Reprinted from THE AMERICAN JOURNAL OF TROPICAL MEDICINE AND HYGIENE
Vol. 26, No. 6, November 1977
Printed in United States of America

EDWARDSIELLOSIS IN MAN AND ANIMALS IN PANAMÁ: CLINICAL AND EPIDEMIOLOGICAL CHARACTERISTICS*

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Abstract. *Edwardsiella tarda* was isolated during etiologic and epidemiologic investigations of diarrheal disease agents in man and for Enterobacteriaceae in various species of wild-caught animals in Panamá. A total of 50 strains were recovered from approximately 14,000 specimens cultured between 1965 and 1972. In this period, *Edwardsiella* was isolated from ten individuals with a clinically diagnosed diarrheal syndrome, while 20 of some 3,000 persons from rural areas were asymptomatic carriers of these organisms. *Edwardsiella tarda* was also associated with two fatal cases of extraintestinal infection in man. In both cases liver abscess was a predominant feature. *Edwardsiella* was not demonstrated in either symptomatic or asymptomatic persons from urban areas. *Edwardsiella tarda* was present among some of the wild fauna of Panamá; various species of animals including snakes, toads, monkeys, and opossums harbored this organism.

Edwardsiella tarda, a recently described type species for a group of Gram-negative motile bacilli conforming to the definitions of the Family Enterobacteriaceae and the tribe *Edwardsiella*,[1] has been incriminated as a possible agent of diarrheal disease in man only infrequently.[2–5]

There have been few reports on *Edwardsiella* in the literature since the group was recognized by Japanese investigators who isolated these bacteria, primarily from reptiles; of the 256 strains reported by Sakazaki, five were recovered from humans with acute gastroenteritis.[6]

Since there seem to be indications that *E. tarda* may be associated with gastroenteritis and diarrheal disease in man, additional information is desirable in order to assess the etiologic relationship of *Edwardsiella* with undifferentiated diarrhea and other infections in man.

During the course of etiologic and epidemiologic investigations of diarrheal disease agents in man and of Enterobacteriaceae in many species of wild-caught animals in Panamá, *E. tarda* was isolated. A few of our *Edwardsiella* isolations have been mentioned in previous reports,[7,8] but the majority of our isolations are described in the present paper.

MATERIALS AND METHODS

Between 1965 and 1972 our laboratory cultured more than 10,000 fecal specimens (rectal swabs and fecal samples) for enteropathogenic bacteria. These were collected from 3,061 diarrheic and asymptomatic persons in rural communities and from 3,365 individuals with and without diarrhea in urban areas. Table 1 shows the composition of the human population studied. In the same period, more than 3,500 animals (primates, mammals, birds, reptiles, and amphibians) were also examined for pathogenic enteric bacteria.

Field and laboratory methods for our human studies have been reported elsewhere;[8,9] briefly, rectal swabs were obtained from children under 10 years of age, and fecal samples from older children and adults. Specimens were examined for enteric bacterial pathogens, including *Edwardsiella*, by methods described previously.[8,9] The primary emphasis was on isolation and identification of the common enteropathogens (*Shigella*, *Salmonella*, enteropathogenic *Escherichia coli* and *Arizona*), but satisfactory identification of *Edwardsiella* became a special concern to us after our first case was identified in 1965.

For our studies, we considered a person to have diarrhea if he, a responsible person, or a physician reported that five or more liquid or semi-liquid stools had been passed within a 24-h period. Blood, pus, or mucus in a single liquid stool, regardless of the number of stools, was also considered as diarrhea. Clinical data including fever, vomiting, dehydration, and abdominal cramps or pain were recorded, as well as the presence or absence of diarrhea during the week previous to the clinical and laboratory examination. The consistency and appearance of the fecal sample was also noted.

Accepted 9 May 1977.

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TABLE 1

Composition of the human population examined for Edwardsiella tarda *by location and socioeconomic conditions, Panamá, 1965–1972*

Population	Age group (yr)	Clinical status*	No. exam.	No. pos.	% pos.
Non-urban					
Rural	All ages	A + D	2,787	34	1.2
Deep forest	Adults	A + D	274	1	0.4
Urban					
Sectors of city:					
High income	All ages	D	554	0	–
Low to middle income	0–12	A + D	126	0	–
Low income	0–2	D	2,150	0	–
Controls (urban only)					
Hospital personnel†	18–40	A	239	0	–
Non-diarrheic infants	0–2	A	296	0	–

* A, asymptomatic; D, diarrheal disease.
† Includes student nurses, hospital aides, and Red Cross volunteers from low, middle, and high income areas of city.

Nonhuman primates were captured in remote forested areas in Panamá by professional trappers and delivered to our laboratory within 48 h.[7] None of the monkeys exhibited overt signs of disease on arrival. Rectal swabs were collected from each monkey upon arrival at the laboratory and an appropriate medium was inoculated with each rectal swab within minutes after the samples were taken.

Reptiles and amphibians,[10] birds, and mammals,[11] were collected alive and brought to our laboratory, where portions of their intestinal tract were aseptically removed after death and cultured for enteric bacteria.

Collections made in remote regions of the country were handled at base camps (Fig. 1), where samples of intestine were placed in separate jars containing buffered glycerol-saline solution. These were delivered to the laboratory in Panama City, and cultures were made usually within 48 h after the animal had been killed.

The procedures recommended by Edwards and Ewing[1] were followed for bacterial cultures isolated and characterized by us as *E. tarda*. Selected strains were sent for confirmation to the Center for Disease Control, Atlanta, Georgia.

RESULTS

A total of 50 *E. tarda* strains were isolated from approximately 14,000 specimens cultured between 1965 and 1972 (Table 2). With the exception of four strains, all were obtained from fecal samples. Of the 50 strains recovered, 35 (70%) came from humans, 14 (28%) were isolated from animals, and 1 (2%) was recovered from the environment.

Infections in Humans

The distribution of clinical illness associated with the presence of *Edwardsiella* is presented in

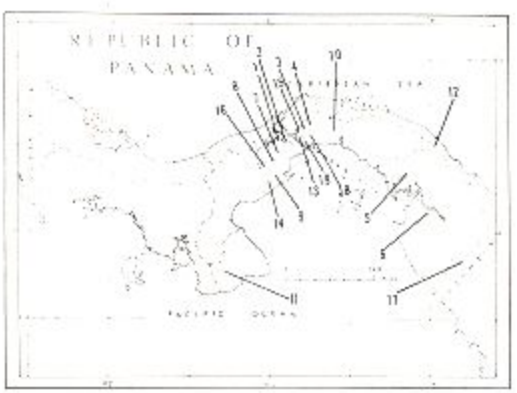

FIGURE 1. Map of Panamá, showing localities where *Edwardsiella tarda* was found in man (No. 1–11), animals (No. 5, 10, 12–18) and in the environment (No. 19). Locality No. 1, Mendoza; 2, Represa; 3, Caimitillo; 4, Calzada Larga; 5, Santa Fé; 6, Chepigana; 7, Zanguenga; 8, Buenos Aires; 9, Capira; 10, Pacora; 11, Tonosi; 12, Sasardí; 13, Cardenas Village; 14, Aguacate; 15, Madden Road; 16, Santa Rita; 17, Cerro Quía; 18, Panama City; 19, Ft. Clayton.

TABLE 2
Sources of Edwardsiella tarda *isolations in Panamá, 1965–1972*

Source	Number of isolates by year								Total
	1965	1966	1967	1968	1969	1970	1971	1972	
Human									
Stool	1	15	15	–	–	–	–	1	32
Liver	–	–	–	–	–	2	–	–	2
Blood	–	–	–	–	–	–	–	1	1
Animal									
Stool	–	1	2	3	1	4	3	–	14
Environment									
Sewage	–	–	–	–	–	1	–	–	1
Total	1	16	17	3	1	7	3	2	50

Table 3. This information was obtained by either a physician's examination of the patient or by direct interview with the patient's parents or the person responsible for the family. In the 7-yr period from 1965 to 1972, *E. tarda* was isolated from 10 individuals with a clinically diagnosed diarrheal syndrome.

Of some 3,000 persons who participated in our surveys for Enterobacteriaceae in rural areas, 20 were found to be asymptomatic carriers of *Edwardsiella*. Agents generally regarded as enteropathogens were not isolated from these carriers. Family members, at the time the fecal specimens were obtained, had diarrhea or a previous history of it. No data for diarrhea were obtainable for 6 of the 20 carriers. *E. tarda* was also recovered twice from an individual with a liver abscess, and once from a patient hospitalized for septicemia.

Intestinal infections

The clinical cases observed in this study revealed that diarrheal illness was characterized by watery stools and intermittent low grade fever (38–38.5°C); vomiting was observed in 7 of the 10 cases. The most common complaint was of intermittent diarrhea, with five or more soft or liquid stools a day lasting from 2 to 3 d, and occasionally 2 to 3 wk. One person from whom *E. tarda* was recovered was a 56-yr-old man who was ill for 6 days with diarrhea. He experienced occasional vomiting, complained of abdominal pain, and passed very liquid stools in up to 10 bowel movements per day.

Of the 10 persons with intestinal symptoms, only 1 patient, a 34-yr-old man from a rural village, manifested a more severe form of diarrheal illness. He gave a history of fever, vomiting,

TABLE 3
Edwardsiella tarda *in symptomatic and asymptomatic persons in Panamá, 1965–1972*

Clinical diagnosis	Specimen source	Total isolations	Number of persons with *Edwardsiella*								
			Sex		Age group (yrs)						
			M	F	<1	1–4	5–9	10–14	15–19	20–69	>70
Acute gastroenterocolitis	Stool or rectal swab	7	4	3	0	4	1	0	0	2	0
Acute enterocolitis	Stool or rectal swab	1	0	1	0	0	1	0	0	0	0
Chronic enterocolitis	Stool or rectal swab	4*	0	2	0	0	0	1	0	1	0
Liver abscess	Liver biopsy (pus)	2†	0	1	0	0	0	0	0	0	1
Septicemia	Blood	1	1	0	0	0	0	0	0	1	0
Asymptomatic	Stool or rectal swab	14	9	5	4	1	1	2	1	5	0
No information	Stool or rectal swab	6	3	3	2	1	1	0	0	2	0
	Total	35	17	15	6	6	4	3	1	11	1

* Includes three isolations from the same individual examined three times at 6-mo intervals.
† Two isolations from biopsies taken 1 mo apart from the same individual.

and bloody stools along with diarrhea, and was hospitalized with overt signs of dehydration. He was infected with both *E. tarda* and *Shigella flexneri* type 3.

One of two females diagnosed as having chronic enterocolitis was a 40-yr-old housewife from a remote rural community; she was examined on three different occasions at 6-mo intervals, and each time she had diarrhea without fever or vomiting. *E. tarda* was isolated from liquid stools, while no other enteropathogen was found.

Extraintestinal infections

A 71-yr-old woman was admitted to the hospital with abdominal pain and fever, and liver abscess was diagnosed. There was no history of recent dysentery. Although laboratory examinations of material from stools were negative for *Entamoeba histolytica*, she was treated for amebic infection. Because the patient did not respond to therapy, pus for bacteriological culture was obtained from the abscess by needle aspiration. *E. tarda* was obtained in pure culture and its susceptibility to antimicrobial agents was tested by the modified Kirby-Bauer method, which revealed in vitro sensitivity to ampicillin, tetracycline, kanamycin, colistin, erythromycin, and chloramphenicol; it was resistant to sulfadiazine, oxacillin, lincomycin, and cloxacillin. Although the patient's condition appeared to improve slightly, antibiotic treatment was unsuccessful. A second sample of pus taken 4 wk later also revealed growth of *E. tarda* with the same antimicrobial sensitivities as the first isolate. Despite revised treatment with antibiotics, the patient continued to deteriorate and died on the 50th hospital day. The cause of death was given as liver abscess and intestinal obstruction.

The second case of extraintestinal infection by *E. tarda* was in a 35-yr-old male farmer who was hospitalized with an acute febrile illness characterized by epigastric pain, vomiting, chills and fever, dark urine, and yellow staining of the conjunctivae of 4 wks' duration.

On admission, physical examination showed a critically ill patient with rapid respiration, hypotension, icterus, and distended abdomen. The liver and spleen were enlarged, and there was edema of the lower extremities. Cultures of blood obtained a few hours after admission yielded *E. tarda*. Examination and culture of fecal specimens were negative for *E. tarda* and other enteropathogenic bacteria, and for *E. histolytica*. Laboratory findings included marked anemia and elevated urea, creatinine, and bilirubin.

Despite treatment, which included antibiotics (penicillin G, gentamicin and ampicillin), the patient deteriorated with symptoms of disseminated intravascular coagulation. Treatment with heparin for consumptive coagulopathy produced only a temporary improvement, and the patient died on the 5th hospital day. The autopsy report listed the causes of death as choledocolithiasis, cholangitis, multiple abscesses of the liver, sepsis, and intravascular coagulation.

TABLE 4

Incidence of Edwardsiella tarda *in Panamanian wild animals, 1965–1972*

Host animal	No. of genera represented	No. animals		% pos.
		Examined	Positive	
Reptiles				
Lizards	20	465	0	–
Snakes	27	100	5	5.0
Amphibians				
Toads	1	34	2	5.9
Frogs	9	92	0	–
Mammals				
Monkeys	5	671	1	0.2
Rats	7	1,068	0	–
Opossums	5	304	5	1.6
Bats	ND*	94	0	–
Sloths	2	21	0	–
Rabbits	1	5	0	–
Squirrels	1	2	0	–
Kinkajou	1	1	0	–
Olingo	1	2	0	–
Armadillo	1	1	0	–
Birds	ND	215	1	0.5

* ND, not determined.

Infections in Wild Animals

Specimens were examined from a total of 3,381 animals representing various reptiles, amphibians, mammals, and birds. *Edwardsiella tarda* was isolated from the intestinal contents of the following animals: snakes (5%); toads (5.9%); monkeys (0.2%); opossums (1.6%); and birds (0.5%). In addition, one of the snakes found positive for *Edwardsiella* also harbored a strain of *Arizona*, and one opossum had a double infection with *Edwardsiella* and *Salmonella*. The highest incidence of infection was observed in toads, while the lowest was in monkeys (Table

TABLE 5

Edwardsiella tarda *infections in various species of Panamanian animals, 1965–1972*

Host animal	Collection site	
	Location	Habitat
Aotus trivirgatus (night monkey)	Pacora	Deep forest
Bufo marinus (giant toad)	Sasardí	Coastal forest
Didelphis marsupialis (common opossum)	Santa Fé	Deep forest
Xenodon sp. (snake)	Panama City	Grassy urban area
Bufo marinus (giant toad)	Cardenas village*	Secondary forest
Leimadophis sp. (snake)†	Sasardí	Coastal forest
Chironius sp. (snake)	Aguacate	Mountain village
Pseudoboa neuwiedi (snake)	Madden road	Secondary forest
Erythrolamprus bisonus (snake)	Aguacate	Mountain village
Sarcorhamphus papa (King Vulture)	Cerro Quia	Deep forest
Philander opossum (four-eyed opossum)	Santa Rita	Secondary forest
Didelphis marsupialis (common opossum)	Santa Rita	Secondary forest
Philander opossum (four-eyed opossum)‡	Santa Rita	Secondary forest
Philander opossum (four-eyed opossum)	Santa Rita	Secondary forest

* Suburban area.
† Double infection, *E. tarda* and *Arizona* 26: 23: 31.
‡ Double infection, *E. tarda* and *S. rubiaw*.

4). No overt signs of disease were observed when the animals were captured, and no significant lesions were noted in their intestine. The greatest number of the animals surveyed were rats, representing seven genera, but no *E. tarda* isolation was made from the 1,068 specimens studied. Other species of animals examined included rabbits, squirrels, kinkajous, olingo, and armadillos, but these were not as abundant or were not as successfully trapped during our studies (Table 4).

Nearly all the animals were collected in deep virgin forest or in isolated rural areas (Fig. 1). The various species of animals positive for *E. tarda* and the type of habitat characteristic of the region where they were trapped are shown in Table 5.

Isolation from the Environment

During studies on the design and use of stabilizing ponds as an economical, simple, and efficient method for treating domestic sewage in the tropics (manuscript in preparation), certain biological parameters that influence the fate of enteropathogenic bacteria were investigated. Weekly samples of sewage were taken from the ponds over a period of 5 yr and cultured for Enterobacteriaceae. Unlike *Salmonella*, which was frequently isolated from both influent and effluent sewage, *E. tarda* was recovered only once from influent sewage.

DISCUSSION

The diagnosis of *E. tarda* infection depends solely upon the isolation and characterization of the organism in the laboratory. Because this bacterium may be missed, not looked for, or confused with other bacteria whose biochemical characteristics are similar to those of *Edwardsiella*, reports of such infections in man are few. We believe that the incidence of edwardsiellosis may be greater than indicated by the literature. However, there is growing evidence that *E. tarda* can and does cause human disease, thus underscoring the pathogenic potential of this organism. In general, *Edwardsiella* has been more commonly associated with acute and chronic diarrheal syndromes,[3–5, 12] or has been detected in the feces of patients without diarrhea. Still less commonly reported are isolations of this agent from blood, wounds, throat abscesses,[13] and from spinal fluid.[14] It is believed that the organism is capable of producing disease patterns similar to those caused by salmonellae.

The present communication on sporadic cases of *E. tarda* associated with acute and chronic diarrhea (Table 3) lends support to the growing evidence of the relative importance of this agent in undifferentiated diarrhea. The more common bacterial pathogens, *Shigella*, *Salmonella*, *Arizona*, and enteropathogenic *Escherichia coli*, have been

accepted for many years as causing gastroenteritis, but these agents and *Entamoeba histolytica*, individually or in association with one or more of the others, ordinarily can be demonstrated in only about 20% of the cases of diarrhea in developing countries. Infrequently, the isolation rate for these organisms reaches 40%. There is need for investigation of agents not ordinarily considered in relation to diarrheal disease, and for further information on potentially pathogenic agents, associated with or causally related to this syndrome, that may account for some of the cases of diarrhea. Based on the work presented here, it is conceivable that *Edwardsiella* may account for a meaningful fraction of diarrheal cases of undetermined etiology.

The geographical distribution of human infections with *E. tarda* in Panamá seems to be confined to the rural and forested areas. *Edwardsiella* was not demonstrated in either symptomatic or asymptomatic individuals in the urban series of our studies (Table 1), which represented population groups drawn from all major socioeconomic sectors of Panama City. A recent survey for enteric pathogens in a cross-section of the general population of Panamá aged 0–10 yr from 31 rural, semi-rural and urban communities revealed that 5 (0.4%) of 1,178 children examined harbored *E. tarda*. All isolates came from different rural areas.[8] Despite the large number of individuals examined during the present study (3,061 from rural and 3,365 from urban areas), *Edwardsiella* was found infrequently (Table 1). This may be due to environmental and host characteristics which influence the host-parasite interaction. That *Edwardsiella* was isolated only from populations in rural areas may be due to greater exposure to environmental risks of infection in rural Panamá.

Human infection with *Edwardsiella*, whether symptomatic or asymptomatic, may remain circumscribed to the intestine or may involve other organs and systems by direct or indirect avenues. Adequate data have been collected elsewhere for specific extraintestinal infections caused by this agent and resulting in overt disease.[4,13,14] In Panamá, so far we have observed two fatal cases of liver abscesses associated with this organism. One of these was a solitary liver abscess; in the other there were multiple liver abscesses with ascending cholangitis, sepsis, and intravascular coagulation.

Edwardsiella tarda has been isolated only rarely from domestic or wild animals. It was first reported in Japan and characterized as the "Asakusa group" in the family Enterobacteriaceae.[6] Most of the isolates were from snakes, two were from seals, and five were from the feces of humans affected with diarrhea. Several infections of cattle and pigs, and one from a panther have been reported.[15,16] *Edwardsiella tarda* was found in association with enteric disease in aquatic species of birds in several instances, in alligators, and in one case of hemorrhagic disease in a fish (largemouth bass).[18] The organism has also been recovered from a sea lion and an Australian skink, and has been reported to have caused diarrhea and severe enteritis in an ostrich.[16,19] Several species of turtles have been found to harbor *E. tarda*, and it has been reported as a pathogen of channel catfish in the United States.[20,21]

The present study revealed that *E. tarda* was present among some elements of the wild fauna of Panamá, and that they might be natural carriers of this potential pathogen. The organism was found in various species captured in dense jungle and in forested rural areas (Table 5). High infection rates were determined in several species of snake and the giant toad, which are widely distributed in rural and forested areas of Panamá. The giant toad (*Bufo marinus*) was the only species of amphibian infected with *Edwardsiella*, and only one night monkey (*Aotus trivirgatus*) of several species of nonhuman primates examined harbored this organism. Although not as commonly infected as toads and snakes, opossums were found to harbor *E. tarda*. Unlike snakes, the species of opossums found infected are usually present in or near areas of human activities in their constant search for food, and could serve as a source of infection for man, his livestock, poultry, and other domestic animals. The foraging habits of opossums also bring them into closer contact with humans in the forest. Of all the avian species sampled, *E. tarda* was isolated only once from a King Vulture (*Sarcorhamphus papa*), captured on a mountain top (800 m elev.) in a remote, dense jungle area.

The natural behavior of animals may influence infection and spread it to other species, including man, in the rural environment. It is possible that they may act as carriers of *Edwardsiella* and contaminate their natural surroundings by fecal spread. *Edwardsiella tarda* may be transmitted

from one host to another in a way similar to the transmission of *Salmonella*, when birds flock and feed together, or when animals live in close proximity in groups, providing the ideal epidemiological conditions for spread of infection. Some species of animals play an important part in the animal food chain, for example in the case of a snake eating an opossum which, in turn, has eaten a smaller animal harboring *Edwardsiella*; or the King Vulture eating a small animal infected with this agent.

In rural areas, where inadequate sanitation prevails, humans may be infected by ingesting food and water contaminated by *E. tarda* from the excreta of animals, thus spreading the agent in the same manner other intestinal pathogens are spread. Children, in playing, also may transmit the potential pathogen from the ground directly to the mouth.

The occasional presence of *Edwardsiella* in sewage is not surprising. Carriers of this organism were detected among persons working and living in rural areas, and the probability that *E. tarda* will occasionally enter and survive in sewage is quite high. Where pollution of surface and underground water by human wastes or sewage is high, health hazards may arise due to contamination of drinking water. Thus, *E. tarda* may contribute to the endemicity of diarrheal diseases in areas lacking sanitary facilities.

Despite the apparently widespread distribution of *E. tarda* in man and animals, clinical human disease associated with it is not common or is not being reported. It may be that special environmental or etiological conditions are necessary to result in overt disease—intestinal or extraintestinal. This report adds to the growing evidence that *E. tarda* is a potential pathogen and can cause serious disease in humans. It is necessary for clinicians and bacteriologists to become more familiar with this organism and to be more aware of the various clinical conditions that infection may cause. Finally, it is within the capability of most laboratories diagnosing enteric infections to isolate and identify *E. tarda*.

ACKNOWLEDGMENTS

The authors are indebted to the Enteric Section of the Center for Disease Control, Atlanta, Georgia, for confirmation of selected strains of *Edwardsiella tarda*.

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