for both the ethanol and water extracts against the tested *Campylobacter* isolates. For the control *Bacillus subtilis* the (MIC) for *Acacial nilotica* water extracts was 125mg/ml while the corresponding value for ethanol extracts was 65mg/ml. MIC for *Vitex doniana* water and ethanol extracts was above 500mg/ml.

Table 4.5. Concentration and pH of the extracts of the plants.

Extracts	pH	Quantity
ANE	4.40	2gm / 5ml
VDE	5.35	2gm / 5ml
VDW	5.30	2gm / 5ml
ANW	4.60	2gm / 5ml

ANE=*Acacia nilotica* ethanol extract
ANW= *Acacia nilotica* water extract
VDE=*Vitex doniana* ethanol extract
VDE=*Vitex doniana* water extract

CHAPTER FIVE

DISCUSSION

The results of this study indicates the overall isolation rate of ovine ***Campylobacter*** to be 3.54% from all the samples examined. The isolation rate of 2.8% from the rectal swabs collected from healthy sheep in Zaria is similar to those of Turkson *et al.* (1988), who reported a prevalence rate of 2.0% from rectal swabs of healthy sheep in Kenya. Olubunmi and Adeniran (1986), reported a prevalence rate of 6.25% in sheep from western Nigeria, and a 6.9% prevalence rate was reported by Abraham *et al.* (1990) in healthy Ghanaian sheep. The results are different from those of Adegbola *et al.* (1991) and Adetosoye and Adeniran (1987) who failed to isolate any *Campylobacter* organism from heathly sheep and goats in Ile-Ife . Similarly, all the isolates of *Campylobacter* obtained from rectal swabs in this study were *C. jejuni* with biotype II the commonest, representing 57% of the total isolates. This disagrees with the findings of Olubunmi and Adeniran (1986), in which *Campylobacter* biotype I accounted for 58% of their isolates, but agrees with the results of Abraham *et al.*,(1990),in which biotype II predominated in their studies. Furthermore, the variation could be a true reflection of the distribution of *Campylobacter* biotypes in these environments.

The isolation of *Campylobacter* from intestinal contents of slaughtered sheep at Kaduna abattoir indicates that *Campylobacter* does colonize the intestinal mucosa of healthy animals as earlier reported by Skirrow and Butzler (1979). This may serve as a source of infection to other animals in the environment.

The isolation rate of 6.8% from the intestinal contents of sheep slaughtered at Kaduna abattoir is higher than the 4.9% reported by Koides (1991) from intestinal contents of healthy sheep in Turkey. The two results also differ in the percentages of *C. jejuni* isolates. In this study, the overall proporation of *C. jejuni* 64% (Table 4.1) in relation to all isolates, is much lower than the 93% reported by Koides (1991). In both findings, other *Camylobacter species* such as *C. coli* were also isolated. In the present study,*Campylobacter* was also isolated from the vagina of apparently healthy ewes from a flock in Shika-Zaria which has no history of abortion. However, the birth of weak lambs that eventually survive was reported by the herdsmen.This study did not however determine the role of *Campylobacter* in the birth of these weak lambs. Isolation of *Campylobacter* organisms from vaginal swabs of healthy sheep after parturition was earlier reported by Montagna *et al.*(1988). The low isolation rate of 2.0% of *Campylobacter* from the vaginal swabs of sheep sampled at the NAPRI farm in Shika, Zaria and the failure to isolate *Campylobacter* from fetuses examined at Kaduna abattoir suggest that vagina and fetuses may not be important sources of the organism for human infections.The fact that all the vaginal isolates of *Campylobacter* belonged to the same biotype and species of *C. jejuni* is in agreement with work reported by Montagna *et al.*, (1988), who reported an isolation rate of 2.5%, and found *C. jejuni* as the major *Campylobacter* species identified in the vagina of ewes.

The gall bladder which is regarded as organ of choice for the isolation of *Campylobacter* organisms (Firchammer *et al.*, 1962). An isolation rate of (4.0%) was found in gall bladder in the present study, which has been regarded as the organ of choice for the isolation of *Campylobacter* species. A similar (3.3%) rate was found by Marsh and

Firehammer,(1953) in two different geographical locations which was taken as indication of a poor rate of isolation of *Campylobacter* from the gall bladder. The majority (67%) of the *Campylobacter* isolates in the report of Marsh and Firehammer,(1953) were classified as *C. fetus* subsp *fetus* whereas the remaining 33% were identified as *C fetus subsp jejuni*. In the present study, most of the isolates (80%) from the gall bladder were *C. jejuni*. The rest were either *C. coli* or *C. laridis*. The reasons for the differences are not clear, but the type of *Campylobacter* in the gall bladder may be related to types of *Campylobacter* most commonly isolated from other anatomical sites in the animal. Furthermore, the variation could be a true reflection of the level of infection in these environments. In this study, the most common *Campylobacter spp* was *C. jejuni*. The failure to isolate *Campylobacter* from the fetuses examined in this study may be attributed to the fact that the fetuses were not obtained from clinical cases of epizootic abortion outbreaks and the methods used may not be adequate for the isolation from fetuses . The most common biotype of *C. jejuni* found in the present study was biotype I which accounted for 40.3% of all the isolates.This is in agreement with the findings of Adegbola *et al.,*(1990) and Varyal *et al.,*(1991) who found the same biotype to predominant in their studies. It is however different from what Adesiyun *et al.*, (1992) reported, who found *C .coli* biotype I as the major biotype from healthy sheep and other farm animals in Trinidad and Tobago. The differences might be due to a number of factors. Part of this may be related to ecologic differences in the areas where the studies were conducted. Similarly, the methodologies used in the laboratories where the studies were conducted could have influenced results obtained. This is illustrated by the works of Adetosoye and Adeniran (1987), Adegbola *et al.*, (1991),compared with the results of Olubunmi and Adeniran (1986), in the

same locality but in different laboratories. *Campylobacter fetus* which is also known to colonize the intestinal tract of domestic animals (Clark and Dufty, 1978; Doyle,1981; Manser and Dalziel, 1985), was not isolated in the present study. Failure to isolate the organism from the samples examined in this study may mean that the ewes examined were not infected with *C. fetus.* In addition, since the technique used in this study for isolating the organism from the samples involved using enrichment procedure and culturing within 4 hours of samples collection, it was unlikely that the organism was present in the samples examined. The isolation of *Campylobacter* organisms from rectal, vaginal, intestinal content, and gall bladder in this study is a clear indication of the presence of *Campylobacter* in sheep in Zaria and Kaduna.

The most common serogroups of *Campylobacter sp* isolated from sheep in this present study were 4, 36 ,9,20, 84, and to lesser extent 29, 6, and 8 accounting for 64% of the total isolates(Table 4.4) . Adegbola *et al.* (1991) reported serogroups 2, 4, 29 and 36 as accounting for 61.7% of all the serogroups found in animals in Ile-Ife. In another study, Adegbola *et al* (1990), reported serogroups 2, 4, 20, 29, 36 and 45 to be predominant among animals in Ile-Ife. In the same study they also found serogroups 2, 4, 29, 36, 45 and 53 to be the most common in humans. Recently, Coker and Adefeso (1994) reported that during a ten year study, serogroups 29 and 36 were the commonest serogroups in humans in Lagos. The serogroups found in sheep in this study are similar to those previously reported in domestic animals (Adegbola *et al.*, 1990;1991) and in humans (Coker and Adefeso, 1994; Alabi *et al.*, 1986) in Nigeria. During slaughter of sheep, goats and cattle, intestinal contents often contaminate meat. Improperly cooked meat contaminated with these organisms may therefore pose risk to

consumers. Meat and meat products should therefore, be properly cooked to destroy the organism so as to prevent possible transmission to man and other animals (Coker and Adefeso, 1994; Adegbola *et al.*,1990) It has been established that sheep is a natural reservoir of *Campylobacter* (Firehammer *et al.*, 1962). The serotypes identified in sheep in this study are similar to those reported previously in humans in Nigeria, which suggest the possibility of cross-transmission of *Campylobacter* from sheep to man and other animals. This assertion has been justified by the detection of *Campylobacter* serogroups in sheep similar to those reported in humans. To our knowledge, this may be the first documentation in sheep in Northern-Nigeria of serotypes that are similar to those that have been previously reported in humans.

The fact that there was no inhibitory effect of ethanol and water extracts of *Vitex doniana* on the tested *Campylobacter* isolates at MIC value of 500mg/ml suggests that this plant may have no therapeutic effect on the *Campylobacter* organisms. The antimicrobial effect of the ethanol and water extracts of the leaf of *A. nilotica* on *Campylobacter* isolates may be related to their pH values of 4.4 and 4.6 respectively. The pH values are lower than the optimal pH range of 6.5 to 7.5 for the growth of *Campylobacter sp.* However, the pH of the *V. doniana* was 5.3 while the ethanol extract had pH of 5.3, which are very close to the optimal growth condition of *Campylobacter*. This may be the explanation for the active effects of *A. nilotica* leaf extracts compared with the bark extracts of *V. doniana*. The susceptibility of *Campylobacter spp* to the extracts of these plants is very significant, considering the world wide phenomenon of antibiotic resistance widely exhibited by this organism (Coker and Adefeso, 1994). It is noteworthy and significant that both water and ethanol extract of the leaf of *A nilotica* had antibacterial activities against *Campylobacter* isolates. Low concentration of

the extract of this plant was inhibitory against 90% *Campylobacter* isolates. This shows the potentials of this plant in the therapy of *Campylobacter* infections and as a candidate for further investigation and possibly commercialization. Although, local herbalists know little or nothing about bacterial etiology of disease, their reported use of *A nilotica* and *V.doniana* for the treatment of diarrhea, dysentery and abdominal pains have been justified by the findings of this study.

5.1. CONCLUSIONS

These results clearly demonstrated the occurrence of *Campylobacter jejuni, C. coli* ,and *C. laridis* in sheep in Zaria and Kaduna.The study also revealed *Campylobacter jejuni* biotype 1 as the commonest biotype in sheep. *Campylobacter* serogroups 4, 20 ,29 and 36 were the predominant serogroups found in the course of the study.

Extrats of locally available plants of *Acacia nilotica* and *Vitex doniana* showed antibacterial properties against Campylobacter isolates *in -vitro*.

The isolation of *Campylobacter* species from sheep, biotypes and serogroups similar to those previously reported in humans in Nigeria suggest possible cross-transimission of *Campylobacter* from sheep to man in this area.

5.2. RECOMMENDATIONS

Based on the results of the present study the following recommendation are made:

1. Detailed epidemiological surveys are desirable to determine the magnitude and spread of *Campylobacter* species in man, animals and birds in Nigeria.
2. Further studies are needed on the sensitivity of the isolates to antimicrobial agents.
3. There is a need to investigate cases of abortion in sheep in order to determine the role played by *Campylobacter* species.
4. There is a need for veterinarians to further strengthen hygienic practices, in the disposal of wastes and during meat inspection procedures to minimize contamination and the risk of human transmission of zoonotic *Campylobacter* .
5. The finding of this study also calls, for increased funding of traditional medicine research with emphasis on toxicology, pharmacology and clinical application of the crude extracts.
6. There is a need to determine the active principle components of *A. nilotica.*
7. Further work is needed in the use of purified extracts rather than crude extracts

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Appendix 1: Antimicrobial effects of the crude water /ethanol extracts of the bark of *V.doniana* and leaf of *A nilotica* using paper disc diffussion method on thirty-nine isolates of *Campylobacter*

	ANE			ANW			VDE			VDW		
Isolates	200	20	2	200	20	2	200	20	2	200	20	2
Bacillus subtilis	24	18	-	24	6	-	6	-	-	-	-	-
$I10_2$	24	12	-	18	6	-	6	-	-	-	-	-
$G15_2$	24	12	-	18	6	-	6	-	-	-	-	-
$G23_1$	24	12	-	18	6	-	6	-	-	-	-	-
$I9_1$	24	12	-	18	6	-	6	-	-	-	-	-
$G15_1$	24	12	-	18	6	-	6	-	-	-	-	-
I26	24	12	-	18	6	-	6	-	-	-	-	-
V11	24	12	-	18	6	-	6	-	-	-	-	-
$G19_1$	24	12	-	12	6	-	6	-	-	-	-	-
$I21_2$	24	12	-	12	6	-	6	-	-	-	-	-
$I18_2$	24	6	-	12	6	-	6	-	-	-	-	-
V12	24	12	-	18	6	-	6	-	-	-	-	-
V16	24	12	-	18	6	-	6	-	-	-	-	-
$G23_2$	24	12	-	12	6	-	6	-	-	-	-	-
$I9_2$	24	12	-	12	6	-	6	-	-	-	-	-
$I10_1$	24	12	-	12	6	-	6	-	-	-	-	-
I22	24	12	-	6	6	-	6	-	-	-	-	-
$G19_2$	24	12	-	12	6	-	6	-	-	-	-	-
V9	24	12	-	18	6	-	6	-	-	-	-	-
F18	24	12	-	18	6	-	6	-	-	-	-	-
F8	24	12	-	18	6	-	6	-	-	-	-	-
I6	24	6	-	18	6	-	6	-	-	-	-	-
V15	24	12	-	18	6	-	6	-	-	-	-	-
I23	24	12	-	18	6	-	6	-	-	-	-	-
F33	24	12	-	18	6	-	6	-	-	-	-	-
I13	24	12	-	18	6	-	6	-	-	-	-	-
$I16_2$	24	12	-	18	6	-	6	-	-	-	-	-
F10	24	12	-	18	6	-	6	-	-	-	-	-

Appendix 1 (continued).

Isolates	ANE			ANW			VDE			VDW		
	200	20	2	200	20	2	200	20	2	200	20	2
F22	12	12	-	18	-	-	6	-	-	-	-	-
F11	24	12	-	18	-	-	6	-	-	-	-	-
$G17_1$	24	12	-	18	-	-	6	-	-	-	-	-
F28	12	12	-	18	-	-	6	-	-	-	-	-
$G17_2$	24	12	-	18	-	-	6	-	-	-	-	-
$I19_1$	24	12	-	18	-	-	6	-	-	-	-	-
G23	24	12	-	18	-	-	6	-	-	-	-	-
$I28_2$	12	12	-	18	-	-	6	-	-	-	-	-
$I14_1$	24	12	-	18	-	-	6	-	-	-	-	-
G9	24	12	-	18	-	-	6	-	-	-	-	-
$I18_1$	12	12	-	18	-	-	6	-	-	-	-	-
I21	12	12	-	18			6					

Key

F = Fecal swabs
V = Vaginal
- =no effecton the isolates
6 = diameter about 2mm
18 = diameter about 4-6mm
ANE = *A nilotica* ethanol extract
ANW = *A nilotica* water extract
VDW =*V. doniana* water extract
G = Gall bladder
I = Intestinal

12 = diameter about 2-4mm
24 = diameter about 6-8mm
VDE = *V.doniana* ethanol extract

Appendix 2 . The minimum inhibitory concentration of crude extract of *A nilotica* and *V. doniana* using agar incorporation method to thirty –nine isolates of *Campylobacter*.

Isolates	ANE					ANW					VDE			VDW		
	500	250	125	80	65	500	250	200	125	65	500	250	125	500	250	125
$I10_2$	-	-	-	-	+	-	-	+	+	+	-	+	-	-	+	-
$G15_2$	-	-	-	-	+	-	-	+	+	+	+	+	-	-	+	-
$G23_1$	-	-	-	-	+	-	-	+	-	+	+	+	-	-	+	-
$I9_1$	-	-	-	-	+	-	-	+	-	+	+	+	-	-	+	-
$G15_1$	-	-	-	-	+	-	-	+	+	+	-	+	-	-	+	-
I26	-	-	-	-	+	-	-	+	-	+	-	+	-	-	+	-
V11	-	-	-	-	+	-	-	+	+	+	-	+	-	-	+	-
$G19_1$	-	-	-	-	+	-	-	+	+	+	-	+	-	-	+	-
$I21_2$	-	-	-	-	+	-	-	+	+	+	-	+	-	-	+	-
$I18_2$	-	-	-	-	+	-	-	+	+	+	-	+	-	-	+	-
V12	-	-	-	-	+	-	-	+	+	+	+	+	-	-	+	+
V16	-	-	-	-	+	-	-	+	+	+	+	+	-	-	+	-
$G23_2$	-	-	-	-	+	-	-	+	-	+	-	+	-	-	+	-
$I9_2$	-	-	-	-	+	-	-	+	+	+	+	+	-	-	+	+
$I10_1$	-	-	-	-	+	-	-	+	+	+	+	+	+	+	+	-
I22	-	-	-	-	+	-	-	+	-	+	+	+	-	+	+	-
$G19_2$	-	-	-	-	+	-	-	+	+	+	+	+	+	+	+	+
V9	-	-	-	-	+	-	-	+	+	+	+	+	-	+	+	+
F18	-	-	-	-	+	-	-	+	+	+	+	+	+	+	+	+
F8	-	-	-	-	+	-	-	+	+	+	+	+	+	+	+	+
I6	-	-	-	-	+	-	-	+	+	+	+	+	-	+	+	+
V15	-	-	-	-	+	-	-	+	+	+	+	+	+	+	+	+
I23	-	-	-	-	+	-	-	+	+	+	+	+	+	+	+	+
F33	-	-	-	-	+	-	-	+	+	+	+	+	+	+	+	+
I13	-	-	-	-	+	-	-	+	+	+	+	+	+	+	+	+
$I16_2$	-	-	-	-	+	-	-	+	+	+	+	+	+	+	+	+
F10	-	-	-	-	+	-	-	+	+	+	+	+	+	+	+	+

Appendix 2 (continued)

Isolates	ANE						ANW				VDE			VDW		
	500	250	125	80	65	500	250	200	125	65	500	250	125	500	250	125
F22	-	-	-	-	-	-	-	-	-	-	+	+	-	+	-	+
F11	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	+
$G17_1$	-	-	-	-	-	-	-	-	-	-	+	+	-	+	-	+
F28	-	-	-	-	-	-	-	-	-	+	+	+	-	-	-	+
$G17_2$	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	+
$I19_1$	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	+
G23	-	-	-	-	-	-	-	-	-	-	+	+	-	+	-	+
$I28_1$	-	-	-	-	-	-	-	-	-	-	+	+	-	+	-	+
$I14_1$	-	-	-	-	-	-	-	-	-	+	+	+	+	+	-	+
G9	-	-	-	-	-	-	-	-	-	+	+	+	-	-	-	+
$I18_1$	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	+
I21	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	+
BS	-	-	-	-	-	-	-	-	-	-						

Key:

F = Fecal swabs
V = Vaginal
- = no growth
ANE = *A. nilotica* ethanol
VDE = V. ethanol extract
VDW = V. doniana water extract
BS = Bacillus subtilis
ANW = *A. nilotica* water extract
G = Gall bladder
I = Intestinal
– = growth

Appendix 3. Butzler's Culture Media Composition:

SR 85:	Bacitracin	25,000 units/litre
	Cyclohexamide	50mg/litre
	Colistin Sulphate	10,000 unit/litre
	Cephalozin Sodium	15mg/litre
	Novobiocin	5mg/litre
SR 84:	Ferrous Sulphate	0.25 g/litre
	Sodium Metabisulphite	0.25g/litre
	Sodium Pyruvate	0.25g/litre