APPLICATION OF STRATIFIED ANALYSIS IN EPIDEMIC INVESTIGATIONS : ANALYSIS OF AN OUTBREAK OF FOOD POISONING

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ABSTRACT

The present study demonstrates the use of stratified analysis when high (or low) risk is observed for two or more food items during the investigation of food poisoning, rather than simply implicating the item showing the highest risk. In this study an outbreak of food poisoning, which exhibited a clinico-epidemiological profile of *Clostridium perfringens* as the possible agent, was investigated using a case-control typology. Evaluation of food histories showed a high and significant odds ratio (OR) for a mutton dish (OR = 6.45, $p < 0.001$) and peas dish (OR = 5.6, $p < 0.001$), while a legume dish showed a protective effect (OR = 0.13, $p < 0.001$) which proved erroneous. Stratified analysis using Mantel-Haenszel procedure indicated that the risk due to mutton persisted even after adjusting for consumption of peas (adjusted OR = 4.22) or legume dish (adjusted OR = 4.25). The use of stratified analysis in investigating epidemics has been recommended.

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KEY WORDS : Food poisoning; Epidemiologic methods; Case-control studies.

Introduction

An outbreak of food poisoning provides a prototype example of any field investigation of epidemics. The principles of investigating such occurrences have been discussed in a number of standard texts [1-5]. In short, the epidemiologist would work out the relative risk (RR) and attributable risk (AR) in a retrospective cohort design or the odds ratios (OR) in a case-control typology, and place high suspicion on the food item showing the highest RR or AR or odds ratio. Examining this finding in conjunction with the clinical, microbiological, circumstantial, and environmental evidence, the investigators would then tentatively identify the food item which was the likely source of the outbreak and accordingly suggest preventive measures.

Often if the RR, AR, or odds ratio of two or more food items are high, as is quite likely to

happen in real situations, the epidemiologist, using the above mentioned conventional techniques, would tend to incriminate the food item exhibiting the highest risk thus overlooking the other food items. This may constitute a potential trap for the investigator. The present study describes such a situation, and the application of stratified analysis in evaluating the risks due to various food items.

Material and Methods

The present study was an observational, analytical, epidemiological approach using the case-control design while investigating an outbreak of food poisoning. This occurred in an Armed Forces unit, located in a semipermanent camp set up in the hot, arid, semi-desert terrain, somewhere in western India, during the early summer season. All the cases ($n=36$) that had occurred after consumption of dinner and a systematic random sample of controls ($n=51$) who did not develop the

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illness, despite consumption of the same dinner, were studied. Exposure to various food items was recorded based on subject's recall within 2 days of the episode (to reduce recall bias) using a predesigned questionnaire. Special biostatistical methods for stratified analysis were used, as described in standard texts. Basically this involved calculation of Mantel-Haenszel adjusted estimates of OR, Mantel-Haenszel chi square, 95% confidence intervals of the adjusted estimate, and comparison with the crude estimates [5-7].

Results

General

The incriminated meal was cooked between 5 p.m. and 7 p.m. and consumed between 8 p.m. and 11.30 p.m. by a total of 126 personnel out of whom 36 developed clinical features (attack rate= 28.6%). The median incubation period was 13 hours (range 11-19 hours). All the cases exhibited 3-4 watery motions and abdominal cramps. None of the patients suffered fever, vomiting, blood in stools, or dehydration. The overall clinico-epidemiological profile was that of *Clostridium perfringens* food poisoning, with *Bacillus cereus* (long incubation type) being another possibility [2,3,9,10]. Microbiological investigations could not be made use of mainly because food samples were not avail-

able and also because microbiological facilities were much restricted in the area of occurrence. Under such circumstances, reliance had to be placed mainly on clinical, and epidemiological findings

Epidemiological analysis of exposure to various food items :

The findings are presented in Table 1. It was observed that the OR for chapati and rice were very high to the extent of being undefined. However, this was a statistical fallacy, since everybody (excepting for one subject each) whether ill or not had consumed these items. Similar fallacious situations have been quoted in standard texts [3].

It was observed that out of the other 3 items, the highest risk was due to mutton curry (OR = 6.45), followed by peas in gravy (OR = 5.60) while moong dal (green gram), interestingly, showed a protective effect (OR = 0.13). The OR's for all these 3 items were highly significant.

Excluding the possibility of rice :

The risk due to rice was likely to be a statistical fallacy, however, since *B. cereus* (which has a predilection for rice) poisoning was also being considered, the data was analyzed to see whether there was any independent effect of rice, or whether the risk was only because of it being consumed alongwith

TABLE 1
Food histories of cases and controls

Food item	Cases (n = 36)		Controls (n = 51)		Odds ratio (95% CI)	'p' value
	Eaten	Not eaten	Eaten	Not eaten		
Mutton curry	31	5	25	26	6.45 (1.96-22.5)	< 0.001
Peas in gravy	32	4	30	21	5.60 (1.56-21.9)	< 0.001
Moong dal (Green gram)	18	18	45	6	0.13 (0.04-0.43)	< 0.001
Rice	36	0	50	1	undefined*	1.00**
Chapati	36	0	50	1	undefined*	1.00**

* Statistical fallacy without much epidemiological relevance. See text and Ref. No.3

** 'p' value (two tailed) by Fisher's exact method = 1.00, indicating a completely non-significant result.

mutton (the main suspected dish). The results are presented in Table 2. It is evident that consumption of rice alone had no risk as compared to when rice was eaten with mutton.

TABLE 2
Risk due to eating only rice as compared to eating both mutton and rice

Food history	Cases	Controls	Total
Ate rice but not mutton	5	26	31
Ate both rice and mutton	31	24	55
Total	36	50*	86*

(* one control had eaten neither rice nor mutton, hence total is less by 1)

Odds ratio (OR) = 0.15; 95% CI = 0.04-0.43

χ^2 (Yates) = 11.59; df = 1; $p < 0.001$

Evaluation of the independent effects of mutton and peas :

As observed from Table 1, while the highest risk was found to be due to mutton (OR = 6.49), consumption of peas also had a very high risk (OR = 5.6). Accepting mutton as the only possible source simply because it carried the highest risk, therefore, would not have been scientific under this situation. For this reason, stratified analysis, using Mantel-Haenszel procedure, to evaluate the independent risk of mutton and peas was undertaken. The results are presented in Tables 3 and 4.

Evaluation of the confounding effect of moong dal on the high OR for mutton :

It was earlier observed from Table-1 that consumption of dal had a significant protective effect (OR = 0.13, $p < 0.001$). It was therefore necessary to evaluate whether this was a real effect or simply due to a confounding effect since most of the dal eaters (vegetarians) were not likely to have eaten mutton, and vice versa. The findings are presented in Table 5.

Discussion

In real situations, it would be often diffi-

TABLE 3
Stratified analysis : risk due to eating peas controlling for the effect of mutton

Exposure to peas	Stratum I		Stratum II	
	<u>Mutton eaten</u>		<u>Mutton not eaten</u>	
	Cases	Control	Cases	Control
Peas eaten	30	23	2	7
Peas not eaten	1	2	3	19
Stratum OR = 2.6 (p = 0.58, NS)		Stratum OR = 1.8 (p = 0.61, NS)		

Crude OR for eating peas = 5.60

Mantel - Haenszel OR (adjusting for the effect of mutton) = 2.10 (95% CI = 0.37 to 12.35)
(not significant, $p > 0.05$)

TABLE 4
Stratified analysis : risk due to eating mutton, controlling for the effect of peas

Exposure to Mutton	Stratum I Peas eaten		Stratum II Peas not eaten	
	Cases	Control	Cases	Control
Mutton eaten	30	23	1	2
Mutton not eaten	2	7	3	19
Stratum OR = 4.57 (exact 'p' = 0.05)		Stratum OR = 3.17 (exact 'p' = 0.42)		

Crude OR for eating mutton = 6.45

Mantel-Haenszel OR (adjusting for the effect of peas) = 4.22 (95% CI = 0.89 to 21.35)

cult to find any single food item showing a clearly high RR, AR, or OR. Quite often, more than one item may show high risk. In such situations, it would be, therefore, scientific to undertake a stratified analysis to assess the independent effect of each such food item rather than simply going by the conventional teachings of identifying the item that exhibits the highest risk.

It was observed from Table 3 that while the overall (crude) OR for eating peas was 5.6, which was highly significant, however after adjusting for the effect of eating mutton, the Mantel-Haenszel OR was only 2.1 and that too was not significant ($p > 0.5$). Eating peas therefore did not have an independent effect and the overall effect observed for peas was actually due to mutton.

TABLE 5

Stratified analysis : risk due to eating mutton, controlling for the effect of moong dal (green gram)

Exposure to peas	Stratum I Dal eaten		Stratum II Dal not eaten	
	Cases	Control	Cases	Control
Mutton eaten	14	20	17	5
Mutton not eaten	4	25	1	1
		Stratum OR = 4.38 (p = 0.034, NS)	Stratum OR = 3.4 (exact 'p' = 0.44, NS)	

Crude OR for eating mutton = 6.45

Mantel-Haenszel OR (adjusting for the effect of dal) = 4.24 (95% CI = 1.18 to 15.70; p < 0.05)

On the other hand, as observed in Table 4, the overall (crude) OR due to mutton which was 6.49 still remained 4.22 after adjusting for the effect of eating peas. Mutton was, therefore, the contaminated vehicle in the present episode.

It was also observed that the overall OR of mutton (6.49) still remained reasonably high at 4.24 even after adjusting for the effect of eating dal. The observed overall protective effect of dal was therefore fallacious and it occurred because most of those who ate dal did not eat the contaminated dish, i.e., mutton (26/63 i.e. 46%) while a large majority who did not eat dal had eaten mutton (22/24, i.e. 92%). Also, most of the cases were those who had eaten mutton, irrespective of whether they had eaten dal or not.

Once a particular item is incriminated after stratified analysis, the epidemiologist is more confident of it being the causative vehicle, and public health efforts can be directed accordingly. The situation would become more interesting if a protective role is observed for any food item (e.g. dal in the present episode). In such situations, besides assessing for any confounding, what is more important is that the epidemiologist should use stratified analysis and procedures like Woolf's test [8] to evaluate whether there is any effect modification or interaction. Such a finding, should it occur, would raise further questions on the

protective or hazardous role of certain food items in the etiology of food poisoning. The present study suggests that some departures from the traditional teachings on investigations of food poisoning [2-4,7] may be considered as regards the following aspects which have been highlighted by the findings of this study : (a) in addition to the traditional retrospective cohort approach, a case-control approach may be more useful in certain situations, (b) before incriminating a particular food item as the source of poisoning, in addition to the laid down procedure of identifying the item with highest attributable risk, stratified analysis is of use to control for the possible confounding effect of other food items.

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