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A cluster of meningococcal disease in western Sydney, Australia initially associated with a nightclub

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SUMMARY

Fourteen cases of meningococcal disease (MD) occurred in August–September 1996 in western Sydney, Australia. Seven of the 10 young adults affected had a direct or indirect link with a local nightclub. Ten of 11 systemic meningococcal isolates had the phenotype C:2a:P1.5 and showed close genetic relationship by pulsed-field gel electrophoresis (PFGE). Organisms of this phenotype have not previously caused outbreaks in Australia, but have been associated with outbreaks and hyperendemic serogroup C MD in Europe, Canada, and the United States.

This is the largest cluster of serogroup C MD reported in urban Australia, and the first involving a nightclub. The strain differentiation results were available rapidly enough to augment epidemiological investigations on a daily basis. Public health staff could thus establish links between cases quickly, follow the spread of new cases in the community, give accurate information to health officials and the press, and utilize existing knowledge about the characteristics of this phenotype to predict likely developments during the outbreak and afterwards. The strain differentiation data was also very helpful when the role of vaccination was considered, and existing guidelines on the management of outbreaks of MD could be used effectively for the first time in western Sydney.

INTRODUCTION

Clusters and outbreaks of meningococcal disease (MD) have been identified only infrequently in Australia. Though serogroup A meningococci caused large outbreaks in indigenous communities in the past, the only recent epidemic occurred in the late 1980s in central Australia [1, 2]. From the early 1990s clusters of serogroup C MD have been reported in both urban and rural communities [3–8]. However, most MD occurs sporadically with annual rates of about 2·1 per 100000 population [9].

Since 1994 a laboratory-based surveillance system, the National Neisseria Network (NNN), has provided national data on characteristics of strains causing sporadic disease and outbreaks. Laboratories perform serogrouping, and from 1996, serotyping and subtyping (phenotyping) for all isolates. In addition, the Network can provide DNA typing using pulsed-field gel electrophoresis (PFGE) when required. Serogroup B strains predominate nationally (63%), with serogroup C strains accounting for 29% of cases [10]. Phenotyping data are available from 1995 in New

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South Wales (NSW), where phenotypes of serogroup B strains have been varied, whereas amongst serogroup C isolates, 45% had the phenotype 2b:P1.5,2 and 30% 2a:P1.5,2 in 1995 [11]. No clusters or outbreaks were reported in that year. National phenotyping data became available in 1996 [10]. Across Australia sporadic cases were caused by diverse phenotypes. There was an increase in occurrence of the phenotype B:4:P1.4 in eastern states; sporadic serogroup C isolates were most commonly C:2b:P1.5,2.

Retrospective phenotyping of strains causing the reported serogroup C MD outbreaks in the 1990s revealed that the outbreak phenotype in all cases was C:2b:P1.2 and strains were genetically homogeneous by PFGE, although from geographically diverse locations [12]. Although strains of the phenotype C:2a:P1.5,2 have been associated frequently with sporadic disease in NSW, there were no substantial clusters identified anywhere in Australia prior to 1996. This phenotype has caused multiple outbreaks of MD with high attack rates and case fatality rates in young adults in Canada and Europe in the 1990s [13–15], followed by an increased number of sporadic cases for several years thereafter [16]. Multilocus enzyme electrophoresis (MLEE) analysis of systemic isolates of serogroup B and serogroup C meningococci usually assigns isolates to 1 of 3 distinct genetic lineages: ET 5 complex, the ET 37 complex and Cluster A4 [17, 18]. Serotype 2a meningococci are most often associated with the ET 37 complex (referred to as cluster B2 in reference [18]) but other phenotypes may also be found within this complex [17, 18]. Differentiation by MLEE indicated that strains causing outbreaks in Canada and the Czech Republic belonged to a new clone of the ET37 complex – ET15 [13, 14]. ET15 strains have also been implicated in at least 2 of the 21 outbreaks of serogroup C MD reported from the United States between 1980 and 1993 [19–21]. Thus it appears that this phenotype has potential to cause outbreaks of MD and continuing activity in a community in the following years.

We report here the first cluster in Australia of cases of MD associated with the C:2a:P1.5 phenotype. The cases occurred over a 6-week period in August and September 1996 during late winter, the peak season for MD in Australia. The first three patients presented within days of each other and all had attended a nightclub on the same evening. The local NNN laboratory was able to provide phenotyping and PFGE results rapidly enough to provide accurate and up to date information to the public on a daily basis in conjunction with the Public Health Unit's epidemiological investigations. It was thus possible to confirm the probable relationship between cases early and to follow published guidelines for management of clusters of MD [22, 23].

METHODS

Setting

The Penrith local government area, where most of the cases occurred, is about 60 km from the central business district of Sydney. It comprises 407 km^2, has a mix of urban and semi-rural development, a small commercial centre and a rapidly growing residential population. The estimated 1996 population was 168400 persons, with 28560 persons in the 15–24 years age group (source: Australian Bureau of Statistics estimated population by age and sex, June 1996 (HOIST), Epidemiology and Surveillance Branch, NSW Health Department). The nightclub was in the commercial section of Penrith.

Outbreak investigation

Case definition

In NSW, general practitioners and hospitals have a statutory obligation to notify their local public health unit by telephone of clinical cases of MD. Laboratories also have a statutory obligation to notify any isolates of *N. meningitidis* from a normally sterile site.

A clinical case of MD is defined as a suspected but unconfirmed case of meningococcaemia or meningococcal meningitis. Criteria for a confirmed case are: (i) isolation of *N. meningitidis* from blood, cerebrospinal fluid or skin lesion or (ii) detection of *N. meningitidis* antigen in blood, urine, joint or cerebrospinal fluid in a clinically compatible case or (iii) detection of intracellular Gram-negative diplococci in a specimen from a clinically compatible case where the organism failed to grow [24].

Outbreak investigation

Notification was received by the Western Sector Public Health Unit of three young adults admitted to a local hospital with MD over a 5-day period in the first week of August 1996. The public health unit considered this to be an unusual cluster of cases since

only 3–4 cases of MD had been notified during August in each of the preceding 5 years. An outbreak investigation was immediately instituted which involved interviews with the first three cases and subsequent cases (or a close relative) to obtain details about the illness, household contacts, and close social and work contacts, and attendance at an institution, e.g. school or child care centre. The attending physician was also interviewed for clinical details. All household and other close contacts were given chemoprophylaxis according to current guidelines [22]. Sexual partners and those where there was the possibility of salivary contact (e.g. kissing or sharing of food and eating utensils) in the 10 days prior to the onset of illness in the index case were defined as close contacts.

The public health unit convened a group of public health staff, epidemiologists, microbiologists, and physicians experienced in the management of outbreaks of MD. This group coordinated the laboratory and public health investigations, determined what outbreak control measures were required and planned further investigations.

All general practitioners, hospitals and laboratories in the local area were informed of the cluster and encouraged to notify suspected or confirmed cases. Active surveillance was also conducted through a well-established statewide public health network. This active surveillance has continued to the present time.

Laboratory investigation

This was undertaken at the NNN laboratory, Department of Microbiology and Infectious Diseases, South Western Area Pathology Service, Sydney. Systemic meningococcal isolates were referred from hospital laboratories in western Sydney.

Identification of N. meningitidis

Referred systemic isolates were stored at −70 °C in tryptose broth containing 20% glycerol and additionally subcultured onto chocolatized horse blood agar plates (Becton Dickinson, MD, USA). Isolates were confirmed biochemically as *N. meningitidis* using the APINH system (BioMerieux, Australia).

Serogrouping, serotyping and serosubtyping

N. meningitidis isolates were serogrouped by slide agglutination using normal saline suspensions and polyclonal antisera against serogroups A, C, W135, X, Y, Z and monoclonal antisera against serogroup B (Murex Diagnostics, UK).

Serotyping to determine the specificity of the class 2 or 3 outer membrane protein (OMP) was performed using monoclonal antibodies with the following specificities: 1, 2a, 2b, 4, 14 and 15. Serosubtyping to determine the class 1 OMP specificity was performed with monoclonal antibodies specific for: P1.1, P1.2, P1.4, P1.5, P1.6, P1.7, P1.9, P1.10, P1.12, P1.13, P1.14, P1.15 and P1.16. Monoclonal antibodies were supplied by Dr J. Poolman, National Institute for Public Health and Environmental Protection, RIVM, The Netherlands.

A dot blot method was used [25, 26] with the following modifications: intact strips of nitrocellulose (Biorad Laboratories, Sydney), protein. A peroxidase conjugate (Cat. P8651 Sigma Chemicals, St Louis, MO, USA) and the substrate 4-chloro-1-napthol (Cat. C7788, Sigma Chemicals, St Louis, MO, USA) were used.

Pulsed-field gel electrophoresis (PFGE)

PFGE was performed following the method of Smith and Cantor [27] with modifications. Briefly, five colonies from a fresh culture were inoculated into 5 ml of tryptose soya broth and incubated at 36 °C overnight. Cells were harvested by centrifuging and the pellet resuspended in TEN solution (10 mM Tris, pH 8·0; 50 mM EDTA, pH 8·0 and 20 mM NaCl) to which an equal volume of molten 2% Seaplaque agarose (FMC products) prepared in TEN was added. This preparation was dispensed into prepared gel moulds (Biorad Laboratories), allowed to solidify and then dispelled into 5 ml of ESP solution (0·5 M EDTA, pH 9·0; 1% SDS and 0·5 mg/ml Proteinase K (Promega, Australia) and incubated for 24 h at 50 °C with occasional gentle agitation.

The ESP solution was discarded and plugs were washed in TE (10 mM Tris; 100 mM EDTA, pH 8·0), including one wash with 1 mM PMSF (Cat. no. P7626 Sigma Chemicals, St Louis, MO, USA). Plugs were stored in 0·5 M EDTA, pH 8·0 at 4 °C until use.

Restriction endonuclease digestion was undertaken with two enzymes, *Spe*I and *Nhe*I (Promega, Australia). One sixth of a plug was equilibrated in 10 mM Tris/0·1 mM EDTA, pH 8·0, on ice for 1 h. This solution was then replaced with 180 μl of sterile distilled deionized water and 20 μl of the appropriate restriction buffer, mixed gently and incubated on ice

for 1 h. Acetylated bovine serum albumin was added and then 36 units of the respective endonuclease. The tube contents were mixed gently and digested at 36 °C overnight. Following overnight digestion, 12 μl of 0·5 M EDTA was added to each tube to halt digestion. Digestion plugs were equilibrated in 0·5 × TBE (45 mM Tris-Borate; 1 mM EDTA, pH 8·3) on ice prior to gel loading. Plug slices were then loaded into wells of a 1·2% Fastlane agar gel (FMC Products) in 0·5 × TBE. PFGE was performed on a CHEF-DRII (Biorad) with the following running conditions: total run time 22 h at 200 V with switch times of 5–30 s for *Nhe*I and 10–25 s for *Spe*I. Chiller temperature was 10 °C. Lambda concatemers (FMC products) were used as size standards. Gels were stained with 0·5 μg/ml ethidium bromide in distilled deionized water for 30 min, destained in distilled deionized water for 30 min, then photographed under ultraviolet trans-illumination using Polaroid 667 film. Isolates were compared visually and relatedness judged using criteria cited recently [28].

RESULTS

Epidemiological investigation of the cluster of MD

Links with the nightclub

The first case became ill on 1 August 1996, and the next two within 6 days. Interviews with these three cases suggested that a common point of exposure may have been attendance at a local nightclub. The nightclub was open for business two nights per week. It attracted 400–500 patrons regularly on a Wednesday night, with a throughput of up to 1000 patrons on Saturday nights. The first three cases were unacquainted with each other, but all three had been at the nightclub on the evening of 31 July 1996 (a Wednesday). Cases 1 and 3 were patrons and case 2 worked at the nightclub. No other household members, work colleagues or social contacts of these three cases had been ill.

In the following week, active case finding through hospitals and laboratories in the region revealed three more new cases (cases 4–6), none with links to the nightclub (Table 1). In the third week of August there were four more cases, all with indirect links to the nightclub through a household member (cases 7 and 8) or social contact (cases 9 and 10) who had attended the nightclub on or around 31 July. From the end of August to mid-September a further four cases occurred, none with links to the nightclub.

In the 6-week period from 1 August 1996 there was a total of 12 cases of MD diagnosed in the Penrith local government area, with two further cases from adjacent local government areas (cases 4 and 9 in Table 1). There were no deaths, but significant morbidity with organ failure and thromboembolic complications in one patient. Eight patients were males, six females. The mean age was 23·8 years (median age: 18·5 years; age range: 2–66 years). The incidence of primary MD in the 15–24 years age group population reached 21 per 100000.

Control measures

Chemoprophylaxis was offered to all household and close contacts of cases. It was also offered to staff of the nightclub and their families. The use of mass vaccination was considered on a number of occasions, but at no stage was there a clearly definable at risk population that could be targeted for vaccination. Mass vaccination was not offered.

Ongoing surveillance following the cluster

MD activity in the Penrith area fell in late September 1996 with five cases notified between October and December 1996, a low incidence period for MD in Australia. However, in 1997 notifications again increased with nine cases notified between January and June 1997, an incidence of 5·3 per 100000 population. The age range of patients affected was 3 months to 32 years (mean age 16·7 years; median age 17 years). No further clustering of cases or links to the nightclub were noted.

Laboratory investigation

Phenotype and PFGE of meningococcal isolates, August–September, 1996

Eleven systemic isolates from the cluster of 14 cases of MD August–September 1996 were confirmed as *N. meningitidis*. Ten of the 11 isolates belonged to the phenotype C:2a:P1.5. The remaining isolate was C:2b:P1.5,2. It caused disease in case 11, a 66-year-old female with no links to the local nightclub (Table 1). The three remaining cases were clinical diagnoses.

All isolates were analysed by PFGE using the two restriction endonucleases, *Spe*I and *Nhe*I. Twelve to 15 fragments were obtained with *Spe*I and 8–16 fragments with *Nhe*I. All C:2a:P1.5 strains yielded

Table 1. *Details of cases in western Sydney MD cluster, 1996*

	Age (years)	Sex	Date of onset of illness	Positive cultures	Typing results	Links with nightclub
Case 1	21	M	1/8/96	Blood	C:2a:P1.5	Attended nightclub on 31/7/96
Case 2	19	M	3/8/96	Nil*	—	Worked at nightclub on 31/7/96
Case 3	18	M	6/8/96	Blood, CSF†	C:2a:P1.5	Attended nightclub on 31/7/96
Case 4	18	F	12/8/96	Nil‡	—	No links with nightclub
Case 5	15	F	13/8/96	Blood	C:2a:P1.5	No links with nightclub
Case 6	2	M	14/8/96	Blood	C:2a:P1.5	No links with nightclub
Case 7	46	M	20/8/96	CSF	C:2a:P1.5	Daughter attended nightclub on 31/7/96 and 10/8/96
Case 8	19	F	20/8/96	Blood	C:2a:P1.5	Sister attended nightclub on 31/7/96 and 7/8/96
Case 9	14	F	23/8/96	CSF	C:2a:P1.5	Social contact of daughter of case 7 who attended nightclub
Case 10	2	M	27/8/96	Nil‡	—	Sister of this case and case 8 are friends. Sister also attended nightclub on 13/7/96, 20/7/96, 3/8/96 and 10/8/96
Case 11	66	F	28/8/96	Blood	C:2b:P1.5,2	No links with nightclub
Case 12	16	F	30/8/96	Blood	C:2a:P1.5	No links with nightclub
Case 13	32	M	4/9/96	Blood	C:2a:P1.5	No links with nightclub
Case 14	45	M	10/9/96	Blood	C:2a:P1.5	No links with nightclub

* Clinical diagnosis, given intramuscular penicillin by general practitioner prior to referral to hospital.
† Cerebrospinal fluid.
‡ Clinical diagnosis, no history of antibiotics prior to blood cultures being drawn.

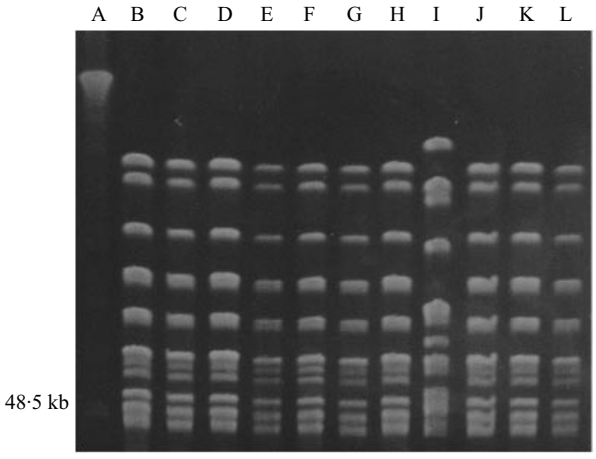

Fig. 1. PFGE analysis using restriction endonuclease *Spe*I of 11 systemic isolates of *N. meningitidis*. Lane A, Lambda concatemers; lanes B–H, *N. meningitidis* C:2a:P1.5; lanes J–L, *N. meningitidis* C:2a:P1.5; lane I, *N. meningitidis* C:2b:P1.5.

identical patterns following digestion by both *Spe*I and *Nhe*I (only *Spe*I results shown; Fig. 1). The C:2b:P1.5,2 strain fingerprint was dissimilar from the outbreak strains with both restriction endonucleases.

Phenotyping and PFGE of meningococcal isolates, October 1996–June 1997

Only one systemic isolate was recovered from the five cases of MD notified between October 1996 and December 1997. This isolate had the phenotype Y:NT:P1.5,2. From January to June 1997, six systemic isolates were recovered from the nine cases of MD notified in the Penrith area. All six isolates were serogroup C. Three had the phenotype C:2a:P1.5, two isolates were C:NT:P1.5 and one C:NT:NT. Isolates of the phenotype C:2a:P1.5 showed patterns on PFGE that were similar to the PFGE patterns of the C:2a:P1.5 organisms causing the cluster of cases in August and September 1996 (T. Shultz, personal communication).

DISCUSSION

This 1996 community cluster of MD in western Sydney is the largest reported in urban Australia, although there have been larger outbreaks of both serogroup A and C MD in remote Aboriginal communities [2, 6]. It is also the first reported instance

in Australia of MD linked to attendance at a nightclub.

Previous clusters of serogroup C MD have involved the phenotype C:2b:P1.5 [12, 29], and only two possible small clusters due to C:2a:P1.5,2 have been reported previously in Australia [8]. Three cases occurred in a Queensland indigenous community over a 5-month period and resulted in a mass vaccination programme. The other cluster of four cases also occurred in Queensland over 1-month period but vaccination was not undertaken. PFGE fingerprinting was not reported. None of the above clusters was associated with a bar or nightclub.

The pattern of MD in the Penrith region due to the C:2a:P1.5 phenotype is very similar to that seen in Canada, Europe, and the United States. The cluster of cases in August and September 1996 initially affected young adults attending a nightclub, then subsequently spread to contacts of nightclub attendees and into the community to persons having no identifiable relationship to the nightclub. Since then, similar strains have caused ongoing sporadic MD at a higher endemicity than seen in previous years.

Preliminary MLEE results indicate that strains involved with the present cluster belonged to the clonal group ET15, a variant found within the ET37 complex [13, 30]. It is unclear for how long this clonal group has been present in Australia, although two C:2a:P1.5 isolates causing MD in a Sydney high school in 1994 were shown to belong to the ET 37 complex/ET 15 subgroup [30]. An extensive vaccination programme was undertaken and there were no further cases at that time [31].

The nightclub environment may have provided unique opportunities for nasopharyngeal acquisition of the C:2a:P1.5 strain and invasive disease followed in a small number of susceptible hosts. Bar patronage associated with a university campus has been the focus of two outbreaks of MD in the United States [20, 21]. MLEE data from these two reports indicated that in each case the outbreak strains were similar to the C:2a:P1.2 strain associated with epidemic MD in eastern Canada in 1991–2. Both reports suggested that cigarette smoking, alcohol consumption, and crowded bars may have contributed to the transmission of pathogenic strains of meningococci.

The later cases in our cluster were older and lived further from the centre of Penrith. There may have been other unidentified environmental factors operating which were conducive to the spread of MD in the community. The incidence rate in young adults aged between 15 and 24 years in our area (defined by local government boundaries) reached 21 per 100000, well above the rate of 10 per 100000 when it is recommended that a community vaccination programme be considered [23]. We did not institute a mass vaccination programme. Although the initial cases were mainly young adults, the later cases were older adults and children, therefore the size of the population at risk was very large and at no stage was there a clearly definable at-risk population that could have been targeted for vaccination. It would have been logistically and financially burdensome to institute a vaccination programme. Similarly, in the months following the initial cluster of cases there has not been an identifiable group to target for vaccination.

The availability within days of results of phenotyping and DNA fingerprinting of isolates was of great assistance in giving accurate and up-to-date information to the public, an integral component of the public health response. Although the public health response was triggered initially by cases associated with the nightclub, DNA fingerprinting confirmed the relatedness of C:2a:P1.5 strains from patients who had not attended the nightclub. Since C:2a:P1.5 has been a relatively common phenotype in cases of sporadic MD in NSW in the past few years, DNA fingerprinting provided additional evidence of cases associated with this cluster in the community. We were able to make an informed assessment on the extent of the outbreak on a daily basis and relay this information promptly to the public, health administrators, and politicians. Although mass vaccination was not undertaken, the rapid updating of information about strains from new cases meant that we were able to have in place a contingency plan for mass vaccination, which could have been implemented had an appropriate target group been identified. The combination of epidemiological methods and supplementary microbiological techniques delivered through the National Neisseria Network was of great assistance in providing accurate and up-to-date information to the public.

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