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Full Length Research Paper

Epidemiological studies of the incidence of pathogenic *Campylobacter* spp. amongst animals in Lagos metropolis

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Faecal specimens were collected from different animal species (puppies, Guinea fowl, chicken and pigs) at different farm locations in Lagos (Inner City - Central urban area; peripheral City – rural/farming houses; Rural - a farming and agricultural zone, where people raised their own domestic animals). In all, 200 animals were sampled out of which twenty (14%) were positive for *Campylobacter*, seventeen (17) of these (60.7) were positive for animals from peripheral zone, nine (9)(32.1%) were found positive for animals from the rural zone and two (2)(7.1%) were positive for animals obtained from the inner zone of Lagos metropolis. The trend of infection by *Campylobacter* as exemplified in this study was pig, two samples (7.1%); chicken, 7 samples (25%); sheep, two samples (7.1%); puppies, 0 (0.0%), and guinea fowl, 17 samples (60.7). The peripheral zone had more incidence of *Campylobacter* with an incidence rate in the order peripheral zone (8.5%» rural zone (4.5%) > inner zone (1.0%). The results of biochemical tests reveal that three (3) samples were positive for *Campylobacter coli* biotype II, seventeen (17) samples were positive for *Campylobacter coli* biotype I and one (1) sample for *Campylobacter jejuni* biotype I. These results are indicative that the epidemic of *Campylobacter* infection is possible amongst handlers of these farm animals.

Key words: Faecal, peripheral, central, inner, puppies, campylobacter, coli, jejuni

INTRODUCTION

Many years ago there had been a marked change in the pattern of infectious diseases. Along with the disappearance or decline of some infections, new infections have been recognized. Some of these have been due to technical advances in laboratory methods for identification of causal pathogens e.g., for bacterial infections like *Campylobacter enteritis* (Skirrow, 1994; Blaser et al., 1983; Hindiyeh et al., 2000; Steinhauserova et al., 2000; Coker et al., 2002; Moore et al., 2005). *Campylobacter jejuni* is naturally found in wild birds, chicken, cattle and sheep, cats and puppies and have been noted to cause sporadic abortion in sheep (Petersen et al., 2001; Hald et al., 2004; Moser et al., 2001; Sandberg et al., 2002; Steinhauserova et al., 2000; Torre and Tello, 1993). Humphrey (1986b) have also argued that *C. jejuni* is sublethally damaged by exposure to low temperatures hence investigation that do not take into cognizance above phenomenon are likely to underestimate the incidence and number of *C. jejuni* in refrigerated milk or food.

Origin of outbreaks can be determined by haemagglutination method (Penner and Humphrey, 1980) and the slide agglutination method of Lior (1984), which are both widely used for serotyping strains of *Campylobacter* species. The importance of serotyping strains from out-

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breaks has been documented (Jones et al., 1985). The biotyping scheme of Preston has been used for the epidemiological typing of strains from outbreaks (Bolton et al., 1984). The biotyping scheme has provided means of differentiating between *C. jejuni, Campylobacter coli* and *Campylobacter laridis*.

Bolton et al. (1984) reported cases of *Campylobacter* enteritis from a litter of 11 puppies in which all but one died and most of the household, which received the puppies, had human cases. In their report nine (9) households were infected and sixteen (16) human cases diagnosed. Strains of cases associated with animals and the environment were diagnosed using the diagnostic scheme of Humphrey (1980), Lior (1984) and the Preston biotyping scheme (Bolton et al., 1984) and recently the PCR identification methods of identification using either the 16S rRNA (Giesendorf and Quint, 1995; Linton et al., 1996), the 23S rRNA (Eyers et al., 1993), *flaA* (fagellin)(Waegel and Nachamkin, 1996), GTP-binding protein (van Doorn et al., 1997), *ceuE* (iron transport protein)(Gonsales et al., 1997), 5' Taq nuclease assay (McMillen et al., 2006) and *hip* (hippuricase)(Linton et al., 1997) as well as genotyping of bovine and humans isolates by amplified-fragment length polymorphism (AFLP) (Johnsen et al., 2006).

C. jejuni have now been recognized as one of the leading bacterial agent of gastro-enteritis in humans whereas hitherto attention was focused on their pathogenicity among domesticated animals (Coker et al., 2002; Moore et al., 2005; Skirrow, 1982). Information from available data have shown few outbreaks in which an animal or animal product were ultimately incriminated/or identified as source. Furthermore, some serotypes of *Campylobacter* that cause disease in humans have been isolated from animals. Blaser et al. (1983) also confirmed that there is *Campylobacter* infection in domestic animals and that most commercially raised poultry have *Campylobacter* in their intestinal flora. Infection may start early in life and that sources of entry into flock includes infection of new born chick from old birds, contaminated feed or contaminated water (Blaser et al., 1983). *C. Jejuni* and other *Campylobacter* species are often present in the stools of healthy dogs and those with diarrhoea. Reported isolated cases vary from herd to herd, but may be due to the use of different methods for isolation of *Campylobacter* (Blaser et al., 1983). Penner and Humphrey (1980) in his study observed that more than half of commercially raised pigs excrete the organism. Isolates of *C. jejuni* are usually susceptible to erythromycin and therapy shortens the duration of faecal shedding of bacteria (Cheesebrough, 1984).

Campylobacter are polarly flagellated. Somatic flagellum and capsular antigens all contribute to the numerous serotypes. Most *Campylobacter* are microaerophilic and members of the genus utilize a respiratory pathway; they do not ferment carbohydrates. *Campylobacter* infection can cause ulcerative, inflammatory lesions in jejunum, ileum or colon. It can cause bacteraemia as seen in children and adults (Strohl et al., 2001). *Campylobacter* because of their wide distribution can be transmitted to humans primarily via the faeco-oral route, through direct contact exposure to contaminated meat (especially poultry or contaminated water supplies (William et al., 2001). *C. jejuni* is a cause of Traveller's diarrhoea and pseudo-appendicitis (Strohl et al., 2001). Complications may include septic abortion, reactive arthritis and Guillain-Barre Syndrome (Strohl et al., 2001. Other enteric *Campylobacters* cause similar clinical presentation. *Campylobacters fetus* causes infection of vascular sites, but may also infect CNS and other local sites. In compromised hosts, campylobacteriosis is more likely to result from infection with *C. fetus* than with *C. jejuni*. *C. fetus* causes spontaneous abortion in domestic animals (McMillen et al., 2006; Tortora et al., 1997).

Campylobacter can be isolated from faeces using special selective media and microaerophilic conditions. Because of their small size bacteriologic filters that hold back most other bacteria do not retain these organisms. Thus, filtration of the faecal suspension may enhance recovery rate. Presumptive diagnosis can be made on the basis of finding curved organisms with rapid, darting motility in wet mount of faeces (William et al., 2001). Diarrhoea caused by *Campylobacter* in United States has surpassed that of *Shigella* and few inoculum doses are necessary to establish infection (Tortora et al., 1997; William et al., 2001).

MATERIALS AND METHODS

Materials and reagents

Materials and reagents used were *Campylobacter* Agar base, 10% blood, disposable, ethanol, selective supplement (SR 85), oxidase strips, hydrogen peroxide, tryptone Soya broth, glycerol, swab stick, membrane filters, 1% sodium hippurate, 3.5% ninhydrin, butanol, DNA test Agar, *Brucella* broth, Na_2HPO_4, KH_2PO_4, Agar-Agar, ferrous sulphate, sodium metabisulphite and sodium pyruvate.

Preparation of *Campylobacter* media

About 19.5 g of *Campylobacter* agar base powder was dissolved in 500 ml of distilled water. This was mixed gently, melted to dissolve in a water bath and sterilized by autoclaving at 121°C for 15 min. The medium was allowed to cool to 50°C after which 10% blood was added and mixed gently. The selective supplement was reconstituted with a prepared mixture of 50% methanol and distilled water and allowed to mix thoroughly. This was dispensed asepticcally in 20 ml portion into sterile petridishes. The surface of the prepared agar, which were to be used immediately were dried in an incubator.

Culture method

Samples from freshly voided stool were collected in sterile container. Each sample after collection was inoculated into Butzler medium, care being taken to avoid contamination after faeces collection. In addition, where it was not possible to collect faeces (in cases of pigs, puppies and sheep) rectal swabs were collected. Such rectal swabs were collected by inserting sterile cotton swabs into the rectum for about 10 s, care being taken to avoid contamina-

Table 1. Result of all tests performed for the identification of the various *Campylobacter* spp. and biotypes in animal faeces.

Test	Biotype							
	C. jejuni				*C. coli*		*C. laridis*	
	I	II	III	IV	I	II	I	II
Chicken	+	Nil	Nil	Nil	+++	++	Nil	Nil
Guinea Pig	Nil	Nil	Nil	Nil	+++++++++++	+	+	+
Sheep	Nil	Nil	Nil	Nil	+	Nil	Nil	Nil
Pig	Nil	Nil	Nil	Nil	+	Nil	Nil	Nil

Nil = None of the biotypes was found; + = positive for the biotype.
The number of + shows the number of faeces samples that were positive for each of the animals

Table 2. Occurrence and distribution of *Campylobacter* in faeces samples collected from different zones

Zone	No. of samples collected	% of samples collected	No. of positive samples	% of positive samples over total samples
Inner	66	33.0	2	1.0
Peripheral	71	35.5	17	8.5
Rural	63	31.5	9	4.5
Total	200	100	28	14.0

tion of specimen collected from the anal skin. Specimens were labeled accordingly with date of collection, type of specimen, location and type of investigation. Specimens were taken to the laboratory, streaked on plates and incubated at 42°C for 48 h at microaerophilic condition for distinct colonies. The cultural methods employed were those of Blaser et al. (1983), Bolton et al. (1984), Endberg et al. (1999), Annan-Prah and Janc, 1988; Hariharan et al., 1996), Adetosoye and Adeniran (1987), Cheesebrough (1984) as well as Skirrow and Benjamin (1980) and Skirrow (1980). Thereafter all presumptive Campylobacter isolates were subcultured into freshly prepared Butzler medium for distinct colonies and easy identification from mixed cultures and then incubated microaerophilically for 48 h at 42°C.

Storage of *Campylobacter*

Into prepared sterile tryptone soya broth supplemented with 20% glycerol was inoculated pure colonies of *Campylobacter*. The inoculated broth was frozen or kept below 4°C.

Biochemical test

Since *Campylobacter* are not carbohydrate fermenting, suspected colonies were subjected to catalase, motility and oxidase test as described by Olutiola et al. (1991). Identification of the various biotypes was done using the schemes of Lior (1984) and Bolton et al., (1984) as well as Nachamkin (1995).

RESULTS AND DISCUSSION

From the faeces of domestic animals analyzed, one (4.8%) from Guinea fowl was positive out of twenty-one samples and was identified as *C. jejuni* biotype I and three (14.2%) from chicken/guinea fowl were identified as *C. coli* biotype II, while seventeen (81%) of all the species of animals understudy except puppies were positive and were found to be *C. coli* biotype I. No *C. laridis* was isolated (see Table 1)

Of the total number of animals sampled during the period of study, twenty-eight (14%) were positive for *Campylobacter* (Table 2), of which, seventeen (8.5%) were found in the peripheral zone, nine (4.5%) were found in the rural zone and two (1.0%) in the inner zone. Only animals that had *Campylobacter* infections alone were included in the analysis.

Table 3 shows the degree of occurrence of *Campylobacter* in the samples of faeces analysed. Seventeen samples (60.7%) were positive for guinea fowl, two (7.1 %) were positive for sheep; seven (25%) were positive for chicken and two (7.1 %) were positive for pigs. All faecal samples for puppies were negative for Campylobacter

Most epidemiological studies in different parts of the world have indicated that domesticated animals constitute a major natural reservoir for *Campylobacter s*pecies (Blaser et al., 1980; Annan-Prah and Janc (1988); Adetosoye and Adeniran (1987). This study has noted that *Campylobacter* coli biotype I is more preponderant amongst chicken and guinea fowl from this study (17; 60.7%) of the total samples analyzed were positive) (Table 3). This observation suggests *C. coli* biotype I was mostly responsible for enteritis in chicken and guinea fowl observed during the field study. This result agrees with the findings of Annan-Prah and Janc (1988) where they observed a high incident rate of spread of *C. jejuni* and *C. coli* among broiler flocks. Blaser et al. (1980) in their own findings observed that most commercially raised poultry

Table 3. Occurrence and distribution of *Campylobacter* in faeces samples collected from different animals

Animal	No. of samples collected	% of samples collected	No. of positive samples	% of positive samples over total samples
Pig	20	10	2	7.1
Chicken	76	38	7	25
Sheep	13	6.5	2	7.1
Puppies	20	10	–	–
Guinea fowl	71	35.5	17	60.7
Total	200	100	28	99.9

have *Campylobacter* in their intestinal flora and said that contamination may be nearly universal and may start early in life although some flocks escape infection completely. This may have been responsible for negative results obtained for faeces obtained from some guinea fowl and chicken. The preponderance of *Campylobacter* infection amongst the guinea fowl samples cannot be explained by an epidemic report; however, it is not inconceivable that the large numbers of these birds, which are kept together in the same cage, could have been responsible for the observed phenomenon. Only three (3) isolates had *C. coli* biotype II (Table 1) and were associated with chicken/guinea fowl. Similar results were obtained on the epidemiology of *Campylobacter* by Lior (1984), Penner and Humphrey (1980) on their work on 11 puppies. Surprisingly, none of the puppies used in this study showed incidence of *Campylobacter* in their stool. Blaser (1980), Moser et al. (2001), Sandberg et al. (2002) have all observed *Campylobacter* infections in domestic animals. In this study only two samples (7.1%) were positive each for sheep and pigs. This finding also agrees with the observation of Penner and Humphrey (1980) that more than half of commercially raised pigs excrete the organisms.

On the basis of zonal occurrence of *Campylobacter* organisms among animals, the peripheral zone had more positive samples for *Campylobacter* (8.5%) (Table 2) compared to rural zone with nine (4.5%) and inner zone two (1.0%). The low degree of positive samples observed in the inner zone correlates with the hygienic nature of central Lagos. The observed differences in the distributional pattern of *Campylobacter* in the different zones could be attributable to the nature of animals that were sampled, their degree of maturity and the presence of mature animals that could discharge faeces and hence enhance spread.

The occurrence of *Campylobacter* species in these animals serves as a potential danger to animal farmers since *Campylobacter* species are contacted faeco-orally. Bolton et al. (1984) observed an outbreak of *Campylobacter* enteritis among humans who had puppies infected with enteritis associated with untreated milk. It is therefore necessary that an epidemiological survey on the distribution of this *Campylobacter* organisms is effected and a database created for the types of *Campylobacter* species in human and animal population not only in Lagos and its surroundings but in the country in general due to the potential nature of the diseases originating from *Campylobacter* infections.

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