Epidem. Inf. (1989), **103**, 143–156
Printed in Great Britain

Leptospiral infection in school-children from Trinidad and Barbados

C. O. R. EVERARD[1], R. J. HAYES[2] AND C. N. EDWARDS[3]

[1]*MRC/Government of Barbados Leptospirosis Project, Veterinary Diagnostic Laboratory, The Pine, St Michael, Barbados,* [2]*Tropical Epidemiology Unit, London School of Hygiene & Tropical Medicine, Keppel Street, London WC1E 7HT,* [3]*Queen Elizabeth Hospital, St Michael, Barbados*

(*Accepted 19 March 1989*)

SUMMARY

A serological survey for leptospiral agglutinins was undertaken between 1980 and 1983 in over 500 Barbadian and 500 Trinidadian school-children aged 7–14 years. The children were selected randomly from urban and rural schools, and examined three times at approximately annual intervals. A total of 12·5% of the Barbadian children and 9·5% of the Trinidadian children were seropositive at a titre of 50 using the microscopic agglutination test. On both islands, sero-prevalence was higher in males than females, the difference being significant in rural schools. There was no evidence of a difference in prevalence between urban and rural schools, or between junior and secondary age-ranges. Analysis of the association of serology with socio-economic and behavioural factors showed a significant association in Trinidad with father's occupation, but most other variables on both islands showed only weak non-significant associations. Fourteen children in Trinidad and three in Barbados seroconverted. Seroconversion in Trinidad occurred at a rate of 1·6% per annum and was significantly associated with livestock contact and with absence of a tapped water supply. In Trinidad, Autumnalis was the most commonly recorded serogroup, but this accounted for less than a quarter of seropositives. In Barbados, Panama accounted for over half the seropositives and was about four times more common than the next most common serogroup, Autumnalis. In Barbados, 39 persons aged 19 or less were hospitalized with leptospirosis between November 1979 and December 1986. Average annual incidence rates were 2·2, 4·9 and 13·3 per 100000 in the 5–9, 10–14 and 15–19 age-groups, respectively.

INTRODUCTION

Leptospirosis was for many years considered to be primarily an infection of adulthood, but since the mid-1950s cases have been reported in children in many parts of the world. In the British Isles, for example, Alston & Broom (1) reported that of all cases recorded between 1940 and 1955, 5% were children aged 9 or under, and 16% were aged 10–19. More recently, Kaufmann (2) reported that of cases recorded in the USA from 1965–74, 10% were aged 0–9, and a further 22% (most of them males) were aged 10–19.

Unfortunately most of the available reports present data only for 10-year age groups, and so it is impossible to determine the relative numbers of cases in infants, pre-school children, primary school-children and in the early and late teens. Moreover, most reports give cases in each age group as a percentage of all recorded cases, rather than as an incidence rate which takes into account the size of the population in each age group. Another problem of interpretation is the possibility that an increasing proportion of cases are children because of improved diagnosis and reporting in this age group, or because incidence rates are falling faster in older groups due to public health or occupational health interventions. Despite these reservations, the evidence suggests that relatively few young children acquire severe leptospirosis, but that severe cases among teenagers, especially males, are more common.

An alternative method of examining the incidence of leptospirosis, which focusses on the number of individuals with evidence of past infection rather than on the number of reported clinical cases, is to conduct a serosurvey of the general population. In 1978, Everard & Fraser-Chanpong (3) conducted a survey for leptospiral agglutinins in samples of school-children from five Caribbean territories and Bermuda. Overall, 11% of 827 children aged 5–14 were seropositive at titres of 100 or greater. This study was based on a relatively small number of children from each island, was cross-sectional, and no data were collected on possible risk factors. To provide further information, a longitudinal survey was conducted between 1980 and 1983 of over 500 Barbadian and 500 Trinidadian school-children aged 7–14 years. The main objectives were to examine the prevalence of seropositivity by age, sex and location (urban or rural); to investigate the association of serology with a range of behavioural, environmental and socio-economic factors; to examine changes in serological titres between surveys and estimate the incidence of seroconversion; to study the relative prevalence of different serogroups; and to compare the findings from the two islands. The results of this survey are presented below. We also report the incidence of hospital cases of leptospirosis in Barbados between 1979 and 1986, among children and young people up to 19 years of age.

METHODS

Survey of school-children

Stratified random sampling was used to select five junior and five secondary schools on each island. In Barbados, a list was made of all government primary and 'all-age' schools, and three rural and two urban schools were randomly selected. Sampling of secondary schools was restricted to government secondary schools, which account for about 70% of all school-children in this age-range; because more secondary schools are located in urban locations, three urban and two rural secondary schools were randomly selected. Similar methods were used in Trinidad, but for practical reasons the survey was restricted to schools in the north-western part of the island. For purposes of analysis, one of the 'urban' schools selected in Barbados was later reclassified as 'rural'. This school was located in a small coastal town which was regarded as essentially rural in character.

A full list of pupils was obtained in each of the selected schools, and a random sample of 16 pupils (8 boys and 8 girls) was chosen from each year group (Junior: 7/8, 8/9, 9/10, 10/11; Secondary: 11/12, 12/13, 13/14), a total of 64 children from each junior school and 48 from each secondary school, giving a total of about 560 children from each island.

The selected children were studied in three consecutive surveys, at approximately annual intervals (during the school years 1980/1, 1981/2 and 1982/3). At each survey, the child was asked to give a blood sample for serological analysis, and a questionnaire was administered to the child concerning parental occupations, living conditions, and a range of leisure and domestic activities. A few children refused to give blood samples. Moreover, at the second and third surveys many of the children were found to have moved to different schools or to have left school. Every attempt was made to trace them at their new school or place of work or at home, but a few could not be traced. In Trinidad, of the 573 children selected for study, serological results were available for 558 (97%) at Survey 1, for 510 (89%) at Survey 2, and for 444 (77%) at Survey 3. The corresponding figures for the 560 children selected in Barbados were 528 (94%), 487 (87%) and 438 (78%).

Laboratory techniques

Blood samples of 5–7 ml were drawn intravenously from each child and taken in a cold box to the laboratory, where they were centrifuged. The sera were stored at −20 °C until examined by the Leptospira Microscopic Agglutination Test (MAT) using a standard microtitre technique (4). Twelve different serogroups were represented by a battery of serovars of *Leptospira interrogans* used in the form of live culture antigens of a standard density. These serovars were: *ballum*, *canicola*, *pyrogenes*, *copenhageni* (Icterohaemorrhagiae), *bataviae*, *grippotyphosa*, *fort-bragg* (Autumnalis), *pomona*, *sejroe*, *georgia* (Mini), *tarassovi* and *panama* (The serogroup was of the same name unless shown in parentheses). Sera were screened initially at dilutions of 1:25 to 1:100. All sera which were positive at a screening dilution of 1:100 were titrated to the end point. Sera were retested if anomalous results were recorded at any of the three surveys.

Hospital patients

All patients aged 19 years and under presenting at the Queen Elizabeth Hospital (QEH) between November 1979 and December 1986 with symptoms consistent with leptospirosis were examined serologically and nearly all bacteriologically. Isolation of *Leptospira* sp. was attempted from blood, dialysate fluid and urine collected at appropriate times. The isolates were identified by cross-agglutinin-absorption techniques (4, 5) and by monoclonal antibodies prepared against the common serovars found in Barbados (6, 7). The serological tests used included the Patoc I (Slide) Agglutination Test (PSAT), the MAT and the ELISA for detecting IgM and IgG antibodies. Details of the methods used are given in references (8–10).

Table 1. *Prevalence of seropositivity by age-range, sex and urban/rural location of school*

	Males	Females	Total
		Trinidad	
Rural schools			
Junior	12/93 (13)*	5/96 (5)	17/189 (9)
Secondary	7/48 (15)	4/48 (8)	11/96 (11)
Urban schools			
Junior	5/68 (7)	7/62 (11)	12/130 (9)
Secondary	7/66 (11)	6/77 (8)	13/143 (9)
Total	31/275 (11)	22/283 (8)	53/558 (9)
		Barbados	
Rural schools			
Junior	14/91 (15)	10/93 (11)	24/184 (13)
Secondary	9/68 (13)	5/66 (8)	14/134 (10)
Urban schools			
Junior	6/51 (12)	10/66 (15)	16/117 (14)
Secondary	7/42 (17)	5/51 (10)	12/93 (13)
Total	36/252 (14)	30/276 (11)	66/528 (12)

* Figures in parentheses are percentages.

RESULTS

Prevalence of seropositivity at Survey 1 by age, sex and location

Children whose sera gave MAT titres of 50 or above were defined as 'seropositive'. The prevalence of seropositivity by age-range, sex and urban/rural location of school is shown for both islands in Table 1.

A higher overall prevalence was recorded in Barbados (12·5%) than in Trinidad (9·5%), but the difference was not statistically significant ($\chi^2 = 2{\cdot}21$, 1 D.F., N.S.). On both islands, the prevalence rate was rather higher in males than in females, but again the difference was not significant (Mantel–Haenszel (MH) test allowing for urban/rural location and age-range gives Trinidad: $\chi^2 = 1{\cdot}58$, 1 D.F.; Barbados: $\chi^2 = 1{\cdot}18$, 1 D.F.). The sex difference was more marked in rural schools, and combining the evidence from both islands the prevalence rate in rural schools was significantly higher in males than females (MH test allowing for island and age-range gives $\chi^2 = 5{\cdot}09$, 1 D.F., $P = 0{\cdot}02$). In urban schools, there was little difference in prevalence overall between the sexes.

There was no evidence in either island of any difference in seropositivity rates between junior and secondary age-ranges (MH test with allowance for sex and location gives Trinidad: $\chi^2 = 0{\cdot}07$, 1 D.F.; Barbados: $\chi^2 = 0{\cdot}27$, 1 D.F.), or between urban and rural schools (MH test with allowance for sex and age-range gives Trinidad: $\chi^2 = 0{\cdot}04$, 1 D.F.; Barbados: $\chi^2 = 0{\cdot}17$, 1 D.F.). When the data were reanalysed with respect to the urban or rural location of the child's home, the results were similar to those obtained on the basis of school location.

When the effect of age was examined in more detail (Table 2), the difference in prevalence between the four age-groups in Trinidad was close to statistical significance (logistic regression with allowance for sex gives $\chi^2 = 7{\cdot}02$, 3 D.F.,

Table 2. *Prevalence of seropositivity by age and sex*

Age in years	Males	Females	Total
		Trinidad	
6–8	8/81 (10)*	6/84 (7)	14/165 (8)
9–10	9/77 (12)	6/71 (8)	15/148 (10)
11–12	5/56 (9)	1/66 (2)	6/122 (5)
13–	9/61 (15)	9/62 (14)	18/123 (15)
		Barbados	
6–8	3/40 (8)	8/54 (15)	11/94 (12)
9–10	9/67 (13)	9/78 (12)	18/145 (12)
11–12	15/89 (17)	9/84 (11)	24/173 (14)
13–	9/56 (16)	4/60 (7)	13/116 (11)

* Figures in parentheses are percentages.

$P = 0{\cdot}07$), but there was no clear trend in the prevalence rates, although the highest rates were seen in the oldest children. Age-related patterns of prevalence appeared to differ between the sexes in both islands, but these differences could easily have occurred by chance (logistic regression for interaction between age and sex gives Trinidad: $\chi^2 = 2{\cdot}78$, 3 D.F., N.S.; Barbados: $\chi^2 = 4{\cdot}03$, 3 D.F., N.S.).

Overall prevalence rates varied substantially between schools (from 2·9 to 16·7% in Trinidad; from 6·7 to 17·7% in Barbados), but sample sizes at each school were relatively small, and these variations could easily have occurred by chance (Trinidad: $\chi^2 = 8{\cdot}17$, 9 D.F., N.S.; Barbados: $\chi^2 = 4{\cdot}77$, 9 D.F., N.S.).

Few of the seropositive sera gave high titres. Amongst the 53 seropositives in Trinidad, 24 gave titres of 50, 19 of 100, 3 of 200 and 7 of 400 (Geometric Mean Titre (GMT) = 91). Of the 66 seropositives in Barbados, 33 gave titres of 50, 21 of 100, 6 of 200, 5 of 400 and 1 of 800 (GMT = 86).

Risk factors for seropositivity at Survey 1

The association between seropositivity and each of the variables recorded on the questionnaire was examined using logistic regression, with allowance for age-group (6–8, 9–10, 11–12, 13+) and sex (Table 3).

In Trinidad, a significant association was found with father's occupation, the prevalence rate being highest in children of labourers and agricultural workers ($\chi^2 = 6{\cdot}02$, 2 D.F., $P < 0{\cdot}05$). Among this group, the highest prevalence was recorded in the small group of children whose fathers were agricultural workers (labourers: 21/163 = 12·9%; agricultural workers: 4/20 = 20·0%).

Most other variables showed a weak association in the expected direction, but none were close to statistical significance. To examine whether exposure to several of these assumed risk factors was associated with seropositivity, a 'risk score' was defined for each child. The score was incremented by one for each of the following activities: bathing in fresh water rivers, streams or ponds; playing in the open countryside; clearing drains; contact with livestock in the yard or garden; walking barefoot (often or sometimes) in the street or lane; and walking barefoot (often or sometimes) in the open countryside. Thus the minimum possible risk score was 0 and the maximum was 6. The prevalence of seropositivity analysed by

Table 3. *Association between seropositivity and questionnaire variables*

Variable	Trinidad Prevalence	Trinidad Corrected χ^2	Barbados Prevalence	Barbados Corrected χ^2
Father's occupation*				
Group 1	21/264 (8)†		15/133 (11)	
Group 2	25/183 (14)	6·02	8/50 (16)	0·86
Group 3	7/111 (6)		43/345 (12)	
Bathing				
Yes	14/132 (11)	0·19	4/22 (18)	0·56
No	39/426 (9)		62/504 (12)	
Playing in countryside				
Yes	40/373 (11)	0·86	45/352 (13)	0·00
No	13/185 (7)		20/175 (11)	
Playing with wild animals				
Yes	2/14 (14)	0·17	2/52 (4)	6·18
No	51/544 (9)		63/475 (13)	
Clearing drains				
Yes	34/323 (10)	0·35	11/70 (16)	0·29
No	19/235 (8)		55/458 (12)	
Walking barefoot				
In yard/garden				
Yes	49/527 (9)	0·32	60/452 (13)	1·20
No	4/31 (13)		6/76 (8)	
In street				
Yes	38/374 (10)	0·17	53/399 (13)	0·49
No	15/184 (8)		13/129 (10)	
In countryside				
Yes	27/237 (11)	0·31	46/329 (14)	1·12
No	26/321 (8)		20/199 (10)	
Livestock in yard/garden				
Yes	17/137 (12)	1·40	31/265 (12)	0·35
No	36/421 (9)		35/263 (13)	
Taps in house				
Yes	38/421 (9)	0·67	56/448 (12)	0·00
No	15/137 (11)		10/79 (13)	
Availability of WC				
Yes	14/199 (7)	2·65	57/427 (13)	1·06
No	39/359 (11)		9/95 (10)	
Risk score (see text)				
0–1	6/124 (5)		13/119 (11)	
2	10/116 (9)		12/100 (12)	
3	15/116 (13)	1·88‡	18/130 (14)	0·30‡
4	11/120 (9)		14/140 (10)	
5–6	11/82 (13)		9/39 (23)	

* Occupational Group 1, Factory-workers, shopworkers, white-collar workers, mechanics, electricians. Group 2, Labourers, agricultural workers. Group 3, Other, retired, unemployed, not given.

† Figures in parentheses are percentages.

‡ χ^2 for trend. All χ^2 are corrected for age and sex.

https://doi.org/10.1017/S0950268800030442 Published online by Cambridge University Press

Table 4. *Children seroconverting between successive surveys*

School no./ location	Age*/sex	Titre at Survey 1	Survey 2	Survey 3	Serogroup
		Trinidad schools			
13 Urban	12 M	−ve	200	200	Panama
	11 F	−ve	−ve	100	Bataviae
14 Urban	12 F	−ve	−ve	100	Ictero
	11 F	−ve	−ve	100	Ictero
	13 M	−ve	−ve	100	Ictero
	12 F	−ve	−ve	100	Ictero
15 Urban	11 M	−ve	−ve	200	Bataviae
	13 F	−ve	−ve	400	Ictero
16 Rural	9 M	−ve	200	100	Autumnalis
17 Rural	10 M	−ve	100	50	Pyrogenes
	8 F	−ve	100	50	Panama
18 Rural	9 F	−ve	100	100	Hebdomadis
19 Urban	7 M	−ve	−ve	400	Hebdomadis
20 Urban	10 M	−ve	200	50	Canicola
		Barbados schools			
2 Rural	11 M	−ve	−ve	200	Panama
5 Urban	10 F	−ve	−ve	200	Bataviae
8 Rural	12 M	−ve	400	200	Ictero

* Age at Survey 1.

risk score is shown in Table 3. There was an upward trend in prevalence with increasing risk score, but this was not statistically significant (logistic regression allowing for age and sex gives $\chi^2 = 1{\cdot}88$, 1 D.F.).

In Barbados, children who said they collected or played with wild animals had a significantly lower prevalence rate than other children ($\chi^2 = 6{\cdot}18$, 1 D.F., $P = 0{\cdot}01$). Given that this represents the one significant result obtained from a large number of significance tests, and given that it is in the opposite direction to that expected from biological considerations, it seems likely that it occurred by chance. Some of the other variables showed trends in the expected direction, but these were nowhere near statistical significance, and two variables (livestock in yard/garden and availability of WC) showed trends in the direction opposite to that expected. When the 'risk score' was calculated in the same way as for the Trinidad children, there was no significant upward trend in prevalence with increasing risk score ($\chi^2 = 0{\cdot}30$, 1 D.F., N.S.). The highest prevalence was observed in children with the highest risk scores, but the sample size in this category was small.

Seroconversion between surveys

Analysis of seroconversions was restricted to children who gave blood samples at all three surveys, 425 in Trinidad and 427 in Barbados. Seroconversion was defined as a change between two successive surveys from negative to $\geqslant 100$, or a fourfold increase to > 100.

In Trinidad there were 14 seroconversions from negative, 6 between Surveys 1 and 2, and 8 between Surveys 2 and 3 (Table 4). The four identical Icterohaemorrhagiae seroconversions taking place at school 14 (an urban secondary school) between Surveys 2 and 3 are of interest. The estimated

Table 5. *Variables significantly associated with seroconversion in Trinidad*

Variable	Incidence	Corrected χ^2*
Taps in house		
Yes	7/320 (2)†	10·62
No	7/63 (11)	
Livestock in yard/garden		
Yes	10/158 (6)	6·59
No	4/225 (2)	

* Corrected for age.

† Figures in parentheses are percentages.

incidence rate of seroconversion was $14/(2 \times 425) = 1{\cdot}6\%$ per annum, with approximate 95% confidence limits of 0·8% and 2·5%. The incidence rate among those aged 6–10 years (1·1%) was lower than in those aged 11+ (2·4%), but there were too few cases for this difference to be statistically significant ($\chi^2 = 1{\cdot}10$, 1 D.F.). There was no appreciable sex difference in incidence (8 seroconversions in males, 7 in females).

In Barbados there were only three seroconversions, one between Surveys 1 and 2, and two between Surveys 2 and 3 (Table 4). All three were initially negative. The estimated incidence rate of seroconversion was $3/(2 \times 427) = 0{\cdot}4\%$ per annum, with 95% confidence limits of 0·1% and 1·0%. There were too few cases to examine whether incidence varied with age or sex.

Characteristics of seroconverters

To obtain additional information on risk factors for leptospiral infection, the 14 children in Trinidad who seroconverted were compared with respect to the questionnaire variables with the 370 children who remained seronegative. There were too few seroconversions to conduct this analysis among the Barbados children. The questionnaire data used were those collected at Surveys 2 and 3, as these described the characteristics of the children during the 2-year study period in which the seroconversions occurred. For each child, the 'worst exposure' during this 2-year period was used in the analysis. Logistic regression was used to correct the analysis for age (6–10, 11+).

Only two variables showed a significant association with the incidence of seroconversion (Table 5). Children living in houses without a tapped water supply were more likely to seroconvert ($\chi^2 = 10{\cdot}62$, 1 D.F., $P = 0{\cdot}001$), as were children having contact with livestock in their yard or garden ($\chi^2 = 6{\cdot}59$, 1 D.F., $P = 0{\cdot}01$). Each variable was still significantly related to seroconversion after allowing for the possible confounding effect of the other (tapped water allowing for livestock and age: $\chi^2 = 9{\cdot}33$, 1 D.F., $P = 0{\cdot}002$; livestock allowing for tapped water and age: $\chi^2 = 5{\cdot}30$, 1 D.F., $P = 0{\cdot}02$).

Changes in titre over time

It is assumed that, following infection, the leptospiral antibody titre declines gradually over time, but little is known about the rate at which this decline occurs. Changes in titre from one survey to the next are shown in Table 6, which combines data recorded between Surveys 1–2 and 2–3.

Table 6. *Year-to-year changes in leptospiral antibody titre*

Initial titre	Titre one year later: Negative	50	100	200	400	800
			Trinidad			
Negative	818	13	9*	4*	2*	—
50	10	23	1	—	—	—
100	4	6	25	—	—	—
200	2	1	1	1	1	—
400	—	—	—	3	7	1
800	—	—	—	—	1	—
			Barbados			
Negative	791	7	1*	2*	1*	—
50	28	29	2	—	1*	—
100	3	13	14	2	—	—
200	1	2	3	4	—	—
400	—	—	3	3	3	—
800	—	—	—	1	—	—

* Seroconversions.

Table 7. *Dominant serogroups of seropositive children at Survey 1*

Serogroup	Trinidad: Rural	Urban	Total	Barbados: Rural	Urban	Total
Panama	4	3	7	20	16	36
Autumnalis	4	9	13	3	5	8
Icterohaemorrhagiae	6	1	7	2	2	4
Canicola	1	2	3	4	3	7
Pyrogenes	4	2	6	—	1	1
Hebdomadis	1	2	3	2	—	2
Ballum	—	—	—	4	1	5
Bataviae	1	—	1	1	—	1
Grippotyphosa	1	—	1	—	—	—
Tarassovi	1	—	1	—	—	—
Mixed	5	6	11	2	—	2
Total	28	25	53	38	28	66

In Trinidad, 56 (64%) of 87 individuals recorded as seropositive at the start of the year showed the same titre the next year, 21 (24%) showed a reduction to the next titre below, 7 (8%) showed a greater reduction in titre and 3 (3%) increased to the next titre above. The corresponding figures for 112 individuals in Barbados were 50 (45%) showing no change, 47 (42%) showing a reduction to the next titre below, 10 (9%) showing a greater reduction and 4 (4%) increasing to the next titre above. In addition there was one fourfold increase from 50 to 200, but this was considered a seroconversion, particularly as the titres were to differing serogroups.

Serogroups of seropositive individuals

Table 7 shows the dominant serogroups of the seropositive children seen at Survey 1. No single serogroup predominated in Trinidad. Autumnalis was the most common, but accounted for less than a quarter of the seropositives. Autumnalis appeared to be more common in urban than in rural schools at this survey, while Icterohaemorrhagiae appeared more common in the rural schools, but these findings need to be interpreted with caution in view of the small number of schools and small number of seropositives in each category.

In contrast to the data from Trinidad, Panama was by far the most common serogroup in Barbados, accounting for over half the seropositives, and being around four times more common than the next most common serogroup, Autumnalis. There were no striking differences between rural and urban schools.

Barbadian hospital patients

Between November 1979 and December 1986, 52 patients aged 19 years or under were admitted to QEH with symptoms consistent with leptospirosis. The disease was confirmed in 4/4 of those aged 5–9, 9/11 aged 10–14, and 26/37 aged 15–19. The youngest confirmed case was 7 years old. Average annual incidence rates were 2·2, 4·9 and 13·3 per 100000 in the 5–9, 10–14 and 15–19 age-groups, respectively.

Ten isolates were obtained from these 39 cases as follows: 5 Autumnalis *bim*, 4 Icterohaemorrhagiae *copenhageni* and 1 Ballum *arboreae*. Data from either serology or isolation indicate that the infecting serogroups in 38 of the 39 cases were as follows: Autumnalis (23), Icterohaemorrhagiae (11), Ballum (1), Autumnalis or Icterohaemorrhagiae (1), Icterohaemorrhagiae or Canicola (1), Ballum or Canicola (1). The remaining case was the only fatality, a 15-year-old from whom only a single acute stage serum sample was available. There was no apparent difference in the predominance of serogroups between the 13 children aged under 15, and the 26 young people aged 15–19 years.

DISCUSSION

Our data demonstrate that leptospirosis is a common infection of children in Trinidad and Barbados. Assuming that, following an infection, antibody levels decline and eventually become unmeasurable, our overall seroprevalence rates of 9% in Trinidad and 12% in Barbados are probably underestimates of the proportions of schoolchildren on these islands that have experienced leptospiral infections. Using the more conventional cut-off for seropositivity of 100, the seroprevalence rates were somewhat lower at 5% in Trinidad and 6% in Barbados. Our reason for additionally including titres of 50 as seropositive was the observation in these longitudinal data of many children giving consistent titres of 50 to the same serogroup in successive years. It seems unlikely that these consistent positive results represent chance laboratory variations.

We were surprised to find no evidence of any difference in seroprevalence between children in urban and rural schools, on either island. Previous studies of adults in Trinidad (11) indicated a generally higher prevalence in rural areas, although there were substantial differences between particular urban and rural settlements, suggesting that local ecological factors may be important. It appears that among children on these islands, there may be little difference in the risk of exposure to leptospires between those living in rural and urban areas. Possibly this is because the lifestyles of children, who spend a lot of time playing outside, show less variation than among adults. In Barbados, an additional factor is that many children with urban homes make frequent visits to the countryside, while many living in rural areas go to school in the town.

There was no clear age-trend in seroprevalence in our study. Assuming that reversion to seronegativity after infection is slow, the seroprevalence rate can be regarded as a rough measure of the cumulative incidence of infection. We would therefore expect the prevalence to increase with age, assuming that exposure and infection continue during the school years. The lack of an age-trend in our data should be interpreted with caution, however, as children in the junior and secondary age-ranges were sampled from different schools. The fact that seroprevalence was already around 10% in our 6–8-year-olds, however, suggests that infection may be common in the first 5 years of life.

Previous studies both of hospital cases of leptospirosis (8, 12) and of seroprevalence in the general population (11) have indicated considerably higher rates of infection in males than in females. We observed a higher seroprevalence in male school-children in both Trinidad (males 11%, females 8%) and Barbados (males 14%, females 11%), but the differences were small and were statistically significant only in rural schools. It is possible that sex differences in risk-related behaviour are less marked among school-children than in adults.

The seroconversion rates of 1.6% per annum in Trinidad and 0·4% per annum in Barbados are probably underestimates of the incidence of new infections, given that only 12 serogroup antigens were used, and the possibility that titres in some seroconverters may have declined by the time of the next annual survey so that the strict criteria for 'seroconversion' would not be fulfilled. The much higher number of seroconversions in Trinidad is not understood, although it is possible that the higher than average rainfall in Trinidad during the 2 years of the study may have led to an increased incidence of infection. The four children who seroconverted at the same school, in the same year, and to the same serogroup (Icterohaemorrhagiae) (Table 4), may represent a common-source exposure at this school.

Analysis of the association of seropositivity or seroconversion with a range of possible risk factors gave few significant results. In Trinidad, seroprevalence was significantly higher in children whose fathers were labourers or agricultural workers. It seems likely that this association reflects the living conditions of the families of such workers, and the environment surrounding their homes. Two more factors were significantly associated with seroconversion in the Trinidad children. Children living in houses with a tapped water supply were less likely to seroconvert. In Trinidad, most households without tapped water obtain their supplies from a standpipe in the road, from roof drainage water stored in 44 gallon drums, or from wells, and the water is subject to contamination during storage. Alternatively, water supply may, like father's occupation, be a proxy variable for the general living standards of the child's family. The significant association between seroconversion and contact with livestock may represent a direct risk of infection. An association between seropositivity and contact with livestock was also recorded in a previous study of adults in Trinidad (11).

None of these associations was significant in the Barbados children. However, the data on father's occupation were unsatisfactory in Barbados, as many children were unable to provide this information. Moreover, few households in Barbados are without tapped water, and those few can usually obtain their water from a standpipe in the road only a few yards away, so that storage of water is uncommon. We have no explanation for the lack of an association with livestock contact; perhaps Barbadian children are less closely involved in 'minding' the family livestock than their more rural Trinidadian counterparts.

Several other factors showed trends in the expected direction, for example bathing, playing in the country, and walking barefoot, but none of these associations was statistically significant. This does not mean, of course, that such factors are unimportant, but it should be recognized that the infection process is multifactorial, and that marked associations with individual risk factors would not necessarily be expected.

Our repeated surveys at annual intervals allowed us to examine year-to-year changes in antibody titre among seropositive individuals. In Trinidad, nearly two-thirds remained at the same titre over a 1-year period, while one-third showed a decline, mostly to the next titre below. The findings in Barbados were a little different, with less than half remaining at the same titre, and half showing a decline. It is possible that slight variations in laboratory techniques and changes in laboratory staff might account in part for these differences. Alternatively, it could be argued that individuals are continually reexposed to infection leading to a boosting of their antibody levels, in which case the difference between the islands could reflect a higher rate of reexposure in Trinidad, possibly related to 2 years of higher than average rainfall. Our data are too sparse to give precise estimates of the rate of decline in titre, or to determine whether this rate changes as the titre falls. They suggest, however, that between a third and a half of seropositive children can be expected to show a fall in titre from one year to the next, mostly to the next titre below, and that very few will show a rise unless they seroconvert to a high titre following a new infection. These findings would imply that a child starting with a titre of 800 after infection would on average take 10–15 years to become seronegative, although this is based on simplifying assumptions, and is very approximate.

The finding of Panama as a major infecting serovar in Barbadian and, to a lesser extent, Trinidadian children poses interesting problems concerning the pathogenicity and source of these infections. The serogroup was recorded as the presumptive infecting serogroup in only 4% of adult patients in Trinidad (12), and in one adult patient in Barbados. None of these cases was confirmed by isolation, and the Barbadian case was doubtful. Further, it has not been recorded in hospital patients under 15 years of age. It therefore seems likely that most Panama infections are mild or subclinical.

Data on hospital cases among children indicate that on both islands the incidence of severe infections rises steeply from age 5 to 19 years. Data reported in this paper give annual incidence rates for Barbados of 2·2, 4·9 and 13·3 per 100000 for the 5–9, 10–14 and 15–19 age groups respectively. Corresponding rates for Trinidad (12) were 0·4 (age 0–9), 7·9 and 21·5 per 100000, but detection of cases in Trinidad is known to be much less complete than in Barbados, and so these latter rates are certain to be underestimates. Thus, although the seroprevalence rates observed on the two islands were very similar, the incidence of severe infections among children and teenagers appeared somewhat higher in Trinidad. This may be due to the predominance of mild Panama infections in Barbadian children. Altogether, 15% of hospital cases of leptospirosis recorded in our study in Barbados were under 20 years of age, and 5% were under 15 years. The corresponding figures for Trinidad were 24 and 6%.

There was a marked excess of males among hospital patients aged 19 years or less on both islands (34/39 = 87% in Barbados, and 34/37 = 92% in Trinidad). The sex difference in the incidence of severe cases thus appears to be much greater than the difference in seroprevalence rates.

Our study has demonstrated that leptospirosis is a common infection of children in Trinidad and Barbados, sometimes resulting in a severe illness requiring hospital treatment, although the incidence of hospital admission is lower among children of school age than in older age groups. It is likely that the pattern is similar in other tropical and sub-tropical countries with comparable climatic and ecological features, and leptospirosis should be considered as a possible diagnosis in both minor and severe febrile illnesses in such populations.

ACKNOWLEDGEMENTS

This study was supported by the UK Medical Research Council (MRC) and Overseas Development Administration, and by the Ministries of Health and Education of Trinidad and Barbados. We are grateful to the PAHO/WHO Caribbean Epidemiology Centre for providing facilities and assistance. We would like to thank G. Fraser-Chanpong, M. Mohammed, G. Webb, D. Jean Marie, D. Carrington, F. B. M. Rodrigues, D. Beckles and S. Daniel for their technical assistance; A. Cumberbatch, R. Samuel, E. Hope, M. Greaves and S. Forde for their work on the field surveys; Drs T. A. Hassell, G. D. Nicholson, H. St. C. White and A. C. Graham, and Ms J. Callender of the Queen Elizabeth Hospital for their help in collecting the data on hospital patients; and D. Carson at the London School of Hygiene & Tropical Medicine for assistance with data processing. The identification of *Leptospira* isolates was undertaken at WHO Reference Laboratories at the Centers for Disease Control, Atlanta, by Dr C. R. Sulzer; and at the Royal Tropical Institute, Amsterdam, by H. Korver.

REFERENCES

1. Alston JM, Broom JC. Leptospirosis in man and animals. Edinburgh: Livingstone, 1958.
2. Kaufmann AF. Epidemiologic trends of leptospirosis in the United States, 1965–1974. In: Johnson RC, ed. The biology of parasitic spirochetes. New York: Academic Press, 1976.
3. Everard COR, Fraser-Chanpong GM. Serological evidence of leptospirosis in Caribbean schoolchildren. Trans R Soc Trop Med Hyg 1979; **73**: 591–3.
4. Sulzer CR, Jones WL. Leptospirosis: methods in laboratory diagnosis. DHEW Publ. No. 1978 (CDC) 79–8275. Washington: Government Printing Office.
5. Wolff JW, Broom JC. The genus *Leptospira* Noguchi, 1917. Problems of classification and a suggested system based on antigenic analysis. Doc Med Geograph Trop 1954; **6**: 77–95.
6. Fazekas de St. Groth S, Scheidegger G. Production of monoclonal antibodies: strategy and tactics. J Immunol Meth 1980; **35**: 1–21.
7. Galfre G, Milstein C. Preparation of monoclonal antibodies: strategies and procedures. In: Houba V, Chan SH, eds. Properties of the monoclonal antibodies produced by hybridoma technology and their application to the study of disease. Proceedings of a symposium held at the National University Singapore, 19–23 October 1981. Geneva: UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases 1982.
8. Everard COR, Edwards CN, Webb GB, White H St C, Nicholson GD. The prevalence of severe leptospirosis among humans on Barbados. Trans R Soc Trop Med Hyg 1984; **78**: 596–603.
9. Faine S, ed. Guidelines for the control of leptospirosis. WHO Offset Publication No. 67. Geneva: World Health Organization, 1982.

10. Terpstra WJ, Ligthart GS, Schoone GJ. ELISA for the detection of specific IgM and IgG in human leptospirosis. J Gen Microbiol 1985; **131**: 377–85.
11. Everard COR, Hayes RJ, Fraser-Chanpong GM. A serosurvey for leptospirosis in Trinidad among urban and rural dwellers and persons occupationally at risk. Trans R Soc Trop Med Hyg 1985; **79**: 96–106.
12. Everard COR, Fraser-Chanpong GM, Everard JD. The incidence of severe leptospirosis in Trinidad. Trop Geogr Med 1987; **39**: 126–32.