

## The "Emerging" Foodborne Bacterial Zoonoses

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Beginning in the 1970's, the food industry and other regulatory agencies became concerned about the emergence of other foodborne pathogens such as *Yersinia enterocolitica*, *E. coli* O157, *Listeria monocytogenes*, in addition to the known principal foodborne pathogen which is *Salmonella* spp. In the late 70's, *Campylobacter* spp. emerged as an additional pathogen. Data from the WHO have shown this pathogen surpassing *Salmonella* spp. as a major pathogen-causing diarrhea in children in developing countries. An overall prevalence of 12.5% was seen in infections caused by *Campylobacter* compared to *Salmonella*, which were only 2.5%. In the year 2000, the National Epidemic Sentinel Surveillance Field Epidemiology Program (NESS-FETP) of the Department of Health (DOH) reported a total number of 866,411 cases with 113.8 rate per 100,000 population due to diarrhea. Diarrhea still ranks number one as the leading cause of morbidity in the Philippines.

In March 1995, a WHO Consultation Meeting was convened in Berlin, Germany in the hope that the work of the Consultation would be instrumental in protecting consumers and achieving the goal of "Safe Food for All". The objective of the meeting was to review current knowledge on the epidemiology, prevention and control of the four "emerging" pathogens, *Yersinia enterocolitica*, *E. coli* O157, *Listeria monocytogenes* and *Campylobacter jejuni*.

Several factors have created new possibilities for the spread of new microorganisms which are pathogenic to man and animals. Changes in microbial populations have led to the evolution of new pathogens, development of new virulence factors for existing pathogens, development of antibiotic resistance, which could make a disease more difficult to treat or to changes in environmental conditions that organisms will normally tolerate. Unlike some other animal and human pathogens, "emerging" foodborne pathogens such as *Listeria* spp. and *Yersinia* spp. not only survive

in the environment but also multiply under favorable conditions. It was apparent for decades that *Campylobacter* spp. were associated only with warm-blooded animals, but their significance as human pathogens was not established because of the lack of detection methods (Norcross *et al.*, 1992). However, recent advances in epidemiology and microbiology have directly resulted in association of *Campylobacter* spp. with human enteric illnesses.

On the other hand, cattle have also emerged as the principal animal reservoir of *E. coli* O157:H7 and outbreaks have been associated with beef and milk products.

Poorly regulated antimicrobial use during animal and aquaculture production can pose human health hazards. Because of globalization of the food supply and increasing international travel, multi-resistant organisms can spread to all parts of the world. A change in human demographics means that there are now more people with increased susceptibility to foodborne and other diseases such as pregnant women, neonates, elderly (over 65 years), residents in nursing homes or related health care facilities, cancer patients, organ transplant patients, AIDS patients, among others. Changes in food production have also created similar problems to those outlined on the migration of people with new or altered pathogens having the potential to be introduced into new populations. There is thus the potential for larger and geographically widespread outbreaks of foodborne disease.

Adherence to Good Agricultural Practices and disease prevention programs should under normal conditions, ensure that pathogens are kept under control as much as possible. However, new situations arise and these need careful surveillance in order to detect new pathogens that have arisen. *Listeria*, *Yersinia*, *Campylobacter* and *E. coli* O157 may present particular problems in this respect.

There is a dearth of information on review of related literature and studies in the Philippines on the four "emerging" bacterial zoonoses. It is the purpose of this paper to provide some necessary information and data available for Filipinos to better understand the etiology, pathogenesis and hopefully prevention and control of diseases caused by these bacterial pathogens.

## The Four "Emerging" Foodborne Zoonoses

### *Campylobacter* spp.

*Campylobacter* spp. is now considered one of the most important enteric pathogen in addition to *Salmonella* spp. and *E. coli*. The disease, campylobacteriosis, is manifested as a form of enteritis and is caused by two related species, *Campylobacter jejuni* and *C. coli* but *C. jejuni* is predominant. The organism has long been recognized as a veterinary problem causing diarrhea in cattle and septic abortion in both cattle and sheep. The two species have only been recognized as an important cause of human illness for only the last 30 years. In humans, the usual manifestation of campylobacter enteritis is a very unpleasant attack of acute diarrhea lasting for about 5 days (Skirrow and Blaser, 1992). The pathology is that of an acute inflammatory enterocolitis and stool microscopy almost invariably shows the presence of cellular exudate, and frank blood which is visible in about one-quarter of patients. Fever and other signs of acute infection are common in the early stages of the disease. With the development of better culture and serologic techniques, it has been possible to define new associations of campylobacter infection with new diseases. Since laboratories began to isolate *Campylobacter* from stool specimens some 20 years ago, there have been many reports of Guillain-Barre syndrome (GBS) following *Campylobacter* infection (Nachamkin et al. 1988). GBS is an autoimmune disorder of the peripheral nervous system characterized by weakness, usually symmetrical, evolving over a period of several days or more (Adams and Victor, 1993).

Published information indicate that campylobacteriosis is a zoonotic disease. Pets, water and contaminated foods, mainly poultry products, unpasteurized milk, are considered the main sources of sporadic infections in humans (Svedhem and Kajiser 1980, 1981; Morgan et al. 1985; Harris et al., 1986; Tauxe et al., 1988; Tauxe, 1992). According to Stern (1992), cross-contamination and improper handling and cooking of foods of animal origin account for the majority of the disease.

Campylobacter enteritis is essentially a foodborne zoonosis and the principal vehicle of infection is raw or undercooked meat. Any raw meat is likely to be contaminated with campylobacter organisms. Poultry are by far the most important source especially broiler chickens and they are consumed in such prodigious quantities. Poultry not only harbor many campylobacters in their rectum but also become heavily cross-contaminated during mass mechanized processing.

To date, very studies have been conducted in the Philippines. There is an on-going study in the University of the Philippines National Institutes of Health (UP-NIH) on the "emerging" foodborne bacterial pathogens where research investigators are trying to isolate and identify *Campylobacter* spp. from susceptible foods.

### *Listeria monocytogenes*

*Listeria monocytogenes* is a Gram-positive, facultative intracellular bacterium that causes severe diseases in both animals and humans. The organism has been implicated as the causative agent in several foodborne outbreaks of listeriosis in North America and Europe. The normal route of entry of *L. monocytogenes* into the host is the gastrointestinal tract via listeria-contaminated food as shown by several outbreaks of listeriosis. These could be traced to contaminated food such as milk, Mexican-style cheeses, meat and meat products and vegetables including coleslaw (Farber and Perkin, 1991).

Since the beginning of 1980's a number of cases of listeriosis caused by consumption of foods such as cheese, meat, ice cream and fish have been reported overseas. *Listeria monocytogenes* serotypes 1/2a, 1/2b and 4b have been almost exclusively implicated in these incidents. Thus, listeriosis became one of the important causes of food-borne infections especially in immunocompromised individuals, as well as among the elderly. Clinical manifestations of listeriosis include septicemia, meningitis, brain stem encephalitis, and liver abscess. The organism also causes abortion and stillbirth in pregnant women. Despite modern antibiotic therapy, neonatal listeriosis still has a mortality rate of about 36% as reported by Evans et al., 1985. To prove that a human case of listeriosis emanates from food, it is necessary to show that *L. monocytogenes* strains isolated from the food and the patient are identical.

There are sporadic cases reported in other countries but the disease is not yet well studied in the Philippines. Cases of listeriosis in the country have been reported as early as 1963 (Garcia and Manahan, 1963; Garcia, 1964) but mode of transmission is still unknown. To date, not much has been known about the disease in the local setting. Perhaps during those times in the early 60's diagnostic methods to identify the causative organism are not yet available which

probably explains why the disease remains undetected or undiagnosed. Since the sources of infection is still unknown and such food-borne outbreaks have not yet been reported in the country, there is a need to study the prevalence of *Listeria monocytogenes* in susceptible food products. In 2001, a group of BS Public Health students under the supervision of Dr. Bungay conducted a prevalence study, which aimed to isolate *Listeria* spp. from locally-produced "kesong puti". Results of their study have shown other species of listeria, which include *L. ivanovi*, *L. welshimeri* and *L. dentificans* but not *L. monocytogenes*. Another group of students is studying the prevalence of these organisms in processed meat products such as corned beef.

*Listeria monocytogenes* is the only species of listeria known to cause foodborne disease in humans. All *L. monocytogenes* strains should be considered potentially pathogenic to humans at present. The reservoir of *L. monocytogenes* is primarily warm-blooded animals and their surroundings. The organism is also frequently present in the environment of processing plants where it can establish as "endemic" strains. Processed foods can be contaminated from raw foods and processing equipment. Cross-contamination of final products from raw products is one of the major factors in the spread of the bacterium. It seemed that it is now appropriate to conduct prevalence studies to determine the extent of the problem, which will later on provide the benefits of safety to both consumers and the food industry alike. Foodborne listeriosis is not only a threat to public health with high mortality rates but may also cause loss of economic productivity. In the advent of international trade, much emphasis has been placed on food safety, and foodborne pathogens like *Listeria monocytogenes* is definitely a public health concern.

#### *Yersinia enterocolitica*

The World Health Organization (WHO) has classified *Yersinia enterocolitica* to be a "rapidly emerging worldwide pathogen" associated with a wide spectrum of clinical and immunologic manifestations. Human *Y. enterocolitica* infection is characterized by enteritis and/or abdominal pain due to mesenteric lymphadenitis, sometimes imitating appendicitis. The disease may be complicated by reactive arthritis, especially in person exhibiting the histocompatibility complex HLA B27. There are reports overseas that septicemic diseases have occurred occasionally among older persons and patients with metabolic or hemolytic illness or other severe compromising disease.

Healthy pigs are known to carry the pathogenic strains of *Y. enterocolitica* (Christensen, 1980; Doyle et al., 1981; Nesbakken and Kapperud, 1985;

Pedersen, 1979). This organism was first isolated in 1939 and has been recognized as a major etiologic agent of gastroenteritis more commonly among children.

Pigs (tonsils and feces) are regarded as an important reservoir for the human pathogenic *Y. enterocolitica* serogroups. It has also been found by previous studies conducted overseas that sources of infections include ingestion of contaminated water, drinking water from streams or wells, foods such as vegetables which have been washed in contaminated water and milk products. Cattle are not reservoirs of pathogenic serogroups of *Y. enterocolitica* but milk and milk products have been implicated in outbreaks. These may have occurred as a result of cross-contamination from other sources such as contaminated water or pigs.

Early reports of isolation of *Yersinia enterocolitica* were mostly in European and other well-developed countries such as Canada (Toma and Lalaur, 1981), Japan (Maruyama, 1987) and the United States (Bottone, 1983; Shayegeani et al., 1981). In a report of the WHO Consultation Meeting in Berlin, it was mentioned in one of the reports that *Y. enterocolitica* infection appears to be confined to moderate climates; reports from tropical areas are scarce (Africa) or absent (South and Southeast Asia) but under-reporting cannot be excluded. Although isolation of the organism is uncommon in tropical and developing countries (Robins-Browne, 1992), it has been isolated in Nigeria (Agbonlahor et al., 1983), Singapore (Ho and Koh, 1981), Trinidad (Adesiyun et al., 1992) and the Philippines (Egos and Bungay, 1999, unpublished).

In the local setting which occurred in 1997, there were two cases of diarrhea in children admitted in the Philippine General Hospital (PGH). The causative agent isolated was *Yersinia* spp. This finding highly suggests that this organism might be an important agent of undiagnosed diarrheal cases in the Philippines.

A link exists between yersiniosis (a disease cause by *Yersinia enterocolitica*) and consumption of undercooked pork, sausages and untreated water (Christensen, 1987; Cornelis et al., 1987; Tauxe et al., 1987). *Y. enterocolitica* has been isolated from a wide variety of foods, such as milk and milk products, raw meats, (beef, pork and lamb), poultry, vegetables and miscellaneous food products (Mollaret, 1979). Because of the association between *Y. enterocolitica* O:3 and pigs, the incidence of this organism in pork and pork products has been widely investigated (Gilmour and Walker, 1988).

Ostroff (1995) in his study, found an association between high incidence of yersiniosis and pork consumption. A higher risk for this organism may be found among Filipinos, majority of whom are pork eaters.

In the local setting, a preliminary study on *Yersinia enterocolitica* among slaughtered pigs in a Metro Manila abattoir was conducted. This can be considered the first local study undertaken to investigate the presence of this bacterial pathogen from freshly slaughtered pigs. The study was a joint collaborative research between the Department of Medical Microbiology, UP College of Public Health (MS in Public Health thesis) and the National Meat Inspection Commission, Department of Agriculture (NMIC-DA). Results showed that 14 out of the 384 paired tonsils (3.6%) examined were positive for *Y. enterocolitica* serogroup O:3 biotype 4, VP (-) variant. This strain is highly associated with human disease and is considered as the Asian strain. A research study is currently underway to determine the prevalence of *Yersinia* spp. from susceptible Philippine foods. (Bungay et al., 2002).

### *E. coli* O157

The enterohemorrhagic *E. coli* (EHEC) is considered as the most important pathogen among the pathogenic species of *Escherichia coli*, other than the enteropathogenic *E. coli* (EPEC), the enterotoxigenic *E. coli* (ETEC) and the enteroinvasive *E. coli* (EIEC). Majority of the studies on EHEC were concentrated on *E. coli* O157:H7, which has been associated with numerous outbreaks and sporadic infections in humans worldwide (Reilly, 1998). The disease caused by Shiga-toxin producing *E. coli* (STEC), also known as verocytotoxin *E. coli* (VTEC) can range from uncomplicated diarrhea to hemorrhagic colitis (HC), to hemolytic uremic syndrome (HUS) (Karmali 1989; Griffin and Tauxe, 1991). Reported cases of HUS in developed countries such as the U.S.A. and U.K. are steadily increasing and HUS is now the single commonest cause of acute renal failure in children. Reported outbreaks of *E. coli* O157 infection have also been very severe, with high mortality rates in the elderly (Chapman, 1999).

In humans, infections due to these pathogens are often caused by contaminated foodstuffs like meat and unpasteurized milk (Dorn, 1988; Karmali, 1989; Salmon et al., 1989). Results of various epidemiological investigations have shown that cattle frequently harbor STEC infection (Montenegro et al., 1990; Orskov et al., 1987; Wells et al., 1991). In fact, the first documented outbreak of HC caused by *E. coli* O157 which occurred in Michigan and Oregon, U.S.A. in 1982, has shown strong epidemiological association between infection and prior consumption of ground beef from one chain of fast-food restaurants.

Although outbreaks of human *E. coli* O157:H7 infection have been most frequently linked with the consumption of undercooked minced beef, waterborne

outbreaks are increasingly recognized as well as person-to-person transmission. There is direct and indirect evidence that the following foods have acted as sources or vehicles of human *E. coli* O157:H7 and non-O157:H7 VTEC infections: beef, pork, lamb chicken, turkey, sausages, salami, hamburgers, fish, shellfish, vegetables, milk, yoghurt, cheese, apple cider, and water, both for human and recreational purposes. The role and importance of these vehicles for transmission has yet to be established. A range of other risk factors has been identified and these include contact with carrier animals and consumption of surface water.

In the local setting, a preliminary study was undertaken by a group of BS Public Health students at the University of the Philippines Manila. *E. coli* O157 was isolated from the feces of freshly slaughtered cattle in a Metro Manila abattoir. The study was however, limited only to identification of serotype O157 because of the non-availability of other typing antisera. Identification of vehicles and measurement of infection rates is often complicated by current poor isolation/detection methods and differences in laboratory techniques and clinician practices.

Surveillance of foods and food animals reveal many Vero-toxin *E. coli* (VTEC)-positive isolates, belonging to a range of *E. coli* serotypes. It has yet to be established which of these are capable of causing infections to humans.

We conducted a two-month collaborative study on isolation and identification of VTEC or Shiga-toxin *E. coli* (STEC) from fecal samples (cattle, goats and carabaos) coming from the Philippines at the Division of Emerging Pathogens, Robert Koch Institute in Berlin, Germany under the supervision of Dr. Lothar Beutin. The laboratory is well equipped with state-of-the-art facilities for studying VTEC. The study was supported by the DAAD (German Academic Exchange Service). The results of this research conform to previous studies conducted by Dr. Beutin and other investigators (Beutin et al., 1993). VTEC or STEC from different sources and geographical areas were found to belong to a large number of serotypes. In this study, VTEC were found to be particularly frequent in 3 different animal sources (cattle, goats and carabaos). The serotypes isolated were found to vary among these 3 ruminant species. The VTEC from cattle were found to be different from the VTEC of goats and carabaos. Based on this research study, certain types of STEC are well adapted to their ruminant hosts. These O:H serotypes such as O82:H+, ONT:H+ (O-nontypable), O73:H+ from cattle; O91:H-, O76:H+, O146:H+ from goats and O22H+ from carabaos have been implicated as human pathogens. *E. coli* O157:H7 was isolated from 2 goats but was found to be VTEC-negative by reversed passive latex agglutination (RPLA) test. Since these ruminants

harbor VTEC or STEC in their feces, they therefore represent an important source of infection for humans and should never be underestimated.

This is a preliminary study for the Philippines where samples were collected from a relatively small geographical area, which is only in Luzon. We intend to continue our study on STEC increasing the sample size and expanding collection of samples from the Visayas and Mindanao areas. We also plan to do research on susceptible foods such as raw ground beef, unpasteurized milk and vegetables. The only drawback of this plan is the lack of an emerging pathogen laboratory for microbiological assays.

## Conclusion

In the Philippines, the threat of "emerging" foodborne bacterial pathogens cannot be underestimated and there is a need to develop active surveillance systems for the foodborne diseases under consideration to:

- determine susceptible food/food products
- identify the risk factors likely to have the greatest impact on the incidence of the disease
- provide a basis for specific control and prevention strategies along the entire food production chain aimed at reducing the incidence of foodborne infection in humans.

There is also a need to adopt internationally validated methods for isolation and identification including rapid methods, which are applicable for all relevant species of pathogens under consideration. The studies should include methods for the examination of clinical, environmental and food samples and should provide the optimum opportunity of isolating the target pathogen.

From time to time, crises on other "emerging" foodborne zoonoses are expected to arise with major effects on food safety and public health, consumer confidence, economy and public policy. Such crises should not cause another food scare to add to the long list of diseases that have dominated the news at regular intervals in the last two decades. The Philippines even as a developing country should by then be fully prepared to handle such crises in the near future.

Individuals can protect themselves from infections due to these bacterial pathogens, as more information become available. Safe food handling practices from farm to fork should ensure food safety and quality.

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