
Edwardsiellosis, common and novel manifestations of the disease: A review

Edwardsiellosis, manifestaciones usuales y nuevas de la enfermedad: Una revisión

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Resumen

Edwardsiella tarda es una bacteria Gram-negativa encontrada comúnmente en ambientes y animales acuáticos donde causa edwardsiellosis o septicemia por *Edwardsiella tarda*. La bacteria tiene una distribución mundial y un alto potencial de infectar a humanos, causando infecciones que van desde una enfermedad gastrointestinal autolimitante en recién nacidos y adultos ancianos hasta infecciones extraintestinales similares a aquellas observadas en peces. Las lesiones incluyen abscesos, piogranulomas y necrosis en tejidos, como el cerebro, el hígado, la piel y los músculos. La distribución sistémica del microorganismo usualmente termina en septicemia. Varios de los cambios patológicos inducidos por *E. tarda* en humanos son consistentemente observados en peces enfermos, y estos animales constituyen un modelo apropiado para el estudio de la patogénesis de edwardsiellosis. En esta revisión, se describen las manifestaciones clínicas, los cambios patológicos macroscópicos y microscópicos comunes y nuevos de la enfermedad en dos especies de peces de importancia comercial: el lenguado japonés (*Paralichthys olivaceus*) y la tilapia híbrida (*Oreochromis spp.*), así como la variedad de infecciones reportadas en humanos.

Palabras clave: *Edwardsiella tarda*, gastroenteritis, abscesos, septicemia, peces, humanos.

Abstract

Edwardsiella tarda is a Gram-negative bacterium commonly found in aquatic environments and in water-borne animals where it causes a disease named edwardsiellosis or *Edwardsiella septicemia*. The bacterium is distributed worldwide and has a high potential to infect humans, causing infections ranging from self-limited gastrointestinal disease particularly in newborns and aged, and a variety of extraintestinal infections similar to those observed in affected fish, including pyogranulomatous inflammation, abscesses and necrosis in different tissues such as the brain, liver, skin and muscles. Systemic dissemination of the micro-organism usually ends in septicemia. Many of the pathological changes induced by *E. tarda* in humans are consistently observed in diseased fish, and these animals seem to be an appropriate model to study the pathogenesis of edwardsiellosis. In this review we describe common and novel clinical, gross and histopathological manifestations of the entity in two commercial fish species, Japanese flounder (*Paralichthys olivaceus*) and tilapia hybrids (*Oreochromis spp.*), as well as the diversity of infections documented in humans.

Keywords: *Edwardsiella tarda*, gastroenteritis, abscesses, septicemia, fish, human.

Introduction

Edwardsiella tarda is a Gram-negative bacterium originally found in humans with and without clinical disease. The microorganism was recognized in the United States and Japan as early as 1959. The initial study in Japan described 5 out of 256 isolates from feces of humans with symptoms of gastroenteritis (Sakazaki, 1965), however was Ewing et al. (1965) who proposed *Edwardsiella tarda* as a new genus and species in the family Enterobacteriaceae, reporting 34 out of 37 strains isolated from humans in USA, Brazil, Ecuador, Israel and Japan. Of those strains, twenty five originated from human feces, five of which manifested diarrhea (Ewing et al., 1965). Two additional species, *E. hoshinae* (Grimont et al., 1980) and *E. ictaluri* were added to the genus but have limited host distribution, particularly *E. ictaluri* was recognized as the causative agent of the enteric septicemia of catfish (*Ictalurus punctatus*) (Hawke et al., 1981), and it may also affect Danio (*Danio devario*) and Blue tilapia (*Tilapia aurea*) (Bullock and Herman, 1985). Despite of the initial association of *E. tarda* with diarrheal disease in humans, the bacterium was subsequently isolated from diseased fish, and the infection in humans was considered accidental or associated with close contact with aquatic environments, diet habits and underlying clinical diseases.

E. tarda is a small, straight rod of about 1 µm in diameter and 2-3 µm in length that forms small round, convex, transparent colonies of approximately 0,5 mm in diameter when grown in common media including trypton soy agar (TSA) for 24 to 48 hr at 26 °C to 30 °C (Inglis et al., 1993). They are usually motile by peritrichous flagella and are facultative anaerobic, catalase-positive, cytochrome oxidase-negative, glucose fermentative, reduces nitrate to nitrite, lactose-negative and indole positive (Table 1). The indole, methyl red, and hydrogen sulfide production and salt tolerance differentiate *E. tarda* from *E. ictaluri* (Wakabayashi and Egusa, 1973). *E. tarda* has been adapted to live in diverse environmental conditions and can be isolated from a wide range of water salinity (0-4% NaCl), pH (4.0-10), and temperatures (14 to 45 °C) (Plumb, 1999), explaining to some extent its capability to cause disease not only to freshwater and marine fish, but also to terrestrial animals. Recently several outbreaks of edwardsiellosis have been diagnosed in tilapia (*Oreochromis* spp.) hybrids in Colombia, indicating an increase in disease prevalence. Here we document novel clinical, gross and histopathological changes in tilapia hybrids as well as the diversity of infections in other aquatic animals and in humans. The data suggests that *E. tarda* induces a very similar type of infection across animal species, providing support to the use of a fish model to understand edwardsiellosis.

Table 1. Biochemical reactions of typical strains of *Edwardsiella tarda*. (Adapted from Wakabayashi and Egusa, 1973).

Test or substrate	Results	Test or substrate	Results
Oxidase	-	Galactose acid	+
Catalase	+	Mannose (acid)	+
Hydrogen sulfide, H ₂ S	+	Sorbose (acid)	-
Urease	-	Lactose (acid)	-
Indole	+	Sucrose (acid)	-
Ammonium	-	Maltose (acid)	+
Methyl red	+	Threhalose	-
Voges-Proskauer	-	Cellobiose	-
Nitrate reduction	+	Raffinose	-
KCN	-	Starch	-
Gelatin	-	Dextrin	-
Starch	-	Glycogen	-
Phenylalanine deaminase	-	Inulin	-
Christensen's citrate	+	Salicin	-
Jordan's tartrate	(+)	Esculin	-
Malonate	-	Glycerol	(+)
Hugh-Leifson's agar	F	Mannitol	-

Glucose (acid)	+	Sorbitol	-
Gas from glucose	+	Dulcitol	-
Arabinose (acid)	-	Adonitol	-
Rhamnose (acid)	-	Erthritol	-
Xylose (acid)	-	Inositol	-
Fructose (acid)	+	Beta-D-galactosidase	-
Ammonium agar	-	Kauffman-Peterson broth,	(+) 25 °C
Citrate	-	Citrate	-37 °C
Glucose	-	D-tartrate	-
Acetate	-	i-tartrate	-
Alginate	-	L-tartrate	-
		Mucate	-
Decarboxylases	+	Lipases	-
Lysine	-	Corn oil	-(25 °C)
Arginine	+	Triacetin	-
Ornithine		Tributylin	

Edwardsiella tarda infection in fish

E. tarda causes a severe disease in freshwater and marine fish in both farmed and wild populations, with serious epizootics reported in North America and Japan (Inglis et al., 1993). It was initially found to cause disease in channel catfish (*Ictalurus punctatus*) in the United States (Meyer and Bullock, 1973), and in eels (*Anguilla japonica*) in Japan (Hoshina, 1962; Wakabayashi and Egusa, 1973). Currently, the disease known as *Edwardsiella* septicemia occurs with high frequency in tropical fish species, which are intestinal carriers and constitute the natural habitat of *E. tarda*. The bacterium is commonly isolated from intestinal samples of commercial fish, which make possible the contamination of fish carcasses during fish processing. These factors, together with consumption of raw fish products, are the most probable source of human diarrhea associated with *E. tarda* in tropical countries (Van Damme and Vandepitte, 1980; Leung et al., 2012)

Regardless of environmental factors characteristic of each aquatic ecosystem, outbreaks of *E. tarda* infection in channel catfish (Meyer and Bullock, 1973), striped bass (*Morone saxatilis*) (Baya et al., 1997), turbot (*Scophthalmus maximus*) (Padros et al., 2006), Japanese flounder (*Paralichthys olivaceus*) (Nakatsugawa, 1983), eels (*Anguilla japonica*) (Wakabayashi and Egusa, 1973), and black mullets (*Mugil cephalus*) (Kusuda et al., 1976), have occurred at water temperature above 30 °C during the summer months and when the organic material in the water augmented considerably. However, an outbreak of edwardsiellosis, which caused up to 80% mortality in Japanese flounders was reported at a water temperature around 18 °C (Kodama et

al., 1987). Handling of infected fish such as harvest and transferring to holding tanks under crowding conditions were associated with a rapid spread of the disease through the fish population.

E. tarda can infect other fish species such as largemouth bass (*Micropterus salmoides*), Japanese red sea bream (*Pagrus major*), yellow tail (*Seriola quinqueradiata*), and common carp (*Cyprinus carpio*) (Plumb, 1999). Japanese flounder and red sea bream are considered to be very susceptible to *E. tarda* infections (Mekuchi et al., 1995); a similar trend of bacterial multiplication in liver, kidney, intestine and blood of experimentally infected fish was observed regardless of the way of exposure, including intraperitoneal injection, oral intubation or immersion methods (Rashid et al., 1997). However, while flounders appear to be susceptible to typical *E. tarda* strains, red sea bream seems to be more susceptible to atypical non-motile strains (Matsuyama et al., 2005). *E. tarda* is associated with warm-water fish species, salmonids are not common hosts, although natural outbreaks of *E. tarda* have been reported in prespawning wild Chinook salmon (*Oncorhynchus tshawytscha*) (Amandi et al., 1982), and in brook trout (*Salvelinus fontinalis*) (Uhland et al., 2000), particularly associated with warm summer months, when the water temperatures range from 17 °C to 20 °C. Epizootic outbreaks of edwardsiellosis were also reported in adult largemouth bass during late summer and early fall (Francis-Floyd et al., 1993). Isolation of *E. tarda* from different fish species, aquatic environments and geographical locations (Wyatt et al., 1979; Rashid et al., 1994; Muratori et al., 2000; Clavijo et al., 2002), may indicate this bacterium has wide distribution in nature, high adaptability and a number of potential reservoirs.

Pathological features of edwardsiellosis

The pathology induced by *E. tarda* is very similar across various fish species and characterized by systemic suppurative abscesses (Miyazaki and Kaige, 1985; Rashid et al., 1997; Plumb, 1999; Darwish et al., 2000). In catfish, small lesions located on the postero-lateral areas of the body progress into abscesses within muscles of the flanks or caudal peduncle; those abscesses rapidly increase in size and are visible as convex, swollen areas on the body surface that when incised, a foul odor is emitted (Meyer and Bullock, 1973; Kusuda et al., 1976). Internally, hyperemia, hemorrhages, swelling of the liver, spleen, kidney, gastrointestinal tract and musculature are also present (Inglis et al., 1993). A distended abdomen due to the presence of abundant

yellowish, sanguineous or fibrino-hemorrhagic ascitic fluid, which can lead to a severe prolapse of the anus and the large intestine is a common manifestation of the natural or experimental infection in Japanese flounder (Figure 1A, 2A), and a similar finding has been reported in turbot and striped bass (Padros et al., 2006). In contrast to the limited lesions developed in experimentally infected striped bass (Baya et al., 1997), experimental infection in Japanese flounder (8-10 g body weight) by a 10-min immersion period in a bacterial solution containing $2,3 \times 10^6$ bacterial cells/mL, a dose very close to the lethal dose 50 percent, LD50% ($3,6 \times 10^6$ CFU/mL) reported previously (Mekuchi et al., 1995), produced almost 100% mortality within a 30 days post-infection period (Figure 2) and reproduced consistently the gross and histopathological changes of the natural disease, including pale and mottled liver with multiples abscesses (Figure 3A, B).

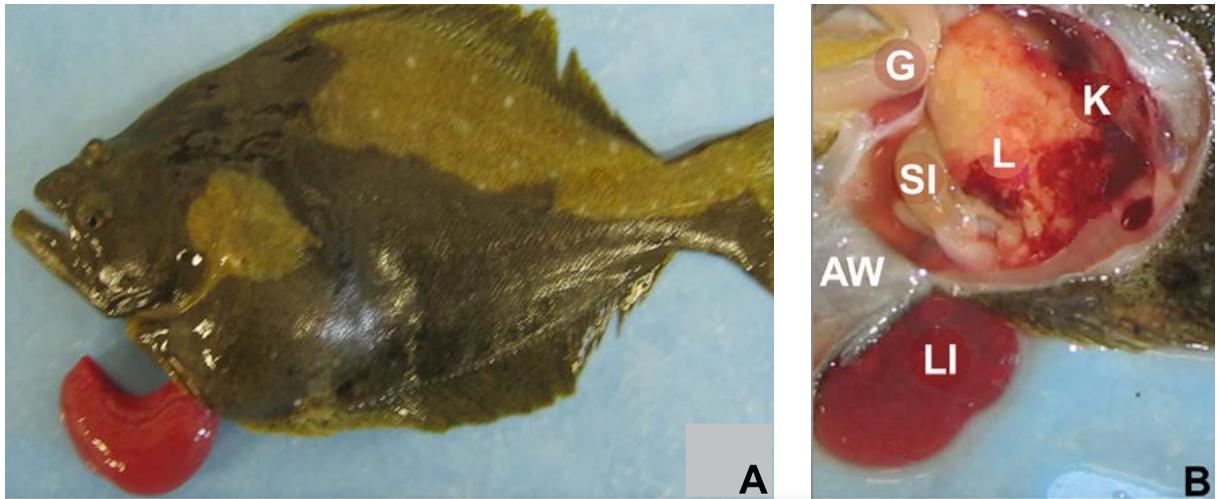


Figure 1. Japanese flounder juvenile naturally infected by *E. tarda*. A. the abdomen is distended and shows prolapsed rectum and large intestine. B. A large amount of sera-sanguineous ascitic fluid accumulated in the abdominal cavity, the liver is severely pale and mottled with multiples abscesses, and it is also covered by a fibrin-hemorrhagic exudate. AW: abdominal wall; G: gill; L: large intestine; SI: small intestine; L: liver; K: kidney.

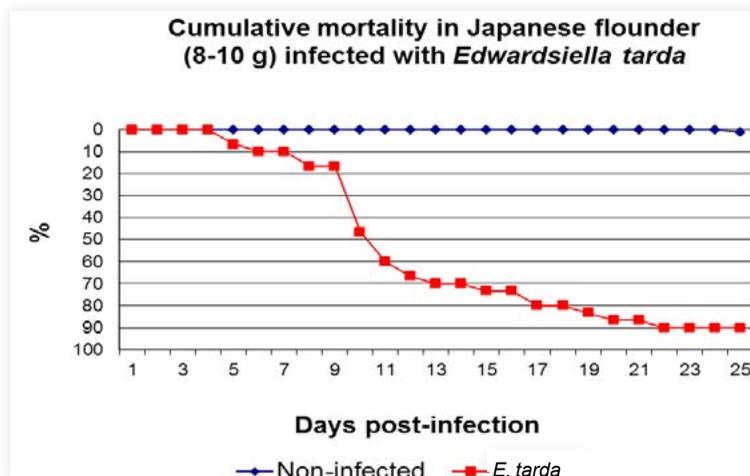


Figure 2. Cumulative mortality in Japanese flounder (*Paralichthys olivaceus*) fingerlings experimentally infected with *E. tarda* by immersion method.

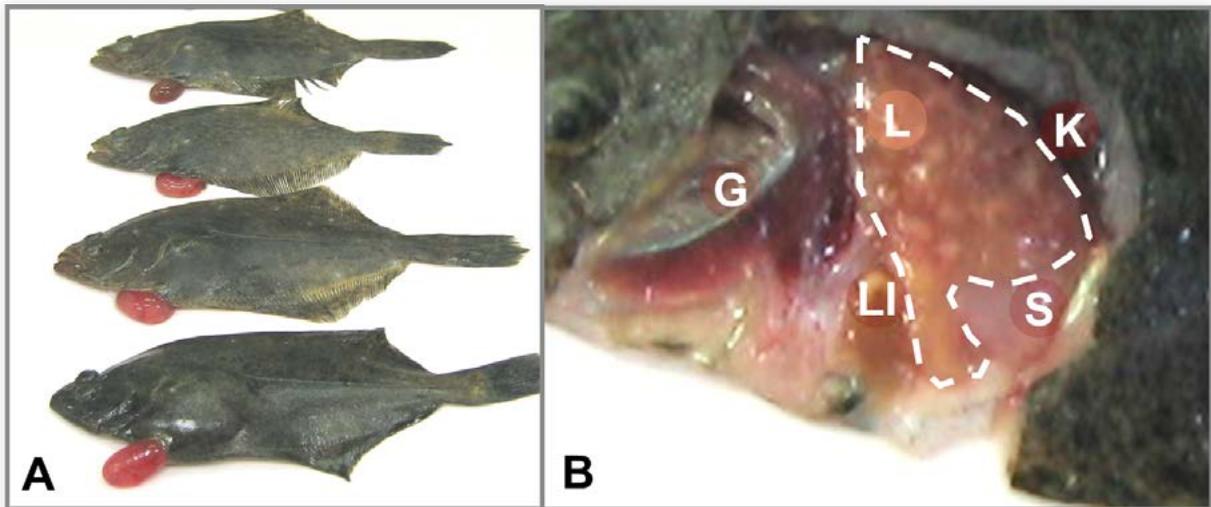


Figure 3. Gross changes in Japanese flounder fingerlings experimentally infected with *E. tarda* by immersion method. A. The abdominal cavity is rounded due to the collection of ascitic fluid and there is an initial prolapse of the rectum and large intestine. B. A dashed area delimits the liver, which is pale and mottled showing multiples abscesses. G: gill; LI: large intestine; L: liver; K: kidney; S: stomach.

Edwardsiellosis was initially documented in young tilapia hybrids cultured in Colombia, where it caused mortalities ranging from 10 to 90% of the population and characterized by systemic inflammation with suppurative abscesses (Rey et al., 2002). More recently, at least 21 outbreaks of edwardsiellosis have been detected in tilapia hybrids, which manifested ocular lesions (i.e., exophthalmia, corneal opacity and eyes losing), abdominal distension, nodules in the caudal fin, and hemorrhagic ulceration of the urogenital orifice. Gross findings such as pallor of the gills and muscle are frequently seen, sanguineous fluid accumulates in the abdomen which is accompanied by peritonitis and pancreatitis. The liver, spleen, and kidney can be enlarged and in most of the cases exhibiting whitish nodules. The gastrointestinal tract shows different degrees of sanguineous content associated with intussusceptions (Figure 4). Those changes resembles the gastrointestinal pathology reported in tilapia hybrids experimentally infected with *Aeromonas hydrophila* (Rey et al., 2009), and suggest a species-specific susceptibility or a common response to bacterial toxins that could persistently stimulate the gastrointestinal nervous system, creating myoelectric patterns of intestinal motility responsible for fluid propulsion (Navarre and Roussel, 1996; Husebye et al., 2001; Tanabe et al., 2004). The persistent contraction of the intestinal muscle might explain in some extent the development of intussusceptions in tilapia and the intestinal prolapse in Japanese flounder, in which the abundant ascitic fluid may also contribute to this condition.

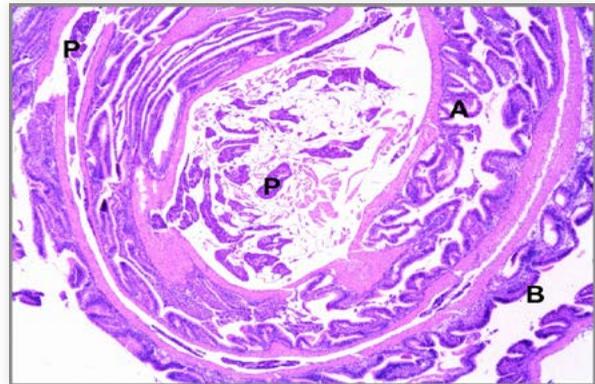


Figure 4. Microscopic appearance of the intestinal tract of a tilapia hybrid (*Oreochromis species*) naturally infected with *Edwardsiella tarda* showing severe intussusceptions. A considerable part of the small intestine has invaginated (A, intussusceptum) into another section of the intestine (B, intussuscipiens). Note the pancreatic tissue (P) relocated between the serosa layers of the intestinal wall and particularly in the lumen formed by the intussusceptum. H&E (40x).

Microscopically, the type of inflammatory response induced by *E. tarda* may differ between fish species (Miyazaki and Kaige, 1985). This may be due to the fish species itself, the phase of infection, the virulence factors produced by different strains of *E. tarda*, as well as the fish condition to mount a weak or strong inflammatory response. The disease in Japanese eel and flounder was described as a suppurative inflammation of the lesions, where the *E. tarda* growth overcame the bactericidal activities of neutrophils and macrophages. In the other hand, the infection in tilapia and red sea bream has been documented more likely to be a type of granulomatous inflammation preceded by macrophage infiltration and a reduced number of bacteria inside the granulomas (Miyazaki and Kaige, 1985). Tissue necrosis of several organs,

including the gill (Figure 5), meningitis, encephalitis and vasculitis with fibrinoid necrosis of the blood vessel wall, as well as the formation of plaque-like structures were the most significant lesions in tilapia manifesting nervous signs (Iregui et al., 2012). Although micro-vascular changes and multifocal necrosis were associated with the granulomas in largemouth bass and wild adult striped bass (Francis-Floyd et al., 1993; Baya et al., 1997), to our knowledge this is the first time intussusceptions and microvascular lesions in the brain are associated with *E. tarda* infection in fish. The lesions in the gastrointestinal tract are mostly necrotic, however, a degenerative process of the intestinal muscular layers characterized by fragmentation and hyaline appearance of the muscle cells (Figure 6), appear to be novel manifestations. Lesions in the liver range from necrotic to granulomatous reactions; other localization of the granulomas is the choroid of the eyes, which is severely infiltrated by leukocytes. In sharp contrast with other Gram-negative bacillary infections in tilapia, *E. tarda* is more often observed inside the granulomas, a finding that although not pathognomonic, is very helpful in cases where the isolation of the microorganism has not been feasible.

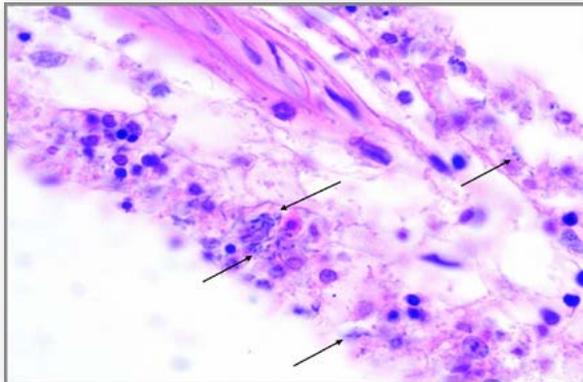


Figure 5. Gill filament of tilapia hybrid. Numerous bacilli (arrows) have already invaded the epithelium of the filament, which is edematous and necrotic and the lamellae are no longer visible. H&E (100x).

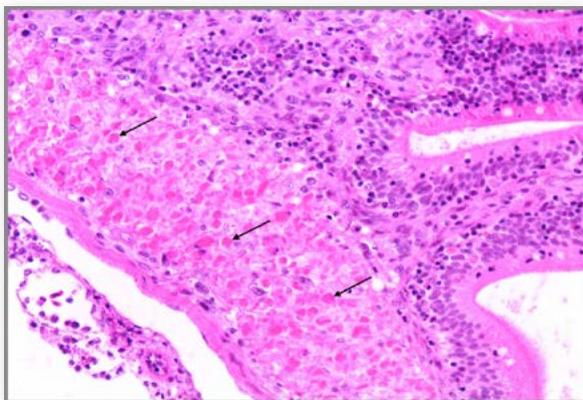


Figure 6. Intestine of a red tilapia. Severe hyaline degeneration of the longitudinal layer of muscle cells (arrows). Leukocytes have infiltrated the submucosa and are also present in a blood vessel and free in the peritoneum. H&E (40x).

E. tarda in other animal species

E. tarda has been adapted to and isolated from a wide variety of animal species, some of them with a concomitant clinical condition and others indicating a carrier state or common gut flora. They include reptiles such as lizards, snakes and alligators, diseased birds such as Ostrich (*Struthio camelus*) with acute intestinal infection, brown pelicans (*Pelecanus occidentalis carolinensis*) and common loons (*Gavia immer*) with hemorrhagic enteritis, and from intestinal samples of healthy turkey vultures (*Cathartes aura*), ring-billed gulls (*Larus delewarensis*) sandhill crane (*Grus canadensis*), bald eagle (*Haliaeetus leucocephalus*), and blue herons (*Ardea herodias*), and less common from sea mammals (Berg and Anderson, 1972; White et al., 1973; Winsor et al., 1981; Martineau et al., 1985). Scavenger birds such as Gulls are considered important reservoir of *E. tarda* and *Salmonella* in seafood and fish meal processing plants (Berg and Anderson, 1972). The bacterium was recovered from frogs, turtles, crayfish and cattle fecal samples sharing the same aquatic environment of catfish, revealing not only potential reservoirs of infection but indicating the possibility of cross-contamination during catfish processing (Wyatt et al., 1979).

Edwardsiella tarda infection in humans

The intestinal disease

Edwardsiella tarda most frequently causes gastroenteritis with acute watery diarrhea predominantly in immunocompromised or immunodepressed patients, older adults and children (Slaven et al., 2001; Wang et al., 2005; Spencer et al., 2008). The intestinal disease caused by *E. tarda* is characterized by watery to bloody diarrhea that could be prolonged or intermittent, anorexia and vomiting are common in neonates and young infants, which are particularly susceptible to the infection due to an immature immunity (Lim, 1978; Vandepitte et al., 1983). In some instances, gastroenteritis has been documented to progress into an ulcerative colitis (Engel and Martin, 2006), and patients receiving immunosuppressive therapy during organ transplantation are susceptible to develop gastroenteritis by *E. tarda* (Spencer et al., 2008). The disease is of high concern in tropical and subtropical regions, where dietary habits such as consumption of raw fish and sea foods are directly associated with clinical manifestations (Tsuji et al., 2008).

The extra-intestinal disease

E. tarda is capable of colonize the maternal birth canal and uterus that often progress into necrotizing soft-tissue and neonatal infection (Mowbray et al., 2003; Mikamo et al., 2003), particularly meningitis in newborns (Sonnenwirth and Kallus, 1968; Okubadejo and Alausa, 1968), that could progress into septicemia (Vohra et al., 1988), or multiple neonatal brain abscesses, that if early detected could be properly diagnosed and treated (Takeuchi et al., 2009). Extraintestinal infections usually originate from wounds associated with fishing, diving or swimming or abdominal trauma (Zigelboim et al., 1992; Slaven et al., 2001), with subsequent escape of the bacteria from the bowel and spreading into adjacent tissues, causing peritonitis, multiple liver abscesses, cholangitis, meningitis, cholecystitis, salpingitis, bronchopneumonia, empyema, skin and genitourinary tract infections (Janda and Abbott, 1993; Mizunoe et al., 2006). In some instances extraintestinal infections ends in septic shock (Clarridge et al., 1980; Funada et al., 1988), particularly in immunocompromised patients (Peyrade et al., 1997), and those with underlying malignancy (Tamada et al., 2009). Hepatobiliary diseases including biliary tract lithiasis, biliary tract infection, malignancy and diabetes mellitus are known to predispose to *E. tarda* infections (Wang et al., 2005). Wound infections by *E. tarda* might also develop into myonecrosis (Slaven et al., 2001), or even osteomyelitis (Ruff et al., 1977). The wide variety of human tissues that *E. tarda* successfully colonize may be associated with the intracellular nature of this pathogen. Since *E. tarda* has been isolated from diseased tilapias, caution must be taken to properly evaluate and diagnose its role in human disease in Colombia.

Molecular pathogenesis of edwardsiellosis

The pathogenesis of *E. tarda* infection as occurs with other members of the *Enterobacteriaceae*, is considered to be due to multiple factors (Leung et al., 2012). The *E. tarda* virulence factors responsible for its pathogenicity includes stable enterotoxin and hemolysins (Chen et al., 1996; Hirono et al., 1997), dermatonecrotic toxin, chondroitinase activity, complement-mediated resistance, hemagglutination mediated by nonfimbrial adhesins, and siderophore production (Kokubo et al., 1990; Janda et al., 1991a), invasive ability and cytotoxicity to HEP-2 cell lines (Janda et al., 1991b; Janda and Abbott, 1993; Darwish et al., 2000; Ling et al., 2000; Rao et al., 2004; Tan et al., 2005; Zheng and Leung, 2007) and a type III (T3SS) and type VI (T6SS) secretion of virulence factors (Janda et al., 1991b; Janda and Abbott, 1993; Darwish et al., 2000; Ling et al., 2000; Rao et al., 2004; Tan et al., 2005; Zheng and Leung,

2007) have been characterized. Recently, full genome analysis of various fish pathogens has offered robust information on the genetic makeup and pathogenic mechanisms used to infect a number of fish species. In the case of *E. tarda*, those studies provide support to an intracellular nature of the pathogen, a better understanding of the multidrug resistance features and its capability to infect diverse animal species (Wang et al., 2009). A recent review highlights a rapid genomic evolution of *E. tarda*, and the adaptive evolutionary strategies of bacterial fish pathogens in different ecological niches (Sudheesh et al., 2012), although, a number of potential vaccines have been developed (Park et al., 2012), it seems that more research is needed before an efficacious vaccine is commercially available, however, some of the structural antigens that might be useful to develop subunit vaccines have been identified (Verjan et al., 2005) and need to be explored deeply. Interested readers are invited to consult a more complete review on the virulence factors and their roles in the pathogenesis of *E. tarda* infections (Leung et al., 2012).

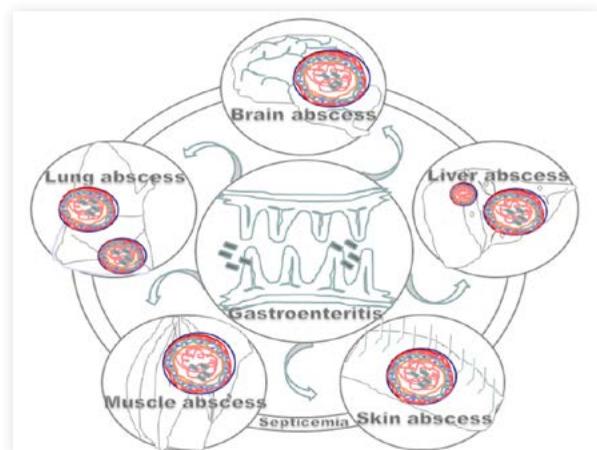


Figure 7. Edwardsiellosis, tissue distribution of *E. tarda* infection in human and fish hosts. Although no abscesses have been found in fish gills (the lung counterpart of fish), severe necrosis of gill epithelium is a common finding (Figure 5).

Conclusions

E. tarda is an enteropathogenic bacterium distributed widely in nature, commonly found in aquatic animals and their environment, where it causes severe disease in wild and commercial fish species with clinical and pathological changes similar to those observed in humans. Although, the bacteria cause mainly diarrheal illness in humans, common features of *E. tarda* infection in fish and humans are the development of local and systemic abscesses, which can progress into severe tissue necrosis and lethal septicemia (Figure 7). The novel

pathological changes described in tilapia including the intestinal intussusceptions and degeneration of intestinal muscular layers, might represent the fish manifestation of the diarrheal illness observed in terrestrial animals, which is very difficult to judge in the aquatic environment. Similarities in the tissue distribution, colonization and pathological changes caused by *E. tarda* in fish and human hosts might support the use of the fish model to study *E. tarda* pathogenesis aimed to develop novel strategies to control edwardsiellosis.

Acknowledgments

We thank to all members at the Genome Science Laboratory, Tokyo University of Marine Science and Technology and the Laboratorio de Patología Veterinaria, Universidad Nacional de Colombia for invaluable assistance and critical discussions.

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