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Study of *Campylobacter* in Raw Cow Milk in Sokoto State, Nigeria

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Abstract: The study was conducted to establish the presence of *Campylobacter* species, determine its prevalence and assess some of the epidemiological variables such as breed and season in the distribution of *Campylobacter* species in raw cow milk in the state. During October, 2007 and September, 2008, a total of 146 raw milk samples were collected from lactating cows in selected dairy herds in the state. The samples were processed for *Campylobacter* isolations by inoculating in Preston Enrichment broth and incubated for 48h at 37ºC, and subsequently spread-plated on mCCDA incubated at 42ºC for 48 h microaerobically. The colonies were subjected to oxidase reaction, hippurate hydrolysis and sensitivity to nalidixic acid and cephalothin for identification. The identified isolates were biotyped using the new extended scheme of Lior. Only 7(4.8%) of the samples were positive and all the positive isolates were identified as *C. jejuni* and *C. jejuni* biotype I. The white Fulani breed of cattle had the highest contamination rate of 2(5.4%) and there was no association between the breed and *Campylobacter* contamination of milk (χ^2 = 0.6997, p>0.005). More contamination rate of 3(6.1%) was observed during the dry hot season than other seasons, there was no association between *Campylobacter* contamination of milk and the seasons (χ^2 = 0.3776, p>0.005). The isolation of *C. jejuni* from milk in this study is of serious public health concern as *C. jejuni* is the most common *Campylobacter* species implicated in human *Campylobacteriosis* worldwide.

Key words: *Campylobacter*, cow, milk, prevalence, seasonal, Sokoto

INTRODUCTION

Food safety, and in particular safety of products of animal origin, is an increasingly important issue concerning human health. With increase in the consumption of products of animal origin the risk of foodborne diseases of humans also increases. The raw food movement, characterized by eating raw rather than cooked food has increased the awareness of consumption of raw food. One product that is commonly distributed in raw form is milk. Raw milk is a known vehicle and medium for pathogens such as *E. coli*, *Mycobacterium bovis*, *Listeria monocytogenes* and species of *Campylobacter*, *Brucella* and *Salmonella* (Leedom, 2006). Milk can become contaminated in many ways. For example, if the dairy cow has a mammary gland infection (mastitis) or a systemic infection, the pathogen can be passed to the milk. Milk can become contaminated by the faeces of the animals and the hand of the milker usually during hand milking procedure or by equipment used for milk collection and storage.

Campylobacter species have been reported in many foods of animal origin (Altekruse *et al*., 1999). Surveys of raw agricultural products support epidemiologic evidence implicating poultry, meat, and raw milk as sources of human infection. Outbreaks of *Campylobacteriosis* are relatively rare (Pebody *et al*., 1997) and often associated with the consumption of unpasteurized milk (Evans *et al*., 1996) or untreated water (Palmer *et al*., 1983). Unpasturized milk is considered next to poultry as the most frequent cause of alimentary infections in people. Studies conducted have indicated that one out of five cases of *Campylobacteriosis* in people, where milk is the source of infection was caused by drinking unpasteurized milk. A prevalence of 6% of *Campylobacter* in unpasteurized milk on sale to the public has been demonstrated in Britain (Humphrey and Hart, 1988). In one study, 12% of raw milk samples from dairy farms in eastern Tennessee were contaminated with *C. jejuni* (Rohrbach *et al*., 1992). Raw milk is presumed to be contaminated by bovine faeces; however, direct contamination of milk as a consequence of mastitis also occurs (Hudson *et al*., 1984).

This study was conducted to establish the presence of *Campylobacter* sp., determine its prevalence and assess some of the epidemiological variables such as breed and season in the distribution of *Campylobacter* sp. in raw cow milk in the state.

MATERIALS AND METHODS

The study was conducted in Sokoto state, Nigeria between October, 2007 and September, 2008.

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Study area: Sokoto state is located to the extreme Northwest of Nigeria between longitudes 4º 8'E and 6º 54'E and latitudes 12ºN and 13º 58'N. It forms boundaries with the Republic of Niger to the North, Kebbi state to the west and southwest and Zamfara state to the east. The state is divided into 23 Local Government Areas (LGA). The state has 4 agricultural zones namely, Sokoto, Isa, Gwadabawa and Tambuwal with average of 5-6 local government areas. Farming is the main stay of the state's economy accounting for a significant proportion of the Gross Domestic Product (GDP) and responsible for about 70% of the total employment (NPC, 1991). Sokoto state ranks second in the nation's livestock population, with estimated 3 million cattle, 3 million sheep, 5 million goats, 4600 camels and variable species of poultry including chickens, guinea fowls, ducks and turkeys (MOCIT, 2002). Consumption of meat, milk, and their products (yoghourt, *nono*, *kilishi*) forms part of the food habits of the inhabitants of the state. Significant percentage (over 75%) of the livestock and poultry are raised or reared in a traditional free-range system.

Sokoto has a tropical continental type of climate dominated by two opposing air masses: the tropical marine from the south and tropical continental from the north. Annual rainfall is about 550 mm with the highest peak in August. Dry season sets in first with the cold harmattan from October to February, and a hot period comes in from March to the end of May when temperatures reach 38ºC during the day with humidity less than 20% and the rain begins in June to September (Sokoto, 2000).

Sample collection: Milk samples were collected from lactating cows in each of the selected herd by directly milking into plain sterile sample bottles. About 15-20 mL was collected midstream from each animal. The bottles were properly labeled and transported to the laboratory. A total of 146 milk samples were collected during October 2008 and September, 2008 across the four agricultural zones of the state.

Media and culture methods: Oxoid media were used for the isolation of *Campylobacter*. Preston Enrichment (PE) broth was used for selective enrichment purposes, while *Campylobacter* blood-free selective agar (modified CCDA-Preston) was used for selective plating. Agar plates were incubated under microaerobic conditions produced by the Campy-Gen™ atmosphere generation system (Oxoid). Medium term storage was achieved by keeping agar plates at 4ºC in anaerobic jars under microaerophilic conditions, by inoculating 10ml of trypticase soy broth subsequently maintained at 37ºC or by freezing at -70ºC

Isolation and Identification: The samples were processed by adding 10 mL of each sample to 90 mL of Preston Enrichment (PE) broth and incubating for 48h before a 0.1 mL volume from this enrichment was spread-plated to selective agar which was in turn incubated for 48 h at 42ºC under microaerophilic conditions. Identification was achieved by testing cultures for sensitivity to nalidixic acid and cephalothin (by placing 30µg discs on Columbia blood agar plates inoculated from a broth culture), oxidase reaction using the dry Slide™ system (Difco), hippurate hydrolysis (Lior, 1984) and observing Characteristic morphology.

Table 1: Breed prevalence of *Campylobacter* in raw milk in the herds in Sokoto state

Breed	Positive	Negative	Total
Sokoto gudali	3(4.8%)	59	62
Red bororo	2(5.7%)	33	35
White Fulani	2(5.4%)	31	37
Friesian cross	0(0.0%)	12	12
Total	7(4.8.0%)	139	146

$\chi^2 = 0.6997$ ($p>0.05$)

Table 2: Seasonal prevalence of *Campylobacter* in raw milk in the herds in Sokoto state

Seasons	Positive samples	Negative samples	Total
Cold dry	1(2.1%)	46	47
Hot dry	3(6.1%)	46	49
Raining season	3(6.0%)	47	50
Total	7(4.8%)	139	146

$\chi^2 = 0.3776$ ($p>0.05$)

Biotying: Biotyping of the isolates was carried out using the new extended scheme described by Lior (1984). The scheme employs three chemical reactions such as rapid H_2S production, Hippurate hydrolysis and DNA detection to differentiate *C. jejuni* into four biotypes (I, II, III and IV), *C. coli* and *C. lari* each into biotypes I and II

Statistical analysis: Data generated from this study were subjected to Chi-square test statistic (Graphpad software) to determine associations between the parameter tested and the variables $p<0.05$ was considered significant in this study.

RESULTS

A total of 146 raw milk samples were collected during the period of the study across the four agricultural zones in the state. Only 7(4.8%) of the sampled milk were positive for *Campylobacter* and all the isolates from the 7 positive samples were *Campylobacter jejuni*. However, all the *C. jejuni* isolates were biotype I. The breed distribution of the *Campylobacter* positive milk samples indicated that white Fulani has higher prevalence rate of 2(5.4%) than the other breeds. The Friesian cross (Sokoto Gudali-Friesian) had a prevalence of 0(0.0%), while Sokoto Gudali had prevalence rate of 3(4.8%) (Table 1). There was no statistical association between the breeds

and rate of *Campylobacter* contamination of milk (χ^2 = 0.6997, p>0.05). Seasonal prevalence of *Campylobacter* isolations showed that milk samples collected in the hot dry and the wet season recorded the highest prevalence rates of 3(6.1%) and 3(6.0%) respectively, while samples collected during the cold dry season had prevalence rate of 1(2.1%) (Table 2). There was no association between the seasons and the infection (χ^2 = 0.3776, p>0.05).

DISCUSSION

The occurrence of human *Campylobacter gastroenteritis* has been largely attributed to the consumption of contaminated food animal products, especially poultry, because of the high prevalence of *Campylobacter* in these food animals (Park and Stankiewicz, 1981; Harris *et al*., 1986; Deming *et al*., 1987; Corry and Atabay, 2001). A growing body of evidence, however, suggests that other vehicles such as contaminated raw meat, surface ground water, and unpasteurized milk may be important sources of these organisms (Garcia *et al*., 1985; Hanninen *et al*., 1998; Peterson, 2003). In the present study *Campylobacter* species were isolated from milk.

The result of milk samples is in agreement with results of Beumer *et al*. (1992) who reported 5% isolation rate from milk. The low rate of isolation of *Campylobacter* spp. in this study is in line with the observations of some authors who reported incidence rates of between 0 and 8.1% of *Campylobacter* spp. in raw milk (Orr *et al*., 1995; Stanley and Jones, 2003; Rosef *et al*., 1983). The prevalence rate of 4.8% in this study was however lower than the 12% reported by Rohrbach *et al*. (1992). *Campylobacters* are normal flora of the GIT therefore; its detection in the milk may be due to contamination by faeces and through direct contaminations of milk as a consequence of mastitis (Hudson *et al*., 1984). The low rate observed in this study is an indication of low faecal contamination of milk in the state.

In the breed specific prevalence rate, the highest prevalence of 5.7% in Red Bororo breed shows that there are more likely chances of faecal contamination of the milk of red Bororo than in other breeds. The 0% isolation rate observed in the Friesian Cross could perhaps be due to the extreme care and observance of hygienic procedures when milking Friesian cross because of their high milk yield as against the local breeds. The 0% isolation rate agreed with observation of Rosef *et al* (1983), Uche *et al*. (1987) and Waterman and Park (1982). There was no statistical association between breeds and rates of *Campylobacter* isolation from milk (p>0.05). The isolation of *Campylobacter* from milk is of serious public health concern in the state considering the feeding habit of the people who consumed milk in its raw form without pasteurizing it. Surveys of raw agricultural products support epidemiologic evidence implicating meat and raw milk as source of human infection (Altekruse *et al*., 1999). The seasonal prevalence showed that the cold dry season had the lowest rate of 2.1%, while the highest prevalence rate of 6.4% was observed during the wet season. This indicates that there are more chances of fecal contamination of milk during the wet and hot season than in the cold dry season. There was no statistical association between the seasons and faecal contamination of milk (p>0.05).

All the isolates from the milk samples were identified as *Campylobacter jejuni* biotype I. The biotyping of the isolates from raw meat showed that *C. jejuni* biotype I was the most common biotype of *C. jejuni*. This is in line with the findings of Pezzotti *et al*. (2003) and Saenz *et al*. (2000), who observed that 78-96% of *C. jejuni* isolates were *C. jejuni* biotype I. The finding also revealed that *C. coli* biotype I was more common than biotype II.

The most common *Campylobacter* species in raw milk in this study was *C. jejuni* biotype I. This implies that *C. jejuni* biotype I is the most frequently isolated *Campylobacter* species from food animals in Sokoto state (Salihu *et al.,* 2009a, b, c, d). Studies have shown that *C. jejuni* is the most common in causing human disease than *C. coli* (Skirrow, 1998; Altekruse *et al*., 1999; Rautelin and Hanninen, 2000). *Campylobacter jejuni* biotype I prevailed in humans as described by Lior (1984) and Varoli *et al*., (1991). The observations in this study may suggest that animals and animal products are the reservoir for human infection.

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