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Epidemic Coxsackie B virus infection in Johannesburg, South Africa

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SUMMARY

A particularly extensive epidemic of Coxsackie B3 virus infection occurred in Johannesburg in the spring and summer of 1984. A total of 142 positive cases were diagnosed by isolation of the virus from stools and other specimens (60) or by serology (82). Coxsackie B3 accounted for 87 % of the isolations and was also the dominant serotype on serology.

The outbreak involved predominantly children and young adults, with no apparent sex differences being noted. The majority of specimens came from the white population and no significant difference in age or sex distribution could be observed between the two race groups. The major clinical presentation in the white group was Bornholm disease followed by cardiac involvement and then meningo-encephalitis. In the black group, however, myocarditis was the major clinical presentation, which is of particular interest taking into account the extremely high incidence of acute rheumatic carditis in this population and the prevalence of chronic cardiomyopathy.

INTRODUCTION

Epidemics of Coxsackie B virus infection have occurred at regular intervals in Johannesburg since the first documented outbreak of neonatal disease in a maternity home in October and November 1952 (Javett *et al.* 1956; Gear, 1967). In temperate countries, as well as in South Africa, these outbreaks occur characteristically in the warmer spring to autumn months in cycles of some 3- to 6-year intervals (Gear, 1967; Melnick, 1982; Banatvala, 1983). With particular Coxsackievirus serotypes no regulation pattern of annual recurrence has been noted and Coxsackie B1 does not in fact appear to show any periodicity at all (Melnick, 1982). Recognition of the cyclical outbreaks of Coxsackie B virus disease is of considerable public health importance and a vigilant surveillance programme is essential for early detection of outbreaks to enable appropriate public health preventive measures to be instituted, especially in maternity homes (Gear, 1967).

In the spring and summer of 1984 a particularly extensive outbreak of Coxsackie

B virus infection took place in Johannesburg and in neighbouring areas, resulting in a significant morbidity in both children and adults and at least one death in a neonate. Early warning of the epidemic was provided both from an active surveillance programme which is operated continually by the National Institute for Virology (NIV) in the Johannesburg area and also from passive case-finding. The latter consisted either of specimens sent in routinely to the laboratory for virological investigation or specimens recruited during investigation of localized outbreaks of disease reported to the laboratory. Laboratory diagnoses were made either by isolation of Coxsackie B virus from patient specimens or serologically on the basis of a 4-fold or greater rise or fall in paired sera or a single titre of 640 or higher. A relatively early diagnosis of the epidemic could thus be made and the central and local public health authorities notified; in addition, local medical practitioners could be alerted to the problem via a regularly published laboratory-based epidemiological bulletin.

METHODS

Collection of specimens

Specimens were collected from three main sources: Firstly, a sentinel sampling network consisting of 2 general practices, 2 pediatric hospitals (1 black and 1 white), a mine hospital (adult blacks), a large network of black primary health care clinics in Soweto (adjacent to Johannesburg) and staff clinics at the NIV and a neighbouring infectious diseases hospital. This sentinel network is used primarily to monitor viral respiratory infections and viral gastroenteritis, but during the Coxsackie epidemic it was also utilized as a source of material for investigation of Coxsackie B virus infections. The second source of specimens were those acquired in response to the notification of various outbreaks. The third source consisted of specimens sent to the diagnostic laboratories of the NIV for routine laboratory investigation.

The epidemic involved the cities of Johannesburg and Pretoria and a number of smaller towns surrounding them. The specimens investigated were obtained mainly from Johannesburg, although a number were also sent from the town of Middelburg, some 80 miles east of Johannesburg, where a particularly extensive epidemic took place.

Virus isolation

The specimens consisting either of suspensions of stools, throat swabs, cerebrospinal fluids, urines or rectal swabs were inoculated into cultures of primary vervet kidney cells (VK) and HeLa cells as well as into suckling outbred Swiss mice of up to 24 h of age. The cell cultures were maintained in Eagle's minimal medium (MEM), incubated at 37 °C and observed daily for 14 days for cytopathic effects (CPE). Positive cultures were passaged in VK cells and neutralization tests carried out for serotyping using Coxsackie B1–6 standard sera obtained from the National Institutes of Allergy and Infectious Diseases, Bethesda, Maryland, USA. Neutralization was carried out by mixing equal volumes of virus (at 100 $TCID_{50}$) with specific antiserum (at four neutralizing units—a neutralizing unit being that dilution able to neutralize 100 $TCID_{50}$ of virus at 50% end-point). After

incubating the mixture for 1 h, it was inoculated into VK cells and observed for a week.

Suckling mice were inoculated with suspensions of stools or undiluted suspensions of other specimens in their transport media, or with undiluted cerebrospinal fluid or urine. They were observed daily for typical signs of Coxsackie infection for up to a week. Mice showing clinical signs of infection were killed and tissue sent for histological confirmation of Coxsackie A or B virus infection. Positive specimens from mice were also serotyped as detailed above.

Microneutralization test

Sera were all inactivated at 56 °C for 30 min before testing for Coxsackie neutralizing antibodies. Standard Coxsackievirus B1–6 strains were obtained from the National Institute of Allergy and Infectious Diseases, Bethesda, Maryland, USA. These strains were mixed at 100 $TCID_{50}$/0·05 ml with an equal volume (0·05 ml) of serum at a screening dilution of 1/10 and incubated for 1 h at 37 °C in 96-well microtitre plates. To each well containing the virus-serum mixture was added 0·1 ml of freshly trypsinized Vero cells at 1×10^5 per ml and incubated at 37 °C in a 5 % CO_2 incubator. Those sera which were positive at 1/10 were then further doubly diluted to 1/640 and the titre of neutralizing antibodies read as the end-point in which only 50 % of the inoculated cells showed cytopathic effect.

RESULTS

The epidemic curve of the 1984 Coxsackie B virus outbreak is shown in Fig. 1. The epidemic was characterized by a 'herald wave' of a few isolates in the late winter (mid-August to early September) with the epidemic proper beginning in the latter part of September and reaching a peak towards the end of October. By the end of December only scattered sporadic cases were occurring and these continued into the new year.

A total of 60 isolates were made from specimens derived from 396 patients during the epidemic, thus giving an isolation rate of 15 %. Passive surveillance provided 303 specimens, 41 of which were positive (14 %) and from active surveillance 93 specimens, of which 19 (20 %) were positive. By far the dominant serotype was Coxsackie B3 which accounted for 52 of 60 isolates (87 %).

A further 82 specimens were positive by the microneutralization test based either on a $\geqslant$ 4-fold rise or fall in titre of paired sera (9 specimens) or a titre of equal or greater than 640 in a single serum (73 specimens). However, in the case of the serological investigations the B3 dominance was not nearly as clearly seen as with virus isolation. The results of seropositive specimens are shown in Table 1 and show a more dispersed distribution of serotypes although B3 was still the most commonly positive serotype. Seropositivity to a single serotype was seen in 49 (60 %) of the 82 positive specimens of which 3 were positive for B1, 12 for B2, 16 for B3, 12 for B4, 5 for B5 and 1 for B6 respectively.

Of the total (by isolation and serology) of 142 positive specimens, 113 came from white patients and 29 from black. This difference probably reflects inadequate sampling in the black community rather than a real difference in prevalance of the disease, although this was, in fact, not definitely established in the black group.

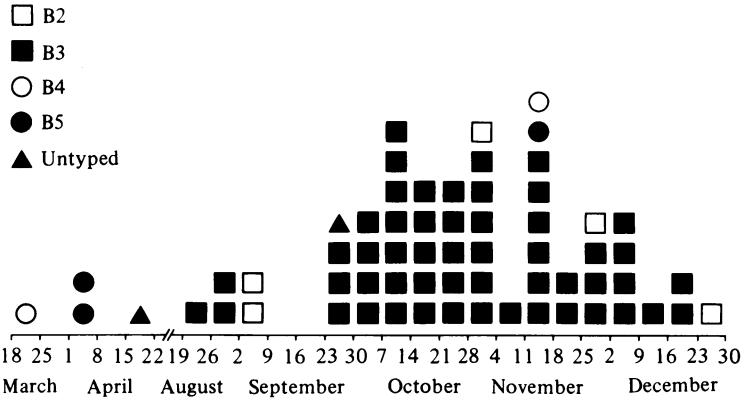

Fig. 1. Epidemic curve of 1984 Coxsackie B virus outbreak in Johannesburg and environs.

Table 1. *Results of positive Coxsackie B neutralization tests*

	Total no. of specimens	Coxsackie neutralization titres					
		B1	B2	B3	B4	B5	B6
⩾ 4-fold rise/fall	9	3	4	4	2	1	0
Titres ⩾ 640	73	12	21	37	26	13	5
Total	82	15	25	41	28	14	5

Table 2. *Sex* distribution of positive† specimens from white & black patients*

	Male	Female	χ^2 test
Whites	45 (47)‡	50 (53)	N.S.§ ($P = 0{\cdot}309$)
Blacks	15 (58)	11 (42)	N.S. ($P = 0{\cdot}422$)
Total	60 (49·5)	61 (50·5)	N.S. ($P = 0{\cdot}985$)

* The sexes of 18 white and 3 black patients were unknown.
† Specimens positive either by isolation or by microneutralization test as defined in test.
‡ Figures in parentheses denote percentages of total for each race group.
§ N.S. = not significant.

In both black and white patients no significant sex difference was noted (see Table 2).

The majority of infections, 78 of 101 (77 %), were found in children and young adults up to the age of 29 years, the incidence being fairly uniformly distributed through the various age groups. After 30 years of age the incidence dropped steeply. There did not appear to be any appreciable differences in the patterns of age distribution between black and white patients (see Fig. 2).

The relationship of isolations to clinical presentations is shown in Table 3. The most common clinical presentation during the outbreak was Bornholm disease, accounting for 30 of the 97 specimens where diagnoses were provided. The isolates obtained from these patients were all B3.

Abdominal pain was reported to be a more prominent feature in the younger

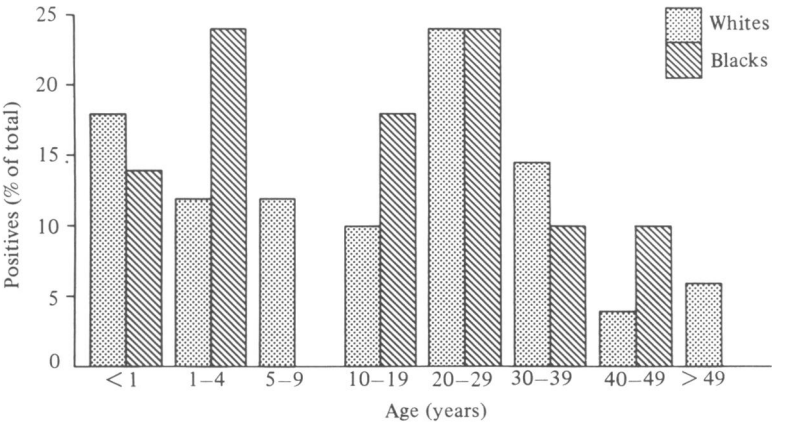

Fig. 2. Comparison of Coxsackie B virus isolations in white and black subjects at various age groups.

Table 3. *Coxsackie B positives—clinical presentation and relationship to serotype*

		Isolation					
	Total	B2	B3	B4	B5	Untyped	Serology
Bornholm disease	30	0	20	0	0	0	10
Myocarditis/pericarditis/ cardiac failure	21	2	5	0	0	0	14
Meningo-encephalitis	15	1	7	0	1	1	5
Polio/paralysis	3	0	2	0	0	0	1
Pyrexia/malaise ± respiratory	12	0	7	0	0	0	5
Assorted diagnoses	16	1	4	0	0	0	11
No diagnosis	45	1	7	1	0	0	36

Table 4. *Clinical presentations of Coxsackie B positives by age and race*

	Total			White			Black		
Age (years)...	< 1*	1–9	⩾10	< 1	1–9	⩾10	< 1	1–9	⩾10
Bornholm disease	1	7	21	1	7	19	0	0	2
Myocarditis	4	5	9	3	2	2	1	3	7
Meningo-encephalitis	8	1	4	8	1	3	0	0	1
Polio/paralysis	1	2	0	0	0	0	1	2	0
Pyrexia	2	2	6	1	2	5	1	0	1

child and chest pain in the adult. Cardiac involvement presenting either as acute myocarditis or pericarditis or acute cardiac failure was the next commonest clinical presentation (21 of 97) where both B3 and B2 isolates were obtained. Meningo-encephalitis featured next in prominence (15 of 97) with the majority of the isolates being B3. Of the more important clinical presentations, Bornholm disease appeared to be more prevalent in the older age group whereas meningo-encephalitis was somewhat more prevalent in young infants (see Table 4). In the black group both Bornholm disease and meningo-encephalitis were relatively uncommon as

compared to the whites whereas myocarditis was the major clinical presenting feature. In addition, the three cases of polio-like paralysis were found only in black children (see Table 4).

DISCUSSION

The laboratories of the Poliomyelitis Research Foundation (the predecessor of the NIV) instituted, in 1950, a longitudinal study to investigate the incidence as well as the medical importance of Coxsackievirus infections. During the following two decades the cyclical appearance of Coxsackievirus outbreaks every few years could be graphically shown with a particular serotype dominating each outbreak (Gear, 1967). During this 20-year period, B2 virus was responsible for 5 outbreaks, B3 and B4 for 3 each, and B1 for 2. In 1982 relatively few Coxsackie B viruses were isolated during the summer season and in 1983 B5 was responsible for 50% of the Coxsackie B isolates. The 1984 epidemic detailed above was clearly dominated by B3 which accounted for 87% of the isolates while B2 made up only 8% and B4 and B5 each 2%. In a 12-year study in the United Kingdom, Bell & McCartney (1984) found B4 and B2 to be the commonest serotypes, whereas during an 8-year survey from 1967 to 1974, conducted by the WHO virus reporting system, B3 and B5 were found to be the most commonly reported serotypes (Grist, Bell & Assaad, 1978).

Isolations of virus commenced in mid-August and it is probable that the epidemic itself started in early August, which in Johannesburg is still late winter. In the United Kingdom and USA enterovirus outbreaks are generally described as peaking in summer and autumn (Banatvala, 1983; Moore & Morens, 1984). It could be speculated that the unusual earliness of the 'herald wave' in this epidemic could have presaged a particularly extensive subsequent epidemic. The one which did follow was indeed extensive and lasted 5 months, tailing off towards the end of December. Unfortunately, as the NIV does not have the resources to carry out comprehensive population investigations, it was not possible to determine attack rates during the epidemic. In addition, the extent of the infection in terms of total numbers infected could also not be determined accurately. Nevertheless, it was apparent from informally obtained information that many hundreds were affected, including a nosocomial outbreak amongst neonates in a maternity hospital affecting 11 neonates (1 of whom died) and some of the staff. A Coxsackie B3 virus was isolated from the index case.

The most common group of clinical presentations associated with Coxsackie B virus infection found in the 8-year WHO virus unit study was CNS diseases, which accounted for 34% of all the reports, the majority of them aseptic meningitis (Grist, Bell & Assaad, 1978). In the Johannesburg outbreak the commonest (31%) clinical diagnosis provided with the specimens was Bornholm disease and related syndromes of severe acute myalgia presenting mainly as abdominal pain in children and chest pain in adults, as has been reported by others (Moore & Morens, 1984). In our epidemic, meningo-encephalitis was only the third most prevalent clinical presentation (15%), coming after cardiac involvement (22%). The only serotype obtained from the Bornholm cases was B3, whereas additional serotypes were found in the cardiac as well as the meningo-encephalitis cases, although the predominant serotype in both of these conditions was still B3.

Unfortunately, considerably fewer specimens and clinical data were collected from the black population, although this does not necessarily indicate that the epidemic was less extensive in this community. Nevertheless, it is of interest that in this group by far the major clinical presentation (over 50 %) was myocarditis, with Bornholm disease and meningo-encephalitis being relatively infrequent. This is particularly noteworthy, taking into account the association of Coxsackie B virus infection with cardiomyopathy and endocarditis (Banatvala, 1983) and the high prevalence in the black population of cardiomyopathy (Beck, 1978) and especially rheumatic heart disease (where the occurrence in the black population of Johannesburg is one of the highest in the world) (McLaren *et al.* 1975). Already it has been recommended that the role of Coxsackie B virus infections in 'acute rheumatic carditis' in developing countries should be further investigated (Banatvala, 1983). It was also interesting to note that the only cases presenting with polio-like paralysis were from black patients.

The age distribution of cases was, as found elsewhere, predominantly in the younger group—77 % falling into the category of children and young adults up to the age of 29 years, with no particularly remarkable clustering in any specific age group under 29 years. Nevertheless the proportion of young children in our group was considerably lower than that reported by the WHO virus unit study (Grist, Bell & Assaad, 1978), who found that 78·7 % of their reports came from children under the age of 14 years as compared to 50 % in the corresponding age group in our study. No significant difference in age distribution was noted between white and black groups. As reported in other parts of the world (Melnick, 1982; Moore & Morens, 1984) we also found that Bornholm disease presented more frequently in older children and adults.

The male–female ratio of incidence of enterovirus disease involving Coxsackie B virus has been variously reported as either showing a distinct male predominance of 1·5 to 2·5:1 (Moore & Morens, 1984) or else no apparent sex difference (Melnick, 1982). During our epidemic, males and females were affected equally in both whites and blacks.

Coxsackie B virus infection is not a notifiable disease in any country in the world and statistics of its prevalence are woefully inadequate. Nevertheless recurrent epidemics of Coxsackie B virus infection are a major public health problem, causing significant mortality, widespread morbidity and to some extent permanent incapacity due to cardiac or CNS involvement. This emphasizes the very important need for public health authorities to maintain both active and passive surveillance programmes to be alerted to an impending outbreak at as early a stage as possible. Surveillance is usually based for convenience on laboratory data, either passively generated from routine diagnostic tests or investigations in reaction to notification of outbreaks. A more active approach should, however, be encouraged to attempt to elicit the earliest possible signals of an impending outbreak. At the NIV, information is obtained not only from passive laboratory data but also by maintaining a network of eight sentinel stations encompassing both white and black population groups, paediatric hospitals and general practices and higher and lower socio-economic groups. These sentinels were established

primarily to monitor viral respiratory and gastroenteric diseases but act also as very useful clinical information sources for other viral diseases in the community. Regular contact is maintained during collection of respiratory and gastroenteritis surveillance material and also by regular epidemiological meetings and a monthly epidemiological bulletin. Although the resources of a laboratory must, of necessity, impose a limitation on the volume of specimens which can be processed as part of a surveillance programme, the extension of the sentinel network to include medical practitioners providing only clinical information on a regular basis is an extremely valuable adjunct to an early detection surveillance programme. In the epidemic described above it was evident by about September 1984 that a major outbreak of Coxsackie B virus was under way and both central and local health authorities could be alerted. Of particular interest were the maternity homes, where strict preventive measures, as outlined by Gear (1967), were instituted.

Much still requires to be elucidated regarding the epidemiology of Coxsackievirus infections, and especially Coxsackie B virus. The propensity to involve the young individuals in the community, apparently regardless of socio-economic or racial factors (although the latter still needs to be definitely established), certainly differs from the patterns seen in poliomyelitis in the pre-vaccine era. The ecology of the virus and its circulation in the community, both during and between epidemics, requires to be investigated and especially the extent of the 'iceberg' of asymptomatic carriers of the viruses and their importance as a reservoir of infectious virus. The behaviour of the different serotypes is also not fully understood, beyond that certain serotypes appear to be more frequently associated with epidemic activity. Clearly more finely tuned techniques of defining strains of Coxsackie B virus are required for epidemiological linkage and tracing studies as is now commonly used with poliomyelitis, e.g. T1 ribonuclease fingerprinting (Kew & Nottay, 1984) or monoclonal antibodies for epitope differentiation (Osterhaus *et al.* 1983). With more updated tools for epidemiological study we may be in a position to understand better the epidemiological vagaries of this group of important human pathogens.

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