ISOLATION AND CHARACTERIZATION OF YERSINIA SPECIES FROM CHICKENS AROUND ZAR1A

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B.Sc. UNIVERSITY OF NIGERIA, NSUKKA 1981

A thesis submitted to Ahmadu Bello University, Zaria in partial fulfilment for the degree of MASTER OF SCIENCE

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DECLARATION

I hereby declare the originality of this work which was carried out by me in the laboratory of the Department of Veterinary Public Health and Preventive Medicine, Ahmadu Bello University, Zaria, under the supervision of Dr. A. A. Adesiyun.

The work of other investigators is duly acknowledged and referred to. No part of this thesis has previously been submitted for a degree or diploma.

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CERTIFICATION

This thesis entitled "ISOLATION AND CHARACTERIZATION OF YERSINIA spp. FROM CHICKENS AROUND ZARIA" by NWOSUH NNENNIA EVELYN (MRS) meets the regulations governing the award of the degree of MASTER OF SCIENCE of Ahmadu Bello University, Zaria and it is approved for its contribution to knowledge and literary presentation.

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DEDICATION

The journey was not that smooth and seemed farfetched, thank God it has come to an end.

Dedicated to the families of Mr. Bennett Kenechukwu Ikeogu and Ichie I.P.O. Nwosuh for all they have done for me. Also to Chika, my husband with all my love.

ACKNOWLEDGEMENT

I am greatly indebted to my major supervisor, Dr. A. A. Adesiyun for his continuous assistance and supervision of every aspect of this study. I quite appreciate his criticisms, suggestions made while writing this thesis and his deep understanding throughout the course of study.

Appreciation is also extended to Dr. J. U. Umoh, a member of the supervisory committee for his time, advise and help through the period of this project.

My sincere thanks to Dr. P. A. Abdu for his time, sustained interest and help throughout the period of sample collection. Without him, sample collection for the study would have been very difficult. My thanks to Mr. Azubike Ezenwe on this aspect too.

I am immensely grateful to Dr. C. D. Ezeokoli for the concern shown, time and help rendered during the surgical aspect of this work.

To all members of staff of the Department of Veterinary Public Health and Preventive Medicine; Mr. Fidelis Onianwa, Okoro Ndimele, Mr. Kevin Iwuanyanwu, Mr. J. E. Okoro and Mr. O. O. Okanlawon, I am grateful for their assistance at various stages of this work. Special mention should be made of Dr. J. K. P. Kwaga, I appreciate your help.

My gratitude also goes to Mr. Lateef Yusuf and Mr. Johnson Leko for typing this thesis.

I wish to express my profound gratitude to Ms. S. Ihenko, Ms. U. Ibeziako, Ms. J. Abii, Ms. L. Gosham and Ms. K. Mebuge for their individual contributions to make this study a success.

I quite appreciate the role my parents have played in my life. I thank them for the love and support they have always given me.

I acknowledge the encouragement and love Chika has always given me. You are the best of all husbands.

My thanks to Dr. A. G. Lamorde, Director of National Veterinary Research Institute, Vom, for making this training possible.

Finally, this study would not have been accomplished without the good works of God, who has spared my life till this day. I acknowledge all He has done for me.

ABSTRACT

The study was conducted to determine the prevalence of Yersinia spp. infection in chickens kept under different management systems in Zaria area using cultural and serologic means. All isolates were fully characterized. In addition, experimental studies were conducted in chickens exposed to Y. enterocolitica by the oral and intravenous routes.

Five (0.63%) strains of Y. enterocolitica were isolated from 800 cloacal swabs of chickens kept under the extensive (free-range), semi-intensive and intensive management systems. All samples were enriched in 0.67m phosphate buffered saline, pH 7.6 for 3 weeks and plated on desoxycholate citrate agar and MacConkey agar. All 5 strains came from chickens kept under the extensive management system ana were of serotype 0:12,25, biotype 3 and ptoagetype X_z.

Biochemically, all reactions of the isolates were typical for Y. enterocolitica but for the utilization of citrate by all and Voges-Proskauer positive results at 37^0 C for two isolates.

Only a strain (ZM12) was positive for all 4 virulence markets assayed for, namely, calcium dependency at 37^0 C, autoagglutination at 37 C, invasiveness in guinea-pig eye (sereny test) and production of heat-stable (ST) enterotoxin as detected by the infant mouse system (IMS). The IMS was found to be more sensitive in detecting ST enterotoxin with 4 of 5

strains being positive, than the rabbit ileal loop system where filtrates of supernatant of the 5 strains were negative.

In vitro activities of the antimicrobial agents tested by the disc and agar dilution metnods demonstrated that neomycin, kanamycin, gentamycin, nalidixic acid, colistin sulphate and sulphamethoxazole-Trimethoprim (SXT) were most effective against Y. enterocolitica strains from chickens.

An overall antibody prevalence of 1.9% was detected against serotypes 0:3, 0:5, 27, 0:8, 0:9 and 0:12,26 screened for in 800 serum samples obtained from the extensive and intensive systems of management. All sere, were negative for agglutinins against serotypes 0:5,27, 0:8 and 0:12,26 but 4(0.5%) and 11(1.4%) were positive for antibodies to serotypes 0:3 and 0:9 respectively.

Experimental inoculation, by the oral route, of 10^9 to 10^{11} cells of 3 serotypes of Y. enterocolitica into day-old broiler chicks did not cause any overt clinical signs. Weight gain in infected chickens was not affected over the study period. Colonization of the gut of infected chickens was minimal with Y. enterocolitica shed in faeces for only a few days. Administration of 10% $NaHCO_3$ had no significant effect on colonization of the gut of chickens by Y. enterocolitica.

Of public health significance was the detection of Y. enterocolitica in the liver, spleen, heart and bile of some

experimentally exposed but apparently healthy chickens,

Three-week old chicks inoculated with 1.0×10^{10} and 2.1×10^{10} cells of Y. enterocolitica by the intravenous route showed initial clinical signs like diarrhoea, depression and loss of appetite but recovery was completed within 24hrs in most chicks.

It was concluded that the rather high environmental temperature in Nigeria, a tropical country coupled with the relatively high normal body temperature of chickens may be responsible, in part, for the low prevalence of Y. enterocolitica infection detected in the present study. This is because the organism has affinity for low temperature.

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INTRODUCTION

The organism now called Yersinia enterocolitica was first reported in 1939 as Bacterium enterocoliticum made up of five human strains (Schleifstein and Coleman, 1939). Of the group of microorganisms formerly described as Y. entero colitica-like (Knapp and Thal, 1973), new species have been described namely, Y. entercocolitica sensu strictb (Bercovier, et al., 1980a), Y. intermedia (Brenner et al., 1980b), Y. frederiksenii (Ursing et al., 1980) and Y. kristensenii (Bercovier et al., 1980b). Agbonlahor (1986) recently advocated for a new group to be called Y. intermedia-like bacteria.

Yersinia enterocolitica has been known to cause diseases in human beings and less frequently in animals but the source of infections is often unknown (Lee et al., 1981). As a human pathogen, it is most frequently associated with acute diarrhoea, terminal ileitis, mesenteric lymphadenitis, pseudoappendicitis, septicaemia, wound infections and other clinical syndromes (Bercovier et al., 1980c; Shayegani et al., 1981). In animals, the commonly reported clinical signs of yersiniosis are chronic enteritis (Krogstad et al., 1972; Farstad, et al., 1976), abdominal cyst, mesenteric adenitis, diarrhoea, infection of anal glands, mastitis, mesenteric lymphadenitis, abortion and endocarditis, though rare (Mollaret et al., 1979). Of all the known species of Yersinia, Y. enterocolitica has been reported by several workers as the most frequently implicated in human and animal diseases (Morris and Feeley, 1976; Bottone, 1977; Shayegani, et al., 1981). The status of Y. intermedia as a pathogen is, however, yet to be resolved particularly with the

reported implication in fatal cases of diarrhoea in patients and in acute gastroenteritis (Agbonlahor et al., 1983; Butler et al., 1984). To date, in Nigeria only two reports exist on gastroenteritis caused by Y. enterocolitica in human beings (Anjorin et al., 1979; Agbonlahor et al., 1983) but no cases have been documented in animals.

The most common serotypes of Y. enterocolitica associated with human diseases are 0:3, 0:8, 0:9 and 0:5,27 (Bottone, 1981) and these have been isolated from animals like pigs, cattle, sheep and horses in several countries particularly in Europe and North America (Mollaret et al., 1979; Bottone, 1981; Shayegani et al., 1981). For sometime, it was generally believed that Y. enterocolitica infections in humans and animals were restricted to temperate environments since the organism has an affinity for low temperatures (Samadi et al., 1982) coupled with the fact that all earlier reports came from the temperate regions. However, reports are now available on isolation of species of Yersinia from tropical and subtropical regions of the world (Samadi et al., 1982; Agbonlahor et al., 1983; Butler et al., 1984). Yersinia enterocolitica may therefore be an important disease agent in Nigeria.

Yersinia enterocolitica has been isolated from a wide range of animal hosts though without clinical signs. Amongst these animals are the cat, dog, galago, chinchilla, mink, cow, pig, beaver, monkey, deer, goose, raccoon, robin, oyster, rabbit, fox, horse, ocelot, sheep, snail, frog, many species of small rodents and pigeons (Mollaret et al., 1979; Bercovier, et al., 1980a). In Nigeria, Yersinia spp. have been isolated from apparently healthy cattle and pigs

(Kwaga et al., 1984; Lombin et al., 1985) while serologic evidence of the infection has been demonstrated in 11 species of animals (Adesiyun et al., 1986b). Worldwide, information on Yersinia infection in chickens is scanty and to date, no reported isolation from chickens in Nigeria exists. Although it is believed that animals generally do not succumb to yersiniosis, they act as sources of human infection through their products such as milk (Schiemann and Toma, 1978; Hughes, 1979, 1980; Hughes and Jansen, 1981), contamination of water in the environment (Shayegani et al., 1981) and their improperly cooked contaminated meat (Leistner et al., 1975; Lee et al., 1981). In addition, isolated serotypes 0:3 and 0:5 from pig tonsils (Hanna et al., 1980) and 0:8 from tongue swabs of pigs (Adesiyun et al., 1985; Lombin et al., 1985) are biochemically identical with the most common human strains of Y. enterocolitica.

The pathogenicity of Y. enterocolitica has been associated with several characteristics such as biotype (Schiemann and Devenish, 1982), serotype (Une et al., 1977), autoagglutination at 37°C (Laird and Cavanaugh, 1980), calcium dependency at 37°C (Gemski et al., 1980), invasiveness in guinea-pig conjunctiva (Boyce et al., 1979) and heat stable (ST) enterotoxin production (Pai and Mors, 1978; Robins-Brown et al., 1979) and production of diarrhoea in mice (Laird and Cavanaugh, 1980). A close association has been demonstrated between autoagglutination, calcium dependency and invasiveness in guinea-pigs and pathogenicity of Y. enterocolitica strain in mice (Schiemann et al., 1981; Mosimabale and Gyles, 1982).

There is presently a dearth of information on these characteristics for strains of Y. enterocolitica from chickens, particularly in Nigeria where there is so much proximity between local chickens kept under the free-range management system and

human beings. It is only when these properties are known that the risk chickens pose to human health, from the view point of yersiniosis, can be defined.

The above-stated research problems have therefore led to a study with the following specific objectives:

1. To determine, culturally and serologically, the prevalence of Yersinia species infection in chicken kept under different management systems in Zaria area.
2. To characterize isolates of Yersinia spp. from chickens as to their biochemical properties, serotypes, biotypes, lysotypes, antibiograms and virulence.
3. To investigate the degree of colonization of the intestine, frequency and length of shedding, appearance and decay of antibody titres in chickens experimentally exposed to Y. enterocolitica.

2. LITERATURE REVIEW

Historical Background

Yersinia enterocolitica, an aetiologic agent of a number of disease syndromes in man was first described and named *Bacterium enterocoliticum* by Schleifstein and Coleman in 1939. They isolated this bacterial pathogen from man and observed that it had some cultural, biochemical and pathogenic features common to both *Yersinia pseudotuberculosis* and *Actinobacillus lignieresi*. Subsequently, this organism was commonly isolated from pseudotuberculoid lesions in man and some animals (mainly chinchillas) and was assigned the name *Yersinia enterocolitica*.

Hassig *et al.*, (1949) isolated strains of bacteria which they identified as human *Pasteurella pseudotuberculosis*, partly because of the nature of pathological changes in their patients. These strains were further studied by Knapp and Thal (1973) who found that they differed biochemically in many respects from *Pasteurella pseudotuberculosis* and concluded that they did not belong to that species.

In the early 1960s, clinical bacteriologists working in the fields of human and veterinary medicine in various countries obtained a number of new isolates of bacteria which they described as *Pasteurella pseudotuberculosis*, *Pasteurella pseudotuberculosis*-like organisms and *Pasteurella* 'Y' (Morris and Feeley, 1976). In 1963, Daniels and Goudzaard described a similar strain as *Pasteurella* 'X' but Frederiksen (1964) did more work on the different strains and found that *Pasteurella* 'X' and the Hassig strains were similar to the strain isolated by Schleifstein and Coleman in 1939, and proposed a new name for the species as *Yersinia enterocolitica*.

Staley (1980) reported that the original isolates were of animal origin and that isolations from human, animal and environmental sources have increased markedly during the last 10 years. Toma and Lafleur (1974) and Mollaret (1972) reported the increase in cases of Yersinia enterocolitica infections from 23 recorded in 1966 to over 4000 in 1974. Morris and Freeley (1976) then concluded that the increase in reported isolations is probably due to greater awareness about this organism and about its potential role in human and animal disease.

The Organism

The organism is now considered as a member of the family Enterobacteriaceae and this classification has been adopted in the latest edition of Bergey's manual of determinative bacteriology (Mollaret and Thal, 1974). It was formerly grouped under Pasteurella but recognised as a distinct species in 1964.

There are several species of the genus Yersinia. The two major ones are Yersinia enterocolitica and Y. pseudotuberculosis. Others like Y. intermedia, Y. frederiksenii, Y. kristensenii and some untypable species have been identified as opportunistic pathogens (Bercovier et al., 1980a). Yersinia enterocolitica infections are more frequent than infections with any other species (Tertti et al., 1984). They are Gram-negative, aerobic and facultative anaerobes often referred to as coccobacillary. They are motile at room temperature not very motile at 37°C. They are capable of growing at refrigeration temperature (4°C) used to store perishable foods and as such are regarded as psychrophiles. Their optimum temperature for growth ranges from 30°C to 37°C but grow better at room temperature (28°C).

This temperature dependence reflects in many of the physiological characteristics. They are oxidase-negative, catalase-positive fermentative coccobacilli. They are asporogeneous, non-capsulated when grown in-vitro. Their motility is via peritrichous flagellation. They are prototrophic when incubated at 28°C. Several workers have reported growth on common laboratory media like MacConkey agar (MCA), Deoxycholate citrate agar (DCA), Nutrient agar (NA), Nutrient broth, Tetrathionate broth and a lot of other media including a selective agar medium Cefsulodin-irgasan-novobiocin (CIN) formulated by Schiemann (1979). Agbonlahor et al., (1982) developed DYS medium and Bercovier et al., (1984) formulated a new isolation medium-BABY 4 - for study of environmental samples (water, vegetables etc). They reported that the medium counterselects Enterobacteriaceae species from Y. enterocolitica in a ratio of 10^{-1} to 10^{-5}, makes quantitative studies of environmental sources possible and also allows the detection after 3-4 days incubation of as few as 10^{-3} Y. enterocolitica per gram of stool.

Colonies of Yersinia appear tiny, clear and non-lactose fermenting on most laboratory media. However, they appear black on Bismuth sulfite agar (Staley, 1980), red colonies on BABY 4 medium (Bercovier et al., 1984), and dark red on DCLS agar because of the fermentation of sucrose (Shayegani et al., 1981). The colonial morphology was described by Staley (1980) as being smooth to granular in appearance on bismuth sulfite agar.

The species enterocolitica is composed of a heterologous group of organisms that can be biochemically separated into biotypes or chemiotypes (Staley, 1980). Each biotype is composed of one or more serotypes (Nilehn, 1969). Wauters' biotyping scheme divides Y. enterocolitica into five biotypes mainly on the basis of the

reactions obtained with tests for indole, lipase and fermentation of aesculin, salicin and xylose. Biotype 1 comprises the environmental strains. Serotype 0:8, a North American human pathogenic strain belongs to this biotype. Biotype 2 contains the human pathogenic serotype 0:9. Biotype 3 consists of mainly opportunist pathogens. Biotype 4 consists mainly of the common pathogenic strain, serotype 0:3. Biotype 5 consists of the leprine strains.

Nilehn (1969) developed three schemes for dividing the species of Y. enterocolitica into biogroups because the organisms show different patterns of biochemical reactions. Recently a modification dividing these biogroups into a total of four named species and two additional unnamed groups based on deoxyribonucleic acid hybridization and biochemical reactions has been proposed (Brenner, 1979; Brenner, et al., 1976, 1980a; Bercovier, et al., 1978, 1980b; Ursing et al., 1980). The name Y. enterocolotica was retained for biochemically typical strains. The atypical Y. enterocolitica-like strains were assigned to three new species, Y. intermedia (Brenner et al., 1980b), Y. frederiksenii (Ursing et al., 1980) and Y. kristensenii (bercovier, et al., 1980b) and two unnamed groups X1 and X2 (Bercovier et al., 1980a). Agbonlahor (1986) proposed a new name Y. intermedia-like bacteria for the isolates obtained from humans. This taxonomy proposal of these workers was on the basis of biochemical reactions of sucrose, rhamnose, raffinose, melibiose and x-methyl-D-glucoside. The characters are as follows - for Y. enterocolitica sucrose-positive, does not ferment any of the other four carbohydrates, for Y. intermedia, ferments all five carbohydrates;

for Y. frederiksenii, ferments sucrose and rhamnose but not the other three, and for Y. kristensenii, does not ferment sucrose or any of the other four sugars. The X1 and X2 strains are also sucrose-negative. The X1 strain is negative in all five sugars as is Y. kristensenii and can be differentiated from this species by a negative Ornithine decarboxylase reaction. The X2 strain ferments only rhamnose. The five strains of Y. intermedia-like proposed by Agbonlahor (1986) are negative for melibiose and raffinose and ferments rhamnose, α-methyl-D-glucoside and positive for Simmon's citrate.

Frequency and Protocols for Isolation of Y. enterocolitica

A number of protocols for isolation and identification of Y. enterocolitica have been described (Schiemann and Fleming, 1981; Shayegani et al., 1981, 1983; Agbonlahor et al., 1983; Samadi et al., 1983). Since the infections caused by Yersinia enterocolitica are of faecal origin, the laboratory specimens taken are faecal specimens. Laboratory specimens are subjected to a cold-enrichment procedure using any of the following: sterile saline, Rappaport's medium, buffered phosphate saline pH 7.6; 1% peptone broth (Staley, 1980) or 30% glycerol buffer (Sheyagani et al., 1981). Sheyagani et al., (1981) suggested the enrichment of faecal specimens at 4°C for three weeks and sub-culture onto agar plates at 1 week and 3 weeks.

Hunter and Hughes (1983) reported that incubation of specimens at 4°C for six weeks and subcultured at three and six weeks while Eiss (1975) reported that incubation at 4°C for two to five days significantly increased the strain isolation. Isolation media mentioned above are incubated at room temperature for 24 hours to 48 hours. Different isolation rates were reported by different workers

using different specimens. Yersinia enterocolitica infections are more common in children between the ages of 3 -5 years than in adults (Morris and Feeley, 1976).

The first isolation of Y. enterocolitica in humans in Czechoslovakia was reported in 1963 and by 1971 a total of 845 cases had been reported (Rakovsky et al., 1973). Sheyagani et al., (1981) reported the isolation rate of 3.2% of Y. enterocolitica and related organism from children in day care centres in New York. Serogroup 0:8 accounted for 2.1% of the isolates obtained from this foodborne outbreak. They also reported that from a survey carried out on human specimens, 22 isolates of serogroup 0:8, 4 of serogroup 0:6,31 and 7 other serogroups and 10 non-groupable were obtained. Varghese et al., (1984) reported the isolation of three strains (0.2%) of Y. enteroco litica from 1470 faecal specimens processed, using unmodified routine techniques. They reported that all the strains were isolated from children in the age group of 3 months to one year suffering from gastroenteritis and that all strains belonged to serogroup 0:4 and lysotype Xz, two of the strains belonged to chimiotype 1 while one as untypable.

Anjorin et al., (1979) and Agbonlahor, et al., (1983) reported isolation of Yersinia species from patients with gastroenteritis in Nigeria, a tropical country. Agbonlahor et al., (1983) reported an isolation rate of 1.3% from 1082 specimens of faeces examined. Six of the isolates were Y. enterocolitica serotypes 0:3, 0:5,27, and 0:9, seven were of Y. intermedia and one of Y. frederiksenii. Samadi et al., (1983) reported the first isolation of Y. intermedia from stool of a 5 year-old healthy child in Bangladesh, a subtropical country.

Staley (1980) reported the isolation rate of 95% from cases of human specimens in Canada. He noted that Y. enterocolitica serotype 0:3 biotype 4 was the most predominant human serotype accounting for 80%, followed by serotypes 0:5,27 (4.4%); 0:8 (3.2%; 0:6,30 (2.6%), 0:4,32 (1%), other different serotypes (3.9%) and nontypable cultures (5%). The author also reported the unusual prevalence of indole-positive Y. enterocolitica cultures and that it corroborated with the findings in United States between 1972 and 1977. In contrast, Toma and Lafleur (1974) reported that serotype 0:3 accounts for about 76% of all human isolates in Canada. Serotype 0:8 has been reported more frequently from humans in the United States (Bisset, 1976; Vandipette et al., 1973) and the western provinces in Canada. This serotype occurs in about 3.4% of all human isolates in New York (Schiemann et al., 1981). The authors also noted that with the exception of serotype 0:9 in Europe, other serotypes of Y. enterocolitica are isolated from humans with relatively low frequencies and suggested that the infrequently encountered serotypes are not pathogenic for humans.

Black et al., (1978) and Groote et al., (1982) have reported the isolation of Y. enterocolitica throughout the year but most frequently isolated during the colder period.

Isolation of Yersinia enterocolitica from Animals

Isolation of Y. enterocolitica has been reported in wide-range of domestic and wild animals including birds. Among meat animals, swine is considered as the most important source of clinically significant types of Y. enterocolitica (Hanna et al., 1980). They reported the isolation of eight positive samples of

Y. enterocolitica from pig tonsils belonging to serogroup 0:5 and assigned to Nilehn biotype 3. Doyle et al., (1981) reported isolations of clinical serotypes 0:8, 0:3 among other serotypes of Y. enterocolitica from tongues of apparently normal, freshly slaughtered pigs in Wisconsin. Toma and Diedrick (1975) reported isolations of serotypes 0:3, 0:5,27 from the caecal content of swine slaughtered at Toronto Abattoirs. Also, Harmon et al., (1984) reported isolation of Yersinia species from swabs of swine carcasses and samples of porcine tongue and trim obtained from abattoir in USA.

In Europe and Japan, Y. enterocolitica serotype 0:3, phage type 8, the most predominant human serophage type in these areas, was isolated from swine, the only animal species which consistently harbours this human serophage type (Esseveld and Goudzaard, 1973; Tsubokura, et al., 1973; Zen-Yoji et al., 1974).

Sheyagani et al. (1981) surveyed a host of animal species and reported the recovery from 6.6% of animal faecal specimens with a total of 75% of animal isolates being Y. enterocolitica, 5 isolates each of Y. intermedia and Y. kristensenii. Ahvonen et al., (1973) reported the isolation of serotypes 0:9 and 0.3 from pigs, dogs and cats. The workers did not report any isolation of Y. enterocolitica from 150 chickens sampled.

Hunter and Hughes (1983) carried out a survey of the incidence of Y. enterocolitica from pigs in the United Kingdom, using pre-enrichment culture methods on colon/caecal contents, faeces and tonsil tissue and were able to isolate 112 (5.8%) strains out of 1931 specimens sampled. Two of the strains were related to biotype 4, human serotype 0:3 and none to biotype 2, human serotype 0:9. Other strains belong to biotype 1 and the predominant serotypes isolated

were 0:6 and 0:5 while 41 strains were untypable.

In Nigeria, Lombin, et al. (1985) reported on isolation rate of 1.6% Yersinia spp. from apparently healthy live pigs. They isolated among others serotypes 0:3 and 0:8 which are known human strains of Y. enterocolitica. Serotype 0:8 had hitherto never been reported in animal species or outside the U.S.A. Agbonlahor et al. (1986) reported the isolation rate of 1.01% from cattle in Nigeria.

Davey and other workers (1983) reported the isolation rate of 50% for Y. enterocolitica and related species from faeces of cows in Scotland using Cefsulodin-irgasan-novobiocin agar. Hughes (1979) reported isolation of the organism from the faeces of healthy cows in Australia. Kwaga et al. (1984) also reported isolation from faeces of healthy cattle slaughtered in abattoir.

The occurrence of Y. enterocolitica in dogs has been reported (Farstad et al., 1976; Pederson, 1976; Kaneko et al., 1977, Fukushima et al., 1984, Papageorges et al., 1984).

Isolation has also been made from wild-living birds and Japanese serows (Yukio Kato et al., 1985), from migratory birds (Kawaoka et al., 1984). Y. enterocolitica has been isolated in different parts of the world from a large number of wild and domesticated animals (Hurvell, 1981; Mollaret et al., 1979).

Isolation from Foods and Environmental Sources

Yersinia enterocolitica has been isolated from numerous food stuffs (Lee et al., 1981) and domesticated water supplies (Hurvell, 1981). The organism was also isolated from raw milk in Canada (Schiemann and Toma, 1978), Australia (Hughes, 1979), Denmark (Christensen, 1981), France (Vidon and Delmas, 1981) and Nigeria (Umoh et al., 1984).

The presence of Y. enterocolitica in milk is of particular concern because of the potential public health hazard it presents. It is likely that the presence of Y. enterocolitica in pasteurized milk reported by Schiemann (1978) and Hughes (1980) resulted from post-pasteurization contamination since the organism is killed by heating at 60°C for 1-3 min (Hanna et al., 1976).

Sheyagani et al., (1981) surveyed environmental specimens (milk, water, soil and food) for presence of Yersinia species and reported that the isolates got were mostly Nilehn biogroups 1 and 2, Wauters biogroup 1 and Knapp and Thal biogroups 2 and 3, also that human isolates could not be differentiated from animal and environmental isolates by biogrouping alone. Included in their isolates are serogroups O:4,33, O:6,31, O:7,8 and O:8 which have been implicated in human infections. Sheyagani et al., (1983) reported isolation of serotypes O:8, O:4,33 and Y. intermedia serogroup O:5 from food and environmental samples (Milk suspensions, Milk dispenser and Turkey chow mein).

Cavazzini et al., (1982; 1983) reported first isolation of Y. enterocolitica from horticultural products. Among the serotypes isolated are O:6,30 from carrots, O:4,32 from curly parsley, O:7,8 and O:6,30 from celery. The authors suggested that environmental strains of Y. enterocolitica may acquire virulence and cause infection in man and observed that serotype O:6,30 had been recovered from four cases of human infection and represent the most frequently isolated serotype from humans in Canada, together with O:3, O:5,27 and O:9. Hughes and Jensen, (1981) reported the presence of Y. enterocolitica strains resembling biotype 1 or 2 in raw Goat's milk in Australia. The isolates fermented rhamnose and showed other a typical reactions.

Schieman (1980) reported isolation rate of 7% of processed and 49% raw retail pork products of toxigenic Y. enterocolitica in Canada. Hanna et al., (1976) also reported isolation of Yersinia species from vacuum-packaged beef and lamb stored for 21-35 days at 1-3°C). Nesbakken et al., (1985) isolated 27.0% of Yersinia species from samples of retail pork products. Doyle and Hugdahl (1983) reported the presence of Y. enterocolitica in already processed meats.

Recovery of Y. enterocolitica from streams and lakes of California was reported by Harvey et al., (1976). Of all the ten strains isolated, only serotype 16 could be biochemically and serologically confirmed. Also Fukushima et al., (1984a) reported the recovery of Yersinia species from surface water in Japan. Yersinia enterocolitica serotype 0:3 was most isolated and was the first documented from surface water. However, Lombin et al., (1986) reported that water samples from ponds, streams and wells in northern Nigeria were all negative for Yersinia enterocolitica and suggested that well water may not be important in the epidemiology of Yersinia enterocolitica infections in animals and human being in northern Nigeria.

Identification of Y. enterocolitica

Biochemical tests and serotyping by the slide agglutination test using O-antisera on suspect isolates are often used for the identification of Yersinia enterccolitica and related species (Lombin et al., 1985).

Cultural Characteristics

Yersinia enterocolitica, like other Yersinia species produces colonies of 0.5 - 1mm in diameter after 24 hours incubation on nutrient agar at temperatures ranging from 22°C to 37°C (Bercovier et al., 1980a).

The authors noted that Y. enterocolitica grows much better at 28°C than at 37°C when cultured on bile salts selective media used to detect pathogens in human stools. The organisms are almost always non-motile at 37°C, motile at 30°C and almost all strains are motile at 25°C. The growth range of Y. enterocolitica strains is from 4°C to 41°C and the optimal temperature for the expression of many phenotypic characters is 29°C (Bercovier, et al., 1979).

Biochemical characteristics

Temperature-dependent biochemical tests include the Voges-Proskauer test and those for β-galactosidase, fermentation of some sugars and ornithine decarboxylase (Bercovier et al., 1980a). These tests are usually positive for cultures incubated at 28°C. (Bercovier et al., 1980a). Yersinia enterocolitica is also characterized by rapid urease activity on urea-indole medium (Bercovier et al., 1980a) and a negative phenylalanine deaminase test. The reactions for amygdalin, salicin, dextrin, esculin and sometimes inositol were weak, delayed or irregular and should not be considered as definitive for biochemical characterization of Y. enterocolitica (Bercovier et al., 1980a).

Several different schemes have been proposed for biotyping Y. enterocolitica strains (Knapp and Thal, 1973; Nilehn, 1969; Wauters, 1973; Bercovier et al., 1979). But Bercovier et al., (1980a) proposed a slightly modified Wauter's biotyping scheme and defined five biotypes as shown in Table 1.

According to Mollaret and Lucas (1965) biotype 5 strains have only been isolated from hares in Europe. They do not reduce nitrate to nitrites and are always trehalose-negative. Bercovier et al., (1979) reported that strains of other biotypes are

trehalose-positive and reduce nitrates to nitrites with a type B nitrate reductase.

Typical Y. enterocolitica are easily differentiated from other Yersinia species and from biotypes X1 and X2 strains on basis of biochemical reactions (Bercovier et al., 1980a) and rarely occurring strains of Y. enterocolitica give atypical reactions in various biochemical tests.

Serotyping

A variety of thermostable O-somatic antigens permit Y. enterocolitica to be sub-divided into sero-groups (Bercovier et al., 1980a). Winblad (1967) established an antigenic scheme with 9 O-antigen groups and this scheme was enlarged by Wauters in 1971 and 1972 (Bercovier et al., 1980a). Wauters (1973) characterized 34 O-antigen and 20 flagellar (H) antigen groups. Toma et al., (1984) reported using 54 O-antisera for slide agglutination and the number is increasing. Bercovier et al., (1980a) characterized Y. enterocolitica sensu stricto strains that share at least one H antigen, whereas other Yersinia species according to Wauters (1972, 1973) have H antigens that differ from those of Y. enterocolitica.

Strains belonging to serogroups 0:3, 0:9 and 0:5,27 are responsible for most gastrointestinal infections, but extra-intestinal infections are caused by strains belonging to other serogroups, usually from biotype 1 (Bercovier et al., 1980a). However, biotype 1, serogroup 0:8 strains have been isolated from patients in United States of America with acute messenteric infections (Gutman et al., 1973). Toma et al., (1984) grouped serotype 0:13a, 13b as a new pathogenic serotype of Y. enterocolitica of non-human primates and humans belonging to biotype 1 and were salicin and esculin-negative. They discovered that there was a common antigenic factor between 0:7,13

and 0:18 using immune sera raised in rabbits against the used representiative non-human primate and human isolates. They were cross-absorbed by the strains used and because there was no reduction in activity after cross-absorption of either antiserum by the heterologous strain, it appeared that serotype 0:18 must contain a component of 0:13b, which they therefore subdivided into 0:13a and 0:13b.

Phage type

Phage typing is also used to subdivide Y. enterocolitica (Bercovier et al., 1980a). They gave example with serogroup 0:3 strains that have different phage types. The strains (0:3) were isolated in Europe as belonging to phage type VIII, Canada as phage type IXb and South Africa as phage type IXa. Shayegani et al., (1981) reported that phage typing was not useful for their isolates and that representative strains of human, cow, milk and water isolates, serogroupable or nongroupable were either phage type Xz (as in American strain) or Xo.

Genetic composition of Yersinia spp.

Several investigators agree that Y. enterocolitica is biochemically an extremely diverse species, and have attempted to define its biochemical boundaries, but none of the proposed definitions were totally acceptable (Wauters, 1970; Knapp and Thal, 1973; Mollaret and Knapp, 1972; Nilehn, 1969). Investigators have therefore looked into the genetic composition of Y. enterocolitica strains and attempted to relate to biochemical characteristics.

Yersinia enterocolitica

Brenner et al., (1976) showed that strains conforming to a genetic definition of Y. enterocolitica (minimum of 70% relatedness in

60°C reactions, percent divergence (%D) below 5, and 60% or more relatedness in 75°C reactions), varied in reactions for indole, esculin, xylose, salicin, lactose, christensen's citrate, cetrimide, l-inositol, Jordan's tartrate, DNase, and B-galactosidase. They also reported that strains atypical in reactions for urease (negative), raffinose (positive) or ornithine decarboxylase (negative), belonged to Y. enterocolitica. In 1980, the same workers worked on a larger number of strains biotype 1 through 4 and discovered that they were highly related. Among the strains were urease-negative, raffinose-positive, lactose-positive strains, Simmon's citrate positive strains and melibiose-positive strains. They therefore concluded that strains exhibiting these atypical biochemical reactions belong to Y. enterocolitica.

Cornelius et al., (1976; 1978) reported the isolation from Y. enterocolitica of metabolic plasmids that specify both raffinose and lactose. Bercovier et al. (1980c) therefore suggested that the gene for citrate utilization also may be plasmid mediated in Y. enterocolitica.

Brenner et al. (1980a) in their work on characterization of Y. enterocolitica and Y. enterocolitica-like strains by DNA relatedness, discovered that Guanine+cytosine content in the DNA of Y. enterocolitica sensu stricto that were melibiose-positive, rhamnose-positive and sucrose negative was 48.5 + 1.5 mol %. Mollaret and Lucas (1965) reported that most biotype 5 strains of Y. enterocolitica isolated from hares in Europe differ from other biotypes in their negative reaction for trehalose and nitrate reductase and are often negative in reactions for sucrose, ornithine decarboxylase, sorbose, i-inositol and β-galactosidase.

But Brenner et al. (1980a) conclusively showed that with DNA relatedness reactions, biotype 5 strains, regardless of variability in sucrose and other reactions are members of Y. enterocolitica. Furthermore, intragroup relatedness within biotype 1 through 4 and biotype 5 strains is some-what higher than intergroup relatedness between biotype 1 through 4 and biotype 5 strains. Sucrose-negative and sucrose-positive biotype 5 strains are essentially identical genetically (Brenner et al., 1980a). Therefore, all the trehalose-negative, metabolically inactive, biotype 5 strains constitute a single group within Y. enterocolitica.

Yersinia kristensenii

Bercovier et al., (1980b) described a group of sucrose-negative Y. enterocolitica-like organisms as Yersinia kristensenii. The reactions most useful in differentiating Y. kristensenii from other yersiniae are: sucrose, Voges-Proskauer, trehalose, rhamnose, melibiose, raffinose, x-methyl-D-glucoside, sorbose, sorbitol, ornithine decarboxylase and cellobiose. Its negative rhamnose reaction alone is sufficient to distinguish Y. kristensenii from Y. intermedia, Y. frederiksenii, Yersinia biotype X2 and Y. pseudotuberculosis. Negative reactions in melibiose, x-methyl-D-glucoside and raffinose also separate Y. kristensenii from Y. intermedia and negative sucrose and Voges-Proskauer reactions help to separate Y. kristensenii from Y. frederiksenii. Positive cellobiose and sorbose reactions, and a negative Voges-Proskauer reaction also distinguish Y. kristensenii from Yersinia biotype X2. Positive ornithine decarboxylase, cellobiose and sorbose reactions separate Y. kristensenii from Y. pseudotuberculosis. Yersinia enterocolitica, Yersinia biotype X1 and Y. kristensenii are all rhamnose-negative

Its negative sucrose and Voges-Proskauer reactions serve to differentiate Y. kristensenii from biotype 1 through 4 of Y. enterocolitica. Its positive reactions for trehalose, sorbitol and negative reaction for Voges-Proskauer separate Y. kristensenii from biotype 5 of Y. enterocolitica. Positive reactions for sorbose, sorbitol and ornithine decarboxylase separate Y. kristensenii and Yersinia biotype X1 (Bercovier, et al., 1980b). Yersinia X1 is negative for sucrose and ornithine decarboxylase while Yersinia biotype X2 is sucrose negative and rhamnose positive (Bercovier et al., 1978; Brener et al., (1980b). Another group described as the fourth group of sucrose-negative yersiniae are the sucrose-negative, trehalose-negative strains in biotype 5 of Y. enterocolitica (Bercovier et al., 1980b).

Bercovier et al., (1980c) also examined the genetic parameters of the sucrose-negative, trehalose-positive strains. They discovered that the G+C content in DNA was 48.5 ± 0.5 mol % in six strains of Y. kristensenii.

Yersinia frederiksenii

Yersinia frederiksenii is rhamnose-positive, which separates it from Y. enterocolitica, Y. kristensenii and Yersinia biotype X1. Negative melibiose, raffinose and α-methyl-D-glucoside reactions separate Y. frederiksenii from Y. intermedia. Its positive sucrose reaction distinguishes Y. frederiksenii from the rhamnose-positive, sucrose- negative Yersinia biotype X2 (Ursing et al., 1980). Bercovier et al., (1980b) had suggested additional tests of differential value between Y. frederiksenii and other Yersinia species. Ursing et al., (1980) reported that 92% of the B-xylosidase-positive Y. frederiksenii strains are Simmon's citrate-positive, and that 12% of the 201 strains they worked on, were

Simmon's citrate-positive, but B-xylosidase negative. They also reported four biotype of Y. frederiksenii: (1) B-xylosidae positive and Simmon's citrate positive. (2) Negative for both reactions. (3) Simmon's citrate positive, B-xylosidase negative. (4) B-xylosidase positive, Simmon's citrate-negative. Ninety-eight of the Y. frederiksenii strains were slide agglutinable. Serogroup 0:16 predominated (24%), followed by serogroups 0:1 (6%) and serogroup 0:10 (4%). The remaining typable strains were distributed among 16 different serogroups (Ursing et al., 1980).

Wauters, et al., (1971) showed that the H antigen of Y. frederiksenii strain 867 differed from that of Y. enterocolitica sensu stricto. Mathew et al., (1977) studying isoelectric focusing patterns of B-lactamase, found different patterns for strain 867 and for Y. enterocolitica sensu stricto.

Ursing et al., (1980) also examined the genetic parameters of the rhamnose positive group and found the G+C content of three Y. frederiksenii strains (867, 5981 and 6175) as determined by optical thermal denaturation to be 48mol %. They also used hybridization reactions between labelled DNA from Y. frederiksenii strains and individual Yersinia strains to determine relatedness among rhamnose positive strains. They found strain 867 was 53-73% related to 11 other rhamnose-positive strains. Average relatedness in 60°C reactions was 64%. Percent divergence (%D) was 9.5-13 and relatedness in 75°C reactions averaged about 30%. They concluded that strain 867 showed a similar level of relatedness to strains of Y. enterocolitica, Y. intermedia and Y. kristensenii.

Yersinia intermedia

Yersinia intermedia has been defined biochemically and genetically as a new species composed of rhamnose-positive, melibiose-positive, x-methyl-D-glucoside positive, raffinose and usually Simmon's citrate positive, (Brenner, et al., 1980b). These positive reactions serve to distinguish Y. intermedia from Y. enterocolitica and Y. kristensenii. Positive melibiose, raffinose and α-methyl-D-glucoside reactions differentiate Y. intermedia from the other new rhamnose-positive species, Y. frederiksenii.

Yersinia intermedia is separated from Y. pseudotuberculosis by its positive reactions for sucrose, indole, cellobiose, i-inositol, D-sorbitol, α-methyl-D-glucoside and ornithine decarboxylase (Brenner et al., 1980b).

Yersinia intermedia is metabolically more active at 22-28 °C than at 35-37 °C (Bottone et al., 1974), Brenner et al., (1980b) reported the different biotypes (1-8) of Y. intermedia. Other Yersinia species have a type B nitrate reductase by Y. intermedia strains have either type B or type A nitrate reductase. Strains of either type have the same level of DNA relatedness and are evenly distributed among the different biotypes. They also reported the G + C content in DNA to be 48.5 ± 0.5mol % in eight strains of Y. intermedia.

Wauters et al., (1971) grouped Y. intermedia under a known serogroup. Serogroup 0:4 and 0:17 being predominant, the other typable strains are found in 25 different serogroups. Most strains are still untypable because Wauters' serotyping scheme (1971) was based mainly on typical Y. enterocolitica strains. Serogroup 0:17

was well represented among Y. intermedia strains H antigens (Wauters et al., 1971) and structural antigens such as B-lactamase (Mathew et al., 1977) from Y. intermedia also differ from those of Y. enterocolitica and other Yersinia species (Bercovier et al., 1980a; 1980b; Mathew et al., 1977).

Transmission of Yersinia infections

The frequency of isolatior of human serotypes from pigs indicates that swine is a major reservoir for human infection with Yersinia enterocolitica. Dogs may play a role as source of infection to humans since human serotypes have been reported in dogs (Kaneko et al., 1977). Wild-living birds have also been thought to play a significant role in maintenance and dissemination of Y. enterocolitica in nature because of their great mobility (Kapperud and Olsvik, 1982; Kapperud and Rosef, 1983). The birds on their movement contaminate pastures and surface water (Yukio-Kato et al., 1985). Migratory waterfowls (tufted ducks and swans) also serve as agents of transmission of Y. enterocolitica infections (Kawaoka et al., 1984). Mollaret et al., (1979) compiled a list of animals that harbour this organism and found that most animals from which Y. enterocolitica was isolated were healthy carriers.

Human infection has also been attributed to drinking contaminated stream water (Keet, 1974; Harvey et al., 1976; Fukushima et al., 1984a).

Food-borne Implications

Contaminated food has been linked bacteriologically and epidemiologically to an outbreak of yersiniosis (Black et al., 1978). Several recent large outbreaks of Y. enterocolitica infections in Canada, Japan and United States of America

(Asakawa et al., 1973; Zen-Yoji et al., 1973; Shayegani et al., 1983) have led to a worldwide investigation of foods as a vehicle of Y. enterocolitica infections. An additional public health hazard is the ability of the organisms to grow at refrigeration temperatures (0 to 5°C) used to store perishable foods. The close association of Y. enterocolitica with domestic livestock has led to a widespread investigation of meats as a possible source of Y. enterocolitica infection (Lee et al., 1981). Y. enterocolitica is fairly common in raw milk, raw meats, raw oysters and water (Lee, 1977).

Lee et al., (1981) reported the isolation of Y. enterocolitica from ice cream and pasteurized milk. Schiemann and Toma (1978) recovered the organisms from pasteurized milk and eggnog in a Canadian Hospital. In Czechoslovakia (Aldova et al., 1975), the organism was recovered from milking machine, raw milk and cream. It was also recovered from raw milk in Australia (Mollaret et al., 1979). Two large outbreaks of Y. enterocolitica infection involving 137 children and 1 adult who had gone on outings were reported in Quebec, Canada (Lee et al., 1981). Two samples of raw milk from the site of the outbreak were found to be contaminated with Y. enterocolitica. However, the serotype of the isolates from milk were not the same as that recovered from children. An outbreak due to Yersinia enterocolitica has also been linked to a food handler in a diet camp in America as reported by Morse et al., (1984). Clinical serotype 0:8 was recovered from the individuals.

In a survey work carried out in Ontario, Schiemann and Toma (1978) recovered 42 strains of Y. enterocolitica from 29 samples of raw milk out of a total of 131 samples tested. Isolation of Y. enterocolitica from raw milk is world wide and milk has been

indicated as a source of human infection if not properly pasteurized, though post pasteurization contamination with the organism may occur (Schiemann and Toma, 1978; Hughes, 1979; Vidon and Delmas, 1981) as the organism does not survive pasteurization (Francis et al., 1980). Isolation of Y. enterocolitica from milk and milk products has also been documented in Nigeria (Umoh et al., 1984).

The presence of Y. enterocolitica in animals suggest that it also may be present in meats and extensive surveys have been conducted to recover Y. enterocolitica from meats.

Cattle and sheep are generally not considered to be carriers of the clinical biotypes of Y. enterocolitica. However, beef sometimes may be consumed without adequate heating and there has been some concern about Y. enterocolitica contamination in beef (Lee et al., 1981). Leistner et al., (1975) in their extensive survey in Germany reported the isolation of 4 strains of Y. enterocolitica and 6 related bacteria from 37 samples of beef. Two of the beef isolates belonged to biotypes that can cause typical human illness, but their virulence was not tested. Inoue and Kurose (1975) also reported the isolation of environmental biotypes of Y. enterocolitica from samples of beef in Japan. Environmental biotypes were also recovered from vacuum packaged beef and lamb (Hanna et al., 1976) and retail ground beef in United States of America (Lee et al., 1981). Leistner et al., (1975) also reported recovery from chicken meat, pork and beef.

Narucka and Westendoorp (1977) reported the recovery of serotype 0:9 from one of the pork livers and serotypes 0:3 from 75 pork tongues out of 142 samples. This showed a remarkable recovery

of potentially pathogenic strains of Y. enterocolitica from a food source.

Seafoods (fish, mussels and Oysters) and vegetables have also been reported as possible sources of virulent strains of Y. enterocolitica (Lee et al., 1981). Poultry has not been proven to be a carrier of clinical biotypes of Y. enterocolitica (Lee et al., 1981) th ough isolations have been made from chicken meats and turkey meat samples (Leistner et al., 1975; Inoue and Kurose, 1975; Capriolli et al. 1978).

Geographical Distribution

Yersinia enterocolitica has been isolated in different countries of the world both temperate and tropical.

In Czechoslovakia, Japan and Canada, serotype 0:3 has been reported to be the dominant cause of human illness (Toma, 1973). Serotype 0:9 is the common cause of illness in Finland, Belgium and the Netherlands while serotype 0:8 is the major cause of human illness in the United States of America (Staley, 1980). The organism has also been recovered in tropical and subtropical areas like India, Bangladesh and Nigeria (Anjorin et al., 1979; Samadi et al., 1982; Butler et al., 1984).

Clinical disease caused by Yersinia spp.

Manifestation in human

Yersinia enterocolitical is associated with human disease which may be manifested as gastroenteritis, terminal ileitis, mesenteric lymphadenitis, septicaemia, meningitis,s skin and eye infections (Winblad, 1973; Bottone, 1977, Anjorin et al., 1979; Shayegani et al., 1981; Agbonlahor et al., 1983).

Hubert et al., (1975) grouped the clinical manifestation of yersiniosis into four forms:-

(a) Acute mesenteric lymphadenitis commonly caused by Y. pseudotuberculosis.

(b) Enteritis commonly caused by Y. enterocolitica which may be severe with signs of toxemia and dehydration.

(c) Erythema nodosum that occurs as a separate clinical entity of in association with mesenteric lymphadenitis.

(d) Septicemia which is the rarest form of the disease.

Other symptoms include low-grade fever, headache, pharyngitis, anorexia, vomiting, arthritis, cutaneous ulceration and abscesses.

Mollaret et al., (1979) summarized the various clinical circumstances in which Y. enterocolitica was isolated from man into three forms.

Group A: - The 'Classical' clinical forms include

Abdominal forms-

- Enteritis:- Familial forms or epidemics chronic forms

  Limited rough forms in healthy carrier.

- Syndrome of right iliac fossa -

  mesenteric lymphadenitis

  Pseudo-appendicts form

  Tumor form

  Latent form

  Terminal ileitis

- Septicemias

- Erythema nodosum

- Polyarthritis

Group B: - Extra-mesenteric forms

- Isolated, deep suppurations

  Splenic abscess

  Hepatic abscess

  Colonic abscess

  Abdominal abscess

  Peritoneal effusion

  Bile

- Isolated bone joint suppuration

- Super infection of minor wounds

- Cutaneous manifestations

- Respiratory tract

Group C:- Healthy Carriers

Manifestation in animals

Animals are considered as reservoirs of Y. enterocolitica for human infections and the organism has been isolated from various animal species (dogs, cats, goat etc) in connection with acute ileitis and appendicitis (Nilehn, 1969; Winblad, 1969; Langford, 1972).

Farstad et al., (1976) reported isolation of the organism from a dog with chronic enteritis. Krogstad et al., (1972) also reported an outbreak of yersiniosis in a goat herd in Norway where the goats suffered from profuse diarrhoea.

Clinical manifestation of the disease has not really been determined in animals. Most of the reported isolations done to establish the presence of the organism are on healthy carriers.

Antimicrobial susceptibility of Yersinia enterocolitica

In spite of increasing reports of isolation of Y. enterocolitica from various clinical conditions in the world literature, there is very little information on its antimicrobial susceptibility pattern (Hammerberg et al., 1977; Raevuori et al., 1978).

Toma (1973) reported that stock cultures and cultures isolated in Ontario, using the Kirby-Bauer technique, were found to be susceptible to polymyxin, streptomycin, gentamicin, kanamycin, chloramphenicol, sulfadiazine (while three cultures were resistant) nitrofurantoin, nalidixic acid and tetracycline (except two cultures) but resistant to penicillin, oxacillin, lincomycin and novobiocin. He also reported that the cultures were resistant or moderately resistant to ampicillin, erythromycin, cephaloridine and carbenicillin.

Hammerberg et al., (1977) tested the antimicrobial susceptibility against clinical isolates of Y. enterocolitica biotype 4, serotype 0:3, using agar dilution and disk diffusion methods. They found that the isolates were susceptible to the six aminoglycosides used: gentamicin, kanamycin, netilmicin, siscomicin, amikacin and tobramycin. Chloramyphenicol was less active and similar to tetracycline and rifampin. All isolates were resistant to ampicillin, carbenicillin, cloxacillin and erythromycin. Susceptibility to cephalothin ranged from moderate resistance (MIC, $\leq$25 ug/ml) to complete resistance (MIC $\geq$100 ug/ml). They concluded from their result that aminoglycosides and co-trimoxazole are the most active antimicrobials in-vitro against Y. enterocolitica. The work of Raevuori et al., (1978) using agar dilution method corroborated with

the observations on aminoglycocides in the work of Hammerberg et al., (1977) except that the former MIC values were slightly less than the latter. The data of Raevuori et al, (1978) on chloramphenicol and tetracycline were comparable with Hammerberg et al., (1977), their data on tetracycline and streptomycin were in accordance with Toma (1973). Raevuori et al. (1978) worked on isolates from human, environmental and animal sources and found they had equal susceptibility patterns, but water isolates showed slightly greater susceptibility than did other strains. They, however, concluded that biochemical and antimicrobial tests do not reliably identify isolates of Y. enterocolitica according to their origin and these tests do not evaluate the homogenecity of this species.

Kohl et al., (1976) worked on clinical isolates of Y. enterocolitica and observed a similar susceptibility pattern as Hammerberg et al., (1977), Raevuori et al., (1978), also Vidon and Delmas (1981) who worked on milk isolates in France. Restaino and Hill (1981) compared the antibiograms of atypical and typical strains of Y. enterocolitica and reported that typical strains displayed greater susceptibility than the atypical strains to antibiotics used. They reported susceptibility of strains of serotype 0:8 to ampicillin, carbenicillin, erythromycin and cephalothin.

Agbonlahor and Odugbemi (1982) determined the antimicrobial susceptibility pattern of 15 strains of Y. enterocolitica isolates from clinical specimens in Nigeria, using microdilution and disc-diffusion techniques. They observed susceptibility of all isolates to amino-glycosides to conform with the findings of Hammerberg et al., (1977), and susceptibility to chloramphenicol,

tetracycline and co-trimoxazole to conform with Toma (1973) and Raevuori et al., (1978). The isolates were resistant to ampicillin, carbenicillin and cephalothin. They also compared the in-vitro activity of amoxycillin with augmentin (amox cillin and clavulanic acid) and found that augmentin had superior effect.

Kwaga et al., (1980) found that 14 strains of Y. enterocolitica from apparently healthy cattle and pigs in Nigeria were susceptible to gentimicin erythromycin, tetracycline, chloramphenicol, nalidixic acid and colistin. Raevuori et al., (1978) reported that gentamicin, tetracycline nitrofurantoin, chloramphenicol and sulphamethoxazole - trimethoprim are most effective antimicrobial agents against yersiniae of animal.

Mechanisms of Pathogenicity of Y. enterocolitica

The pathogenic mechanisms of Y. enterocolitica are not fully known (Pai and Mors, 1978). Carter (1975) reported an experimental infection of Y. enterocolitica in mice, which closely resembled the naturally acquired human infection. He demonstrated that intravenous inoculation of mice with Y. enterocolitica gave rise to a systemic, pyogenic infection involving the spleen, liver and lungs and that there was massive neutrophil infiltration of these organs in the early period of the infection, which eventually led to large abscesses and pulmonary consolidation. He also reported that mice infected intragastrically showed neutrophil infiltration in the Peyer's patches of the distal ileum followed by local abscesses in the medullary regions.

The virulence of Y. enterocolitica has been associated with several characteristics notably, the ability to produce heat stable (ST) enterotoxin (Pai and Mors, 1978), invasiness of guinea-pig conjunctiva (Gemski et al., 1980; Zink et al., 1980), autoagglutination at 37°C (Laird and Cavanaugh, 1980; Vesikari et al., 1981), calcium dependency at 37°C (Gemski et al., 1980), invasion of Hela cells (Zink et al., 1980; Portnoy et al., 1981), possession of 42-megadalton plasmid (Gemski et al., 1980; Portnoy et al., 1981), and the presence of WA-SAA antigen (Doyle et al., 1981).

1. Heat stable (ST) enterotoxin

Yersinia enterocolitica ST enterotoxin was earlier thought to be involved in pathogenicity like the heat stable enterotoxin of E. coli (Pai and Mors, 1978; Robins-Brown et al., 1979). Pai and Mors (1978) reviewed some cases of childhood Y. enterocolitica enteritis in Canada and reported that the clinical symptoms are much milder than would be expected from the pathological findings in the mouse model. Furthermore they found that a significant number of cases had profuse watery diarrhoea which was suggestive of a similarity between gastroenteritis due to Y. enterocolitica and that caused by enterotoxigenic strains of E. coli. Robins-Browne et al., (1979) suggested that the role of enterotoxin in the pathogenesis of intestinal Y. enterocolitica infection may be similar to that of heat-stable in E. coli diarrhoea.

Schiemann and Devenish (1982) however found that there was no association between virulence and the ability to produce ST enterotoxin measured by the infant mouse assay. Mosimabale and

Gyles (1982) similarly found that heat-stable enterotoxin plays no role in pathogenicity. Also Pai and Mors (1980) failed to detect ST enterotoxin in faeces of rabbits that were infected with Y. enterocolitica and had diarrhoeal symptoms. Aulisio et al., (1983) detected ST enterotoxin in both virulent and avirulent strains of Yersinia and concluded that ST enterotoxin test may characterize a strain but ST enterotoxin production does not contribute to pathogenicity.

A number of tests to detect ST enterotoxins have been described. Notable amongst these are the infant mouse system (Pai and Mors (1978), rabbit ileal loop test (Pai and Mors, (1978) and Pig gut test (Mosimabale and Gyles (1982).

(a) Infant Mouse Test

Pai and Mors (1978) demonstrated enterotoxin production by Yersinia enterocolitica using three-day-old suckling mice which were fed orally with Yersinia enterocolitica culture filtrate and sacrificed four hours post-inoculation. The authors concluded that majority of Y. enterocolitica was capable of producing enterotoxin of which the characteristics resemble those of heat-stable enterotoxin of E. coli. The test separates enterotoxigenic from non-enterotoxigenic strains (Pai and Mors, 1978; Mosimabale and Gyles, 1982). Schiemann (1981) reported that all strains of Y. enterocolitica they used for ST production were negative when he injected the culture filtrate directly into the stomach of 2 to 4-day old mice. Heat-stable enterotoxin produced in vitro by some strains of Y. enterocolitica and measured by the infant mouse assay is not necessary for pathogenesis (Schiemann, 1981). Robins-Browne et al., (1979) reported that the three clinical isolates of Y. enterocolitica

they worked with gave positive results in the infant mouse test and concluded that the enterotoxin produced by Y. enterocolitica share a number of biological properties with ST of E. coli and both substances stimulate fluid accumulation in infant mice and rabbit ileal loops within the same time interval.

Many human and environmental isolates of Y. enterocolitica and Y. enterocolitica-like bacteria produce heat-stable enterotoxin in vitro at 20°C to 30°C (Pai et al., 1978; Kapperud, 1980) but the highest prevalence of enterotoxin production has been reported among human clinical isolates, though none has been shown to produce enterotoxin at human body temperature (Kapperud, 1982). Nesbakken (1985) demonstrated the production of enterotoxin by Yersinia spp. isolated from porcine tonsils and pork products, at different temperatures 4°C, 22°C and 37°C using infant mouse essay. He discovered that no strain was positive at all 3 temperatures, 37 out of 71 strains tested produced enterotoxin at 22°C, 3 at 4°C and 22°C and 1 at 22°C and 37°C. Furthermore, the highest prevalence of enterotoxin production at 22°C was detected in serotype 0:11 (80%), followed by 0:3 biotype 4 (74.2%) and 0:12 (66.7%). Adesiyun et al., (1986) reported the ST enterotoxin production in seven out of fourteen isolates studied using infant mouse assay.

The influence of several factors affecting detection of Y. enterocolitica heat-stable enterotoxin by infant mouse test was studiedby Serafin et al., (1983). They found that Pai-Mors medium was most suitable for ST enterotoxin preparation, and that the gut/carcass weight ratios obtained with lysed supernatants and

with supernatants from whole cultures of Y. enterocolitica were similar, suggesting that most of the ST enterotoxin produced by this organism in broth cultures is extracellular. The amount of ST enterotoxin produced as well as the ambient temperature at which inoculated mice were kept were inter-related, such that for weakly enterotoxigenic strains there were no significant differences among gut/carcass weight ratio for the conventional and modified infant mouse tests carried out at 18°C or 25°C, but at 30°C the values in the modified test were higher for most ST enterotoxin preparations with Pai-Mors medium.

The infant mouse test was reported to distinguish two populations of Y. enterocolitica clearly separating the positive and negative groups (Pai and Mors, 1978; Mosimabale and Gyles, 1982).

(b) Rabbit ileal loop

Pai and Mors (1978) and Robins-Browne et al., (1979) reported heat stable enterotoxin activity of Y. enterocolitica using rabbit ileal loop assay. They determined the volume/length ratio of each filtrate injected loop at 6 and 18 hrs post-injection and found no difference in time. The strain tested was positive at both intervals. The time taken for fluid to accumulate in infant mouse and rabbit ileal loop models is similar with respect to Y. enterocolitica and E. coli enterotoxins but different from that associated with cholera toxin (Robins-Browne et al, 1979). Adesiyun et al., (1986a) reported that the in-vivo test for ST enterotoxin using rabbit ileal loop appeared to have low sensitivity in detecting ST enterotoxin produced by Y. enterolitica.

(c) Pig gut

Mosimabale and Gyles (1982), detected ST enterotoxin production by Y. enterolitica strain using ligated pig gut. The authors used broth culture supernatant and culture filtrates grown in tryptic soy broth enriched with yeast extract (TSB-YE) and incubated in a shaker bath for 24hr. to inject the loops .. and observed that none of the strains grown in a shaker bath was positive but the culture supernatants induced fluid accumulation in the ligated guts. They then concluded that ligated pig intestine was not a good model for enterotoxigenicity studies of live cultures of Y. enterocolitica. Adesiyun et al., (1986a) obtained similarly unsatisfactory results using 1-week and 3-week-old ligated pig gut.

2. Invasiveness of conjunctiva of Guinea-pig

The invasiveness of Y. enterocolitica, as demonstrated by Séreny tes (Séreny 1955) depends on the presence of 41-megadalton plasmid (Zink et al, 1978) and 42.2 ± 1.1-megadalton plasmid (Germski et al., 1980) and calcium dependency at 35°C (Schiemann and Devenish, 1980). Schiemann et al. (1981) described the correlation calcium dependency with autoagglutination and the ability to produce both guinea pig conjunctivitis and mouse diarrhoea.

Schiemann and Devenish (1982) reported a positive reaction on guinea-pig conjunctivitis which persisted after the first day and the organism recovered at 2 days or thereafter. The animals were observed for 7 days for signs of inflammation and swelling of the conjunctivae, eyeball depression, fluid and mucous accumulation and clouding of cornea. The authors observed the variation in reactions in the guinea-pig eye between serotypes, with strains of serotypes 0:8 and 0:21 producing the most severe infections.

Boyce et al. (1979) also demonstrated positive Séreny tests among strains of serotype 0:8. Schiemann and Devenish (1982) reported uniform negative Séreny test results in all isolates tested and suggested that there was no serotype 0:8 of human origin among the isolates studied hence the result. The authors then explained that it could be that the isolates had lost their capacity to invade epithelial cells.

3. Autoagglutination at 37°C

The autoagglutination test is a simple procedure for screening for potential virulence; however, because this property is apparently lost easily with subculture, negative results must be interpreted with some caution (Schiemann et al., 1981). A positive test as described by Laird and Cavanaugh (1980) consisted of agglutinated cells concentrated along the lower wall and bottom, with a nearly clear medium in the upper portion of the tube after overnight incubation at 35°C and a nearly uniform turbid suspension without agglutination at 22°C. Doyle et al., (1981) reported the positivity of serotypes 0:3 and 0:8 of Y. enterocolitica when tested for autoagglutination at 37°C. Nesbakken and Kapperud (1985) also reported that 67 (45.9%) of the 146 isolates of serotype 0:3 biotype 4 studied were autoagglutination positive.

Laird and Cavanaugh (1980) while studying virulence factors of Yersinia species, discovered that strains which were virulent when fed to adult mice autoagglutinated when grown at 36°C in tissue culture media. Strains which failed to agglutinate were found to be avirulent and this observation aided in the development of a simple test for distinguishing virulent and avirulent strains of yersiniae.

The authors therefore, found a correlation between virulence in Y. pestis, Y. psudotuberculosis and Y. enterocolitica and the ability to autoagglutinate in tissue culture medium at 35°C. In addition to its value in preparing isogenic derivatives needed for studies of pathogenesis, the test is reliable for the rapid screening of large numbers of yersiniae from clinical and food sources (Laird and Cavanaugh, 1980).

Schiemann and Devenish (1982) reported that isogenic pairs for autoagglutination were selected from one strain of serotype 0:8, and that autoagglutination-negative culture was positive for calcium dependency at 35°C, indicating that the plasmid that codes for calcium dependency at 35°C (Gemski et al., 1980) also codes for autoagglutination, and that this plasmid was present in one and absent in the other culture of this isogenic pair. The authors then concluded that when the plasmid coding for autoagglutination and calcium dependency is lost, the virulence is also lost and that the identification of virulent strains of Y. enterocolitica by these two tests is not always dependable, since the plasmid coding for these properties can easily be lost through subculture.

4. Calcium dependency at 37°C

The same plasmid coded for a calcium requirement for growth at 37°C was determined with the magnesium oxalate (Mox) agar developed by Higuchi and Smith (1961) for identifying virulent strains of Yersinia pestis (Schiemann and Devenish, 1982). Carter et al., (1980) showed that the virulence of Y. enterocolitica in mice was related to the ability to produce V and W antigens which were immunologically identical to virulence antigens of Y. pestis and Y. pseudotuberculosis.

Higuchi and Smith (1961) determined the presence of avirulent cells in a predominatly virulent cell population using surface plating on a differential agar medium which inhibited the growth of virulent cells. The medium, magnessium oxalate agar prepared aseptically promotes the growth of avirulent cells present in the virulent cultures.

Agbonlahor (1986) reported four strains out of five of Yersinia species to be calcium-dependent using the interpretation that growth on TSA without growth on magnesium oxalate agar or heavy growth on TSA and few colonies on magnessium oxalate agar was taken as evidence of calcium dependence for growth at 37°C.

5. Invasion of Hela cells

Schiemann and Devenish (1982) tested the distribution of Hela cell infectivity indices for each serotype among 69 strains and found a clear seperation at the index value of 3.5. Consequently, the index was chosen as the dividing value between Hela-positive and Hela-negative cultures. They also detected auto-agglutination-positive strains among Hela-positive strains. They demonstrated also that Hela cell infectivity is not altered with loss of the plasmid coding for autoagglutination and calcium dependency. Mosimabale and Gyles (1982) observed that there was a correlation between the results of the Hela cell test and the magnessium oxalate test and that the two tests are likely to relate to invasive aspects of virulence rather than diarrhoeal aspects of Y. enterocolitica infections.

Serological Tests

Significant titres in serological studies on Y. enterocolitica infections have been shown to demonstrate exposure experience to the

agent which may be current (Krogstad, et al., 1972; Hurvell, 1981). Reports exist showing failure to isolate the organism even though significant titres of antibodies to the serotypes were detected (Krogstad, et al., 1972). An initial problem with the serological studies on Y. enterocolitica was the cross-reaction detected between Y. enterocolitica serogroup 0:9 and Brucella abortus. It is therefore necessary to pay attention to Y. enterocolitica serogroup 0:9 infection when the serological examinations for brucellosis are performed (Sunaga et al., 1983). Antigenic relationships among type strains of Y. enterocolitica and those of Escherichia coli, Salmonella spp and Shigella spp have also been reported (Thomas et al., 1983).

Serological tests used to detect Y. enterocolitica antibody include slide agglutination (Agbonlahor et al., 1983), passive hemagglutination test (Gutman et al., 1973; Chiesa et al, 1984), enzyme-linked immunosorbent assay (ELISA). (ELISA) (Carlsson, et al., 1976), serum agglutination and complement fixation tests (Corbel, 1973; Sunaga et al., 1983). Chiesa et al. (1984) found that out of 221 cord blood samples assessed for antibodies to Y. enterocolitica by whole cell agglutination test, only four showed anti-yersinia antibodies. None of these four sera cross-reacted by this method, but when tested by passive hemagglutination, differences in titre and cross reactivity were noted. Analysis of the distribution of hemagglutinin titer in umblical cord sera against heat-extract from Y. enterocolitica 0:3, 0:5, 27, and 0:8 showed a similarity among the three Y. enterocolitica serogroups.

Their findings that Y. enterocolitica antibodies were only detected in four (1.8%) of 221 newborn sera despite the high percentage (33%) of agglutinating antibodies in sera of pregnant women (especially against Y. enterocolitica 0:3 (17%) and 0:8 (14%) ranging in titre from 4 to 32, indicated that the mean level of Y. enterocolitica agglutinins as assessed by whole cell bacterial agglutination, in sera of pregnant women was insufficient to appear in detectable titre in cord blood. These findings corroborated with previous work done to antigens of E. coli, Salmonella and Shigella (Felsen and Osofsky, 1937; Neter et al., 1955).

Heterologous HA antibodies active in cord sera against Y. enterocolitica could, in the newborn period, serve as potential mediators of resistance to infection not only by many enterobacterial species, but to Y. enterocolitica as well (Chiesa et al, 1984). Sera from pregnant women have IgG antibodies to Y. enterocolitica, which by transplacental passage could also provide a threshold level of antibodies that act alone or in concert with the heterologous antibodies demonstrated by hemagglutination (Chiesa et al, 1984).

In the study by Bitzan et al., (1983), 17 out of 24 patients (71%) tested demonstrated significant agglutinin titres one to 13 weeks after clinical onset. The significant titres were assumed, if the reactions were positive against Y. enterocolitica in serum dilutions 1:40. They also observed that the maximum titres were already developed within two or three weeks after clinical onset in patients. They then concluded, that a reliable diagnosis rests on the demonstration of specific antibodies in significant titres,

preferable with four-fold rise or decrease. Also, 18 members of the families in an inter-familial outbreak of Y. enterocolitica enteritis, demonstrated a positive response by the passive hemagglutination test. Eight persons had titres of 1:512 or greater, and all had symptomatic illness (Gutman et al., 1973). Morse et al, (1984) in an investigation of a Yersinia camp outbreak linked to a food handler, reported the positive reciprocal titres of 256 and 128 in a serologic testing using Y. enterocolitica serogroup 0:8 antigens prepared from the outbreak isolates. They concluded that serologic test may not be diagnostic for an acute Yersinia infection but consistent with previous exposure and the involvement of a food handler.

Krogstad (1972) determined the titre of antibodies to serogroup 0:2 infection in Norwegian goats while Fukushima et al, (1984a) studied the antibody response in pigs experimentally infected with Y. enterocolitica.

In Nigeria, Adesiyun et al. (1986b) reported that 36% of 688 animals tested were seropositive for antibodies against either serogroups 0:3, 0:8 or 0:12,26 using a cut-off point for definition of seropositive of 1/40. The highest antibody prevalence was detected in polo horses (67.5%) and the lowest in pigeons (6.7%).

3. MATERIALS AND METHODS

3.1 Isolation of Yersinia enterocolitica from chickens

(a) Source of samples

A total of eight hundred cloacal swabs were collected from live chickens from three different management systems defined as follows:

Extensive (Free-range) system: owned by a family, predominantly consisting of local breed of chickens. They are not under any form of confinement and usually roam about the environment to return at dusk.

Semi-intensive (Backyard) system: chickens were under confinement in cages. Population of less than 3,000 chickens was classified in this group.

Intensive management system: chickens were under confinement in deep litter or cages. Population above 5,000.

(b) Collection of samples

Cloacal swab samples were obtained by inserting a sterile swab soaked in sterile M/15 phosphate buffered saline (PBS), pH 7.6 into the cloaca of each chicken, rotating it to collect the faecal material and returning it into 5mls of sterile PBS. The samples were taken to the laboratory ice-cooled for cold enrichment in PBS at $4^{\circ}C$ for 3 weeks as described by Schiemann (1980).

(c) Plating Procedure

All enriched samples were plated on both desoxycholate citrate agar (DCA) and MacConkey agar (MCA) after one week and three weeks. Inoculated plates were incubated at room temperature for 24-48hrs after which non-lactose fermenting

colonies were carefully picked for further identification.

3.2 Identification of Y. enterocolitica isolates

All tiny non-lactose fermenting colonies were inoculated into Triple Sugar Iron (TSI) and Kliger Iron Agar (KIA) by streaking the slant and stabbing the butt. All isolates that gave acid/acid (A/A) with or without gass and without hydrogen sulphide production on TSI and alkaline/acid (K/A) with or without gas and without hydrogen sulphide production on KIA were inoculated onto urea agar slants to demonstrate the ability of the organism to produce urease enzymes capable of splitting the urea. Isolates that were urease-positive were subjected to phenylalanine deaminase activity test, motility at 25°C but not very motile or non-motile at 37°C were presumptively identified as Yersinia spp. These isolates were further confirmed using the following available biochemical tests - lysine decarboxylase at 37°C, arginine dihydrolase (37°C), ornithine decarboxylase (37°C), indole activity at 25°C and 37°C, methyl red and Voges-Proskauer at 25°C and 37°C, hydrogen sulphide production, citrate utilization, fermentation of rhamnose, mannitol, lactose, dulcitol, glucose and sucrose. All these were determined using the method described by Bercovier et al., (1978).

3.3. Serotype, Biotype and Phage type determination

Morphologically and biochemically suspect Y. enterocolitica strains were sent to Prof. H. H. Mollaret of the Pasteur Institute, Paris for confirmation, serotyping, biotyping and phage typing.

3.4 Determination of the antibiograms of Y. enterocolitica strains by in-vitro methods

(i) Using the Disc diffusion method

The antimicrobial discs (DIFCO and OXOID) and their concentrations used were as follows:

tetracycline (30 ug), penicillin G (10 units), neomycin (30 ug), gentamicin (10 ug), ampicillin (10 ug), erythromycin (15 ug), methicillin (5 ug), kanamycin (30 ug), streptomycin (10 ug), chloramphenicol (30 ug), and polymixin B (300 units).

A modification of the disc diffusion method of Bauer et al. (1966) was used. The organisms were plated on DCA for isolation from where some colonies were inoculated in sterile tryptic soy broth and incubated at 25°C for 6hrs. Sterile cotton swabs on wooden applicator sticks were dipped into the standardized suspension of the isolates and streaked evenly over the entire surface of Mueller-Hinton agar plates. The plates were allowed to dry and the discs placed on the surface of the plates with sterile forceps. Five to six antimicrobial discs were placed on each plate and the plates were incubated at room temperature for 24 hours. The diameter of the zones of inhibition surrounding each disc to the nearest millimeter were measured with a ruler and the results recorded as sensitive or resistant by referring to the interpretative chart of zone size with the FDA standardized Disc method (Wick et al., 1971).

(ii) Using the Plate Method

The plate method was used to determine the minimal inhibitory concentrations of eleven antimicrobial agents to the five strains of Y. enterocolitica and a [illegible] reference strains of Escherichia coli (K12). The

gentamicin, colistin, cloxacillin, erythromycin, streptomycin, clindomycin, lincomycin, nalidixic acid and sulphamethoxazole - trimethoprim.

The plate dilution method on Mueller Hinton agar using two-fold serial dilutions of each antimicrobial agent as described by Ericson and Sherris (1971) was used with some modification. The procedure is as shown on the table below:

Antibiotic solution		+ Sterile water	= Intermediate concentration		Final concentration at 1:10 agar plates
Volume	ug/ml or I.U/ml	Volume	ug or I.U/ml	ug or I.U/ml	Log concentration
6.4	2,000	3.6	1280	128	7
2	1,280 (from above)	2	640	64	6
1	1,280 "	3	320	32	5
1	1,280 "	7	160	16	4
2	160 "	2	80	8	3
1	160 "	3	40	4	2
1	160 "	7	20	2	1
2	20 "	2	10	1	0
1	20 "	3	5	0.5	-1
1	20 "	7	2.5	0.25	-2

Two millilitres of the appropriate intermediate concentrations were added to 18mls of the molten Mueller Hinton agar to obtain the final concentrations in the plates. The test organisms were streaked on tryptic soy agar plates, then

subcultured into tryptic soy broth incubated for 24 hrs at room temperature. A 10^{-4} dilution of the growth of each isolate was made in sterile distilled water from which inocula consisting of 0.025ml were delivered with an Eppendorf pipette onto the antimicrobial agent containing plates. Tryptic soy agar plates without antimicrobial agents were inoculated with the test organisms and used as controls. All plates were incubated overnight at room temperature after which they were read and the minimum inhibitory concentration (MIC) was the least antimicrobial concentration that yielded no visible growth.

3.5 Assay for virulence markers

(i) Calcium dependency for growth at 37°C

A procedure similar to that described by Agbonlahor (1986) was used. To prepare magnesium oxalate agar, 2.68g of sodium oxalate and 4.067g of magnesium chloride were added to 1 litre of tryptic soy agar (TSA), The final pH was adjusted to 7.6.

Cultures of Y. enterocolitica were streaked on deoxycholate citrate agar and incubated for 24 hours at room temperature to obtain discrete colonies. Five individual colonies of each isolate were picked into 2ml of phosphate buffered saline and then diluted 10^{-2}. The resulting cell suspensions served as inocula. Two plates comprising magnesium oxalate agar (MOA) and tryptic soy agar (TSA) were used for each isolate. Five drops of each of the cell suspensions were spotted onto five separate areas using sterile Pasteur pipettes and the plates were allowed to dry. The plates were, thereafter, inverted and incubated at 37°C for 24 hours.

Growth on TSA without growth on MOA or heavy growth on TSA and few colonies on MOA was taken as evidence that the particular colony selected from the deoxycholate citrate agar (DCA) plates was calcium dependent for growth at 37°C.

(ii) Autoagglutination test at 37°C

A slight modification of the procedure described by Agbonlahor (1986) was used. Isolated colonies of Y. enterocolitica cultures on DCA plates after overnight incubation at 25°C were inoculated into a pair of duplicate tubes each containing 5mls of methyl red-Voges-Proskauer broth. A set of tubes was incubated at 37°C and the other was incubated at room temperature overnight. Uninoculated sterile MR-VP broth served as a negative control. The organisms was considered autoagglutination positive when the tubes at 37°C showed aggregated cells along the lower wall and bottom of the tube with a nearly clear medium above and when the tube at 25°C showed nearly uniform turbidity.

(iii) Séreny test for invasiveness

The ability of Y. enterocolitica strains to invade tissues were determined using the method described by Mosimabale and Gyles (1982). Each isolate was grown on tryptic soy broth and incubated at room temperature for 24hrs after which o.3ml of each was inoculated onto tryptic soy agar plates in duplicate to achieve confluent growth. The plates were incubated at room temperature for 24hrs and then harvested in sterile normal saline. The cell suspensions were washed twice in saline through centrifugation.

The saline suspensions of the organisms were adjusted through dilution to contain 4.26×10^{11} cfu/ml. Drops of each of these suspensions were put under the upper and lower lids of an eye of each guinea-pig. One eye each of two different guinea-pigs were inoculated with each isolate. Two negative controls - Y. enterocolitica serogroup 0:3 and normal saline were included for comparison. The test was conducted on different sets of guinea-pigs at two different times. The guinea-pigs were examined daily for five days for evidence of keratoconjunctivitis as shown by ocular discharges, matted hair around eye lids or frank opacity of the cornea.

(iv) Heat-stable (ST) enterotoxin

A slight modification of the procedure described by Mosimabale and Gyles (1982) was used. Twenty millilitre of tryptic soy broth (TSB) were each dispensed into 250ml Erlenmeyer flasks and sterilized at 121°C for 15 mins. The broth was supplemented with millipore-filtered 0.6% yeast extract. Overnight growth of test cultures and controls in TSB at room temperature served as inocula. Flasks containing inoculated TSB were incubated on a gyrator shaker (250rpm) for 18 hours at 25°C in a chamber with temperature regulation. Growth was then spun down in a refrigerated centrifuge (4°C) for 20 mins at 17,000 x g and the supernatant millipore-filtered (0.45 μ). The supernatant was subsequently heated for 30 mins at 65°C in a water bath, cooled and frozen at -20°C till needed.

(a) Detection of ST enterotoxin using the Rabbit ileal loop test

A slight modification of the protocol described by Robins-Browne et al. (1979) was used to detect the presence of ST enterotoxin in culture filtrates. Adult rabbit was anaesthesized using 1ml of sodium pentobarbitone administered intracardially. An incision was made along the linea alba wide enough to expose most of gastro-intestinal tract. The ileum was isolated and cleaned with sterile PBS, pH 7.6. Starting at the ileo-caecal junction, six loops were made measuring 5-8cm in length by ligation using surgical silk. The first five loops were inoculated with 1ml of filtrate of the test cultures (i.e. one culture per loop) using a hypodermic syringes with fine needles (22G). One millilitre of sterile saline was inoculated into the sixth loop to serve as negative control. Thereafter, the loops were carefully returned into the abdominal cavity and the line of incision sutured up using cat gut material for the subcutaneous tissue and non-absorbable silk for the skin. The rabbit was kept at room temperature for 6hrs. It was then sacrificed and the gut exposed. The volume of fluid which accumulated in each loop was accurately measured and the length of the loop measured. Results were expressed as the ratio of volume (ml) to length (cm). A ratio of 1.0 or greater was considered as positive as suggested by Guerrant et al, (1978).

(b) Detection of ST enterotoxin using the infant mouse system (IMS)

The procedure of Nunes and Ricciardi (1981) was used. Heat-stable enterotoxin was detected in culture filtrates earlier prepared by adding a drop of 1% Evans blue dye in normal saline to approximately

6mls of supernatant. Each member of a group of three 2-5 day old suckling mice which were fed with milk (to make stomach visible) was injected with 0.1ml of the coloured supernatant of each isolate. The mice were held at room temperature (25°C) for 4 hrs and then sacrificed using chloroform. The intestines of the three mice per group were removed, pooled and weighed after the body weight had been determined. The ratio of the gut weight values of 0.085 or greater were considered as positive as recommended by Gyles (1979).

3.6 Determination of Prevalence of Y. enterocolitica antibody in chickens

(i) Source of samples

A total of 800 serum samples were collected from slaughtered chickens. Four hundred samples were obtained from those kept under intensive management system and the remaining 400 from Sabon Gari and Samaru markets in Zaria.

(ii) Collection of samples

Blood samples from the slaughtered chickens were collected into sterile test tubes, left to clot and sera collected were stored at -20°C until needed.

(iii) Preparation of agglutinating antigens

Strains of Y. enterocolitica serotypes 0:3, 0:5,27, 0:8, 0:9 kindly provided by Dr. Devenish of Ontario, Canada and 0:12,26 previously isolated from slaughtered cattle around Zaria (Agbonlahor et al., 1986) were used. The first four serotypes were chosen because they are known human pathogens while serotype 0:12,26 was the predominant serotype isolated from cattle and pigs in Zaria area.

Pure discrete colonies of each of the cultures were inoculated into 3 mls of tryptic soy broth and incubated for 24 hrs at room temperature. Three hundred microlitre culture growth of each serotype was inoculated onto five thick blood agar plates and incubated at room temperature for 48 hrs (to achieve very confluent growth). The organisms were then harvested with sterile normal physiological saline into sterile test tube and washed twice in saline. The washed suspension were then steamed at 100°C for 2½ hrs after which they were again washed twice in buffered formol saline (BFS). These were then diluted in BFS to give a final concentration similar to the standard of 10^9 cfu/ml of E. coli. The suspensions were kept at 4°C for use.

To prepare BFS, a buffer composed of 1.44g of NaH_2PO_4, 6.25g of Na_2HPO_4 anhydrous dissolved in 50mls of distilled water was made. Stock BFS solution was prepared by adding 1ml of formalin (40% Formaldehyde) to 0.5ml of the buffer and made up to 50ml with sterile physiological saline. The stock BFS was thereafter diluted 1:8 with saline to give the washing BFS used above.

(iv) Tube Agglutination Test

All serum samples were first screened at 1:20 dilution made by adding 0.1ml of each serum to 1.9ml of BFS and mixed properly. Subsequently 0.05ml of antigen was added. Each serum samples was screened for antibodies to serotypes 0:5,27, 0:3, 0:9, 0:8 and 0:12,26 using tube agglutination test.

Dilutions of all tentative positive serum samples were done at 1:20 by adding 0.1ml of each serum sample to 1.9mls of BFS in different tubes. One millilitre aliquots of BFS was pipetted to a set of different round bottom tubes used for the experiment. The tubes were arranged in rows to give dilutions 1:40 to 1:2560. 1ml aliquots of 1:20 serum dilution were transfered to the first row of tubes containing BFS to give 1:40 dilution. This was mixed properly and 1ml aliquots transfered till the last 1ml was discarded after the last dilution tube. To each tube was added 0.05ml of the standardized concentration of antigen suspension. The tubes were shaken properly and incubated at 37°C for 2 hrs after which they were left overnight at 4°C. The tubes were subsequently left on the bench at room temperature for 2hrs after which each tube was gently shaken and examined under flourescent light for agglutination. The last tube that showed fine clumps was taken as the end point titre and a titre of 1:40 and above was considered as positive. The same procedure was repeated for the different antigens. For every test run, positive control tubes containing specific antisera to serotype kindly provided by Prof. G. Wauters of Belgium and negative controls tubes made up of the antigen and BFS were used.

Screening for Brucella abortus antibodies

All serum samples were screened for Brucella abortus agglutinins by the rose bengal plate agglutination test (RBPT). The RBPT-positive sera were further subjected to the standard tube agglutination test (S.A.T.) as described by Morgan et al. (1971). All samples positive for Brucella abortus

agglutinins by S.A.T. were eleminated from the study.

3.7 Experimental Inoculation of Broiler Chickens with strains of Yersinia enterocolitica

(a) Source of chickens

One hundred and fifty day-old starbro-broiler chicks bought from a commercial hatchery in Zaria were used. The chicks were immediately transported to the animal room of the department of Veterinary Public Health and Preventive Medicine and kept in well-heated and illuminated partially open box. Commercial feeds and clean water were supplied ad libidum. All chicks to be inoculated with Y. enterocolitica orally were so exposed within 2hrs of arrival in the laboratory.

The study on experimental inoculation was conducted in two phases, the first phase involved oral exposure of strains of Y. enterocolitica to 70 chicks at day-old while another group of 80 chicks were kept till they were 3 weeks old when some were similarly inoculated orally in phase II.

(b) Growth of Strains of Y. enterocolitica used in experimental inoculation of chicks

Three strains of Y. enterocolitica serotypes 0:3, 0:8 and 0:9 amongst the five earlier used to detect antibodies to Y. enterocolitica in chickens were used. These three serotypes have been shown to be human pathogens. Each culture was grown in five tryptic soy agar (TSA) plates for 48hrs at room temperature after which they were harvested, washed twice in saline through centrifugation at 3,000rpm for 10mins and diluted in PBS to give a standard concentration equivalent to

10^9 cfu/ml of E. coli. These suspensions were subsequently plated on DCA after serial dilutions to determine the actual number of viable Y. enterocolitica cells. A portion of these cultures suspensions was used immediately for the phase I study and the other portion kept at $-20^{\circ}C$ for the second phase of the study.

(i) Phase I of Experimental Inoculation of Chicks with Y. enterocolitica

Primarily, this aspect of the study was designed to investigate:

The degree of colonization of the intestine of day-old-chicks by Y. enterocolitica, if any; production of diarrhoea or other side effects in exposed chicks; the possible effect of strain variation on ability of Y. enterocolitica to clonize the intestine of day old chicks; the possible effect of maternal immunity on colonization; the effect of administration of 10% sodium bicarbonate on colonization; effect of Y. enterocolitica on weight gain of chicks. Seventy day-old chicks were randomly distributed into seven experimental groups of 10 chicks each and inoculated as shown in Table 3.

Each group was inoculated orally with one millilitre of cell suspension and 3 drops of 10% sodium bicarbonate ($NaHCO_3$) were administered orally to chicks in experimental groups to be so treated immediately following administration of 0.1ml. of cell suspensions.

To determine the effect of infection on weight gain, immediately after inoculation, all chicks were weighed and this exercise was repeated daily up to 13 days post-inoculation. The weight gain by chickens over the study period was subjected to statistical analysis (Colton, 1974).

To investigate the frequency of excretion of Y. enterocolitica in faeces of infected chicks, approximately 50% of chicks in each experimental group was randomly selected and cloacal swabs obtained from each daily for 5 days and then on day 8, 10 and 13 post inoculation. The cloacal swabs were first streaked directly on DCA for isolation. In addition, the cloacal swabs were enriched in M/15 PBS for 3 weeks at 4°C. For quantitative enumeration of Y. enterocolitica following direct plating of swabs on DCA, the plates were incubated at room temperature for 48hrs. Thereafter, the number of Y. enterocolitica present were counted after testing representative colonies with specific antisera. Scores were then awarded to average counts based on the following criteria:

Score 0 = No growth
1 = 1-9 colony forming units
2 = 10-19 colony forming units
3 = 20-29 colony forming units
4 = 30-39 colony forming units
5 = 40-49 colony forming units
6 = $\geq$ 50 colony forming units

In addition to taking cloacal swabs, all chicks were examined for evidence of diarrhoea or loose faeces till day 13 post-inoculation.

Enriched cloacal swabs plated out after 1 and 3 weeks as earlier described. Results on Y. enterocolitica detection were qualitatively reported as presence or absence.

For the study on histopathological evidence of colonization of infected chicks immediately after oral inoculation, and daily thereafter till day 5, one randomly picked chick from each group was sacrificed. Sections of the ileum, approximately 10cm from the ileo-caecal junction and the caecum were removed and immediately fixed in 10% formalin in saline for histopathological study. Each section in duplicate, was processed and stained in haematoxylin and eosin and Gram stains.

The slides were subsequently examined. On day 13 post-inoculation, two randomly selected chicks were slaughtered and samples were collected for cultural studies (quantitative and qualitiative) and histopathological studies as earlier described. In addition, the number of Y. enterocolitica per gram of caecal material was determined on DCA.

The remaining two chicks per groups were reared and the cloacal swabs samples were taken on days 35, 49 and 70 post-inoculation and enriched in PBS for 3 weeks as earlier described after which they were subcultured onto DCA for detection of Y. enterocolitica. Also, on these days, blood was collected from their wing vein and the serum obtained used for serological study to detect antibodies to the serotype administered (see Appendix I for details).

(ii) Phase II of Experimental Inoculation of 3 week-old chicks with Y. enterocolitica

In addition to the stated objective of phase I, this aspect of the study was to investigate:

The possible effect of native gut microflora on colonization by Y. enterocolitica; the appearance and decay of antibody titre to inoculated serotypes; the duration of excretion of Y. enterocolitica in faeces; the localization of Y. enterocolitica in organs of infected chicks.

A total of 50 three-week old broiler chickens earlier kept in separate rooms from those infected with Y. enterocolitica (Phase I) were used. The procedure for growth of organism, administration of culture suspensions were as described in Phase I. The serotypes of Y. enterocolitica, dose administered with or without prior oral inoculation of 10% $NaHCO_3$ and number of chickens per experimental group are shown in Table 4.

A procedure similar to that used for Phase I day-old chicks between days 0 and 5 were also used here, but for the fact that 3 week-old chickens were used here (see Appendix 2). Cloacal swabs were also obtained on days 14 and 28 for quantitative and qualitative (after enrichment) detection of Y. enterocolitica and on day 49 post inoculation (i.e. 70 day-old chickens), when cloacal swab was collected prior to slaughter and enrichment in M/15 PBS prior to enrichment at $4^{o}C$ for 3 weeks. Also on days 1, 3, 5, 14 and 49 post inoculation, the number of Y. enterocolitica per gram of caecal contents were determined. To determine antibody titre in sera, chickens were bled from the wing vein

on days 14, 28 and 49 post-inoculation. At the termination of the experiment on day 49 post-inoculation, remaining chickens were sacrificed and sections of the spleen, liver, bile and heart were taken and cut into pieces before being enriched in M/15 PBS at 4°C for 3 weeks. Chickens left from phase I (day 70 post inoculation, at day-old) were similarly treated.

(iii) Experimental Inoculation of Chickens with *Y. enterocolitica* by the intravenous route

Growth of Organisms

Yersinia enterocolitica serotypes 0:9, 0:3 and 0:8 were used in this study. Overnight growth of each culture in TSB were seeded onto three plates of TSA each and incubated at 25°C for 24hrs. Cells were harvested in saline and washed three times through centrifugation. Cell suspensions were finally suspended in saline and the optical density at 540nm adjusted to same value through dilution. The number of *Y. enterocolitica* per ml of cell suspension which was used as inoculum was enumerated on DCA.

Inoculation of chickens

Three-week old chickens previously not exposed to *Y. enterocolitica* were used. Hundred microlitres (100 µl) of cell suspension of each of the three serotypes (0:3; 0:8 and 0:9) was injected intravenously by the wing vein into each of 3 chickens per experimental group. The number of yersiniae administered to each chicken was enumerated on DCA and determined to be 2.1×10^{10} cfu for 0:9, 1.8×10^{10} cfu for 0:8 and 2.0×10^{10} cfu for 0:3. Three chickens served as uninoculated controls.

Prior to exposure, the cloacal temperatures of each chicken per group was determined and thereafter injection, they were all regularly examined up till 48 hrs specifically looking for diarrhoea, disposition, rise in cloacal temperature and death. In addition, cloacal swabs were taken and plated directly on DCA to detect Yersinia enterocolitica.

Gross pathological examination was performed on all chickens that died within 48 hrs. post inoculation and those slaughtered on termination of study. In addition, sections of the ileum of the chickens were studied for histopathological changes after H & E and Gram's staining.

3.8 In vitro effect of temperature on survival and multiplication of three serotypes of Yersinia enterocolitica

Yersinia enterocolitica serotypes 0:3, 0:8 and 0:9 were plated out for isolation on DCA after which they were incubated overnight at room temperature. Four plates of tryptic soy agar per isolate were seeded with each serotype and incubated for 24 hrs at room temperature. Cells were then harvested with saline and washed twice using centrifugation. The cells were resuspended in 5ml of saline and the OD_{540} of 1:1000 dilution determined. The concentrations of cell suspensions were then adjusted to same value through dilution before making a 1:100 dilution of each suspension in PBS.

Twenty millilitres of PBS (pH 7.6) was inoculated with 0.2mls of each cell suspension and serial dilutions were immediately made and plated on DCA. For each serotype, two 10ml portions of inoculated PBS were put in universal bottles with one incubated

at room temperature (25°C) and the other at 41.8°C (mean body temperature of chickens used in the study). At 0, 4, 8 and 24hrs post inoculation respectively, 0.1ml portions were removed for plating. Enumeration of colonies on DCA plates was done after incubation at room temperature for 48hrs, following confirmation of representative colonies as Y. enterocolitica using specific antisera. The possible effect of the body temperature of chickens on survival and multiplication of strains of Y. enterocolitica was therefore assessed when compared with values of samples incubated at room temperature.

4. RESULTS

1. Frequency of Isolation and Characteristics of Y. enterocolitica isolates

A total of five isolates of Y. enterocolitica representing an isolation rate of 0.63% were obtained from 800 cloacal swab samples of chickens all of which were from chickens under extensive management (Table 5).

Table 6 shows the biochemical reactions of the five isolates. All the five isolates exhibited acid/acid on TSI slant and alkaline/acid on KIA slant. None of them produced H_2S on these slants. All the isolates were motile at 25°C and all except one at 37°C. All five isolates were positive for methyl red test at 25°C and 37°C. Three of the isolates (ZB3, ZB19 and ZM7) were positive for Voges - Proskauer test at 25°C and two (ZB19 and ZM7) positive at 37°C. They were all positive for citrate production and negative for lysine decarboxylase test. Three isolates (ZB3, ZB19, and ZM7) were positive for arginine dihydrolase while all were positive for ornithine decarboxylase. All the isolates utilized glucose, sucrose, mannitol, dulcitol and rhamnose fermentatively.

All five strains of Y. enterocolitica isolated were classified as belonging to chemiotype (biotype) 3, serotype 0:12,25 and lysotype (phage-type) Xz (Table 7).

II. Antibiogram Studies

(i) Antimicrobial susceptibility testing by the disc method

The results of the antimicrobial susceptibility test by the disc diffusion method are shown in Table 8. All the five isolates were susceptible to neomycin, gentamicin, kanamycin and polymixin B. Four (80%) isolates were susceptible to streptomycin whereas three (60%) were susceptible to tetracycline and chloramphenicol. One (20%) isolate was susceptible to ampicillin. All the five isolates were resistant to penicillin G, erythromycin and methicillin.

(ii) Minimal inhibitory concentration (M.I.C.)

The minimal inhibitory concentrations of the five isolates and a reference E. coli strain against 11 antimicrobial agents, using agar dilution method are shown in Table 9. Four (80%) of 5 strains of Y. enterocolitica tested were susceptible to gentamicin and nalidixic acid. Three of 5 strains of Y. enterocolitica were susceptible to tetracycline, while only 2 of Y. enterocolitica isolates were sensitive to colistin sulphate, streptomycin and sulphamethoxazole - Trimethoprin (SxT). Only a strain (ZB3) was susceptible to cloxacillin. All the five isolates were resistant to ampicillin, erythromycin, lincomycin at M.I.C. of 128 ug/ml except for ZB3 (32 ug/ml), ZM7 (64 ug/ml) and ZM12 (64 ug/ml) with ampicillin.

(iii) Virulence Markers

Isolate ZM12 was positive for calcium dependency test at 37°C, autoagglutination test at 37°C, Sereny test and infant mouse test. ZB3 was positive for Sereny and infant mouse tests.

ZB19 and ZA8 were positive for infant mouse test only. ZH7 was negative in the tests for virulence markers. No isolate was positive in the rabbit ileal loop test (Table 10).

IV. Prevalence of Yersinia enterocolitica antibodies in chickens kept under different management systems

The overall antibody prevalence rate was 1.9% amongst 800 chickens screened for serotypes 0:3, 0:8, 0:9, 0:12,26 and 0:5,27 (Table 11). Six (1.5%) of 400 and 9 (2.3%) of 400 chickens kept under the extensive and intensive management systems respectively were sero-positive. The difference was not statistically significant ($P > 0.05$; X^2).

The prevalence of antibodies to serogroups 0:3, 0:8, 0:9, 0:12,26 and 0:5,27 in chickens is shown in Table 11. All 800 chickens tested were seronegative for antibodies against serotypes 0:5,27, 0:8 and 0:12,26. Four (0.5%) chickens were sero-positive for 0:3 antibodies while 11(1.4%) had antibodies against serotype 0:9. The difference in seropositivity rates between the extensive and intensive management systems was not statistically significant ($P > 0.05$; X^2).

Table 12 contains the antibody titres amongst seropositive for antibodies to serotype 0:3, 3 had a titre of 1/40 while 1 was 1/80. Amongst the 0.9 seropositive chickens, 4 had a titre of 1/40, 4 with 1/80 and three had a titre of 1/160.

V. Experimental Inoculation of Yersinia enterocolitica into chickens

(a) Inoculation into day-old chickens

(i) Qualitative and Quantitative detection of Y. enterocolitica

All inoculated chickens were shedding the organism between day 1 and 5 with an apparent decline in counts per gram of faeces but were all negative at 35 days post-inoculation (Table 13).

Detection of Y. enterocolitica after enrichment showed that all inoculated chickens were negative for the organism by 35 days and remained so up to 70 days. All chickens inoculated were positive on days 8, 10 and 13. For both quantitiative and qualitative detection presence of 10% $NaHCO_3$ did not appear to have any significant effect (Table 13).

(ii) Detection of Y. enterocolitica in caecal contents of chickens inoculated at day old

Between 3.0×10^3 and 3.0×10^7 yersiniae/gm of caecal contents were detected 13-day post-inoculation. On the 70th day, all caecal content tested for Y. enterocolitica were negative (Table 14).

(iii) Detection of Y. enterocolitica in organs of chickens inoculated at day old

The liver, spleen, bile and heart of chicken inoculated orally at day-old with Y. enterocolitica were positive for the organism. For the two serotypes used, all 4 heart samples cultured, 2 samples each of spleen and liver, and

All samples of control chicken cultured were negative (Table 15).

(iv) Histopathological studies on the ileum of chickens inoculated at day-old

The histopathological studies on the ileum of chickens inoculated at day-old with Y. enterocolitica serotype 0:9 with 10% $NaHCO_3$ is shown in Table 16. There was no significant finding with the specimens stained with H & E until day 13 when there was hyperplasia of Peyer's patches in the mucosa. There were no bacteria found except at days 4 and 13 with the specimens stained by Gram's method.

(v) Antibody titres in chickens inoculated to Y. enterocolitica orally at day-old

By 35 days post-inoculation, the highest antibody titres were observed and remained so till day 70 for chickens inoculated with serotypes 0:3 and 0:9 (Table 17). For chickens inoculated with serotype 0:8, a decline in antibody titre between days 35 and 70 was evident.

(vi) Effect of inoculation of day-old chicks with Y. enterocolitica on weight gain

Fig. I shows that over a 13 day period, oral inoculation of day-old chicks with Y. enterocolitica did not have any significant effect ($P > 0.05$; X^2) on weight gain when compared with the controls. The serotype and presence or absence of 10% $NaHCO_3$ did not also have any significant effect on weight gain.

(b) Inoculation of Y. enterocolitica into chickens

(i) Quantitative and Qualitative detection of Y. enterocolitica in cloacal swabs

Table 18 shows the quantitative and qualitative detection of Y. enterocolitica in faeces of chickens inoculated orally at 3 weeks old. For all chickens exposed, Y. enterocolitica was detected quantitatively between days 1 and 5 while they were all negative when sampled on days 14 and 28. Following enrichment of samples for qualitative detection, all chickens were negative for Y. enterocolitica when sampled on days 14, 28 and 49. The presence of 10% $NaHCO_3$ did not appear to have any effect on isolation of the organism from faeces.

(ii) Detection of Y. enterocolitica in caecal contents of chickens inoculated at 3 weeks old

Between 6.0×10^5 and 7.0×10^7 yersiniae per gram of caecal contents were detected 3 days post-inoculation in chickens (Table 19). The number of Y. enterocolitica declined with time till no detection was made in any of the chickens 49 days post inoculation. The presence of $NaHCO_3$ did not appear to have any significant effect on number of Y. enterocolitica detected in caecal contents.

(iii) Detection of Y. enterocolitica in organs of chickens inoculated orally at 3 weeks old

No isolation was made from the heart in all chickens inoculated at 3 weeks old (Table 20). Only 1 chicken each had Y. enterocolitica isolated from the liver, spleen and bile.

(iv) Histopathological studies on the ileum of chickens inoculated at three weeks-old

The histopathology on the ileum of chickens inoculated at three weeks-old with Y. enterocolitica serotype 0:9 with 10% $NaHCO_3$ shows no significant findings with the H & E staining. Bacteria (Gram-negative coccobacilli) were found only on day 3 and 14 post-inoculation.

(v) Antibody titres in chickens inoculated orally with Y. enterocolitica at 3 weeks old

By 14 days post-inoculation, the antibody titres have peaked in chickens inoculated with serotypes 0:9 and 0:3 with or without 10% $NaHCO_3$ (Table 21). A two-fold decline in titre was observed in some chickens between days 14 and 49.

(vi) Effect of oral inoculation of 3-weeks old chicks with Y. enterocolitica on weight gain

Fig. 2 shows that over the 49 day study period, oral administration of Y. enterocolitica into 3-week old chicks did not significantly ($P > 0.05$; X^2) affect the weight gain when compared to control chicks. Similarly, the serotype and presence of 10% $NaHCO_3$ had no significant effect on weight gain in inoculated chicks.

(c) (i) Clinical manifestation in chickens inoculated Y. enterocolitica intravenously

The clinical picture observed in chickens intravenously (i/v) injected serotypes of Y. enterocolitica is shown in Table 22. Diarrhoea was evident in chickens injected the 3 serotypes of Y. enterocolitica within 3 hours post-inoculation.

Chickens inoculated 9.4×10^3 and 9.4×10^5 Y. enterocolitica serogroup 0:8 did not show any clinical signs. Of 3 chickens given 2.0×10^{10} cells of Y. enterocolitica serogroup 0:3, 1 died by 3 hrs post-inoculation while another died about 6hrs post-inoculation. The surviving chicken in the group and others given serogroup 0:9 and 0:8, 2.1×10^{10} and 1.8×10^{10} cells recovered from apparent sickness by 24hrs post-inoculation.

(ii) Pathological findings in chickens inoculated intravenously with Y. enterocolitica

Table 23 shows gross and histopathological findings in chickens inoculated with Y. enterocolitica serogroup 0:9, 0:8 and 0:3. The spleen, liver, lungs and kidney showed pathological conditions in all inoculated chickens. The ileum of all inoculated chickens showed no significant histopathological lesions.

(d) In vitro effect of temperature on survival and multiplication of Y. enterocolitica

Regardless of the serotype used at 25°C a 10 to 100 fold increase in Y. enterocolitica population was observed within 24 hrs (Table 24). For serogroups 0:3 and 0:9, counts, however, declined from 5.1×10^6 to 10^2 and 3.6×10^6 to 10^2 respectively between 0hr and 24hrs at 41.8°C. With YE661 (serogroup 0:8) counts decreased from 9.6×10^6 (0hr) to 1.3×10^3cfu (24hrs) also at 41.8°C.

Table 1: Wauter's Biotyping Scheme of Yersinia enterocolitica (Bercovier et al., 1980a)*

Test	Biotype				
	1	2	3	4	5
Lipase	+	-	-	-	-
DNase	-	-b	-b	+	+
Indole	+	+	-	-	-
D-xylose	+	+	+	-	-b
Sucrose	+	+	+	+	v
D-Trehalose	+	+	+	+	-
No_3 reduction to No_2	+	+	+	+	-

+ = 90% or more positive between 4 and 7 days;

v = reaction varies in different biotypes;

- = less than 10% positive after 72hrs.;

b = Some strains give a delayed positive reaction (after 72hr).

Table 2: Characteristics of cultures used in experimental infection of chickens with Yersinia enterocolitica (Schiemann and Devenish, 1982)*

Culture 1 No.	Serotype	Biotype	Auto-agglu. at 37°C	Calcium dep. at 37°C	Hela[b]	Infant mouse sys.[c]	Sereny test[d]	Symptoms/Faecal Count/g in mice in days 2	5	9	14
E661	O:8	I	+	-	4.73	0.121	+/+	+/1.0x10^9 +/1.0x10^9	Dead Dead		
E674	O:3	II	-	+	4.14	0.08	-/-	-/1.0x10^3 -/1.0x10^3	-/1.0x10^3 -/1.0x10^3	-/1.0x10^3 -/1.0x10^3	-/1.0x10 -/1.0x10
E705	O:9	III	+	-	4.89	0.091	+/+	+/6.0x10^8 +/3.0x10^3	+/4.0x10^7 +/2.0x10^7	+/1.0x10^7 +/8.0x10^6	-/3.0x10^7 -/5.0x10^6

[1] All from human beings

[a] Growth on MOX agar,

[b] HeLa cell infectivity index, 3.5 considered positive.

[c] Infant mouse system, 0.083 considered positive

[d] Guinea-pig conjunctivitis in animal No. 1/animal No. 2.

[e] Data are given for animal nos. 1 & 2.

Table 2: Characteristics of cultures used in experimental infection of chickens with Yersinia enterocolitica (Schiemann and Devenish, 1982)*

Culture 1 No.	Serotype	Biotype	Auto-agglu. at 37°C	Calcium dep. at 37°C	Hela[b]	Infant mouse sys.[c]	Sereny test[d]	Symptoms/Faecal Count/g in mice in days 2	5	9	14
E661	0:8	I	+	-	4.73	0.121	+/+	+/$1.0x10^9$	Dead		
								+/$1.0x10^9$	Dead		
E674	0:3	II	-	+	4.14	0.08	-/-	-/$1.0x10^3$	-/$1.0x10^3$	-/$1.0x10^3$	-/$1.0x10^3$
								-/$1.0x10^3$	-/$1.0x10^3$	-/$1.0x10^3$	-/$1.0x10^3$
E705	0:9	III	+	-	4.89	0.091	+/+	+/$6.0x10^8$	+/$4.0x10^7$	+/$1.0x10^7$	-/$3.0x10^7$
								+/$3.0x10^3$	+/$2.0x10^7$	+/$8.0x10^6$	-/$5.0x10^6$

[1] All from human beings

[a] Growth on MOX agar, [b] HeLa cell infectivity index, 3.5 considered positive.

[c] Infant mouse system, 0.083 considered positive

[d] Guinea-pig conjunctivitis in animal No. 1/animal No. 2.

[e] Data are given for animal nos. 1 & 2.

Table 3: Experimental Protocol used for inoculating day-old chicks in Phase I study

Treatment	Dose given[a] (cells/chick)	No. of chickens
0:8 alone	1.58×10^{11}	10
0:8+10% $NaHCO_3$[b]	1.58×10^{11}	10
0:3 alone	1.35×10^{11}	10
0:3+10% $NaHCO_3$	1.35×10^{11}	10
0:9 alone	8.0×10^{10}	10
0:9+10% $NaHCO_3$	8.0×10^{10}	10
Control	Uninoculated	10

[a] In 0.1ml cell suspension of *Y. enterocolitica*

[b] 3 drops of 10% $NaHCO_3$

Table 4: Experimental Protocol used for inoculating 3 week -old chickens in Phase II study.

Treatment	Dose given[a] (cells/chicken)	No. of chickens
0:3 alone	4.1×10^9	10
0:3+10% $NaHCO_3$[b]	4.1×10^9	10
0:9 alone	7.1×10^9	10
0:9+10% $NaHCO_3$	7.1×10^9	10
Control	Uninoculated	10

[a] In 0.1ml cell suspension of Y. enterocolitica

[b] 1ml of 10% $NaHCO_3$

Table 5: Prevalence of *Yersinia enterocolitica* in chickens kept under different management systems.

Type of Management	No. of chickens sampled	No. positive for Yersinia	Percent positive for Yersinia
Extensive (free-range)	400	5	1.3
Semi-intensive	200	0	0
Intensive	200	0	0
Total	800	5	0.63

Table 6: Biochemical reactions of five Yersinia enterocolitica strains isolated from chickens in Zaria.

Characteristic[a]	Reactions by isolates				
	ZB3	ZB19	ZA8	ZM7	ZM12
Triple Sugar Iron A/A (25°C)	+	+	+	+	+
Kliger Iron Agar K/A (25°C)	+	+	+	+	+
Urease production (25°C)	+	+	+	+	+
Phenylalanine deaminase	-	-	-	-	-
Motility at 25°C	+	+	+	+	+
37°C	+	+	-	+	+
H_2S production (SIM)	-	-	-	-	-
Voges-Proskauer at 25°C	+	+	-	+	-
37°C	-	+	-	+	-
Methyl-red at 25°C	+	+	+	+	+
37°C	+	+	+	+	+
Citrate production	+	+	+	+	+
Lysine decarboxylase	-	-	-	-	-
Arginine dihydrolase	+	+	-	+	-
Ornithine decarboxylase	+	+	+	+	+
Oxidase	-	-	-	-	-
Catalase	+	+	+	+	+
Indole	-	-	-	-	-
Acid from glucose	A[b]	A	A	A	A
Sucrose	A	A	A	A	A
Mannitol	A	A	A	A	A
Dulcitol	A	A	A	A	A
Rhamnose	A	A	A	A	A

[a] Tests were conducted at 37°C unless otherwise indicated

[b] Acid production through fermentation

Table 7: Serotypes, chemiotypes and lysotypes of Y. enterocolitica isolates from chickens.

Strain Number	Source[a]	Species	Chemio-type	Serotype	Lyso-type
ZB3*	Extensive	Y. enterocolitica	3	0:12,25	Xz
ZB19*	Extensive	Y. enterocolitica	3	0:12,25	Xz
ZA8	Extensive	Y. enterocolitica	3	0:12,25	Xz
ZH7**	Extensive	Y. enterocolitica	3	0:12,25	Xz
ZH12**	Extensive	Y. enterocolitica	3	0:12,25	Xz

[a]Type of management

*Both from same flock

**Both from same flock

Table 8: Antibiograms of Yersinia enterocolitica isolates by the disc method.

Antimicrobial agent	Concentration per disc	Zone diameter (mm) sensitivity/Resistance* of isolates					No. (%) Susceptibility
		ZB3	ZB19	ZA8	ZM7	ZM12	
Tetracycline	30 ug	26(S)	22(S)	30(S)	9(R)	09(R)	3(60)
Penicillin G	10 units	0(R)	0(R)	0(R)	0(R)	0(R)	0(0)
Neomycin	30 ug	18(S)	20(S)	20(S)	18(S)	18(S)	5(100)
Gentamycin	10 ug	19(S)	17(S)	21(S)	21(S)	21(S)	5(100)
Ampicillin	10 ug	9(R)	10(R)	12(S)	0(R)	0(R)	1(20)
Erythromycin	15 ug	0(R)	0(R)	0(R)	0(R)	0(R)	0(0)
Methicillin	5 ug	0(R)	0(R)	0(R)	0(R)	0(R)	0(0)
Kanamycin	30 ug	22(S)	19(S)	24(S)	20(S)	20(S)	5(100)
Streptomycin	10 ug	15(S)	11(R)	16(S)	16(S)	16(S)	4(80)
Chloramphenicol	30 ug	24(S)	22(S)	22(S)	0(R)	0(R)	3(60)
Polymixin B	300 units	20(S)	13(S)	21(S)	14(S)	14(S)	5(100)

*R = Resistant

S = Sensitive

Table 9: Minimal inhibitory concentrations (MIC) of 11 antimicrobial agents to 5 isolates of Y. enterocolitica.

Isolate Number	Antimicrobial agents and their MIC (ug or units/ml)											No. of drugs to which isolate was resistant
	PN*	TE	G	CO	OB	E	S	CM	LM	NA	SXT	
ZB3	32R	1S**	0.25S	8S	4S	128R***	8S	128R	128R	2S	8S	4
ZB19	128R	128R	>128R	128R	8R	128R	8S	128R	128R	4S	32R	9
ZA8	128R	2S	1S	128R	128R	128R	128R	128R	128R	128R	128R	9
ZM7	64R	2S	1S	8S	16R	128R	16R	128R	128R	16S	8S	6
ZN12	64R	128R	1S	16R	16R	128R	16R	128R	128R	16S	128R	9
E. coli K12 (control)	128R	128R	2S	16R	128R	128R	128R	128R	128R	16S	128R	9
Percentage susceptibility of all isolate to:	0.0	50.0	83.3	33.3	16.7	0.0	33.3	0.0	0.0	83.3	33.3	

* Ampicillin (PN) at 10ug/ml, Tetracycline (TE) at 30ug/ml, Gentamicin (G) at 10ug/ml, Colistin sulphate (CO) at 10 ug/ml, Cloxacillin (OB) at 5 ug/ml, Erythromycin (E) at 15 ug/ml, Streptomycin (S) at 10 ug/ml, Clindamycin (CM) at 2 ug/ml, Lincomycin (LM) at 15 ug/ml, Nalidixic acid (NA) at 30 ug/ml and Sulphamethoxazole-Trimethoprim (SxT) at 23.75 ug/ml.

** Susceptible

*** Resistant

Table 10: Virulence markers of 5 strains of Y. enterocolitica isolated from chickens.

Isolate No.	Ca^{2+} dependency at 37°C	Autoagglutination at 37°C	Sereny test	ST enterotoxin detection	
				IMS*	Rabbit ileal loop
ZB3	-	-	+	+	-
ZB19	-	-	-	+	-
ZA8	-	-	-	+	-
ZM7	-	-	-	-	-
ZM12	+	+	+	+	-

*IMS = Infant mouse system

Table 11: Prevalence of antibodies to serogroups 0:3, 0:8, 0:9, 0:12,26 and 0:5,27 in chickens under two management systems.

Type of Management	No. of chickens tested	No (%) Positive[a] for serogroups:					Total
		0:3	0:5,27	0:8	0:9	0:12,26	
Extensive (Free-range)	400	3(0.8)	(0.0)	0(0.0)	3(0.8)	0(0.0)	6(1.5)
Intensive	400	1(0.3)	0(0.0)	0(0.0)	8(2.0)	0(0.0)	9(2.3)
Total	800	4(0.5)	0(0.0)	0(0.0)	11(1.4)	0(0.0)	15(1.9)

[a] A titre of 1:40 or above considered as positive.

( ) percent

Table 12: Antibody titre of seropositive[a] chickens

Type of Management	Serogroup of *Y. enterocolitica* 0:3 No. Positive	0:3 Titre[b] 40	0:3 Titre[b] 80	0:9 No. Positive	0:9 Titre[b] 40	0:9 Titre[b] 80	0:9 Titre[b] 160
Extensive (Free-range)	3	2	1	3	2	1	0
Semi-Intensive	1	1	0	8	2	3	3
Total	4	3	1	11	4	4	3

[a] All chickens tested were sero-negative for *Y. enterocolitica* serogroups 0:8, 0:5,27 and 0:12,26.

[b] Reciprocal of titres

Table 13: Quantitative and Qualitative detection of Yersinia enterocolitica in faeces of chicken[a] exposed orally to different serotypes at day-old.

Treatment	Dose administered (cells)	Quantitative detection: Scores[b] of Yersinia in faeces at days: 0	1	2	3	4	5	35	Qualitative (Enrichment) detection: Presence of Yersinia in faeces at days 8	10	13	35	49	70
0:8+$NaHCO_3$	1.58×10^{11}	0	6	3	1	1	2	0	+	+	+	-	-	-
0:8 alone	1.58×10^{11}	0	6	3	2	2	3	0	+	+	+	-	-	-
0:9+$NaHCO_3$	8.0×10^{10}	0	6	2	5	3	1	0	+	+	+	-	-	-
0:9 alone	8.0×10^{10}	0	6	2	3	4	2	0	+	+	+	-	-	-
0:3+$NaHCO_3$	1.35×10^{11}	0	6	2	2	3	2	0	+	+	+	-	-	-
0:3 alone	1.35×10^{11}	0	6	2	3	2	1	0	+	+	+	-	-	-
Control	Not infected	0	0	0	0	0	0	0	-	-	-	-	-	-

[a] Each experimental group had 10 chickens

[b] Average Score: 0 = no growth; 1 = 1-9 colonies; 3 = 20-29 colonies; 4 = 30-39 colonies
5 = 40-49 colonies; 6 = ≥50.

+ = Yersinia enterocolitica detected in enriched samples

- = Yersinia enterocolitica not detected in enriched samples.

Table 14: Quantitation of *Yersinia enterocolitica* in caecal contents of chickens[a] exposed at day old.

Treatment	Dose administered (cells)	Number of *Yersinia* per gm of caecal contents at:	
		13 days post-exposure	70 days post-exposure[b]
0:8+$NaHCO_3$	1.58×10^{11}	3.0×10^{3}	0
0:8 alone	1.58×10^{11}	2.0×10^{6}	0
0:9+$NaHCO_3$	8.0×10^{10}	3.0×10^{7}	0
0:9 alone	8.0×10^{10}	1.7×10^{7}	0
0:3+$NaHCO_3$	1.35×10^{11}	2.0×10^{7}	0
0:3 alone	1.35×10^{11}	ND	0
Control	Not infected	0	0

[a] Each experimental group had 10 chickens

[b] In addition to plating dilutions of caecal contents in PBS, caecal materials were plated directly on DCA

ND – Not done as caecum was too tiny

Table 15: Detection of Yersinia enterocolitica in organs of chickens[a] exposed orally at day-old.

Serotype administered	Identification of chickens	Isolation of Y. enterocolitica from:			
		Liver	Spleen	Bile	Heart
0:9	2	+	+	+	+
	3	-	-	-	+
0:3	4	-	-	-	+
	7	+	+	-	+
Control	0	-	-	-	-

[a]Cloacal swabs of all chickens were negative for Yersinia enterocolitica

[b]All chickens were slaughtered on day 70 post-exposure.

Table 16: Histopathological studies on the ileum of day-old chicks orally administered *Y. enterocolitica* 0:9 per Os with 10% $NaHCO_3$.

No. of Days Post-inoculation	Treatment administered: Serotype 0:9 and 10% $NaHCO_3$	
	H & E stain	Gram's stain[a]
0	NSF	NBF
1	NSF	NBF
2	NSF	NBF
3	Pronounced Peyer's Patches but NSF	NBF
4	Tips of villi had undergone coagulation necrosis. No inflammatory cells seen	Gram-negative coccobacilli in clumps in the lumen
5	NSF	NBF
13	Hyperplasia of Peyer's patches (mononuclear cellular proliferation in the mucosa	Gram-negative coccobacilli in clumps in the lumen and along the surface of the epithelium

NSF = No significant finding; NBF = No bacteria found

[a]All control chickens not inoculated had NBF and NSF throughout the duration of the study.

Table 17: Titres of Yersinia enterocolitica antibodies in chickens exposed orally at day-old.

Serotype administered	Identification of chickens	Dose of organism given (cells/ chicken)	Reciprocal of titres post-exposure on days:			
			0	35	49	70
0:9	2	8.0×10^{10}	< 20	320	320	320
	3	8.0×10^{10}	< 20	320	320	320
0:3	4	1.4×10^{11}	< 20	1280	1280	1280
	7	1.4×10^{11}	< 20	1280	1280	1280
0:8	1	1.6×10^{11}	< 20	640	640	320
	8	1.6×10^{11}	< 20	640	320	320
Control	C+0	Not exposed	< 20	< 20	< 20	< 20

Table 18: Quantitative and Qualitative detection of Yersinia enterocolitica in faeces of chickens exposed orally to different serotypes at 8-weeks old.

Treatment	Dose administered (cells)	Quantitative detection: Score[b] of Yersinia in faeces at days 0	1	2	3	4	5	14	28	Qualitative (Enrichment) detection: Presence of Yersinia in faeces at days: 14	28	49
0:9+$NaHCO_3$	7.1×10^9	0	2	3	1	6	4	0	0	-	-	-
0:9 alone	7.1×10^9	0	5	5	1	1	1	0	0	-	-	-
0:3+$NaHCO_3$	4.0×10^9	0	2	4	4	6	6	0	0	-	-	-
0:3 alone	4.0×10^9	0	5	5	5	1	1	0	0	-	-	-
Control	Not exposed	0	0	0	0	0	0	0	0	-	-	-

[a] Each treatment group had 10 chickens

[b] Average Score: 0 = no growth; 1 = 1-9 colonies; 2 = 10-19 colonies; 3 = 20-29 colonies; 4 = 30-39 colonies; 5= 40-49 colonies; 6 =≥50.

\+ = Yersinia enterocolitica detected

\- = Yersinia enterocolitica not detected

Table 19: Enumeration of Yersinia enterocolitica in caeca of chickens exposed orally at 3 weeks old.

Treatment	Dose administered (cells)	Counts per g of caecal contents post-exposure on days: 1	3	5	14	49
0:9+$NaHCO_3$	7.1×10^9	$< 10^6$	4.0×10^7	3.0×10^5	$< 10^3$	0[b]
0:9 alone	7.1×10^9	$< 10^6$	6.0×10^5	1.4×10^4	7.0×10^3	0
0:3+$NaHCO_3$[a]	4.0×10^9	$< 10^6$	4.0×10^7	4.5×10^6	$< 10^3$	0
0:3 alone	4.0×10^9	$< 10^6$	7.0×10^7	4.0×10^5	1.0×10^3	0
Control	Not exposed	0[b]	0	0	0	0

[a] 1ml of 10% $NaHCO_3$ fed

[b] Also negative for Y. enterocolitica following enrichment

Table 20: Detection[a] of Y. enterocolitica in organs of chickens exposed orally at 3 weeks old.

Serotype administered	Isolation[c] of Y. enterocolitica from:			
	Liver	Spleen	Bile	Heart
0:9 alone	-	-	+	-
0:9+$NaHCO_3$	-	+	-	-
0:3 alone[b]	+	-	-	-
Control	-	-	-	-

[a]Cloacal swabs of all chickens were negative for Y. enterocolitica

[b]Chickens administered 0:3+10% $NaHCO_3$ died before termination of experiment and carcass decomposed before detection

[c]All chickens were slaughtered on day 49 post-infection.

Table 21: Titres of Yersinia enterocolitica antibodies in chickens exposed orally at 3 weeks old

Treatment	Dose of organism given	Reciprocal of antibody titres post-exposure on days:		
		14	28	49
0:9 alone	7.1×10^9	640	640	640
0:9+$NaHCO_3$	7.1×10^9	640	320	320
0:3 alone	4.0×10^9	1280	1280	640
0:3+$NaHCO_3$	4.0×10^9	320	Died[a]	-
Control		<20	<20	<20

[a]All five chickens in the group died before 28 days post-exposure.

Table 22: Clinical manifestation in chickens intravenously exposed to *Y. enterocolitica* serogroups: 0:3, 0:9 and 0:8.

Experimental Group	Serogroup	Dose	No. of Chickens	Clinical manifestation[a] at 3hr	6hr	18hr	24hr	48hr
A	0:9	2.1×10^{10}	3	Chickens depressed and off-feed, evidence of diarrhoea. Mean temp. 42°C.	Depressed, diarrhoea present. Mean Temp. 42°C	Good appetite, still with diarrhoea. Mean temp. 42°C.	Good appetite, diarrhoea Mean temp. 42°C.	Good appetite and very acti but still with diarrheoa. Mean temp. 42°C.
B	0:8[b]	1.8×10^{10}	3	Chicks depressed and off feed. Evidence of diarrhoea. Mean temp. 41.8°C.	Depressed, fair appetite. Mean temp. 41.7°C.	Good appetite, still with diarrhoea. Mean temp. 41.9°C.	Good appetite Mean temp. 41.9°C.	Good appetite, still with diarrhoea but very active. Mean temp. 41.9°C.
C	0:3	2.0×10^{10}	3	Chicks depressed and off-feed. Evidence of diarrhoea. Mean temp. 42°C.	Depressed and off-feed. Evidence of diarrhoea. Mean temp. 42.8°C.	Apparent recovery in surviving bird. Mean temp. 42°C.	Good appetite Mean temp. 42°C.	Good appetite and active Mean temp. 42°C.
D	Control(Not exposed)		3	Looking normal Mean temp. 41.7°C	Normal Mean temp. 41.8°C	Mean temp. 41.8°C	Mean temp. 41.9	Mean temp. 41.8

[a] No deaths were recorded for chicks given *Y. enterocolitica* serogroups 0:8, 0:9 and the controls throughout the observation period. However, 2 of 3 chicks given serogroup 0:3 died by 3 hrs and 6hrs post-exposure.

[b] Doses of 9.4×10^{3} and 9.4×10^{5} cfu earlier given to a set of 5 chicks each also did not cause death. Chicks administered the higher dose were however off-feed and depressed for approximately 6 hrs.

Table 23: Experimental exposure of chickens with Yersinia enterocolitica by intravenous route: pathological findings.

Experimental Group	Sero-group	Dose(cells)	No. of chickens	Pathological[a] findings	
				Gross	Histopathological[b]
A	0:9	2.1×10^{10}	3	Liver pale and streaked. Spleen enlarged and congested. Haemorrhagic and pale kidney. Lungs congested. Slight haemorrhage in leg and thigh muscles.	NSF
B	0:8	1.8×10^{10}	3	Liver pale and slightly enlarged with necrosis. Spleen pale, enlarged and congested. Enlarged and haemorrhagic kidney. Unabsorbed yolk sac congested and coloured dirty green, slight haemorrhage on thigh and leg muscles.	NSF
C	0:3	2.0×10^{10}	3	Enlarged liver and spleen. Heart pale, covered with gelatineous material. Lungs congested. Coronary fat absent. Kidney enlarged and haemorrhagic. Bursa of fabricus enlarged with petechial haemorrhage. Evidence of enteritis.	NSF
D	Control(Not exposed)		3	No Gross pathological lesions	NSF

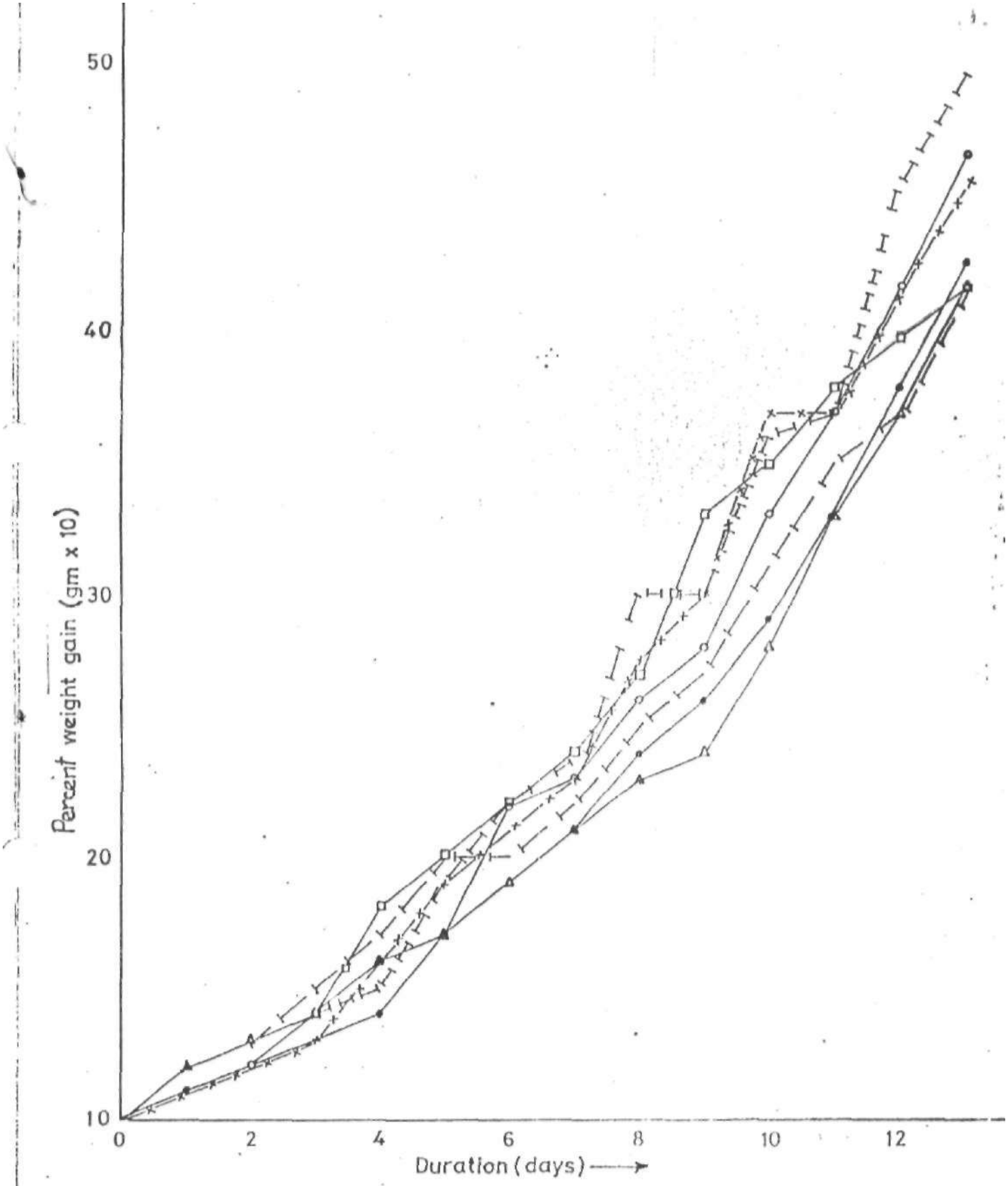
[a] Chickens that did not die from infection were sacrificed after 48hrs for gross and histopathological studies.

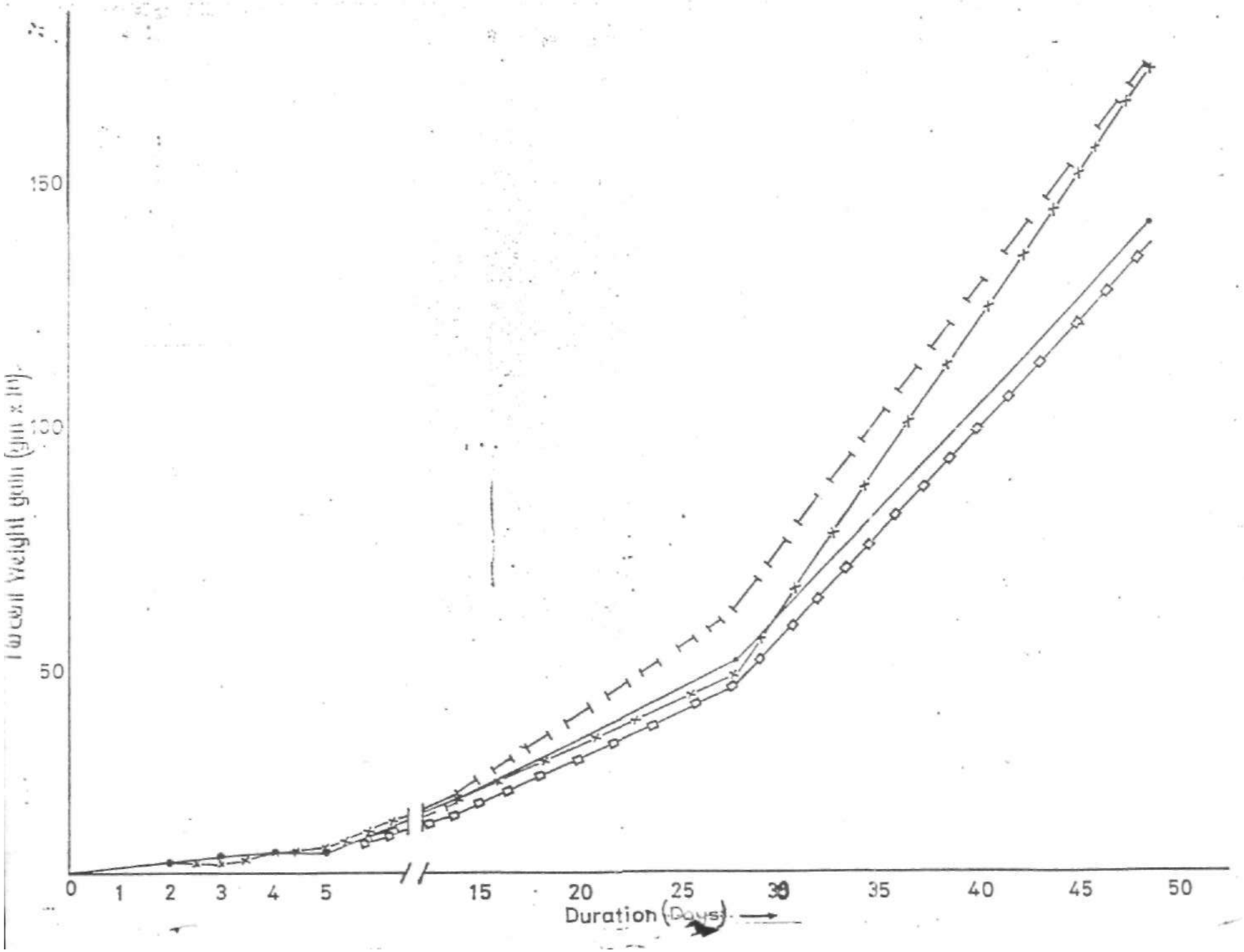
[b] Histopathological study was done only on the ileum. NSF - No significant findings. They were also all negative for Gram-negative coccobacilli.

Table 24: *In vitro* effect of temperature on survival and multiplication of three serotypes of *Yersinia enterocolitica* in phosphate buffered saline (pH 7.6).

Isolate No.	Serotype	Temp.(°C)	Counts of *Y. enterocolitica* per millilitre of PBS after: 0hr.	4hr.	8hr.	24hr.
YE674	0:3	25.0	5.1×10^6	2.5×10^7	3.0×10^7	9.8×10^7
		41.8[a]	5.1×10^6	2.8×10^4	3.3×10^3	$<10^2$
YE561	0:8	25.0	9.6×10^6	2.4×10^7	7.0×10^7	1.2×10^8
		41.8	9.6×10^6	6.3×10^6	2.5×10^6	1.3×10^3
YE705	0:9	25.0	3.6×10^6	1.5×10^7	2.0×10^7	9.0×10^7
		41.8	3.6×10^6	2.0×10^4	4.5×10^2	$<10^2$

[a]Mean body temperature of a group of chickens used in the *in vivo* study.


Percent weight gain (gm x 10)
50
40
30
20
10
0
2
4
6
8
10
12
Duration (days) ⟶

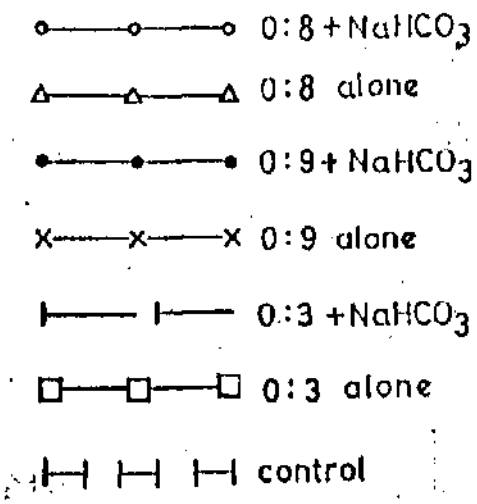

150
50
Weight gain (gm x 10)
0
1
2
3
4
5
15
20
25
30
35
40
45
50
Duration (Days)

5. DISCUSSION

The frequency (0.63%) of isolation of Y. enterocolitica in chickens in Zaria and environs as detected in this study is quite low. It is, however, in agreement with an isolation rate of 0.69% reported for Y. enterocolitica in cattle, pigs and raw milk in the same environment (Agbonlahor et al., 1986). Hitherto, there is no information in the literature on the prevalence of Y. enterocolitica infection in live chickens either in tropical and temperate countries. Generally, higher isolation rates have been reported for Y. enterocolitica in other animal species in temperate countries ranging from 20 to 80.5% in swine and cattle (Schiemann and Fleming, 1981; Shayegani et al., 1981; Fukushima et al., 1984b; Nesbakken, 1985).

The low isolation rate of the organism in chickens in a tropical environment like Nigeria is not surprising since temperature has been documented to have some effect on survival of Y. enterocolitica (Nesbakken, 1985). In fact, it was not until recently that Y. enterocolitica infections were documented in subtropical and tropical regions in human beings and animals. The isolation rates of Y. enterocolitica have been reported to be low (Samadi et al., 1982; Agbonlahon et al., 1983; 1986). In addition to the relatively high temperature in a tropical region

Fig. 1: *Percent weight gain in chickens exposed to three serotypes of Y. enterololitica at day-old.*



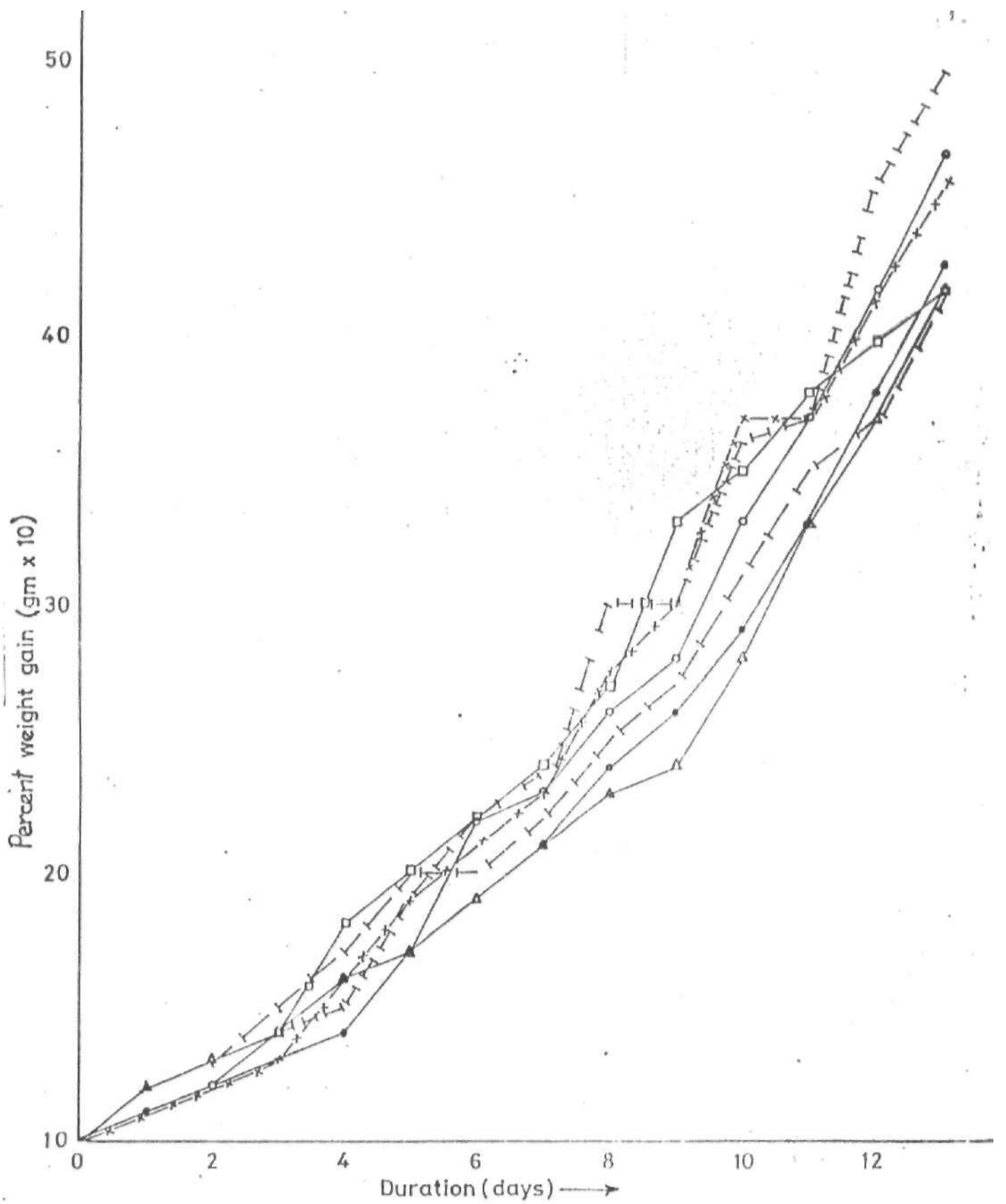
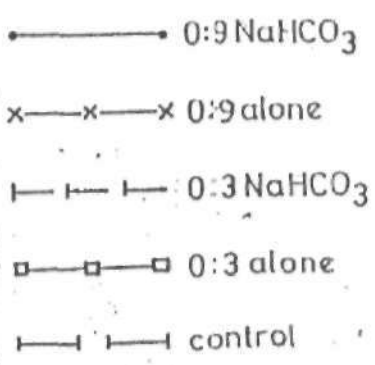
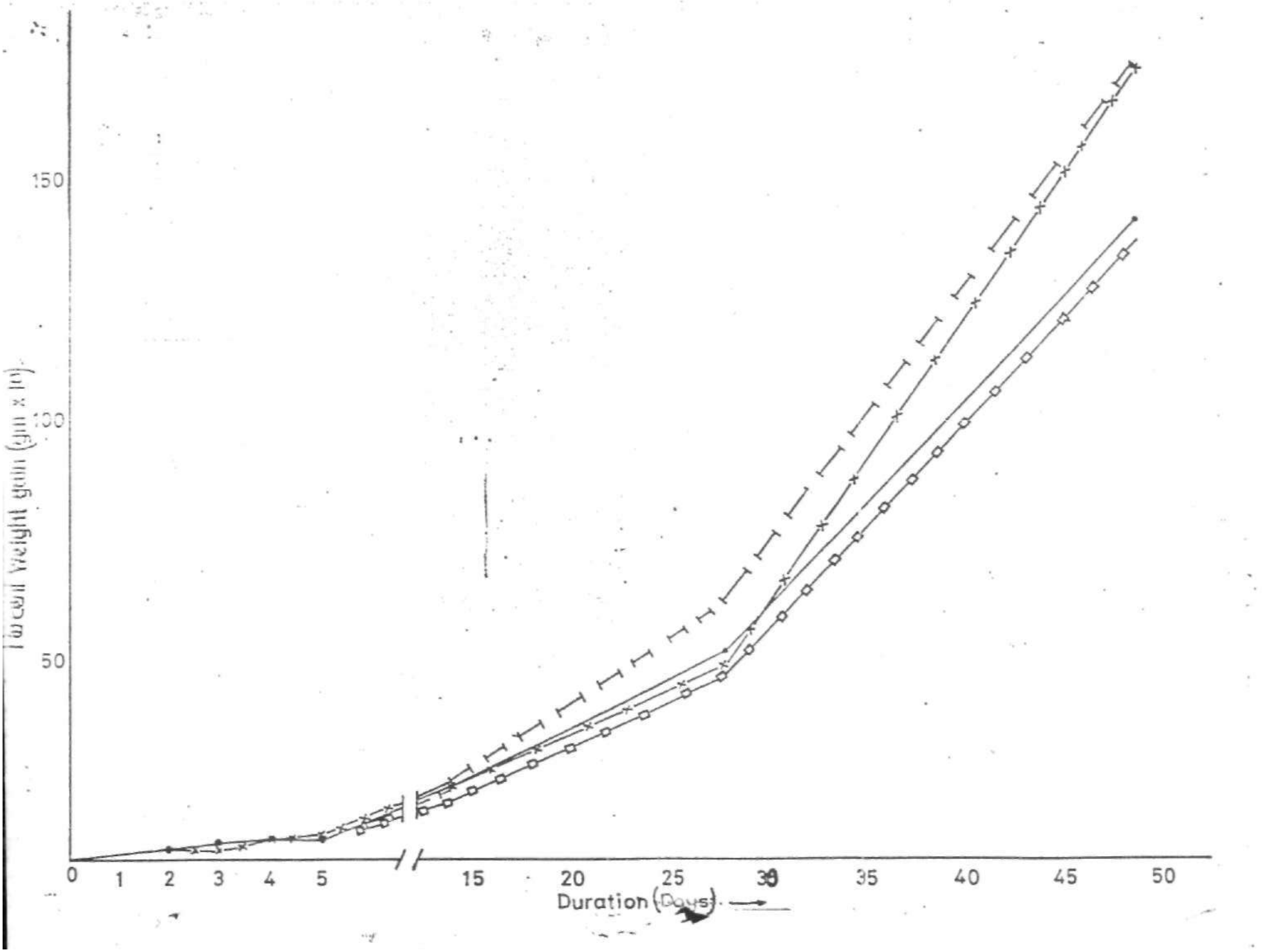

Percent weight gain (gm x 10)
50
40
30
20
10
0
2
4
6
8
10
12
Duration (days) ⟶

Fig. 2: *Percent weight gain in chickens exposed to two serotypes of Y. enterocolitica at three weeks old.*




Tadpole weight gm (gm x 10)
150
100
50
0
1
2
3
4
5
15
20
25
30
35
40
45
50
Duration (Days) →

5. DISCUSSION

The frequency (0.63%) of isolation of Y. enterocolitica in chickens in Zaria and environs as detected in this study is quite low. It is, however, in agreement with an isolation rate of 0.69% reported for Y. enterocolitica in cattle, pigs and raw milk in the same environment (Agbonlahor et al., 1986). Hitherto, there is no information in the literature on the prevalence of Y. enterocolitica infection in live chickens either in tropical and temperate countries. Generally, higher isolation rates have been reported for Y. enterocolitica in other animal species in temperate countries ranging from 20 to 80.5% in swine and cattle (Schiemann and Fleming, 1981; Shayegani et al., 1981; Fukushima et al., 1984b; Nesbakken, 1985).

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like Nigeria, the high body temperature of chickens ($104^{o}F$) is an important factor to be considered in explaining the rather low prevalence of infection.

In this study, it is noted that the role of management system on prevalence of infection by Y. enterocolitica in chickens is very important. There was no isolation of Y. enterocolitica in chickens kept in the intensive or semi-intensive management whereas five isolates of Y. enterocolitica were made from chickens kept under the free-range system of management. Free-ranging chickens are in constant contact with other livestock and human beings in the environment and therefore more likely to be exposed to Yersinia spp. Studies in the same environment (Agbonlahor et al., 1986) have documented Y. enterocolitica infection in pigs and cattle. Chickens on free-range system of management are repeatedly exposed to human, cattle and pig faeces, thus the risk of contracting the organism is high.

The finding that all five strains of Y. enterocolitica isolated from chickens are of serotype O:12,25 is of significance as this serotype had hitherto, never been isolated from any animal species in Nigeria. In earlier studies in cattle

and pigs in this environment the most commonly encountered serotype was O:12,26 (Agbonlahor et al., 1986) which is close serologically to those isolated from the present study. The significance of the phage type for all five isolates determined to be $\overline{X}_Z$ cannot be readily ascertained because all previous isolates of Y. enterocolitica reported in Nigeria were not phage-typed. The same phage type has, however, been isolated from human patients with gastroenteritis in India (Varghese et al., 1984) and from diarrhoeal faeces of humans and cat, and intestinal contents of a pig, mixed with lysotype $\overline{X}_Z$ (Pramanik et al., 1980). Further studies need to be done on isolates of Y. enterocolitica in Nigeria to be able to define the predominant phage types.

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from human patients with gastroenteritis in India (Varghese et al., 1984) and from diarrhoeal faeces of humans and cat, and intestinal contents of a pig, mixed with lysotype $\overline{X}_Z$ (Pramanik et al., 1980). Further studies need to be done on isolates of Y. enterocolitica in Nigeria to be able to define the predominant phage types.

In this study one strain, ZM12, serotype O:12,25, phage type X_Z was found to be positive for autoagglutination at 37°C, calcium dependency at 37°C, Serenytest and ST enterotoxin production. This finding agrees with earlier reports that close relationship exist between these virulence markers since their properties were reported to be encoded on the same plasmid (Gemski et al., 1980).

The infant mouse system (IMS) was found to be more sensitive in detecting ST enterotoxin that the rabbit ileal loop test. This is because four of the five strains of Y. enterocolitica were positive for ST enterotoxin by the IMS while all were negative by the rabbit ileal loop test. Adesiyun et al., (1986a) have earlier reported a similar finding on strains of Y. enterocolitica isolated from cattle and pigs in Nigeria. Although the IMS for the detection of ST enterotoxin has been shown to be a good model with excellent reproducibility (Gianella, 1976; Mosimabale and Gyles, 1982), the exact role of ST enterotoxin in Yersinia pathogenicity is not yet known but there are indications that it may play no significant role (Schiemann, 1981). The detection of ST enterotoxin from four isolates in this study by the IMS but none by the rabbit ileal loop test may indicate the involvement

of type 1 ST (Moon et al., 1970; Burgess et al., 1978). Aulisio et al. (1983) have reported the detection of ST enterotoxin production by a strain may be used in characterization but does not contribute towards pathogenicity.

The in vitro activities of different anti-microbial agents by the disc method on the five strains of Yersinia show that they were all susceptible to neomycin, gentamycin, kanamycin and polymixin B as earlier found in some other studies (Toma, 1973; Hammerberg et al., 1977; Raevuori et al., 1978; Agbonlahor and Odugbemi, 1982). Similarly, the finding that four of five strains were susceptible to streptomycin, three (60%) to tetracycline and chloramphenicol agrees with the work of others (Toma, 1973; Gutman et al., 1984). One strain of Y. enterocolitica was, however, susceptible to ampicillin, a finding at variance with the work of Toma (1973) and Kohl et al. (1976) who reported that all the Yersinia enterocolitica strains tested were resistant to the antibiotic. It was not surprising that all 5 strains tested were resistant to penicillin G, erythromycin and methicillin as found by Toma (1973) since these are routinely used on Gram-positive microorganisms.

The sensitivity patterns detected for the five strains by the disc method correlate well with those by the quantitative agar dilution method where similar antimicrobial agents were used. The only exception detected was strain ZB19 which was resistant to gentamycin (MIC>128 ug) by the agar dilution method but not by the disc procedure. The reason for this discrepancy is not obvious.

Again, the MIC for gentamycin, nalidixic acid, colistin sulphate, streptomycin and suphamethoxazole - Trimethoprim (SXT) are in consonance with published work (Hammerberg et al., 1977; Haevuori et al.,1978; Agbonlahor and Odugbemi, 1982; Kwaga et al., 1986).

Overall, the most effective antimicrobial agents against Y. enterocolitica isolates from chickens are neomycin, kanamycin, gentamicin, nalidixic acid, colistin sulphate and SXT. The limitation of the few (5) isolates studied cannot, however, be ignored. Hammerberg et al. (1977) have shown that SXT or aminoglycosides may have clinical usefulness in the therapy of Y. enterocolitica infections.

The overall prevalence of Y. enterocolitica antibodies against serotypes O:3, O:8, O:9, O:5,27 and O:12,26 of 1.9% found in chickens kept under the extensive and intensive management systems was low. This is an indication that Y. enterocolitica infection in chickens around Zaria is low.

However, a limited serological survey around Zaria was reported to detect Y. enterocolitica antibodies against serotypes O:3, O:8 and O:12,26 in 2(8.7%) of 23 free-ranging chickens in Zaria area, in 8(25.0%) of 32 free-ranging chickens in nomadic fulani settlements and in 3(7.5%) of 40 chickens kept under semi-intensive management systems (Adesiyun et al., 1986b). The discrepancy in results obtained from that study and the present one may be explained, in part, by the rather few chickens studied by Adesiyun et al. (1986b).

It is, of interest to observe that although no Y. enterocolitica strain was isolated from all chickens tested under the intensive and semi-intensive management systems, 2.3% of the chickens had antibody titres against the serotypes screened. Serological evidence of infection is suggestive of exposure experience of the agent which

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may not be current.

The finding that antibodies were detected against only two (0:3 and 0:9) of the five serotypes screened agrees in part with the work of Adesiyun et al. (1986b) where antibodies were detected only against serotype 0:3 amongst two others (0:8 and 0:12,26) screened although 0:9 was not included.

The prevalence of antibody to serotype 0:12,25, the only type isolated from chickens is not presently known because these were not determined in the present study.

Inoculation of chickens with 10^9 to 10^{11} cells of Y. enterocolitica strains known to be virulent for mice, by the oral route at day-old or 3 weeks old did not cause any overt clinical signs. Similar findings have been reported in chickens in another study (Soerjadi-liem et al., 1984).

Of economic relevance is the demonstration that, in addition to not causing overt clinical signs, infection of broiler chickens with Y. enterocolitica either at day-old or 3 weeks old had not significant effect on weight gain.

In the present study, regardless of serotype and dose of Y. enterocolitica administered orally, 10% sodium bicarbonate did not have any effect on colonization of the gut. Adesiyun et al. (1986c) had reported a similar finding in experimentally infected sheep. Fukushima et al. (1984b) have however demonstrated that 10% $NaHCO_3$ aids colonization of the pig's gut by Y. enterocolitica. Factors that must be considered in explaining this discrepancy are, the pH of the gut, the species of animal host, the amount of 10% $NaHCO_3$ administered relative to the weight of the animal and possible variation in susceptibility to colonization of the gut by serotypes of Y. enterocolitica used.

Colonization of the gut of chickens at the two age groups of inoculation (day old and 3 weeks old) appears to be minimal since the organism was only detected for a few days in cloacal swabs post-inoculation and negative thereafter throughout the investigation. The fact that Y. enterocolitica was isolated from sites like the spleen, liver, bile and heart in addition to detection of antibodies against specific serotypes of Y. enterocolitica administered suggest that some of the organism penetrated the gut mucosa to get systemic. Histopathological studies on the ileum at intervals after infection however failed to demonstrate colonization or penetration of the mucosa as Y. enterocolitica was only seen in the lumen. The abrupt cessation of Y. enterocolitica found in inoculated chickens in this study is similar to what had been demonstrated in pigs (Fukushima et al., 1984b) and sheep (Adesiyun et al., 1986C). Soerjadi-Liem et al., (1984) reported colonization of the gastrointestinal tract of newly hatched monoxenic (conventionally reared) chicks following administration of Y. enterocolitica. The authors failed to detect colonization in gut of holoxenic chicks given native microflora prior to exposure to Y. enterocolitica. In the present study, administration of Y. enterocolitica to chickens either at day old or 3 weeks old did not significantly affect duration of shedding, colonization or multiplication in the gut lumen and antibody titres in infected chickens.

The possible effect of relatively high body temperature of chickens on survival and multiplication of Y. enterocolitica was again obvious by the failure of 10^{10} cells administered intravenously

to cause acute death as earlier demonstrated in sheep (Adesiyun et al., 1986c). Although clinical signs were observed in most chickens, all inoculated chickens recovered within 24hrs but for the death of two of three chickens administered serotype 0:3. Data from in vitro study on effect of temperature on survival and multiplication of Y. enterocolitica in phosphate buffered saline (PBS) support the concept of adverse effect of body temperature since within 24hrs, the counts of all serotypes incubated at 41.8°C (mean body temperature of chicks studied) dropped significantly to less than 10^2 to 10^3 cells per ml from 10^6 while multiplication was observed in cells kept at 25°C.

6. CONCLUSION

Yersinia enterocolitica infection in chickens is for the first time documented in Nigeria. Cultural and serologic studies have demonstrated that the prevalence of Y. enterocolitica infections in chickens in Zaria and environs is low. Management system had an effect on frequency of isolation of Y. enterocolitica from chickens with all isolations made from chickens kept under the extensive (free-range) management and none from either the semi-intensive or intensive management systems although serologic evidence of infection by the organism existed.

Yersinia enterocolitica serotype O:12,25 biotype 3, phage type Xz appears to be the predominant type infecting chickens in this environment. Based on possession of virulence markers, only one of the five strains of Y. enterocolitica isolated had the potential of being pathogenic having been positive for autoagglutination at 37°C, calcium dependency at 37°C, invasiveness (Sereny test) and production of ST enterotoxin.

The isolates were susceptible to a number of commonly used antimicrobial agents. Gentamicin, neomycin, kanamycin, nalidixic acid, colistin sulphate and sulphamethoxazole - Trimethoprim (SMT) are therefore possible drugs of choice for treatment of yersiniosis.

Experimental studies on Y. enterocolitica infection in chickens demonstrated amongst other things that, colonization of the gastrointestinal tract appears minimal with shedding stopping

abruptly and of short duration. Administration of 10% $NaHCO_3$ did not aid colonization of the gut of chickens by all the strains of Y. enterocolitica used. Of economical significance was the demonstration that Y. enterocolitica infection in broiler chickens had no adverse effect on weight gain. Finally, no obvious difference was detected in responses of chickens to administration of Y. enterocolitica at day old or 3 weeks old, an indication that maternal immunity may play no significant role in Y. enterocolitica infection in chickens.

The high environmental temperature experienced in Nigeria coupled with high body temperature of chickens may have been responsible, in part for the low prevalence of Y. enterocolitica infection in chickens. Chickens may therefore not be an important source of Y. enterocolitica infection for humans and other animals in this environment.

7. REFERENCES

Adesiyun, A. A.; Bisnu, G.; Adegboye, D. S. and Abdu, P. A. (1984). Serological survey of Salmonella pullorum antibody in chickens around Zaria, Nigeria. Bulletin of Animal Health and Production, 32: 81-85.

Adesiyun, A. A.; Lombin, L. H.; Kwaga, J. K. P. and Agbonlahor, D. E. (1985). Some features of the first isolate of Yersinia enterocolitica serogoup O:8 from a piglet in Nigeria. Nigerian Journal of Microbiology, 4: 97-100.

Adesiyun, A. A.; Agbonlahor, D. E., Lombin, L. H. and Kwaga, J. K. P. (1986a). Occurrence of virulence markers in species of Yersinia isolated from animals in Nigeria. Veterinary Microbiology, 12: 289-294.

Adesiyun, A. A.; Lombin, L. H. P. and Agbonlahor, D. E. (1986b). Prevalence of antibodies to Yersinia enterocolitica serogroups O:3, O:8 and O:12,25 in domestic animals in Nigeria. British Veterinary Journal, 142: 381-388.

Adesiyun, A. A.; Lombin, L. H. and Kwaga, J. K. P. (1986c). Experimental infection of sheep with Yersinia enterocolitica: I. Clinical, cultural studies. Bulletin of Animal Health and Production (in press).

Agbonlahor, D. E.; Odugbemi, T. O. and Lasi, Q. (1981). Isolation of Yersinia enterocolitica from stools of acute gastro-enteritis cases in Lagos, Nigeria. East African Medical Journal, 58: 520-524.

Agbonlahor, D. E.; Odugbemi, T. and Dosunmu-Ogunbi, O. (1982). Differential and selective medium for isolation of Yersinia enterocolitica from stools. Journal of Clinical Microbiology, 15(4): 599-602.

Agbonlahor, D. E. and Odugbemi, T. O. (1982). Yersinia enterocolitica isolates in Lagos, Nigeria. Nigerian Medical Journal, 12(2): 175-179.

Agbonlahor, D. E., Odugbemi, T. O. and Dosunmu-Ogunbi, O. (1983). Isolation of species of Yersinia from patients with gastroenteritis in Nigeria. Journal of Medical Microbiology, 16: 93-96.

Agbonlahor, D. E. (1986). Characteristics of Yersinia intermedia-like bacteria isolated from patients with diarrhoea in Nigeria. Journal of Clinical Microbiology, 23(5): 891-896.

Agbonlahor, D. E.; Adesiyun, A. A.; Kwaga, J. K. P. and Lombin, L. H. (1986). Colonial, Biochemical and Serological Characteristics of Yersinia species isolated from animals in Nigeria. Rev. Elev. Med. Pays Tropical, 38:416-422.

Ahvonen, P.; Thal, E. and Vasenius, H. (1973). Occurrence of Yersinia enterocolitica in animals in Finland and Sweden. In: Contributions to Microbiology and Immunology, 2; Yersinia, Pasteurella and Francisella, pp. 135-136.

Aldova, E.; Cerna, J.; Janeckova, M. and Pegrimkova, J. (1975). Yersinia enterocolitica and its demonstration in foods. Czechoslovakia Journal of Hygiene, 20: 395-398.

Anjorin, F. I.; Sturrock, R. D.; Subbuswamy, S. G.; Lawande, R. V. and Fakunle, Y. M. (1979). Yersinia enterocolitica infection from West Africa - a case report. Transactions of the Royal Society of Tropical Medicine and Hygiene, 73: 634-635.

Asakawa, Y.; Akahame, S.; Kagata, N.; Noguchi, M.; Sakazaki, R. and Tamura, K. (1973). Two community outbreaks of human infection with Yersinia enterocolitica. Journal of Hygiene, 71: 715-722.

Aulisio, C. G.; Hill, W. E.; Stanfield, J. T. and Sellers, R. L. Jr. (1983). Evaluation of virulence factor testing and characterisitics of pathogenicity in Yersinia enterocolitica. Infection and Immunity, 40: 330-335.

Awunor-Renner and Lawande, R. V. (1982). Yersinia and chronic glomerulopathy in the savannah region of Nigeria. British Medical Journal, 285-1464.

Bauer, A. W.; Kirby, W. M. M.; Sherris, J. C.; and Turck, M. (1966). Antibiotic susceptibility testing by a standardized single disk method. American Journal of Clinical Pathology, 45: 493-496.

Bercovier, H.; Brault, J.; Barme, N.; Treignier, M.; Alonso, J. M. The Mollaret, H. H. (1978). Biochemical, serological and phage typing characteristics of 459 Yersinia strains isolated from a terrestrial ecosystem. Current Microbiology, 1: 353-357.

Bercovier, H.; Alonso, J. M.; Bentaiba, Z. N.; Brault, J. and Mollaret, H. H. (1979). Contribution to the definition and the taxonomy of Yersinia enterocolitica. Contributions to Microbiology and Immunology, 5: 12-22.

Bercovier, H.; Brenner, D. J.; Ursing, J.; Steigerwalt, A. G.; Fanning, G. R.; Alonso, J. M.; Carter, G. P. and Mollaret, H. H. (1980a). Characterization of Yersinia enterocolitica sensu stricto. Current Microbiology, 4: 201-206.

Bercovier, H.; Ursing, J.; Brenner, D. J.; Steigerwalt, A. G.; Fanning, G. R.; Carter, G. P. and Mollaret, H. H. (1980b). Yersinia kristensenii: a new species of Enterobacteriacea composed of sucrose - negative strains (formerly called atypical Yersinia enterocolitica or Yersinia enterocolitica-like). Current Microbiology, 4: 219-224.

Bercovier, H.; Mollaret, H. H.; Alonso, J. M.; Brault, J.; Fanning, G. R.; Steigerwalt, A. G. and Brenner, D. J. (1980c). Intra and Inter species relatedness of Yersinia pestis by DNA Hybridization and its relationship to Yersinia pseudotuberculosis. Current Microbiology, 4: 225-229.

Bercovier, H.; Brault, J.; Cohen, S.; Melis, R.; Lambert, T. and Mollaret, H. H. (1984). A new isolation medium for the recovery of Yersinia enterocolitica from environmental sources. Current Microbiology, 10: 121-124.

Bisset, M. L. (1976). Yersinia enterocolitica isolates from humans in California 1968-1975. Journal of Clinical Microbiology, 4: 281-285.

Bitzan, M.; Knapp, W.; Mauffi, G.; and Pulverer, G. (1983). Significance of Yersinia enterocolitica isolates and antibody titres. A prospective study in patients with enteritis and healthy controls under Bacteriological, Serological, Epidemiological and Clinical Aspects. Zentralbatt fur Bacteriologie Mikpobiologie Und Hygiene A, 254: 78-88.

Black, R. E.; Jackson, R. J.; Tsai, T.; Medvesky, M.; Shayegani, M.; Feeley, J. C.; Macleod, K. I. E. and Wakelee, A. M. (1978). Epidemic Yersinia enterocolitica infection due to contaminated chocolate milk. New England Journal of Medicine, 298: 76-79.

Bottone, E. J.; Chester, B.; Malowany, M.; Allerhand, J. (1974). Unusual Yersinia enterocolitica isolates not associated with mesentery lymphadenitis. Journal of Applied Microbiology, 27: 858-861.

Bottone, E. J. (1977). Yersinia enterocolitica. A panoramic view of a charismatic micro-organism Critical Review of Clinical Laboratory Science, 5: 211-241.

Bottone, E. J. (1978). Atypical Yersinia enterocolitica: clinical and epidemiological parameters. Journal of Clinical Microbiology, 7: 562-567.

Bottone, E. J. (1981). Yersinia enterocolitica: An approach to laboratory identification with reference to deoxyribonucleic acid hybridization groups and biochemical characteristics. In: Yersinia enterocolitica ed. Bottone, E. J. pp. 17 - 29. Boca Raton, Florida: CRC Press.

Boyce, J. M.; Evans, D. J. Jr.; Evans, D. G. and Dupont, H. L. (1979). Production of heat stable, methanol-soluble enterotoxin by Yersinia enterocolitica. Infection and Immunity, 25:532-537.

Brenner, D. J.; Steigerwalt, A. G., Falcao, D. P.; Weaver, R. E. and Fanning, G. R. (1976). Characterization of Yersinia enterocolitica and Yersinia pseudotuberculosis by deoxyribonucleic acid hybridization and by biochemical reaction. International Journal of Systemic Bacteriology, 26: 180-194.

Brenner, D. J. (1979). Speciation in Yersinia. In: Contributions to Microbiology and Immunology, vol. 5, S, Karger Basel, pp.33-43.

Brenner, D. J.; Ursing, J.; Bercovier, H.; Steigerwalt, A. G.; Fanning, G. R.; Alonso, J. M. and Mollaret, H. H. (1980a). Deoxyribonucleic acid relatedness in Yersinia enterocolitica-like organisms. Current Microbiology, 4: 195-200.

Brenner, D. J.; Bercovier, H.; Ursing, J.; Alonso, J. M.; Steigerwalt, A.G.; Fanning; G. R.; Carter, G. P. and Mollaret, H. H. (1980b). Yersinia intermedia: a new species of Enterobacteriaceae composed of rhamnose-positive, melibiose-positive, raffinose-positive strain (formerly called Yersinia enterocolitica-like). Current Microbiology, 4: 207-212.

Burgess, M. N., Bywater, R. J., Cowley, C. M., Mullan, N. A. and Newsome, P. M. (1978). Biological evaluation of a methanol-soluble, heat-stable Escherichia coli enterotoxin in infant mice, pigs, rabbits and calves. Infection and Immunity, 21(2): 526-531.

Butler, T.; Islam, M.; Islam, M. R.; Azad, A. K.; Huq, M. I.; Speelman, P. and Roy, S. K. (1984). Isolation of Yersinia enterocolitica and Y. intermedia from fatal cases of diarrhoeal illness in Bangladesh. Transactions of the Royal Society of Tropical Medicine and Hygiene, 78, 499-450.

Caprioli, T.; Drappeau, A. J. and Kasatiya, S. (9178). Yersinia enterocolitica: Serotypes and biotypes isolated from humans and the environment in Quebec, Canada. Journal of Clinical Microbiology, 8: 7-11.

Carlsson, H. E.; Hurvell, B. and Lindberg, A. A. (1976). Enzyme-linked immunosorbent assay (ELISA) for titration of antibodies against Brucella abortus and Yersinia enterocolitica. Acta Pathologica Microbiologica Scandinavica Sect. C, 84: 168-176.

Carter, P. B. and Collins, F. M. (1974). Experimental Yersinia enterocolitica infection in mice: kinetics of growth. Infection and Immunity, 9(5): 851-857.

Carter, P. B. (1975). Pathogenicity of Yersinia enterocolitica for mice. Infection and Immunity, 11(1): 169-170.

Carter, P. B. R.; Zshorchak, J. and Brubaker, R. R. (1980). Plague virulence antigens from Yersinia enterocolitica. Infection and Immunity, 28: 638-640.

Cavazzini, G.; Caccherini, R.; Prati, L. and Rausa, G. (1982). Primo isolamento di Yersinia enterocolitica in alcuni prodotti ortensi destineti al consumo prevalentemente allo stato fresco. Boll. Ist. Sieroter Milan, 6(5): 428-431.

Cavazzini, G.; Ceccherini, R.; Bolognesi, L.; Brandi, A. and Rausa, G. (1983). Yersinia enterocolitica: Biotipi e sierotipi isolati da prodotti orticoli. Boll. Ist. Sieroter Milan, 62(4): 317-322.

Chiesa, C.; Bottone, E. J. and Hodes, H. L. (1984). Status of immunity of newborns against Yersinia enterocolitica: A preliminary report. American Journal of the Medical Sciences, 287(2): 18-20.

Christensen, S. G. (1981). The Yersinia enterocolitica prevalence in slaughter animals, water and raw milk in Denmark. In: Psychrotrophic microorganisms in spoilage and pathogenicity. ed. Roberts, T. A., Hobbs, G., Christian, J. H. B. and Skovgaard, N., pp. 439-445. London: Academic Press.

Colton, T. (1974). Statistics in Medicine. ed. Little, Brown and Company. Boston, Massachusetts 02106.

Corbel, M. J. (1973). The nature of the antibody response to Yersinia enterocolitica serotype IX in cattle. Journal of Hygiene, 71: 309-322.

Cornelius, G.; Bennett, P. M.; Grinsted, J. (1976). Properties of pGCI, a lack plasmid originating in Yersinia enterocolitica 842. Journal of Bacteriology, 127: 1058-1062.

Cornelius, G.; Luke, R. K. J.; Richmond, M. H. (1978). Fermentation of raffinose by lactose-fermenting strains of Yersinia enterocolitica and by sucrose-fermenting strains of Escherichia coli. Journal of Clinical Microbiology, 7: 180-183.

Daniels, J. J. H. M. and Groudzwaard, C. (1963). Some strains of an unidentified species of microorganism resembling Pasteurella pseudotuberculosis isolated from rodents. Diergeneeskundig Jaarboekje, 88: 96-102.

Davey, G. M., Bruce, J., Drysdade, E. M. (1983). Isolation of Yersinia enterocolitica and related species from the faeces of cows. Journal of Applied Bacteriology, 55: 439-443.

Doyle, M. P.; Hugdahl, M. B. and Taylor, S. L. (1981). Isolation of virulent Yersinia enterocolitica from porcine tongues. Applied and Environmental Microbiology, 42(4): 661-666.

Doyle, M. P.; Hugdahl, M. B.; Chang, M. T. and Berry, J. T. (1982). Serological relatedness of mouse-virulent Yersinia enterocolitica. Infection and Immunity, 37(3): 1234-1240.

Doyle, M. P. and Hugdahl, M. B. (1983). Improved procedure for recovery of Yersinia enterocolitica from meats. Applied and Environmental Microbiology, 45(1): 127-135.

Liss, J. (1975). Selective culturing of Yersinia enterocolitica at a low temperature. Scandinavia Journal of Infectious Diseases, 7(4): 249-252.

Erricsson, H. H. and Sherris, J. C. (1971). Antibiotic sensitivity testing, reports of an international collaborative study. Acta of pathologica and microbiologica scandinavica sect. B., supplement No. 217.

Esseveld, H. and Goodzwaard, C. (1973). On the epidemiology of Y. enterocolitica infections. Contributions to Microbiology and Immunology, 2: 99-101.

Farstad, L.; Landsverk, T. and Lassen, J. (1976). Isolation of Yersinia enterocolitica from a dog with chronic enteritis. Acta Veterinaria Scandinavica, 17: 261-263.

Felsen, J. and Osofsky, A. G. (1937). Susceptibility of the newborns to acute bacillary dysentery. Serologic data on the placental transmission of antibodies to bacillus dysenteriae. American Journal of Diseases of Children, 53: 975-984.

Francis, D. W.; Spaulding, P. L. and Loveth, J. (1980). Enterotoxin production and thermal resistance of Yersinia enterocolitica in milk. Applied and Environmental Microbiology, 40: 174-176.

Frederiksen, W. (1964). A study of some Yersinia pseudotuberoulosis-like bacteria (Bacterium enterocoliticam and Pasteurella X). In: Proceedings of the 14th Scandinavian Congress of Pathology and Microbiology, Oslo, Universitets for laget, pp. 103-104.

Fukushima, H.; Saito, K.; Tsubokura, M. and Otsuki, K. (1984a). Yersinia spp. in surface water in Matsue, Japan. Zentralblatt fur Bakteriologie Mikrobiologie und Hygiene, 179: 235-247.

Fukushima, H.; Nakamura, R.; Ito, Y. and Saito, K. (1984b). Ecological studies of Yersinia enterocolitica II. Experimental infection with Yersinia enterocolitica in pigs. Veterinary Microbiology, 9: 375-381.

Fukushima, H.; Nakamura, R.; IItsuka, S.; Tsubokura, M., Otsuki, K. and Kawaoka, Y. (1984c). Prospective systematic study of Yersinia spp. in Dogs. Journal of Clinical Microbiology, 19)5): 616-622.

Gemski, P.; Lazore, Jr. and Casey, T. (1980). Plasmid associated with pathogenicity and calcium dependency of Yersinia enterocolitica . Infection and Immunity, 27: 682-685.

Gianella, R. A. (1976). Suckling mouse model for detection of heat stable Escherichia coli enterotoxin: characteristics of the model. Journal of Infection and Immunity, 14: 95-99.

Groote, G., Vandepitte, J. and Wauters, G. (1982). Surveillance of human Yersinia enterocolitica infections in Belgium: 1963-1978. Journal of Infection, 4: 189-197.

Guerrant, R. L.; Lahita, R.G.; Winn, W. C. and Roberts, R. B. (1978). Campylobacteriosis in man: Pathogenic mechanisms and review of 91 blood stream infections. American Journal of Medicine, 65: 584-592.

Gutman, L. T.; Ottensen, E. A.; Quan, T. J.; Noce, P. S. and Katz, S. L. (1973). An inter-familial outbreak of Yersinia enterocolitica enteritis. New England Journal of Medicine,: 1372-1377.

Gyles, C. L. (1979). Limitation of the infant mouse system for Escherichia coli heat stable enterotoxin. Canadian Journal of Comparative Medicine, 43: 371-379.

Hammerberg, S.: Sorger, S. and Marks, M. I. (1977). Antimicrobial susceptibilities of Yersinia enterocolitica biotype 4 serotype 0:3. Antimicrobial Agents and Chemotherapy, 11(3): 566-568.

Hanna, M. O.; Zink, D. L.; Carpenter, Z. L. and Vanderzant, C. (1976). A research note - Yersinia enterocolitica-like organisms from vacuum-packaged beef and lamb. Journal of Food Science, 41: 1254-1256.

Hanna, M. O.; Smith, G. C.; Hall, L. C.; Vanderzant, C. and Childrers, A. B. Jr. (1980). Isolation of Yersinia enterocolitica from pigs tonsils. Journal of Food Protection, 43(1): 23-25.

Harmon, M. C.; Swaminathan, B. and Forrest, J. C. (1984). Isolation of Yersinia enterocolitica and related species from porcine samples obtained from an abattoir. Journal of Applied Bacteriology, 56: 421-427.

Harvey, S.; Greenwood, J. R.; Pickett, M. J. and Mah, R. A. (1976). Recovery of Yersinia enterocolitica from streams and lakes of California. Applied and Environmental Microbiology, 32(3): 352-35[illegible].

Hassig, A.; Karrer, J. and Pusterla, F. (1949). Vber Pseudotuberkulose beim menschem. Schweizerische Medizinische Wochenschrift, 79: 971-973.

Hubert, W. T.; McCulloch, W. F. and Schwarrenberger, P. R. (1975). Diseases transmitted from animals to man. 6th edition. Charles C. Thomas, Springfield Illincis, pp.

Hughes, D. (1979). Isolation of Yersinia enterocolitica from milk and a dairy farm in Australia. Journal of Applied Bacteriology, 46: 125-130.

Hughes, D. (1980). Repeated isolation of Yersinia enterocolitica from pasteurized milk in a holding vat at a diary factory. Journal of Applied Bacteriology, 48: 383-385.

Hughes, D. and Jensen, N. (1981). Yersinia enterocolitica in raw goat's milk. Applied and Environmental Microbiology, 41(1): 309-310.

Huguchi, K. and Smith, J. L. (1961). Studies on the nutrition and physiology of Pasteurella pestis. VI. A differential plating medium for the estimation of the mutation rate to avirulence. Journal of Bacteriology, 81: 605-608.

Hunter, D. and Hughes, S. (1983). Isolation of Yersinia enterocolitica from pigs in United Kingdom. Veterinary Record, 112: 322-323.

Hurvel, B. (1981). Zoonotic Yersinia enterocolitica infections: Host range, clinical manifestations and transmission between animals and man. In: E. J. Bottone (ed) Yersinia enterocolitica, CRC Press, Inc. Boca Raton, Fla. pp. 145-160.

Inuoe, M. and Kurose, M. (1975). Isolation of Yersinia enterocolitica from cow's intestinal contents and beef meat. Japanese Journal of Veterinary Science, 39: 91-93.

Kapperud, G. (1980). Studies on the pathogenicity of Yersinia enterocolitica and Y. enterocolitica-like bacteria. I. Enterotoxin production at 22°C and 37°C by environmental and human isolates from Scandinavia. Acta Pathologica Microbiologica Scandinavica Sec. B., 88: 287-291.

Kapperud, G. (1982). Enterotoxin production and Y. enterocolitica-like bacteria. Acta Pathologica Microbiologica Scandinavica Sect. B., 90: 185-189.

Kapperud, G. and Olsvik, O. (1982). Isolation of enterotoxigenic Yersinia enterocolitica from birds in Norway. Journal of Wildlife Diseases, 18: 247-248.

Kapperud, G. and Rosef, O. (1983). Avian wildlife reservoir of Campylobacter fetus subsp. jejuni, Yersinia spp. and Salmonella spp. in Norway. Applied and Environmental Microbiology, 45: 375-380.

Kawooka, Y.; Otsuki, K.; Mitani, T.; Kubota, T.; Tsubokura, M. (1984). Migratory waterfowl as flying reservoirs of Yersinia species. Research in Veterinary Science, 37: 266-268.

Keet, E. E. (1974). Yersinia enterocolitica septicemia. Source of infection and incubation period identified. New York State Journal of Medicine, 74: 2226-2228.

Knapp, W. and Thal, E. (1973). Differentiation of Yersinia enterocolitica by biochemical reactions, In: S. Winblad (ed), Contributions to Microbiology and Immunology, 2, S. Karger, Bassel, pp. 10-16.

Kohl, S., Jacabson, J. A. and Nahimias, A. (1976). Yersinia infections in children. Journal of Pediatrics, 89(1): 77-79.

Krogstad, O.; Teige, J. Jr. and Lassen, J. (1972). Yersinia enterocolitica type 2 associated with disease in goat. Acta Veterinaria Scandinavica, 13: 594-596.

Kwaga, J. K. P.; Agbonlahor, D. E.; Lombin, L. H.; Adesiyun, A. A. and Olatunde, A. O. (1984). Isolation of Yersinia spp. from cattle and milk around Zaria: A preliminary report. In: National Veterinary Research Institute Diamond Jubilee Celebrations/ Proceedings of National Conference on Diseases of Ruminant, pp. 149-158.

Kwaga, J. K. P.; Agbonlahor, D. E.; Adesiyun, A. A. and Lombin, L. H. (1986). The sensitivity to antimicrobial agents of species of Yersinia isolated from cattle and pigs in Nigeria. Veterinary Microbiology, 12: 383.

Laird, W. J. and Cavanaugh, D. C. (1980). Correlation of autoagglutination and virulence of Yersiniae. Journal of Clinical Microbiology, 11(4): 430-432.

Langford, E. V. (1972). Yersinia enterocolitica isolated from animals in the fraser valley of British Columbia. Canadian Veterinary Journal, 109-113.

Lee, W. H. (1977). Two plating media modified with Tween 80 for isolation of Yersinia enterocolitica. Applied and Environmental Microbiology, 33: 215-216.

Lee, W. H., Vanderzant, C. and Stern, N. (1981). The occurrence of Yersinia enterocolitica in foods. In: Yersinia enterocolitica (ed). Bottone, E. J., Boca, Raton, Florida: CRC Press, pp. 161-171.

Leistner, L., Hecholmann, H., Kashiwazaki, M. und Albertz, R. (1975). Nachweis von Yersinia enterocolitica in faeces und Fleisch von Schweinen, Rindern und Gefhigel. Sonderdruck aus Die Fleischwirtschaft, 55(1): 1599-1602.

Lombin, L. H.; Adesiyun, A. A.; Agbonlahor, D. E. and Kwaga, J. K. P. (1985). Isolation of Yersinia species from pigs in Nigeria. Veterinary Record, 117: 364.

Lombin, L. H.; Adesiyun, A. A.; Haruna, M.; Kwaga, J. K. P. and Agbonlahor, D. E. (1986). Survey for Yersinia enterocolitica in water from ponds, streams and wells in Northern Nigeria. Microbiologica, 9: 95-100.

Mathew, M.; Cornelius, G.; Wauters, G. (1977). Correlation of serological and biochemical groupings of Yersinia enterocolitica with the beta-lactamases of the strains. Journal of General Microbiology, 102: 55-59.

Mollaret, H. H. and Lucas, A. (1965). Sur les particularites biochemiques des souches de Yersinia enterocolitica isolates chez les li'ever. Annales de l'Institute Pasteur, 108: 121-125.

Mollaret, H. H. (1972). Unddomaine pathologique nouveau: L'infection a Yersinia enterocolitica. Annales de Biologie Cliniques, 30: 1-6.

Mollaret, H. H. and Knapp, W. (1972). International committee on systematic bacteriology subcommittee on the taxonomy of Pasteurella, Yersinia and Francisella. International Journal of Systemic Bacteriology, 22: 401-405.

Mollaret, H. H. and Thal, E. (1974). Yersinia. In: Bergey's Manual of determinative bacteriology, 8th (ed), Baltimore, Williams and Wilkins pp. 330.

Mollaret, H., Bercovier, H. and Alonse, J. M. (1979). Summary of the data received at the WHO reference centre for Yersinia enterocolitica. Contribution to Microbiology and Immunology, 5: 1740 174-184.

Nonno, R.; Balacco-Cabrieli, C. and Palmisar , C. (1982). Yersinia enterocolitica and eye infection: A case report. Boll 1st Sieroter Milan, 61(6): 510-512.

Moon, W. H.; Whipp, S. C., Engstrom, G. W. and Baetz, A. L. (1970). Response of the the rabbit ileal loop to cell-free products from Escherichia coli enteropathogenic for swine. Journal of Infectious Diseases, 121; 182-187.

Morgan, W. J., Mackson, D. J., Gill, K. P.; Gower, S. G. and Morris, P. W. (1971). Standard laboratory techniques for diagnosis of Brucellosis. Weybridge, England: MAFF Central Veterinary Laboratory.

Morris, G. K. and Feeley, J. C. (1976). Yersinia enterocolitica: a review of its role in food hygiene. Bulletin of World Health Organization, 54: 79-85.

Morse, D. L.; Shayegani, M. and Gallo, R. J. (1984). Epidemiologic investigation of a Yersinia camp outbreak linked to a food handler. American Journal of Public Health, 74(6): 589-592.

Mosimablae, F. and Gyles, C. L. (1982). The pathogenicity of Yersinia enterocolitica strains isolated from various sources in four test systems. Canadian Journal of Comparative Medicine, 46: 70-75.

Narucka, U. and Westerndoorf, J. F. (1977). Studies for the presence of Yersinia enterocolitica and Yersinia pseudotuberculosis in clinically normal pigs. Tiddschrift Voor Diergeneerskunde, 102: 299-317.

Nilehn, B. (1969). Studies on Yersinia enterocolitica with special reference to bacterial diagnosis and occurrence in human acute enteric disease. Acta Pathologica Microbiologica Scandinavica, 206: 1-48.

Nesbakken, T. (1985). Enterotoxin production at 4°, 22° and 37°C by Yersinia enterocolitica and Y. enterocolitica-like bacteria isolated from porcine tonsils and pork products. Acta Veterinary Scandinavica, 26: 13-20.

Nesbakken, T. and Kapperud, G. (1985). Yersinia enterocolitica and Y. enterocolitica-like bacteria in Norwegian slaughter pigs. International Journal of Food Microbiology, 1: 301-309.

Neter, E.; Westphal, O. and Luderitz, O. (1955). Demonstration of antibodies against enteropathogenic Escherichia coli in sera of children of various ages. Pediatrics, 16: 801-808.

Nunes, M. F. and Ricciardi, I. D. (1981). Detection of Yersinia enterocolitica heat-stable enterotoxin by sucklinj mouse bioassay. Journal of Clinical Microbiology, 13: 783-786.

Okamato, K., Ichikawa, H., Kawamoto, Y., Myama, A. and Yoshii, Y. (1980). Heat-stable enterotoxin produced by Yersinia enterocolitica isolated from patients. Microbiology and Immunology, 24: 401-408.

Olbrych, [illegible]; [illegible]; [illegible]; Traeger, [illegible] and Tan, J. S. (1984). Bullous lesions associated with Yersinia enterocolitica septicemia. American Journal of the Medical Sciences, 287(3): 38-39.

Pai, C. H. and Mors, V. (1978). Production of enterotoxin by Yersinia enterocolitica. Infection and Immunity, 19(3): 908-911.

Pai, C. H., Mors, V. and Toma, S. (1978). Prevalence of enterotoxigenicity in humans and non human isolates of Yersinia enterocolitica. Infection and Immunity, 22(2), 334-338.

Pai, C. H.; Mors, V. and Seemayer, T. A. (1980). Experimental Yersinia enterocolitica enteritis in rabbits. Infection and Immunity, 28: 238-244.

Papageorges, M.; Higgins, R. and Gosselin, Y. (1983). Yersinia enterocolitica enteritis in two dogs. Journal of American Veterinary Medical Association, 182(16): 618-619.

Pederson, K. B. (1976). Isolation of Yersinia enterocolitica from Danish swine dogs. Acta Pathologica Microbiologica Scandinavica Sect. B., 84: 317-318.

Portnoy, D. A., Moseley, S. L. and Falkow, S. (1981). Characterization of Plasmids and plasmid-associated determinants of Y. enterocolitica pathogenesis. Infection and Immunity, 31: 775-782.

Pramanik, A. K.; Bhattacharyya, H. M.; Chatterjee, A. and Sengupta, D. N. (1980). Isolation of Yersinia enterocolitica from human and animal sources in the same family. Indian Journal of Animal Health, 19: 79-82.

Raevuori, M.; Harvey, S. M.; Pickett, M. J. and Martizi, W. J. (1978). Yersinia enterocolitica: In vitro antimicrobial susceptibility. Antimicrobial Agents and Chemotherapy, 13(5): 888-890.

Rakovsky, J.; Pauckova, V. and Aldova, E. (1973). Human Yersinia enterocolitica infections in Czechkoslovakia. Contributions to Microbiology and Immunology, 2: 88-93.

Ratman, S.; Morcer, E.; Picco, B.; Parsons, S. and Butler, R. (1982). A nosocomial outbreak of diarrhoeal disease due to Yersinia enterocolitica serotype 0:5, biotype 1. Journal of Infectious Diseases, 145(2): 242-247.

Restaino, L. and Hill, W. M. (1981). Antibiotic susceptibility patterns of Yersinia enterocolitica. Journal of Food Production, 44(2): 124-127.

Robins-Browne, R. M.; Still, C. S.; Miliotis, M. D. and Koornhof, H. J. (1979). Mechanism of action of Yersinia enterocolitica enterotoxin. Infection and Immunity, 25(2): 680-684.

Samadi, A. R., Wachsmuth, K., Huq, M. I., Mahbub, M.; and Agbonlahor, D. E. (1982). An attempt to detect Yersinia enterocolitica infection in Dacca, Bangladesh. Tropical and Geographical Medicine, 34: 151-154.

Samadi, A. R., Huq, M. I., Monsur, K. A. and Kibriya, A. K. (1983). First isolation of Yersinia intermedia from stool during an epidemiological investigation in Bangladesh. Tropical and Geographical Medicine, 35: 231-233.

Schiemann, D. A. (1978a). Association of Yersinia enterocolitica with the manufacture of cheese and occurence in pasteurized milk. Applied and Environmental Microbiology, 36: 274-277.

Schiemann, D. A. (1978b). Isolation of Yersinia enterocolotica from surface and well waters in Ontario. Canadian Journal of Microbiology, 24: 1048-1052.

Schiemann, D. A. and Toma, S. (1978). Isolation of Yersinia enterocolitica from raw milk. Applied and Environmental Microbiology, 35: 54-58.

Schiemann, D. A. (1979). Synthesis of a selective agar medium for Yersinia enterocolitica. Canadian Journal of Microbiology, 25: 1298-1304.

Schiemann, D. A. (1980). Isolation of toxigenic Yersinia enterocolitica from retail pork products. Journal of Food Protection, 43(5): 360-365.

Schiemann, D. A. and Devenish, J. A. (1980). Virulence of Y. enterocolitica determined by lethality in mongolian gebrils and by the sereny test. Infection and Immunity, 29: 500-506.

Schiemann, D. A. (1981). An enterotoxin - negative strain of Yersinia enterocolitica serotype O:3 is capable of producing diarrhoea in mice.. Infection and Immunity, 32(2): 571-574.

Schiemann, D. A. and Fleming, C. A. (1981). Yersinia enterocolitica isolated from throats of swine in Eastern and Western Canada. Canadian Journal of Microbiology, 27: 1326-1333.

Schiemann, D. A., Devenish, J. A. and Toma, S. (1981). Characteristics of virulence in human isolates of Y. enterocolitica. Infection and Immunity, 32(1): 400-403.

Schiemann, D. A. and Devenish, J. A. (1982). Relationship of HeLa cell infectivity to biochemical, serological and virulence characteristics of Yersinia enterocolitica. Infection and Immunity, 35(2): 497-506.

Schieifstein, J. and Coleman, M. B. (1939). An unidentified microorganism resembling B. liquieri and Pasteurella pseudotuberculosis and pathogenic for man. New York State Journal of Medicine, 39: 1749-1753.

Serafim, M. B.; Monteiro, A. R. and Decastro, A. F. P. (1983). Factors affecting detection of Y. enterocolitica heat-stable enterotoxin by the infant mouse test. Journal of Clinical Microbiology, 17(5): 799-803.

Sereny, B. (1955). Experimental Shigella kerato-conjunctivitis: A preliminary report. Acta. Microbiology Academy of Science, Hungary, 2: 293-294.

Shayegani, M.; DeForge, L.; Mcglynn, D. M. and Root, T. (1981). Characteristics of Yersinia enterocolitica and related species isolated from human, animals and environmental sources. Journal of Clinical Microbiology, 14(3): 304-312.

Shayegani, M.; Morse, D.; DeForge, L.; Root, T.; Parsons, L. M. and Maupin, P. S. (1983). Microbiology of a major foodborne outbreak of gastroenteritis caused by Yersinia enterocolitica serogroup O:8. Journal of Clinical Microbiology, 17(1): 35-40.

Staley, L. G. (1978). Yersiniosis (Y. enterocolitica). In: CRC handbook series in zoonoses, (ed). James, H. Steele, CRC Press Inc.

Soerjadi-Liem, A.S.; Snoeyenbos, G. H. and Weinack, O. M. (1984). Establishment and competitive exclusion of Yersinia enterocolitica in the gut of monoxenic and holoxenic chicks. Avian Diseases, 28(1): 256-260.

Sunaga, Y.; Tani, F. and Makai, K. (1983). Detection of Yersinia enterocolitica O:9 infection in camels serodiagnosed as brucellosis. Japanese Journal of Veterinary Science, 45(2): 247-250.

Tertti, R.; Granfors, K.; Lehtonen, O. P.; Mertsola, J.; Makela, A.; L., Valimaki, I.; Hanninem, P. and Toivanem, A. (1984). An outbreak of Yersinia pseudotuberculosis infection. Journal of Infectious Diseases, 149(2): 245-250.

Thomas, L. V.; Gross, R. J., Cheatry, T.; Shipp, C. R. and Rowe, B. (1983). Antigenic relationships among type strains of Yersinia enterocolitica and those of E. coli, Salmonella spp. and Shigella spp. Journal of Clinical Microbiology, 17: 109-111.

Toma, S. (1973). Survey on the incidence of Yersinia enterocolitica in the province of Ontario. Canadian Journal of Public Health, 64: 477-487.

Toma, S. and Lafleur, L. (1974). Survey on the incidence of Yersinia enterocolitica infection in Canada. Applied Microbiology, 28: 469-473.

Toma, S. and Deidrick, V. R. (1975). Isolation of Yersinia enterocolitica from swine. Journal of Clinical Microbiology, 2(6): 478-481.

Toma, S.; Wauters, G.; McChure, H. M.; Morris, G. K. and Alice, S. W. (1984). O:13a, 13b, a new pathogenic serotype of Yersinia enterocolitica. Journal of Clinical Microbiology, 20(5): 843-845.

Tsubokura, M.; Otsuki, K. and Itagaki, K. (1973). Studies on Yersinia enterocolitica. I. Isolation of Y. enterocolitica from swine. Japanese Journal of Veterinary Science, 3: 419-424.

Umoh, V. J.; Dangana, A. and Umoh, J. U. (1984). Isolation of Yersinia enterocolitica from milk and milk products in Zaria, Nigeria. International Journal of Zoonoses, 11: 223-228.

Une, T., Zen-Yoji, H., Marayama, T. and Yanagawa, Y. (1977). Correlation between epithelial cell infectivity in-vitro and O-antigen groups of Yersinia enterocolitica. Microbiology and Immunology, 21: 727-729.

Urbano-Marquez, A., Estruch, R., Agusti, A.; Jimenez-De-Anta, M. T., Ribalta, T.; Grau, J. M. and Roxman, C. (1983). Infectious endocarditis due to Yersinia enterocolitica. Journal of Infectious Diseases, 148(5): 940.

Ursing, J.; Bercovier, H.; Brenner, D. J.; Fanning, G. R.; Steigerwalt, A. G.; Brault, J. and Mollaret, H. H. (1980). Yersinia frederiksenii: a new species of Enterobacterioaceae composed of Rhamnose-positive strains (formerly called atypical Yersinia enterocolitica or Y. enterocolitica-like). Current Microbiology, 4: 213-217.

Vandipette, J., Wauters, G. and Isebaert, A. (1973). Epidemiology of Yersinia enterocolitica infections in Belgium. Contributions to Microbiology and Immunology, 2: 111-119.

Varghese, A.; Ramachandran, V. G. and Agarwal, D. S. (1984). Isolation and characterization of Yersinia enterocolitica from patients of gastro-enteritis. Indian Journal of Medical Research, 79: 35-40.

Vesikari, T.; Nurmi, T.; Maki, M.; Skurnik, M.; Sundgvist, C.; Granfors; K. and Gronroos, P. (1981). Plasmids in Yersinia enterocolitica serotype 0:3 and 0:9: Correlation with epithelial cell adherence in vitro. Infection and Immunity, 33: 870-876.

Vidon, D. J. M. and Delmas, C. L. (1981). Incidence of Yersinia enterocolitica in raw milk in Eastern France. Applied and Environmental Microbiology, 41(2): 355-359.

Wauters, G. (1970). Contribution a l'etude de Yersinia enterocolitica. Ph.D. Thesis, Vander Louvain, Belgium.

Wauters, G., LeMinor, L., Chalon, A. M. (1971). Antigenes somatiques et flagellaires de Yersinia enterocolitica. Annales de l'Institut Pasteur, 120: 631-642.

Wauters, G.; LeMinor, L.; Chalon, A. M. and Lassen, J. (1972). Supplement au schema antigenique de Yersinia enterocolitica. Annales de l'Institute Pasteur, 122: 951-954.

Wauters, G. (1973). Improved methods for the isolations and the recognition of Yersinia enterocolitica. Contributions to Microbiology and Immunology, 2: 68-79.

Wick, W. E.; Preston, D. A.; Hawley, L. C. and Griffith, R. S. (1971). Laboratory Testing of Bacterial Susceptibility to Antibiotic: Theory and Practice. Lilly Research Laboratories, Indianapolis, Indiana, pp. 3-51.

Winblad, S. (1967). Studies on serological typing of Yersinia enterocolitica. Acta Pathologica et Microbiologica Scandinavica Supplement, 187: 115-119.

Winblad, S. (1969). Erythema nodusum associated with infection with Yersinia enterocolitica. Scandinavian Journal of Infectious Diseases, 1: 11-16.

Winblad, S. (1973). The clinical panorama of human Yersinia enterocolitica. Contributions to Microbiology and Immunoloty, 2: 129-132.

Yukiokato, Kimiko, I.; Yoko, K.; Tsutomu, M.; Kan-ichi, K. and Masuo, C. 1985). Occurrence of Yersinia enterocolitica in wild living birds and Japanese serows. Applied and Environmental Microbiology, 49(1): 198-200.

Zen-Yoji, H.; Sakai, S.; Yanagawa, Y. and Vanderzant, C. (1973). An outbreak of enteritis due to Yersinia enterocolitica occurring at a junior high school. Japanese Journal of Microbiology, 17: 220-222.

Zen-Yoji, H.; Sakai, S.; Maruyama, T. and Yanagawa, Y. (1974). Isolation of Yersinia enterocolitica and Yersinia pseudotuberculosis from swine, cattle and rats at an abattoir. Japanese Journal of Microbiology, 18: 103-107.

Zink, D. L.; Feeley, J. C.; Wells, J. G.; Vanderzant, G.; Vickery, J. C. and O'Donovan, G. A. (1978). Possible plasmid-mediated virulence in Yersinia enterocolitica. Trans-Gulf Coast Molecular Biology Conference, 111: 155-163.

Zink, D. L.; Feeley, J. C.; Wells, J. G.; Vanderzant, C.; Vickery, J. C.; Roof, M. D. and O'Donovan, G. A. (1980). Plasmid-mediated tissue invassiveness in Yersinia enterocolitica. Nature, 283: 224-226.

Appendix 1: Experimental Design for Phase I study

Y. enterocolitica serotype	Dose	No. of chicks	Administration of 10% $NaHCO_3$	Oral inoculation	Age of chicks in days													
				0	1	2	3	4	5	6	7	8	9	10	11	12	13[a]
					Daily													
O:3	10^{11}	10	+	Cloacal swabs taken before exposure	(1) Weight determine (2) Looks for evidence of diarrhoea. (3) Cloacal swabs taken for direct plating and enrichment. (4) One chick sacrificed from each group. (5) Section of small intestine taken for histopathology and gram staining from each group. (6) Number of *Yersinia enterocolitica* in caecum could not be quantified because caeca were too tiny.					(1) Weight recorded daily (2) Cloacal swabs taken for enrichment at days 7, 10, 13. (3) Looked for evidence of diarrhoea.							(1) Cloacal swabs taken for enrichment. (2) Two chicks sacrificed per group after weighing (3) Number of *Y. enterocolitica* in caeca quantified.
	10^{11}	10	-															
O:8	10^{11}	10	+															
	10^{11}	10	-															
O:9	10^{10}	10	+															
	10^{10}	10	-															
Control	-	10	-															

[a] The remaining chicks per group were reared, weighed fortnightly and bled for serology till day 70.

Appendix 3: Mean weights of chicks after exposure to Y. enterocolitica at day-old

Treatment/ days	Mean weights (g) on Days													
	0	1	2	3	4	5	6	7	8	9	10	11	12	13
0.8+$NaHCO_3$	42.7	48.3	52.0	60.2	66.9	72.4	93.0	97.0	109.5	119.5	141.2	158.7	179.7	[illegible].0
0.8 alone	39.9	46.6	50.1	55.7	63.0	67.0	76.9	82.5	92.0	97.5	112.5	132.4	146.6	166.0
0.9+$NaHCO_3$	40.7	44.6	46.2	51.3	57.6	67.5	79.1	86.7	99.5	107.7	119.3	134.2	154.8	175.5
0:9 alone	39.5	45.3	48.7	52.7	62.5	75.4	84.6	90.0	110.8	118.8	129.5	147.9	165.4	183.4
0.3+$NaHCO_3$	39.5	47.1	49.8	58.4	67.3	77.8	80.3	87.8	100.0	108.2	121.5	131.2	148.8	[illegible].3
0:3 alone	38.4	44.9	48.3	55.1	69.4	75.5	85.2	93.2	103.0	125.0	133.3	144.8	153.8	162.5
Control	37.8	43.6	50.3	54.4	57.8	71.3	82.3	89.4	112.4	114.2	136.0	140.2	174.2	190.0

Appendix 5: Mean weights of chickens after exposure to Y. enterocolitica at 3 weeks old

Treatment	Mean weights (g) on days								
	0	1	2	3	4	5	14	28	49
0.9+$NaHCO_3$	287.6	307.0	345.8	378.8	391.7	412.6	706.0	1515.1	4091
0.9 alone	276.2	310.9	320.3	34..9	396.2	406.8	697.9	1363.6	4773
0.3+$NaHCO_3$	291.3	312.4	325.2	375.0	388.4	410.3	688.8	-	-
0.3 alone	328.9	352.1	358.1	407.7	437.6	450.6	732.8	1591	4545
Control	287.6	305.1	332.5	347.5	410.4	414.3	716.0	1818.2	5000

Appendix 6: Percent weight gain in chickens exposed to Y. enterocolitica at 3 weeks old

	Percent weight gains on days								
Treatment	0	1	2	3	4	5	14	28	49
0.9+$NaHCO_3$	100.00	106.7	120.2	131.7	136.2	143.5	245.5	526.8	1422.5
0.9 alone	100.00	112.6	116.0	128.8	143.4	147.3	252.7	493.7	1728.1
0.3+$NaHCO_3$	100.00	107.2	111.6	128.7	133.3	140.9	236.5	-	-
0.3 alone	100.00	107.1	108.9	124.0	133.0	137.0	222.8	483.7	1381.9
Control	100.00	106.1	115.1	120.8	142.7	144.1	249.0	632.2	173[illegible].5