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Pathogenic *Yersinia enterocolitica* 2/O:9 and *Yersinia pseudotuberculosis* 1/O:1 strains isolated from human and non-human sources in the Plateau State of Nigeria

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ABSTRACT

Foodborne yersiniosis, caused by enteropathogenic *Yersinia*, especially *Yersinia enterocolitica*, is an important cause of diarrhea in developed countries, especially in temperate zones. Since studies concerning the presence of enteropathogenic *Yersinia* in humans and foods are rare in developing countries and tropical areas, human and non-human samples were studied in Plateau state of Nigeria to obtain information on the epidemiology of *Y. enterocolitica* and *Yersinia pseudotuberculosis*. Surprisingly, *ail*-positive *Y. enterocolitica* and *inv*-positive *Y. pseudotuberculosis* were isolated in Plateau state of Nigeria from several samples of human and non-human origin. Bioserotype 1/O:1 was the only *Y. pseudotuberculosis* type found. *Y. enterocolitica* belonging to bioserotype 2/O:9 was the dominating type found in most samples. Bioserotype 4/O:3 was isolated only from one pig and one sheep. Using PFGE, 5 genotypes were obtained among 45 *Y. enterocolitica* 2/O:9 strains with *NotI*, *ApaI* and *XhoI* enzymes and 3 among 20 *Y. pseudotuberculosis* 1/O:1 strains with *NotI* and *SpeI* enzymes. All human *Y. pseudotuberculosis* 1/O:1 strains were indistinguishable from pig, sheep or food strains. The dominating genotype of *Y. enterocolitica* 2/O:9 strains among humans was also found among strains isolated from pig, fermented cow milk and traditional intestine pepper soap samples.

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1. Introduction

Foodborne diseases are a widespread and growing public health problem in developed and developing countries (Schlund, 2002). The high prevalence of diarrheal diseases in many developing countries, including Nigeria, suggests major underlying food safety problems (Ehiri et al., 2001). Foodborne yersiniosis is caused by enteropathogenic *Yersinia enterocolitica* and *Yersinia pseudotuberculosis* (Fredriksson-Ahomaa et al., in press). Although *Y. enterocolitica* is a frequent and important cause of human disease in developed countries, especially in temperate zones (Anonymous, 2007), *Y. enterocolitica* infections have also sporadically been reported in Nigeria (Agbonlahor et al., 1983; Onyemelukwe, 1993; Okwori et al., 2007). Human *Y. pseudotuberculosis* infections are rare; however, several *Y. pseudotuberculosis* outbreaks among humans have been reported in Finland, Russia and Japan (Tsubokura et al., 1989; Nuorti et al., 2004; Jalava et al., 2004, 2006; Anonymous, 2005–2008; Rimhanen-Finne et al., 2008). In France, sudden increase of human *Y. pseudotuberculosis* infections was reported in 2004–2005 (Vincent et al., 2008). No information is available of the presence of *Y. pseudotuberculosis* in tropical area.

All *Y. pseudotuberculosis* strains are potentially pathogenic for humans and for a wide range of animal species, unlike *Y. enterocolitica* strains (Fredriksson-Ahomaa et al., in press). One reliable indicator of *Y. enterocolitica* pathogenicity is biotype. Serotype alone is not a reliable virulence marker because several serotypes are common among both pathogenic and non-pathogenic strains. *Y. enterocolitica* strains belonging to bioserotypes 2/O:9, 2/O:5,27 and 4/O:3 are commonly associated with human infections. The *ail* gene found in the chromosome of pathogenic *Y. enterocolitica* has been widely used to identify pathogenic strains, while the chromosomal *inv* gene has been utilized to identify *Y. pseudotuberculosis* strains (Nakajima et al., 1992; Fredriksson-Ahomaa et al., 2006a).

Pigs have been shown to be an important reservoir of pathogenic *Y. enterocolitica*, particularly for strains of bioserotype 4/O:3 (Fredriksson-Ahomaa et al., 2006b). Strains of bioserotypes

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2–3/O:5,27 and 2–3/O:9 have also been sporadically isolated from cattle, sheep and goats (Gourdon et al., 1999; McNally et al., 2004). Pathogenic *Y. enterocolitica* of serotypes O:3 and O:8 have been isolated from pigs and cattle, respectively, in Nigeria (Adesiyun et al., 1986). The host range of *Y. pseudotuberculosis* is broad, but the principal reservoirs are believed to be wild animals (Fukushima et al., 1998; Fredriksson-Ahomaa et al., in press). Bioserotype 2/O:3 has frequently been isolated from slaughter pigs in Finland (Niskanen et al., 2002, 2008; Laukkanen et al., 2008) and from cattle and buffalos in Brazil (Martins et al., 2007).

Food has been proposed to be the main source of intestinal yersiniosis, although pathogenic isolates have seldom been recovered from food samples. Indirect evidence suggests that food, particularly pork, is an important link between the pig reservoir and human *Y. enterocolitica* infections (Fredriksson-Ahomaa et al., 2006a). A positive correlation between the consumption of raw or undercooked pork or drinking of untreated water and the prevalence of yersiniosis has been demonstrated in case-control studies (Tauxe et al., 1987; Ostroff et al., 1994). Concerning *Y. pseudotuberculosis* outbreaks, fresh produce and untreated surface water has shown to be potential infection sources (Tsubokura et al., 1989; Nuorti et al., 2004; Jalava et al., 2004, 2006; Rimhanen-Finne et al., 2008).

Very little information about the presence of enteropathogenic *Yersinia* in human and non-human sources, and about contamination routes in foods is available in developing countries and tropical regions. Thus, the presence of pathogenic *Y. enterocolitica* and *Y. pseudotuberculosis* was studied in Nigeria and strains isolated from different sources were characterized phenotypically and genotypically in order to clarify possible contamination routes of these pathogens.

2. Methods

The presence of *Y. enterocolitica* and *Y. pseudotuberculosis* was studied in 500 human, 750 animal and 750 food samples collected in the Plateau State of Nigeria between 2002 and 2004 (Table 1). The human fecal samples originated from adults with diarrhea. About 1 g per sample was inoculated into 10 ml of phosphate-buffered saline (PBS) (Life Technologies Ltd., Paisley, Scotland), which was incubated at 4 °C for 21 days. Alkali treatment in 0.25% KOH solution for 20 s immediately before plating on cefsulod in–irgasan–novobiocin (CIN, Oxoid, Basingstoke, UK) and MacConkey (Oxoid) agar plates was used to reduce competitive bacterial flora. The plates were incubated under aerobic conditions at 30 °C for 18–48 h. Putative *Yersinia* colonies were identified with oxidase, Kligler iron (Biotec, Suffolk, UK) and Christensen's urea (Oxoid) tests. Oxidase-negative, glucose-positive, H_2S-negative and urease-positive colonies were finally identified with API 20E strips (Bio-Mérieux, Marcy l'Etoile, France). Polymerase chain reaction (PCR) targeting the *ail* gene in the chromosome of pathogenic *Y. enterocolitica* and the *inv* gene in the chromosome of *Y. pseudotuberculosis*, according to Nakajima et al. (1992), was used to identify pathogenic *Y. enterocolitica* and *Y. pseudotuberculosis* isolates.

Y. enterocolitica and *Y. pseudotuberculosis* isolates were biotyped and serotyped. *Y. enterocolitica* isolates were biotyped using pyrazinamidase, esculin, salicin, tween, indole, xylose and trehalose tests according to Wauters et al. (1987) and *Y. pseudotuberculosis* isolates using melibiose, raffinose and citrate tests according to Tsubokura and Aleksic (1995). Serotyping was carried out with a slide agglutination test using commercial antisera O:1, O:2, O:3, O:5 and O:9 (Denka Seiken, Tokyo, Japan) for *Y. enterocolitica*, and antisera O:1, O:2, O:3, O:4, O:5 and O:6 for *Y. pseudotuberculosis* (Denka Seiken).

Pathogenic *Y. enterocolitica* and *Y. pseudotuberculosis* isolates were characterized with PFGE according to Fredriksson-Ahomaa et al. (2003) and Niskanen et al. (2002), respectively. The DNA was restricted with *NotI, ApaI* and *XhoI* enzymes (New England Biolabs, Beverly, Massachusetts, USA) for *Y. enterocolitica* (Fredriksson-Ahomaa et al., 2003) and with *NotI* and *SpeI* enzymes (New England Biolabs) for *Y. pseudotuberculosis* (Niskanen et al., 2002, 2008).

Table 1
Number of Nigerian human and non-human samples contaminated with enteropathogenic *Yersinia enterocolitica* (YE) and *Yersinia pseudotuberculosis* (YP).

Sample		Number of samples	*ail*-positive YE (47)[a]		*inv*-positive YP (20)
			2/O:9 (45)	4/O:3 (2)	1/O:1 (20)
Human					
	Feces	420	27	0	6
	Appendix	80	2	0	0
Animal					
Pig	Feces	200	6	1	4
	Tonsils	150	3	0	0
Sheep	Feces	200	1	1	1
Dog	Feces	200	0	0	0
Food					
Cow milk	Fermented	250	3	0	4
Soup[b]	Heated	100	3	0	0
Fish	Raw	50	0	0	2
	Roasted	100	0	0	3
Lettuce		250	0	0	0

[a] Number of positive samples in parenthesis.
[b] Soup called *kayanchik* or intestine pepper soap is made from pig, cow and sheep intestines.

3. Results and discussion

Both *ail*-positive *Y. enterocolitica* and *inv*-positive *Y. pseudotuberculosis* were isolated in Plateau state of Nigeria from several human, animal and food samples (Table 1). While yersiniosis has traditionally been associated with countries with temperate climate, present results demonstrate that pathogenic *Y. enterocolitica* and *Y. pseudotuberculosis* are a common finding in feces of humans with diarrhea in tropical region. It is the first time *Y. pseudotuberculosis* was isolated from patients in Nigeria. Additionally, *Y. enterocolitica* was isolated twice from human appendix. Right-sided abdominal pain and fever can be the predominant symptoms of yersinioisis and may be confused with appendicitis. Unnecessary appendectomies have thus been linked to yersinosis patients (Nuorti et al., 2004).

Y. enterocolitica belonging to bioserotype 2/O:9 was surprisingly the only pathogenic type found in human samples collected in the Plateau State of Nigeria (Table 1). *Y. enterocolitica* of bioserotype 4/O:3 is most frequently isolated from humans globally (Fredriksson-Ahomaa et al., in press). In earlier studies, *Y. enterocolitica* of bioserotype 4/O:3 and bioserotype 2/O:5,27 have been isolated from children with gastroenteritis in Nigeria (Agbonlahor et al., 1983; Onyemelukwe, 1993). In this study, bioserotype 4/O:3 was only found in the fecal samples of one pig and one sheep. While *Y. enterocolitica* bioserotype 2/O:9 seemed to predominate in pigs in the Plateau State of Nigeria, in Europe this type has frequently been isolated in pigs, cattle and sheep only in the United Kingdom (UK) (McNally et al., 2004), and in cattle and goats in France (Gourdon et al., 1999).

All Nigerian *Y. pseudotuberculosis* strains from humans, animals and foods were of bioserotype 1/O:1 (Table 1). Serotype O:1 has been shown to be the most common serotype in human infections and animal sources in Europe, followed by serotype O:3 (Aleksic et al., 1995; Vincent et al., 2008). Several recent outbreaks of

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