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PONTIAC FEVER: ISOLATION OF THE ETIOLOGIC AGENT (*LEGIONELLA PNEUMOPHILA*) AND DEMONSTRATION OF ITS MODE OF TRANSMISSION

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Pontiac fever, a unique epidemiologic form of legionellosis, is characterized by a short (one- to two-day) incubation period and a self-limited grippe-like illness without pneumonia. In 1968, the first documented outbreak of this syndrome affected persons who had entered a health department building in Pontiac, Michigan. Epidemiologic analyses clearly implicated an airborne agent and suggested that evaporative condenser water aerosols being disseminated by a defective air conditioning system played a key role in the outbreak. Guinea pigs that were exposed in the building and to laboratory aerosols of evaporative condenser water developed bronchopneumonia. *Legionella pneumophila* (serogroup 1) was isolated from the exposed guinea pigs' lungs. Paired acute and convalescent serum specimens from 37 patients were tested by the indirect fluorescent antibody technique using *L. pneumophila* serogroup 1 antigen, and 31 (84%) had rises in titer from <32 to ≥64.

air conditioning; air microbiology; guinea pigs; Legionnaires' disease

An acute febrile illness affected persons who had entered a health department building in Pontiac, Michigan, in July and August, 1968 (1). The disease, subsequently designated Pontiac fever, had a one- to two-day incubation period and was characterized by fever, chills, myalgia, malaise and headache. Respiratory symptoms such as a slight cough, chest pain or constrictive sensations were common, but no radiographic evidence of pulmonary disease was found. The illness was self-limiting, typically lasting two to five days, and caused no permanent sequellae or deaths.

The health department building, which was built in 1956, is located in an office park of county service buildings. The health department building is not connected to other buildings and has ground-level and basement floors. The ground-level floor contains offices, clinic rooms,

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Abbreviations: EC, evaporative condenser; IFA, indirect fluorescent antibody; SPG, sucrose-phosphate-glutamate.

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storage rooms and an auditorium. The basement contains a laboratory, storage and utility rooms, and the equipment room where the air-conditioning machinery is located. The air-conditioning system had been in use without modification since the building was opened.

Epidemiologic studies established early in the course of the investigation that Pontiac fever was an airborne disease, but person-to-person transmission could not be documented (1). The building's air-conditioning system was suspected as the source and mechanism of spread. Defects in the air-conditioning system allowed water from its evaporative condenser to enter the general air circulation ducts. However, extensive laboratory studies of specimens from patients or the environment at the time of the epidemic failed to implicate any known infectious or toxic agent (1).

On the chance that they might be susceptible, six Hartley strain guinea pigs were placed in the building in mid-July, 1968. They developed fever within two or three days. At necropsy, their lungs were studded with varying numbers of small firm gray nodules due to focal bronchopneumonia. The cause of the pneumonia could not be documented. More guinea pigs as well as mice, rabbits and rhesus monkeys were placed in the building in late July and early August, 1968. Only the guinea pigs developed fever and pneumonia. Guinea pigs placed in other buildings as controls did not become ill and had no evidence of pneumonia upon necropsy.

Because of the above observations, a series of experiments utilizing guinea pigs were conducted. In August, 1968, a controlled exposure of guinea pigs in the health department building indicated the presence of an airborne microorganism that could produce bronchopneumonia and was, at least partially, susceptible to antibiotics. In the period September, 1968, to March, 1971, aerosolization experiments at the Centers for Disease Control demonstrated 1) that guinea pigs exposed to evaporative condenser water aerosols developed bronchopneumonia, 2) that the disease-producing potential of the water was eliminated by both filtration and autoclaving, and 3) that the disease-producing potential of the water declined during storage. Concurrent attempts to isolate the microorganism causing bronchopneumonia in the guinea pigs and demonstrate its etiologic significance in Pontiac fever were unsuccessful. In 1977, tests of stored specimens demonstrated that *Legionella pneumophila* was the agent of Pontiac fever.

Controlled guinea pig exposure in the health department building

Materials and methods

A controlled exposure of guinea pigs in the health department building was initiated on August 14, 1968, about six weeks after the epidemic began and two weeks after onset of the last recognized human case. No changes had been made in the air-conditioning system before or during the controlled guinea pig exposure period. Hartley strain guinea pigs weighing 250 to 300 g were used in this study. The guinea pigs were divided into four groups: 1) a control group of 15 animals housed in a nearby animal-shelter building that was not air-conditioned; 2) a test group of 25 exposed in the health department building with no protective measures; 3) a test group of 30 similarly exposed but given intraperitoneal injections of 2 mg of streptomycin and 2 mg of tetracycline every 12 hours during the exposure period; and 4) a test group of 29 housed in flexible plastic-film chambers that were maintained under positive pressure by air passed through high-efficiency fiberglass filters. The plastic-film chambers receiving filtered air were intended to eliminate exposure to airborne microorganisms without affecting exposure to filterable air components. Unfortunately, the high

daytime temperature in the building required us to leave the chamber entry ports open to prevent heat prostration in the guinea pigs. Six animals in this group died of heat prostration before the problem was discovered. The guinea pigs in the chambers thus had exposure to unfiltered air, but presumably had less exposure than the other groups.

The three test guinea pig groups were exposed in one room in the office area of the health department building. To assure maximum exposure to aerosols generated by the air-conditioning system, they were placed under air-duct outlets at desk-top level. The air-conditioning system, however, was only operated between 5:30 p.m. and 7:30 a.m. to protect persons entering the building during the day. All persons entering the building were also required to wear surgical masks.

The rectal temperatures of all guinea pigs were measured twice a day. Because the temperature of guinea pigs rises in a hot environment, the marked fluctuation of temperature in the building between day and night resulted in a comparable fluctuation of the guinea pigs' temperature. For this reason, we could not determine whether the animals had fever.

Results

Five animals from each group were sacrificed after five days' exposure. None of the animals had clinical illness or necropsy evidence of pneumonia by gross and microscopic examination.

The remaining animals were exposed for another two to four days before being shipped to Atlanta. Unfortunately, 15 guinea pigs died in transit and another 12 lost their identifying ear tags. All surviving guinea pigs that still had their ear tags in place were sacrificed two weeks after initial exposure. None of these animals appeared ill. However, nodules were found in the lungs of 14 of 15 guinea pigs exposed with no protection, in the lungs of seven of 12 exposed but given antibiotics, in the lungs of one of eight held in the chambers in the exposure area, but not in the lungs of 10 housed in the animal-shelter building near the health department (table 1). When compared to the unprotected group, each of the other groups had significantly reduced frequency of nodular lesions (Fisher's exact test, one-tailed: $p = 0.043$, 0.0002, and 0.0000001, respectively). The firm, gray nodules protruded from the lung surface and were less than 1 mm to 15 mm in diameter. The number of nodules per animal varied from few to many; they were scattered throughout all lobes of the lungs. The character, number and distribution of the lung nodules were similar for the three affected exposure groups.

When hematoxylin and eosin stained sections were examined, the nodular lesions were found to be the result of

TABLE 1

Key results of the controlled guinea pig exposure in the health department building, Pontiac, Michigan, 1968, initiated six weeks after Pontiac fever epidemic began

Exposure group	Exposure history (building, protective measures)	Pneumonia incidence*		Lung culture results†	
		No. necropsied	No. with pneumonia	No. cultured	No. positive for *L. pneumophila*
1	Health department, no protection	15	14	10	4
2	Health department, antibiotics	12	7		
3	Health department, air filtration‡	8	1		
4	Animal shelter, no protection	10		9	

* Pneumonia incidence in guinea pigs that were necropsied two weeks after start of the seven to nine day exposure period.

† Lung specimens from only 19 guinea pigs were still available at the time of culture in 1977.

‡ Exposed to unfiltered air as well as air passed through high efficiency fiberglass filters.

bronchopneumonia. Their morphology varied according to their apparent age. With an acute lesion, the bronchioles were filled with neutrophils, and the adjacent alveolar spaces were filled with various proportions of neutrophils and mononucleated phagocytic cells. The center of the large lesions was often necrotic. With resolving lesions, mononucleated phagocytes predominated in the infiltrate. Balls or bundles of fibrin were frequently present in the lesions as well as in the alveoli at their periphery. The lesions were usually sharply demarcated from the surrounding normal lung tissue. In some sections, the lesions were undergoing encapsulation with fibrous connective tissue. Small, pleomorphic, Gram-variable bacterial rods were seen in impression smears of the nodules. Similar bacteria were occasionally seen in phagocytic cells in hematoxylin and eosin and Giemsa-stained sections. No known etiologic agent could be seen in lung sections stained according to the following techniques: Brown and Brenn, acid-fast, Gomori's methenamine silver, and the periodic acid-Schiff reaction. Electron-microscopic examination of three lung nodules revealed no causative agent.

Nodular lesions from affected guinea pigs and lung wedge sections from unaffected guinea pigs were cultured for microorganisms with various techniques in 1968. Several streptococcal species (*S. bovis, S. uberis, S. salivarius,* and unidentified alpha-hemolytic streptococci), *Bacillus* spp., and *Bordetella bronchiseptica* were isolated but not in a pattern that suggested a causative role. Similar organisms were isolated from the lungs of guinea pigs in all four exposure groups with no evidence of bronchopneumonia. No viral, rickettsial or fungal agents were isolated.

Aerosol studies at the Centers for Disease Control

Pontiac fever and the bronchopneumonia of the guinea pigs were both clearly related to exposure in the health department building. If both diseases were caused by the same agent, an ongoing source of infection had to be present at the building. The evaporative condenser water seemed the most likely source. In the period 1968–1971, a series of aerosolization experiments to test the role of such water were conducted at the Centers for Disease Control.

Materials and methods

Portions of a sample of evaporative condenser water collected August 2, 1968, and stored under refrigeration were used for all the key experimental studies.

Guinea pigs weighing about 400 g were used in the aerosol studies. They were held in a room reserved for nonexposed animals for one to two weeks before being exposed to evaporative condenser water aerosols. Baseline rectal temperatures were determined during this period. Guinea pigs exposed to the aerosols were housed in a separate room during and after exposure to prevent potential cross-infection of unexposed control animals.

The test animals were confined in individual wire cages and placed in a 385 l chamber for aerosol exposure. The chamber was located in a well-ventilated isolation room, with air that entered and left the room being passed through high efficiency filters. The aerosols were generated by a baffled nebulizer. Droplet diameter in the primary aerosol generated by this nebulizer was $<5\ \mu$ as determined by measurement of droplets on coated slides. After each exposure, the chamber was cleaned and disinfected with peracetic acid.

The rectal temperature of exposed and control guinea pigs was determined each day they were exposed and up to 14 days after. A rectal temperature $\geq$40 C was considered evidence of a febrile response.

Results

Reproduction of guinea pig pneumonia. In the period September–Decem-

ber, 1968, preliminary experiments demonstrated that guinea pigs exposed to evaporative condenser water aerosols developed bronchopneumonia. Four guinea pigs were initially exposed in the aerosol chamber for 15 hours over a two-day period. Within 24 hours after termination of exposure, all four guinea pigs were febrile. On the next day, they were still febrile, and two were sacrificed for necropsy. The other two animals were febrile for one more day and then were afebrile until sacrificed six days later. All four animals had bronchopneumonia at various stages of development.

Four other guinea pigs were exposed to an aerosol of sterile distilled water under the same conditions. None of these animals developed fever or had evidence of bronchopneumonia at necropsy 14 days after termination of exposure.

The effect of reduced dose was then evaluated. Six guinea pigs were exposed to an aerosol of evaporative condenser water diluted 1:10 with sterile distilled water. The total exposure time was 20 hours over a three-day period.

In the 10-day post-exposure observation period, a biphasic incidence of fever occurred in the group. None of the animals had fever during the exposure period, but four of the six were febrile on the first post-exposure day. All six were afebrile from then on. At necropsy on the tenth day following exposure, all six guinea pigs had the characteristic pulmonary nodules caused by bronchopneumonia.

Exposure to filtered and autoclaved evaporative condenser water. In February, 1969, the effect of filtration and heat sterilization on the disease-producing potential of evaporative condenser water was evaluated. Three test groups of 11 guinea pigs each were exposed to the following aerosols: 1) untreated evaporative condenser water diluted 1:10 with sterile distilled water; 2) similarly diluted water filtered through a membrane with an 0.22 μ pore size; and 3) similarly diluted water that was autoclaved for 20 min at 121 C. All three test groups were exposed to their respective aerosol for 18 to 20 hours over a three-day period. Fifteen unexposed guinea pigs (five per test group) served as source group controls.

Of the 11 guinea pigs exposed to untreated evaporative condenser water, all but one had fever during or after exposure (table 2). A biphasic incidence of fever was observed. Ten of the 11 animals were febrile on the first post-exposure period day, four on the second, three on the third, and eight on the fourth. Only one guinea pig was febrile the last four days of the observation period. All 11 guinea pigs had bronchopneumonia at necropsy on the eighth day following termination of exposure.

TABLE 2

Results of guinea pig exposure to untreated, filtered and autoclaved evaporative condenser (EC) water aerosols, Centers for Disease Control, 1969

Type of EC water aerosol*	No. exposed	Illness after exposure		Lung culture results	
		No. with fever	No. with pneumonia†	No. cultured‡	No. positive for *L. pneumophila*
Untreated	11	10	11	10	6
Filtered	11			8	1
Autoclaved	11				
None	15			7	

* Filtered = passed through membrane with 0.22 μ pore size; autoclaved = heated for 20 min at 121 C; none = no aerosol exposure.

† As determined by both gross and histologic examination of lungs at necropsy on 8th day after termination of exposure.

‡ Specimens from only 25 guinea pigs were still available at the time of culture in 1977.

Source control guinea pigs and those exposed to filtered and autoclaved evaporative condenser water aerosols did not have fever and did not have bronchopneumonia at necropsy.

Duration of evaporative condenser water infectivity. After the experiment to evaluate the effect of filtration and autoclaving on evaporative condenser water, guinea pigs were exposed to aerosols of the August, 1968, evaporative condenser water sample in January, 1970, May, 1970, and March, 1971. The water sample had been refrigerated throughout this time period.

In January, 1970, all 16 guinea pigs exposed to evaporative condenser water aerosols developed bronchopneumonia. In May, 1970, 14 of 18 exposed guinea pigs developed bronchopneumonia, but their disease was much less extensive than that of animals exposed earlier. In March, 1971, none of 35 guinea pigs had either fever or bronchopneumonia after being exposed.

Microbiologic studies in 1968–1971. Extensive efforts were made to isolate the microorganism that had caused the guinea pigs' bronchopneumonia. The bronchopneumonia was thought to have been caused by a bacterial or, less likely, a rickettsial organism. Fungi could not be demonstrated in the tissues of guinea pigs exposed in the health department building. Antibiotics given guinea pigs exposed in the health department building had an apparent protective effect, and filtered evaporative condenser water aerosols did not cause the guinea pigs to have bronchopneumonia.

Both guinea pig tissues and evaporative condenser water were examined. No viral, rickettsial or fungal agents were isolated from the guinea pigs in tests involving a wide range of isolation media. The isolated lung flora of the exposed and control guinea pigs, however, was comparable.

The water contained numerous bacterial types which were predominately *Bacillus* spp., *Corynebacterium equi*, *Flavobacterium* spp., *Mycobacterium rhodochrous*, and *Pseudomonas* spp. None of these organisms were isolated with any significant frequency from guinea pigs exposed to the health department building or to evaporative condenser water aerosols. Guinea pigs were exposed to pure culture aerosols of *Bacillus* spp., *C. equi*, *M. rhodochrous*, *P. alcaligenes* and *P. stutzeri* strains from the water. Despite an inhaled dose of 1×10^5 to 1×10^7 of each of these organisms, none of the exposed guinea pigs developed the characteristic bronchopneumonia.

Both evaporative condenser water and material from lung lesions from guinea pigs that were exposed to the health department building and to evaporative condenser water aerosols were injected into the yolk sacs of embryonated chicken eggs. A small Gram-negative bacterium was observed only in yolk sacs that had been injected with material from lesions. Indirect fluorescent antibody (IFA) examinations of yolk sac material using paired acute and convalescent sera from seven Pontiac fever patients produced equivocal results, and a clear-cut pattern of antibody titer changes was not observed. Whether the bacterium was a growth variant of other organisms being isolated from the eggs on plating media or a different species could not be determined; its true significance was determined eight years later.

Attempts to isolate the causative agent were discontinued in 1971.

Isolation and identification of the agent

Serologic clues

In July and August, 1976, an outbreak of Legionnaires' disease occurred in Philadelphia (2). The illness differed strikingly from Pontiac fever. Legionnaires' disease had a longer incubation period (two to 10 days), was associated

with pneumonia in 90 per cent of cases, and had a case-fatality rate of 15 per cent. Nonetheless, on the chance that the two diseases might be caused by related organisms, sera from Pontiac fever patients were tested for antibody to *L. pneumophila*.

Serum specimens included in the evaluation were paired acute and convalescent sera from 37 Pontiac fever patients and paired sera from 10 unaffected persons who worked in another health department building in the same county. The acute serum specimens were obtained during or shortly after the patients' illness, and the convalescent serum specimens were obtained about 14 days later. The serum specimens from the healthy persons were obtained at the time of the outbreak with an interval of about 14 days between the paired collections.

All the Pontiac fever patients whose sera were tested had the most characteristic (grade I) syndrome associated with the outbreak (1). Their acute illness consisted of three or more of the following symptoms: 1) fever and/or chills, 2) generalized myalgia, 3) malaise, and 4) headache.

Serum specimens were tested with the IFA technique using a strain of serogroup 1 *L. pneumophila* isolated from a patient associated with the 1976 Philadelphia outbreak (3). Of the 37 Pontiac fever patients, 31 (84 per cent) had seroconversions from acute phase titers of <32 to convalescent phase titers of ≥64 (table 3). Of the remaining six patients, one had a stable titer of 256 in both acute and convalescent specimens and the others had negative serologic results (titers ≤32). None of the 10 healthy persons who worked in the other county health department building had serologic evidence of infection.

The serologic results strongly suggested that Pontiac fever was caused by *L. pneumophila* or an antigenically related organism.

Culture of stored specimens from earlier experiments

Fortunately, key specimens from the guinea pig experiments had been stored at −70 C. These included: 1) lung tissue from 10 guinea pigs exposed without protection in the health department building and nine control guinea pigs that had been housed in a nearby building during the controlled August, 1968, exposure; 2) lung tissue from 14 guinea pigs exposed during various evaporative condenser water aerosol experiments in the laboratory; 3) 64 yolk sacs from embryonated chicken eggs that had been injected with lung tissue from 10 guinea pigs exposed to evaporative condenser water aerosols, eight guinea pigs exposed to filtered evaporative condenser water aerosols, and seven control guinea pigs; and 4) yolk sacs from 20 embryonated chicken eggs that had been injected with evaporative condenser water. In addition, evaporative condenser water from the health department building, a water specimen from a drinking water cooler in the health de-

TABLE 3

Results of indirect fluorescent antibody tests with L. pneumophila *serogroup 1 of serum specimens from Pontiac Fever patients*

Serologic category	No. of patients	Highest titer observed for each patient						
		<32	32	64	128	256	512	>1024
Seroconversion*	31			5	9	9	5	3
Positive†	1					1		
Negative	5	3	2					

* Acute serum specimen titer was <32 for all 31 patients.

† Stable titer in acute and convalescent serum specimens.

partment, and an evaporative condenser water specimen from a building adjacent to the health department were available. The water specimens had been refrigerated for more than eight years.

The lung tissue and yolk sacs that had been injected with lung tissue were macerated and diluted to roughly 10 per cent suspensions using sucrose-phosphate-glutamate (SPG) buffer, pH 7.2. The yolk sacs injected with lung tissue from a single animal were pooled. One ml of the lung tissue or yolk sac pool suspensions were injected intraperitoneally into each of two guinea pigs. The guinea pigs were sacrificed on the second day they had fever (0.3 C rise in rectal temperature above baseline) or, if no febrile response occurred, on the fifth day following injection. Their spleens were removed aseptically, macerated, and prepared as a 10 per cent suspension in SPG. The spleen suspension was streaked on Mueller-Hinton agar supplemented with 1 per cent hemoglobin and 1 per cent IsoVitaleX (BBL Microbiology Systems, Cockeysville, MD), and injected into a guinea pig for a second passage. Tissue from culture-negative spleens of animals that had had fever for two days was injected into the yolk sacs of 12 embryonated chicken eggs. The spleen from second passage guinea pigs was streaked on supplemented Mueller-Hinton agar and injected into embryonated chicken eggs. Yolk sacs from eggs whose embryos had died four to 11 days post-injection were harvested and stained according to the Gimenez method for evidence of *L. pneumophila* infection. Yolk sacs containing morphologically and tinctorially compatible organisms that were IFA test positive for *L. pneumophila* were cultured on supplemented Mueller-Hinton agar. A flow diagram of the schema for culturing these specimens is presented in figure 1.

The yolk sacs from eggs that had been injected with evaporative condenser water in 1969 were individually streaked on supplemented Mueller-Hinton agar without further passage in guinea pigs. In addition to direct streaking, the yolk sacs were combined into seven pools and injected into embryonated chicken eggs.

The water specimens were first passed through a membrane with an 0.22 μ pore size. The material trapped on the membrane was eluted in 3 ml of SPG, and 1 ml of the eluate was then injected intraperitoneally into each of two guinea pigs. These guinea pigs were evaluated in a manner identical to that used for those injected with the tissue suspension.

Legionella pneumophila serogroup 1 (4–6) was isolated from the specimens in a pattern consistent with the organism having been in the air of the health department building as the result of dissemination of evaporative condenser water via the air conditioning system. It was isolated from lung specimens of four of 10 guinea pigs housed in the building without protection in August, 1968, but from none of the lung specimens of nine guinea pigs housed in the nearby animal-shelter building (table 1).

The presence of *L. pneumophila* in the evaporative condenser water was documented when it was isolated from the lung tissue of three of 14 guinea pigs exposed to various evaporative condenser water aerosol experiments in the laboratory and when it was isolated from yolk sacs injected with lung tissue from six of 10 guinea pigs exposed to untreated water aerosols in early 1969 (table 2). *L. pneumophila* was also isolated from yolk sacs injected with lung tissue from one of eight guinea pigs exposed to filtered water aerosols in early 1969 (table 3). None of the other specimens including those from seven control animals for the early 1969 aerosol experiments yielded *L. pneumophila*.

Discussion

The Pontiac fever investigations provided the best documentation available to

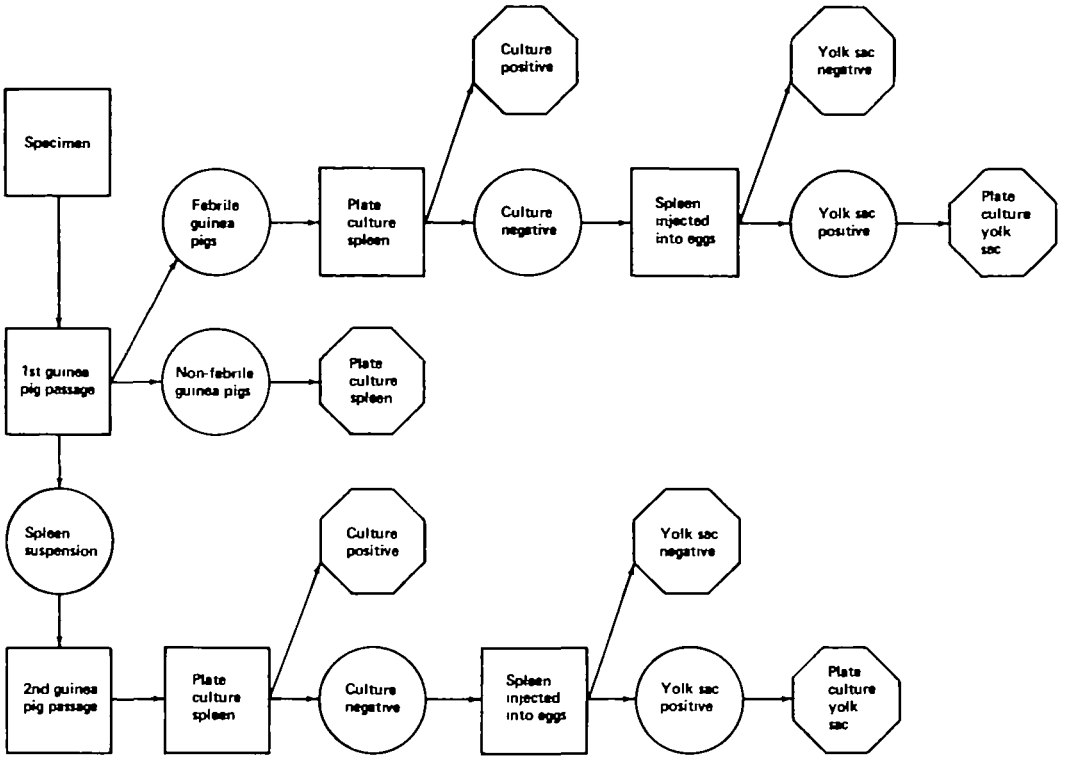

FIGURE 1. Flow diagram of schema for culturing specimens from guinea pig experiments in Pontiac fever investigation.

date for airborne transmission of *L. pneumophila* from environmental sources. The epidemiologic studies clearly implicated an airborne agent and suggested that evaporative condenser water aerosols disseminated by a defective air-conditioning system had a key role in the outbreak (1). Guinea pigs that were exposed to the building and to laboratory aerosols of evaporative condenser water developed bronchopneumonia, and *L. pneumophila* serogroup 1 was isolated from their lungs. Unexposed guinea pigs were not infected. Paired acute and convalescent serum specimens from 37 patients were tested with the IFA technique using *L. pneumophila* serogroup 1 antigen and a high proportion (84 per cent) had rises in titer from $\leq$16 to $\geq$64. Serum specimens from 10 unaffected health department employees who had not entered the building during the epidemic were negative in IFA tests. The observed serologic response of the patient group was compatible with recent *L. pneumophila* serogroup 1 infection (3, 7, 8).

The single result apparently at odds with the overall findings was the isolation of *L. pneumophila* from the yolk sacs injected with lung tissue from one of eight guinea pigs exposed to the filtered evaporative condenser water aerosols in early 1969 (table 2). None of these animals had gross or histologic evidence of pneumonia, and they were never housed in the same room with animals infected with *L. pneumophila*. Also, *L. pneumophila* has never been isolated from many hundreds of control guinea pigs examined at the Centers for Disease Control. The filtration process may not have been totally effective or some other technical problem may have resulted in subclinical infection of the guinea pig. No

satisfactory explanation for this observation has been found.

The reasons why the IFA tests in 1969 did not successfully identify the etiologic agent in the yolk sacs are uncertain. The IFA tests in 1977 were facilitated by the standardization of procedures and reagents during the investigation of the Philadelphia outbreak of Legionnaires' disease.

As an epidemic form of legionellosis, Pontiac fever is clearly different from Legionnaires' disease. The two Pontiac fever outbreaks reported to date have been characterized by short incubation periods (five to 66 hours), high attack rates (95–100 per cent), self-limited non-pneumonic illness, and no deaths (1, 9, 10).

The basis for the epidemiologic and clinical differences is not known. No important differences have been found to date between the Pontiac strains and other serogroup 1 strains in regard to growth characteristics, antigens, antibiotic sensitivity, or pathogenicity for chicken embryos and guinea pigs. Differences in inhaled dose, whether higher or lower, would not seem compatible with the combination of the shorter incubation period and the milder disease observed in Pontiac fever patients.

In the two Pontiac fever outbreaks, the median patient age was lower than that in Legionnaires' disease outbreaks (11). The median age of the Pontiac fever patients, however, reflected the age distribution of persons exposed to the epidemic sites. Patient age alone might explain the difference in clinical severity of the two diseases but would not adequately explain differences in incubation period and attack rate.

Although it seems unlikely, an allergic response by previously hypersensitized individuals cannot be totally ruled out as a mechanism for Pontiac fever. The high attack rate even for persons who had no prior exposure to the building or the geographic area (1) is not typical of hypersensitivity reactions. Also, only 45 of 117 patients re-exposed to the building in the epidemic period claimed recurrent symptoms, and most had atypical illness (1).

Another hypothesis is that Pontiac fever results from the toxic effects of a large number of dead *L. pneumophila* organisms (10). Whether or not this hypothesis is correct, initial exposure and illness appear to provide protection on re-exposure, as indicated by the illness and exposure histories of the 117 patients discussed above. The toxic substance would also have to be non-filterable and heat-labile. Guinea pigs exposed to filtered and autoclaved cooling tower water did not have fever. Guinea pigs receiving aerosol doses of 2100 heat-killed *L. pneumophila* (Philadelphia 1 strain) organisms do not have fever or an antibody response whereas the same dose of viable organisms causes both fever and an antibody response (12). The total dose of live and dead organisms inhaled by Pontiac fever patients is unknown, but they did have an antibody response.

Two groups of guinea pigs exposed to evaporative condenser water aerosols had a biphasic incidence of fever. One possible explanation for this observation is that the peak on the first post-exposure day resulted from a toxic effect and the second peak on the fourth post-exposure day resulted from onset of clinical bronchopneumonia. Whether or not a toxic effect was involved, the febrile response must have been due to inhaling live *L. pneumophila* organisms because the infectivity of the evaporative condenser water decreased over time. Guinea pigs exposed to evaporative condenser water aerosols in March, 1971, developed neither fever nor bronchopneumonia.

A toxic effect from inhaling live organisms seems the most probable pathogenetic mechanism for Pontiac fever. Certain clinical features of Legionnaires' disease, such as obtundation out of proportion to

hypoxemia or metabolic disturbances, suggest organism-related toxic effects. Endotoxin-like activity has been associated with *L. pneumophila* (13, 14). The endotoxin-like substance is heat-stable. Although highly reactive in the limulus lysate test, it is weakly pyrogenic for rabbits (14). *L. pneumophila* also has hemolytic activity in vitro, a property shared by various bacteria that produce toxins affecting cell membranes, but evidence for hemolytic activity in vivo is equivocal (15). Further amplification of these early studies is clearly needed.

The importance of synergistic interaction between the epidemiologist and laboratorian was demonstrated by these studies. The epidemiologic findings focused the laboratory investigations, and innovative approaches were supplied by the laboratorians in designing the experimental epidemiologic studies. Without this interaction, the Pontiac fever investigation would not have reached its highly successful conclusion.

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