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Antimeningococcal herd immunity in the Czech Republic – influence of an emerging clone, *Neisseria meningitidis* ET-15/37

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SUMMARY

For many years, invasive meningococcal disease in the Czech Republic occurred sporadically and was caused mainly by meningococci of serogroup B. In 1993, when a new clone (ET-15/37) emerged, the only phenotype found was C:2a:P1.2,5. In 1995, an antigenic variation of the ET-15/37 clone, B:2a:P1.2,5, occurred. The results of immunological surveys conducted in 1989 and 1996 were compared. A significantly higher proportion of 1996 sera than those collected in 1989 showed bactericidal antibodies against *N. meningitidis* B:2a:P1.2,5 (19·7 *vs.* 5·1%) and *N. meningitidis* C:2a:P1.2,5 (15·9 *vs.* 7·4%), consistent with increased herd immunity due to the spread of the new clone in the Czech Republic. There were differences in the age distribution of the positive sera.

INTRODUCTION

Invasive meningococcal disease used to occur only sporadically in the Czech Republic and was caused mainly by strains of serogroup B [1]. This situation changed in 1993 when a new meningococcal clone C:2a:P1.2,5, ET-15/37, never found here before, appeared, and caused a new epidemiological and clinical situation [2]. In 1995, an antigenic variant of ET-15/37 strains was detected with the appearance of the B:2a:P1.2,5 phenotype. Nevertheless, its frequency was low in comparison with the serogroup C variant [3].

Herd immunity, which is decisive in the spread of the causative agent of invasive meningococcal disease, was found to be very low against both antigenic variants of the ET-15/37 complex in an immunological survey conducted in 1989 [4, 5] and there was an opportunity for the spread of the ET-15/37 complex in the Czech Republic. The incidence of invasive meningococcal disease increased from 1993 to 1996 [3].

In 1996, a further serological survey of antimeningococcal bactericidal antibodies in the healthy population was conducted to find out how the epidemiological situation had changed, and in particular, whether the spread of a newly emerging ET-15/37 clone expressing either the C:2a:P1.2,5 or the B:2a:P1.2,5 phenotype, was reflected in herd immunity.

MATERIALS AND METHODS

Antimeningococcal immunity

Human sera

Sera collected in the Czech Republic by the National Serum Bank of the Centre of Epidemiology and

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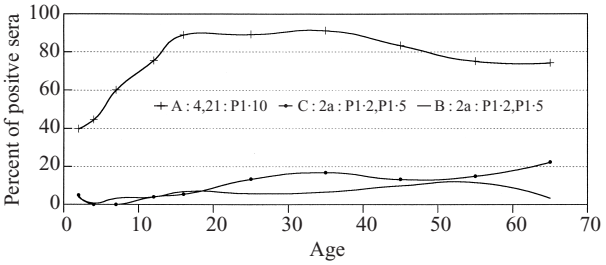

Fig. 1. Meningococcal bactericidal antibodies, Czech Republic, 1989. Healthy population (No. of sera = 770)

Microbiology, National Institute of Public Health (NIPH), were investigated. A human serum sampling strategy for immunological surveys was introduced in 1971 according to the WHO guidelines for Serum Reference Banks [6]. Sera are sampled every year (within 14 days in September) in 10 districts of the Czech Republic. Every year, these are selected at random from the total of 80 districts. In each district, 20 paediatricians or general practitioners are selected at random to sample individuals of varying ages and of either gender to be included in the study. Individuals with known acute respiratory infection, antibiotic treatment or immunodeficiency are excluded [6]. From one district, 750 samples of sera were obtained from all age groups of the population (males and females sampled equally). The totals of the sera investigated in the immunological surveys in 1989 and 1996 were 768 and 982, respectively. The sera were collected in various regions of the Czech Republic (in 1989, three regions; in 1996, four regions). The results of blind testing of the coded sera were submitted to the Department of Statistics of the NIPH for analysis. The percentages of sera testing positive for different antibodies were stratified by age and gender.

Serum bactericidal assay (SBA)

The bactericidal antibody microassay was used [7–9] in our laboratory modification [4, 5]: 50 μl of Brain Heart Infusion (BHI, Oxoid) was dispensed into each well of a sterile 96-well microplate; 50 μl of the serum to be tested (inactivated at 56 °C for 30 min.) was put into the first well, from which twofold dilutions in BHI were performed; 50 μl of living *N. meningitidis* culture in BHI at a working dilution (checked by c.f.u. counting) was added to each well; 50 μl of baby-rabbit serum (source of complement) was placed into each well; except those containing culture and serum controls. Each test included relevant controls (positive, negative, culture, complement, serum). Microplates were covered with lids, put into plastic bags and incubated overnight at 37 °C. After incubation, the results were visualized by adding 50 μl of triphenyltetrazoliumchloride (TTC, Merck) solution, which serves as a germination indicator, changing from colourless to red if the meningococci divide. Microplates with TTC were incubated for 30 min at room temperature and results read with a Dynatech reader. A dilution of 1:6 or more was considered positive.

Antigens

The reference meningococcal strains used for bactericidal tests in l989 and 1996 were: A 1027–A:4, 21:P1.10; B 16B6–B:2a:P1.2,5; C 7606–C:2a:P1.2,5.

N. meningitidis serogroup A and serogroup C are used for the production of the A+C polysaccharide meningococcal vaccine. The non-capsular antigens of the new Czech clone ET-15/37 of *N. meningitidis* (expressing either serogroup B or serogroup C) are identical. The strains B16B6 and C7606 belong to the complex ET-15/37, as confirmed by multi-locus enzyme electrophoresis.

Statistical analysis

The significance of differences in percentages of sera reacting positively in individual age groups were compared using the χ^2 test. The results of the immunological surveys in 1989 and 1996 were compared. The expected percentages of age-stratified positive sera in 1996 were calculated from the percentages of comparable age-stratified positive sera collected in 1989. The significance of differences in proportions were tested by χ^2 test at the level of significance $P \leqslant 0.01$.

Meningococcal carriage data

Two nationwide carriage studies were conducted in collaboration with districts' epidemiologists and microbiologists. Nasopharyngeal swabs of healthy persons of all age groups (in 1996) and those aged up to 24 years (in 1980) were cultured on selective Thayer Martin medium. Serogroups of *N. meningitidis* were identified in the field laboratories. Results of these studies performed in 1989 (i.e. before the ET-15/37 complex emerged in the Czech Republic) and in 1996 (when the second immunological survey was conducted) are presented here.

Table 1. *Antimeningococcal bactericidal antibodies in individual age groups, Czech Republic, 1989 (n = 768)*

Age group (years)	No. of sera	Percentage of positive sera		
		Anti-A:4,21:P1.10	Anti-B:2a:P1.2,5	Anti-C:2a:P1.2,5
0–1	103	39·8	4·0	4·9
2–4	130	44·6	0·8	0
5–9	60	60·0	3·3	0
10–13	49	75·5	4·1	4·1
14–19	72	88·9	6·9	5·5
20–29	119	89·1	5·9	13·4
30–39	57	91·2	6·7	16·9
40–49	60	83·3	10·0	13·3
50–59	60	75·0	11·7	15·0
60+	58	74·1	3·4	22·4

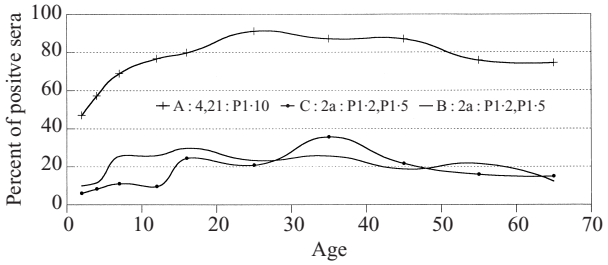

Fig. 2. Meningococcal bactericidal antibodies, Czech Republic, 1996. Healthy population (No. of sera = 982)

Antigenic and genetic characterization of N. meningitidis

Serogroups were determined by slide agglutination with polyclonal antisera to serogroups A, B, C, D, X, Y, Z, W-135 and 29E (Diagnostic Pasteur, Murex). Serotypes and subtypes were determined by whole-cell enzyme-linked immunoassay (WCE) with monoclonal antibody reagents (RIVM, NIBSC) [10]. Genotype analysis for enzyme electrotype (ET) determination was carried out by multi-locus enzyme electrophoresis (MLEE) [11].

RESULTS

Antimeningococcal immunity

Immunological survey in 1989

The percentages of sera testing positively for anti-A:4,21:P1.10 antibodies were significantly higher than for anti-B:2a:P1.2,5 and anti-C:2a:P1.2,5 antibodies. The levels of anti-A:4,21:P1.10 antibodies increased relatively quickly in children (from 39·8% in the age group 0–1 years to 75·5% in the age group 10–13 years) and more than 90% of adult sera tested positive. Bactericidal antibodies against *N. meningitidis* C:2a:Pl.2,5 and B:2a:Pl.2,5 were found in sera of 20% of adults only and children remained almost universally unprotected against this clone (Fig. 1, Table 1).

Immunological survey in 1996

The percentages of sera testing positively for anti-A:4,21:P1.10 antibodies were significantly higher than for anti-B:2a:P1.2,5 and anti-C:2a:P1.2,5 antibodies. The levels of anti-A:4,21:P1.10 antibodies increased relatively swiftly with age in children (from 47·0% in the age group 0–1 years to 76·7% in the age group 10–13 years) and more than 90% of adult sera tested positive. In contrast, the levels of anti-B:2a:P1.2,5 and anti-C:2a:P1.2,5 antibodies increased very slowly in children and adolescents. In the adult population, the sera testing positively did not reach 40% (Fig. 2, Table 2).

There were differences in the age distribution of positive sera. Reactivity against *N. meningitidis* B:2a:P1.2,5 started to increase in the child population, whereas that against *N. meningitidis* C:2a:P1.2,5 was highest in the adolescents and adult population.

Immunological surveys in 1989 and 1996

The results obtained in 1996 were compared with the previous serological survey carried out in 1989, before the emergence of the new meningococcal clone in the

Table 2. *Antimeningococcal bactericidal antibodies in individual age groups, Czech Republic, 1996 (n = 982)*

Age group (years)	No. of sera	Percentage of positive sera		
		Anti-A:4,21:P1.10	Anti-B:2a:P1.2,5	Anti-C:2a:P1.2,5
0–1	100	47·0	10·0	6·0
2–4	180	57·2	11·7	8·3
5–9	90	68·9	25·6	11·1
10–13	73	76·7	26·0	9·6
14–19	94	79·8	29·8	24·5
20–29	158	91·1	23·4	20·9
30–39	70	87·1	25·7	35·7
40–49	69	87·0	18·8	21·7
50–59	74	75·7	21·6	14·9
60+	74	74·3	12·2	14·9

Table 3. *Antimeningococcal bactericidal antibodies, Czech Republic, 1989 vs. 1996 (total percentage of positive sera)*

	1989			1996		
	No. tested	No. positive	% positive	No. tested	No. positive	% positive
Anti-A:4,21:P1.10	768	532	69·3	982	719	73·2
Anti-B:2a:P1.2,5	768	40	5·2	982	194	19·7
Anti-C:2a:P1.2,5	768	67	8·7	982	156	15·9

Table 4. *Antimeningococcal bactericidal antibodies anti-A:4,21:P1.10, Czech Republic, 1989 vs. 1996 (percentage of positive sera in individual age groups)*

Age group (years)	1989 (% of positive)	1996 (% of positive)	χ^2
0–1	39·8	47·0	1·14
2–4	44·6	57·2	3·61
5–9	60·0	68·9	0·69
10–13	75·5	76·7	0·01
14–19	88·9	79·8	0·75
20–29	89·1	91·1	0·05
30–39	91·2	87·1	0·11
40–49	83·3	87·0	0·09
50–59	75·0	75·7	0·00
60+	74·1	74·3	0·00

Table 5. *Antimeningococcal bactericidal antibodies anti-B:2a:P1.2, 5, Czech Republic, 1989 vs. 1996 (percentage of positive sera in individual age groups)*

Age group (years)	1989 (% of positive)	1996 (% of positive)	χ^2
0–1	4·0	10·0	3·71
2–4	0·8	11·7	13·20**
5–9	3·3	25·6	11·66**
10–13	4·1	26·0	9·04**
14–19	6·9	29·8	12·67**
20–29	5·9	23·4	15·57**
30–39	6·7	25·7	8·01**
40–49	10·0	18·8	2·47
50–59	11·7	21·6	2·72
60+	3·4	12·2	3·68

** $P < 0.01$.

Czech Republic (Tables 3–6, Figs 3–5). As in the earlier survey, in 1996 antibodies against *N. meningitidis* A:4,21:P1.10 were found in a high percentage of sera tested, whereas those against *N. meningitidis* B:2a:P1.2,5 and *N. meningitidis* C:2a:P1.2,5 were detected only in a low percentage. The percentages of the sera positive against *N. meningitidis* A:4,21:P1.10 were very similar in 1989 and 1996 (69·3 and 73·2%). In contrast, the percentages of sera positive against *N. meningitidis* B:2a:P1.2,5 and *N. meningitidis* C:2a:P1.2,5 were significantly higher in 1996 than in

Table 6. *Antimeningococcal bactericidal antibodies anti-C:2a:P1.2,5, Czech Republic, 1989 vs. 1996 (percentage of positive sera in individual age groups)*

Age group (years)	1989 (% of positive)	1996 (% of positive)	χ^2
0–1	4·9	6·0	0·21
2–4	0	8·3	10·79**
5–9	0	11·1	6·66**
10–13	4·1	9·6	1·54
14–19	5·5	24·5	10·61**
20–29	13·4	20·9	3·20
30–39	16·9	35·7	5·64
40–49	13·3	21·7	1·95
50–59	15·0	14·9	0·00
60+	22·4	14·9	2·19

** $P < 0.01$.

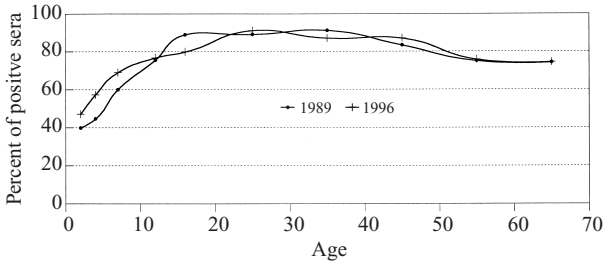

Fig. 3. Bactericidal antibodies – *N. meningitidis* A:4,21:P1.10, Czech Republic, 1989 and 1996.

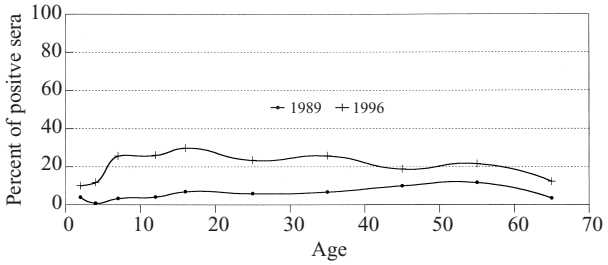

Fig. 4. Bactericidal antibodies – *N. meningitidis* B:2a:P1.2,P1.5, Czech Republic, 1989 and 1996.

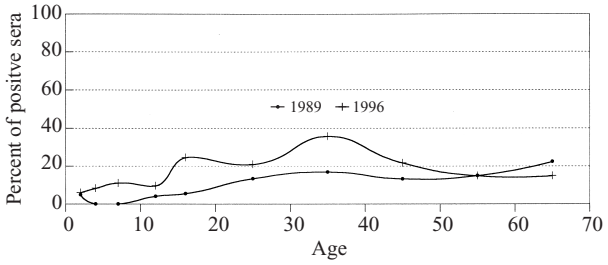

Fig. 5. Bactericidal antibodies – *N. meningitidis* C:2a:P1.2,P1.5, Czech Republic, 1989 and 1996.

1989 (19·7 *vs.* 5·2%, and 15·9 *vs.* 8·7% respectively). This increase in herd immunity is consistent with the spread of the new clone in the Czech Republic. There was no significant difference between the prevalence of antibodies against *N. meningitidis* A:4,21:P1.10 in 1989 and 1996 (Table 4), whereas against *N. meningitidis* B:2a:P1.2,5 and *N. meningitidis* C:2a:P1.2,5 the percentages of reactive sera were significantly higher in 1996 than in 1989 in the age range 3–39 years (Table 5) and 3–19 years (Table 6) respectively ($P < 0.01$).

Meningococcal carriage data

In a 1980 study, 2932 healthy persons were investigated for carriage of *N. meningitidis*; 10.2% of the 0–24 years age group were carriers (Table 7) [1]. The highest percentages of carriage were reached in those aged 20–24 years (21·7%) and 15–19 years (15·4%). Among meningococcal strains in which the serogroup had been identified, those of serogroup B were most prevalent (43·9%), followed by those of serogroup C (15·3%); 33·2% of strains remained non-groupable (predominantly polyagglutinable). In the age group 0–10 years, serogroup B carriers were more frequent (3·8% of the subjects tested), while serogroup C carriers occurred infrequently (0·6% of the subjects tested) (Table 9). Adolescents and young adults, i.e. 15- to 24-year-olds, were more frequent carriers of serogroup B and C than the children's group, with carriage of serogroup B 1·3 times higher (5·1% of subjects tested) and carriage of serogroup C 4·7 times higher (2·8% of subjects tested).

In the 1996 study, 862 healthy persons were tested for meningococcal carriage. The overall positivity rate (all age groups) was 20·1% (Table 8). The highest percentages were in the 20–24 years (36·1%) and 15–19 years (30·3%) age groups. Of meningococcal strains in which serogroup had been identified, there was a higher prevalence of serogroup C than B (45·8 and 35·1%, respectively). Only 10·2% of strains were non-groupable. In the 1–9 age group years serogroup B was more frequent (7·2%) with serogroup C showing a lower occurrence (5·9%) (Table 9). In adolescents and young adults, i.e. those aged 15–24 years, carriers of serogroup B and C were more frequent than in the children's group. Carriage of serogroup B was 1·5 times higher (10·6%) and carriage of serogroup C was 2·5 times higher (14·9%). In 1996, the percentage of serogroup C carriers was higher, when compared with

Table 7. *Healthy carriers of* N. meningitidis *in the Czech Republic, 1980*

Age group (years)	No. of subjects	No. of carriers	% carriers	Serogroup of *N. meningitidis*							
				B	C	Y	Z	29E	W135	NG	nd
0–3	400	30	7·5	15		1				6	8
4–6	508	25	4·9	13	3					3	6
7–10	514	34	6·6	14	4					5	11
11–14	495	25	5·0	7	3	4				9	2
15–19	550	85	15·4	25	16	4	1	1	4	26	8
20–24	465	101	21·7	12	4					16	69
Total	2932	300	10·2	86	30	9	1	1	4	65	104
			Per cent …	43·9	15·3	4·6	0·5	0·5	2·0	33·2	

NG, non-groupable.
nd, not done.

Table 8. *Healthy carriers of* N. meningitidis *in the Czech Republic, 1996*

Age group (years)	No. of subjects	No. of carriers	% carriers	Serogroup of *N. meningitidis*						
				B	C	X	Y	29E	NG	nd
1–4	59	14	23·7	8	4				2	
5–9	93	10	10·7	3	5				2	
10–14	46	5	10·9	1	2				1	1
15–19	231	70	30·3	24	33		3	2	8	
20–24	119	43	36·1	13	19	2	6	2	1	
25–34	122	18	14·7	6	9				2	1
35–44	70	6	8·6	3	3					
45–54	76	5	6·6	1	1				1	2
55–64	25	2	8·0		1					1
65+	21	0	0							
Total	862	173	20·1	59	77	2	9	4	17	5
			Per cent …	35·1	45·8	1·2	5·3	2·4	10·2	

NG, non-groupable.
nd, not done.

Table 9. *Influence of age on prevalence of serogroups of* N. meningitidis *among healthy carriers, Czech Republic, 1980 and 1996*

Carrier study in 1980						Carrier study in 1996					
Age group (years)	No. of subjects	Serogroup B carriers*		Serogroup C carriers*		Age group (years)	No. of subjects	Serogroup B carriers		Serogroup C carriers	
		No.	%	No.	%			No.	%	No.	%
0–10	1422	54	3·8	9	0·6	1–9	152	11	7·2	9	5·9
15–24	1015	52	5·1	28	2·8	15–24	350	37	10·6	52	14·9

* The numbers of expected serogroup B and C carriers were calculated from those of 'nd' in Table 7, to be added to the serogroup B and C carriers found.

1980, consistent with the emergence of ET-15/37 complex in the Czech Republic.

DISCUSSION

A modification of the serum bactericidal assay (SBA) was used in our study. Our participation in a CDC multilaboratory study [9] allowed us to validate this modification to the standardized method recommended by the CDC. Methodological differences were discussed (WHO Workshop – Atlanta 1992, Obernai 1993); it was agreed that they did not influence the results substantially. The results obtained using TTC as a germination indicator are comparable with those obtained by colony counting. Our modification of the SBA involves use of baby rabbit complement for serogroup B. In our laboratory, the SBA with both baby rabbit and agammaglobulinaemic complements gave statistically comparable results. However, in this study the percentages of positive sera (i.e. reaction at a dilution 1:6 or more), not the geometric mean were compared. We do not experience problems with either high levels of anti-serogroup B antibodies or a high percentage of sera with anti-serogroup B positivity.

The increase in herd immunity against *N. meningitidis* B:2a:P1.2,5 was higher than against *N. meningitidis* C:2a:P1.2,5, in contrast with the lower frequency of strains belonging to the serogroup B variant of the ET-15/37 clone. There is evidence that for serogroup B meningococci, bactericidal antibodies are elicited by non-capsular rather than capsular antigens. This could be of interest in development of a new meningococcal vaccine based on non-capsular antigens and is consistent with our previous findings [5].

Differences were found in the age distribution of the positive sera. Sera positive against *N. meningitidis* B:2a:P1.2,5 increased in the child population whereas sera reactive with *N. meningitidis* C:2a:P1.2,5 increased in the adult population. These age variations are consistent with the observed epidemiology of the two phenotypic variants of the ET-15/37 clone in the Czech Republic. *N. meningitidis* B:2a:P1.2,5 is more frequent in the child population, whereas *N. meningitidis* C:2a:P1.2,5 is more frequent in adolescents.

The increase in herd immunity against *N. meningitidis* B:2a:P1.2,5 and *N. meningitidis* C:2a:P1.2,5 may be caused by asymptomatic carriage, since the methodology of immunological surveys would have identified individuals with a history of invasive meningococcal disease and no such individual was found in the study. Increased antimeningococcal immunity after asymptomatic carriage was found in our previous study and has been noted in other countries [12, 13].

N. meningitidis strains with the phenotype C:2a:P1.2,5 or B:2a:P1.2,5 that were isolated from cases of invasive meningococcal disease or from healthy carriers showing were characterised by MLEE in our laboratory. All belonged to the ET-15/37 complex [2, 3]. These strains have begun to be more heterogeneous but they still belong to ET-15/37 complex as confirmed by MLEE.

Recently, the heterogeneity of Czech strains of ET-15/37 complex has been studied by *pilA* and *pilD* polymorphism analyses and genetic exchanges between epidemic and endemic strains, involving mostly the surface structures, have been observed [14].

A high percentage of sera testing positive against *N. meningitidis* A:4,21:P1.10 contrasts with a very low frequency of serogroup A meningococcal carriage and disease in the Czech Republic. These antibodies could be elicited by non-capsular antigens, i.e. serotypes 4, 21 and subtype P1.10, which are frequent in this country (10.8% of carriage strains typed). Alternatively, a high percentage of sera testing positive against *N. meningitidis* A:4,21:P1.10 could be due to cross-reactivity with other bacterial species.

A strategy of targeted vaccination was adopted because of the serious epidemiological and clinical situation in the country caused by the ET-15/37 clone [15]. Nevertheless, this targeted vaccination has not influenced herd immunity (unpublished data) because it was conducted on a small scale. Herd immunity may continue to increase, finally play a role in terminating the spread of the *N. meningitidis* ET-15/37 clone in the Czech Republic.

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