BOVINE CAMPYLOBACTERIOSIS IN THE INDIGENOUS CATTLE OF SOME STATES IN NORTHERN NIGERIA

BY

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DECLARATION

I hereby declare that this thesis is original and has been written by the author. It is a record of my own research work and has not been presented before to any Institution or body for any award or honours.

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CERTIFICATION

This thesis entitled Bovine Campylobacteriosis in the Indigenous cattle of some states in Northern Nigeria by Elias Kambai Bawa meets the regulations governing the award of the degree of Master of Science of Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

This thesis is dedicated to my Grand Parents Bawa, Sheyin, Bafuwai and also to my late father-in-law Abiriyi Dodo and my Parents Reverend Kambai Bawa and Mrs. Assatu Kambai Bawa, who gave me the education that I now cherish. To them all I say bravo and thank you.

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ABSTRACT

Three Northern states (Kaduna, Kano and Borno) were surveyed for bovine campylobacteriosis in breeding bulls and cows. Six hundred and eighty nine breeding cattle were sampled. They were made up of 585 breeding bulls and 104 breeding cows. Campylobacter fetus Subsp. venerealis was isolated from 12 of the bulls, while Campylobacter fetus Subsp. fetus, was isolated from 3 of them

One and four of the cows yielded Campylobacter fetus Subsp. venerealis, and Campylobacter fetus Subsp. fetus. respectively. The overall prevalence of campylobacteriosis in the three states was 2.9 per cent (20/689). A herd that was naturally infected with campylobacteriosis was characterised by irregular returns (12.8%), infertility (36.01%) and abortions (2.3%). The estrous cycle lengths of the irregular breeding cows ranged from 15 to 80 days. Thirty per cent of the cycles ranged from 28 to 80 days which may be suggestive of embryonic mortality. In the second part of the study, 2 bulls were used, one experimentally infected with C. fetus Subsp.

fetus and the other not infected. They were used to serve two groups of virgin heifers of breeding age (3½ - 4 yrs). The group with the infected bull formed the experimental group, while the group with the non-infected bull formed the control. Estrus, services and pregnancy were monitored for 16 weeks. Blood samples were also collected from the heifers every other day for progesterone determination.

Two heifers became pregnant and three did not despite repeated services and thus classified as irregular returns (breeders). The estrous cycle lengths of the 3 irregular breeding heifers were irregular and ranged from 13 to 107 days. Each heifer was served 2 - 5 times without conception. The length of estrous cycles of the 2 non-irregular breeders were regular and ranged from 19 to 22 days and each had 2 to 3 services before conception.

The susceptibility of the five heifers to infection with campylobacter varied as indicated by the differences in the lengths of time before establishment of infection, which was 14, 63, 60, 58 and 56 days respectively after first services by the infected bull. Two infected heifers

that became pregnant after the 2nd or 3rd service by the infected bull stopped shedding the organism, 31 and 41 days after conception. Campylobacter could not be isolated from one of the infected irregular breeding heifers (2879), 70 days from the date she was first diagnosed as infected and she did not become pregnant.

The cervicitis observed in the study occurred in those heifers that had irregular estrous cycles and also failed to become pregnant, one of the irregular breeders developed both cervicitis and vaginitis.

Histologically, pathogenic lesions observed in the infected irregular breeder's genital tracts were limited to only congestion of endometrium of the uterine horns, cervix and the mucosa-submucosa of the vagina.

The progesterone profiles of the infected irregular breeding heifers were irregular confirming the irregularity of the estrous cycles.

The estrous cycle lengths of the control group heifers were regular and varied from 20 - 22 days, the progesterone profiles pattern were also regular. All became pregnant after the first or second service, non developed cervicitis or vaginitis.

In the third part of the study, semen from an infected bull with C. fetus Subsp. fetus was extended with egg-yolk citrate buffer and antibiotics added. The result indicated that the standard treatment of semen with Penicillin G at 1000iu/ml and streptomycin at 1000ug/ml of the extended semen as adopted at our artificial insemination Centre is effective in controlling C. fetus Subsp. fetus infection in semen.

The present study confirms the occurrence of both varieties of Campylobacter fetus (C. fetus Subsp. fetus and C. fetus Subsp. venerealis) in Nigerian cattle.

The results also indicated the consequent high infertility rate in cows despite the low prevalence of the organism among bulls.

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CHAPTER 1

INTRODUCTION

Northern States of Nigeria have 9.0 million out of the 10.5 million cattle population in the country (Adams and Mackey, 1966). Hence 99 per cent of the cattle population is in the North and mostly in the hands of the nomadic Fulani herdsmen.

Low reproductive performance have been reported for the indigenous Nigerian cattle (Nuru, 1974; Osori, 1976; and Oyedipe et al., 1982). This has been attributed to a number of factors including age at first calving (Oyedipe et al., 1982). Long calving interval (Akpokodje and Bolarinwa, 1974), calves losses (Nuru, 1974), seasonality in sexual activity (Osori, 1976; Zakari, 1981; and Voh et al., 1984), irregular estrous cycles and anestrous (Zakari, 1981). Some of these reports have blamed nutrition, management, diseases and their interactions to be responsible for this low reproductive efficiency of cattle indigenous to Nigeria (Nuru, 1974; Oyedipe, 1982).

Losses of National revenue from various disease have not been evaluated in Nigeria, while some diseases such as rinderpest and contagious bovine pleuropneumonia cause economic losses by sporadic outbreak, others cause debilitation and occasional deaths. Reproductive diseases of livestock however, are insidious in nature and of great economic significance and also are losses due to infertility, calf mortality, and lowered milk yield.

A variety of organisms have been incriminated in causing bovine abortions. They include Brucella, Campylobacter, Trichomonas (Dennis, 1969; Laing, 1970), Trypanosomes (Nuru, 1974), Salmonella, Fungi and viruses (Dennis, 1969).

Infectious forms of infertility and abortions reported in Nigeria have included brucellosis (Bale and Kumi-Diaka, 1981), Leptospirosis (Eze, 1981), Infectious rhinotracheitis (I.B.R) and Para influenza virus-3 (Taylor et al., 1977) and Trypanosomes (Ogwu, 1983).

Reports of the prevalence of bovine abortions and infertility from among several causative factors in Nigeria are very scanty.

As early as 1909 (Campylobacter fetus Subspecies; were incriminated as a cause of epizootic abortions in cattle and sheep in Great Britain (McFadyean and Stockman, 1909). The disease is venereally transmitted in cattle and is characterized by infertility and occasional abortions (Lawson and Mackinnon, 1952; Plastridge, 1955; Lawson, 1959 and Hoerlein, 1968). The causative agents Campylobacter fetus Subsp. venerealis and Subsp. fetus are world wide in distribution and have been shown to cause infertility in cattle (Buxton and Frazer, 1977).

The first diagnosis of Campylobacterosis in Nigeria was in a herd of 30 south Devon cattle introduced in Kano State (Nuru, 1974). Also findings from rectal examinations

of cattle and herd histories (abortions, retained foetal membranes and infertility) in herds visited by Akpokodje (1984) tend to indicate that campylobacteriosis might be present in Nigeria. In many parts of the world it is recognized that campylobacteriosis and trichomoniasis are important factors contributing to herd infertility (Akpokodje, 1984).

Detailed investigation on the prevalence, breeding consequences and pathology of campylobacteriosis among Nigerian cattle is lacking. This study was therefore designed:

1. To study the prevalence of campylobacteriosis among cattle in some states of Northern Nigeria.

2. To determine the rate of infection, infertility and pathology of cows bred by a bull artificially infected with C. fetus Subsp. fetus.

3. To determine the effects of semen treatment with antibiotics on the viability of C. fetus Subsp. fetus using some recommended dosages for semen treatment in Artificial Insemination Unit, NAPRI - Shika, Zaria.

CHAPTER 2

LITERATURE REVIEW

2.1 History

In 1909 a committee formed in Great Britain to investigate epizootic abortion in cattle and sheep issued a report incriminating a spirillum which they did not name (McFadyean and Stockman, 1909).

Infection of the reproductive tract of both cattle and sheep was first associated with microaerophilic vibrios Campylobacter fetus Subspecies, by McFadyean and Stockman in(1913).

Their findings were supported by Smith and Taylor (1919) who isolated a spirillium from aborted bovine foetal fluids and named it Vibrio fetus. Thereafter, it was clear that the organism was associated with epizootic abortion in sheep and cattle.

The microaerophilic vibrios were later assigned to genus Campylobacter (from the Greek word Campylo "curved" and bacter "Rod") by Sebald and Veron (1963); when they were found to be different from the true Vibrio such as Vibrio cholerae. Sebald and Veron (1963) found that the molecular weight percentage of guanine and cytosine DNA content of V. fetus averaged 34.40 per cent while that for the classical Vibrio cholerae and halophilic species of vibrio averaged 47.20 per cent.

Furthermore V. fetus differs from other species of Vibrio by the inability to ferment or oxidize carbohydrates and the requirement of microaerophilic condition for growth.

2.2 Classification and Taxonomy

Between 1959 and 1960, Florent studied V. fetus and divided the speices into two subspecies: V. fetus venerealis and V. fetus intestinalis based on their pathogenicity in cattle and biochemical tests such as hydrogen sulphide production, growth in one per cent glycine and 3.5 per cent sodium chloride. V. fetus venerealis is venereally transmitted, causes abortion in cattle, is hydrogen sulphide negative (using lead acetate strip) and will not grow in 1% glycine or 3.5% sodium chloride. V. fetus intestinalis is not considered venereally transmitted. It causes sporadic abortion in cattle and is hydrogen sulphide positive and will grow in 1% glycine but not in 3.5% sodium chloride (Berg et al., 1971).

Bryner et al., (1962) divided V. fetus into three biotypes based on hydrogen sulphide production and growth in 1% bile, 1% glycine but not in 3.5% sodium chloride containing medium. This classification of campylobacter stood until Sebald and Veron (1963) re-classified it, giving it the new genus campylobacter. Campylobacters have since then undergone several classifications. C. fetus Subsp. fetus as classified by Veron and Chatelain, is classified as C. fetus Subsp. intestinalis in Bergey's manual and as V. fetus intestinalis by Florent (1960).

C. fetus Subsp. venerealis in Veron and Chatelain classification, is classified as C. fetus Subsp. fetus in Bergey's manual, and as V. fetus Subsp. venerealis in Florent's classification.

C. fetus Subsp. venerealis biotype intermedius in Veron and Chatelain (1973) classification, is not recognized in Bergy's manual. C. coli and C. jejuni classified as separate species in Veron and Chatelain classification are classified as a single specie. C. fetus Subsp. jejuni in Bergy's manual. C. fetus Subsp. jejuni in Bergey's manual is similar to V. jejuni described by Jones and Little (1931).

Smibert (1978) classified the genus campylobacter with C. fetus Subsp. fetus the same as C. fetus Subsp. venerealis of skerman et al., (1980). Also C. fetus Subsp. intestinalis as C. fetus Subsp. fetus of Skerman et al., and C. fetus Subsp. jejuni to encompass both C. jejuni and C. coli which Skerman et al., classified as separate species.

The classification of C. sputorum Subsp. sputorum; C. sputorum Subsp. bubulus and C. fecalis were similar by both Smibert (1978) and Skerman et al., (1980).

The different classifications caused confusion until the publication of approved list of bacterial names in International Journal of Systematic bacteriology 30,225 (1980). There were two main classifications of campylobacter, the French (Veron and Chatelain, 1973) and American (Smibert, 1974).

In the approved list of bacterial names (1980) which follows the classification of Veron and Chatelain (1973), the genus campylobacter is divided into two groups according to the ability of the organism to produce catalase. The catalase positive campylobacters were further divided into two groups based on their optimal temperature for growth. The C. fetus group which includes C. fetus Subsp. venerealis in Bergey's manual grows at 25°C to 38°C but not at 43°C.

The thermophilic group which include C. fetus Subsp. jejuni in Bergey's manual will not grow at 25°C but will at 43°C. This group of C. fetus Subsp. jejuni was further divided into three groups as C. jejuni biotype 1 and 2; C. coli and nalidixic acid resistant group still un-named.

2.3 The organism

Campylobacters are Gram-negative, non-sporing, micro-aerophilic, oxidase positive bacteria. They are generally 0.2 to 0.4 um wide and 1.5 to 3.5 um long. They are curved or spiral shaped rods and also appear ribbon shaped made up of a chain of single curved cells (Smibert, 1978). Buxton and Frazer (1977) described campylobacter as being characteristically comma or s-shaped and often described as having the appearance of a "flying seagull". Sometimes longer forms of the organism consist of four or five cells. They also reported that in tissue and young cultures, the

shorter forms are more usually seen, but in older cultures, chains of organisms appearing as long spirals may be seen together with coccoid forms. The organism have a single polar flagellum at one or both ends. Morphological differences between groups of Campylobacter were first observed by Florent (1959) and later confirmed by King (1962), who noted that the undulations of related Vibrios tended to be closer together than those in Vibrio fetus. Smibert (1978) described King's related Vibrios (C. coli and C. jejuni), morphologically as short curved rods which are frequently S-shaped with tapering ends. While fetus group of Campylobacters are generally long with blunt ends, although short curved forms with blunt ends are often seen.

Karmali et al., (1981) measured the wavelength and amplitude of representive strains and reported that C. jejuni, C. fetus, Subsp. fetus and C. fetus Subsp. venerealis may be distinguished from each other on morphological grounds by direct microscopy.

Cells of old cultures tend to become coccoid and this has been described as the degenerative form of the organism (Smibert, 1978). Cultures of C. jejuni undergo coccal transformation more rapidly than C. fetus Subsp. fetus or C. fetus Subsp. venerealis in normal atmospheric air (Karmali et al., 1981). The organisms are actively motile with a characteristic cock-screw-like motion,

whereby cells quickly move across a microscopic field twirling or rotating in a spiral-like motion on their axis. Motility and rotation of th cells are so rapid that the curvature of the cells may be overlooked. Skirrow (1981) described the motility of campylobacter on wet preparation as highly motile; spinning on axis; rapid darting and oscillating movements.

2.4 Transmission and pathogenesis

The bull:

The possibility that the bull might be implicated in campylobacteriosis was first suggested by Plastridge and Williams (1943); who reported high agglutination titres from five of six bulls, out of two of seven herds using natural service. The natural habitat of the organism is the genital tract of cattle. Infection is venereal. Healthy bulls may become infected during coitus with diseased cows or from contaminated beddings (Buxton and Frazer, 1977). The site of infection is the epithelium of the prepuce and such animals show no clinical signs of disease and their semen may be normal. Sjollema et al., (1949) observed no abnormalities either in the semen or genital organs of naturally infected bulls. Transmission between bulls kept together have been reported when they mount each other (Wagner et al., 1965). The organism is confined to the preputial cavity and particularly the mucosa

of the glands penis, prepuce and the distal portion of the urethra (Clark, 1971). The greatest number of the organism is however found in the fornix of the prepuce (Samuelson and Winter, 1960; Finlay et al., 1985). Wagner et al., (1965) reported that the susceptibility of young bulls to both natural and experimental infection is remarkably low, whereas older bulls (above five years) are highly susceptible. Samuelson and Winter (1960) associated the hightened susceptibility of older bulls to infection with the increase in size and number of crypts in the epithelium of the penis. The latter also indicated that the greatest concentration of the organism was found within the lumen of the epithelial crypts an environment probably ideal for the organism.

Sjollema et al., (1949) reported that out of 49 cows inseminated artificially with semen of infected bulls, only three conceived. Out of these two aborted and C. fetus Subsp. fetus was recovered from the foetuses. Adler et al., (1952) stated that the use of semen from infected bulls in artificial insemination was a means of spreading the infection. Philpott (1968) reported that venereal campylobacteriosis is perpetrated principally by chronically infected bulls. Clark and Dufty (1978) stated that bulls are the principal carriers of bovine campylobacteriosis.

The cow

Transmission in the cow is through coitus with an infected bull and artificial insemination with infected semen (Clark, 1971). In heifers and cows, the sites of infection are the lumen of the vagina, cervix, uterus and oviducts (Clark, 1971).

Attempts to infect cows by vulva contamination have not been successful (Shepler et al., 1963; Mellick et al., 1965).

During coitus, the organism is transmitted. This results in the development of either an acute or chronic infection of the uterus depending upon the immune status of the cow (Buxton and Frazer, 1977). One week after infection, campylobacter invades the uterus and incites a subacute endometritis (Frank et al., 1962; Estes and Bryner 1966). The inflammatory changes and the resulting embryonic death are in part a function of endotoxin elaborated by the bacterium. Irregular estrous cycles and infertility problems follow these inflammatory changes (Roberts, 1958; Osborne, 1965; and Estes and Bryner, 1966).

Sullivan et al., (1987) reported that C. fetus Subsp. fetus when injected intravenously in mice impaired both implantation and foetal growth. It also causes foetal resorption of mouse embryos, a similar effect was observed on the embryos following injection of the heat-killed organism. Sullivan et al., (1987) thus concluded that campylobacters are capable of affecting the development

of the foetus and the results are similar to those following the injection of both Escherichia coli endotoxin and cholera-endotoxin (Gasic and Gasic, 1972). It seems probable therefore that the pathogenic effects of Campylobacters are due to similar substances.

Inflammatory changes may also be observed in the cervix (Newsam and Peterson, 1965). Hoerlein (1968) attributed the temporary infertility seen in campylobacteriosis to the effect of mild endometritis and salphingitis. Acute infection causes inflammatory changes in the uterus and subsequent death of the fertilized ovum. The latter is followed by either expulsion or resorption of the embryo in the early stages of development, probably within three to four weeks of fertilization. It has been reported that in cows with some immunity to C. fetus the development of the embryo may proceed to later stages in pregnancy before the inflammatory reactions of the uterus and placenta cause death and abortion of the foetus. This usually takes place at about the fifth month of pregnancy (Buxton and Frazer, 1977). Infection of the uterus and oviducts could persist for about two months. Thereafter, the infection is progressively eliminated so that by the third month, it is confined to the cervix and vagina (Adler, 1957; Peterson and Newsam, 1964 and Vandeplassche et al., 1963).

Furie (1982) reported that cows and heifers that have had no previous contact with C. fetus are highly

susceptible to infection. However, one or two individuals in a group of 15 to 25 heifers may not become infected even when enormous numbers of viable organisms are introduced into the reproductive tract. Also the susceptibility of the cow does not appear to change with increasing age (Clark, 1971). Frank et al., (1964) reported that the disease in the female is self-limiting due to the development of immunity.

Rikard-Bell (1954) reported that some cows appear to develop some degree of tolerance and settle to the bull after continual services of 3 to 5 times. Webster and Thorp (1950) explained that susceptible herds may become pregnant with little trouble because of the natural resistance of the bovine to the organism. Hoerlein (1968), reported that some infected pregnant cows may deliver normal calves but may be carriers of C. fetus Subsp. fetus for sometime and a source of infection for the next breeding season.

2.5 Diagnosis

Shepler et al., (1963) and Mellick et al., (1965) stated that the diagnosis of campylobacteriosis in a herd can be confirmed by the examination of the preputial washings collected from the herd's bull by both cultural and immuno-flourescence techniques. Winter et al., (1967) and Dufty (1967) both reported that for definitive

diagnosis of campylobacteriosis in the bull, C. fetus Subsp. fetus and C. fetus Subsp. intermedius must be isolated from the preputial sac and identified. Dufty and McEntee (1969) reported that sampling of preputial smegma by pipette method was superior to washing for the diagnosis of campylobacter infection in the bull. Barlett et al., (1947) and Tedesco et al., (1977) also reported that sampling with the pipette was superior than other methods.

Philpott (1968) described the direct flourescent antibody test (FAT) for the diagnosis of bovine campylobacteriosis and mentioned some of advantages the technique has as a screening test on preputial smegma. They included the specificity of the test and ability to detect dead campylobacters, as such, samples can be stored overnight without affecting the result of the test.

Bacteriological methods have been used to diagnose bovine vibriosis in heifers that have been test mated with bulls or exposed to experimental infection (Clark et al., 1969). The sites of infection with V. fetus (venerealis are the cervix (Adler, 1957) and the anterior vagina Vandeplassche et al., (1963).

Contamination from the vaginal flora is largely avoided by the method advocated for the collection of cervical mucus (Adler, 1957). The method of collecting mucus from the anterior vagina (Anon, 1955) has the advantage of being rapid and simple.

Lawson and Mackinnon (1952) reported that V. fetus (venerealis) could readily be isolated from the vaginal mucus of infected cattle at oestrus than at other stages of the oestrous cycle. Stegenga and Terpstra (1949); Szabo (1951) reported the use of the vaginal mucous agglutination test (VMAT) for diagnosis of campylobacteriosis in the female, among other tests. This detects agglutinins that appear in the female following genital infection with C. fetus.

Newsam and Monsbourgh (1967) stated that although the mucus bacterial agglutination test has been used extensively, there was the need for the standardization of the antigen in order to obtain reproducible results. Clark et al., (1970) stated that although agglutinins against C. fetus Subsp. intestinalis were low in titre, they produced false reactions in the VMAT.

Boy and Reed (1960); Newsam and Monsbourgh (1967) reported that the incidence of false reaction in the VMAT were less than one per cent when samples were collected by the pipette method. Clark et al., (1970) reported that repeated collection of vaginal mucus samples from heifers provoked false suspicious and positive reactions due to minor trauma to the posterior vaginal tract produced by the collection technique. The trauma also appeared to cause increased transfer of plasma derived agglutinins against C. fetus Subsp. intestinalis, thus producing

misleading results. Florent (1954) and Mylrea (1962) both reported that false positive reactions in the VMAT were obtained with cows infected with bovine trichomoniasis. The latter explained that this false reaction was due to the antigenic relationship between Trichomonas fetus. However, Clark et al., (1970) explained that the false reactions in the VMAT to be due to the leakage of agglutinins against C. fetus Subsp. intestinalis and were associated with the inflammatory lesions of the genital tract caused by Trichomonas fetus.

Hewson et al., (1985) used Enzyme Linked Immunosorbent Assay (ELISA) to detect antibodies to C. fetus Subsp. venerealis in bovine vaginal mucus. The result of the testing of 168 samples from experimentally infected animals, field cases and controls, showed that ELISA was more sensitive than the vaginal mucus agglutination test. It also detected antibodies in earlier stages of infection.

ISOLATION AND BIOTYPING

2.6.1 Isolation of the organism

The isolation of campylobacters require special selective technique because of its microaerophilic nature, fragility and the many contaminating organisms usually found in the specimens. For definitive diagnosis of campylobacteriosis in the bull, culture of preputial

samples on solid selective medium is difficult when samples contain few numbers of C. fetus and a large number of resistant contaminating organisms such as pseudomonas. Shepler et al., (1963), and Dufty (1967) thus suggested that such contaminating organisms could be removed from the samples by filtration. However Dufty (1967) reported that filtration technique reduce the numbers of C. fetus in the sample.

To overcome these two difficulties, Clark and Dufty, (1978) reported the use of an enrichment medium which will selectively increase the numbers of C. fetus prior to culture on selective media.

Various selective media have been used for the isolation of campylobacter. These contain various base media and antibiotics, among which are the followings:

(a) Nutrient agar was used in selective media by (Laing (1960); Dufty and McEntee (1969) and Winter et al. 1965, 1967).

(b) Blood agar base was used in selective media by Skirrow (1977); and Butzler and Skirrow (1979).

(c) Thioglycollate agar base was used in Butzler's medium (Lauwer's et al., 1978); and in Butzler and Skirrow (1979) medium.

Blaser's medium (Blaser et al., 1978) contains brucella medium as base medium. Veal infusion broth (Difco) is used in Lander and Gills selective and

enrichment medium (Lander and Gills, 1980). While in Preston medium (Bolton and Robertson, 1982), nutrient broth No. 2 (Oxoid solidified with 2 per cent New Zealand agar or Columbia agar base (Oxoid) is used. Bolton and Coates (1983) medium, used nutrient broth No. 2 (Oxoid) solidified with 2% New Zealand agar. This selective medium when supplemented with ferrous bisulphite will give the highest recovery rate of campylobacters from samples compared to other media. The blood content of these media varied from 5 to 15 per cent.

Bolton et al., (1983) reported the development of a blood free medium for the isolation of C. fetus. The medium contained nutrient broth No. 2 (Oxoid) at 25 g/litre; New Zealand agar at 12 g/litre; bacteriological charcoal (Oxoid) at 4 g/litre; Casein hydrolysates (Oxoid) at 3 g/litre; Ferrous sulphate (BDH) at 0.25 g/litre and sodium pyruvate (BDH) at 0.25 g/litre.

These ingredients were autoclaved at 121^{o}C for 15 minutes, allowed to cool and the selective agents added at final concentrations of: vancomycin 50 ng/ml, polymyxin 150 iu/ml, nalidixic acid 60 ug/ml and actidione 100 ug/ml. He tested this medium with pure culture of C. fetus Subsp. fetus (12 strains); C. fetus Subsp. venerealis (9 strains); C. fetus Subsp. intermedius (3 strains). They all grew from low inocula on the selective media when incubated under microaerophilic

atmosphere at 37°C for 72 hours. The media did not allow the growth of C. jejuni or C. coli. It supported the growth of C. lardis (NARTC) strains. Bolton et al., (1983) also reported that this medium was suitable for the isolation of C. fetus strains from heavily contaminated sources.

2.6.2 Oxygen requirement

Campylobacters require microaerophilic conditions for growth and optimum oxygen concentration of 6% and carbondioxide concentration of 10% (Kiggens and Plastridge, 1958). The aerotolerance of C. fetus could be increased by the addition of ferrous sulphate, sodium bisulphite, sodium pyruvate and haematin (Bowdre et al., 1974). The aerotolerance of a strain of campylobacter was reported to be greatly increase by dihydroxyphenyl ferric binding compounds such as norephinephrine or high concentration iron salts (ferric or ferrous) added to Brucella agar medium (Bowdre et al., 1976).

Bolton and Coates (1983) stated two aspects of aerotolerance viz:

(a) the ability of supplements to sustain the viability of campylobacters seeded onto culture plates left on the bench for up to 6 hours, before microaerophilic incubation;

(b) the ability of the supplements to facilitate the growth of campylobacters at increasing oxygen tension (6, 10 and 17 per cent oxygen).

A combination of 0.4% charcoal, 0.025% ferrous sulphate and 0.025% sodium pyruvate was found to be as effective as blood in both tests.

2.6.3 Cultural characteristics

Colonies are non-hemolytic and vary in appearance from small round convex to medium mucoid almost transparent colonies which resemble droplets of water sprayed on the medium, in the case of C. fetus. For C. jejuni and C. coli, the colonies are non-hemolytic often resemble droplets of water sprayed on the plate and have the tendency to coalesce and grow along the line of streak (Smibert, 1978). "Related campylobacters" colonies are typically flat, glossy and effuse with a tendency to spread along the direction of streak to confluent growth (Butzler and Skirrow, 1979). When well spread out the latter resemble droplets of fluid that have been splattered on the agar.

Smibert (1965) described two types of colonies on primary isolation. One is low flat, grew, finely, granular with an irregular edge. The latter spreads along the direction of streak and tends to coalesce. The other

is round 1 to 2 um in diameter, raised, convex, entirely smooth and glistering. It has a translucent edge with a darker, dirty, brownish slightly opaque centre. With continued incubation, the growth thickens and most strains develop a metallic sheen not unlike that seen on cultures of Pseudomonas aeruginosa (Skirrow and Benjamin, 1980).

Laing (1970) morphologically characterized campylobacter colonies into four as follows:

1. Small, delicate, circular and opaque colonies.
2. Mixture of small and large colonies which may coalesce to produce a large mucoid mass that may also be elongated along the line of inoculation.
3. Colonies that may produce a frosted appearance over the surface of the medium.
4. Shiny, pale-grey colonies or pale-grey mucoid mass.

2.6.4 Biochemical characteristics

Campylobacter fetus is neither an oxidizer nor a fermen-ter in oxidation fermentation medium. It is catalase and oxidase positive. It does not utilize carbohydrates as source of energy. The organism reduces nitrate to nitrites and can not hydrolyse gelatin, urea and casein. It is indole negative and does not produce hydrogen sulphide on triple sugar iron slant. There is

only an alkaline reaction. Hydrogen sulphide is however produced in a medium containing cysteine with lead acetate impregnated strip as a method of detection (Smibert, 1978). Campylobacter fetus grow in semi-solid medium containing 0.12 to 0.2 per cent agar when incubated under aerobic condition. Growth in semi-solid medium occurs only within the first few millilitres below the surface of the medium. C. fetus and C. jejuni can be differentiated based on their ability to grow at 25°C and 45°C. C. jejuni will grow at 43°C while C. fetus will not, however C. fetus will grow at 25°C but C. jejuni will not. Sensitivity to nalidixic acid is also used to differentiate C. fetus from C. jejuni; C. fetus shows no zone of inhibition around the disc while C. jejuni does. Tolerance to 0.5 per cent and 1 per cent glycine are also used to differentiate campylobacter species (Florent 1960; Bryner et al., 1962; Buxton and Frazer, 1977). Harvey (1980) used hippurate hydrolysis test to distinguish between campylobacter species and subspecies. None of the 16 strains of C. fetus Subsp. fetus;five strains of C. fecalis and one strain of C. sputorum tested hydrolysed hippurate. However, 18 out of 20 strains of C. fetus Subsp. jejuni hydrolyse hippurate. C. coli and C. jejuni have also been divided into four biotypes according to their sensitivity to nalidixic acid, hippurate hydrolysis and H_2S production in iron containing medium (Skirrow

and Benjamin, 1980). Those campylobacters which are sensitive to nalidixic acid but fail to hydrolyse hippurate are classified as C. coli.

Analysis of fatty acid contents by gas liquid chromatography have been used to differentiate between subspecies of campylobacter, Blaser et al., (1980) showed that strains of C. fetus Subsp. jejuni contain C-19-cyclopropane which was not present in strains of other species of C. fetus. The C-19-cyclopropane was identified as lacto-bacillic acid (Cis-1-12 methylene acto-adecanoic acid). Krausse and Ullmann (1985) examined 19 to 56 human strains of C. jejuni; C. coli; C. laridis; C. fetus Subsp. fetus; C. fetus Subsp. venerealis and C. fecalis; C. sputorum spp. sputorum; mucosalis and bubulus were examined for the production of volatile and non-volatile short chain fatty acids using high performance liquid chromatography. Five biotypes of C. fetus were characterized by the latter method. The most common biotype produced succinic acid, acetic acid and butyric acid.

Differences also existed in the production of pyruvic, malonic, formic and isobutyric acids. C. jejuni could rapidly and clearly be differentiated from C. coli, C. fetus and the catalase negative Campylobacter species. No qualitative differences were found between the subspecies of C. fetus; C. sputorum Subsp. sputorum. C. sputorum Subsp. mucosalis and C. fecalis were characterized by the presence of fumaric and malonic acid respectively.

Curtis (1983) reported that C. coli, C. jejuni C. fetus Subsp. fetus, C. fetus Subsp. venerealis, C. sputorum Subsp. bubulus and C. Sputorum Subsp. mucosalis produce eight principal fatty acids with some producing additional acids. Veron and Chatelain (1973) used sensitivity to 1 mg triphenyltetrazolium chloride (TTC) per millilitre to differentiate Campylobacters. The latter found that non of the 18 strains of C. fetus Subsp. intestinalis grew on blood agar plates containing 1 mg of TTC per ml in contrast to 9 strains of C. coli and one strain of C. jejuni which grew on the medium.

Butzler and Skirrow (1979) recommended decreasing the concentration of TTC from 1 mg/ml to 100 ug/ml for this test and measured by strip diffusion method. Skirrow and Benjamin (1980) confirmed the usefulness of this test for differentiating strains within C. jejuni and C. coli.

2.7 Biotyping of C. fetus

Berg et al., (1971) used the sensitive hydrogen sulphide and glycine tolerance tests to separate V. fetus into three biochemical groups.

Type 1 Glycine negative and hydrogen sulphide negative

Type 2 Subtype 1 glycine negative and hydrogen sulphide positive

Type 3 Glycine positive and hydrogen sulphide positive.

Also by agglutinating heat stable antigens (O antigens) with antisera produced against whole cell antigens, Berg et al., (1971) revealed three ('O' serotypes) namely A, B, C for V. fetus. By serotyping 53 V. fetus isolates with 7 specific antisera, Berg et al. (1971) revealed that some antisera have antibodies for specific heat labile antigens. These they gave arabic numbers 1, 2, 3, 4, 5 and 6.

When the results of biochemical types (H_2S and glycine), heat labile antigens; heat stable antigens ('O' antigens) and whole cell serotypes were compared, Berg et al., (1971) were able to divide V. fetus into five groups viz:

(a) Group 1. Consisted of serotype A isolates that were glycine negative and hydrogen sulphide negative, they named it as Group A-1 (serotype A biotype 1) and identified as V. fetus Subsp. venerealis, the only recognised cause of Vibrionic infertility in cattle. The latter cannot survive in the intestinal environment and have never been isolated from naturally infected sheep. This group also grows more slowly, is more difficult to grow, produces smaller colonies and generally has fewer heat-labile antigens than A-subtype 1.

(b) Group 2. Consisted of serotype A that were glycine negative but produced H_2S. These were named A-sub 1 (Serotype A biotype 1) and indentified as V. fetus var. venerealis. The latter seem to occur less frequently than A-1, and is also recognized as a cause of vibrionic infertility in cattle and does not survive in the intestinal environment.

(c) Group 3. Consisted of isolates that were glycine positive and H_2S positive. These were named Group A-2 (serotype A-biotype 2) and identified as V. fetus var. intestinalis. It can survive in the intestinal environment and can cause abortion in sheep. The latter occasionally causes abortion in cattle but most of these isolates originated from sheep.

(d) Group 4. Consisted of serotype - B isolates which were glycine positive and H_2S positive. These were named Group B (serotype B) and identified as V. fetus var. intestinalis capable of surviving in an intestinal environment. All V. fetus var. intestinalis isolates that have been reported earlier (Florent, 1963; Park et al., 1962) capable of venereal transfer and survive in genital tract of cattle were serotype B. Some were isolated from bulls in herds with infertility problems.

(e) Group 5. Consisted of serotype C isolates which were H_2S positive and glycine variable. These were named group C (serotype C). These serotype C isolates are readily differentiated by whole cell serotyping system (Serotyp 1) and have been recognised as a distinct group since 1953 (Marsh and Firehammer, 1953). In addition to having specific 'O' antigen and heat labile antigens they constitute a specific temperature tolerance group. They have been found to be the only V. fetus isolates that will grow at 45°C. They also have morphologic differences (Coccoid forms) and different growth requirements from other V. fetus (Ogg, 1962; Tritz and Ogg, 1967). Many serotype-C isolates become non-viable after 1 week storage in a semi-solid medium at ambient room temperature. Whilst other isolates remain viable longer than 3 weeks. They are also the only isolates having delayed catalase reaction. The latter have been reported as the most frequent cause of vibriosis in sheep in Western United States. Serotype-C have never been isolated from bovine genital tract and are most often isolated from ovine foetuses and often from the gall bladder of adult sheep and cattle.

The whole cell serotyping system divides V. fetus into four groups, but fails to differentiate between glycine negative serotype-A isolates that produce H_2S and those that did not (Firehammer et al., 1961).

The occurrence of multiple antigens on individual isolates in all the serotypes except serotype C, makes a clear differentiation by this method impossible. Similarly, the occurrence of multiple heat-labile antigens on individual isolates makes clear differentiation on the basis of specific heat-labile antigens difficult.

Berg et al., (1971) also reported that classifying C. fetus into two varieties was inadequate because it reflected neither the similarities nor the differences between the five distinct group of V. fetus because it divides serotype-A isolates into two varieties but fails to differentiates the glycine positive isolates (Group A-2 and B) which are serologically and apparently immunologically distinct.

Furthermore, serotype-B isolates regarded as V. fetus var intestinalis, survive in either an intestinal or venereal environment and have heat-labile antigens 2, 6 and 7 in common with isolate V. fetus var venerealis. In addition bacterial phages can be cross-propagated between isolates of the two varieties.

The finding of a glycine negative group of V. fetus that produces H_2S and transmitted by venereal routes (A-biosubtype 1) prevent the differentiation of the two varieties on the bases of H_2S test.

Mike Ansfield (1985) (Personal Communication - see Appendix used the following criteria for the biotyping of campylobacters. Viz; catalase, relative size, swarming on moist media, rapid cocal formation, H_2S production from (FBP broth, TSI and cystein containing medium with lead acetate strip as an indicator), hippurate hydrolysis, growth at 25°C and 43°C, nalidixic acid sensitivity (30 ug disc), cephalothin sensitivity (30 ug disc) and 1% glycine tolerance. Based on these criteria, he divided campylobacters into the following species: C. fetus Subsp venerealis, C. fetus Subsp. fetus, C. jejuni biotype 1, C. jejuni biotype 2, C. coli, NARTC and C. fecalis.

2.8 Distribution of Campylobacter fetus in nature

Campylobacter fetus Subspecies are world wide in distribution and have been associated with disease in animals. The most important of these is C. fetus Subsp. fetus (formerly V. fetus Subsp. venerealis which has been shown to cause infertility and abortion in cattle (Buxton and Frazer, 1977). The natural habitat of the organism is the genital tract of cattle.

Venereal campylobacteriosis is perpetrated principally by chronically infected bulls, Philpott (1968) which also are the principal carriers of the disease (Clark and Dufty, 1978). There is no clinical manifestation of the disease in the bull. In cows however, the disease manifests in the form of infertility and abortion.

Diagnosis of the disease in herd is however confirmed by the isolation of C. fetus from the preputial washings of the herd's bull (Shepler et al., 1963; Mellick et al., 1965; Winter et al., 1967 and Dufty, 1967).

Baez-Kohn (1984) reported that from examination of 2,910 specimens of preputial swabs, vaginal discharges placental fluids and stomach contents of aborted foetuses, C. fetus was isolated from 71 of 151 herds (47%). Garcia et al., (1983) using a transport enrichment medium examined in the preputial washings of 105 bulls at an Artificial Insemination (A.I) Unit, by culture and immunofluorescence, reported 16% of the bulls to be possitive or C. fetus. Finlay et al., (1985) isolated no campylobacter from 90 preputial samples collected from 90 bulls in two Ontaria A.I. Units. However, he insolated one C. fetus Subsp. venerealis from the fornix area of the distal genitalia collected at a slaughter house.

Krippner et al. (1985) reported the frequent isolation of C. fetus Subsp. intestinalis in the preputial swabs of young bulls.

The latter also reported that in different years the proportion of bulls aged 9-18 months that harbour C. fetus Subsp. intestinalis in the prepuce ranged from 2.3-21%. He made one isolation of C. fetus Subsp fetus from the prepuce. He reported that the isolated C. fetus Subsp. intestinalis did not become established in the female genital tract and did not cause abortion when inoculated into the cervix of pregnant heifers.

About 66-75% (Roberts, 1958), 40% (Leaver and Hart, 1960) and 60% (Swane Poel et al., 1975) infertility problems have been reported as due to campylobacteriosis. Abortion incidences also vary; Plastridge et al., (1947) estimated the incidence of abortion in cattle to vary from 4% to 20%. Incidence rates of between 2% to 10% in susceptible herds have been reported by Lawson (1959); Hoerlein (1968); Dennis (1969); Kirkbride et al., (1973); Radoslavov and Puskarov (1985) and Bajmocy et al., (1985).

Reports on the time of abortion in bovine campylobacteriosis vary. Plastridge et al., (1943) reported abortion to occur during, the 5th, 6th and 7th month of gestation. Rikard-Bell (1954) reported between 3 to 6 months; Dennis (1969) and Roberts (1971) both reported abortion to occur between the 4th and 7th month of gestation, while Hubbert et al., (1973); Watson and Laing (1970) and Buxton and Frazer (1977) reported abortion to occur at any stage of pregnancy.

2.9 Sensitivity of Campylobacter fetus to antibiotics

The range of therapeutic agents suitable for the treatment of Campylobacter infections is limited by the frequent occurrence of resistance to the more readily available antibiotics (Butzler et al., 1974; Vanhoof

et al., 1978; Karmali et al., 1981; Vanhoof et al., 1982 and Michel et al., 1983). Currently, the antibiotic generally recommended for therapy of infections caused by thermophilic campylobacter species is erythromycin (Butzler and Skirrow, 1979; Ricketts and Hope, 1984). Shutte (1969) has also recommended erythromycin for local 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 and Secker, 1983). Streptomycin treatment of semen from V. fetus carrier bulls prevents vibriosis from being transmitted via A.I. (Orthey and Gilman, 1954; Adler, 1966; McEntee et al., 1954 and 1959). Morgan et al., (1959) however, showed that streptomycin treatment of semen extended with egg yolk was ineffective in controlling the infection by A.I. But in milk it was effective. Albertsen (1957) showed that the marked effectiveness of streptomycin against V. fetus, B. abortus and Corrynebacterium pyogenes was greatly diminished by egg yolk. Corbel et al., (1984) tried the activity of amoxycillin, apramycin sulphate, chloramphenicol, erythromycin, flavomycin, furazolidone, gentamycine, lincomycin, oxytetracycline, spectomycin dihydrochloride, streptomycin sulphate and tylosin tartrate against 60 strains of C. coli: 40

strains of C. fetus Subsp. venerealis, 59 strains of C. jejuni biotype 1 and 10 strains of C. jejuni biotype 2.

Amoxycillin was used as augmentin(R) - a clavulanate potentiated amoxycillin (Beecham Product). Only augmentin(R) inhibited growth of all campylobacter strains at therapeutically attainable concentrations. The minimum inhibitory concentration of augmentin(R) for 90% of the strains was 4 ug/ml for C. fetus Subsp. fetus, C. fetus Subsp. venerealis and C. jejuni biotype 1 and 2. For C. coli the minimum inhibitory concentration for 90% of strains was 6 ug/ml. All the strains tested were inhibited at 8 ug/ml.

In contrast when a non-potentiated amoxycillin was used, the minimum concentrations was 20 ug/ml or greater for 30% of C. fetus Subsp. fetus 43% of C. fetus Subsp. venerealis, 88% of C. coli, 55% of C. jejuni biotype 1 and 70% of C. jejuni biotype 2 strains. The highest minimum inhibitory concentration of amoxycillin was 400 iu/ml for most resistant strains. Corbel et al., (1984) were unable to attribute the greater inhibitory action of augmentin(R) than amoxycillin to either the potentiation of the activity of amoxycillin by the clavulanic acid component or to an antibacterial action exerted by the clavulanic acid.

2.10 Experimental infections of C. fetus in heifers

Clark et al., (1969) test-mated 37 virgin heifers with bulls carrying C. fetus Subsp. venerealis and also challenged them with the organism intra-cervically or intra-uterinely after the first service. All heifers returning to service were not artificially challenged again, but allowed natural service with the infected bull. During the trial which lasted 4 to 7 months, all 37 heifers became infected. However, only 32 (86%) became pregnant with an average number of 3 services per conception and an average time of 55 days from first service to conception. Only 5 (14%) heifers conceived at first service.

In a similar experiment by Newsam (1960); 106 cows were bred once to a bull infected with C. fetus Subsp. venerealis. Among the heifers that developed campylobacteriosis at first service, only 21 per cent conceived at this first service whereas among the heifers that escaped infection, the conception rate was 67 per cent at first service. The heifers which escaped the infection were among the last animals served, which meant that continous and active use of infected bulls reduced their infection potential. However, the bulls did not become entirely free from the infection because the heifers served on the last day also became infected.

Newsam (1960) reported that many cows escaped infection at first exposure, but they became infected on on re-exposure. The interval from service to the first recovery of the pathogen varied from 1 to 115 days.

Clark et al., (1969) reported that the isolation rates of C. fetus before and after conception were significantly different. They reported 60 and 28 per cent respectively of isolation rates before and after conception. The mean isolation rate was 40 per cent during the first two weeks after conception, thereafter it declined to 24 per cent until 12 weeks after conception when it further declined to 15 per cent. This decline in isolation rate before and after conception was attributed to the development of immunity by the heifers.

CHAPTER 3

PREVALENCE OF BOVINE CAMPYLOBACTERIOSIS IN INDIGENOUS CATTLE OF KADUNA, KANO AND BORNO STATES IN NORTHERN NIGERIA

INTRODUCTION

Reports on prevalence of abortions and infertility from infectious agents and other causes in Nigerian cattle are scanty.

Campylobacter fetus Subspecies have been incriminated as a cause of epizootic abortions in cattle and sheep (McFadyean and Stockman, 1909). The most important subspecies which cause infertility and/or abortion in the bovine are C. fetus Subsp. venerealis and C. fetus Subsp. fetus.

Infection in cattle is venereal and transmission is by bulls. The site of infection in the bulls is the epithelium of the prepuce and such bulls show no clinical signs of the disease but may remain chronically infected as carriers. The disease is perpetrated by such infected bulls (Philpott 1968; Clark and Dufty, 1978).

In the cow infection with the disease result in either an acute or chronic inflammation of the uterus depending on the immune status of the cow. The inflammatory changes in the uterus leads to the subsequent death of the fertilized ovum, followed by either expulsion or resorption of the embryo in the early stages of development probably within 3 to 4 weeks of fertilization (Buxton and Frazer, 1977). Development of the embryo may proceed to later stages of pregnancy before the

inflammatory reactions of the uterus and placenta set in, leading to the death and abortion of the foetus. In the cow the disease is therefore characterized by either infertility and/or abortion. However, it is self-limiting in the cow due to the development of immunity from a previous infection (Plastridge and William 1943; Rikard-Bell, 1954; Frank et al., 1964). It is also reported that the cow has some natural resistance to the disease (Webster and Thorp, 1950).

The diagnosis of the disease in a herd is confirmed by the examination of the preputial washings of infected bulls for the isolation of C. fetus Subsp. fetus and C. fetus Subsp. venerealis (Shepler et al., 1963; Mellick et al., 1965 and Winter et al., 1967; and Dufty, 1967) and also by the isolation of C. fetus from the vaginal mucus samples of infected cows. There is a dearth of information on the occurrence of the disease in Nigerian livestock. The following investigation was therefore conducted to determine the prevalence of C. fetus Subsp. fetus and Subsp. venerealis in indigenous cattle of Kaduna, Kano and Borno States.

MATERIALS AND METHODS

3.2.1 Study area and animals

A total of 689 cattle consisting of 585 breeding bulls and 104 breeding cows were sampled from Kaduna, Kano and Borno States.

The cattle sampled were from Livestock Improvement and Breeding Centres (LIBC), Government and Private ranches, Institutional farms, nomadic Fulani herds and Private herds. Animals were also sampled from the Zango and Sabo Gari Zaria abattoirs in Kaduna State.

3.2.2 Sample collection

The bulb and pipette (Barlett et al., 1947) was used for collecting preputial samples from bulls. The apparatus consisted of a glass-rod 45cm long and 0.8cm internal diameter. One end of the glass-rod was bevelled and the other not. The unbevelled end was attached to the bulb, and the free bevelled end was introduced into the preputial cavity.

For cervical/anterior vaginal mucus sampling in the cow, the syringe and pipette method (Phatak et al., 1980) was used. This consisted of an insemination pipette 44cm long and 0.5cm internal diameter, one end of the pipette was attached to an adaptor which is also attached to a 10 ml syringe.

All breeding bulls in herds visited were sampled, whereas only cows with history of abortion, infertility or with pathologic findings such as vaginitis, cervicitis or pyometra on rectal palpation were sampled. Each animals was sampled once a week at weekly interval for three weeks in cases of Kaduna and Kano States; whereas cattle from Borno State were sampled thrice at monthly interval.

In sampling the bulls, 10 mls of pre-measured sterile phosphate buffered saline (PBS , pH7.2) was aspirated into the sampling pipette by means of a bulb attached to the unbevelled end of the pipette. The free bevelled end of the pipette was introduced deep into the preputial cavity and the mucous membrane of the preputial cavity and fornix area was gently scrapped by a backward and forward movement of the pipette as described by Tedesco et al., (1977). The fluid and smegma from the prepuce was then aspirated into the pipette and withdrawn. The preputial smegma obtained was dispensed into labelled test tubes in racks in an iced-cooled coleman box.

In sampling the cow, 10 mls of PBS was aspirated by means a syringeand adaptor into the pipette. The free end of the pipette was then introduced into the vagina as far as the cervix or anterior vagina. This area was then flushed with PBS and scrapped by gentle forward and backward movement of the pipette . The dislodged cervical or vaginal mucus was then aspirated back into the pipette and withdrawn from the cervix and vagina.

The collected cervical or vaginal mucus was dispensed into labelled test tubes in racks in an ice-cooled coleman box. A separate sampling apparatus was used for each cow and bull.

Samples collected in Pbs from locations where culturing on selective media was possible within 2 to 8 hours of collection were transported in the Pbs in an ice-cooled coleman box to the laboratory. Samples collected in Pbs from places where culturing was not possible within 8 hours of collection were innoculated immediately after collection on a campylobacter enrichment and transport medium (TEM) described by Clark and Dufty (1978) but without 5-fluorouracil (Appendix 2); and transported to the laboratory for incubation at 37°C. Although 5-fluorouracil was not added, Campylobacter fetus inoculated into the medium survived for over 7 days at room temperature.

3.2.3 Culturing

The samples collected in Pbs were centrifuged on a bench centrifuge at 2000 rpm for 5 minutes. The supernatant was then aspirated by means of a sterile Pasteur pipette. Two drops of it were streaked in Butzler's selective medium* prepared by adding 20gm of Oxoid blood agar No. 2 to 500 mls of distilled water and just brought to boiling over a bunsen burner, then autoclaved at 121°C at 15 lb/sq in pressure for 15 minutes.

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*Butzler selective medium (BR 85 Oxoid Ltd Basingstoke Hampshire, England).

The autoclaved medium was placed in water bath, the temperature of the water bath had earlier been adjusted to between 50 - 55°C. The temperature of the medium was allowed to equilibrate with that of the water bath. At this temperature (50-55°C) the selective antibiotic supplement (SR 85 Oxoid)was added to the medium after reconstituting it with 3.0 mls of 50/50 v/v ethanol/water asceptically.

The growth supplement (FBP supplement SR 84 Oxoid)** was also reconstituted with 2.0 mls of sterile distilled water and added asceptically to the 500 mls medium at the same temperature. The temperature of the water-bath with the medium was then adjusted to 37°C and then 7.0 lysed horse blood added asceptically to the medium.

The medium was then poured into sterile Petri-dishes and allowed to settle. The plates were stored in the fridge at 4°C until use. Each time the plates were to be used, they were first dried in the incubator before inoculation of samples.

The samples in the transport and enrichment medium (TEM) were incubated for three days, after which the supernatant that formed on top of the TEM was aspirated by means of sterile Pasteur pipette and two drops of it inoculated and streaked on the selective media.

The inoculated plates were incubated under microaerophilic conditions in an anaerobic jar without

**Ferrous Bisulphite Supplement (FBP) SR 84 Oxoid Limited Basingstoke, Hampshire, Endlang.

catalyst, but with gas generating kits (Oxoid code BR 52, No BR 38, Oxoid) at 37°C. Plates were read after 72 hours incubation.

3.2.4 Identification and biotyping of isolates

The identification of the campylobacter isolates was based on cultural, morphological and biochemical criteria. Non-hemolytic colonies that were small, round, convex mucoid and almost transparent, resembling droplets of water sprayed on the medium were picked as suspected C. fetus colonies. Non-hemolytic colonies that have the tendency to coalesce and grow along the line of streak were also suspected. Such colonies were subjected to biochemical tests in the following order. Oxidase and catalase test. The Oxidase and catalase positive colonies were Gram-stained and the morphology examined. Gram-negative curved or spiral shaped rods, were further examined for motility using the wet preparation. Organisms, that spun on axis with rapid darting and oscillatory movement were further tested for the followings:

a) Nitrate reduction

b) Indole production

c) Hippurate hydrolysis

d) Nalidixic acid sensitivity using 30 ug disc

e) Fermentation of carbohydrates using oxidation fermentation (OF) medium

f) 3.5 per cent sodium chloride tolerance test

g) 1.0 per cent glycine tolerance test

h) Growth at 25°C and 43°C

i) Growth on blood agar plates incubated at 37°C aerobically.

Based on the above tests the confirmed Campylobacter fetus isolates were typed into C. fetus Subsp. venerealis and C. fetus Subsp fetus.

3.3 RESULTS

Table 3.1 gives details of the prevalence of C. fetus isolated from bulls and cows in different locations. Out of the 689 animals, 20(2.9 per cent) were positive for C. fetus. Aborted foetuses were 100 per cent positive for the organism; cows were 4.8% per cent positive and bulls only 2.56 per cent. None of the bulls from Kano State was positive whilst bulls from Borno and Kaduna States were 6.0 per cent and 2.4 per cent positive respectively. The very few cows sampled from Borno State were negative for the organism but cows from Kano and Kaduna States were 7.6 per cent and 5.0 per cent positive respectively.

Table 2.2 shows the different Subspecies of C. fetus isolated from sampled animals.

C. fetus Subsp. venerealis almost doubled (1.9%). C. fetus Subsp. fetus (1.0%) in frequency of isolation.

C. fetus Subsp. venerealis was more common in the bulls than C. fetus Subsp. fetus. The reverse was the case in the cows. C. fetus was also isolated from the aborted foetuses.

3.4 DISCUSSION

The low prevalence of 2.9 per cent of campylobacteriosis recorded in this study agreed with the results of Finlay et al., (1985) who had a prevalence of 0.50 per cent in 200 bulls. The result is however lower than that of Roslanowski and Wysyżanowski (1983) who reported a prevalence of between 4.3 to31.7 per cent. The difference might be due to a number of factors such as the breed and type of animals sampled. The study however confirms Nuru's (1974) and Akpokodje's (1984) reports of the presence of campylobacteriosis in Nigeria.

Despite this apparent low prevalence of isolation of Campylobacter, the infertility problems in herds can be grossly high especially among cows. For example, long calving intervals, anestrus, purulent vaginal discharges and generally low pregnancy rates (compared to the normal 60%) were common feature of herds in which even a single bull was diagnosed positive.

The finding of higher prevalence of the organism among cows than bulls is due to the fact that fewer cows than bulls were samples and were from herds with problems of infertility and abortions. Four of the

five isolates from cows were from a herd with infertility problem (36.04%), irregular returns (12.8%) and abortions (2.3%). The vasectomized bull in the herd was also positive for C. fetus.

The fact that higher number of bulls than cows were sampled is due to the fact the disease is more prevalent in bulls than cows, because bulls are chronically infected (Philpott 1968; Clark and Dufty, 1978) whilst infected cows shed-off the infection after sometime (Adler, 1957; Peterson and Newsam, 1964; and Vandeplassche et al., 1963). The present study confirmed the chronicity of the infection in bulls than in cows, because in most herds isolation was made only from the bulls and none from the cows despite previous histories of abortions and irregular returns.

Lack of the organism in bulls from Kano State in this study is unrealistic since only 5 bulls from that State were sampled and in the light of the fact that 7.6 per cent of the cows sampled had the infection.

Since cows contact the infection only venereally (Philpott, 1968; Buxton and Frazer, 1977), it meant that the bull from the herd from which C. fetus was isolated was also infected.

The presence of infection in Borno bulls and lack of it in the cows suggest that the few cows sampled might have shed-off the infection since the infection is self-limiting in the cow (Frank et al., 1964). It also depended on the stage of the estrous cycle of the

cow when the samples were collected; there are higher chances of isolating the organism during pro-estrus and estrus, than other stages of the estrous cycle (Lawson and Mackinnon, 1952).

The presence of the organism in both bulls and cows in Kaduna State represents the real situation since more bulls and cows were sampled in this State. It suggests that infection by the organism occurs in both bulls and cows in this part of Nigeria.

The 100 per cent prevalence of the organism in aborted foetuses obtained in this study is not reliable since only two aborted foetuses sampled, and from a "problem herd". The presence of the organism in both the aborted foetuses and their dams however is a clear indication of calf losses due to the infection in Nigeria. The result of this study indicate that the ratio of C. fetus Subsp. venerealis to C. fetus Subsp. fetus amongst infected cattle in the three States examined is very high (2:1). C. fetus Subsp. venerealis is thus the main cause of campylobacteriosis in indigenous cattle of Northern Nigeria.

Table 3.1 Prevalence of (Campylobacter fetus) in Kaduna, Kano and Borno States

State Location	BULLS			COWS			ABORTIONS			Total No. of animals	Total No. of Isolates	%
	Sampled	Isolates	%	Sampled	Isolated	%	Cases	No Isolates	%			
Kaduna	547	13	2.56	79	4	5	2	2	100	626	17	2.7
Kano	5	-	-	13	1	7.6	-	-	-	18	1	5.5
Borno	33	2	6.0	12	-	-	-	-	-	45	2	4.4
Total	585	15	2.56	104	5	4.8	2	2	100	689	20	2.9

Table 3.2 Different subspecies of Campylobacter fetus isolated from animals sampled

Isolates	BULLS (n=585) No. of isolates*	%	COWS (n=104) No. of isolates	%	Abortions(n=2) Case isolates	Total isolates	Percentage
C. fetus Subsp. fetus	3	0.5	4	3.84	2*	7	1.0
C. fetus Subsp. venerealis	12	2.1	1	0.96	-	13	1.9
Total	15	2.6	5	4.80	2	20	2.9

*Isolated both from the aborted foetuses and the dams

CHAPTER 4

INFECTION, CONCEPTION, ESTROUS CYCLE PATTERN AND PROGESTERONE PROFILES OF ZEBU HEIFERS SERVED BY A BULL EXPERIMENTALLY INFECTED WITH C. FETUS SUBSP. FETUS.

INTRODUCTION

Campylobacteriosis is venereally transmitted by chronically infected bulls or by semen improperly treated with antibiotics. Infected bulls show no clinical signs, the organism lives symbiotically in the penis and the preputial fold (Furie, 1982).

The bovine has natural resistance to the disease and some cows though served by an infected bull may escape the infection even though enormous numbers of the organism might have been deposited in their reproductive tract (Clark, 1971). Infected cows return to service with inter-service intervals prolonged from 28 to 35 days or even more (Clark, 1971; Roberts, 1958).

In an outbreak, a high proportion of cows return to service with only 25 to 40 per cent being pregnant after two services, the average time from first service to establishment of pregnancy often exceeding 60 days (Clark, 1971). Roberts (1958) reported that herds affected with campylobacteriosis return to estrus at regular intervals, although many herds contain odd cows with oestral cycles of irregular lengths varying from 8 days to 3 months.

Examination of cows ovaries through rectal palpation is an intergal part of diagnosis of infertility and also of planned breeding programme (Dawson, 1975; Boyd and Munro, 1979).

In non-pregnant cows the differentiation of those that are acyclic (true anestrus) from those that are cycling but not observed in estrus, could be done by rectal palpation of the ovaries. Detection of corpus luteum indicates that the cow is cyclic (Edwards, 1965). In addition to accurate diagnosis of the corpus luteum and its stage of development, could be used to predict the time interval between the examination and the next expected estrus (Zemjanis et al., 1969).

Detection of estrus in Zebu cattle is more difficult than in Bos taurus due to their weaker estrous signs (Zakari et al., 1981) and the shorter duration (Johnson and Gambo, 1979).

The development of radioimmunoassay procedures have provided a very sensitive and reliable technique for measuring blood levels of reproductive hormones. The levels of progesterone in all domestic animals are less than 1.0 ng/ml of serum during follicular phase of estrous cycle. During the luteal phase plasma progesterone levels range from 4.0 ng/ml (Hafez, 1974).

Oyedipe et al., (1986) reported on the endocrine profile of Nigerian cattle during normal estrous cycles and early pregnancy. Plasma progesterone concentrations greater than 1.0 ng/ml indicated the presence of functional luteal tissue in the ovaries of Zebu cattle.

The endocrine profile of Zebu cattle infected with reproductive diseases such as campylobacteriosis have not been reported.

The range of therapeutic agents suitable for the treatment of campylobacter infections is limited by the frequent occurrence of resistance to the more readily available antibiotics (Butzler et al., 1974; Vanhoof et al., 1978; Karmali et al., 1981; Vanhoof et al., 1982; Michel et al., 1983). Shutte (1969) has recommended a combination of penicillin and streptomycin for the treatment of C. fetus Subsp. venerealis. Orthey and Gilmann (1954); Adler (1966); McEntee et al., (1954 and 1959) all reported streptomycin to be effective in preventing C. fetus Subsp. venerealis from being transmitted via Artificial Insemination. However, Morgan et al., (1959) and Albertsen (1957) showed that streptomycin treatment of semen extended with egg yolk was ineffective or, the effect is greatly diminished in controlling C. fetus in semen.

Combination of penicillin G and Streptomycin sulphate is usually adopted for the treatment of semen for A.I. at National Animal Production Research Institute (NAPRI).

In a previous investigation (Chapter 3) the occurrence of campylobacter among Nigerian cattle was demonstrated. In order to be able to plan adequate control measures, the pattern of infection, the consequences to fertility need investigation. The present study was therefore designed to determine

infectivity, isolation, plasma progesterone profiles and reproductive performance of heifers bred to a bull artificially infected with C. fetus Subsp. fetus. This will assist in understanding the pathogenesis, epidemiology and control of the disease. The study was also intended to determine if the addition of penicillin at 1000 iu/ml and streptomycin at 1000ug/ml of extended semen was effective in controlling campylobacter in semen processed for A.I.

4.2 MATERIALS AND METHODS

Ten heifers aged 42 to 48 months and 2 bulls aged between 3.5 - 4 years that had not been used for breeding were used in this study.

All animals were certified in good physical condition. The heifers were rectally palpated to ensure a well-developed genitalia and ovaries, while the libido of the bulls was tested by the use of a teaser bull. Their semen quality was evaluated and confirmed to be within normal range (over 30 million spermatozoa per ml).

The heifers and bulls were screened for campylobacteriosis, brucellosis and trichomoniasis by cultural isolation. The bulls and heifers were kept separated thereafter.

One of the bulls was later removed to a separate pen and artificially infected with C. fetus Subsp. fetus. Ten milliliters of broth culture of C. fetus Subsp. fetus containing 8×10^7 to 3×10^8 organisms per ml of broth

was infused into the fornix area of the penis of the bull using a syringe and pipette. The preputial orifice was then held tightly closed and the prepuce massaged for 5 minutes. The process was repeated on two other consecutive days. Seven and 14 days after the last day of infection the preputial smegma of the bull was collected by the bulb and pipette method (Barlett et al., 1947) and cultured on selective media plates, for the isolation of C. fetus Subsp. fetus. C. fetus was isolated from the bull during both samplings, thus confirming the bull infected with the organism.

All the heifers in both experimental and control groups were rectally palpated and those that had corpora lutea were given 5.0 mls (25mg) of prostaglandin F_2 alpha (PGF_2alpha) intramuscularly to synchronize their estrus. The heifers in the experimental group were housed with the infected bull, while the heifers in the control group were similarly housed with the un-infected bull.

The animals in both groups were observed for estrus (heat) continously from 6.00 a.m to 6.00 p.m. An animal was assumed to be on heat when observed to stand to be mounted by the bull or other heifers in the group. The heifers were served by the bulls as they came on heat.

Fourteen days following first estrus and service by the infected bull, the anterior vaginal mucus of the heifers was collected and cultured an campylobacter selective media for the isolation of C. fetus to

determine infectivity. Repeated sampling of the heifers were performed thereafter at one week interval except on one occasion when sampling was done after two weeks.

Blood samples were also collected from the experimental and control heifers on every other day starting from the day the heifers were housed with the bulls. The separated sera samples were decanted, labelled and stored at -20^{O}C until assayed for progesterone.

Radioimmunoassay of progesterone

Radioimmunoassay of plasma progesterone was done on all the ten heifers (control and experimental) using the "Coat-A-Coat" progesterone kit (Diagnostic products, Coportation U.S.A.) as described by Pathiraja et al., (1986). To antibody coated tube, 100 ul of Standards (1. to 40.0 ng/ml) or sample and 1.0 ml ^{125}I labelled progesterone solution, were added. The mixture was incubated for 3 hours at room temperature, the liquid phase discarded and the radioactivity bound to the antibody-coated tube was counted. The potencies of the samples were estimated using a linear logit-log dose response curve. Plasma progesterone concentration for each of the 5 infected heifers were graphically represented individually. The mean progesterone concentrations ($\pm$ SD) of the 5 un-infected heifers in the control group were determined andgraphically represented as a single graph.

Serum progesterone concentrations > 1.0 ng/ml was taken to indicate the presence of a functional luteal tissue in the ovaries (Oyedipe et al., 1986).

Semen was collected by rectal m ssage from the bull infected with C. fetus Subsp. fetus at weekly interval for 16 weeks, the collected semen was examined for volume and color and microscopically under low power (x 10) for gross motility. The IMV spectrophotometer (France) was used to determine semen concentration.

2.9 grams of tri-sodium citrate was dissolved in 100 ml of sterile distilled water. 20 mls of egg-yolk was added to 80 mls of the prepared tri-sodium citrate, thus making 100 mls of egg-yolk citrate diluent. Streptomycin sulphate at 1000 ug/ml and sodium penicillin-G at 1000 IU/ml were added to the diluent to give a final concentration of each of the antibiotics as 1000 ug/ml and 1000 IU/ml of the diluent respectively. The egg yolk citrate diluent was then used in extending the semen.

Campylobacter selective media were innoculated with both the extended antibiotic treated semen and untreated semen as control. Plates were incubated as earlier described.

4.3 RESULTS

Table 4.1 shows the infection, clearance and conception rates of the heifers following services by a bull experimentally infected with C. fetus Subsp. fetus.

After synchronization with PGF_2alpha, two heifers 3142 and 2869 came on estrus (heat) the second day following treatment and introduction of the bull to the heifers (Day 1). The remaining three heifers, 2834, 2879 and 2950 came on heat at 5, 8, and 8 days respectively. The heifers were served by the bull as they came on heat. The length of time between first service and isolation of C. fetus from the heifers' was 14, 60, 58, 56 and 63 days for heifers 3142, 2869, 2834, 2950 and 2879 respectively.

Two heifers, 3142 and 2950 became infected following first service. Heifers 2869 and 2834 became infected following second service. Heifer 2879 became infected following third service. The prepatent period for heifer 2879 was the longest and she was the only heifer not pregnant that shed-off the infection.

Two heifers 2834 and 2869 became pregnant following 2nd and 3rd services respectively; they also shed off the infection at 41 and 31 days respectively following conception.

The remaing three infected heifers, 3142, 2879 and 2950 did not become pregnant up to the termination

of the study. Only one (2879) lost her infection before the termination of the study.

Table 4.2 shows the pattern of estrous cycle lengths (in days) and pregnancy rates, while Figures 1 to 5 shows the progesterone profiles of the Zebu heifers following services by the infected bull. While Figure 6 shows the mean progesterone concentration ($\pm$ SD) profile of Zebu heifers served by a non-infected bull.

The two heifers that became pregnant had normal estrous cycle lengths (Table 4.2). The 3 non-pregnant heifers had irregular estrous cycle lengths (Table 4.2) and their progesterone profiles showed irregular pattern (Figures 1, 4 and 5), characterized by short luteal phases of progesterone production.

Heifer 3142 estrous cycles were characterized by marked irregularity as confirmed by progesterone profiles (Fig. 1). The cycle lengths were 32, 17, 32 and 43 days, following the first infertile service. During the estrus that followed that in which she was served by the infected bull (E1), there was no appreciable increase in plasma progesterone, until day 14 after estrus (day 0 = estrus). Progesterone level increased from day 14 and reached a peak level of about 3.5 ng/ml on day 24 and declined thereafter to less than 1 ng/ml on day 32. During the following

estrous cycle which lasted 17 days (E2) there was an increase in progesterone level to about 6 ng/ml on day 2 after estrus, followed by a sharp decline to about 0.5 ng/ml on day 8, then a gradual rise to about 1 ng/ml on day 10 and declined gradually thereafter to non-detectable levels on day 17.

During the following estrous cycle (E3) which lasted 32 days, there was no appreciable increase in progesterone level until on day 25 after the last estrus and reached a peak level of 1.6 ng/ml on day 31 and then declined to non-detectable on day 32.

The following estrous cycle (E4) was also prolonged as shown by the progesterone profile. The latter estrous cycle lasted 43 days.

The estrous cycle lengths following first service for heifer 2869 were normal and regular being 22 and 19 days respectively. She became pregnant after the third service, although she was infected.

The plasma progesterone profile (Fig. 2) had a regular pattern compared to that of (Fig. 1). The estrous cycle (E1) that followed the first service by the infected bull showed a rise in plasma progesterone from day 2 after estrus, and gradually reached a peak of 5.7 ng/ml on day 10 and thereafter declined to non-detectable levels on day 22. The follwing estrous cycle (E2) which lasted 19 days, was also normal and progesterone level increased from Day 1-4 after estrus and reached a peak level of 6 ng/ml on day 8 and thereafter declined to <1 ng/ml (0.45 ng/ml) on day 19.

Heifer 2869 became pregnant at the 3rd estrus (E3) or service. From conception, progesterone level increased from Day 1-3 after estrus to about 4.1 ng/ml and remained elevated thereafter.

Heifer 2834 estrous cycle length following first service (E1) was normal (21 Days) and as confirmed by the plasma progesterone profile (Fig. 3). Plasma progesterone rose from Day 1 after estrus (E1) and reached a peak level on day 5 and declined thereafter to non-detectable levels on day 21. During the second estrus (E2) and service, heifer 2834 become pregnant and plasma progesterone remained elevated thereafter until the end of the experimental period.

Heifer 2879 estrous cycle lengths were irregular being 28, 13, 36 and 23 days for the 1st, 2nd, 3rd and 4th estrous cycles following first infertile service (E1) on introduction of the infected bull.

The progesterone profile (Fig. 4) also showed an irregular pattern. The estrous cycle (E1) following that in which she was first served by the infected bull lasted 28 days. The subsequent cycles (E2 and E3) showed irregular progesterone profiles which were characterized by short luteal phases of progesterone production. The heifer was not diagnosed pregnant throughout the experimental period. She shed off the infection 70 days from the date she was first diagnosed infected (C. fetus could not be isolated from her again).

Heifer 2950 became infected at first service, appeared to have gone into complete anestrus thereafter.

The progesterone profile (Fig. 5) of the estrous cycle (E1) following the first estrus in which she was first served by the infected bull was non-detectable until 68 days after first estrus (E1). During the latter period, progesterone rose to about 4.7 ng/ml on day 100 and declined henceforth to non-detectable levels on day 107.

The heifers in the control group had normal estrous cycle lengths (21 ± 2 days). They all became pregnant after the 1st or 2nd services with the non-infected bull.

The progesterone profile (Figure 6) showed a regular pattern characterized by gradual rise and declined. The progesterone levels remained elevated after conception.

C. fetus Subsp. fetus was isolated from all the cultured untreated extended semen samples, but was not isolated from the cultured semen treated with the two antibiotics.

4.4 DISCUSSION

The successful infection of all the experimental heifers by the use of a bull infected with C. fetus Subsp. fetus showed the importance of a carrier bull as a main vehicle of transmission of this organism amongst heifers.

The infertility patterns of the heifers confirmed earlier findings by Newsam (1960) which showed that animals first served by an infected bull would become infected and those which escaped infection at first service would be infected in subsequent services. From this study of the heifers 3142 and 2869 served on the second day on introduction of bull, only heifer 3142 which was first served became infected at this service, the other became infected after the second service. Similarly, heifers 2879 and 2850 served on day 8 by the infected bull, only heifer 2950 became infected at this service, heifer 2879 became infected after three services.

The variability of susceptility of the heifers to campylobacteriosis was further demonstrated by the differences in the length of time before establishment of infection and first recovery of C. fetus from the infected heifers, which was 14, 56, 58, 60 and 63 days respectively. The interval from first service to first recovery of C. fetus from heifers varied from 14 to 63

days. Similar observation was reported by Newsam (1960) who reported an interval of 1 to 115 days from service to first recovery of the pathogen.

The active use of an infected bull reduced his ability to infect heifers, but since the bull did not get rid of his infection the infection built up later significantly enough to infect heifers that escaped in earlier services. Furthermore the result indicated that infected cows may become pregnant and some though infected may give birth to normal calves as observed by Hoerlein (1968).

The finding that infected but pregnant heifers later lost the infection after pregnancy agreed with Clark et al., (1969) who observed that infected heifers shed off the infection after pregnancy.

The inability of the other 3 infected heifers to become pregnant and the prolonged irregular estrous cycles exhibited by them are characteristics of campylobacteriosis in cows (Roberts, 1958 and Clark et al., 1969). This further confirmed campylobacteriosis as a disease of infertility rather than abortion in cows (Roberts, 1958 and Clark, 1971).

The conception rates obtained in this study after the first service was zero as compared to Newsam (1960), 21 per cent and Clark et al (1969), 14 per cent conception rates after similar experiment.

The differences were probably due to either differences in breed of animals used or the species of C. fetus or both. It could also be due to the different numbers of experimental animals used.

Progesterone plays a major role in maintainance of pregnancy and there is evidence to show that blood progesterone levels may influence the survival of the early embryo (Staples and Hansel, 1961). Oyedipe et al., (1986) reported that plasma progesterone concentration >1.0 ng/ml indicate the presence of functional luteal tissue in the ovaries of zebu cattle. From this study observed increases in levels of progesterone to 6.0 ng/ml, 4.5 ng/ml and 4.5 ng/ml in Fig. 1, 4 and 5 respectively indicated early pregnancy and functional corpora lutea. The decline from these elevated levels to less than 1.0 ng/ml or non-detectable levels indicated embryonic death and corpus luteum luteolysis. The longer estrous cycle lengths observed in this study also indicated that embryonic death might have occurred. The levels of plasma progesterone during early pregnancy are similar to those found during the luteal phase of estrous cycle (Hendricks et al., 1971 and Guthrie, 1973). However as pregnancy progresses the levels of progesterone in circulation tends to increase as observed in this study from the heifers that became pregnant (Figs. 2, 3 and 6).

returns: showed short (13 days) and prolonged (>23 days) estrous cycle lengths as a result of the infection, as indicated by progesterone profiles (Figs. 1 and 4).

The mechanism controlling the regularity of the estrous cycles of infected cows are not very clear, Kindahl et al., (1986) reported that various uterine infections alter the reproductive function, through changing of the estrous cycle length, or causing foetal resorption or abortion. These effects on the reproductive function seems to be mediated through endotoxins release. Endotoxins seem to be a very potent biological compound in releasing prostaglandins.

Uterine infections are capable of changing estrous cyclicity. Shortening of the estrous cycle has been observed in the mares with spontaneously or experimentally infected uteri (Hughes et al., 1977 and Neely et al., 1979).

The short estrous cycle lengths (13 days) and the rapid decline in progesterone levels (short luteal phases) observed in this study from the irregular returning heifers could be attributed to C. fetus endotoxin release accompanied by endometritis and prostaglandin release, thereby resulting in luteolysis of the corpus luteum (Kindahl et al., 1986).

In cases of chronic uterine infections in the mare the estrous cycles are in most cases prolonged

(Hughes et al., 1979) most likely because the bacterial infections cause progressive destruction of the endometrial elements with little or no concomitant repair. Thus reducing the capacity for synthesizing prostaglandins in quantities required for luteolysis. The observed prolonged anestrus (107 days), absence of luteal development, low and irregular plasma progesterone concentrations in heifer 2950 (Fig. 5), following injection of PGF_2alpha and subsequent estrus and service by the infected bull is in agreement with earlier reports by Voh et al., (1987). He observed periods of anestrus, poor luteal development low and irregular progesterone concentrations following synchronization of some Zebu heifers with PGF_2alpha.

Sullivan et al., (1987) reported that C. fetus Subsp. fetus endotoxin impaired inplantation in mice. Kindahl et al., (1986) have shown that endotoxin infusions into the uterus cause the release of prostaglandin F_2alpha, a luteolysin due to the irritation of the endometrium by the endotoxins and the accompanied inflammatory changes in the endometrium. This could be pathway controlling the irregularity of estrous cycles following C. fetus Subsp. fetus infection.

The isolation of C. fetus Subsp. fetus from the extended semen in egg-yolk citrate diluent without the addition of antibiotics but its absence in the extended semen with sodium penicillin G at 1000 IU/ml and

streptomycin sulphate at 1000 ug/ml of diluent, shows the effectiveness of these two antibiotics in controlling C. fetus Subsp. fetus infection in semen. This finding agreed with Shutte (1969); who reported that the combination of penicillin and Streptomycin was effective against C. fetus Subsp. fetus. However it disagreed with Albertsen (1957) and Morgan et al., (1959) who reported that Streptomycine treatment of semen extended in egg-yolk was ineffective or diminished in controlling C. fetus infection by A.I. This study confirmed that the Standard procedure adopted for semen processing in the National Animal Production Research Institute, Artificial Insemination Unit was adequate in controlling C. fetus Subsp. fetus infection in semen.

The infertility (60%) and irregularity of estrous cycles and irregular breeding due to embryonic death as a result of C. fetus Subsp. fetus infection in heifers observed in this study demands that Campylobacteriosis should be controlled if good fertility rates are to be maintained in Zebu cattle.

Table 4.1 Infection, clearance and conception rates of Zebu heifers bred to a bull experimentally infected with C. fetus Subsp. fetus

Services by infected bull	No. of heifers served at each service	Infection and pregnancy at each service				Cummulative infection rates
		No. Pregnant		No. not pregnant		
		Infected	Not Infected	Infected	Not Infected	
1st	5	-	-	1	4	40%
2nd	4	1	-	3*	1	80%
3rd	3	2	-	3	-	100%
4th	2	1	1	3	-	80%
5th	2	-	2	2	1	40%

*2950 positive for C. fetus at 2nd service without repeat service, making the cummulative infection rate after 1st service 40%. She became anestrus after 1st service until 5th service.

**Figures not cummulative vertically.

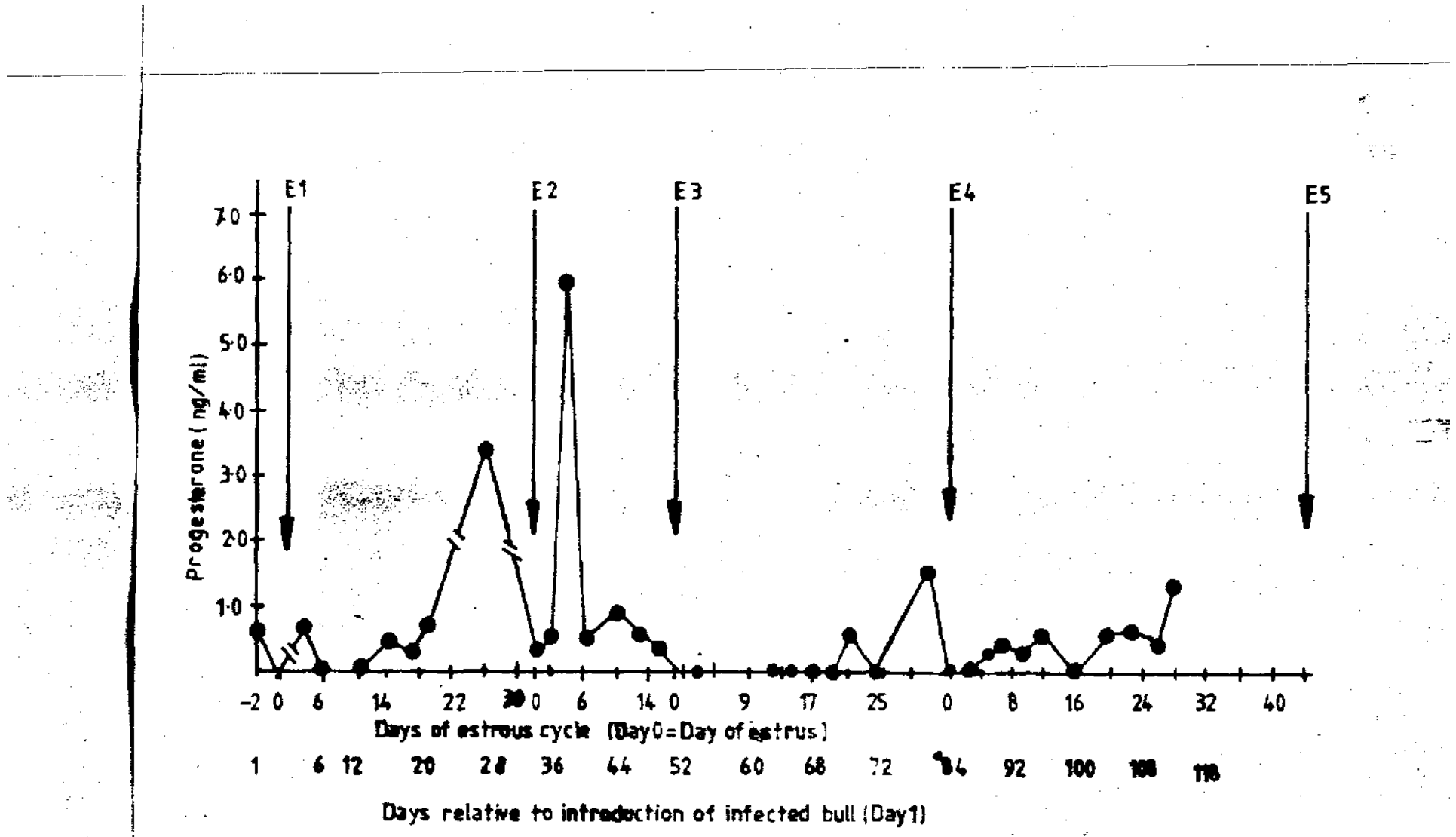
1st service - All 5 heifers served, C. fetus isolated from one

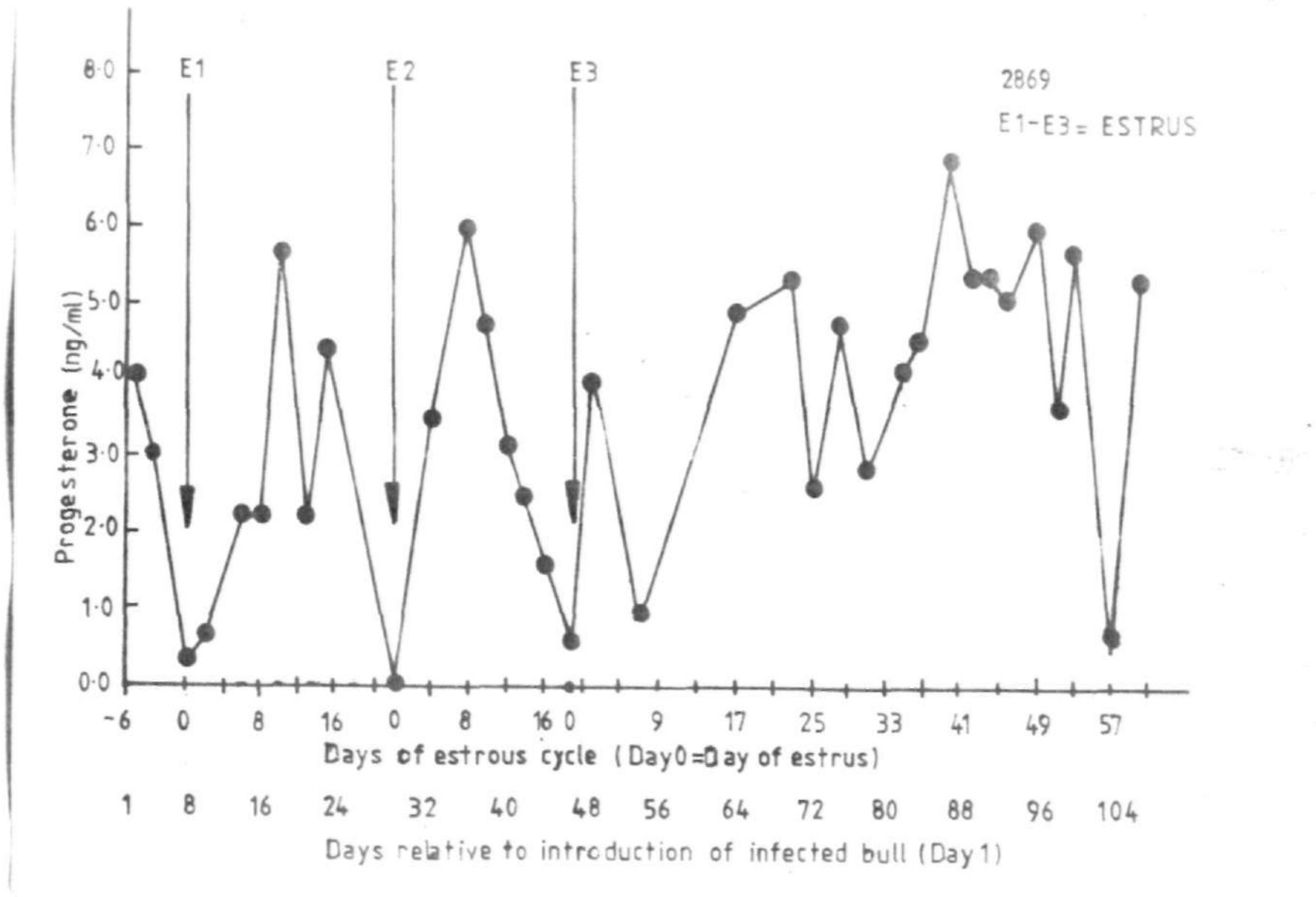
2nd service - 4 heifers served, C. fetus isolated from 3 (i.e including the anestrus 2950); one heifer became pregnant.

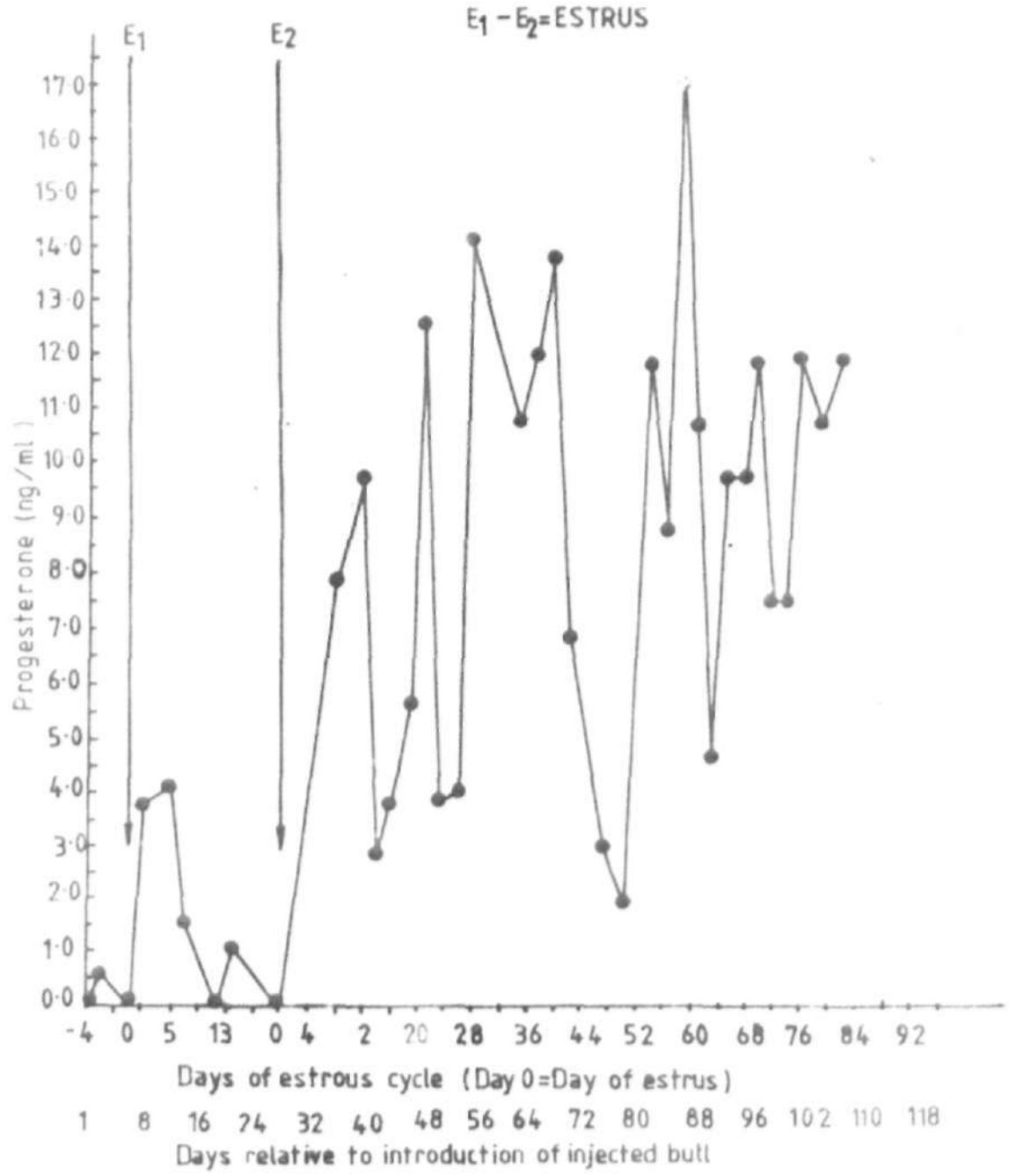
3rd service - 3 heifers served C. fetus isolated from last heifer previously not infected, another heifer became pregnant (total 2).

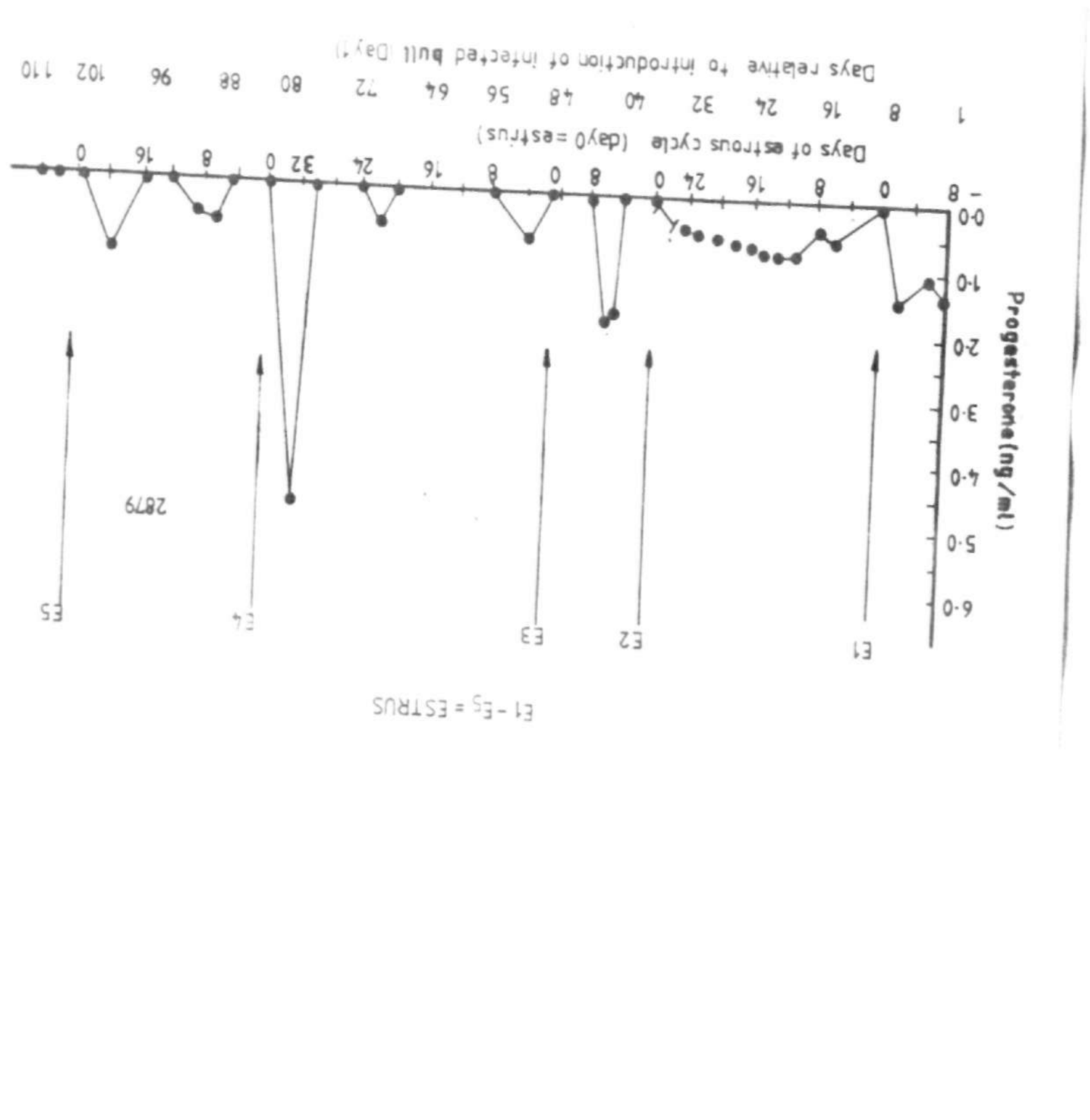
4th service - 2 heifers served, one pregnant heifer shed-off the infection.

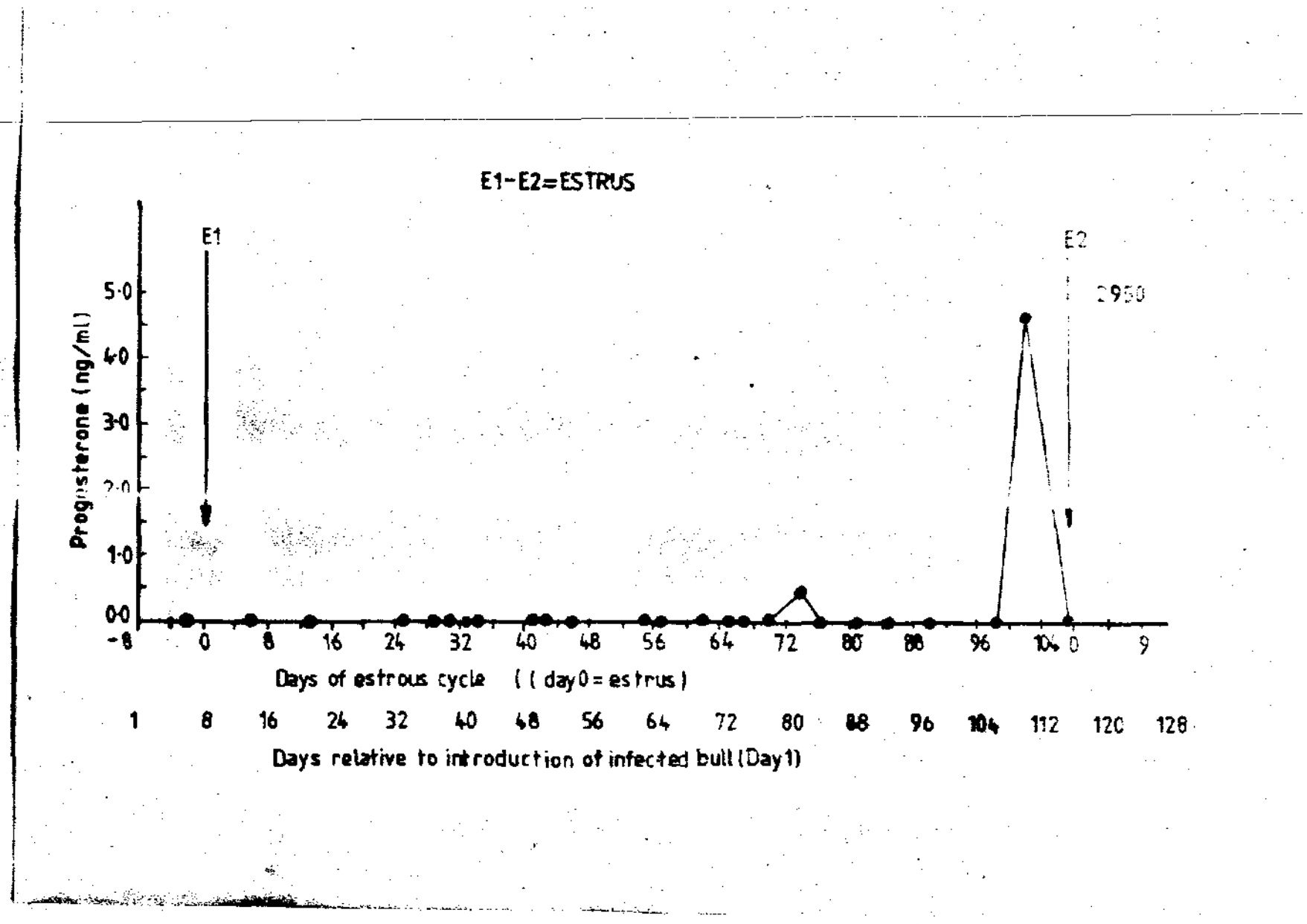
5th service - 2 heifers served, the other pregnant heifer and last non-pregnant heifer to be infected shed-off the infection.

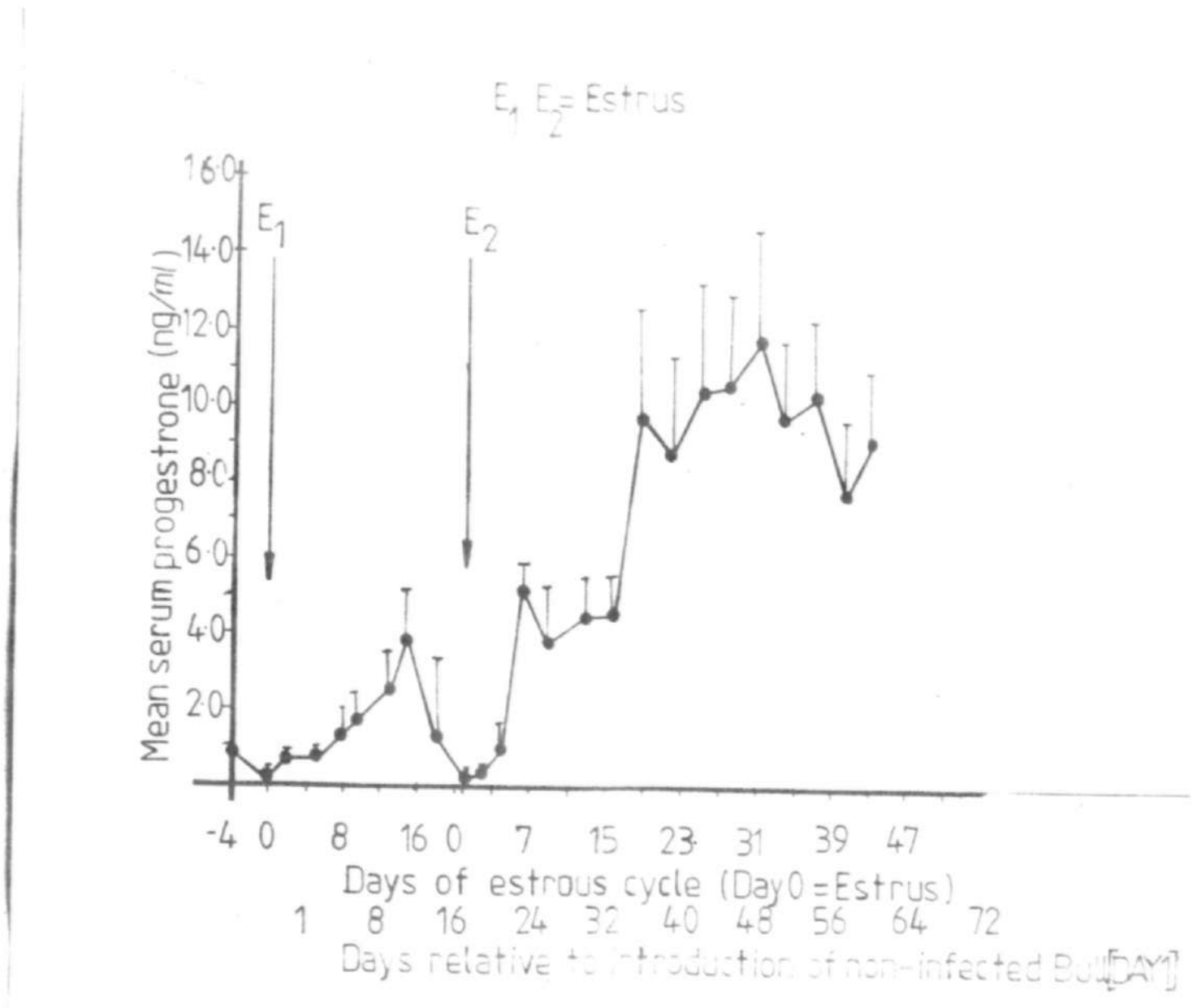

E1
E2
E3
E4
E5
7·0
6·0
5·0
4·0
3·0
2·0
1·0
Progesterone (ng/ml)
-2 0 6 14 22 30 0 6 14 0 9 17 25 0 8 16 24 32 40
Days of estrous cycle (Day 0 = Day of estrus)
1 6 12 20 28 36 44 52 60 68 72 84 92 100 108 116
Days relative to introduction of infected bull (Day 1)


E1
E2
E3
2869
E1-E3 = ESTRUS
8·0
7·0
6·0
5·0
4·0
3·0
2·0
1·0
0·0
Progesterone (ng/ml)
-6 0 8 16 0 8 16 0 9 17 25 33 41 49 57
Days of estrous cycle (Day 0 = Day of estrus)
1 8 16 24 32 40 48 56 64 72 80 88 96 104
Days relative to introduction of infected bull (Day 1)


E1 – E2 = ESTRUS
E1
E2
Progesterone (ng/ml)
17·0
16·0
15·0
14·0
13·0
12·0
11·0
10·0
9·0
8·0
7·0
6·0
5·0
4·0
3·0
2·0
1·0
0·0
-4 0 5 13 0 4 2 20 28 36 44 52 60 68 76 84 92
Days of estrous cycle (Day 0 = Day of estrus)
1 8 16 24 32 40 48 56 64 72 80 88 96 102 110 118
Days relative to introduction of injected bull


Progesterone (ng/ml)
0·0
1·0
2·0
3·0
4·0
5·0
6·0
E1
E2
E3
E4
E5
2879
E1 - E5 = ESTRUS
Days of oestrous cycle (day0 =estrus)
0 8 16 24 0 8 16 24 32 0 8 16 24 32 40 48 56 64 72 80 88 96 102 110
Days relative to introduction of injected bull (Day1)


E1-E2=ESTRUS
E1
E2
2950
Progesterone (ng/ml)
5·0
4·0
3·0
2·0
1·0
0·0
-8
0
8
16
24
32
40
48
56
64
72
80
88
96
104
0
9
Days of estrous cycle ((day 0 = estrus)
1
8
16
24
32
40
48
56
64
72
80
88
96
104
112
120
128
Days relative to introduction of infected bull (Day 1)


E1 E2= Estrus
E1
E2
16·0
14·0
12·0
10·0
8·0
6·0
4·0
2·0
Mean serum progestrone (ng/ml)
-4 0 8 16 0 7 15 23 31 39 47
Days of estrous cycle (Day 0 = Estrus)
1 8 16 24 32 40 48 56 64 72
Days relative to introduction of non-infected Bull [DAY]

CHAPTER 5

PATHOLOGY OF THE GENITALIA OF ZEBU HEIFERS SERVED BY A BULL ARTIFICIALLY INFECTED WITH *C. fetus* Subsp. *fetus*

5.1 INTRODUCTION

The examination of cows ovaries through rectal palpation is an intergral part of diagnosis of infertility and also of planned breeding programme (Dawson, 1975, 1975; Boyd and Munro, 1979). Examination of the genitalia by rectal palpation does not necessarily easily present pathological findings that may be present. Reports of pathognomic lesions due to Campylobacteriosis in the bovine in are scanty. Frequently observed are inflammatory reactions of the reproductive tracts which include salphingitis, endometritis and cervicitis (Newsam and Peterson, 1965; Clark, 1971; Buxton and Frazer, 1977 and Furie, 1982).

Abortions are not a regular feature of the disease. When they occur the foetus is autolytic and placenta is leathery and thickened by a grey-white cheesy exudate (Furie, 1982).

V. fetus Subsp. venerealis is not always associated with histological alterations in the genital tract, a frequent finding is mild endometritis. Less frequently, mild inflammatory changes can be observed in the cervix and oviduct.

Histologically the main lesions observed are lymphocytic and plasmocytic reactions of the endometrium which are often more focal than diffuse

(Clark, 1971). The pathology of the reproductive tract of temperate breeds of cattle have been reported (Clark, 1971; Buxton and Frazer, 1977; Furie, 1982).

The present study was designed to study the pathology of the reproductive tracts of infected zebu heifers that failed to conceive following services by a bull infected with C. fetus Subsp. fetus.

5.2 MATERIALS AND METHODS

The heifers used in the previous experiment (Chapter 4) were also rectal palpated at weekly interval to check ovarian activity on both experimental and control heifers, and specifically at 60 days following first service for pregnancy diagnosis (Zemjanis, 1970).

Those heifers that failed to conceive and had either vaginitis or cervitis were slaughtered at the end of the experiment for gross and histopathological examination.

The genital tracts were physically examined for gross pathological lesions and histopathological sections taken from the oviducts uterus, cervix and vagina. Sections of the ovaries were taken also in 10% buffered formalin and prepared routinely for histopathological examination (Humanson, 1967).

5.3 RESULTS

All the irregular breeding heifers developed cervicitis at different periods of the experiment following services by the bull infected with C. fetus Subsp. fetus. Furthermore they did not become pregnant throughout the experimental period. The heifers in the control group did not developed cervicitis following services by the non-infected bull. They became pregnant after the 1st and 2nd services.

Heifers 3142 from the experimental group was the first to be served on the 2nd day of introducing the infected bull into the group. She developed cervicitis and vaginitis 51 days following confirmation of infection. She recovered from the cervicitis/vaginitis 63 days following diagnosis by rectal palption.

Examination of her genitalia after slaughter revealed no gross pathological lesions except induration observed in the cervix. No histological lesions were observed from either the oviducts, uterus, cervix, vagina or ovaries. C. fetus Subsp. fetus was isolated from the cervix at slaughter.

Heifer 2879 was served on day 9 of introducing of the infected bull. She was diagnosed, infected 63 days following first service by the bull. She developed cervicitis 19 days after confirmation of infection, and 82 days after the first service by the infected bull.

The hard enlarged cervix (cervicitis) reduced in size considerably by 60 days following diagnosis by rectal palpation. At the termination of the experiment the cervicitis was diagnosed as mild through rectal palpation.

Examination of the genitalia after slaughter revealed no gross pathological lesion except induration of the cervix. Histological examination showed only congestion of the cervix (Fig. 7). However, the ovaries, oviducts, uterus and vagina had no pathological lesions. C. fetus Subsp. fetus was not isolated from any part of the genital tract at slaughter.

Heifer 2950 was also served on day 8 after the introduction of the infected bull. She became infected following first service, this was confirmed by the isolation of C. fetus from the genitalia 56 days after first service.

She developed severe cervicitis, 26 days following confirmation of infection; and 82 days after first service. She was the only heifer that had a cervix that was enlarged and also bent posteriorly.

She appeared to have recovered from the cervicitis within 60 days after diagnosis by rectal palpation. Examination of the genitalia after slaughter revealed no gross pathological lesions except induration of the cervix. Histological examination showed only congesttion of the endometrium of the uterine horns (Fig 8) and the mucosa-submucosa of the vagina (Fig 9).

She had persistent infection up to the termination of the experiment as C. fetus Subsp. fetus was isolated from the uterus and vagina after slaughter.

5.4 DISCUSSION

The inability to become pregnant and the irregular estrous cycles exhibited by the heifers which developed cervicitis agreed with Roberts (1971) conclusion that herds affected with C. fetus could return to regular normal estrus

cycles but where this did not occur repeat breeding and increased number of services appear to be the common feature. Repeat breeding as a result of infectious agents have been reported by (Laing, 1970; Buxton and Frazer, 1977). Usually these were accompanied by some or all of the following: Salphingitis, cervicitis endometritis and vaginitis leading to infertility.

The cervicitis and vaginitis observed in the irregular breeding heifers might have been as a result of C. fetus Subsp. fetus endotoxin or the invasion of the endometrium by C. fetus Subsp. fetus leading to inflammatory changes and cellular destruction.

Hughes et al., (1979) reported that most bacterial infections of the uterus most likely cause progressive destruction of the endometrial element with little or no concomitant repair.

Histologically, pathologic lesions observed in the present study were limited to any congestion of the cervix, uterine horns and the mucosa and submucosa of the vagina. Similar inflammatory changes have been observed by Newsam and Peterson (1965), Hoerlein (1968); Buxton and Frazer (1977).

Lack of any pathology in the genital tracts of heifers 2950, 2879 and 3142 suggested they have recovered from the cervicitis earlier diagnosed, within 60 to 63 days post infection. This finding agreed with those of other workers like Adler (1957), Peterson and Newsam, (1961) and Vandaplassche et al., (1970) who reported that infection of the uterus persist for about 60 days. Thereafter the infection is progressively eliminated.

Heifer 2879 was diagnosed with mild cervicitis through rectal palpation at the termination of the study, this agreed with the congestion observed in the cervix on histological examination.

The consequences of Campylobacter infections in cattle appear to culminate in tissue destruction (cervicitis and vaginitis) short luteal phase, failure of conception and early embryonic or foetal death. Therefore if good fertility rates of Zebu cattle are to be maintained, screening for C. fetus would appear highly necessary.

Fig. 7 CONGESTION OF THE CERVIC OF INFECTED ZEBU HEIFER 2879 BRED TO A BULL INFECTED WITH *C. fetus* Subsp. *fetus*
Mag. x 40

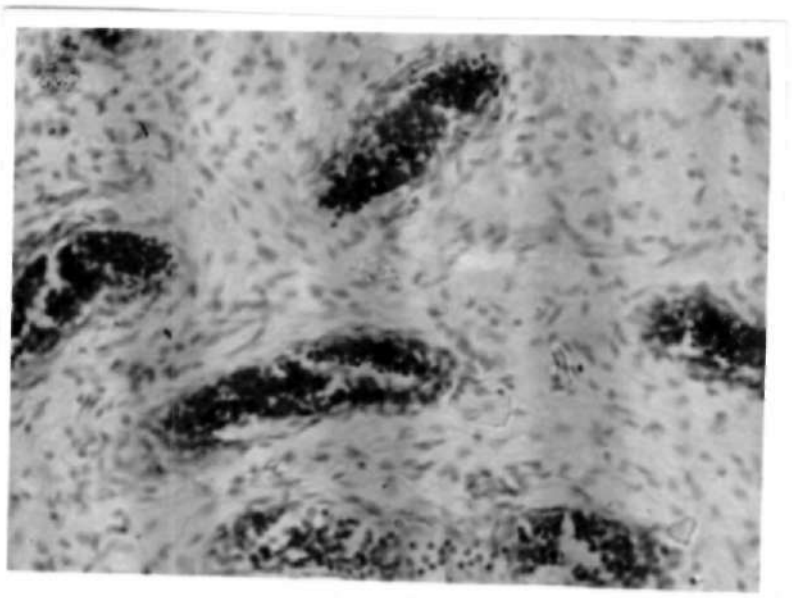


Fig. 8 CONGESTION OF THE ENDOMETRIUM OF UTERINE HORNS OF INFECTED ZEBU HEIFER 2950 BRED TO A BULL INFECTED WITH C. fetus Subsp fetus. Mag. x 40

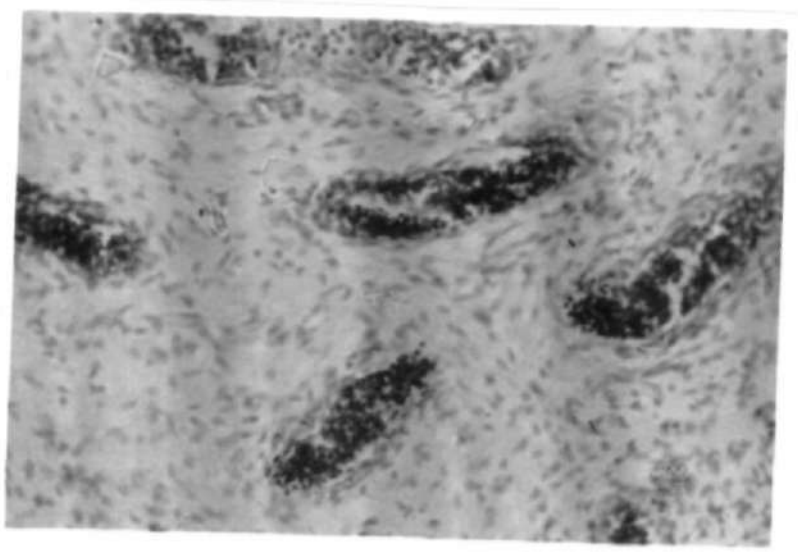
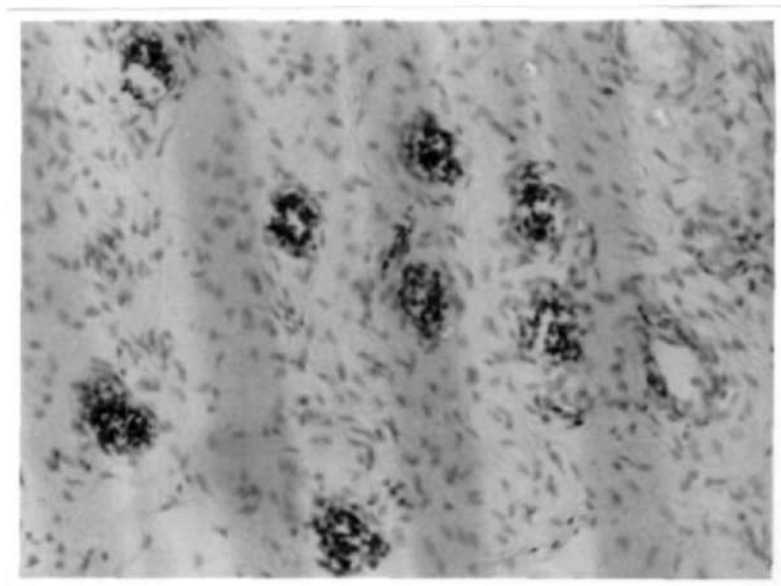


Fig. 9 CONGESTION OF THE MUCOSA--SUBMUCOSA OF THE VAGINA OF INFECTED ZEBU HEIFER 2950 BRED TO A BULL INFECTED WITH C. fetus Subsp. fetus

Mag. x 40



CHAPTER 6

GENERAL DISCUSSION

The prevalence of campylobacteriosis caused by C. fetus Subsp fetus and C. fetus Subsp venerealis in Nigerian cattle as obtained in this study from three states was 2.9 per cent.

Most importantly the present study confirms the occurrence of campylobacteriosis in Nigerian bulls and cows. The isolation from Nigerian cattle of the two Campylobacter subspecies reported world-wide as causes of infertility and/or abortion in cattle, implied that the two subspecies might be implicated in the infertility and abortion problems in Nigerian cattle. The isolation of C. fetus Subsp. fetus from a naturally infected herd with infertility 36.04 per cent, abortions (2.3 per cent), irregular breeding (12.8 per cent) further confirms that campylobacteriosis is responsible for some of the infertility and abortion problems in Nigerian cattle.

The higher isolation of C. fetus Subspecies from bulls than cows indicated the importance of the bull as the carrier of the infection.

The prevalence of the infection might have been different (probably higher) if Borno and Kano States were covered extensively as Kaduna State.

The infertility rate of 60.0 per cent recorded in the infected experimental heifers agreed with the 40 - 75 per cent reported for naturally infected herds by (Roberts, 1958; Lenver and Hart, 1980 and Swane Poel et al., 1975).

Observed in the study was thatcampylobacteriosis is characterized in Zebu cattle by tissue destruction in the form of cervicitis and vaginitis resulting in infertility. However there was self recovery from the vaginitis and cervicitis and also the elimination of the infection in some irregular returns and all the pregnant heifers. Therefore infering that campylobacteriosis is self-limiting in Zebu cattle.

The time interval between service by infected bull and recovery of C. fetus from the heifers varied from 14-56 days, although this interval is shorter than that reported by Newsam, 1960) it still stresses the need for the adoption of quick and reliable diagnostic tests for campylobacteriosis which can pick up the infection early enough in cows for effective control measures to be adopted.

Most of the irregular breeders in the experimental group repeated 2 - 5 times without conception, also in the naturally infected herd with C. fetus Subsp. fetus the latter repeated 5 - 10 times without conception. This findings indicated the serious infertility problems economic set backs in terms of low calf crop, lowered milk yield campylobacteriosis can cause in the indigenous cattle.

Another finding of this study was the confirmation by progesterone profiles the clinically observed irregularity of estrous cycles. Therefore using progesterone radio-immunoassay the accurate patterns of estrous cycles of the infected and irregular breeding heifers was determined.

Measurement of progesterone and to a lesser extent of estrone sulphate has found a practical application as method of improving reproduction in Farm animals. Examples are diagnosis of (non-) pregnancy and confirmation of (non-) estrus is possible on the basis of progesterone concentration in body fluids. This is because progesterone levels are low around the time of ovulation and high during the luteal phase of estrous cycle or during pregnancy.

Measurement of progesterone by RIA has been used for early diagnosis of (non-) pregnancy in cattle, pigs, buffaloes, goats; for confirmation of estrus in cattle and buffaloes, and monitoring of fertility in cattle, goats, buffaloes and horses, (Van de WIEL. et al., 1986).

The sequential monitoring of progesterone can be used to detect sexual maturity, postpartum period, subsequent ovulations, proportion of cycling females in a group, and time when inseminations were performed whether during the follicular phase.

Similarly information on ovarian activity, pregnancy rates following breeding and fertilization failures can be obtained by progesterone radioimmunoassay. Thus progesterone assay is an important tool in planned breeding programmes.

This study clearly showed that the local control measures adopted by herdsmen in form of removal and disposal of bulls and cows suspected of infertility problems is inadequate for the control of campylobacteriosis in infected herds. Because infected cows could deliver normal cows, thereby will be considered not infected by herdsmen, however it is shown that

such cows could be infected and be a source of infection for the next breeding season when natural service is used (Hoerlein, 1969).

For adequate control measures, a fast reliable test such as Enzyme Linked Immunosorbent Assay (ELISA), which has been shown by (Hewson et al., 1985) to pick up the infection earlier in cows should be used as a tool for diagnosis.

Infected bulls should also be removed and disposed off; and artificial insemination should be instituted to replace natural service. The infection is self-limiting in cows, therefore all animals should be routinely checked for the infection Using ELISA until confirmed free before instituting natural service with proven bulls free from campylobacteriosis and other reproductive diseases.

Finally the present study highlights the occurrence of campylobacteriosis and the accompanied infertility estrous cycle patterns and pathology of the infection in Nigerian Zebu cattle. Proper screening of all breeding bulls (both in natural and artificial services) for the disease is highly advocated for Nigerian cattle.

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Appendix I BIOTYPING OF CAMPYLOBACTERS ACCORDING TO MIKE ANSFIELD*

Campylo-bacter Species	Catalase	Relative	Swarming on moist	Rapid coccal forma-tionin	H_2S Production FBP broth	H_2S From TS1	Hippurate Hdrolysis	Growth at 25°C
C. fetus ss Venere-alis	+	Long	-	-	-	-	-	+
C. fetus ss fetus	+	Medium	-	-	-	+on lead acetate strip	-	+
C. jejuni biotype 1	+	Short S-shaped	+	+	-	-	+	-
C. jejuni biotype 2	+	Short S-shaped	+	+	-	-	+	-
C. coli	+	Short	-	-	-	-	-	-
NARTC	+	-	-	-	+slow	-	-	-
C. fecalis	+	Medium	-	-	-	+	-	-

Appendix I Cont'd

Campylobacter Species	Growth at 43°C	Nalidixic acid sensitivity (30 ug disc)	Cephalothin sensitivity (30 ug disc)	1% glycine
C. fetus ssp *veneralis*	-	R	S	-
C. fetus ssp *fetus*	-	R	S	+
C. jejuni biotyp 1	+	S	R	-
C. jejuni biotype 2	+	S	R	
C. coli	+	S	R	
NARTC	+	R	R	
C. fecalis	+	R	-	

R = Resistant; S = Sensitive; + = Positive; - = Negative

* = Mike Ansfield; Worcester Veterinary Investigation Centre, Ministry of Agriculture, Fisheries and Food England.

Appendix II Transport Enrichment Medium (TEM)

Polymyxin B Sulphate	100 units/ml
Brilliant green	50 ug/ml
Nalidixic acid	30 ug/ml
Cycloheximide	100 ug/ml

The medium was prepared as described by Clark and Dufty (1978), omitting 5-Flourouracil because it was not available.

Appendix III Selective Media

Bacitracin	12,000.0	units
Cyloheximide	2.5	mg
Colistin Sulphate	5,000.0	units
Cephazolin Sodium	7.5	mg
Novobiocin	2.5	mg
Sodium pyruvate	0.125	g
Sodium metabisulphite	0.125	g
Ferrous Sulphate	125.0	g

The first five were supplied in ampoules as freeze dried antibiotic supplement for the isolation of C. fetus (Butzler Code SR 85) The last three were supplied as FBP broth Code SR 84.