

FLIES AS A SOURCE OF ENTERIC PATHOGENS IN A RURAL VILLAGE IN THAILAND

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OBJECTIVE : To determine if flies are important disseminations of enteric pathogens in a rural village in Thailand.

BACKGROUND : The village of Ban Pong in northeastern Thailand was studied from January through December 1981 to determine the importance of flies as sources of enteric pathogens. The number of flies, that were predominantly *Musca domestica*, increased in kitchens and animal pens in the hot dry spring at a time of the year when the incidence of diarrhea was highest in the village. Enterotoxigenic *Escherichia coli* (ETEC), *Shigella*, non-01 *Vibrio cholerae* and *Vibrio fluvialis* were isolated from 69 percent of fly pools from yards, 38 percent from animal pens, 35 percent from bathrooms, and 8 percent from kitchens. ETEC were isolated from one fly pool in May and another in June when the incidence of ETEC infections was highest in the village. Flies often carry and presumably disseminate enteric pathogens in rural Thailand.

METHODS : Ban Pong, a small farming village of 625 inhabitants living in 125 homes, is located in Amphur Soongnern, Changwat Nakornrajsima, approximately 240 km northeast of Bangkok. This village is 15 km from a paved highway and is accessible only by dirt road. There are no rivers in Ban Pong and inhabitants obtain water either from tube wells, a small pond at the Wat (temple), or rain water. The chief livelihood of the village is subsistence farming and most families keep animals (pigs, cows, buffalo, ducks, or chickens) in pens adjacent or under their homes. The majority of homes have "suams" (concrete encased bathrooms) which are flushed with water drawn from wells or the pond. From January through December 1981 inhabitants of Ban Pong were interviewed six days/week and stools were collected from individuals with diarrhea to determine the prevalence of enteric pathogens. Temperature and humidity were measured each morning at the same location in the village.

Variation of fly density by month : Fly density was determined between ten and eleven o'clock in the morning during the first week of every month. The number of flies alighting on a standard wooden grid (11) (90cm x 90cm in animal pens, yards, and "suams" and 44cm x 44cm in kitchens) in three 30 second time periods were recorded. Grids were placed for one minute before counting was started and were picked up and repositioned between each count. Fly densities were determined in three animal pens, three yards, three "suams", and three kitchens. The same areas were used each month. The results were expressed as the mean of nine 30 second counts performed at each of the four different locations. Counts on the smaller grid were multiplied by the ratio of the surface area of the larger grid divided by the surface area of the smaller grid (x4.18).

Identification and culturing of flies : Every two weeks approximately 75 flies were captured with a net in animal pens, yards, or "suams", anesthetized by exposure to dry ice, and visually separated to species on a clean paper surface. The number of each species was counted and one of every five flies of a particular type was saved for later microscopic identification. The remaining flies were cultured as separate pools for each species. Because catching flies in kitchens with a net was impractical, fly paper (Tat fly paper, Walco-Linch Corp., Clifton, NJ) was hung for 24 hours. Flies were removed with sterile forceps and either cultured or saved in vials of xylene for later identification (a preliminary study indicated that the glue that entrapped the flies was not bactericidal).

Flies were cultured by first washing them in five ml of brain heart infusion broth (BHIB) and immediately inoculating the broth onto MacConkey, Hektoen, and thiosulfate citrate bile salts sucrose (TCBS) media (Difco, Detroit, MI). BHIB was inoculated into Hajna broth, alkaline peptone water (APW, pH = 8.0), and phosphate buffered saline (PBS, pH = 7.6). APW was incubated for six hours at 37°C and subcultured onto TCBS media. To identify *Yersinia enterocolitica*, BHIB washes were inoculated into PBS which was held at 4°C for 21 days and then subcultured on MacConkey and *Salmonella-Shigella* agar at 25°C for 48 hours. Hajna broth was incubated at 37°C for 24 hours and subcultured on Hektoen and desoxycholate media. Cultures were examined for *Salmonella*, *Shigella*, *Vibrio*, *Yersinia*, and *Aeromonas* by standard procedures and with the use of the API-20E system (4,6,15). Ten lactose positive colonies, selected from the MacConkey media, were stored on nutrient agar stab cultures, and tested within one month of isolation for heat-labile (LT) and heat-stable (ST) toxin with the Y-1 adrenal (17) and suckling mouse (7) assays simultaneously. Non-O1 *V. cholerae* were tested for cholera-like toxin production by the GM-1 ELISA (18) and for heat-stable toxin in the suckling mouse assay (7).

Starting in July 1981 the fly washings were inoculated onto nitrocellulose paper placed on a MacConkey plate, and incubated at 37°C overnight. The filters were then successively treated with 0.5 N NaOH and 1.0 M ammonium acetate - 0.02 N NaOH, air-dried, baked at 65°C overnight, and examined for nucleotide sequences coding for LT and ST with the DNA hybridization assay as previously described (13).

Fly population : Approximately 90 percent of the flies were identified as *Musca domestica*, Linnaeus, six percent as *Musca domestica*, Wiedemann, two percent as *Phaenicia cuprina* (Wiedemann), and two percent were distributed among six other types of flies (*Lymanophora* spp., *Chrysomya megacephala* (Fabricius), *Sarcophagidae* spp., *Sepsidae* sp., *Tabanidae* sp., and *Stomoxys calcitrans* (Linnaeus)). The relative proportion of each species of fly found at different locations each month did not differ appreciably throughout the year. *Musca domestica* was consistently dominant, comprising 96 percent (262/274) of flies caught from kitchens, 92 percent (341/371) from animal pens, 87 percent (306/350) from "suams", and 87 percent (311/359) from yards.

The mean number of flies counted at 12 different locations (three kitchens, three "suams", three yards, and three animal pens) each month are shown in Figure 1. Flies increased in kitchens in March, remained prevalent in April

and then declined in May. Flies in animal pens increased in April, were highest in June, and decreased in July. These increases in fly numbers were not associated with an increase in the prevalence of any single species. The temperature and humidity recorded each month in Ban Pong are shown in Table 1.

Bacteriological results : As shown in Table 2, ETEC, *Shigella flexneri*, non-01 *Vibrio cholerae*, and *V. fluvalis* were isolated from 69 percent of the fly pools caught from yards, 38 percent from animal pens, 35 percent from "suams", and eight percent from kitchens. *Salmonella* and *Y. enterocolitica* were not isolated from the house fly, *M. domestica*. Two of 15 non-01 *V. cholerae* produced cholera-like toxin, as determined by the GM-1 ELISA. Although *E. coli* were isolated from 88 percent of pools of flies from yards, 85 percent from animal pens, 81 percent from "suams", and 68 percent from kitchens, ETEC were isolated only from pools of flies caught from one yard in June and from one kitchen in May. ETEC from these two pools produced LT alone and were of different serotypes, O?:K?:H- and O6:K12:H?. DNA encoding for LT or ST was not found in 32 fly pools collected after July 1, 1981. *Aeromonas hydrophila*, bacteria of uncertain enteropathogenicity, was isolated from 63 percent of the fly pools.

The number of inhabitants with diarrhea in Ban Pong and the percent of individuals with diarrhea infected with different enteric pathogens per month is shown in Figure 2. ETEC were isolated from 11 percent, *Shigella flexneri* from eight percent, non-01 *V. cholerae* from 1.5 percent, and *A. hydrophila* from 34 percent of 132 inhabitants with diarrhea in Ban Pong in 1981. *Vibrio fluvalis* was not isolated from any inhabitants with diarrhea (Echeverria, P., C. Tirapat, C. Charoenkul, S. Yanggratoke, and W. Chaicumpa. Epidemiology of bacterial enteric pathogens in rural Thailand. in Takeda, Y. (ed.) Proceedings of the International Symposium on Bacterial Diarrheal Diseases, Marcell Dekker, New York, 1982).

The common house fly, *Musca domestica*, was recently documented as the most abundant species of fly in market places, garbage, slaughter houses, and animal sheds in the northern, northeastern, and central parts of Thailand (21). This species was by far the most abundant fly throughout the year in the kitchens, yards, animal pens, and "suams" of Ban Pong village during our study. Furthermore, the densities of this and other species of fly increased significantly in kitchens and animal pens during the hottest, driest season of the year when diarrheal disease was most prevalent in the villagers.

Bacteria that have previously been associated with episodes of diarrhea were isolated from *M. domestica* in all locations in Ban Pong. Flies caught in yards were more often colonized with enteric pathogens (69%) than those from animal pens (38%), "suams" (35%), or kitchens (8%). ETEC was only isolated from one fly pool in May and another in June when the incidence of ETEC infections were highest in inhabitants with diarrhea. Although only a small proportion (2 of 144) of the pools examined contained flies which carried ETEC, this study demonstrates that flies carry and presumably disseminate this pathogen. Further studies are necessary to determine the importance of flies as carriers of ETEC.

Vibrio fluvalis was the most common bacterial enteric pathogen isolated from *M. domestica*. These organisms have been implicated as a cause of diarrhea worldwide (1,8,10,12,19), and have been isolated from a variety of environmental sources (19). Four patterns of biological activity have been found in non-O1 *V. cholerae* isolated from patients with diarrhea in Bangladesh (20); those that produce a cholera-like toxin, those that produce a heat-stable toxin as measured in the suckling mouse assay, those whose whole cultures produce enteritis in infant rabbits, and others that demonstrate no activity at all. Spira and Daniels (19) found that six of 18 strains isolated from patients with diarrhea, but none of an equal number of environmental isolates produce a cholera-like toxin. The enteropathogenicity of non-O1 *V. cholerae* is not fully understood. However 13 percent (2/15) of isolates from flies produced a cholera-like toxin and were presumably capable of causing diarrhea in man.

Vibrio fluvalis which have been isolated from patients with severe cholera-like diarrhea in Bangladesh (5), and *Shigella flexneri*, a well accepted enteric pathogen, were cultured from flies in Ban Pong. Flies have previously been implicated in the dissemination of *Shigella* in crowded, unsanitary conditions (23), but environmental sources of *Vibrio fluvalis* are unknown. *Aeromonas hydrophila*, that has been suggested, but not proven to be an enteric pathogen (15), was commonly isolated from flies. The significance of the high prevalence of *A. hydrophila* in fly pools is unknown. However, data in Figure 2 suggest a direct correlation between *A. hydrophila* isolates from inhabitants and the number of inhabitants with diarrhea.

Although it was not possible to determine the relative importance of flies vs other routes in the dissemination of enteric pathogens, several conclusions can be drawn from this study. One, the majority of flies in yards, animal pens, "suams", and kitchens in Ban Pong were *M. domestica*; two, both the fly population and the incidence of diarrhea increased in the hot dry season; three, 29 percent of fly pools collected in the village carried enteric pathogen; four, flies caught in yards, "suams", or animal pens carried enteric pathogens more often than those caught in kitchens; five ETEC were isolated from flies when ETEC infections were most frequent among inhabitants with diarrhea in the village. Efforts to decrease the fly population, or reduce their numbers in areas where people live and eat would be a reasonable approach to decreasing diarrheal disease in rural Thailand. Prospective studies performed before and after active interventions to reduce the fly populations may be required to determine the importance of flies in disseminating enteric pathogens. This study suggests that such a study would be worthwhile.

FIGURE 1. MEAN NUMBER OF FLIES COUNTED IN THREE ANIMAL PENS, THREE YARDS, THREE BATHROOMS AND THREE KITCHENS EACH MONTH IN BAN PONG, IN 1981.

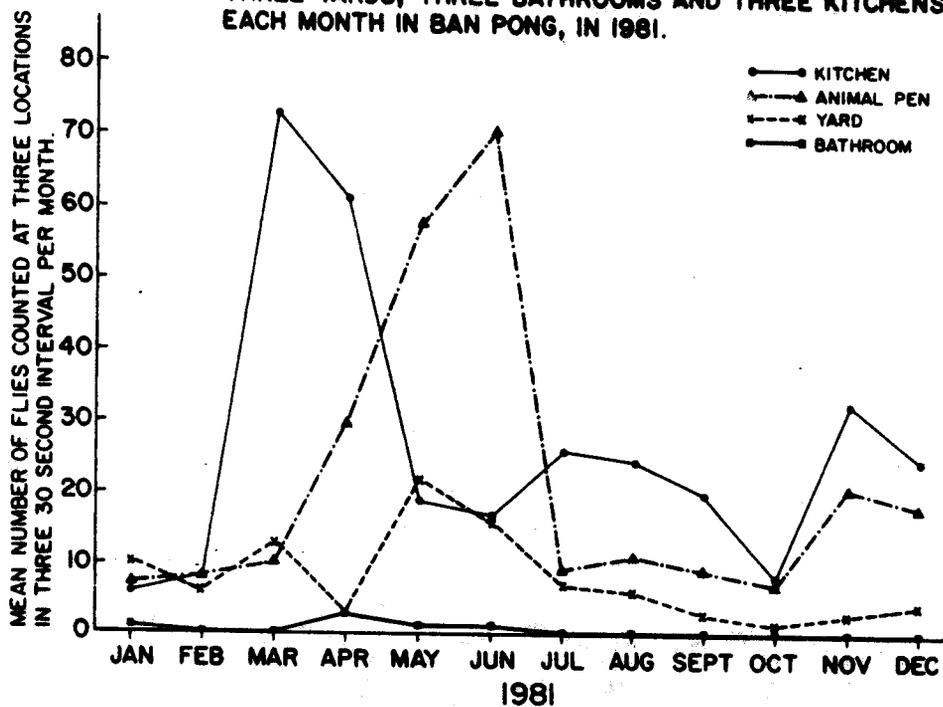


FIGURE 2. INHABITANTS WITH DIARRHEA ASSOCIATED WITH DIFFERENT ENTERIC PATHOGENS, IN 1981.

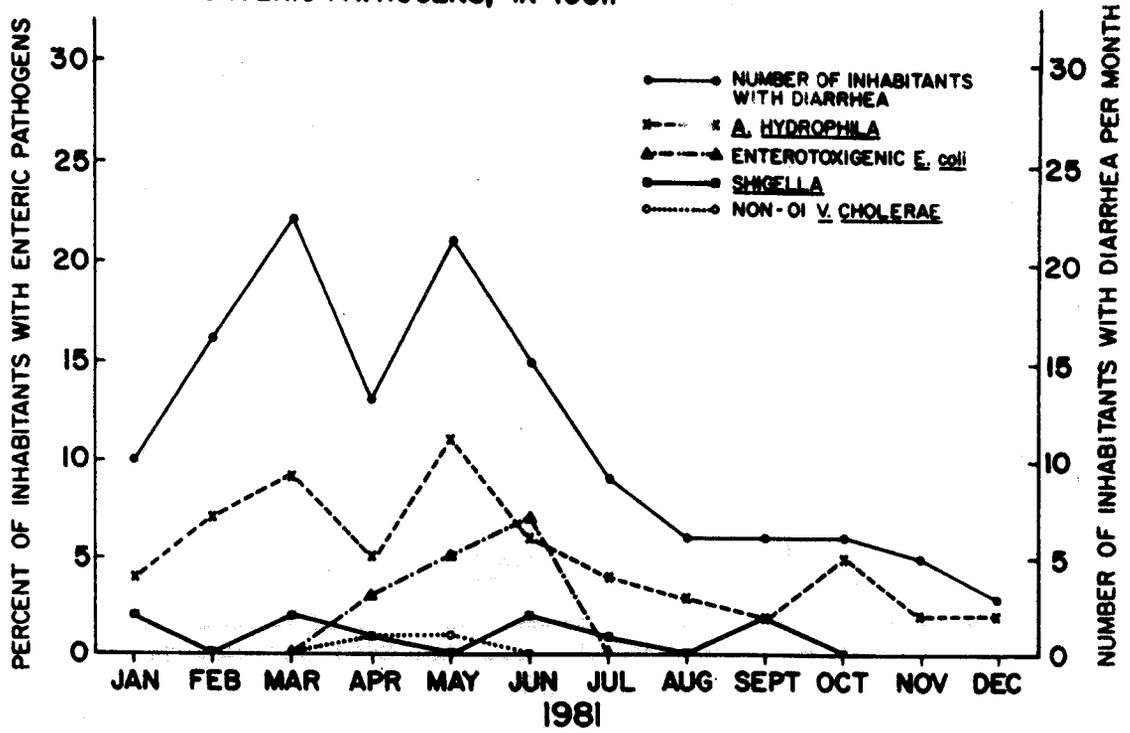


Table 1. Mean monthly temperature and humidity in Ban Pong in 1981

<u>Month</u>	<u>Temperature (°F)</u>	<u>Humidity (%)</u>
January	64	68
February	79	58
March	94	48
April	92	55
May	84	70
June	81	74
July	81	79
August	81	81
September	79	83
October	75	83
November	67	67
December	66	66

Table 2. Pools of flies containing enteric pathogens

<u>Enteric pathogens</u>	<u>Source and number of pool cultured</u>				
	<u>Yard</u>	<u>Animal pen</u>	<u>Bathroom</u>	<u>Kitchen</u>	<u>All sites</u>
	(26)	(26)	(26)	(66)*	(144)
ETEC	1	0	0	1	2
<i>Shigella</i>	0	1	0	0	1
Non-O1 <i>V. cholerae</i>	7	4(1)**	3(1)	1	15
<i>Vibrio fluvalis</i>	10	5	6	3	24
Total	18(69)+	10(38)	9(35)	5(8)	42(29)
<u>Possible enteric pathogen</u>					
<i>A. hydrophila</i>	20	17	22	32	91(63)

* More pools of flies were collected in kitchens than at other locations since it was often necessary to hang fly paper on two or three occasions to collect 75 flies every two weeks.

** Number of non-O1 *V. cholerae* which produced *V. cholera* -like toxin as determined by the GM-1 ELISA.

+ Percent of pools containing an enteric pathogen.

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