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Seroprevalence of *Borrelia* IgG antibodies among young Swedish children in relation to reported tick bites, symptoms and previous treatment for Lyme borreliosis: a population-based survey

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ABSTRACT

Background Lyme borreliosis (LB) is the most common tickborne infection in Sweden and the seroprevalence of *Borrelia* immunoglobulin G (IgG) antibodies varies between 2% and 26%. The seroprevalence in young Swedish children is unknown and the relation to clinical data has not been previously studied.

Objective To determine the seroprevalence of *Borrelia* IgG antibodies in serum of young Swedish children and to relate it to gender, geographical location, reported tick bites, symptoms and previous treatment for LB.

Methods 2000 healthy 5-year-old children (n=2000) were randomly selected from among participants of a larger prospective population-based study, the ABIS (All Babies in Southeast Sweden) study. Serum samples were collected and a *Borrelia* specific ELISA test (Dako) were performed for IgG antibody detection. Clinical data were collected from questionnaires completed by the parents.

Results The seroprevalence of *Borrelia* IgG antibodies was 3.2% (64/2000). Previous tick bite had been noted in 66% of these seropositive children but the majority (94%) had not previously been treated for LB. In addition, another 55 children reported a history of LB but were negative to *Borrelia* IgG antibodies in serum. Many of these seronegative children had received treatment for erythema migrans (n=24), which is a clinical diagnosis. Whether children were correctly treated or overtreated for LB is however unknown. No differences in gender, geographical location or reported tick bites were found when comparing *Borrelia*-seropositive children (n=64) and seronegative children with previous LB (n=55).

Conclusion This population-based study demonstrates a *Borrelia* IgG antibody seroprevalence of 3.2% in young Swedish children. Very few of these seropositive children report previous symptoms or treatment for LB. Thus the findings suggest that exposure to the *Borrelia* spirochaete (with subsequent antibody response in serum) does occur in young children, mostly without giving rise to clinical LB. Future studies on cell-mediated immune responses are needed to investigate explanatory immunological mechanisms.

What is already known about this topic

- Lyme borreliosis is the most common tickborne infection in children in Sweden.
- The seroprevalence of *Borrelia* immunoglobulin G (IgG) antibodies varies between 2% and 26% among adults, but the seroprevalence in young Swedish children is unknown.

What this study adds

- This population-based study demonstrates a *Borrelia* IgG antibody seroprevalence of 3.2% in young Swedish children. Very few of these children report a history of Lyme borreliosis (LB).
- Our findings suggest that exposure to the *Borrelia* spirochaete (with subsequent antibody response in serum) does occur among young children, mostly without giving rise to clinical LB.

INTRODUCTION

Lyme borreliosis (LB) is the most common tickborne infection in Sweden. The infectious agent is the spirochaete *Borrelia burgdorferi sensu lato*, of which four subspecies are known to be pathogenic to humans: *Borrelia afzelii, Borrelia garinii, Borrelia burgdorferi sensu stricto* and *Borrelia spielmanii*.[1] The infection may give rise to many different symptoms by affecting different organs such as the skin, joints, heart muscle or nervous system. The most common manifestation of LB is the skin lesion erythema migrans (EM). This presents as a circular red skin lesion 1–2 weeks after a tick bite. EM can resolve spontaneously but is normally treated with antibiotics. The risk of contracting LB from a tick bite has been estimated at 1/221 (0.5%) in southern Sweden[2] and many tick bites remain unnoticed.[3]

In southern Sweden, the overall annual incidence of LB is reported to be 69 cases per 100<TS thin space>000 inhabitants (range 26–160/100<TS thin space>000) with a peak in childhood at 5–9 years of age and in the older people at 60–74 years of age.[4] In Europe and the USA a similar incidence has been reported with the highest rates in the coastal areas of the Baltic Sea and along the north-eastern coast of the USA.[5–7]

The population-based seroprevalence of *Borrelia* immunoglobulin G (IgG) antibodies among adults varies between 2% and 26% in Scandinavia.[8–10] There is a female dominance, a clear increase with

age, and the highest rates occur in high endemic regions (up to 53% at >70 years of age).[10] The seroprevalence in children (0–13 years) has been reported to be 2.6% in Lower Saxony in Germany[5] and 15% in Slovenia (children ≤5 years of age were not included).[11] In a high incidence endemic area in Sweden, only 4–7% of the *Borrelia* seropositive inhabitants (mainly adults) reported previous antibiotic treatment for LB,[8] suggesting that asymptomatic LB occurs to a great extent among adults and that elevated antibody titres can remain after antibiotic treatment. The population-based *Borrelia* seroprevalence among young Swedish children in relation to clinical features of LB has not been previously studied.

The aim of this study was to determine the seroprevalence of *Borrelia* IgG antibodies in serum of young Swedish children and to relate it to gender, geographical location, reported tick bites, symptoms and previous treatment for LB.

MATERIAL AND METHODS

Sample

From a population of 21<TS thin space>700 children born between 1 October 1997 and 1 October 1999 in southeast Sweden, 17<TS thin space>055 children (79%) agreed to participate in the prospective ABIS study (All Babies in Southeast Sweden), a study with the main purpose of finding risk factors for type 1 diabetes and other immune-mediated diseases.[12] Children were prospectively followed from birth. At the 5-year check-up, 7356 families completed questionnaire and serum sampling of the child. These serum samples were drawn throughout the years 2002–2004, in all seasons and as close as possible to the child's fifth birthday. All geographical areas in southeast Sweden were represented. Two thousand (n=2000) children were randomly selected (ie, including every third to fourth sample arriving at the laboratory throughout the collection period). Baseline data have previously been studied for the participating families at the 5-year follow-up (n=7356) compared to the initial cohort (n=17<TS thin space>055), and the material remains representative.[12] Our randomly selected 2000 children are therefore considered representative of the population of 5-year-old children in southeast Sweden.

Informed consent was obtained from all families participating in the ABIS study. The study was approved by the regional ethical committee at the Faculty of Health Sciences, Linköping, Sweden (Dnr 02-042).

Questionnaire

The 5-year questionnaire was completed by 7356 families, from which 2000 children took part in our *Borrelia* seroprevalence study as described above. Information on gender, geographical location, history of tick bites, symptoms and previous treatment for LB were collected from the questionnaires.

Laboratory method

All serum samples (n=2000) were screened for *Borrelia* IgG antibodies. A commercial ELISA kit, based on the *Borrelia* specific protein flagellin, was used according to the manufacturer's instructions (IDEA *Borrelia burgdorferi* IgG kit; DakoCytomation, Glostrup, Denmark and Oxoid, Hampshire, UK).[13] The method has a good diagnostic performance[14] and is used for routine serology of LB in Sweden.[15] However, antibody levels are low in early LB (ie, patients with EM) and serology is not recommended owing to low sensitivity.[14 16] Furthermore, the current ELISA method is not 100% specific but the best among different assays tested.[14 15] The ELISA test was considered positive if the optic density value exceeded the cut-off value given by the manufacturer. Borderline cases were not included. Subject specimens were analysed as single samples.

Statistics

The software SPSS (Version 15.0) was used for statistical calculations. The non-parametric tests (Kruskal-Wallis and Mann-Whitney U test) were used for comparing continuous data between groups. χ^2 and Fisher's exact test were used for non-continuous data. Level of significance was $p<0.05$.

RESULTS

Out of 2000 children in the study, 1881 reported no history of LB and had no *Borrelia* IgG antibodies in serum (table 1). However, positive *Borrelia* IgG antibody titres were found in 64 out of 2000 children, corresponding to a seroprevalence of 3.2%. Among these seropositive children, sex distribution was equal and 36% lived in rural areas (table 2, group A). One or several tick bites had been noticed during the previous 2.5 years in the majority of children (66%) and all parts of the body were affected including the head and neck region. The vast majority (94%) reported no known EM, arthritis, meningitis or other neurological symptoms and had not previously been treated for LB. Four of the *Borrelia*-seropositive children (7%) reported previous treatment for LB (EM n=2, facial nerve palsy n=1, Lyme meningitis n=1) (table 2, group A).

Another 55 children reported a history of LB but were negative for *Borrelia* IgG antibodies in serum (table 1). Sex distribution was equal and 25% lived in rural areas (table 2, group B). Tick bites were reported by 73% and they were distributed equally over the body. Reported symptoms of LB are shown in table 2, group B. In cases with 'unspecified symptoms' (n=16), no closer description of the child's symptoms were given by the parents in the questionnaire.

In order to show differences and/or similarities between the *Borrelia* IgG-seropositive children (group A) and the *Borrelia* IgG-seronegative children with reported previous LB (group B), results with p values are shown in table 2. No differences between the groups were found concerning gender, geographical location, number or localisation of tick bites. Differences in symptoms and treatment for LB are shown in table 2.

Of the remaining 1881 children in the study population (*Borrelia* IgG-seronegative children with no history of LB) 52% were male, 35% lived in rural areas and 52% had noted a previous tick bite. They are not described in more detail since this was not the aim of the study.

DISCUSSION

The seroprevalence of *Borrelia* IgG antibodies in young Swedish children has hitherto been unknown. In this population-based study, serum samples from 2000 healthy 5-year-old children

Table 1 History of Lyme borreliosis and occurrence of *Borrelia* IgG antibodies in sera* of 2000 young Swedish children

	Borrelia IgG antibodies*	
History of LB	+	–
Yes	4	55
No	60	1881

*ELISA (Dako) kit, based on the *Borrelia*-specific flagella antigen.[13]
IgG, immunoglobulin G; LB, Lyme borreliosis.

Table 2 Comparison between *Borrelia* IgG seropositive (group A) and seronegative children with previous Lyme borreliosis (group B)

	Group A (seropositive children) (n=64)	Group B (seronegative children with previous LB) (n=55)	
	No (%)	No (%)	p Value
Male	35 (55)	29 (53)	NS
Rural living	23 (36)	14 (25)	NS
Tick bite, one or more†	42 (66)	40 (73)	NS
Head and neck	12 (19)	15 (27)	NS
Upper body and arms	22 (34)	20 (36)	NS
Lower body and legs	18 (28)	16 (29)	NS
Previous LB, self-reported	4 (7)	55 (100)	<0.001
EM	2 (3)	24 (43)	<0.001
Facial nerve palsy	1 (2)	9 (16)	<0.01
Meningitis	1 (2)	4 (7)	<0.05
Affected joint	0 (0)	3 (5)	<0.05
Unspecified symptoms	0 (0)	16 (29)	<0.001
No previous LB	60 (94)	0 (0)	<0.001
Borrelia IgG antibodies in serum*	64 (100)	0 (0)	<0.001
Antibiotic treatment for LB	4 (7)	55 (100)	<0.001

Results are shown as number (No) and percentage (%).
*ELISA (Dako) kit, based on the *Borrelia* specific flagella antigen.[13]
†Several patients reported tick bites on more than one part of the body (n=24).
EM, erythema migrans; IgG, immunoglobulin G; LB, Lyme borreliosis; NS, non-significant.

living in southeast Sweden showed a seroprevalence of 3.2%. This can be compared to similar data in a German population-based study in which *Borrelia* IgG antibodies were found in 2.6% of children[5] and to a Belgium study that reported a seroprevalence of 2%.[17] In Sweden, among healthy adult blood donors living in Linköping (an inland municipality in the same region as our study),[18] the seroprevalence was higher (5.8%) but comparable to our results considering the age differences. A good knowledge of the seroprevalence in a specific population is crucial for understanding the predictive value of diagnostic tests and we believe our results can be useful when testing young Swedish children with non-specific symptoms and suspected LB.

Since our questionnaire data were based on self-reported symptoms and previous treatment for LB, details on diagnostic criteria are not available. Consequently, we have not described our data as LB prevalence or incidence figures. However, our population-based study, including both serum samples and questionnaires, gives us the opportunity to put the seroprevalence data into a clinical perspective. This is a clear advantage and enables us to discuss demographic data, symptoms of LB and *Borrelia* IgG antibody serum reactivity in relation to each other.

Sixty-four children (n=64) had raised *Borrelia* IgG antibody titres in their sera but very few (n=4) reported symptoms or previous treatment for LB (tables 1 and 2). Admittedly, one cannot fully exclude the possibility that a number of the seropositive children may have false positive antibody results. This is, however, less likely since the flagellin ELISA has previously been shown to have high specificity and the best performance among different serology assays.[14 15]

Our finding that a large number of *Borrelia* IgG-seronegative children (n=55) reported symptoms or previous treatment for LB (group B) in the questionnaire is interesting. The major reason is probably the low sensitivity of the testing method.[14] In addition, many children had EM (n=24), which is a clinical diagnosis and laboratory investigation is normally not recommended owing to low antibody titres in early LB.[16] Furthermore, facial nerve palsy (n=9) is a manifestation of early disseminated LB and antibody production may have been delayed.[19] Children with 'unspecified LB' (n=16) could have had other conditions and received treatment for LB on empirical grounds. Whether patients were correctly treated or overtreated for LB cannot be clarified in this study.

Our findings in this population-based study suggest that exposure to the *Borrelia* spirochaete (with subsequent antibody response in serum) does occur among young children, mostly without giving rise to clinical LB. This suggestion is based on data from questionnaires where parents reported no symptoms or previous treatment for LB, in combination with raised titres of *Borrelia* IgG antibodies in serum. Tick bites were reported in the majority of cases (66%). We believe our data are solid and that it is an important finding. However, *why* some individuals get an antibody response when being exposed to the *Borrelia* spirochaete without developing symptoms of LB is not clear.

Gender, geographical location, number or localisation of tick bites did not differ when comparing *Borrelia*-seropositive (group A) and *Borrelia*-seronegative children with previous LB (group B) in our study. The pathogenic properties or the inserted amount of the *Borrelia* spirochaetes might be of importance or possibly the properties of the elicited immune systems in the host.[18] An earlier study showed that both adult asymptomatic *Borrelia*-seropositive individuals and adult patients with LB have a similar *Borrelia*-specific interferon-γ response in peripheral blood mononuclear cells.[20] This finding cannot explain why some individuals have an antibody response when being exposed to the *Borrelia* spirochaete without developing symptoms of LB and why some individuals get clinical LB. However, children are known to have a Th2-skewed immunity and a different inflammatory response to LB compared to adults.[21] Indeed, children with LB showed a stronger interleukin-4 response to *Borrelia* than adults with LB,[21] suggesting that the quality of the cell-mediated response is of importance. Thus further studies of the cell-mediated immune response in children and adults with LB are desirable to investigate this issue.

CONCLUSION

This population-based study demonstrates a *Borrelia* IgG antibody seroprevalence of 3.2% in young Swedish children. Very few of these children reported symptoms or previous treatment for LB. Our findings suggest that exposure to the *Borrelia* spirochaete (with subsequent antibody response) does occur among young children, mostly without giving rise to clinical LB. Future studies on cell-mediated immune responses are needed to investigate explanatory immunological mechanisms.

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Competing interests None.

Ethics approval This study was conducted with the approval of the regional ethics committee at the Faculty of Health Sciences, Linköping, Sweden (Dnr 02-042).

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