Division of Bacterial Disease,
Center for Disease Control, NIOSH,
Atlanta, Georgia, EPI 81-82-2,
20 pages, 27 references

FOR ADMINISTRATIVE USE
LIMITED DISTRIBUTION
NOT FOR PUBLICATION

PUBLIC HEALTH SERVICE-CDC-Atlanta
EPI 81-82-2 September 20, 1983

To: Director, Centers for Disease Control

From: Division of Bacterial Diseases
Center for Infectious Diseases

Subject: Pontiac fever in an automobile manufacturing plant, Windsor, Canada

On August 27, 1981, Arthur L. Reingold, M.D., Medical Epidemiologist, Special Pathogens Branch (SPB), Bacterial Diseases Division (BDD), Center for Infectious Diseases (CID), received a telephone call from Frank Viola, M.D., the company physician of a motor company in Windsor, Ontario, about an outbreak of febrile illness among employees of the plant. Approximately 260 of 4,200 employees had developed fever, myalgia, headache, and dry cough during the week of August 17-21. Illness was self-limited and resolved in all cases within 3 or 4 days of onset. None of the individuals had evidence of pneumonia, and there were no fatalities. Because of the diagnosis of Pontiac fever was entertained, environmental sampling of the plant was undertaken, and preliminary results indicated that 2 of 18 samples contained Legionella pneumophila.

After discussions that involved Dr. Reingold, Claire V. Broome, M.D., Chief, SPB, Loreen A. Herwaldt, M.D., EIS Officer, SPB*; Rogert A. Feldman, M.D., Director, BDD*, Richard Keenlyside, M.D.* and Gary Liss, M.D.*, Hazard Evaluation and Technical Assistance Branch, Division Surveillance, Hazard Evaluation and Field Studies, National Institute for Occupational Safety and Health, Centers for Disease Control (CDC); Dr. Viola, Anne Robinson, Ph.D., Ministry of Labor for Ontario; J.R. Jones, M.D., Regional Health Officer for Windsor, J. Carlson, M.D., Ministry of Health for Ontario; P. Clayton, M.D., Ministry of Health, Ottawa; and M. Silverstein, M.D., United Auto Workers, it was concluded that an epidemiologic and environmental investigation was warranted. Drs. Robinson, Carlson and Clayton extended an invitation to CDC to participate in the investigation. Accordingly, Drs. Herwaldt and Reingold left for Windsor on September 9 to assist in the investigation. Results of the investigation are reported in the attached manuscript.

*These organization designations reflect the organization at the time the request for assistance was received.

Loreen A. Herwaldt, M.D.
Respiratory and Special
Pathogens Epidemiology Branch
Division of Bacterial Diseases
Center for Infectious Diseases

Claire V. Broome, M.D.
Chief, Respiratory and Special
Pathogens Epidemiology Branch
Division of Bacterial Diseases
Center for Infectious Diseases

Roger A. Feldman, M.D.
Director
Division of Bacterial Diseases
Center for Infectious Diseases

Distribution:
Mailing keys WF-1,2,3
James C. Feeley, Ph.D., Chief, Field Investigations Lab Section, RSPLB, DBD, Building 1, Room B353
John C. Feeley, Ph.D., Assistant Director for Laboratory Science, DBD, Building 1, Room 5041
Donald R. Hopkins, M.D., Assistant Director for International Health, Building 1, Room 2122
Richard Keenlyside, M.D., National Institute for Occupational Safety and Health, CDC, 4686 Columbia Parkway, Cincinnati, Ohio 45226
Gary Liss, M.D., National Institute for Occupational Safety and Health, CDC, 4676 Columbia Parkway, Cincinnati, Ohio 45226
Hazel Wilkinson, Ph.D., Chief, Special Immunology Section, RSPLB,DBD Building 5, Room 313

PONTIAC FEVER IN AN AUTOMOBILE ENGINE ASSEMBLY PLANT CAUSED BY A NEW LEGIONELLA SPECIES: LEGIONELLA FEELEII, SP. NOV.

Loreen A. Herwaldt, M.D.[1*]
George W. Gorman, B.S.[1]
Teresa McGrath, M.D.[2]
Sandu Toma, M.D.[3]
Bonnie Brake[1]
Allen W. Hightower, M.S.[1]
J. Jones, M.D.[4]
Arthur L. Reingold, M.D.[1]
Peter A. Boxer, M.D.[5]
Patrick W. Tang M.Sc. [3]
C. Wayne Moss, Ph.D.[1]
Hazel Wilkinson, Ph.D.[1]
Don J. Brenner, Ph.D.[1]
Arnold G. Steigerwalt, B.S.[1]
Claire V. Broome, M.D.[1]

RUNNING HEAD: Pontiac fever

From: [1]Division of Bacterial Diseases, Center for Infectious Diseases, Centers for Disease Control, Atlanta, Georgia;
[2]Ministry of Labor, Toronto, Ontario;
[3]Ministry of Health, Toronto, Ontario;
[4]Windsor-Essex County Health Unit, Windsor, Ontario;
[5]Hazard Evaluation and Technical Assistance Branch, NIOSH, Cincinnati, Ohio

Please address reprint requests to: Centers for Disease Control
ATTN: Loreen A. Herwaldt, M.D.
Division of Bacterial Diseases
Center for Infectious Diseases
Atlanta, Georgia 30333

*Current address: Division of Infectious Diseaaes
Department of Internal Medicine
Washington University School of Medicine
660 South Euclid Avenue
Box 8051
St. Louis, Missouri 63110

The Field Investigation Team

Frank Crosswell, UAW health and safety representative for plants 1 and 2; William Simmonds, David Rattray, William Sproat, M.D., Frank Viola III, M.D., David Sugano, DR.P.H., and Roger L. Wabeke of Corporation A; Shiela Barker R.N., P.H.N., Martin J. Strong C.P.H.I. of the Windsor-Essex County Health Unit; Evelyn Wallace, M.D. of the Ontario Ministry of Health; Nicholas R. Paul, M.Sc. of the Windsor Public Health Laboratory

ABSTRACT

From August 15-21, 1981, 317 engine assembly plant workers in Windsor, Ontario, were affected by an explosive outbreak of Pontiac fever (nonpneumonic legionellosis). Diagnostic serologic testing was negative for Mycoplasma, Chlamydia, respiratory viruses, and previously recognized legionellae. A gram-negative rod-shaped organism, designated WO-44C, was isolated from the water-based coolant in the piston department which, like other legionellae, did not grow on blood agar, required cysteine for initial growth, and contained large amounts of branched chain fatty acids. However, it did not react with antisera against any previously characterized legionellae, and by DNA-DNA hybridization the organism was less than 10% related to all previously described Legionella species. Geometric mean titers by indirect fluorescent antibody testing to the organism were significantly higher in ill employees than in controls ($p=.0001$). Attack rates by department showed a linear decrease from 100% to 5% with increasing distance from the implicated coolant system. Smoke candle studies and prevailing wind direction were consistent with the hypothesis that an aerosol from a contaminated coolant system could spread throughout the plant. We conclude that this outbreak was caused by a new Legionella species; we propose the species name Legionella feeleii, sp. nov. This is the first outbreak of nonpneumonic legionellosis in which the etiologic agent was not Legionella pneumophila, serogroup 1.

INTRODUCTION

Pontiac fever is a severe influenza-like illness characterised by fever, headache, myalgia, and malaise. Unlike Legionnaires' disease (pneumonic legionellosis), which has been recognized in numerous outbreaks and as sporadic cases (1-6), Pontiac fever has been recognized only retrospectively in 2 outbreaks (7, 8). The first occurred in the Oakland County, Michigan, Health Department in August 1968 and was caused by airborne spread of Legionella pneumophila, serogroup 1, from a contaminated air-conditioning system. The organism was retrospectively isolated in 1977 from frozen samples of both condenser water and lung tissue from guinea pigs exposed to aerosols of evaporative condenser water (9). In addition, seroconversion to L. pneumophila serogroup 1 was demonstrated in ill individuals but not in controls. The second outbreak occurred in 1973 and affected 10 men who used compressed air to clean a steam turbine (8). The etiologic agent was not isolated but 5 of the 10 men demonstrated seroconversion to L. pneumophila, serogroup 1. In both outbreaks the mean incubation period was approximately 36 hours, and the attack rates were 95% and 100%, respectively. The illness was self-limited and there were no fatalities.

We report the clinical, epidemiologic, microbiologic, and serologic characteristics of an outbreak of Pontiac fever caused by a Legionella (isolate WO-44C) which is distinct from all previously described legionellae.

BACKGROUND

Corporation A operates a large site in Windsor, Ontario, which includes 2 engine assembly plants, (plants 1 and 2) that produce V-8 engines from precast iron or aluminum parts which are ground and machined to the proper specifications on the production lines and assembled into completed engines on the assembly line (Figure 1a). The production departments and the assembly line are serviced by a number of systems which produce aerosols or exhaust humidified air into the plant or onto the roof--compressed air lines, parts washers, "wet" air cleaners, and coolant systems (Figure 1b). The coolant systems lubricate, cool, and clean the grinding and machining surfaces. The individual coolant systems do not interconnect and they range in size from 1000 to 30000 gallons. The coolants are made up of water (88%-99%) and oil (1%-12%). Caustics are added as needed to keep the pH between 8.5 and 9.5 (except systems servicing aluminum machining for which the pH should not exceed 9), and biocides are added when the bacterial count exceeds 10^5-10^6 organisms per milliliter. The coolant, which also contains metal shavings, dirt, and other debris, is mechanically circulated through underground troughs from a main tank to the machines and back to the main tank. As the coolant is applied to the grinding or machining surface, large drops of coolant splash on and around the machine and a very fine aerosol--oil mist--is generated and remains suspended in the air.

Because production exceeded demand for the engines produced in plant 2, it was shut down August 8-16 (Figure 2), except for the crankshaft and stamping lines. At 0800 on August 17, plant 2 resumed production. By 0800, August 19, it was apparent that most of the men on the crankshaft, camshaft, and piston lines were ill and were complaining of headaches, severe body aches, high fever, and extreme fatigue. The 3 lines were promptly shut down, and an investigation was begun. By August 20, the investigation revealed that men who worked on other production lines and men who had worked on the engine assembly line in plant 2 on August 17 and 18 and shifted to plant 1 on August 19 were also becoming ill, but men who had worked only in plant 1 were not ill.

As was the weekly routine, the coolant systems were tested for total bacteria count and pH on August 19, and biocides were added to all coolant systems on August 21.

METHODS

Surveys. Several questionnaire surveys were conducted as part of the investigation. Workers in plant 2 were questioned about underlying medical problems, use of tobacco and alcohol, department and job description, contact with aerosol sources (compressed air, parts washers, and coolant systems), areas within plant 2 other than their work stations which they most likely visited on August 17-19, and symptoms which they experienced. They were also asked about the severity and duration of their illness and whether family members had become ill. A supplementary questionnaire was administered to employees who worked in engine line sections G to K.

Laboratory studies. Environmental samples were obtained on August 19, September 15, and October 28 from systems capable of producing aerosols or

exhausting humidified air in the plant or on the roof. Initially, environmental samples were inoculated intraperitoneally into guinea pigs. Because chemical components of the fluids were toxic to the guinea pigs all environmental specimens were subsequently processed by direct plating (10).

Isolates requiring L-cysteine for growth were tested by direct fluorescent antibody (DFA) against the 9 previously described Legionella species and a number of other Legionella-like organisms. Organisms which did not stain with any of these sera were further evaluated by biochemical testing, DNA-DNA hybridization, and guanine-cytosine content (11,12). The fatty acid composition was determined by gas liquid chromatography (GLC) (13,14).

Antimicrobial susceptibility testing was performed using an agar dilution method (15) on a medium consisting of yeast extract, oxoid agar, cysteine, and charcoal. Buffered charcoal yeast extract agar (BCYE) was not used because ferric pyrophosphate antagonizes tetracyclines and the pH of 6.9 inhibits erythromycin activity.

A serosurvey of plant 2 employees was begun on September 1. Three sets of sera were obtained at approximately 2-week intervals. Sera from 330 employees who worked in plant 2 were screened by indirect fluorescent antibody (IFA) using 3 antigen pools which together included 10 Legionella antigens--L. pneumophila serogroups 1-6, Legionella bozemanii, Legionella dumoffii, Legionella micdadei, and Legionella gormanii (16). Sera from 25 ill persons and 11 well persons were tested by complement fixation (CF) against influenza A, influenza B, parainfluenza 1, 2, and 3, adenovirus, Chlamydia, and Mycoplasma pneumoniae. Sera from 20 of the ill persons and the 11 well persons were also tested by complement fixation against respiratory syncytial virus and Herpes simplex virus (17).

One serum sample was also obtained from each of 104 controls, 2 groups of Corporation A employees with no exposure to plant 2. One group of 64 individuals had exposure to coolant systems, while the other group of 40 individuals had no exposure to coolant systems.

All sera were tested by IFA for titers against WO-44C using heat killed WO-44C as the antigen.

Only 1 serum specimen was obtained from all controls and 72 of the 208 cases who had serum specimens drawn, while several serum specimens were obtained from the remaining cases. Therefore, the titer to WO-44C from the first serum drawn on or after September 7 (21 days after the peak of the outbreak), was arbitrarily used to calculate the geometric mean titers (GMT) and the titer distributions. Since the GMT's and the titer distributions of the two control groups to isolate WO-44C were not statistically different, the two groups were combined into 1 control group for the following analyses.

Additional studies. Smoke candle studies were performed to determine the extent of spread of production-generated aerosols from various points in the plant. Meterologic data for Windsor, Ontario from August 13 through August 23, 1981, were obtained from Environment Canada, Atmospheric Environment Service, Downsville, Ontario.

Analysis. A case was defined as a worker who experienced at least three of the following four symptoms: fever, chills, headache, and myalgia during August 1981. A well person was one who answered "no" to the question, "Were you ill in August?" Individuals who were ill but did not meet the case definition were considered possible cases.

The initial analysis of the questionnaire and serologic data was done with routine statistical tests which are cited in the text when appropriate. Workers who were "possible cases" or who could not be assigned to a specific

production line or to the assembly line were excluded from department specific attack rate calculations and from risk factor analysis. Multivariate risk factor analysis was done by chi-square automatic interaction detection (CHAID) which first identifies by univariate analysis the factor most closely associated with illness. The program then divides the study population into risk groups based on this factor. Within each risk group the program identifies additional risk factors (18).

RESULTS

Survey results. Six hundred ninety-five (80%) employees from plant 2 completed the questionnaire. Of these, 317 (46%) met the case definition; 270 (39%) were not ill; and 108 (16%) were ill but did not meet the case definition.

The illness had a mean maximum incubation period of 46 hours and was characterized by fever ranging from 99.5-104° F, severe myalgia, headache, and extreme fatigue (Table 1). The illness was short (median duration - 3 days) but severe enough to cause nearly 30% of the workers to miss work (median days sick leave - 2 days). There were no fatalities, and only 4 of the workers reported similar illness in family members within 72 hours after the onset of the workers illness.

Attack rates varied significantly by department (Figure 1b). In general the attack rates by department decreased progressively from north to south. This trend was particularly striking in the stamping and the engine assembly departments, which are divided into working sections (Figures 1a, 1b). The departments with the highest attack rates were along a line extending from the piston, camshaft, and crankshaft departments in the northwest to the cylinder head and assembly department in the southeast.

The attack rate gradient suggested an association between the location in which an employee worked on August 17 and 18 and his risk of developing illness. The association was confirmed by the CHAID risk factor analysis ($p=6.4 \times 10^{-28}$). Several other factors, including visiting the piston ($p=.005$), crankshaft ($p=.032$), and the cylinder block ($p=.030$) departments were also closely associated with illness by univariate analysis. However, when the individual risk areas (Figure 1b) were analyzed by multivariate analysis, risk factors other than where one worked were not identified for employees in the four highest risk areas. Only in the very lowest risk area did factors other than location seem to be associated with the development of illness. In this area, individuals were at higher risk of illness if they had visited the connecting rod department which is located in a higher risk area ($p=.012$). Of those who did not visit the connecting rod department, individuals who consumed more than 10 alcoholic beverages in a week were at higher risk of illness than those who drank less ($p=.026$). In addition, analysis of the supplementary engine line questionnaire revealed that ill persons working in the low risk sections of the engine line had a slightly higher risk of developing illness if they visited engine line sections A, C, and D ($p=.039$, $p=.034$, $p=.0023$ respectively, Fisher's exact test, 2-tailed). However, there were 24 workers who worked in the lowest risk area who had no exposure to any of the higher risk departments yet became ill.

Since 99.7% of the respondants were male, and 97% were white, consistent with the sex and racial makeup of the plant, sex and race could not be evaluated as risk factors. Other factors not associated with illness were age, shift worked, underlying medical illness, regular use of medication,

cigar and cigarette smoking, water consumption, showering at work, working with or near compressed air, washer, and coolant systems.

Culture results. A Legionella-like organism, designated WO-44C, was isolated from a sample of coolant obtained August 19 from system 17 in the piston department (Figure 1a). No other legionellae or Legionella-like organisms were isolated from any of the other environmental samples. Unfortunately, coolant samples obtained on August 19 were available only from systems 7, 10, and 17.

Phenotypic characteristics of WO-44C are similar to those for previously reported legionellae (Table 2). However, WO-44C is the only Legionella species which does not produce gelatinase and only 1 of 2 to hydrolyse hippurate. The organism grows well at 25°C and at 35°C but not at 42°C. Like other legionellae, it is sensitive in vitro to rifampin, erythromycin, and several other antimicrobics (Table 3).

WO-44C is antigenically distinct from other legionellae since it does not stain with DFA sera against the 9 previously described species and other undescribed legionellae or Legionella-like organisms. By DNA-DNA hybridization, WO-44C is less than 10% related to all named species (Table 4) and less than 20% related to all strains tested (data not shown). The guanine-cytosine content is 45.7%.

Qualitative and quantitative cellular fatty acid results are similar to other legionellae with the characteristic features of relatively large amounts of branched-chain acids-i-14:0, a-15:0, i-16:0, and a-17:0 acids-the presence of relatively large amounts of 16:0 and 16:1 straight-chain acids, and the absence of hydroxy acids (Figure 3) (13-14, 26-27). No cyclopropane acids were detected at concentrations greater than 1%.

Serologic results. Testing of sera from cases and well persons against influenza A, influenza B, parainfluenza 1,2, and 3, adenovirus, respiratory syncytial virus, herpes simplex, Chlamydia, and Mycoplasma pneumoniae was nondiagnostic. IFA testing of sera from cases, well persons, and possible cases against 10 Legionella antigens, including L. pneumophila serogroup 1 were also nondiagnostic.

IFA testing of all available sera against WO-44C revealed 28 cases (21% of the cases with paired sera) with seroconversions (>4-fold rise in IFA titer) to WO-44C. In addition, 1 possible case and 4 well persons (5% and 33% of those with paired sera) seroconverted to WO-44C.

The IFA titers to WO-44C for cases (GMT=296) were significantly different by statistical testing than those for well persons (GMT=165, $p = .0006$), possible cases (GMT=128, $p = .007$), and controls (GMT=71, $p < .0001$). The IFA titer distribution is illustrated in Figure 4. GMT's for well persons and possible cases were not statistically different from each other, but were significantly higher than the GMT for controls ($p < .001$, $p = .0152$). The well persons and the possible cases had been in plant 2 at the time of the outbreak and may have been exposed to the etiologic agent; whereas, the controls had no recent contact with plant 2. Since case definition was closely correlated with both location of work and IFA titer, the serologic data were stratified by case definition to determine whether IFA titer was merely a function of case definition or varied independently with location of work and, therefore, with dose. Ill persons (cases) who worked in the 3 highest risk areas (departments closest to system 17) had a higher GMT (345) than did cases who worked in the 2 lowest risk areas (GMT= 154) ($p=0.0339$ Wilcoxon rank sums). There was no statistical difference between the GMT's for well persons who worked in the higher and the lower risk areas.

Smoke candle studies showed that regardless of the point of release the smoke particles spread horizontally to cover a large portion of 3-4 production lines with a visually detectable level of particles. The particles also left the plant through roof windows and re-entered the plant several production lines distal to the source.

DISCUSSION

The outbreak in engine plant 2 was very similar to the 2 previously reported outbreaks of Pontiac fever. It was characterized by a self-limited, severe flu-like illness with a short incubation period, a high attack rate among workers nearest the presumed source, no evidence of secondary spread, and no fatalities. The epidemic curve is consistent with an explosive common-source outbreak (Figure 2). Food and waterborne spread were ruled out by the epidemiologic survey, which documented no common source of food or water for the ill employees. Airborne spread from one or more of the coolant systems, including system 17, was the most likely mode of transmission in this outbreak. It is likely that there was a continuing exposure for at least 1 full working day, as workers from all 3 shifts became ill.

The attack rate gradient was also consistent with airborne spread of the etiologic agent. The departments with the highest attack rates were along a line from northwest to southeast, which was along the same axis as the wind direction from 0700 to 1200 on August 17. Wind direction is a major determinant of air flow in plant 2, which in the summer uses natural ventilation through windows and large doors some of which will accomodate trains and trucks.

The smoke candle results demonstrated that smoke released from a point source could spread over a significant portion of 4 production lines at visually detectable concentrations. Further from the source the smoke particles are diluted by an increasing volume of air but they remain suspended until they impinge on solid objects, flocculate, are filtered, or serve as condensate nuclei. Since smoke particles (0.01-2 diameter) and aerosols generated by machining and grinding operations (99% are ≤ 3 in diameter and 50% ≤ 1) are similar in size, their behavior in air currents may also be similar (personal communication Roger Wabeke), suggesting that coolant aerosols could be spread over a comparable area in relatively high concentration.

The results of the epidemiologic survey strongly suggested that the etiologic agent was airborne and spread throughout the entire plant from a source in the northwest production lines. Aerosols from system 17, from which WO-44C was isolated, most likely were responsible for the outbreak. This system was not being circulated during the shut-down period from August 8-16, 1981, because the piston department was not operating. Although the data necessary to prove this hypothesis were not available, one can speculate that during the period of disuse, bacterial overgrowth, which is known to occur more readily when coolants are not circulated, decreased the pH of the coolant and led to separation of the oil-water emulsion (personal communication Cynthia Trudell, Ph.D.). The separation could have been favorable for the growth of WO-44C. With the resumption of production on August 17, the machines serviced by system 17 may have generated a contaminated aerosol which was spread throughout the plant.

Risk factors other than location of work could not be identified in the 4 highest risk areas, but workers in the lowest risk areas who visited the connecting rod department and who drank more than 10 alcoholic drinks per week were at an increased risk of illness. This suggests that individuals exposed

to a large dose developed illness regardless of their underlying state of health while those exposed to a lower dose developed clinical illness more readily if they had an additional risk factor, such as a history of heavy alcohol consumption.

The significant difference in GMT's to WO-44C between the cases, the well workers exposed to plant 2, and the controls, the seroconversions to WO-44C, and the absence of diagnostic titers to other possible etiologic agents indicates that WO-44C was the etiologic agent of the outbreak. It is also of note that the GMT's and the titer distributions of the well persons and the possible cases who were exposed to plant 2 were significantly higher than those for the controls who had no exposure to plant 2. Most of these plant 2 employees worked in lower risk areas, suggesting that a low dose may have been sufficient to cause an antibody response but was not adequate to cause clinical illness. Antibody response in the absence of disease has been previously reported by Haley and coworkers in an outbreak of nosocomial Legionnaires' disease (6) but was not recognized in the 2 previous outbreaks of Pontiac fever.

The epidemiologic and serologic results of this investigation strongly suggest that WO-44C was the etiologic agent of a large outbreak of Pontiac fever and the results of the laboratory studies have shown that it is distinct from all previously described Legionella species. We feel that WO-44C should be now recognized as a new Legionella species and we propose the name Legionella feeleii (fe' lei i) sp. nov. (feeleii modern Latin genitive noun) in honor of James C. Feeley, Ph.D. who has participated in the laboratory investigation of all reported outbreaks of Pontiac fever and who pioneered work on artificial media capable of supporting the growth of legionellae. As a result of work which Dr. Feeley and his coworkers started, the technology was developed which made possible the isolation of WO-44C. The direct plating procedure was particularly important in this investigation because toxic components of the fluids prevented isolation in guinea pigs.

The type strain of L. feeleii is WO-44C (ATCC 35072). A description of L. feeleii is found in the text and in Tables 2-4 and Figure 3. L. feeleii is one of the few Legionella species that can be identified phenotypically: only L. feeleii and L. pneumophila hydrolyse hippurate, and L. feeleii is the only described Legionella species which does not produce gelatinase.

Acknowledgments

The authors acknowledge the indispensible laboratory support from Ms. Mary Ann Schott, Mr. Harold McEachern, Mr. Barry Fields, Dr. Herta Wulff, Dr. Roger McKinney, Dr. Robert Weaver, and Ms. Linda K. McDougal and the enthusiastic participation of Dr. Michael Silverstein of the UAW International and the members of UAW local 200. We thank Professor Thomas O. MacAdoo from Virginia Polytechnic Institute and State University and Dr. Harold W. Rossmoore from Wayne State University for their technical advice. We thank Drs. G. Martin, R. Andreychuck, and J. Carlson of the Ontario Ministry of Health and Dr. Richard A. Condon for their encouragment; Dr. Donald Krogstad for his critical review; Ms. Frances Wilson for graphics; and Ms. Burnese Harper and Ms. Iris Lansing for their untiring secretarial support.

REFERENCES

1. Fraser DW, Tsai TF, Orenstein W, et al. Legionnaires' disase: description of an epidemic of pneumonia. N Engl J Med. 1977;297:1189-97.
2. England AC III, Fraser DW, Plikaytis BD, Tsai TF, Storch G, Broome CV. Sporadic legionellosis in the United States: the first thousand cases. Ann Intern Med. 1981;94:164-70.
3. Broome CV, Goings SAJ, Thacker SB, et al. The Vermont epidemic of Legionnaires' disease. Ann Intern Med. 1979;90:573-7.
4. Storch G, Baine WB, Fraser DW, et al. Sporadic community-acquired Legionnaires' disease in the United States. A case-control study. Ann Intern Med. 1979;90:596-600.
5. Band JD, LaVenture M, Davis JP, et al. Epidemic Legionnaires' disease. Airborne transmission down a chimney. JAMA. 1981;245:2404-7.
6. Haley CE, Cohen ML, Halter J, et al. Nosocomial Legionnaires' disease: a continuing common-source epidemic at Wadsworth Medical Center. Ann Intern Med. 1979;90:585-6.
7. Glick TH, Gregg MB, Berman B, et al. Pontiac fever: an epidemic of unknown etiology in a health department. I. Clinical and epidemiologic aspects. Am J Epidemiol. 1978;107:149-60.
8. Fraser DW, Deubner DC, Hill DL, et al. Non-pneumonic, short-incubation-period legionellosis (Pontiac fever) in men who cleaned a steam turbine condenser. Science. 1979;205:691-2.
9. Kaufmann AF, McDade JE, Patton CM, et al. Pontiac fever: isolation of the etiologic agent (Legionella pneumophila) and demonstration of its mode of transmission. Am J Epidemiol. 1981;114:337-47.
10. Gorman GW, Feeley JC. Procedures for the recovery of Legionella from water. Laboratory Manual, Centers for Disease Control, 1982.
11. Cherry WB, Gorman GW, Orrison LH, et al. Legionella jordanis: a new species of Legionella isolated from water and sewage. J Clin Microbiol. 1982;15:290-7.
12. Brenner DJ, Steigerwalt AG, Weaver RE, McDade JE, Feeley JC, Mandel M. Classification of the Legionnaires' disease bacterium: an interim report. Curr Microbiol. 1978;1:71-5.
13. Moss CW. Gas-liquid chromatography as an analytical tool in microbiology. J Chromatogr. 1981;203:337-47.
14. Moss CW, Weaver RE, Dees SB, Cherry WB. Cellular fatty acid composition of isolates from Legionnaires' disease. J Clin Microbiol. 1977;6:140-3.
15. Orrison LH, Cherry WB, Fliermans CB, Dees SB, McDougal LK, Dodd DJ. Characteristics of environmental isolates of Legionella pneumophila. Appl and Environ Microbiol. 1981;42:109-15.
16 Wilkinson HW, Fikes BJ, Cruce DD. Indirect immunofluorescence test for serodiagnosis of Legionnaires' disease: evidence for serogroup diversity of Legionnaires' disease bacterial antigens and for multiple specificity of human antibodies. J. Clin Microbiol. 1979;9:379-83.
17. Guide to the performance of the standardized diagnostic complement fixation method and adaptation to micro test. Department of HHS, PHS publication 1228, revised 1981 by the CDC Laboratory Branch Task Force.
18. Sonquist JN, Baker EL, Morgan JA. Searching for structure (Alias - AID III). Michigan: Institute for Social Research, University of Michigan. 1971.
19. Brenner DJ, Steigerwalt AG, McDade JE. Classification of the Legionnaires' disease bacterium: Legionella pneumophila, genus novum, species nova, of the family Legionellaceae, familia nova. Ann Intern Med. 1979;90:656-8.

20. Brenner DJ, Steigerwalt AG, Gorman GW, et al. Legionella bozemanii sp. nov. and Legionella dumoffii sp. nov.: classification of two additional species of Legionella associated with human pneumonia. Curr Microbiol. 1980;4:111-6.
21. Morris GK, Steigerwalt A, Feeley JC, et al. Legionella gormanii sp. nov. J Clin Microbiol. 1980;12:718-21.
22. McKinney RM, Porschen RK, Edelstein PH, et al. Legionella longbeachae species nova, another etiologic agent of human pneumonia. Ann Intern Med. 1981;94:739-43.
23. Hebert GA, Moss CW, McDougal LK, Bozeman M, McKinney RM, Brenner DJ. The Rickettsia-like organisms TATLOCK (1943) and HEBA (1959): bacteria phenotypically similar to but genetically distinct from Legionella pneumophila and the WIGA bacterium. Ann Intern Med. 1980;92:45-52.
24. Orrison LH, Cherry WB, Tyndal RL, et al. Legionella oakridgensis: an unusual new species isolated from cooling tower water. Appl Environ Microbiol. 1983;45:536-45.
25. Edelstein PH, Brenner DJ, Moss CW, Steigerwalt AG, Francis EM, George WL. Legionella Wadsworthii species nova: a cause of human pneumonia. Ann Intern Med. 1982;97:809-13.
26. Moss C, Karr DE, Dees SB. Cellular fatty acid composition of Legionella longbeachae sp. nov. J Clin Microbiol. 1981;14:692-4.
27. Moss CW, Dees SB. Further studies of the cellular fatty acid composition of Legionnaires' disease bacteria. J Clin Microbiol. 1979;9:648-9.

Fig.1a PRODUCTION AND ASSEMBLY DEPARTMENTS, ENGINE PLANT 2, WINDSOR, ONTARIO, AUGUST 1981

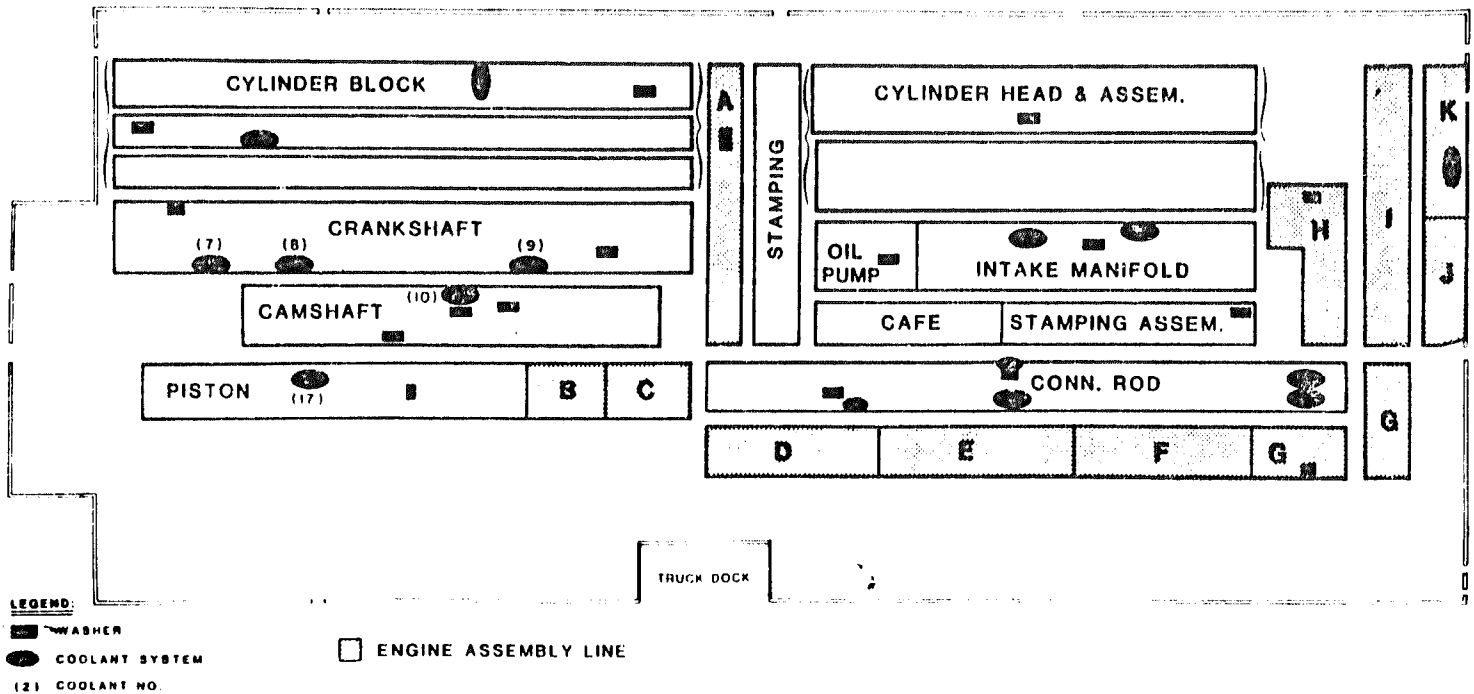

Fig.1b PONTIAC FEVER BY WORKERS' DEPARTMENT, ENGINE PLANT 2, WINDSOR ONTARIO, AUGUST 17, 1981

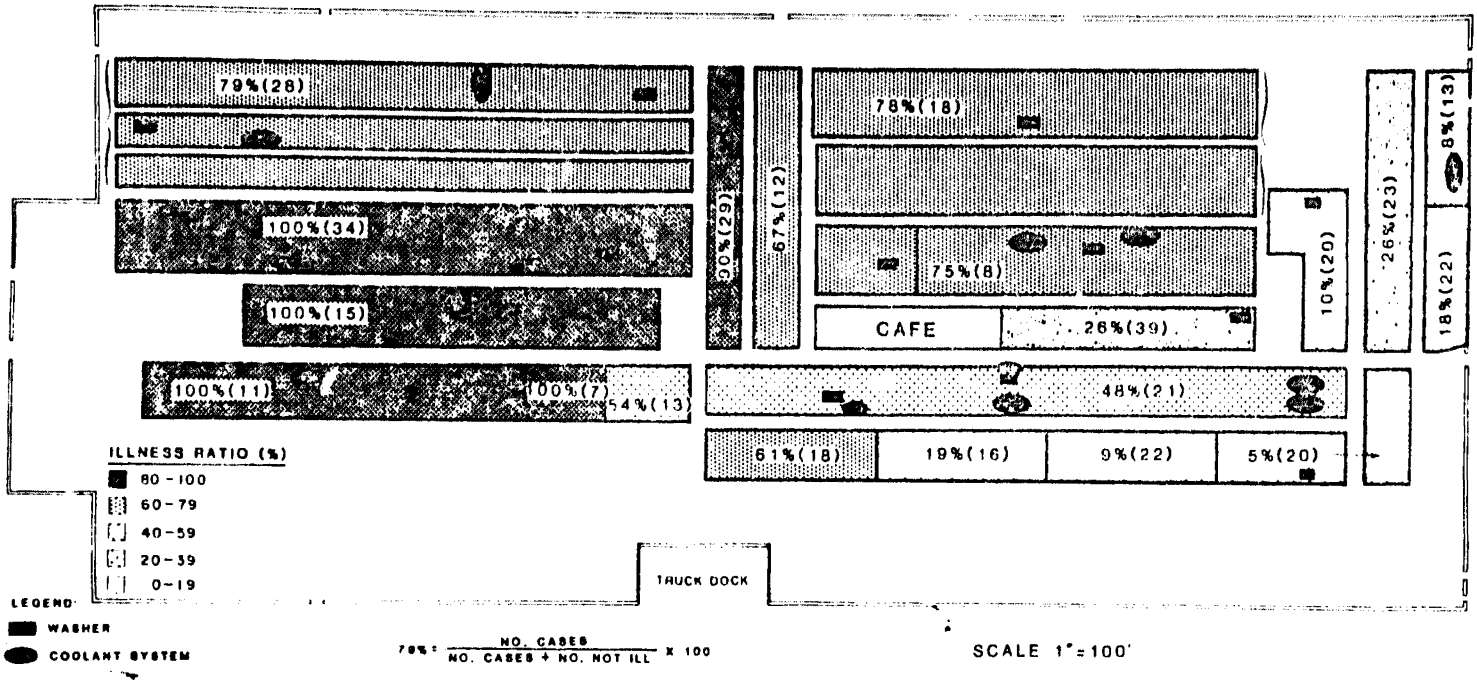

Figure Legend

Figure 3. Gas chromatogram of esterified fatty acids (as methyl esters) of saponified whole cells of WO-44C after 48 hours growth on charcoal yeast extract agar. Analysis was made on a 50-m x 0.2-mm fused silica OV-1 capillary column. Peak designation: numbers to the left of the colon refer to the number of carbon atoms, numbers to the right refer to number of double bonds; i- indicates a methyl branch at the iso carbon; a- indicates a methyl branch at the anteiso carbon atom.

Fig. 2 PONTIAC FEVER CASES, BY DAY OF ONSET, WINDSOR, ONTARIO, AUGUST 1981

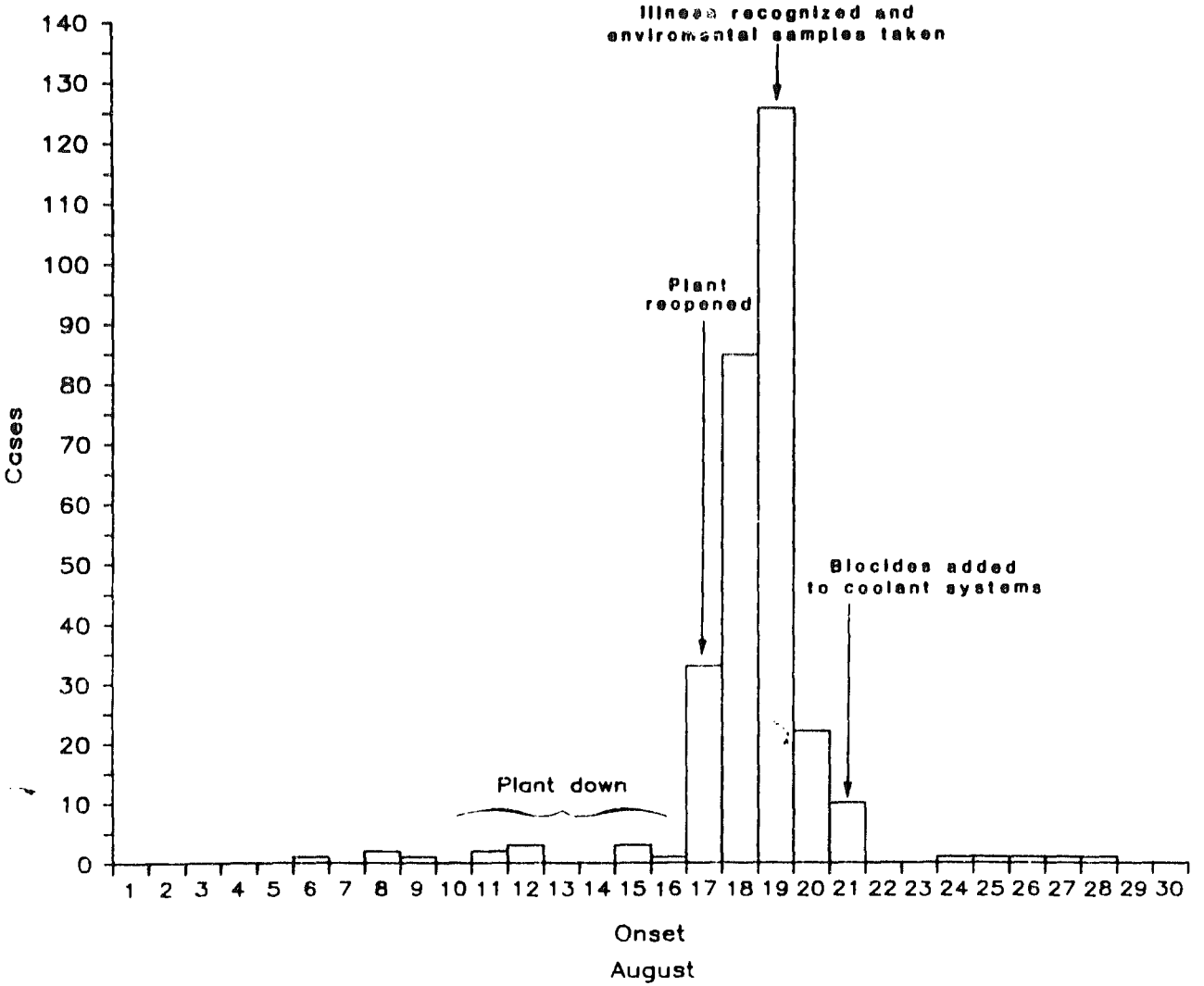

Fig. 4 DISTRIBUTION OF PEAK IFA TITERS TO WO–44 BY EXPOSURE TO PLANT

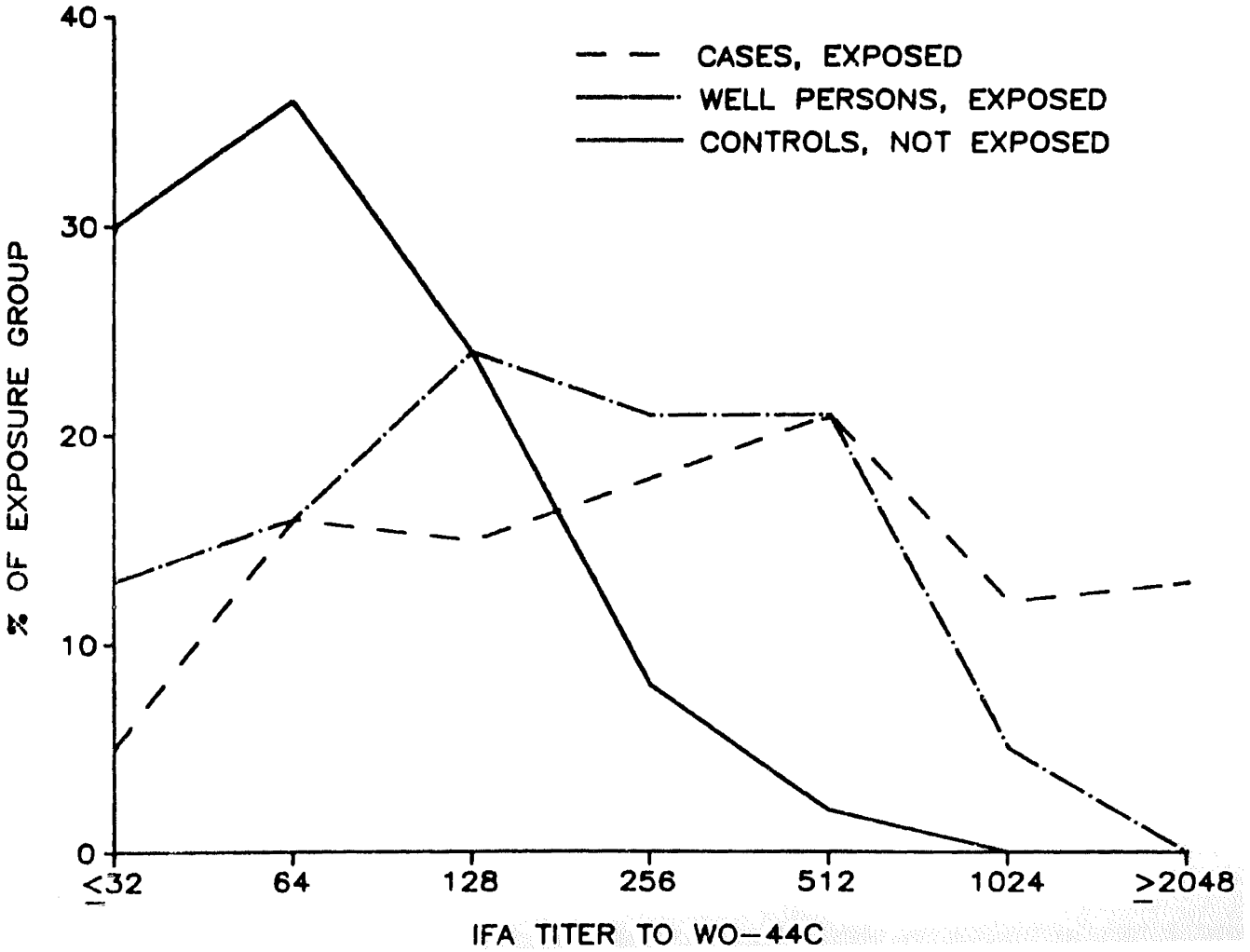

Fig. 3

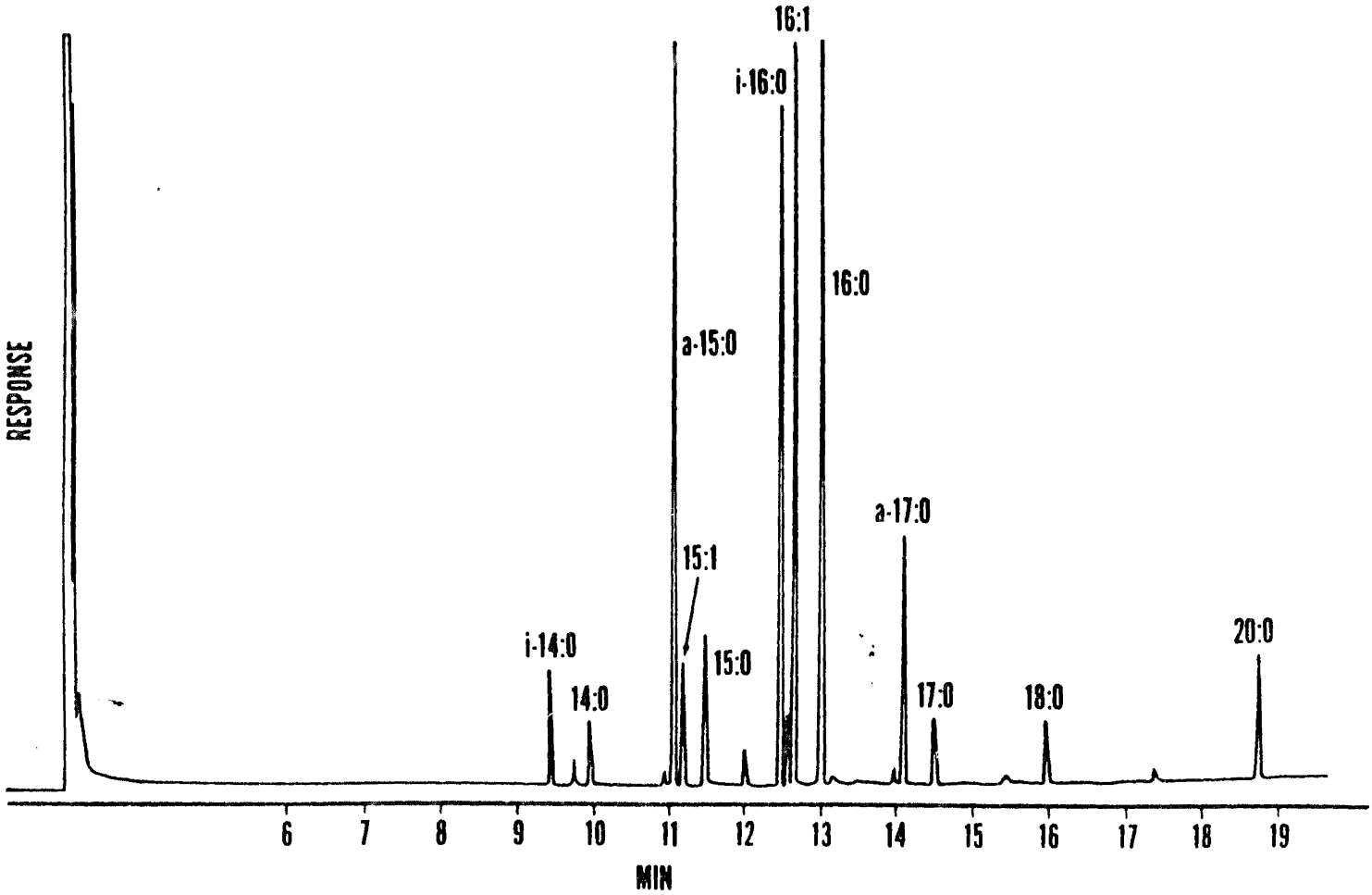
16:1
i-16:0
16:0
a-15:0
a-17:0
15:1
i-14:0
15:0
14:0
17:0
18:0
20:0
RESPONSE
6
7
8
9
10
11
12
13
14
15
16
17
18
19
MIN

Table 1

SYMPTOMS EXPERIENCED BY ILL WORKERS

N = 317

Symptom	%
myalgia*	93
chills*	92
fever*#	91
malaise	89
headache*	88
dizziness	68
nausea	51
chest pain	36
cough	32
abdominal pain	30
shortness of breath	28
coryza	20
vomiting	15
diarrhea	11

*case definition required at least 3 of 4 symptoms with asterix
#subjective or objective evidence of fever was accepted

Table 2

PHENOTYPIC CHARACTERISTICS OF WO-44C AND OTHER LEGIONELLA SPECIES

Characteristics	WO-44	L. pneumophila	L. bozemanii	L. dumoffii	L. gormanii	L. jordanis	L. longbeachae	L. micdadei
Growth on:								
blood agar	-	-	-	-	-	-	-	-
BYCE agar	+	+	+	+	+	+	+	+
BYCE without L-cysteine	-	-	-	-	-	-	-	-
Fluorescence	-	dull yellow	blue-white	blue-white	blue-white	?	dull-yellow	dull-yellow
Oxidase	-	+	-	-	-	+	+	+
Catalase	+	+	+	+	+	+	+	+
Gelatinase*	-	+	+	+	+	+	+	
+								
Urease	-	-	-	-	-	-	-	
-								
NO3 NO2	-	-	-	+	-	-	-	
-								
Acid from carbohydrates	-	-	-	-	-	-	-	
-								
-lactamase	-	+	+	+	ND	-	+ or -	
-								
Hippurate hydrolysis*	+	+	-	-	-	-	-	
-								

*Phenotypic characteristics which help differentiate WO-44C from other legionellae

Table 3

MIC OF SELECTED ANTIMICROBIALS AGAINST WO-44C

rifampin	.06 g/ml
penicillin	.25 g/ml
erythromycin	$.5^{-1}$ g/ml
cefoxitin	.5 g/ml
doxycycline	$\leq$ .5 g/ml
gentamicin	1.0 g/ml
chloramphenicol	2.0 g/ml
sulfamethoxazole-trimethoprim	4.8/0.25 g/ml

Courtesy of Linda K. McDougal

Table 4

DNA RELATEDNESS OF WO-44C TO OTHER LEGIONELLA SPECIES

Source of unlabeled DNA		Source of labeled DNA (WO-44C) RBR (60° C)
WO-44C		100[b]
L. pneumophila	(Philadelphia 1)	2
L. bozemanii	(WIGA)	3
L. micdadei	(TATLOCK)	6
L. dumoffii	(NY-23)	3
L. gormanii	(LS-13)	5
L. longbeachae	(LB-4)	5
L. wadsworthii	(81-716A)	3
L. jordanis	(BL-540)	3
L. oakridgensis	(OR-10)	4

[a]RBR = relative binding ratios = (% heterologous DNA bound to hydroxyapatite)/(% homologous DNA bound to hydroxyapatite) X 100

[b]All reactions run in duplicate. Reassociation of WO-44C DNA in homologous reactions was between 51% and 71% (59% average). These are arbitrarily designated as 100% and heterologous reactions are normalized to them. Control reactions containing only labeled DNA showed 0-1.5% of labeled DNA was bound to hydroxyapatite. This background binding was subtracted from heterologous reaction results before normalization.