Centers for Disease Control and Prevention
Case Studies in Applied Epidemiology
No. 901-N11

L-Tryptophan and Eosinophilia-Myalgia Syndrome

Participant's Guide

Learning Objectives
After completing this case study, the participant should be able to:

- ☐ Discuss issues related to rapid establishment of national surveillance for a newly recognized and little understood disorder.
- ☐ Discuss development of case definitions.
- ☐ Describe the elements of design and the advantages and disadvantages of case-control versus cohort studies.
- ☐ Discuss some of the biases that might have affected these studies.
- ☐ Calculate and interpret a relative risk, odds ratio, and attributable risk percent.
- ☐ List and evaluate the criteria for causation.

This case study was developed by Leslie Swygert and Richard Dicker in 1990, and has been revised over the years with input from EIS Summer Course instructors.

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service

PART I

On October 30, 1989, the New Mexico Department of Health and Environment was notified by a local physician of three patients with marked peripheral eosinophilia (increase in a particular type of white blood cell) and severe myalgias (muscle pains). All three patients had been taking oral preparations of L-tryptophan (LT). Despite extensive clinical evaluation and testing, these patients' illnesses were not consistent with any known clinical condition. Public announcement of the cluster prompted reports of similar cases in other states. Active case finding was initiated in New Mexico, and the CDC was invited to participate in the investigation on November 8, 1989.

LT is an essential amino acid normally ingested as a constituent of dietary protein. Manufactured preparations have been used for a variety of reasons for many years (for example, as nutritional supplements or for disorders such as insomnia, anxiety, depression, and premenstrual syndrome). Despite its frequent use for therapeutic reasons, LT was classified as a food supplement and, as such, was essentially unregulated by the Food and Drug Administration (FDA).

Question 1: On the basis of the information above, is national surveillance appropriate? If yes, what are the advantages of a state-based system versus a system where the reports come to CDC? Should the system be active or passive?

Table 1 below, taken from the front page report in CDC's *Morbidity and Mortality Weekly Report* of November 17, 1989, describes the clinical features of 14 New Mexico residents reported to have a similar syndrome.

Table 1. Clinical features of first 14 patients with EMS, New Mexico, October – November 1989

	#	Percentage
Eosinophilia > 2000 cells per mm^3* (mean = 2300 cells per mm^3; range = 2064 to 12,100 cells per mm^3)	14	(100%)
Myalgias	14	(100%)
Weakness	11	(79%)
Fever (temperature of ≥99.7°F)	11	(79%)
Arthralgias	11	(79%)
Shortness of breath	9	(64%)
Rash	8	(57%)
Peripheral edema	8	(57%)
Clinical pneumonia	5	(36%)

* normal range, = 50 - 350 cells per mm^3

Question 2: Develop a surveillance case definition. Would you include L-tryptophan (LT) use in the case definition?

Question 3: What information would you collect on the case report form?

PART II

By November 15, a surveillance system had been established. By November 17, 287 EMS cases, including a well publicized fatality in New York, had been reported from 37 states and the District of Columbia. More than 98% of patients had used LT prior to illness onset.

Question 4: Now that the surveillance system is effectively accumulating data from throughout the country, by what means and how frequently might you close the feedback loop and disseminate information? To whom would you send the information?

Cases continued to be reported. Preliminary analysis of the first 235 case reports received at CDC revealed the following:

- Ages of patients ranged from 14 to 76 years, with a median of 46.
- 97% were white, 2% were black, and 1% were Hispanic.
- 83% were female.
- Cases had been reported from all regions of the United States. (Although they were not counted as part of national surveillance, reports of cases in Canada, Europe, and the Middle East had also been received.)
- 88% of patients reported onset of symptoms during or after July 1989.
- 99% had a history of LT ingestion preceding the onset of symptoms.

Question 5: At this point, what type of study design would you propose to test the hypothesis that LT is associated with EMS? Why?

PART III

Having identified several cases of EMS in their states, New Mexico and Minnesota epidemiologists quickly conducted case-control studies to assess possible exposures associated with the development of illness.

Eosinophilia-myalgia syndrome continued to be a front-page story in the United States.

Question 6: What effect might the publicity about EMS and LT have had on case-control studies?

Question 7: If you were designing one of these case-control studies, from what population would you select your control group?

PART IV

In the New Mexico case-control study, cases (n=12) were ascertained by chart review. All case-patients and two (8%) controls had used products containing LT (odds ratio not calculable, $p=6.9 \times 10^{-6}$). Illness was not associated with any of 32 other potential risk factors studied.

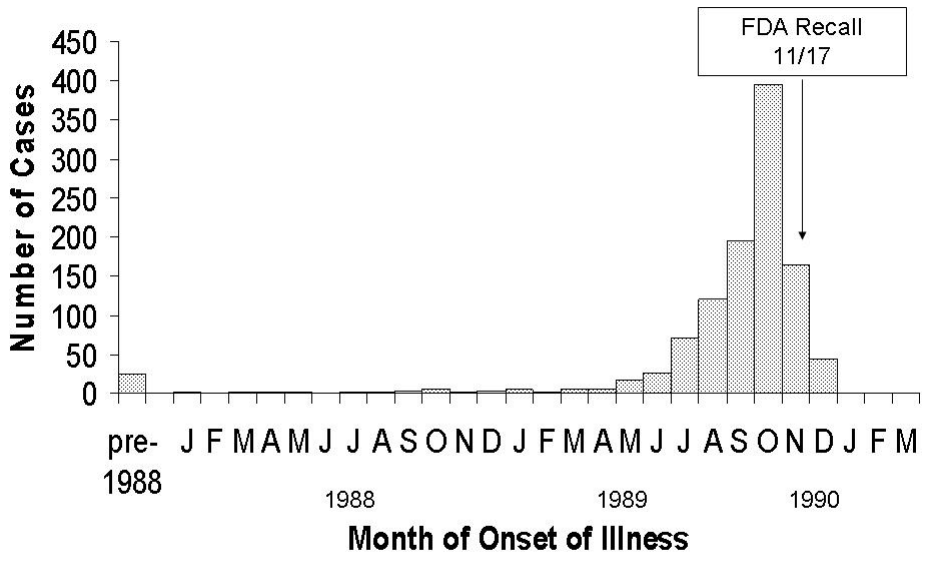
In Minnesota, all cases (n=12) and no controls had used LT (odds ratio not calculable, $p=8 \times 10^{-4}$) during the month before illness onset for case-patients and during a similar time period for matched controls. Nine (75%) case-patients and four (33%) controls were taking some type of prescription medication (not statistically significant after adjustment for LT). Illness was not associated with consumption of vitamins and health-food products, wild game, under-cooked meat or fish products, or nonprescription medications.

Question 8: Interpret these data.

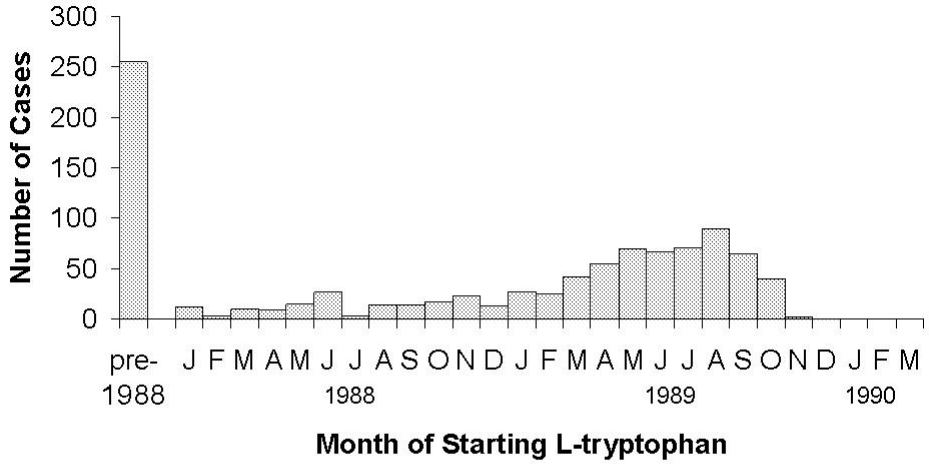
On the basis of results of national surveillance and these two state-based case-control studies, FDA recalled LT on November 17, 1989. The data suggested a causal relationship between EMS and the ingestion of products containing LT, but the exact reason for this association was unclear. Almost 200 different brands of LT were reported by EMS patients nationwide, so no point source of LT associated with disease was readily apparent. Preliminary results from case-control studies in Oregon and Minnesota, however, suggested association of EMS with specific but different brands of LT in those geographic regions.

The following figures display month and year of illness onset and month and year of beginning LT use associated with cases of EMS reported nationally.

Number of reported cases of Eosinophilia-Myalgia Syndrome by month and year of onset, United States, pre-1988 to March 1990



Number of reported cases of Eosinophilia-Myalgia Syndrome by month and year of start of L-tryptophan use, United States, pre-1988 to March 1990



A variety of hypotheses about the cause of EMS were suggested, including LT itself, an impurity or contaminant of LT products, or an excipient (inert ingredient) added during manufacturing.

Question 9: Which of these hypotheses seem most plausible?

Question 10: How would you test these hypotheses?

PART V

In Oregon, case-patients who had used LT were compared with controls who were asymptomatic users of LT. The following table shows the relationship between EMS and trace-backs to a specific Japanese manufacturer.

Table 2. Eosinophilia-myalgia syndrome by use of LT produced by Company X versus all others, Oregon, 1989

	Case-Patients	Controls	
LT manufactured by Company X	45	18	
LT manufactured by other-than-Company X	1	23	P value = 2×10^{-8} 95% CI = 7.8, 2419.1
Total	46	41	

Question 11: Calculate the appropriate measure of association. Qualitatively interpret the measure of association, P value, and 95% confidence interval.

The following table shows the relationship between trace-backs to a specific Japanese manufacturer and EMS from a New York-based case-control study.

Table 3. Eosinophilia-myalgia syndrome by use of LT produced by Company X versus all others, New York, 1989

	Case-Patients	Controls	
LT manufactured by Company X	68	28	Odds ratio not calculable, $p < 0.01$
LT manufactured by other-than-Company X	0	16	
Total	68	44	

These data were thought to be most consistent with the hypothesis of a contaminant originating during manufacture in Japan. Had an excipient been the culprit, a variety of over-the-counter supplements should have been involved or a formulator of LT-containing products in the United States would have predominated, and neither of these was the case. The lack of illness associated with other manufacturers of LT was evidence against LT as the sole etiologic agent. The implicated Japanese manufacturer had been using the bacterial fermentation method of production for several years, but changed its manufacturing process during late 1988. This change was thought to have played a role in the epidemic of EMS in 1989.

Question 12: On the basis of the information at hand, can you calculate an LT-user's risk of developing EMS? If not, what information do you need in order to do this calculation?

PART VI

As luck would have it, a South Carolina physician had been a strong advocate of the therapeutic uses of LT and had prescribed it to 505 of the 654 patients he had seen during 1989. Of the 505 patients who used LT in 1989, 418 (83%) were enrolled in a cohort study of LT-users (87 either could not be reached or refused participation). Of these 418 persons, 47 fit the CDC surveillance case definition for EMS, although another 68 partially met the CDC case criteria. In addition, 45 of the 47 persons with clear-cut cases had used a specific brand of LT (Brand A); of the 418 patients in the cohort, 157 had used that same brand.

Question 13: Would you continue to use the surveillance case definition, or would you consider another case definition for the case-control study?

Question 14: Using the CDC surveillance case definition, calculate the attack rate for the cohort of LT-users and the attack rate for users of Brand A. What do these data suggest about exposure and your etiologic hypotheses?

The following table summarizes preliminary data from the South Carolina cohort study:

Table 4. Eosinophilia-myalgia syndrome by dose of L-tryptophan among patients of a single medical practice, South Carolina, 1989

Dose of Brand A (mg/day)	# Cases of EMS	Number at risk	Risk	Relative Risk	Risk Difference	Attributable Risk Percent
0	2	261	_____	referent	referent	referent
1 - 1,500	5	37	_____	_____	12.74	_____
1,501 - 3,000	10	47	_____	_____	20.51	_____
3,001 - 4,000	12	35	_____	_____	33.52	_____
>4,000	18	38	_____	_____	46.60	_____
All brand A	45	157	28.66/100	_____	27.89	_____
Total	47	418	11.24/100	--	--	--

Question 15: Assuming that users of Brand A are more likely to be exposed to the etiologic agent than users of other brands of LT, compute risk of EMS, risk ratios, and attributable risk percents for each exposure category. What does each of these measures mean?

The following data are also from the South Carolina cohort study and illustrate the effect of age on case status for high vs. low dose exposure to Brand A:

Table 5. Eosinophilia-myalgia syndrome among patients of a single medical practice by dose of L-tryptophan and age group, South Carolina, 1989

Dose of Brand A (mg/day)	Age Group	# Cases of EMS	Number at risk	Risk	Risk Ratio
≤4000	≤ 45 years	8	200	_____	referent
> 4000	≤ 45 years	10	20	_____	_____
≤ 4000	> 45 years	21	180	_____	_____
> 4,000	> 45 years	8	18	_____	_____
Total		47	418	11.24/100	--

Question 16: Calculate risk (attack rate) and risk ratio (relative risk) for the above categories. What do these data suggest about the effect of age on development of EMS?

Question 17: For each of the following features, indicate whether a case-control study or cohort study is advantageous.

	Case-control	Cohort
Sample size		
Costs		
Study time		
Rare disease		
Rare exposure		
Multiple exposures		
Multiple outcomes		
Progression, spectrum of illness		
Disease rates		
Recall bias		
Loss to follow-up		
Selection bias		

Question 18: Why do you think case-control studies were conducted first? Why conduct additional case-control studies? Why conduct a cohort study?

Question 19: Generate a list of criteria for causality. Indicate whether each criterion is met by the evidence presented from these studies of EMS and LT.

PART VII – CONCLUSION

By August 1991 more than 1500 cases of EMS, including 36 deaths, had been reported to the CDC by state and territorial health departments. Occasional cases were still being reported and usually involved patients who had continued to take products containing manufactured LT despite media publicity and the FDA recall. Such recalls can be difficult to enforce, and "black market" sources of LT have apparently been available and may still be causing illness.

Clinical investigation into the spectrum of illness associated with ingestion of LT-containing products has been one primary area of research. Because this was a new and poorly defined illness, the original surveillance case definition stressed specificity rather than sensitivity, but the spectrum of illness caused by LT was expected to be broader than initially apparent. Present knowledge confirms that there is a wider range of clinical manifestations in disease associated with LT than would be included in the CDC case definition of EMS, and there were probably many more cases of disease related to consumption of LT-containing products than were counted in CDC surveillance figures (perhaps two to three times as many). Many patients have developed chronic sequelae affecting multiple organ systems, and information regarding the natural history of LT-associated EMS is still accumulating. Clinical follow-up studies report high prevalences of persistent disability related to continuing myalgia, muscle cramping, neuropathy, fatigue, shortness of breath (often due to chest wall muscle weakness but sometimes related to primary pulmonary disease), scleroderma-like skin changes, and cognitive dysfunction. The 1989 EMS epidemic produced considerable morbidity and mortality, and ongoing clinical studies suggest that this is a multisystemic disorder which represents an immunologic reaction to an environmental toxin and continues to threaten the lives and function of its victims.

Once the source of the toxic LT causing EMS was identified, scientific investigation began to focus on identifying the precise toxin(s) associated with EMS. Several peaks are consistently eluted on chromatography, and work has been directed toward identifying the components of these peaks. Scientists at CDC have found more than 60 minor contaminants in the toxic LT and have identified at least six contaminants potentially associated with EMS. Two substances, known by the abbreviations EBT and PAA, have been isolated from case-associated lots of LT and have been the focus of several scientific studies. These substances were present in product from Company X prior to 1989, but their concentrations had generally been less than that found in lots of LT associated with the EMS outbreak. Furthermore, the case-control study from Minnesota reported several changes made during late 1988 and early 1989 in the bacterial fermentation method of LT production used by Company X which appear related to the sudden outbreak of EMS in 1989: (1) introduction of a new strain of bacillus in December 1988; (2) reduction in the amount of powdered carbon used as a method of purification in the fermentation process; (3) a decision to bypass a filtration step employing a reverse-osmosis membrane for certain lots of LT. Animal experiments conducted by a variety of investigators have shown that case-associated LT from Company X causes EMS-like symptoms in test animals, and studies focusing on EBT and PAA suggest that they both may be contributing to various abnormalities involved in EMS. Although further studies will be necessary to sort out unanswered questions regarding the specific cause(s) and pathogenesis of EMS, the tremendous body of scientific evidence already accumulated documents toxic LT, rather than LT per se, as the cause of the 1989 EMS epidemic. Efforts to refine in vitro and animal models for testing of candidate etiologic agents continue.

Finally, this epidemic has had important regulatory implications for FDA. The toxic LT episode suggests that production of food supplements (and possibly drugs) from genetically engineered organisms in fermentation processes may lead to low concentrations of biologically active impurities. A better understanding of this episode is needed to improve our ability to prevent similar occurrences in the future.

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