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A *Staphylococcus aureus* Food Poisoning Outbreak in Four Junior High Schools, Taichung County

Abstract

On March 18, 1997, 1,948 teachers and students of four junior high schools in Taichung county, after having had boxed lunch prepared by food factory X of Taichung city, had developed a large scale food poisoning outbreak. A retrospective cohort study was conducted and a structured questionnaire was used to interview teachers and students of the Junior High School A. Information on their demographic backgrounds, food items eaten, symptoms, time of onset, and medical care was collected to identify the pathogenic agents, the food responsible, and the causes of the outbreak.

Analysis of questionnaires collected from 362 students who had the boxed lunch showed that their clinical symptoms were (by order of percentage): abdominal pain 70.7%, watery diarrhea 68.8%, nausea 8.6%, vomiting 3.6%, fever 1.1%, and others 3.6%. Of them, 204 met the criteria of a case, giving an attack rate of 56.4%. Their incubation periods ranged from less than one hour to 6 hours, with a median of 3 hours and a mode of 2 hours. These findings indicated a *Staphylococcus aureus* infection. The Central Branch Laboratory of the National Institute of Preventive Medicine had succeeded in isolating *Staphylococcus aureus* in 45 of the rectal swabs collected from students and cooks. Among them, 12 were enterotoxin A-genic, 2 were B-genic, and 3 were C-genic. It was therefore concluded that *Staphylococcus aureus* was the pathogenic agent of the incident.

Statistical analysis of food items showed that the risk of food poisoning for those who had the sauteed diced chicken were 1.65 times higher than those who

didn't have it (95% confidence interval at 1.17-2.34). The risk of those who had the pork with pickles were 1.41 times higher than those who didn't have it (95% confidence interval at 1.12-1.78). Both food items could have been the causes of the outbreak, though no *Staphylococcus aureus* was isolated from food specimens. Materials for the diced chicken were prepared the night before and left unfrozen. If they had been contaminated by *Staphylococcus aureus*, during the time they were left unfrozen, they could have produced enough bacteria and enterotoxins. The pork with pickles was prepared the night before, and was kept in the pot without cover. It was not sufficiently heated again the next day before use. It could also have been contaminated and produced enough amount of pathogenic agents. It could have also been the cause of the outbreak.

Introduction

At about noon of March 18, 1997, of the 1,948 teachers and students of four junior high schools in Taichung county, after having had the boxed lunch supplied by food factory X of Taichung city, 1,147 had developed food-poisoning symptoms such as diarrhea, abdominal pain, and vomiting. Some students were so seriously ill to take a day off or be treated at hospital. The Taichung County Health Bureau, upon notification of the high schools, visited the schools to collect human specimens for laboratory testing by the Central Branch Laboratory of the National Institute of Preventive Medicine. Remains of the boxed lunches were sent to the Central Branch Laboratory of the National Laboratories of Foods and Drugs for testing. Since the outbreak involved a large number of victims and was a concern of the public, the Bureau of Food Sanitation of the Department of Health soon requested trainees of the Field Epidemiology Training Program (FETP) to collaborate with the Taichung County Health Bureau in an epidemiological investigation to understand the causes of the incident, the food items responsible, and the possible pathogenic agents.

Materials and Methods

The retrospective cohort study[1,2] was used in the investigation to first establish the relationship between the incident and the boxed lunch supplied by food factory X. The possible food items responsible were then identified from the students who had the boxed lunch. The likely pathogenic agents were then deduced from the distribution of incubation periods, laboratory findings of human and food specimens, and process of food preparation and storage. The process of the investigation is as follows:

Lunch Supply at Four Schools

Table 1 shows the number of students appeared at the four schools on March 18, 1997. The sources of lunch, number of boxed lunch ordered, and number of students infected by the boxed lunch supplied by food factory X are also presented.

Visit to High School A and Questionnaire Survey

The four schools are similar in their geographic environment and students' backgrounds. Since lunches of High School A students were either brought by themselves or ordered from three factories, the School was adequate for investigating the association between the incident and the boxed lunch supplied by different food factories. Both human and food specimens had been collected from this School by the Taichung County Health Bureau, findings of laboratory testing could help in defining cases and thus to identify the causes and pathogenic agents of the incident. In addition, High School A was the first school to report the outbreak and it was willing to join the investigation. Hence, it was decided to choose the High School A for the investigation.

The structured questionnaire contained questions on the demographic backgrounds of the students, food items eaten, symptoms, time of onset and medical care. Face to face interview began after a briefing in the auditorium to the teachers by the principal of the School. Students were interviewed in classes. The returned questionnaires were checked for accuracy by the staff of the Taichung County Health Bureau and the FETP trainees. From 1,290 students, 1,212 copies of the questionnaire were collected, giving a response rate of 94%.

The school environment and water supply (including drinking water) were also inspected to see if the outbreak had either spread to the entire school or clustered around certain classes or areas. Records of the health center and records of students' sick-leaves were also checked, focusing particularly on records before March 18 and after March 20 to see if the incident in fact was an outbreak or a result of other infections.

Visit to Food Factory X

The boxed lunch associated with the present incident was supplied by Food Factory X. The Factory is located in Taichung city and has been accredited by the Taiwan Provincial Health Department as Class A food manufacturer. The Factory, with good facilities, has a staff of 70-80. On March 18, the Factory had supplied 36 schools in Taichung City and County with 18,842 boxed lunches. The sanitation of the kitchen were examined, focusing on the manufacturing process, time of processing, sources of materials, packaging, transportation of the boxed lunches prepared on March 18, and the health status and personal hygiene of the cooking staff.

Investigating the Fried Egg Factory

The fried eggs of the day were bought from the Chang's egg shop. It was a small factory without license. On March 18, a total of 19,050 fried eggs was supplied to Factory X. Preparation began at 6 o'clock in the morning. The fried eggs making machine has two production lines, each can produce 130 fried eggs per round. Each egg took about 85 seconds to fry. The whole lot was

finished by 9 o'clock and delivered in four batches to the Factory. The distance between the egg shop and Factory X was about 15-20 minutes drive. The first batch of 2,000 some eggs arrived at the Factory about 7:30.

Collection of Specimens

The Taichung County Health Bureau collected 30 rectal swabs from School A, 25 from School B, 66 from School C and 30 from School D for laboratory testing. Testing was done by the Central Branch Laboratory for *Staphylococcus aureus* (including enterotoxins), *Bacillus cereus, Vibrio parahaemolyticus, Salmonella, Vibrio cholerae, Salmonella typhi (paratyphi),* and *Bacillus dysenteriae.* Rectal swabs were also collected from 53 cooking staff of Factory X and 7 employees of the egg shop for laboratory testing.

Remaining boxed lunches kept by schools and Factory A were sent to the Central Branch Laboratory of the National Laboratories of Foods and Drugs for testing of *Staphylococcus aureus* (including enterotoxins), *Bacillus cereus, Vibrio parahaemolyticus, Salmonella,* and *pathogenic E. coli.* Additionally, 14 environmental specimens were also collected (two each of the surface water, water on chop boards, drain water, water on the iron gate, water on pushing cart, and oil stains).

Results

Visit to High School A

Survey of the school environment and water supply (including drinking water) showed that most cases clustered in classes where the boxed lunches were supplied and not throughout the school. It was concluded that the incident was not associated with the school environment or drinking water. Records of the health center and students' sick-leaves did not show any increase in the number of students taking leaves either before March 18 or after March 20, though a sudden increase on March 19 and 20. The only reason in the sudden increase of the number of students taking leave on these two days was the consumption of the boxed lunches supplied by Factory X. It was therefore decided that the present food poisoning incident was an outbreak of common exposure and not a result of common cold or other infections.

Questionnaire Survey

1,211 (612 male students, 50.5%; 599 female students, 49.5%) of the 1,212 copies of questionnaire collected from School A were effective. By sources, 29.9% (362/1,211) of the lunches on March 18 were ordered from Factory X, 12.3% (149/1,211) from Factory C, 37.4% (453/1,211) from Factory D, 12.5% (151/1,211) brought by students themselves, and 7.9% (96/1,211) either shared with others or did not indicate source of lunches.

Of the 362 students who had boxed lunches supplied by Factory X, their

major complaints were (by order of percentage): abdominal pain 70.7%(256/362), diarrhea (more than twice a day) 68.8%(249/362), nausea 8.6%(31/362), vomiting 3.6%(13/362), fever 1.1%(4/362), and others 3.6%(13/362). Most symptoms were not serious, and none was cared in hospital. A case was then defined as one who had the boxed lunch supplied by Factory X on March 18 and had developed diarrhea (twice or more a day) and abdominal pain. 204 of them met the criteria, giving an attack rate of 56.4% (204/362).

Analysis by the weighted least square analysis[3] showed that the proportion of becoming ill by having the boxed lunch supplied by Factory X (56.4%) was significantly higher than that of having either boxed lunches supplied by other factories or brought by students themselves (see Table 2, all p values smaller than 0.05). This finding verified the hypothesis that the food poisoning incident of March 18 was associated with the boxed lunches supplied by Factory X. Table 3 shows classes which had ordered boxed lunch from Factory X, number of students in each class, number of boxed lunch ordered, number of students meeting the criteria of a case, and attack rates. These figures indicated that students who had the boxed lunches supplied by Factory X and students of lower grades had higher attack rate.

Epidemiological curve (Figure 1) shows the incubation periods ranging from less than one hour to 6 hours, with a median of 3 hours and a mode of 2 hours.

Food Items

Food items of the lunch included: sauteed diced chicken, pork with pickles, fried egg, vegetarian chicken, stir-fried vegetable, creamed corns and rice. Food items consumed by 204 cases and 158 non-cases were compared and tested by χ^2 test to see which food item was associated with the incident (Table 4). It was found that the diced chicken, pork with pickles, fried egg, vegetarian chicken, and stir-fried vegetable were all significantly associated with the food poisoning (all p values smaller than 0.01); whereas creamed corns and rice were not associated with (all p values larger than 0.05). The relative risks of having or not having a certain food item were: 1.65 times for diced chicken, 1.41 times for pork with pickles, 1.54 times for fried egg, 1.36 times for vegetarian chicken, and 1.31 times for vegetable. Further multi-variable cross analysis of these food items showed that only diced chicken, pork, and vegetable were significantly associated with the food poisoning (all p values smaller than 0.05).

Process of Food Preparation

With the exception of pork with pickles, which was prepared the night before, the other food items were prepared on the morning of the day. The boxed lunches associated with the food poisoning were packed on the second of the three production lines and were the first batch to have been packed. One production line could pack 40 boxed lunches every minute.

Except the fried eggs, which were bought from the Chang's egg shop, the

other food items were prepared by the Factory X. Chickens for making the diced chicken were bought from a store in Taiping City of Taichung County. The diced chicken was added soy sauce, sugar, pepper on the night of March 17, and added flour on the morning of March 18 for frying. One frying pot could prepare 700-800 servings one time. The minced pork for pork with pickles came from a butcher's in Taichung City; the pickles came from a factory in Fangyuan Township of Changhua County. They were prepared according the following procedures: oil was first put in the pot, heated, added onion and soy sauce; meat was then fried in it, and more flavors added; finally, pickles were put in and mixed with the meat. The food was then left in the pot overnight without cover. It was heated again before use the next day. The vegetarian chicken was made from flour, soy sauce and sugar. The flour came from a shop in Erhlin Township. Vegetable was stir-fried. Cream was heated in a pot, then corns, beans, carrots and meat were cooked in it. The corns came from the Chang's of Taichung City; beans from a frozen food factory; carrots from a vegetable supplier; and meat from another supplier. Ten some rice cookers were there in the factory. Each cooker could cook in 40 minutes rice for 50 people.

The boxed lunches were delivered in closed vans. One van was for delivery to schools A and B; another van for schools C and D. Lunches were delivered to schools about 11:30 for students to eat at noon. The Factory has ten some vans. The vans are not equipped with either heating or frozen facilities.

Laboratory findings

Of the 151 rectal swabs collected from students, *Staphylococcus aureus* was isolated in 29 of them, including 11 of enterotoxin A-genic, and two each of type B and type C-genic, giving a positive isolation rate of 19.2%. Of the 53 rectal swabs collected from the cooking staff, *Staphylococcus aureus* was isolated in 16 of them, including one enterotoxin A-genic and 3 type C-genic, giving a positive isolation rate of 30.2%. No pathogenic agents were isolated from the 7 rectal swabs collected from the cooking staff of the fried egg shop. In total, *Staphylococcus aureus* was isolated in 45 of all rectal swabs, including 12 enterotoxin A-genic, 2 type B-genic, and 5 type C-genic.

Laboratory findings of food specimens are shown in Table 5. From remaining boxed lunches collected from the four schools, *Bacillus cereus* ranging from 2.0×10^3 to 4.0×10^3 CFU/g was isolated from the rice. In boxed lunches collected from two schools, *Bacillus cereus* of 2.0×10^3 and 2.0×10^4 CFU/g respectively was isolated from the vegetable, though not from others. From the boxed lunches kept by the Food Factory, *Bacillus cereus* of 2.0×10^4, 2.0×10^3, 2.0×10^4 CFU/g was isolated respectively from the rice, fried egg and vegetable. Of the environmental specimens, *Bacillus cereus* (6.0×10^3 CFU/g) was isolated from one drain water specimen and not from others.

Discussion and Conclusion

A total of 1,147 teachers and students of four junior high schools in Taichung County, after having had boxed lunches supplied by Food Factory X, developed gastro-intestinal symptoms, giving a high attack rate of 58.8%. By the definition of a case, the attack rate of School A was as high as 56.4%. Data collected from questionnaire interview showed that the attack rates declined with age (see Table 3, 63.0% for the first grade, 37.5% for the second grade, and 54.0% for the third grade), suggesting that older students were more resistant to the pathogenic agents. Symptoms were moderate, including more diarrhea and abdominal pain, but less vomiting and nausea. Though the attack rate was as high as 56.4%, only 21.6% (44/204) were cared medically, and none was hospitalized.

From the incubation periods and the distribution of symptom frequencies, the incident was more likely a *Staphylococcus aureus* infection[4]. The Central Branch Laboratory of the National Institute of Preventive Medicine had isolated *Staphylococcus aureus* in 29 human specimens collected from students and in 16 specimens collected from the cooking staff of the Factory X, giving a positive rates of 19.2% and 30.2% respectively. The *Staphylococcus aureus* isolated from the 45 specimens was capable of producing three types of enterotoxins, type A (12 cases), type B (2 cases) and type C (5 cases). Some cooks could be *Staphylococcus aureus* carriers to have contaminated the food. It was therefore concluded that *Staphylococcus aureus* was the pathogenic agent of the present incident. Since the amount of the bacilli was not pathogenic enough yet ($>10^5$ CFU/g) and the major symptom was not vomiting, also considering the incubation period of this outbreak, *Bacillus cereus* should not be the pathogenic agent of the present incident.

Boxed lunches supplied to the four schools were packed on the second production line in the Factory X. They were also the first products of that line (they were packed by 10 a.m.). Workers of this production line wore working clothes, gloves, hats and masks. The possible reason that a large-scale food poisoning could have been induced in such a short period of time was that a certain food item was contaminated and left unprotected for too long for pathogenic agents to grow in large number or to produce exterotoxins. Data from epidemiological surveys and statistical analysis showed that the diced chicken, port with pickles, fried egg, vegetarian chicken and fried vegetable were all associated with the poisoning. This means that there was cross contamination among food in the box, resulting in all food items being contaminated by the pathogenic agents. The creamed corns were in semi-jelly shape. They were less likely to be contaminated and therefore were not associated with the poisoning. The vegetarian chicken and fried vegetable were prepared on the morning of that day, their chances of being contaminated or pathogenic agents growing to large number were little, they were less likely to induce food poisoning of such large scale. The diced chicken was processed with soy sauce, sugar and pepper on the night of March 17. If it was contaminated by *Staphylococcus aureus*, under room temperature, the pathogenic agent would have

sufficient time to grow in large number or to produce enterotoxins. Though the diced chicken was heated again the next morning, the temperature was not high enough to destroy enterotoxins. Since the relative risk for the diced chicken was as high as 1.65 (95% confidence interval at 1.17-2.34), the diced chicken was most likely the cause of the poisoning. The pork with pickles was also suspicious, as the relative risk was also as high as 1.41 (95% confidence interval at 1.12-1.78). It was prepared the night before and left uncovered in the pot. The next morning, for cooking other food under a tight schedule, the pork with pickles was heated in a hurry, removed from the pot for packing on the second production line, and result in the food poisoning. When fried eggs were delivered to the Factory X, the time they were left unprotected was not long enough for the pathogenic agents to grow in large number. In summary, the diced chicken and pork with pickles were more likely to be the causative food items of the present incident.

In food remains, only *Bacillus cereus*, though not in large number, was isolated (Table 5). The boxed lunches that the Factory gave the schools to keep were different from the boxed lunches supplied to the students. They were packed in polyethylene sheet. This was why no other pathogenic agents were isolated from the boxed lunches kept by the schools, and why laboratory findings of food remains were different from those of human specimens. Laboratory findings of food remains though helped in the investigation, were not used in identifying the pathogenic agents.

Recommendations

For the prevention of similar incidents in the future, the following recommendations are made:

1. Personal hygiene of cooking staff is most important[6]. Hands must be kept clean, and without wounds. While working, gloves, hats and masks must be worn. Regular physical examination is required. Individuals with wounds on hands or carriers should not be allowed to process and manufacture food.
2. Kitchen utensils, containers, and clothes, gloves, hats and masks of cooking staff should be kept clean to avoid the growth of pathogenic agents[6].
3. Food to be left overnight should be kept properly (frozen under 4 ℃, for instance) or heated again thoroughly[7] to avoid the growth of pathogenic agents.
4. Local health authorities, upon their inspection of food factories, should be more careful. For example, if a food factory is not equipped with egg frying machines, it must purchase fried eggs somewhere else. Buying food from other sources is in violation of regulations.
5. Local health authorities should organize regular training programs on food safety for food handlers.

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Medicine, and the Central Branch Laboratory of the National Laboratories for Foods and Drugs for their cooperation.

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Figure 1. Distribution of Incubation Periods

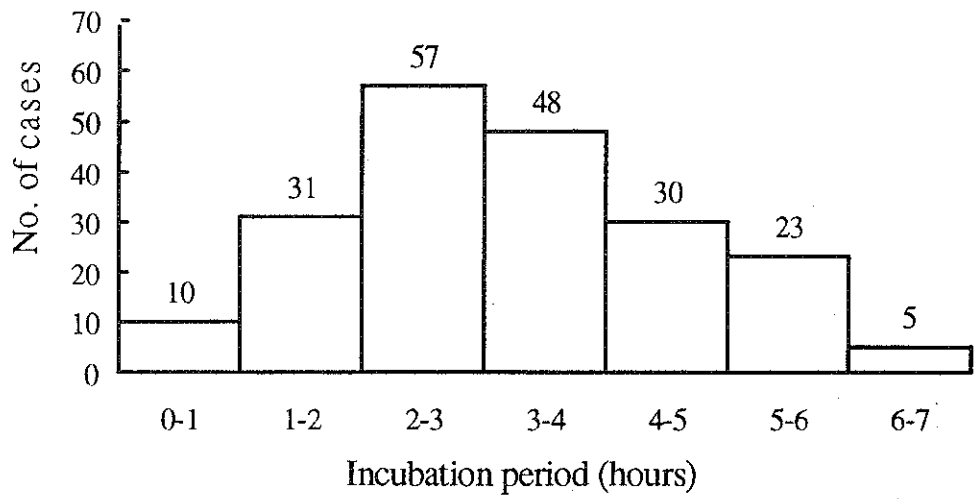

Table 1. Lunch Supply at Four High Schools, March 18, 1997

School	No. of Students	Source of Lunch	No. Lunch Boxes Ordered	No. of Cases (%)
A	1,290	Factory X	362	270 (75%)
		Factory C	176	0
		Factory D	503	0
		Brought by Self	249	0
B	1,601	Factory X	847	640 (76%)
		Brought by Self	754	0
C	3,207	Factory X	434	66 (15%)
		Factory A	1,063	0
		Factory B	314	0
		Brought by Self	1,391	0
D	3,845	Factory X	305	171 (56%)
		Factory E	71	0
		Factory D	1,178	0
		Brought by Self	2,291	0

Table 2. No. of Cases and Attack Rates by Sources of Lunch, School A

Source	No. Lunch Boxes Ordered	No. of Cases	Attack Rate (%)
Factory X	362	204	56.4
Factory C	453	5	1.1
Factory D	149	1	0.7
Brought by Self	151	1	0.7
Others	96	2	2.1
Total	1,211	213	17.6

Table 3. No. of Cases and Attack Rates by Class, School A

Class	No. of Students	No. Lunch Boxed Ordered	No. of Cases	Attack Rate (%)
First Grade:				
Class 1	42	39	19	48.7
Class 4	40	32	19	59.4
Class 5	41	38	19	50.0
Class 7	43	40	35	87.5
Class 9	43	38	25	65.8
Second Grade:				
Class 1	44	40	15	37.5
Third Grade:				
Class 2	45	1	1	100.0
Class 3	43	41	24	58.5
Class 4	41	30	18	60.0
Class 8	39	33	15	45.5
Class 10	40	30	14	46.7

Attack rates by grade: 63.0% for the first grade (117/187); 37.5% for the second grade (15/40); 54.0% for the third grade (72/134).

Table 4. Attack Rates and Relative Risks by Food Items, School A

Food	No. Eaten			No. Not Eaten				
	Sick (1)	Not Sick (2)	Attack Rate (%) (3)=(1)/(1)+(2)	Sick (4)	Not Sick (5)	Attack Rate (%) (6)=(4)/(4)+(5)	Relative risk (3)/(6)	95% Confidence Interval
Diced chicken*	182	118	60.7	22	38	36.7	1.65	1.17-2.34
Pork with pickles*	155	94	62.3	49	62	44.1	1.41	1.12-1.78
Fried egg*	176	113	60.9	28	43	39.4	1.54	1.14-2.09
Vegetarian Chicken*	131	74	63.9	72	81	47.1	1.36	1.12-1.65
Stir-fried Vegetable*	106	57	65.0	97	99	49.5	1.31	1.10-1.57
Corns	155	110	58.5	47	46	50.5	1.16	0.92-1.45
rice	200	146	57.8	4	12	25.0	2.31	0.98-5.43

* Statistically significant, χ^2 test, $p<0.01$.

Table 5. Results of Laboratory Testing of Food Items

Food	Source	*S. aureus* Enterotoxin CFU/g	*B cereus* CFU/g	*Salmonella* CFU/g	Pathogenic *E coli* CFU/g	*V. parahaemo-lyticus* CFU/g
Rice	School A	negative/not isolated	$2.0x10^3$	negative	negative	negative
	B	negative/not isolated	$4.0x10^3$	negative	negative	negative
	C	negative/not isolated	$2.0x10^4$	negative	negative	negative
	D	negative/not isolated	$2.0x10^4$	negative	negative	negative
	Factory X	negative/not isolated	$2.0x10^4$	negative	negative	negative
Diced chicken	School A	negative/not isolated	negative	negative	negative	negative
	B	negative/not isolated	negative	negative	negative	negative
	C	negative/not isolated	negative	negative	negative	negative
	D	negative/not isolated	negative	negative	negative	negative
	Factory X	negative/not isolated	negative	negative	negative	negative
Pork with pickles	School A	negative/not isolated	negative	negative	negative	negative
	B	negative/not isolated	negative	negative	negative	negative
	C	negative/not isolated	negative	negative	negative	negative
	D	negative/not isolated	negative	negative	negative	negative
	Factory X	negative/not isolated	negative	negative	negative	negative
Fried egg	School A	negative/not isolated	negative	negative	negative	negative
	B	negative/not isolated	negative	negative	negative	negative
	C	negative/not isolated	$3.0x10^3$	negative	negative	negative
	D	negative/not isolated	negative	negative	negative	negative
	Factory X	negative/not isolated	$2.0x10^3$	negative	negative	negative
Stir-fried Vegetable	School A	negative/not isolated	negative	negative	negative	negative
	B	negative/not isolated	negative	negative	negative	negative
	C	negative/not isolated	negative	negative	negative	negative
	D	negative/not isolated	$2.0x10^3$	negative	negative	negative
	Factory X	negative/not isolated	$2.0x10^4$	negative	negative	negative
Corns	School A	negative/not isolated	negative	negative	negative	negative
	B	negative/not isolated	negative	negative	negative	negative
	C	negative/not isolated	negative	negative	negative	negative
	D	negative/not isolated	negative	negative	negative	negative
	Factory X	negative/not isolated	negative	negative	negative	negative