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Invited review

Recent trends in research on congenital toxoplasmosis ☆

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1. The european research network on congenital toxoplasmosis

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The start of the European Research Network on Congenital Toxoplasmosis was a seminar in Copenhagen on 17 January 1992, with more than 100 participants from Europe and the United States (Lebech and Petersen, 1992). It was realised that there were many aspects of congenital infection with *Toxoplasma gondii* that were not known, or where the existing data dated back more than 30 years. The seminar resulted in an application to the European Union for funding, and the network received funding for a concerted action in the autumn of 1992, officially commencing operation on January 1st 1993. From the beginning the idea was to create a network open to individuals interested in all aspects of congenital toxoplasmosis, who were interested in contributing to research projects which would create good quality data, on the basis of which, sound decisions on case definition, prevention, screening, diagnosis and treatment could be taken.

The first meeting took place in Grenoble and working groups were established on serological and non-serological diagnostic methods, ophthalmology, epidemiology, quality control and quality assurance, treatment, and health education. Later meetings took place in Bonn, Warsaw and a final open congress on congenital toxoplasmosis was held in Vienna in June 2000. The submissions making up this review are the highlights of the Vienna meeting.

Some working groups have been more successful than others. It proved particularly difficult to provide common guidelines on health education, for instance, although a review of preventive measures in different European countries was made (Ho-Yen et al., 1995). From the start the working group on epidemiology concentrated on studies of risk factors of infection with a study from Naples, Italy (Buffolano et al., 1996). Later a European multicentre study showed that risk factors vary from area to area, but that overall, infection from meat can explain approximately two-thirds of all infections (Gilbert et al., 2000). This was the first study from Europe that actually provided sound data on the proportion of infections transmitted through meat and is important when future strategies for prevention are decided.

The serological working group started with an ambitious project comparing 17 different diagnostic assays for *T. gondii*-specific IgG-, IgM- and IgA-antibodies. The working group collected sera from the centres where the time of seroconversion and thus the time of infection was known and was able to establish a bank of sera where the date of infection was known within weeks. Using these well-characterised sera, it was possible to show that no method, either single or in combination, could predict the time of infection within the first year after infection. The study is in still in the process of being published.

A second study compared Western blot with ELIFA for the diagnosis of prenatal infection in newborns without detectable *T. gondii*-specific IgM- or IgA-antibodies at birth. The study again took advantage of collecting well-characterised sera from newborns who were born to mothers with *Toxoplasma*-infection during pregnancy and who were followed-up to 1 year of age. The results of the study are still in the process of being published. A retrospective multicentre study in newborns born to mothers treated during pregnancy showed that the diagnostic sensitivity of *T. gondii*-specific IgM- and IgA-antibodies varied between 41 and 66%. However, it should be noted that the results can not be extended to children born to mothers not treated during pregnancy (Naessens et al., 1999).

The working group on standardisation and quality control was very concerned with the quality of different diagnostic tests and performed two large quality assurance studies where test sera kindly provided by the French National

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Quality Assurance Programme were sent to more than 200 European laboratories (Petithory et al., 1996). Another study looked at the Sabin–Feldmann dye test (SFDT), which is still performed in a few laboratories. The SFDT still has its place in acute diagnosis as a supplement to other tests (Reiter-Owona et al., 1999).

The working group on non-serological diagnostic methods rapidly decided to try and evaluate the newly developed direct detection of *T. gondii* DNA by the PCR. This new method was performed in many laboratories but no external quality control scheme existed, protocols varied widely, and there was great uncertainty on how reliable and reproducible the results were. The working group managed to organise two European multicentre studies which beyond doubt showed a wide variation in the quality of different PCR assays and helped focus on the quality and the need to have a certain number of tests in the laboratory to maintain expertise (Guy et al., 1996; Pelloux et al., 1998).

Another group discussed a common case definition and agreed on guidelines classifying congenital infections into certain, probable, possible and no infection. The guidelines provide case definitions which could be used to standardise data from different studies to allow comparisons (Lebech et al., 1996).

The ophthalmology working group started working on the protocol for a blinded study of retinal changes with the aim of providing data on the proficiency of clinical diagnosis. However, the protocol proved very difficult in practical terms and the study was eventually abandoned. A planned randomised study comparing traditional daily treatment with sulfadiazine and pyrimethamine with weekly sulfadoxine and pyrimethamine (Fansidar®) also had to be abandoned due to lack of support from the manufacturers of the drugs.

Through the years it became more and more apparent that the data from the 1950s and 1960s on transmission and clinical severity may not be valid any longer. A retrospective pilot study had shown that treatment during pregnancy may reduce clinical symptoms in congenitally infected children (Foulon et al., 1999), but it was realised that new data from a prospective study on the natural history of infection and the long term consequences in congenitally infected children were much needed.

The working group on the epidemiology of congenital toxoplasmosis has performed several projects looking at existing databases in European centres. One particular large study was a retrospective evaluation of all infected women from Lyon, France, which provided precise data on transmission and clinical symptoms in children with congenital toxoplasmosis related to gestational age at infection (Dunn et al., 1999). A collaborative study with the European study group working with HIV infection in pregnancy showed that congenital toxoplasmosis was not a problem in HIV-positive pregnant mothers (Dunn et al., 1996).

The most ambitious project of the network started in 1996 with the planning of the European Multicentre Study on Congenital Toxoplasmosis (EMSCOT), coordinated from the Institute of Child Health in London. The study started in 1997 and recruitment ended in 2000 when more than 1300 infected women and their newborns from more than 14 European centres had been recruited into the study. The study now focuses on the follow-up of 3-year-old children in particular with regard to new cases of retinochoroiditis despite treatment and neurological symptoms. A further follow-up at 6 years of age is also being prepared based on the hypothesis that slight damage in infected children may not be apparent before school age.

2. Neonatal screening for congenital toxoplasmosis

2.1. Early evaluation of the postnatal treatment efficacy in infants with congenital toxoplasmosis from West Poland

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2.1.1. Introduction

Infection with *T. gondii* acquired during pregnancy may cause intrauterine damage and sequelae in the newborn. Up to 87% of congenitally infected infants are asymptomatic at birth (Foulon et al., 1999), and the infection may pass unnoticed until childhood or adolescence, when ocular lesions and neurodevelopmental disorders may develop (Foulon et al., 1999; Dunn et al., 1999; Wilson et al., 1980; Koppe et al., 1986). Anti-parasitic chemotherapy is believed to reduce the risk of sequelae and should be initiated as soon as the infection is diagnosed, but the impact of treatment during pregnancy on materno-foetal transmission is still controversial (Foulon et al., 1999; Wallon et al., 1999; Couvreur et al., 1993; Stray-Pedersen, 1992).

In Poland, like in most European centres, we currently use a combination of pyrimethamine with sulfadiazine alternating with spiramycin for 12 months. Other centres, particularly in France, prefer a continuous therapy combining pyrimethamine and sulphadoxine (Fansidar®) for 2 years (Villena et al., 1998). In Poland, in the absence of nationwide screening of pregnant women, the early postnatal treatment of all newborns with congenital toxoplasmosis was performed in Poznan since 1996, when the regional screening programme detecting *Toxoplasma*-specific IgM antibody at birth was introduced (Paul et al., 2000).

The aim of the present study has been to evaluate the effectiveness of serological screening in newborns followed

by postnatal anti-parasitic therapy for the prevention of clinical and immunological recurrences in patients with confirmed congenital *T. gondii* infection in early infancy.

2.1.2. Materials and methods

Patients: Fifteen infected infants (eight males and seven females, all singletons) born before November 1998 were included. Thirteen of 15 patients were diagnosed during the first days of life by detection of anti-*Toxoplasma* IgM from Guthrie cards eluates; two remaining infants from the study area, with no positive specific IgM ELISA at birth were recognised only by the typical triad of clinical signs: hydrocephalus, intracranial calcifications, chorioretinitis, and later confirmed serologically. The study was approved by the University of Medical Sciences Ethical Council (Poznan, Poland).

Clinical investigations: The children were systematically evaluated by the multidisciplinary group consisting of a neonatologist, an infectious disease specialist, an ophthalmologist, a paediatric radiologist and an immunologist. Symptomatic cases were additionally surveyed by a paediatric neurologist, managed under the rigorous care of a physical therapist and/or an audiologist. The paediatric follow-up, including an assessment of psychomotor and growth development with a routine neurological examination was performed soon after birth and again every 2 months during the first year of life, and then every 4 months after anti-parasitic therapy was discontinued.

At each paediatric visit, a blood sample was taken for specific antibody measurement. Transfontanel ultrasound of the brain was repeated, when feasible, every 2 months for 1 year. The general ophthalmic assessment, followed by direct and indirect ophthalmoscopy after pupil dilatation, was usually done before 4 weeks of age and repeated every 3 months, and at each immunological rebound. Hearing investigation was performed during the neonatal period by the Echo-Screen apparatus (Fischer–Zoth GmbH) as well as cranial radiography done in the lateral and sagittal projections. The imaging investigation was completed by computed tomography scan near 12 months of age.

Laboratory diagnosis: The non-commercial immunocapture ELISA, adapted for testing of specific IgM in dried blood neonatal samples collected at birth has been previously described (Lebech et al., 1999; Cazenave and Bessières, 1992). When the screening result was positive, the suspected infection was verified by a mother and child comparative immunological profile analysis (CIP-WB) using the Western blot assay (LDBIO Diagnostics) and conventional serology by ELISA for anti-*Toxoplasma* IgG, IgM and IgA antibodies (PLATELIA TOXO, Sanofi Diagnostics Pasteur, VIDAS TOXO, bioMérieux), and Immunosorbent Agglutination Assay (ISAGA) for IgM and IgA (ISAGA PLUS IgA/IgM, bioMérieux), according to the manufacturer's standard procedures.

Age of maternal primary infections during pregnancy was estimated on the basis of the kinetics of specific IgG, IgM and IgA antibodies by different serological techniques performed in the post partum period (Guerina et al., 1994).

Treatment management: Children with subclinical infection or non-specific signs at birth were treated with pyrimethamine (1 mg/kg body weight per day) plus sulfadiazine (100 mg/kg body weight per day) completed with folinic acid (5 mg three times per week) for 4 weeks with spiramycin (375 000 IU/kg body weight per day) for 4 weeks. For infants with clinical toxoplasmosis, the continuous therapy with pyrimethamine, sulfadiazine and folinic acid (Lederfolat) was prescribed for 12–24 months, according to the severity of infection and immunological status of the patient. Leucocyte and thrombocyte counts were controlled twice per month during the periods of the pyrimethamine/sulfadiazine administration. Infants with neurological abnormalities were managed by regular rehabilitation therapy using Voyta's or Balott's methods.

2.1.3. Results

In June 1996, the regional screening programme for congenital *T. gondii* infection was developed for all neonates from the Grand Poland Province. During the first 28 months of the study period, 27 516 liveborn infants were tested for anti-*Toxoplasma* IgM using peripheral blood spotted on neonatal Guthrie cards (Lebech et al., 1999). Fifteen children born with congenital toxoplasmosis in the study area, gave an incidence of one per 1834 live births (0.55 per 1000), which is relatively higher than other countries conducting similar preventive management of newborns (Guerina et al., 1994; Malm et al., 1999; Pawlowski et al., 1994). A seroprevalence of *Toxoplasma* antibodies in pregnant women in Poland of 58.9% in the early 1990s (Couvreur et al., 1984) and 44% in 1998–2000 is also elevated. Therefore, passively transmitted antibodies make the confirmation of neonatal screening results more difficult.

Clinical pattern of infection: Eleven of 15 children with congenital *T. gondii* infection were followed-up clinically and serologically between 25 and 47 months (mean 34.6 ± 8.4 months). The remaining four children were lost for follow-up between the age of 3 and 16 months. Two families (patient nos. 3 and 5) refused treatment and/or further follow-up. Two postnatal deaths occurred at 3 and 12 months, respectively; one of severe staphylococcal sepsis (patient no. 13), and one (patient no. 6) from respiratory insufficiency (Table 1).

There were 11 children born at term (73.3%), and four premature babies delivered between the 32nd and 35th week of pregnancy (patients nos. 3, 6, 14 and 15). The birthweight ranged from 1560 to 3920 g (mean 2983 ± 776 g), and the head circumference of neonates measured from 29 to 38 cm (mean 32.9 ± 3.0 cm) (Table 2).

One of 13 infected newborns (patient no. 6) identified by serological screening developed hydrocephalus, brain calci-

Table 1
Clinical expression of congenital *Toxoplasma gondii* infection in 15 infants during the follow-up period[a]

Patient no.	Age of the last follow-up months	Signs or symptoms recognised after the neonatal period
1	47	Transitory muscular hypertonia by 3 months
2	44	Asymptomatic
3	13	Moderate ventricular enlargement without neurodevelopmental complications
4	41	Anaemia, slight dilatation of the both lateral ventricles without neurological impairment
5	16	Asymptomatic
6	12	Progressing hydrocephalus, ventriculoperitoneal shunt, cerebral atrophy, multiple intracranial calcifications, seizures, muscular hypotonia, unilateral microphthalmia and cataract with blindness (left eye), severe developmental delay, death from respiratory insufficiency at 1 year[b]
7	32	Asymptomatic
8	28	Asymptomatic
9	28	Asymptomatic
10	27	Asymptomatic
11	26	Asymptomatic
12	25	Asymptomatic
13	3	Dilatation of sagittal suture and anterior fontanel, death of severe staphylococcal sepsis at 3 months
14	40	Severe hydrocephalus at the 6th week of life, ventriculoperitoneal shunt, multiple intracranial calcifications, unilateral and peripheral retinochoroidal scar (right eye), strabismus convergent of the right eye, slight asymmetry of eyes size, suspicious changes in EEG
15	43	Unilateral and peripheral retinochoroidal scar (left eye), multiple intracranial calcifications, moderate dilatation of the both lateral ventricles, anaemia, no neurological impairment

[a] Cases 1–13: Identified solely by the neonatal screening programme detecting *Toxoplasma*-specific IgM in filter-paper blood specimens; cases 14 and 15: recognised from clinical signs and confirmed by serology.
[b] This patient had abnormal results of foetal ultrasonography at 27 weeks of gestation (intracranial calcifications, hypotrophy) but was not treated in utero.

fications, microphthalmia and hepatosplenomegaly and five neonates had non-specific clinical abnormalities at birth, undetectable later in life, resulting from prematurity or adaptative respiratory disorders. In one infant (patient no. 4) previously classified as asymptomatic at birth, some additional clinical signs for intrauterine infection became obvious after the neonatal period (anaemia at 2 months and slight dilatation of lateral ventricles at 12 months). The remaining six IgM-positive children had no signs or symptoms of *Toxoplasma* infection, especially no signs of vision impairment due to retinochoroiditis.

Two infants (patient nos. 14 and 15) with a lack of specific IgM ELISA at birth and probably infected in the early intrauterine period demonstrated a typical triad of clinical signs recognised during the first year of life. One of them necessitated an introduction of ventriculoperitoneal shunt due to a suddenly increasing ventricular size at 6 weeks (normal at birth) and its revision at 11 months of age. Although only two episodes of febrile seizures occurred, the infant received periodic cover of anti-convulsion drugs because of suspicious changes in the EEG. His psychomotor development investigated with the Infants Developmental Scale of the National Institute of Mother and Child was normal at 3 years of age. Generally the children demonstrated substantial symptoms or signs, if the maternal infection took place in the first half of pregnancy (patients no. 6, 14 and 15). During the follow-up period, no clinical relapses were reported through detailed paediatric, ophthalmic and imaging surveillance (Table 1). Signs of the classic triad were found in five cases (33.3%): (i) one case of progressing hydrocephalus and cerebral atrophy, blindness in one eye, multiple intracranial calcifications and profound neurological disorders, leading to death at the age of 1 year; (ii) one case of hydrocephalus, brain calcifications, unilateral chorioretinal scar without visual impairment; (iii) one case of moderate dilatation of ventricular system without hydrocephalus, brain calcifications, unilateral chorioretinal scar without vision loss; (iv) two cases of isolated slight enlargement of lateral ventricles without any symptoms of hydrocephalus or neurological dysfunction (Table 3).

Four patients exhibited some congenital malformations such as unilateral microphthalmia with cataract (patient no. 6), slight asymmetry in eye size, bilateral inguinal hernia with hydrocele (patient no. 14), ependymal cyst of the brain (patient no. 12), and disturbed skull sutures development (patient no. 13).

Specific antibodies monitoring: The sensitivity of the detection of specific IgG, IgM and IgA from filter-paper eluates at birth has been previously described (Lebech et al., 1999). Paired sera were available for a confirmatory analysis between the 2nd day and 11 weeks after birth (mean 4.6 ± 3.4 weeks). Specific IgM and IgA were less frequently detected after the first week of life; there were no differences in sensitivity of ISAGA and ELISA (nine of 13 were positive). During the first weeks of life before the treatment, seven infants were positive for IgM and IgA antibodies (53.8%), two children had only positive values of IgM (15.4%), one patient was only positive for IgA (7.7%), and two cases had borderline levels of IgM or IgA (15.4%).

Table 2
Newborns maturity parameters and estimated age of maternal infection in relation to individual treatment recommendations for 15 congenitally infected infants[a]

Patient no.	Sex	Gestational age at delivery (weeks)	Birth weight (g)	Head circumference (cm)	Apgar score	Delivery mode	Estimated maternal infection (weeks)	Treatment regimen	Age when treatment started (days)	Duration of the treatment (months)	Side effects
1	M	37	3300	32	10	C	35–36	P + S/R	23	14	None
2	F	40	3240	35	10	S	37–39	P + S/R	37	13	None
3	F	35	1560	28	9	C	28–32	P/S	89[b]	10[b]	None
4	M	42	3050	33	6/8/9	S	28–32	P + S/R	50	15	None
5	F	40	2700	32	9	S	36–40	P + S/R	81[b]	14	None
6	F	32	1800	28	7/7/8	S	12–20	P/S	1[c]	12	None
7	F	38	3150	34	9	S	21–25	P + S/R	23	13	None
8	M	40	3700	34	10	S	Periconceptional	P + S/R	17	13	None
9	M	41	3820	36	10	S	28–32	P + S/R	23	14	None
10	F	40	3150	32	10	S	32–36	P + S/R	28	12	None
11	M	41	3920	37	7	S	25–30	P + S/R	26	12	Skin rash
12	M	37	3880	38	4/9/10	S	26–30	P + S/R	35	12	Crystalluria
13	M	40	3260	34	6/7/7	C	Periconceptional	P + S/R	20	3[d]	None
14	M	32	1790	29	2/5/7	S	< 8	P/S	61	24	Crystalluria
15	F	34	2430	31	9	C	Unknown	P/S	360	24	None

[a] Presumptive dates of infections estimated on maternal serology (Foulon et al., 1999).M, male; F, female; S, spontaneous; C, Caesarean section; P/S, pyrimethamine and sulfadiazine; P + S/R: pyrimethamine and sulfadiazine alternated with spiramycin.
[b] Treatment and follow-up refusal by parents.
[c] Death of severe staphylococcal sepsis.
[d] Cover with spiramycin during the 1st day of life.

Table 3
Incidence of clinical findings typical for congenital *T. gondii* infection among 15 infected infants untreated prenatally

Clinical sign	Number of affected children
1. Unilateral retinchoroiditis	$N = 2$
Pigmented scar	2
Fresh lesion	0
2. Intracranial calcifications	$N = 3$
Perivendular region alone	0
Cerebral cortex and periventricular	3
3. Dilatation of ventricualr system	$N = 5$
Severe with hydrocephalus	2
Mild or moderate	3
4. More than one clinical sign	3

The comparative mother and child Western blot assay allowed the detection of actively synthesised IgM in 14 of 15 (93.3%) cases examined at admission; 13 children produced IgG of different antigenic specificity but the difference was not statistically significant.

During the first year of life, a significant increase of specific IgG was observed in three cases (patient nos. 1, 9 and 11), all treated and a short reappearance of IgM and IgA was documented in another patient (no. 4). *Toxoplasma*-specific IgG showed decreasing titres in the infants when treated. In three cases, mostly with severe clinical damage, specific antibodies were temporarily undetectable. Specific IgG antibodies reappeared at the age of 10 months (patient no. 8) or 5 months after the cessation of treatment (patient no. 14). The third patient (patient no. 6) was IgM- and IgA-positive for 4 weeks after birth and then synthesised IgG of different antigenic specificity to the mother.

Nine of 11 children (81.8%) with complete serological follow-ups showed serological rebounds of specific IgG (two cases) or IgG and IgA (seven cases) between 3 and 10 months after the cessation of treatment (mean 6.6 ± 2.1 months) at the age of 16.5 to 34 months (mean 24.1 ± 5.4 months) without any signs of clinical reactivation (Fig. 1).

Anti-parasitic treatment regimen and its tolerability: for 13 IgM-positive neonates the anti-parasitic chemotherapy was initiated between the first day to 11 weeks of life (mean 4 weeks); for two other children (patients nos. 14 and 15) not identified by the screening programme, the treatment was administered at the age of 7 weeks and near 12 months, respectively, after recognition of clinical symptoms and serological confirmation.

Considering the estimated date of maternal infection, the postnatal treatment of IgM–positive infants was initiated between 4.5 and 42 weeks after the presumed *T. gondii* infection of the mothers (mean 18.0 ± 11.5 weeks). The duration of the specific anti-*Toxoplasma* therapy ranged from 3 to 24 months (mean 13.7 ± 5.0 months). No important side-effects giving indications for the interruption of treatment were seen. Mild skin rash, probably related to the treatment, was observed in one infant, and transitory crystalluria occurred in two cases; one of them (patient no. 14) had hyperuricaemia and a family predisposition to renal lithiasis (Table 2).

2.1.4. Discussion

Thirteen of 15 neonates with proven congenital toxoplasmosis were identified solely by serological screening, and appeared healthy at birth. Guerina et al. (1994) observed that all infants diagnosed by serological screening were judged by neonatologists on routine clinical examination to have no clinical symptoms, but careful investigations showed neurological and/or ocular abnormalities in about 40% (19 of 48) of the newborns. Couvreur et al. (1984) found neurological abnormalities in 33% of infants diagnosed as asymptomatic by their primary physicians.

The prevalence of typical clinical signs or symptoms in our patients was 33% (five of 15) and rather exceeds the data reported by previous studies, where 80–87% of newborns were free of symptoms (Foulon et al., 1999; Villena et al., 1998; Lebech et al., 1999; Gratzl et al., 1998; Pratlong et al., 1994). Such discrepancies are difficult to explain but may be due to a limited number of cases, to the observation period, or possibly to the *Toxoplasma* strain or inoculum size. Except for one case with severe neurological sequela and a poor prognosis at birth, the clinical pattern was stable or improved through intensive treatment. In the New England screening trial (Guerina et al., 1994), new retinochoroidal lesions causing vision impairment occurred in 10% of cases in early infancy (four patients) and a severe persistent neurological deficit was observed in one patient.

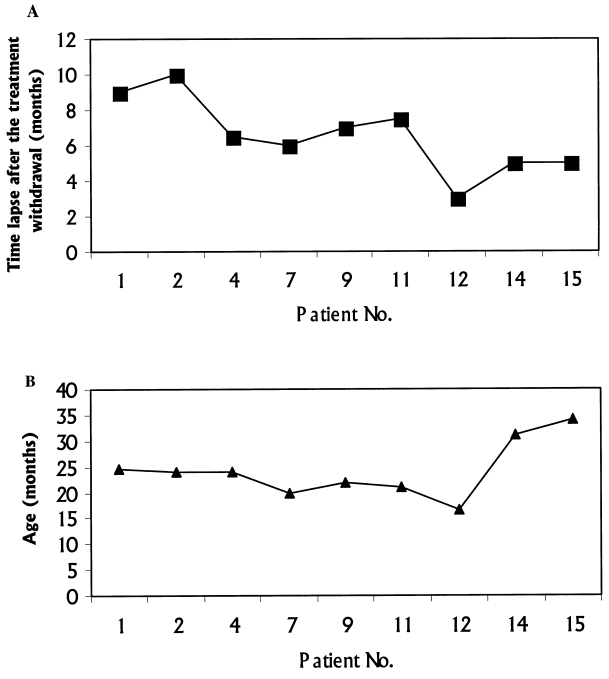

Fig. 1. Occurrence of immunological rebounds in relation to (A) the time lapse after stopping the treatment and (B) age of infected children.

In contrast, in the Chicago collaborative study following 44 congenitally infected infants, there were few asymptomatic patients with biological signs of infection only and no systematic prenatal or neonatal screening (McAuley et al., 1994). A higher risk for clinical abnormalities in early infancy due to *Toxoplasma* was also mentioned by Berrebi et al. (1993), who found 41% (nine of 22) of symptomatic children during a mean observation period of 32 months, although half of them were treated prenatally. The incidence of typical clinical findings was also high in a total group of 103 newborns (56 symptomatic cases) born to mothers treated during pregnancy (Couvreur et al., 1993).

Sequelae were diagnosed in two children with a treatment delay, but probably existed already prenatally without being diagnosed at birth. In only two children, progressing or spontaneous hydrocephalus needed neurosurgical intervention. Three infants identified by regional screening only had typical clinical findings but no retinochoroidal lesions. The incidence of *Toxoplasma* retinochoroiditis in newborns varies between studies. Villena et al. (1998) reported four cases with inactive eye lesions at birth of 78 newborns (5.1%), whose mothers were screened during pregnancy, and some of them treated in utero. The new ocular sequelae developed in 12 infants (15.4%) during the first year of life or in early post-treatment period. Couvreur et al. (1985) observed retinal lesions in 24% (26 of 108) of newborns at initial examination after birth with a secondary apparition of new ocular complications between 4 months and 7.5 years in 8% of them who remained untreated, but not in patients receiving intensive postnatal chemotherapy.

The significant diminution or resolution of intracranial calcifications during intensive therapy as well as an improvement of psychomotor development in symptomatic cases is now unquestionable (Roizen et al., 1995).

The serological rebounds following treatment occurred frequently (81.8%) in our patients, but never had clinical implications. Among 63 infants followed by Fortier et al. (1997) during the first 2 years of age, clinical recurrences were noted in five patients, and four of them were preceded by a serological rebound. So far, a mechanism of this phenomenon is not known. Some authors recommend Fansidar® treatment for 4–6 months following an initial treatment of pyrimethamine/sulfadiazine (Couvreur, 1993), but we believe that only clinical relapses justify a further treatment (Stray-Pedersen, 1992; Villena et al., 1998). Garin (1988) reported that secondary synthesis of antibodies occurred frequently 3–4 months after therapy withdrawal, usually close to the end of the 2nd year, and he proposed a treatment prolongation until this age.

Negative serology especially in immature infants with small birth weight or hypotrophy is not surprising and has already been reported by others (Villena et al., 1998; Fricker-Hidalgo et al., 1996). Some authors observed symptomatic infants or infants with parasitological proven infection without specific IgG (McAuley et al., 1994).

The study provides more information on the outcome of specific treatment given to children with a postnatal diagnosis of *Toxoplasma* infection soon after birth. Although the long-term follow-up of the patients is necessary to make an accurate evaluation of the benefit within adolescence and adult life, the actual prognosis of infected patients remains satisfactory.

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2.2. Screening for Congenital Toxoplasmosis in Turkey

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2.2.1. Introduction

Toxoplasmosis is usually asymptomatic in immunocompetent subjects. However, *T. gondii* infection acquired during pregnancy can lead to infection of the foetus, which may result in foetal loss or lesions that usually involve the brain and the eyes (Naessens et al., 1999; Roizen et al., 1995; Lynfield and Guerina, 1997).

The major influences on the spread of *Toxoplasma* to humans include hygienic routes, contamination of vegetables, fruit, and unfrozen meat, and association with cats, but handwashing, rinsing of vegetables and fruit, and the handling of cats are unlikely to have altered very much over the years.

Stray cats are one of the major problems that face the Turkish community in this regard. Tradition is another challenge for the Turkish people who like consuming raw meat balls or consuming uncooked salami or sucuk (which is a meat filling with spices). On a large scale and as a developing country, most people consume fresh meat which is not kept in the freezer. Meat frozen at −20°C for 2–3 days kills most of the *T. gondii* bradyzoites, so this does not occur.

The prevalance of antibodies against *T. gondii* in fertile

women differs widely with geographic location. Risk factors for vertical transmission from an infected mother to her children also differ in different studies. The estimated incidence of congenital *Toxoplasma* infection is as high as 1–4 per 1000 births in certain European countries. In Turkey there is no obligation for TORCH screening before marriage except for AIDS recently. The serological screening of pregnant women for toxoplasmosis and the follow-up until delivery, is not a routine procedure and there is no obligation to report verified cases of toxoplasmosis in Turkey.

Accurate serological tests for screening of Toxoplasma infections can only be done in the big cities (such as Istanbul, Ankara, Izmir, etc.) and in certain laboratories (mostly parasitology laboratories of the universities). In most of the private laboratories different commercial kits are used and of course the results obtained are difficult to compare. Many gynaecologists do not realise the importance of *Toxoplasma* infection and hence they usually do not recommend testing for toxoplasmosis during pregnancy. Due to geographic and financial problems no programme has ever been planned for this purpose. As a result there are no good data concerning the prevalence of parasitic diseases, as well as toxoplasmosis, in Turkey. Some local studies have been performed but these can not be applied all over the country and so far the incidence of congenital toxoplasmosis in Izmir is unknown. Because antenatal screening was not available, the aim of this study was to examine the children and mothers who were affected by prenatal toxoplasmosis after delivery.

2.2.2. Materials and methods

Samples: Between 1998 and 1999, altogether 953 cord blood samples were collected from all pregnant women who delivered at Ege University Medical Faculty Hospital. The serological proceedings (ELISA, IFA) were performed after all cord bloods were collected. IgM immunocapture and IgM ISAGA were performed on sera of suspected patients. The mean age of the study population was 26.3 years (range 16–42).

Antigens: Antigen preparations were made from tachyzoites of the TRH strain (RH like strain; personal information from Dr. Sibley) of *T. gondii*. Tachyzoites were obtained from the peritoneal exudates of mice infected 2 days earlier. These antigens have been routinely used for the serological *Toxoplasma* tests in our laboratory (Ertug et al., 2000; Kuman et al., 1999).

Enzyme Linked Immunosorbant Assay (ELISA). The wells in microtiter plates were sensitised with the *Toxoplasma* antigen overnight at 4°C. The plates were then given three washes each of 3 min in PBS containing 0.05% Tween 20 and 100 μl diluted serum was added to each well and incubated for 1 h at 37°C. The plates were washed as before in PBS/Tween 20. One hundred microlitres anti-human IgG or IgM labelled with alkaline phosphatase conjugate (Sigma A3187, Sigma A-3437) was added to each well and incubated for 1 h at 37°C and 100 μl enzyme substrate was added. The enzymic hydrolysis of substrate was stopped after 30 min by the addition of 100 μl NaOH. Samples with absorbance values greater than two or usually three times of the absorbance values of negative control are considered as positive. Values between two and three times are considered as suspected positive and further investigated.

Indirect immunofluorescence antibody (IFA) test: IFAT was used in the detection of *T. gondii* IgG and IgM in our laboratory. Briefly, multiwell slides were coated with a suspension of *T. gondii,* adjusted to contain 100 organisms per 5 μl, air dried and stored at −70°C until used. For the test, parasite smears were incubated at 37°C for 30 min with serial dilutions of the sera to be tested, washed three times with PBS, and air dried. The slides were then incubated with a 1/300 dilution in PBS of fluorescein-labelled goat anti-human immunoglobulin IgG or IgM (bioMerieux 75692, bioMerieux 75672) at 37°C for 30 min. After three washes with PBS, the slides were mounted with glycerol. The test slides were viewed with a fluorescence microscope. Reactions were considered positive when >50% of the organisms showed complete peripheral fluorescence. Dilutions of 1:16 and higher were evaluated as positive (Ertug et al., 2000).

IgM Immunocapture: Microtitre plates were coated with 100 μl rabbit anti-human IgM (DAKO A 0425) and kept at 4°C overnight, washed three times with PBS (pH 7.2) with 0.0005% Triton X-100. Serum samples (100 μl) diluted 1:100 were tested in duplicate. The microtitre plates were incubated at 37°C for 1 h, washed three times. *T. gondii* antigen (100 μl) was applied and incubated at 37°C for 1 h, washed and 100 μl mAb (S13) was added per well and incubated at 37°C for 1 h. Rabbit anti-mouse immunoglobulins (100 μl) (DAKO D 0314) with conjugated alkaline phosphatase was applied and incubated at 37°C for 1 h and washed. Enzyme substrate [p-nitrophenyl phosphate] (100 μl) were applied and incubated for 30 min before the reaction was stopped. Greater than 8 IU were regarded as positive. WHO International standard sera (Statens Seruminstitut, Denmark) were used as negative and positive controls (Lebech et al., 1993).

IgM immunosorbent agglutination assay (ISAGA): IgM ISAGA (bioMerieux) was used. The ISAGA index was interpreted as follows: 0–5 negative reaction; >5 positive reaction (Duffy et al., 1989).

2.2.3. Results

Nine hundred and fifty-three cord bloods were analysed for IgG and IgM anti-*Toxoplasma* antibodies by ELISA and IFAT. Specific IgG anti-*Toxoplasma* antibodies were found in 375 (39.34%) cord bloods. Five cord bloods were evaluated positive by IgM-ELISA and -IFAT. ISAGA and Immunocapture were performed on these five sera and one of them was found positive by these tests. This child was

followed up until 2 years of age but no signs of sequelae, either neurological or ophthalmological were found.

2.2.4. Discussion

The reasons for differences in the prevalence of *Toxoplasma* infections in different countries or regions, as noted also in Turkey, have been attributed to variations in, for example, human behaviour such as cooking habits and the number of cats living outdoors, but these are still not fully understood (Gilbert et al., 2000; Frenkel et al., 1995; Rey and Ramalho, 1999).

The relationship of toxoplasmosis with feeding habits, domestic animals, and urban and rural settlements have been studied and higher *Toxoplasma*-seropositivity has been found in people living in urban areas. But there was no significant difference between their culinary habits (eating raw meat or rare cooked meat, uncooked vegetables, drinking raw or pasteurised milk) as well as their relations with domestic animals (cats, dogs, birds, etc.) (Kuman et al., 1996).

No programme has ever been planned for detection of congenital toxoplasmosis in Turkey and only local studies have been performed. According to these local serological studies the seroprevalence found is between 17.3 and 78% (Table 4) (Altintas et al., 1997; Altintas et al., 1998; Altintas, 1996)

A seroconversion rate between 0.2 and 1% was found (Table 5) (Dilmen et al., 1990; Dabakoglu et al., 1995)

In another study in Izmir in 1990, the sera of 300 mothers were tested with ELISA and 136 (45.33%) and five (1.66%) were found positive for *Toxoplasma*-IgG and –IgM, respectively, and the rate of prenatal toxoplasmosis was determined to be three in the 300 cord blood samples by testing for IgM antibodies (1%) (Table 6) (Sasmaz et al., 1990).

It has been suggested that there are 13 million reproductive women and 1.5 million pregnancies in Turkey. According to our laboratory records the seropositivity of toxoplasmosis is 55% in pregnant women but in this study we could not follow up the pregnant women and so we do not have a serologic profile for them. We can only say that the seropositivity found was 39.34% in pregnant women and seroconversion was 0.1%.

The data from the present study in the Izmir area confirms that seroconversion to *T. gondii* during pregnancy is not a rare event. In order to determine the incidence of congenital toxoplasmosis in children in Izmir a study will be performed, but a Turkish nationwide screening program should be set up to identify women at risk of *Toxoplasma* infection during pregnancy. The risk to the foetus does not correlate with whether the infection in the mother was symptomatic or asymptomatic during gestation. Therefore, neonatal screening for toxoplasmosis is important to identify infection and start early treatment of congenitally *Toxoplasma*-infected children.

3. Toxoplasmosis in central Europe

3.1. Toxoplasmosis Prevalence in Eastern Romania

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3.1.1. Introductionn

Toxoplasma gondii is common in Romania, but there is no systematic serological screening in pregnant women so toxoplasmosis diagnosis is often a problem of physician inspiration.

The aim of this study was to estimate toxoplasmosis

Table 4
Results of *T. gondii* IgG and IgM antibodies in various cities of Turkey (seroprevalence)

Year of survey	Province	Number of patients	Name of the test	Positive (%)	
				IgG	IgM
1954	Ankara	687	Skin test	40.3	
1954	Istanbul	687	Skin test	31.27	
1971	Adiyaman	500	Skin test	60.6	
1980	Ankara	550	Sabin–Feldman	51.8	
1981	Kayseri	1232	IHA, ELISA	25	
1985	Izmir	18233	IFAT	24.12	
1985	Kayseri	4603	IHA	17.3	
1988	Adana	4200	IFAT	48.88	
1989	Ankara	1772	IHA, ELISA	65	
1993	Konya	1423	ELISA	39	13.4
1995	Istanbul	110	ELISA	34	0.9
1995	Malatya	510	ELISA, SF	22.6	1.1
1995	Izmir	9410	IFAT, ELISA	49.4	
1998	Izmir	1865	IFAT, ELISA	23.1	

Table 5
T. gondii IgG antibodies and seroconversion rates obtained in different studies

Year of survey	Number of pregnant women	IgG positive (%)	Seroconversion (%)
1980	1772	65.0	1.00
January 1988 –July 1995	11170	41.5	0.20

prevalence among the human population in eastern Romania and to study its consequences.

3.1.2. Materials and methods

Study population: The study was performed in 1996–1999 in Eastern Romania. The study population included pregnant women, newborns, and HIV infected patients and patients with eye disease and lymphadenopathy.

The pregnant women included 810 healthy women and 378 women with spontaneous abortion in the first trimester of pregnancy. Sero-negative healthy women were considered at risk of acute acquired toxoplasmosis and were serologically followed up until birth.

Cord blood from 1226 newborns that were analysed for specific *Toxoplasma* IgM- and IgG-antibodies and newborns with suspected infection were followed until they became seronegative or the diagnosis was confirmed.

In addition we studied the seroprevalence of antibodies against *T. gondii* in 102 HIV infected children (mean age 14.5 years), 50 chorioretinitis patients, 17 uveitis patients, and 43 patients with other ocular pathology (strabismus, cataract, microphthalmia, etc) and 343 children (mean age 15.8 years) with lymphadenopathy.

Antibody analysis: The following tests were used for serologic diagnosis of *T. gondii* infection: IFAT, 2-mercapto-ethanol agglutination test for *Toxoplasma* IgG, and ISAGA for *Toxoplasma* IgM and IgA (Thulliez et al., 1986; Dannemann et al., 1990; Desmonts et al., 1981).

Positive diagnosis of acute acquired toxoplasmosis was considered when all of the following criteria were fulfilled: positive IgG, IgM, and IgA, high level or significant increase of specific IgG-antibodies. Chronic toxoplasmosis was considered when IgG only was positive. Equivocal serologic pictures were cleared up by repeatedly testing at 2–3 week intervals.

A risk of congenital toxoplasmosis in newborns were considered if we found a high level of IgG, positive/equivocal IgM and/or IgA. A child that remained seropositive after first year of life, with or without signs and symptoms of disease was considered congenitally infected.

Table 6
Results of sera from 300 mothers and the corresponding cord blood samples

Working group	Toxo-IgM		Toxo-IgG		Number of total patients
	+	%	+	%	
Mothers	5	1.67	136	45.3	300 (100 %)
Cord bloods	3	1.0	136	45.3	300 (100 %)

3.1.3. Results

Pregnant women: The prevalence of *Toxoplasma*-speciifc IgG-antibodies in toxoplasmosis in pregnant women was 41%. There were no significant variation between the clinically healthy women group and the group suffering abortion (Table 7). The serologic screening of the apparently healthy women revealed 15 cases (1.9%) of asymptomatic, IgM-positive women who could have acquired the infection during pregnancy, compared with 16 cases (4.2%) with IgM-antibodies among the women with spontaneous abortion.

Newborns: *Toxoplasma gondii* antibodies were found in 44.5%. The majority of cases (43.8%) were uninfected newborns with maternal transplacental antibodies. Nine newborns (0.7%) with *Toxoplasma*-specific IgM- and or IgA-antibodies were followed up during the first year of life and one infant was confirmed as having congenital toxoplasmosis by persistent IgG-antibodies at 1 year of age.

Toxoplasma prevalence among HIV infected patients was 34.3% and no patient showed either specific IgM- or IgA-antibodies. *Toxoplasma*-specific IgG-antibodies varied between 4 u.i./ml and 6400 u.i./ml.

In patients with chorioretinitis 68% had specific Toxoplasma antibodies and patients with other eye pathology had a seroprevalence of 37.3%.

The seroprevalence in patients with lymphadenopathy was 34.1% and acute infection was confirmed in 44 cases (12.8%).

3.1.4. Discussion

The results show that *T. gondii* is common in Eastern Romania. The seroprevalence of 41% in pregnant women with an average age of 26 years shows that *Toxoplasma* seroprevalence is at the same level as in France and Austria

Table 7
Toxoplasma seroprevalence among pregnant women

T. gondii serologic status	Healthy pregnant women (810 cases)		Women with abortion (378 cases)	
	No.	%	No.	%
Seropositive	335	41.4	155	41.0
Toxoplasma IgG alone	320	39.5	139	36.8
Suspected infection during pregnancy	15	1.9	16	4.2
Seronegative	475	58.6	223	59.0

and thus that the situation in Romania is comparable with other central European countries.

The level of IgM-antibodies do not necessarily show that these women were infected during pregnancy as IgM-antibodies may persist for years.

One confirmed case of congenital toxoplasmosis in Romania was found in 1226 newborns which show that the birth prevalence of congenital toxoplasmosis is at the same level as that in Poland (Paul et al., 2000).

The level of 68% toxoplasmosis seroprevalence among chorioretinitis patients compared to the pregancy cases, HIV-positive children and patients with other eye diseases may indicate that infection with *T. gondii* may be an important risk factor for chorioretinitis. However, the seroprevalence need to be adjusted for age before a direct comparison can be made. Patient and physician restraint regarding ocular fluid testing made it difficult to definitively confirm toxoplasmosis as a cause for all tested chorioretinitis.

3.2. Toxoplasma in the Czech Republic 1923–1999: first case to widespread outbreak

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3.2.1. Introduction

Research into human toxoplasmosis originated in Bohemia: *T. gondii* was identified as a serious human pathogen by Janků (1923) in Prague. Subsequently it became clear that toxoplasmosis constitutes the most significant parasitosis in this region and the disease was subjected to very close attention (Kouba et al., 1974; Jíra and Rosický, 1983). Since 1955, cases of the disease are subject to mandatory reporting and its prevalence is repeatedly monitored by means of serological surveys. Although a great amount of data have been gathered, the majority were published for internal use only, or not at all. The extensive 1994 epidemic of toxoplasmosis in the Czech Republic resulted in further information, as collected by the existing reporting system. The aim of this report is to evaluate all hitherto unused data and further existing knowledge of the epidemiological aspects of toxoplasmosis in central Europe.

3.2.2. Materials and methods

Data sources: The National Reference Laboratory for Toxoplasmosis (NRLT) continuously gathers and evaluates data from two major sources: data concerning seroprevalence of toxoplasmosis gained from irregular serological surveys and incidence, gained from the database of reported cases of clinical toxoplasmosis. Supplementary information on the 1994 epidemic is based on epidemiological examinations conducted in hygiene stations Brno and Olomouc and the Mikrochem Laboratory in Olomouc. Demographic data concerning population figures or toxoplasmosis-associated mortality are provided by the Czech Statistical Office and the Institute of Health Information and Statistics of the Czech Republic.

Serological surveys: The programme for monitoring seroprevalence of selected infections (Multi-purpose immunological surveys of infection in the Czech Republic) includes monitoring of the prevalence of toxoplasmosis. Samples of sera for the purposes of this monitoring were collected in each case by district and paediatric practitioners from 20 to 30 randomly-selected medical districts in selected regions. Each participating medical practitioner was specifically instructed as to which age-group, category and number of samples were to be collected (always from subjects without acute clinical symptoms). A random selection of samples from the complete collection of sera was examined, encompassing in equal measures gender and number of subjects in all nine age-groups.

In 1971, 1977, 1983, 1985, 1987, 1990 and 1996 the NRLT examined a total of 5431 samples of sera taken from 21 regions of the Czech Republic (Table 8). A total of 1775 samples were collected in towns with a population exceeding 100 000 inhabitants (urban areas) and 3656 in predominantly rural regions. Data associated with the samples contain the district of domicile, gender, age, month and year of birth and the results of serological examinations for other specific infectious diseases. None of the regions were examined repeatedly with the exception of various Prague districts.

Surveillance of toxoplasmosis: Data concerning cases of acute toxoplasmosis were taken from centralised databases (systems ISPO, Epidat) and reviewed at NRLT. Cases of serologically confirmed clinical toxoplasmosis are reported by the practitioner concerned. Reports include place of residence, gender and age, date of first symptoms, clinical form and epidemiological case history. HIV-positive patients are not included in the survey.

The 1994 epidemic: Data include reported cases and the results of epidemiological surveillance. All patients with confirmed toxoplasmosis in Brno (257) and Olomouc (148) during the period December 1993 to April 1994 responded to a questionnaire detailing clinical complaints, contact with animals and soil and dietary habits. The percentage of positive answers to individual questions was calculated. The number of persons with acute toxoplasmosis among members of one family was also followed up.

Serological tests: Sera were examined by complement-fixation test - CFT (Pokorný et al., 1972b) using antigens from tween-ether extraction (Pokorný et al., 1972a). Titres of 8 and higher were evaluated as positive. Reaction using the antigen supplied since 1972 by SEVAC (now SEVAPHARMA) Prague, managed by the same procedure throughout the monitoring period, is still the most wide-

Table 8
Results of the Multipurpose serological surveys of infection in the Czech Republic 1971–1996: Seroprevalence of toxoplasmosis in selected districts with predominatly urban or rural character of residence

Year	District	Character	Men			Women			Total		
			Tested	Positive	%	Tested	Positive	%	Tested	Positive	%
1971	Prague 1, 4, 5, 7, 8, 9	Urban	148	6	4.1	160	16	10.0	308	22	7.1
	Benešov	Rural	29	2	6.9	27	3	11.1	56	5	8.9
	Pelhřimov	Rural	61	14	23.0	59	11	18.6	120	25	20.8
	Tábor	Rural	73	13	17.8	85	18	21.2	158	31	19.6
	Southern Moravia[a]	Rural	66	63	95.4	87	77	88.5	153	140	91.5
1977	Beroun	Rural	125	45	36.0	126	67	53.2	251	112	44.6
	Strakonice	Rural	131	68	51.9	131	90	68.7	262	158	60.3
	Semily	Rural	132	48	36.4	128	71	55.5	260	119	45.8
	Brno	Urban	125	34	27.2	120	57	47.5	245	91	37.1
1983	Teplice	Rural	133	38	28.6	132	43	32.6	265	81	30.6
	Gottwaldov	Rural	129	54	41.9	134	69	51.5	263	123	46.8
	Prague 3, 7	Urban	130	41	31.5	124	49	39.5	254	90	35.4
1985	Trutnov	Rural	99	56	45.5	108	67	62.0	207	123	59.4
1987	Písek	Rural	150	30	20.0	148	32	21.6	298	62	20.8
	Cheb	Rural	148	16	10.8	150	17	11.3	298	33	11.1
1990	Prague 1, 2	Urban	110	19	17.3	110	21	19.1	220	40	18.2
	Jindřichův Hradec	Rural	127	34	26.8	128	45	35.2	255	79	31.0
	Třebič	Rural	133	35	26.3	131	39	29.8	264	74	28.0
1996	Prague 2, 4, 7, 8, 9	Urban	311	37	11.9	291	45	15.5	602	82	13.6
	Hradec Králové	Urban	57	6	10.5	69	13	18.8	126	19	15.1
	Chrudim	Rural	73	14	19.2	66	17	25.8	139	31	22.3
	Jičin	Rural	75	12	16.0	74	20	27.0	149	32	21.5
	Náchod	Rural	78	11	14.1	68	20	29.4	146	31	21.2
	Svitavy	Rural	62	16	25.8	70	23	32.9	132	39	29.5
Total			2705	712	26.3	2726	930	34.1	5431	1642	30.2

[a] Southern Moravia: samples collected from neighbouring districts Břeclav, Gottwaldov, Hodoníin, Znojmo.

spread screening system in use and form the basis of immunological surveys. In the 1980s, the NRLT began using the ELISA test for IgG (Pokorný et al., 1989) and double-sandwich ELISA for specific IgM (Pokorný et al., 1990). Since the early 1990s, both NRLT and regional diagnostic laboratories also use commercial immunoenzyme tests on anti-toxoplasma IgG, IgM and IgA for confirmation of results and determination of the phase of infection (Sanofi Diagnostics Pasteur, Captia Centocor, Abbott IMX and Test-Line Brno).

Data processing: All available data from serological surveys were combined for analysis. The Poisson regression model (for categorical variables) with multiplicative risk, which links a count and a rate multiplier with a set of fixed covariate values, was used for analysis of the count of toxo-positive subjects from immunological surveys by gender, domicile (rural/urban) and age-group in the EPICURE software package. Chi-square test was used for comparison of percentages in the contingency table in Epi-Info programme. From the data on reported clinical cases, morbidity per 100 000 population and month was calculated. All statistical tests were carried on 5% significance level.

3.2.3. Results

Prevalence of toxoplasmosis in the Czech Republic: Of 5431 persons examined as part of the immunological survey, 30.2% were CFT-positive. Seroprevalence in women (34.1%) was significantly higher than in men (26.3%). The ratio of seropositive persons has a marked fluctuation from 7.1% (Prague) to 91.5% (southern Moravia) (Table 8).

The percentage of positivity correlates with the type of environment: In urban regions an average of 19.6% persons (16.2% men, 23% women) from our sample were positive, while in rural areas average positivity was 35.3% (31.2% men, 39.4% women). These differences are statistically significant.

The seroprevalence of toxoplasmosis increases with age (Table 9). Increasing seropositivity is marked in children of both sexes up to 4 years of age and continues until age 9. In higher age-groups the increase is less marked. For women, seroprevalence generally increases in a linear manner up to age 50 when peak values are reached; in higher age-groups positivity declines. For men, seroprevalence begins to increase in the over 20 years age-groups, while in persons older than 60 years it is lower than in previous age-groups.

Analysis of the effects of domicile and gender on seroprevalence in the various age-groups: Seroprevalence in the individual age-groups is strongly influenced by place of residence (Fig. 2). While in urban inhabitants of both sexes it increases at a more or less even rate, in the rural

Table 9
Seroprevalence of toxoplasmosis by age-group and gender in the Czech Republic

Age-group (years)	Men		Women	
	Tested	Prevalence (%)	Tested	Prevalence (%)
0	23	0.0	23	0.0
1	70	11.4	80	10.0
2	102	13.7	84	27.4
3	108	11.1	109	25.7
4	125	24.8	129	24.8
0–4	428	15.2	425	21.4
5–9	416	27.9	418	30.1
10–14	299	20.4	294	27.9
15–19	282	25.5	325	31.1
20–29	401	28.7	351	38.5
30–39	215	27.4	240	46.3
40–49	224	34.4	214	46.7
50–59	211	34.6	234	41.0
60+	229	32.3	225	39.1
Total	2705	26.3	2726	34.1

population there is a very high seroprevalence from age 9 onwards. In men, seroprevalence remains practically unchanged in the higher age-groups while in women there is a slight increase up to age 40–49 years (52.3% positive); seroprevalence decreases in subsequent age-groups. The resultant Poisson regression model for assessment of toxoplasmosis-positivity prevalence in the monitored subgroups expresses the influence of residence character (urban/rural) as dependent on age. For the effects of gender, uniform coefficient is sufficient (Table 10). The effect of gender is similar in all age-groups (no statistically significant differences); prevalence in women in a given sub-group is therefore calculated as 1.293-fold the prevalence in the corresponding male sub-group. This increase is statistically significant. For instance, in the up to 4 years age-group, the estimated prevalence in men from rural areas exceeds 20 % (0.212×100), while in the city it is 0.247-fold this figure

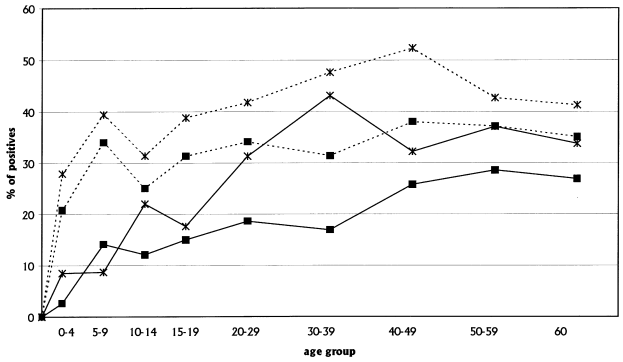

Fig. 2. Comparison of seroprevalence by age and gender in urban and rural districts of the Czech Republic. Solid square: males; Cross: females; Solid line: urban; Dotted line: rural.

and only reaches 5.24%, which is significantly lower. However, in the higher age-groups the differences between urban and rural areas diminish. In age-groups over 50 years the multiplication factor reaches about 0.8 and the differences are no longer statistically significant (the confidence interval covers the value 1).

Antibody titres of examined sera were generally low and diagnosed toxoplasmosis can be evaluated as latent or chronic. For all sera with a CFT titre of 64 and higher, collected in 1990 ($N = 23$) and 1996 ($N = 23$), specific IgM antibodies were determined. Two samples from 1990 were positive and the infection was confirmed as acute.

Reported cases of acute toxoplasmosis: Prior to 1945, only one case of toxoplasmosis is on record (Janků, 1923), and until the mid-1960s the disease was diagnosed sporadically (Table 11).

The creation of new diagnostic centres (from three prior to 1966 to 82 in 1999) contributed to the increase in numbers of confirmed cases. The number of reported cases rose slightly during the periods 1971–1975, 1979–1983 and 1987–1993, with a decrease in the interim periods. Average morbidity during 1982–1991 was 0.538 per

Table 10
Influence of urban and rural environments on prevalence of toxoplasmosis by age-group and gender

Age-group	Baseline relative prevalence (males in rural regions)		Effect of urban area	
	Estimate	95% CI[a]	Estimate	95% CI[a]
0–4	0.212	(0.178, 0.253)	0.247	(0.149, 0.409)
5–9	0.320	(0.277, 0.370)	0.312	(0.211, 0.459)
10–14	0.246	(0.202, 0.300)	0.607	(0.418, 0.883)
15–19	0.305	(0.256, 0.365)	0.464	(0.321, 0.670)
20–29	0.332	(0.285, 0.386)	0.646	(0.484, 0.862)
30–39	0.346	(0.288, 0.414)	0.780	(0.549, 1.108)
40–49	0.393	(0.331, 0.468)	0.643	(0.446, 0.927)
50–59	0.347	(0.289, 0.418)	0.826	(0.586, 1.164)
60+	0.333	(0.276, 0.401)	0.798	(0.563, 1.131)
Effect of female gender			1.293	(1.173, 1.426)

[a] CI, confidence interval.

Table 11
Cases of clinical toxoplasmosis reported in the Czech Republic 1923–1999 including the three most commonly specified clinical forms and mortality

Year	No. of cases	Men	Women	Proportion of men (%)	Morbidity per 100 000 pop. and month	No. of deaths	Clinical form		
							Lymphadenopathy	Congenital	Ocular
1923	1					1			
1945–1950	4								
1951–1955	45								
1956–1960	40								
1961–1965	61								
1966–1968	486								
1969	100				0.084	3			
1970	101				0.086	4			
1971	103				0.087	3			
1972	155				0.131	2			
1973	212				0.178	3	61	7	3
1974	342				0.285	3	96	10	11
1975	431				0.357	3	100	7	27
1976	402				0.331	1	149	11	19
1977	368				0.301	0	167	8	22
1978	388				0.316	2	198	3	17
1979	291				0.236	0	155	9	12
1980	346	114	232	32.9	0.279	0	231	12	20
1981	476	150	326	31.5	0.385	0	313	9	19
1982	578	215	363	37.2	0.467	0	444	10	18
1983	747	273	474	36.5	0.603	0	560	13	22
1984	635	230	405	36.2	0.512	0	481	14	18
1985	706	266	440	37.7	0.569	0	536	12	39
1986	667	232	435	34.8	0.538	0	528	19	17
1987	513	193	320	37.6	0.413	1	412	5	16
1988	601	240	361	39.9	0.484	0	515	4	13
1989	560	220	340	39.3	0.450	0	458	4	16
1990	746	267	479	35.8	0.600	0	609	10	18
1991	706	255	451	36.1	0.571	1	563	10	14
1992	823				0.665	0			
1993	860	275	585	32.0	0.694	0	596	3	22
1994	2056	716	1340	34.8	1.658	0	1558	12	26
1995	1514	475	1039	31.4	1.221	0	1065	8	44
1996	1217	413	804	33.9	0.983	0	952	7	30
1997	952	328	624	34.5	0.770	0	696	11	37
1998	777	258	519	33.2	0.629	0	595	2	30
1999	857	291	566	34.0	0.695	0	664	3	41

100 000 inhabitants per month. In 1994, certain regions were struck by a widespread epidemic of toxoplasmosis resulting in a threefold increase of average nationwide morbidity against the previous average.

The 1994 Toxoplasmosis epidemic started simultaneously in all districts. Most of the patients reported first symptoms in January 1994 (in some areas at the end of December 1993), a smaller proportion in February to March 1994 (Fig. 4). From April onwards the number of new cases declined rapidly. Nevertheless, the number of cases remained above average for some time and pre-epidemic status was regained in 1997 (Table 11).

A transitional increase in numbers of cases was recorded in many areas of the Czech Republic, particularly in the eastern regions. The greatest increase was in ten Moravian districts, where monthly morbidity exceeded ten times the 1982–1991 national average (Table 12, Fig. 4). The epidemic did not spread to other parts of the Czech Republic or neighbouring Slovakia and Austria.

The outbreak had a focal character: whereas certain districts reported an abnormal incidence of toxoplasmosis in 1994, neighbouring districts had no such increase. Prevalence of the disease varied widely within the epidemic area (Fig. 3).

The number of afflicted persons is unknown. The ten most heavily affected districts had 722 reported cases (Table 12) within the first 3 months of 1994 (in contrast to 91 cases during the same period of the previous year). As a result of the epidemic, the total number of cases throughout the Czech Republic in 1994 rose by 1196 cases as compared with the previous year – an increase of 2.4-fold. The majority of cases were amongst inhabitants of large cities such as Brno and Olomouc.

In Brno and Olomouc, less than 25% of patients queried

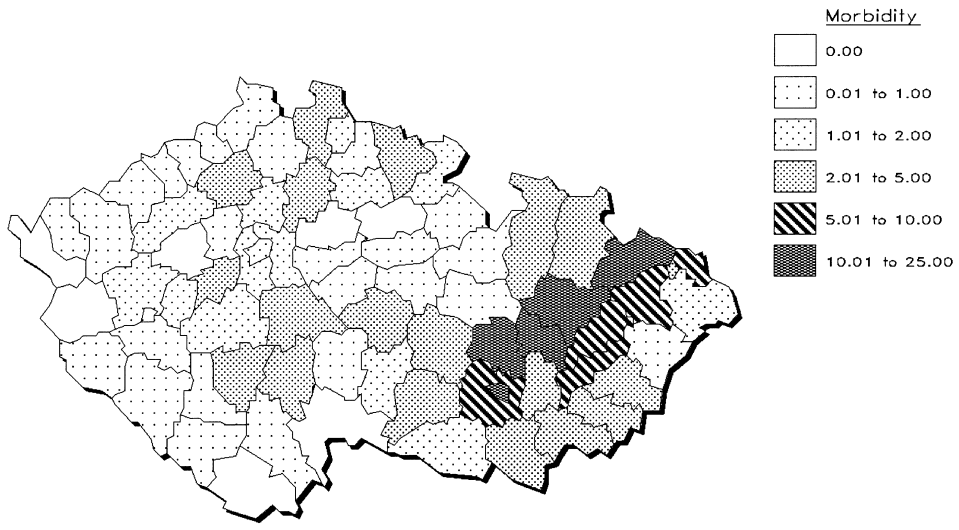

Fig. 3. The geographical distribution of the monthly morbidity from Toxoplasma gondii-infection per 100 000 population and month during the peak epidemic period January–March, 1994, Czech Republic.

consumed raw meat or cereals, kept pet cats, ate fast-food or visited a rural location regularly. Less than 50% consumed the products of hog-killing or had contact with sand or soil. The following foods were frequently consumed: raisins (80%), peanuts (85%), mayonnaise, ham (93%), milk (pasteurised) and milk products (95%), oranges and apples (99.2%). Vegetarians constituted 2.2%, three-quarters of who eat ham occasionally. Drinking water for individual areas is locally supplied.

Profile of persons with clinical toxoplasmosis: The majority of all patients infected during 1980–1999 were women (Table 11), the proportion of men fluctuated from 31.4% (1995) to 39.9% (1988). Acute toxoplasmosis was recorded mainly amongst the younger population: of all reported male patients 50% were under 16 years of age and 75% under 25 years. Amongst the female patients 50% were under 23 years and 75% under 32 years and 66% were 18–45 years of age (data from the period 1995 to 1999). A similar trend was recorded during the epidemic.

Of all cases reported in the years 1993 and 1995–1999, from 5% (1999) to 7.8% (1993) are recorded as familial incidence, e.g. associated with families where at least two cases of acute toxoplasmosis were recorded simultaneously.

In 1994, the nationwide percentage of familial incidence rose to 13.2%. In certain areas, over 60% of cases during the epidemic qualify as familial (Table 13). The infection was detected among male and female patients, both children and adults. An unusual case was that of a family of eight in

Table 12
Ten districts in Moravia (Czech Republic) where monthly morbidity exceeded 5.52 per 100 000 population (ten times the nationwide average for 1983–1993) during the peak of the epidemic (Jan–Mar 1994). The urban character of the epidemic is highlighted by comparison of morbidity data for Brno-city and Brno-rural districts

District	No. of inhabitants	Cases Jan–Mar 1994	Morbidity per 100000 population and month Jan–Mar 1994	Mean 1982–1991
Brno-city	390112	250	21.36	0.26
Olomouc	226091	133	19.61	0.83
Opava	181976	85	15.57	1.61
Blansko	107885	40	12.36	0.23
Prostějov	111490	37	11.06	0.25
Karviná	285903	65	7.58	2.36
Přerov	138472	30	7.22	0.98
Nový Jičín	160526	34	7.06	1.07
Kroměříž	108917	20	6.12	0.35
Brno–rural	155208	28	6.01	0.30
Total	1866580	722	12.89	0.91

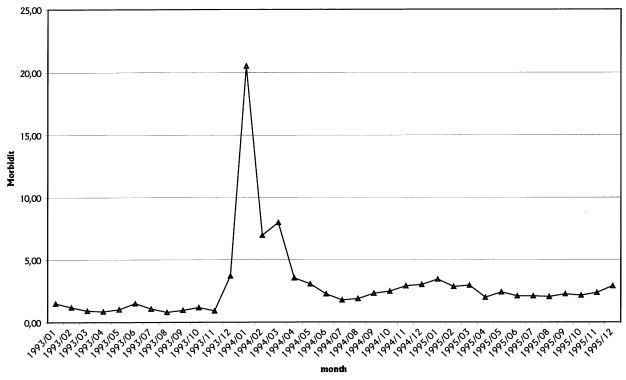

Fig. 4. Morbidity per month and 100 000 in ten Moravian districts (Czech Republic) with most apparent outbreak of toxoplasmosis, 1993–1995.

Olomouc, of which six members suffered from lymphadenopathy and one child from subclinical toxoplasmosis; the father was uninfected.

Clinical presentation: The most frequent symptom of toxoplasmosis is lymphadenopathy; in the past 15 years 76–88% of cases reported were the lymphatic form. The number of reported cases of congenital toxoplasmosis continues to be low. Cases of the ocular form multiplied following the 1994 epidemic (Table 11). Lymphadenopathy was also prevalent amongst epidemic patients (86% Brno, 89.9% Olomouc). Two thirds of patients reported 'protracted angina' with subfebrility and lassitude. A total of 212 children were hospitalised in Brno clinics for infectious diseases. Laboratory tests revealed elevated gamma globulin and slight increase in ALT in 7% of child and 12% of adult patients. Drug therapy was successful in all but two cases where the therapy had to be repeated (Černý et al., 1995).

Mortality: In the Czech Republic 1969–1999, 26 deaths from toxoplasmosis are recorded (Table 11). Child mortality (boys/girls) was as follows: up to 1 year age (10/9), age 1 year (2/2), age 3 years (1/0) and 5–9 years (2/0). The last reported mortality was in May 1991, a 2-month old male with generalised toxoplasmosis and hydrocephalus. NRLT isolated a cyst-forming strain from the tissue.

3.2.4. Discussion

Since 1971, NRLT has examined sera from a randomly selected cross-section of the population as part of a program of immunological surveys. Although large-scale population groups were examined annually, there were not enough individuals in each group for accurate analysis when distributing by age, gender or residence. In view of the fact that the main screening test is still the CFT, which remains a satisfactory test with no changes in technique, and because there is no reason to suppose marked changes of toxoplasmosis prevalence in the Czech Republic during the monitored period, we have combined the results of all seven serological surveys. This yielded a group of 5431 examined persons and enabled in-depth analysis.

Of the examined group, 30.2% were positive. This does not significantly differ from older available data. Jírovec (1971) detected 37.7% positivity in 15 972 persons in the former Czechoslovakia by intradermal testing (IDT). Similar seroprevalence was detected in the Czech Republic in HIV-positive patients (27.5%) (Pospíšlová et al., 1997); (29.8%) (Sýkora et al., 1992). However, average counts tell us little about the pattern of toxoplasmosis seroprevalence in the population, which varies widely from region to region. For instance, in southern Moravia seroprevalence was recorded as 91.5 against 7.1% in Prague. Local differences in prevalence are quoted by a number of authors who examined pregnant women (Prague: 24.8%, Jindřick ův Hradec: 44.8% (Ziteck, 1998), České Budějovice district: 37% (Hejlízek et al., 1999); Karviná district: 40% (Palička et al., 1998) and blood donors (Strakonice district: 24.6% (Hejlíček and Literák, 1993a) Nový Jičín region: 32.1% (Svobodová and Literák, 1998). The great differences between individual regions are due to a number of factors: Prevalence of toxoplasmosis may be influenced by climate and height above sea-level (Shevkunova, 1980) and the epidemiological situation in previous years. The governing factor is, in all likelihood, whether the area is urban or rural. In the Czech Republic, prevalence in the rural population is significantly (1.8 times on average) higher than in the urban population. These differences are far more marked in the lower age-groups. For instance, seroprevalence amongst male children up to 4 years of age in rural areas is approximately four-fold higher than in their urban counterparts. Rural children have earlier contact with the infection and their prevalence increases at a more rapid rate than in the city. In the over 10 years age-group, the prevalence in rural-dwelling boys practically tails off, and rises only slightly in girls of the same age. In contrast, urban children who have less opportunity to become infected exhibit a less rapid rate of seroprevalence for a longer period. In the over 50 age-groups the difference between urban and rural populations is practically nil.

Table 13
Familial cases of toxoplasmosis in Brno (reported) and in Olomouc (diagnosed at Mikrochem laboratory) 1994

City	Cases in 1 family							Familial occurrence	Families	Total cases	Percentage of family cases
	1	2	3	4	5	6	7				
Brno	183	24	13	1	1	0	0	96	39	279	34.4%
Olomouc	50	14	6	5	3	0	1	88	29	138	63.8%

The above data show that the risk of acquiring toxoplasmosis in pregnancy is paradoxically higher for the urban population of women who have a sharp rise of serprevalence at a fertile age, than in rural women who, for the most part, were infected at an earlier age. Moreover, in young mothers the disease causes psychological changes (Flegr and Havlíček, 1999).

The source of infection, apart from contaminated soil or sand, can be certain types of meat. For instance, 14% of pigs (Hejlízek and Literák, 1993b) and 44–84% of rabbits (Hejlíček and Literák, 1994) from domestic breeding are *Toxoplasma* positive. Contact with raw meat, but not cats, in the family appears to be a major risk factor (Flegr et al., 1998). Women appear to be in contact with these sources to a greater extent with a prevalence 1.3 times higher than men. This ratio is independent of age, being practically identical in all age-groups.

The majority of *Toxoplasma*-positive persons in the Czech Republic become infected at age 9 years or younger. If we compare prevalence year by year we can see that all examined children under 1 year of age were negative. In 1–4 year olds, particularly from rural regions, there is a sharp rise. A further increase in prevalence, particularly in women, is seen between the ages of 15 and 40 during peak fertility.

Toxoplasma survives in the infected host until the death of the host (Krahenbuhl and Remington, 1982). Hence the increase of prevalence is cumulative. However, in our group there was a decline in seroprevalence in the over 50 age-groups, more marked in women, particularly those from rural areas. Decreased prevalence in the highest age-groups (unfortunatelly uncommented) is apparent from IDT data by Jírovec (1971) and Shevkunova (1980), but not by Sandow et al. (1989) who noted a continual increase leading to 100% prevalence in persons over 67 years using the IFAT.

A possible explanation for this decline is that the general rule of life-long seropositivity is not absolute and that in certain individuals immune response may fade after a long period p.i. NRLT has recorded two cases: a man and a woman who were seronegative 20 and 15 years following hospitalisation for confirmed acute toxoplasmosis (Kodym-unpublished data). Another explanation would be the lower seroprevalence in the past, although this variant does not fit with established data. The third explanation for the decrease of seropositive individuals in higher age-groups-that they have died-is adamantly rejected by the authors, some of whom are seropositive themselves.

Clinical toxoplasmosis in the Czech Republic manifests itself as a disease affecting children and persons (mainly women) of fertile age. The most frequent symptom is lymphadenopathy, and following the outbreak in 1993/1994 there was an increased incidence of ocular toxoplasmosis. There is an unexpectedly low incidence of reported congenital toxoplasmosis (no increase was detected even during the epidemic). The expected annual number should be about 100, if estimating from the frequency of 1.2 cases per 1000 in Karviná district (Palička et al., 1998) and approx 90 000 births annually. In the Czech Republic there is no screening for toxoplasmosis apart from several districts (Karviná and western Bohemia) and many cases are unrecognised; the problem is also due to lack of discipline in the reporting of cases.

Up to 1976, 1–4 deaths from toxoplasmosis were reported annually. The virulent P-Cz strain was isolated from the brain of a girl aged 2 months who died of congenital toxoplasmosis in 1963 (Kouba et al., 1974; Literák et al., 1998).

It is difficult to ascertain whether there are any long-term changes of incidence and prevalence of toxoplasmosis in the Czech Republic. The increasing trend of reported cases is probably due to greater access to improved diagnostic methods. Figures for prevalence recorded in serological surveys from past years are influenced more by actual site of collection and locality (urban versus rural) than by the time factor. Hejlíček and Literák, 1993a) found no changes among blood donors in the Strakonice district over the period 1980–1990. Repeated examinations were performed only on the population of Prague, showing low prevalence and no dramatic changes between the first CFT examinations in 1960 (14.2%) (Seeman, 1960) and the serological survey in 1996 (13.6%).

The epidemic started at the end of December 1993. The greatest increase in morbidity (14 times the long-term average) was recorded in ten Moravian districts containing 18% of the Czech Republic population. At the peak of the epidemic (January–March 1994) the disease was confirmed in these districts in a total of 722 patients.

The largest local outbreak of toxoplasmosis was documented by Bowie et al. (1997) in 1995 in Victoria, Canada, where 2900–7700 cases were estimated; outbreaks, described by Radulovic et al. (1990): (78 persons); Benenson et al. (1982): (38 persons) and Teutsch et al. (1979): (37 persons), were much smaller. Other epidemics mentioned in the literature involved four to ten persons, on a local level. An epidemic of toxoplasmosis affecting hundreds of people over such an extensive area is a unique phenomenon.

Morbidity declined after the epidemic subsided but remained at elevated levels for a period of 3 years. Aside from increased circulation of the infectious agent this fact may be due to increased diagnostic attention and reporting around the critical period.

Sources of the infection are given as oocysts excreted by cats contaminating water (Bowie et al., 1997; Benenson et al., 1982), food from army kitchens (Radulovic et al., 1990), riding school environments (Teutsch et al., 1979), sandpits (Stagno et al., 1980) or farm soil (Coutinho et al., 1982); or bradyzoites/tachyzoites from animal food products, unpasteurised goat's milk (Sacks et al., 1982), uncooked meat from arctic mammals (McDonald et al., 1990), traditional kibbi from raw mutton (DeSilva et al., 1984) or hamburgers (Kean et al., 1969).

Eight cases of toxoplasmosis have been reported in genetic laboratory personnel infected by tissue cultures (Markvart et al., 1978). Epidemiological investigations have eliminated all of the above as sources of the epidemic described in this paper.

In view of the fact that we have eliminated contact with soil (winter epidemic), cats or other animals and common water supplies as causative factors; the urban character of the epidemic (occurring simultaneously in isolated foci during the Christmas period) and with a high familial incidence, denotes an alimentary route of infection.

The majority of patients reported consumption of foodstuffs that may potentially have contained tissue cysts (ham) or may have been contaminated during manufacture or storage by oocysts from cat faeces (nuts, fruit). Infected foodstuffs, imported or domestic, were probably distributed to shops in various towns prior to the Christmas period with resulting infection of the local population. Isolation of *T. gondii* developmental stages in foods or the environment during the epidemic was unsuccessful. As such, this has occurred in only three of the 11 above-mentioned epidemics: from mice and cats captured in the infested riding school (Dubey et al., 1981), farm soil (Coutinho et al., 1982a,b) and drinking water (Isaac-Renton et al., 1998). The exact route of transmission of the Moravian epidemic in 1993/1994 will probably remain unsolved.

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4. Coinfection between HIV and *Toxoplasma gondii*

4.1. Coinfection between HIV and Toxoplasma gondii in an african population – an underestimated threat?

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4.1.1. Introduction

Although AIDS is widespread in several African countries, the influence of opportunistic infections for the outcome of the disease is unknown. Tuberculosis is thought to be one of the most important causes of death in HIV-infected patients in Africa (Lucas et al., 1993), whereas in Europe other opportunistic pathogens such as *Pneumocystis carinii* or *T. gondii* have been shown to account for a large number of deaths (Clumeck, 1991). However, since autopsies are not regularly performed in Africa and facilities for the accurate diagnosis of these opportunistic pathogens are only rarely available, the definite cause of death in AIDS patients often is unclear.

In order to determine whether toxoplasmosis might be important in the progression of AIDS in Africa as well, we analysed in this study 668 serum samples from Nigerian individuals.

4.1.2. Materials and methods

The samples were collected between January and June 1994 and consisted of 380 sera from female individuals (average age 30.1 years) and 288 sera from male individuals (average age 32.6 years). The majority (365) of serum samples were from outpatients with various ailments, such as typhoid fever, diabetes, and malaria fever. Additional samples (137) were from antenatal patients and 166 sera were from apparently healthy individuals, 65 of whom were blood donors.

The serum samples were examined for *T. gondii*-specific antibodies by the direct agglutination test (DA, Toxo-Screen), the CFT, and the SFDT, and for antibodies to HIV I and II by an enzyme immunoassay (EIA, Enzygnost Anti-HIV 1/2 plus, Behring). EIA HIV-positive and borderline results were retested by an immunoblot (New LAV BLOT I&II, Sanofi Diagnostics Pasteur, Freiburg, Germany). A serum was considered positive for *T. gondii* antibodies if it was reactive in at least both DA and SFDT; and for HIV antibodies if it was reactive in both EIA and immunoblot.

4.1.3. Results

The overall prevalence of 25.9% for *T. gondii* antibodies in the general population recorded in this preliminary study is surprisingly low (Table 14), compared with studies in other parts of Nigeria, which demonstrated a seroprevalence of 72.2% (Olurin et al., 1971), especially as the dietary habit of the sampled population (Olusi et al., 1994) and the abundance of stray cats particularly expose the population to infection with *T. gondii*. The HIV infection rate of 5.7% was expected since promiscuity is common in Nigeria. Interestingly, the seroprevalence of HIV antibodies in Nigeria, all of them specific for HIV I, is significantly

Table 14
Seroprevalence of HIV/*Toxoplasma gondii* co-infections in Benue state of Nigeria.

Infection	Number tested	Number positive	Percent positive
HIV I	668	38	5.7
Toxoplasma gondii	668	173	25.9
HIV/*Toxoplasma gondii*	668	10	1.5

lower than found in neighboring Cameroon and five other central African countries (Kanki et al., 1987).

4.1.4. Discussion

No significant difference was demonstrated for the seroprevalence of *T. gondii*-specific antibodies between HIV-positive and -negative individuals. This is perhaps not surprising because HIV infection has not been shown to be a risk factor for infection with *T. gondii*. However, the number of HIV-positive individuals chronically infected with *T. gondii* is significantly higher in Nigeria compared with Europe and thus might be important to know in view of the fact that reactivation of chronic *Toxoplasma* infection might cause fatal toxoplasmic encephalitis.

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5. Toxoplasmosis in southeastern Brazil: an alarming situation of highly endemic acquired and congenital infection

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5.1.1. Introduction

The organisms belonging to the *Toxoplasma* genus were described independently in 1908 in Brazil by Splendore (1908) in rabbits, which he named *T. cuniculi*, and by Nicolle and Manceaux, 1908, 1909) in Tunisia in gondii (*Ctenodactylus gondii*) a South African rodent, which they named *T. gondii*. Although toxoplasmosis is undoubtedly an ancient disease it was recognised as a specific entity only after the parasite was discovered. The first epidemiologic studies in Brazil came after the introduction of the SFDT in 1948. Busacca et al. (1953) reported that 23% of posterior uveitis had *T. gondii* as an etiological agent. Subsequently Abreu Fialho (1953), Pereira et al. (1965) and Martins et al. (1969) reported seropositivity rates of 38, 30 and 68%, respectively, in patients with posterior uveitis in Brazil (Oréfice and Bonfioli, 2000). In 1988 it was shown in the World uveitis symposium in São Paulo, Brazil, that there existed a high prevalence of acquired toxoplasmic uveitis and some peculiarities of the ocular disease caused by *T. gondii* infection in the Rio Grande do Sul state (Melamed, 1989). However, an unusually high prevalence of ocular toxoplasmosis in southern Brazil that was reported at the beginning of the 1990's has been the classical reference citation of acquired ocular toxoplasmosis all over the world (Glasner et al., 1992).

5.1.2. Epidemiology of toxoplasmosis in Campos dos Goytacazes: a cross-sectional study

In Brazil, serologic prevalence of *T. gondii* infection ranges from 50 to 80% of the healthy adult population, the highest values being found in some northern and southern states. The lowest values tend to occur in some southeastern states (São Paulo and Minas Gerais) (Oréfice and Bonfioli, 2000). The highest incidence of posterior uveitis seems to occur in southern of Brazil where it can reach a prevalence of 95% (Melamed, 1989). In Rio de Janeiro state there is a paucity of information on the epidemiology of toxoplasmosis. In 1987 a survey in the capital of the state (Rio de Janeiro city) showed the following prevalence: 30% for children younger than 5 years, 59% in the age range from 6 to 10 years, 69% for those between 11 and 15 years and 71% for persons ranging in age from 16 to 20 years (Souza et al., 1987).

Campos dos Goytacazes, which is located at north of Rio de Janeiro is the third city in terms of importance in the state. Enormous social disparities are seen in the region where about 70% of the population depends on the public health system, which cannot provide for even the basic necessities for the entire population. Local infectious disease specialists and ophthalmologists have expressed their concern regarding the great number of cases of acute toxoplasmosis in children as well as posterior uveitis caused by toxoplasmic infection. This concern led to an epidemiologic survey on toxoplasmosis, in Campos dos Goytacazes the results of which we present herein, as an initiative of a new University (the University of the North of Rio de Janeiro State-UENF).

5.1.3. Material and methods

Study population: The study population consisted of (A) individuals from schools and slums or poor communities participating in a survey for toxoplasmosis; (B) children assisted on a outpatient basis by a county health public programme for assistance to children and teens for the pilot study of seroprevalence of toxoplasmosis in neonates and young children; (C) neonates screened for toxoplasmosis based on specific IgM testing of filter paper blood samples, as described in detail below.

(A) The individuals participating in this survey belong to three different economic and social strata defined as follows: persons living in slums or very poor communities under precarious sanitary and poor social conditions ($N = 316$),

population 1 P1; lower middle-class children attending public school and the adult school's staff ($N = 564$), population 2 P2; and upper middle-class children attending private schools and the adult school's staff ($N = 337$), population 3 P3. The schools and slums or poor communities are located in four different geographic regions of the city, with urban, suburban or rural characteristics predominating. Only those who provided written consent were included in this study.

(B) Children who received assistance on a outpatient basis from a county public health program for assistance to children and teens were included in the pilot study of seroprevalence of toxoplasmosis in neonates and young children: a total of 100 neonates (from 7 to 28 days) and children (from 1 month to 6 years) who were randomly selected during 1998 for a pilot study of seroprevalence of toxoplasmosis in neonates and young children were included. Blood samples from babies and children were collected for routine examination as part of the protocol of the program for assistance to children and teens from the county public health service.

(C) Neonates screened for toxoplasmosis based on specific-IgM testing of filter paper blood samples. A total of 2550 neonates aged 3–20 days during April 1999 to June 2000 were tested for specific IgM in filter paper blood samples which were collected for evaluation of phenylketonuria and hypothyroidism in a county-funded program. The babies participating in the screening were randomly selected using days of the week as criteria for selection. Written informed consent was obtained from patients or their guardians according to the guidelines of the Ethical Committee from the FIOCRUZ (Ministry of Health, Brazil) for serological and filter paper testing, ocular examinations and interview surveys.

Serological assays: For the survey from P1, P2 and P3 and for the pilot study to investigate seroprevalence in neonates and young children the sera were tested for toxoplasmosis using a commercial ultramicro ELISA for specific IgG with fluorometric detection (UMELISA Toxoplasma, Havana, Cuba) at the Núcleo de Apoio Diagnostico (NUPAD) from the Federal University of Minas Gerais (UFMG). The correlation between the UMELISA and the SFDT, as well as the quality control of the UMELISA's system were evaluated by exchanging blind sera samples between NUPAD and the Serology Laboratory, Department of Immunology and Infectious Diseases, Research Institute, Palo Alto Medical Foundation. All the samples exchanged agreed 100% in terms of the value of titre and with regard to positivity or negativity for toxoplasmosis.

IgG and IgM measurements for confirmatory serological tests which are done for positive filter paper blood samples were performed using the kit VIDAS-TOXO-IgM (ELFA-Enzyme Linked Fluorecent Assay, Biolab-Merieux). The mothers of IgM positive babies were also tested for IgM and IgG. These tests were done in a private laboratory in Campos.

Neonatal screening assay: All assays were performed by one laboratory with expertise in toxoplasmosis neonatal screening (Centro de Triagem Neonatal in Porto Alegre RS) using a commercial capture *T. gondii* IgM fluorometric enzyme immunoassay (FEIA) (Labsystems)

Ophthalmic examinations: after pupil dilatation individuals were examined by a team of nine ophthalmologists from the UFMG, Federal Fluminense University in Niterói (UFF) and Campos dos Goytacazes. Indirect ophthalmoscopy was used to investigate the presence of posterior uveitis. At the time of the examination the ophthalmologists were unaware of the serologic status of the individuals. Active inflammatory retinal lesions as well as pigmented retinal or retinochoroidal lesions consistent with healed retinochoroiditis were observed. Every retinochoroidal lesion suggestive of an uveitic process, active or not, was notified. The size, number, and location of lesions, and inflammatory and vascular ocular disease were documented in a standardised protocol. All the healed and active lesions were classified independently of the serological results. According to their morphological characteristics, the retinal lesions were classified as type A, B and C, type A and B being those with the greatest probability of being caused by toxoplasmic infection. The signs taken into account for the classification were the degree of involvement of retina and choroid, judged mainly by spatial features of pigment mobilisation, gliosis and tissue atrophy. For babies with congenital toxoplasmosis ocular examination was carried out under anaesthesia by two ophthalmologists from Campos participating in this study.

Neonatal examinations took place according to a protocol which includes: routine general physical examination, neurological and ophthalmologic evaluations.

Analysis of data: The data were entered into the computer and analysed using Epi-info.

5.1.4. Results

We observed an increase in the seroprevalence by age for all population groups as shown in Fig. 5. The prevalence levels for P1 and P2 in the age range of 6–25 years is substantially higher in comparison with P3. The seroprevalence and the prevalence of ocular disease for the three populations (without considering age of individuals) are shown in Fig. 6. The overall prevalence of ocular disease for the three populations ranges from 10 to 12%, however, some communities within each strata that defined a population (see Section 5.1.2) showed differing prevalence of ocular disease which ranged from 8 to 14%. For instance, the lowest prevalence of ocular disease (8%) occurred in an urban slum community (with 110 invididuals) in which seroprevalence for toxoplasmosis is higher than 90% for individuals older than 25 years. On the other hand the highest prevalence of ocular disease (14%) was observed for a poor community (with 104 individuals) living in a predomi-

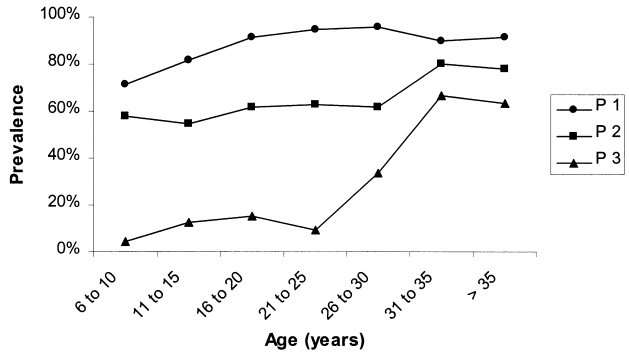

Fig. 5. Toxoplasmosis serum prevalence according to the age of three populations living under different sanitary and social conditions in Campos dos Goytacazes RJ. Population 1 (P1) comprised individuals living under precarious sanitary and poor social conditions ($N = 316$); population 2 (P2) comprised lower middle-class, individuals ($N = 564$); population 3 (P3) comprised individuals living under good sanitary and social conditions ($N = 337$).

nantly rural area in which the seroprevalence is close to 90% for persons older than 25 years (not shown).

In the preliminary data analysis of all three groups, factors associated with infection included (1) drinking unfiltered water; (2) drinking water from wells; and (3) cat ownership. Drinking filtered or spring water was protective. Only within the lower-middle class population (P2) was eating undercooked beef a risk factor (the very poor are unable to afford meat). Among the upper class population, no risk factors were identified.

The IgG seroprevalence for 100 neonates and children who were randomly selected during the year of 1998 for a pilot study of seroprevalence of toxoplasmosis (see Section 5.1.2) is shown in Fig. 7. About 40% of the babies had anti-*Toxoplasma* antibodies until the age of 5 months, from 6 to 36 months the prevalence of IgG anti-*Toxoplasma* decreases to 5%, which was expected because of the clearance of maternal IgG occurring by this age, and 22% of the babies ranging in age from 37 to 72 months had IgG against *T. gondii* antigens. This is in agreement with data for P1 and P2 (Fig. 3) where respectively 40 and 30% of the children by

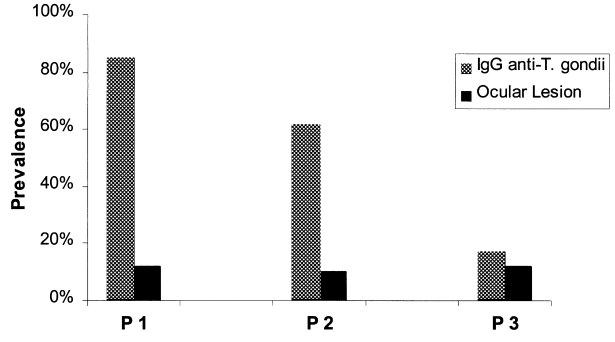

Fig. 6. Seroprevalence and toxoplasmic ocular lesions in the three populations, P1, P2, and P3, without considering the age of individuals.

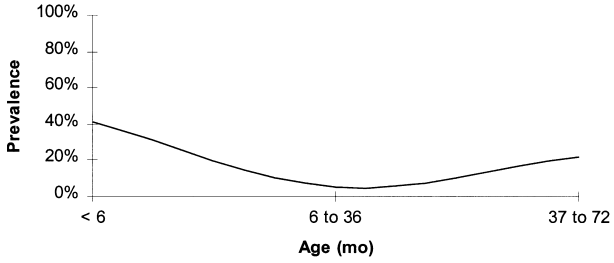

Fig. 7. Seroprevalence in neonates (3–28 days) and children in the age range of 1–5 months (30 individuals), 6 to 36 months (35 individuals) and 37–72 months (35 individuals). Neonates and children who have been randomly selected during the year of 1998 for a pilot study of seroprevalence of toxoplasmosis (see Section 5.1.2).

the age of 6 years are seropositive for toxoplasmosis. In fact, the children in the outpatient basis from the county public health program belong to the social strata P1 or P2. Two babies with toxoplasmosis presenting with symptoms were identified in the pilot study (Table 15) and during 1999, two neonates (not participating in the neonatal screening) were referred to the paediatrician of our group for examination for suspected suffering congenital toxoplasmosis; the serological IgM tests confirmed the disease, however both died even before treatment was initiated. They had systemic illness. One of them presented splenomegaly and enlarged lateral ventricles; the other had microcephaly and microphthalmy (not shown).

Screening of neonatal filter paper blood spots for *Toxoplasma*-specific IgM followed by confirmatory testing during April 1999 to June 2000 showed that 5/2550 babies had congenital toxoplasmosis. This study is still ongoing and the number of babies screened (2550) represents about 25% of the total births in Campos during that period. The clinical status of the babies is shown in Table 15. All the babies with congenital disease, independent of whether they had symptoms, have been treated according to regimens described by Remington et al. (1995).

5.1.5. Discussion

These data suggest that social and economic conditions influence risk factors for toxoplasmosis in the Rio de Janeiro state. The contribution of congenital toxoplasmosis for the total/global prevalence of toxoplasmosis in Campos is ongoing and data have revealed that birth prevalence of congenital toxoplasmosis is also elevated.

The lower seroprevalence of toxoplasmosis observed for P3 (17%) and P2 (61%) in comparison with P1 (86%) contrasts with the similar ocular prevalence found for the three populations namely, P3 (12%) P2 (10%) and P1 (12%). The P1, P2 and P3 prevalence curves (Fig. 5) suggest that the risk of women getting infected during pregnancy is high for those under better economic and social conditions (P3) in Campos. This gives rise to the question whether the ocular lesions found in the three populations are mainly

Table 15
Clinical status of babies with congenital toxoplasmosis in Campos

Baby Code	Age therapy begun (months)	Age Evaluation (months)	Signs/symptoms observed[a]	Treatment
1[b]	1	1	No symptoms or sign	PS
2[b]	1	1	No symptoms or sign	PS
3[b]	1	1	No symptoms or sign	PS
4[b]	1	1	Macular retinochoroidites (unilateral)	PS
5[b]	1	1	No symptoms or sign	PS
6[c]	4	4	Strabism, nistagmus, macular retinochoroidites (bilateral) cerebral calcifications	PS
7[c]	6	6	Abnormal cognitive development, mild motor development	PS

[a] Evaluation included general physical examination, neurologic (including computer tomography of the head) and ophthalmic evaluation. These children are participating in a 5-year follow-up which started 1999.
[b] Babies participating in the neonatal screening.
[c] Babies assisted in the pilot study (see Section 5.1.2).P, Pyrimethamine; S, sulfadiazine.

caused by congenital or acquired infection. However it is important to note that the profile of the P3 seroprevalence curve has to be interpreted carefully in terms of its value to estimate risk for persons living in Campos under good social and economic conditions to become infected with *T. gondii,* in particular for those older than 25 years. For the past 10 years a great number of persons from different regions of Brazil have migrated to Campos because of the oil exploration in the region. The demographic data of the P3 individuals must be carefully taken into account to help to clarify the profile of P3 seroprevalence curve. This is not applicable for P2 and P3 who are individuals not linked and not attracted to the oil exploration activities.

It is interesting that drinking unfiltered water appears as the first risk factor when analysis is done for the three populations together as well as when it is done for P1 and P2 separately. Attempts to isolate parasites from water reservoirs from Campos and surrounding have recently been implemented. An interesting and curious report from 1977 describing a case of a dolphin with disseminated toxoplasmosis, which was found dead in the Guanabara bay, in Rio de Janeiro capital (Melamed et al., 1989), opens the question of contamination of water reservoirs in the Rio de Janeiro state which should be carefully pursued. The dolphin species, *Sotalia guianensis* lives in estuaries which put them in contact with sources of fresh water potentially contaminated with oocysts of *T. gondii*.

The high prevalence of ocular disease (14%) in a rural poor community (with 104 individuals) as compared with the prevalence observed for another community (with 110 individuals) living in an urban region in which 8% of ocular lesions was found, is also an interesting finding since both communities belong to the same social and economic strata (P1). Anecdotal reports suggest higher prevalence of ocular disease for individuals living in rural areas (without an association with the habit of eating raw or undercooked meat) in comparison with their neighbors from urban regions in south of Brazil (Melamed personal communication.). It raises the question whether contact with soil contaminated with *T. gondii* oocysts would be an important risk factor for the development of ocular disease. It might be possible considering the fact that many variables such as host genetics, parasite strain, inoculum size, host age, sex hormones and immune status clearly modulate and affect outcome of experimental infections (Melamed et al., 1989). In this sense the chances of an individual to be infected with a large oocyst inoculum (concentrated in faeces of felines spread in soils of rural regions) is potentially higher in comparison with those not exposed to constant contact with soil from urban regions.

The prevalence of congenital infection in Campos is higher in comparison with that recently reported in Brazil estimated in a 3-year prospective neonatal screening study in which the prevalence was 1 per 3000 live births (Bandoli and Basilio de Oliveira, 1977) and those reported previously (Paul et al., 2000; Guerina et al., 1994; McLeod et al., 1996; Camargo-Neto et al., 2000). The long term follow-up of babies participating in this study will help to clarify the impact of asymptomatic congenital infections in the life of individuals. The social and economic profile of women having infants with congenital toxoplasmosis in Campos is still unknown, however it will be of vital importance for the implementation of health public preventive measures.

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6.1. The activity of Fansidar® in chronic murine toxoplasmosis-a combined serological and morphological study

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6.1.1. Introduction

Previous studies indicate that children with prenatal *Toxoplasma* infection benefit from prolonged therapy (Roizen et al., 1995). The standard therapeutic regimen is a combination of pyrimethamine and sulfadiazine given in treatment courses of 3 or 4 weeks separated by spiramycine treatment during the first year of life. However, rising antibody titres (serological rebounds) which are frequently observed in children after the end of a long-term therapy signal that the antiparasitic effect is only limited and temporary. Serological rebounds are rarely accompanied by clinical recurrences and do not occur during adequate therapy. There is evidence that in prenatally infected, postnatally treated children the antibody decline is faster than in untreated individuals (WHO, 1988). These findings support the probability that a release of drug pressure allows reactivation of the parasite. However, the findings do not exclude that an immunosuppressive effect of the chemicals or the parasite itself could prevent antibody maturation during therapy.

In general, our knowledge of the efficacy of the standard chemotherapeutic agents on *Toxoplasma* cysts is limited. In vivo studies with different *T. gondii*/mouse models have demonstrated that the number of *Toxoplasma* brain cysts decreases early after therapy but that cysts are not completely eliminated (Djurkovic-Djakovic et al., 2000; Couvreur et al., 1991; Araujo et al., 1993). The survival of *Toxoplasma* parasites during therapy and the subsequent reactivation may be due to either a limited anti-bradyzoite activity of the drugs or to the limited amount of chemicals which reach the brain.

Only a few in vivo studies give information on the antibody response of chronically infected mice during *Toxoplasma* therapy. To our knowledge long-term studies or studies on the antibody response after cessation of therapy (serological rebound) are not available. The aim of our study was to demonstrate the effect of a long-term, pyrimethamine/sulfadoxine (Fansidar®) application on brain cysts of chronically infected mice and to study the immune response during and after *Toxoplasma* therapy.

6.1.2. Materials and methods

Parasite: The cyst forming *T. gondii* strain 'Gail', a human isolate (1959) maintained in mice by continuous i.p. passage of brain cysts, was used for infection. According to the classification of Gross et al. (1991) it is an avirulent strain.

Mice: Thirty-eight female NMRI mice (own laboratory breeding), 6 weeks of age, were infected orally with brain cysts from five chronically infected mice. The brains were dissected, ground with pestle and mortar and resuspended in a sterile solution of 0.9% NaCl. The mean value of cysts detected in 10×10 μl of homogenate was used to calculate the approximate total cyst number. The homogenate was then diluted with 0.9% NaCl to get an infection dose of seven cysts per 200 μl per mouse. Three months after *Toxoplasma* infection all animals were tested for *Toxoplasma* antibodies by SFDT and proved to be positive with titres ranging from 1:4000 to 1:64 000.

Five female mice from the same age-group were kept uninfected until treatment started (therapy control group). Blood samples were collected in vivo by retro-orbital puncture or from the heart at autopsy as indicated in Table 16.

Treatment: Before treatment (3 months after *Toxoplasma* infection) the mice were randomly divided into four groups (Table 16). Groups A–C received therapy for a period of maximum 30, 60 and 90 days, respectively. Five uninfected mice were treated continuously for 90 days (therapy control animals). The remaining five *Toxoplasma* infected mice (group 4) were kept untreated as *Toxoplasma* control animals.

Fansidar® (Roche) was diluted in a solution of 4% BSA in PBS, pH 7.2, to give a final solution of 0.55 mg pyrimethamine and 11 mg sulfadoxine per 100 μl. Every 3 days each mouse received 100 μl of the solution s.c. corresponding to an average dose of 18.3 mg of pyrimethamine and 367 mg of sulfadoxine per kg of body weight. The mean body weight of the mice was 30 g when treatment started.

Parasite detection: Tissue and blood samples were recovered from animals during (subgroup a) or after (subgroup b) treatment as indicated in Table 16. At autopsy the brain of each mouse was immediately removed for microscopic and histologic studies. At first, the weight of the whole brain was determined. Thereafter, the brain was cross-sectioned and the caudal part cut longitudinally into two pieces. The weight of the resulting two caudal pieces was recorded.

Cyst count: The left caudal piece of each brain was homogenised in 0.3 ml of 0.9% NaCl. The whole homogenate was examined microscopically for the presence of cysts. The right caudal piece was cut into two sections. Each section was squeezed between two slides, the number of tissue cysts was determined microscopically and the result of both sections added. Finally, the total brain cyst number of one animal was calculated in relation to the weight of the respective caudal brain piece and the weight of the total brain.

Histology, electron microscopy: the frontal brain part was cut longitudinally. The left part was immediately fixed in 4% formaldehyde, the right part in 2% glutaraldehyde. The

Table 16
Treatment and observation periods of chronically *T. gondii*-infected NMRI mice[a]

Group	Fansidar® treatment	Mouse no.	Autopsy[b]	Blood Sample[b]
Aa	Up to 30 days	4	18	0, 18
Aa		9	27	0, 27
Aa		15	18	0, 18
Aa		20	27	0, 12, 27
Ab		1	76	0, 38, 56, 68, 76
Ab		2	110	0, 38, 56, 68, 76, 110
Ab		3	76	0, 38, 56, 68, 76
Ab		5	110	0, 38, 56, 68, 110
Ab		6	55	0, 38, 55
Ab		7	46 em	0, 38
Ab		8	46	0, 38
Ab		10	55	0, 38, 55
Ab		11	140	0, 49, 140
Ba	Up to 60 days	13	49	0, 49
Ba		16	49	0, 49
Ba		21	41	0, 38, 41
Ba		22	41	0, 12, 41
Bb		12	111	0, 49, 111
Bb		14	111	0, 49, 111
Bb		17	140	0, 49, 140
Bb		18	75	0, 49, 75
Bb		19	75	0, 49, 75
Ca	Up to 90 days	29[c]	88	0, 49, 70, 88
Ca		30[c]	88	0, 49, 70, 88
Ca		31[c]	63 em	0, 12, 49, 63
Ca		32[c]	77 em	0, 12, 49, 70, 77
Ca		33[c]	63	0, 12, 49, 63
Cb		23[c]	161 em	0, 70, 90, 113, 138,161
Cb		24[c]	119	0, 49, 70, 90, 113, 119
Cb		25[c]	182 em	0, 70, 90, 113, 138, 165, 182
Cb		26[c]	103 em	0, 49, 70, 90, 103
Cb		27[c]	103	0, 49, 70, 90, 103
Cb		28[c]	119	0, 49, 70, 90, 113, 119
4	No	34[c]	85	0, 54
4		35	161 em	0, 54, 113, 139, 161
4		36	32 em	0, 32
4		37[c]	139	0, 54, 113, 139
4		38	4 em	0, 4

[a] em, brain tissue examined by electron microscopy; (a), mouse autopsied during treatment; (b), mouse autopsied after treatment.
[b] Days after onset of therapy.
[c] Parasite detection by PCR.

material was then submitted to standard embedding as described earlier (Reiter-Owona et al., 1996). Paraffin sections were cut 4–6 μm thick, stained by HE, or used for immunohistochemistry. Electron microscopic studies were performed as indicated in Table 16.

Immunohistochemistry was performed on selected, serial paraffin sections after clearance in xylene and acetone. A rabbit hyperimmune anti-*Toxoplasma* serum (rabHS) diluted 1:300 in PBS (pH 7.6) was applied as primary antiserum. The rabbit antibody was detected by an avidin-biotinylated horseradish peroxidase (HRP) complex technique with the Vectastain® kit (Camon Labor-Service). Visualising of the peroxidase-labelled secondary antibody was by diaminobenzidine (Liquide-DAB). Slides were counterstained with haemalaun.

Serology: Antibodies to *T. gondii* were determined by SFDT as described earlier (Reiter-Owona et al., 1998). *Toxoplasma* antibodies of the IgM and IgA class were detected by an ISAGA described elsewhere (Saathoff and Seitz, 1991) with some variations. Plates were coated with a polyclonal sheep anti-mouse IgM or IgA antibody (Serotec Ltd.) diluted 1:200 or 1:250 in 0.1 M carbonate buffer (pH 9.8). *Toxoplasma* antigen (Antigen Toxo AD, bioMérieux) was diluted 1:12 and 1:20, respectively. Antibodies to BSA were detected by ELISA. Plates were coated with a 10% BSA/carbonate buffer (pH 9.8) solution. Mouse serum samples (first dilution 1:64) and goat anti-mouse HRP conjugate (1:1000, Southern Biotechnology) were diluted in a solution of 10% human serum in PBS (pH 7.2). Visualisation was by OPD at 450 nm after 10 min of incubation.

PCR: DNA was extracted from 200 μl of mouse blood by means of the QIAamp blood kit (QIAGEN) following the manufacturer's instructions. In some cases the volume had to be adjusted to 200 μl with physiological saline. Amplification was based on the 35-fold repetitive B1 gene of *T. gondii* (Burg et al., 1989), making use of the primers TOXOB22 and TOXOB23 as described (Bretagne et al., 1993) as well as of the primers 1 and 2 (Eggers et al., 1995). With each DNA specimen two separate PCR assays were run with the different sets of primers, thereby one amplification result serving as a specificity control for the other.

Reaction mixtures consisted of 200 μM of dNTPs each, 10 pmol of each of the two primers (either TOXOB22/TOXOB23 or 1/2), 10 mM KCl, 10 mM Tris–HCl, pH 8.3, 2 mM $MgCl_2$, 1.25 units of AmpliTaq DNA-polymerase Stoffel-fragment (Perkin–Elmer), 10 μl of DNA solution, and sterile water to give a final volume of 50 μl. Negative controls (sterile distilled water instead of DNA solution as well as QIAamp blood kit processed physiological saline) and a positive control (QIAamp blood kit extracted *Toxoplasma* DNA, strain BK, corresponding to three tachyzoite DNA equivalents) were included.

Samples were cycled as described (Bretagne et al., 1993). Amplification products were separated on a 1.8% agarose gel, ethidium-bromide stained, and analysed under UV light.

6.1.3. Results

The Fansidar® application was well tolerated by all short- and long-term treated animals without severe side effects. The total brain cyst number recorded was generally higher in the tissue smear probes than in the tissue homogenate examined from each animal in parallel. Therefore, the results are generally expressed as mean values of the results from both methods. A significant reduction in the total brain cyst numbers of treated animals was recorded early after therapy (Fig. 8). The concentration of *Toxoplasma* cysts

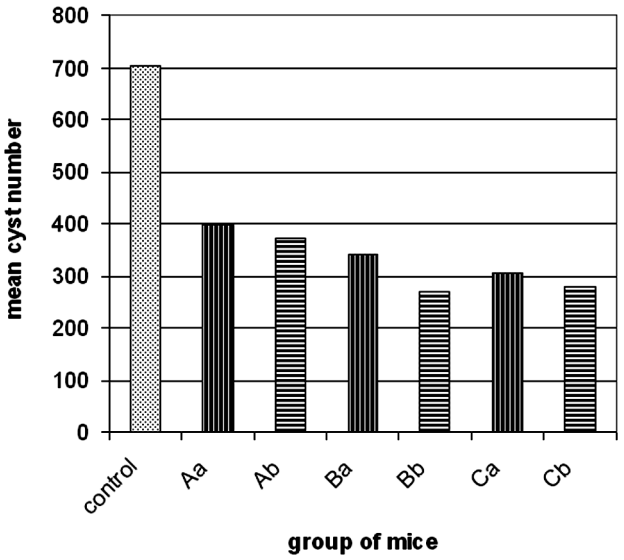

Fig. 8. Reduction in *T. gondii* brain cyst numbers during Fansidar® therapy. Duration of therapy: (A) up to 30 days; (B) up to 60 days; (C) up to 90 days. (a) Mice autopsied during therapy; (b) mice autopsied after therapy.

detected in the brains of mice examined during Fansidar® treatment was 56% (group Aa), 48% (group Ba), and 43% (group Ca) in comparison with the control group (group 4). The concentration of brain cysts in mice examined after cessation of therapy was 52% (group Ab), 38% (group Bb), and 39% (group Cb), respectively (Fig. 8). Histological examinations revealed little inflammatory response in short- or long-term treated mice and very few signs of inflammation in the control animals. In some of the immunolabelled sections inflammatory nodules surrounding amorphous *Toxoplasma*-positive material (Fig. 9a) or degenerating cysts with invading glia cells (Fig. 9b) were found. There was no sign for cyst disruption or infection of the brain tissue by extra-cystic parasites. The distribution of differently sized tissue cysts was similar in treated and non-treated animals.

The ultrastructural study of brain tissue from treated animals during and after therapy was difficult due to the low number of tissue cysts present. Bradyzoites in the cysts of control animals were lying in close contact within the electron-dense ground substance, whereas in cysts of treated animals bradyzoites tended to be loosely packed within a electron-lucent ground substance. Within the *Toxoplasma* cysts detected, degenerating bradyzoites (mice nos. 7, 26), chromatin aggregations at the border of the bradyzoite nucleus (mouse no. 32) as well as alterations of the cyst wall structure (mice nos. 26, 32) were seen (Fig. 10a,b). In some cysts the wall appeared collapsed and thickened as if several sections of the limiting membrane were lying on top of each other. In mice nos. 25 and 26, autopsied 92 and 13 days after the end of a 90-day treatment, multiplying bradyzoites (endodyogeny) were detected (Fig. 11).

The anti-*Toxoplasma* SFDT and IgM-/IgA-ISAGA antibody responses remained stable in the control group and in the short-to medium-term treatment groups A and B. In group C, however, there was a slight decline in the SFDT (Fig. 12) and IgM-/IgA-ISAGA antibody response in animals treated more than 60 days. After cessation of the 90 day treatment course there was a slight increase of the antibody response of one (mice nos. 23, 24, 26, 27, 28) or two (mouse no. 25) dilution steps (Fig. 12, SFDT). In parallel, there was a steep rise of the anti-BSA antibody response

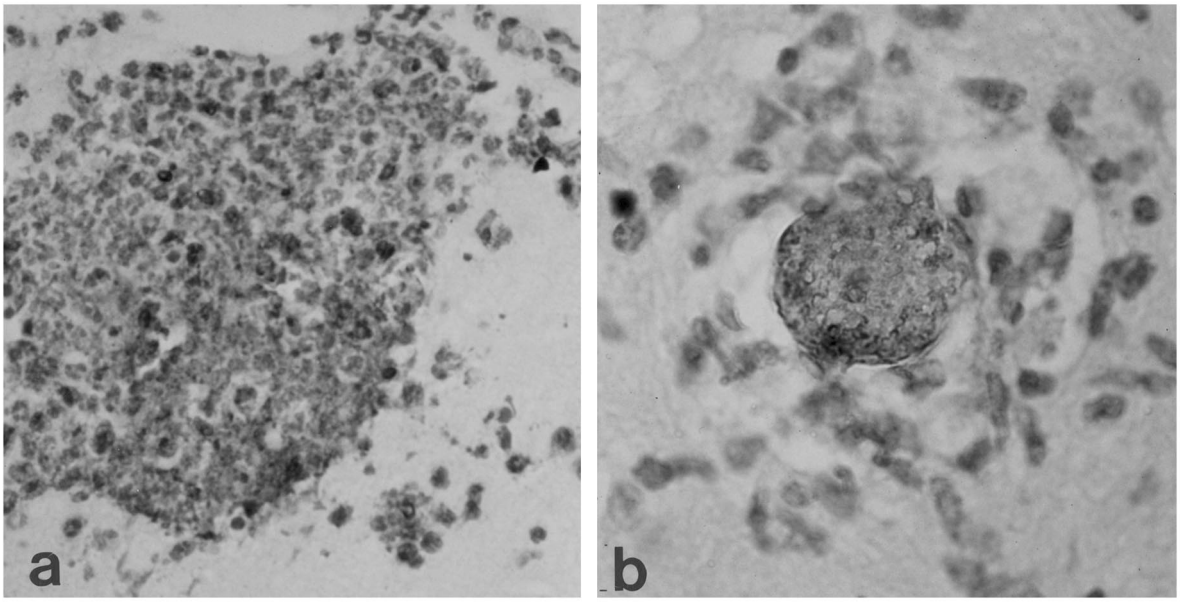

Fig. 9. Immunoreaction in brain tissue sections after labelling with a polyclonal *Toxoplasma* antibody (rabHS, brown label) and counterstaining with haemalaun. (a) Inflammatory nodules surrounding amorphous *Toxoplasma*-positive material (mouse no. 24, autopsy 29 days after a 90-day treatment cycle; 462×); (b) degenerating cyst with invading glia cells (mouse no. 20, autopsy 27 days after Fansidar® treatment; 693×).

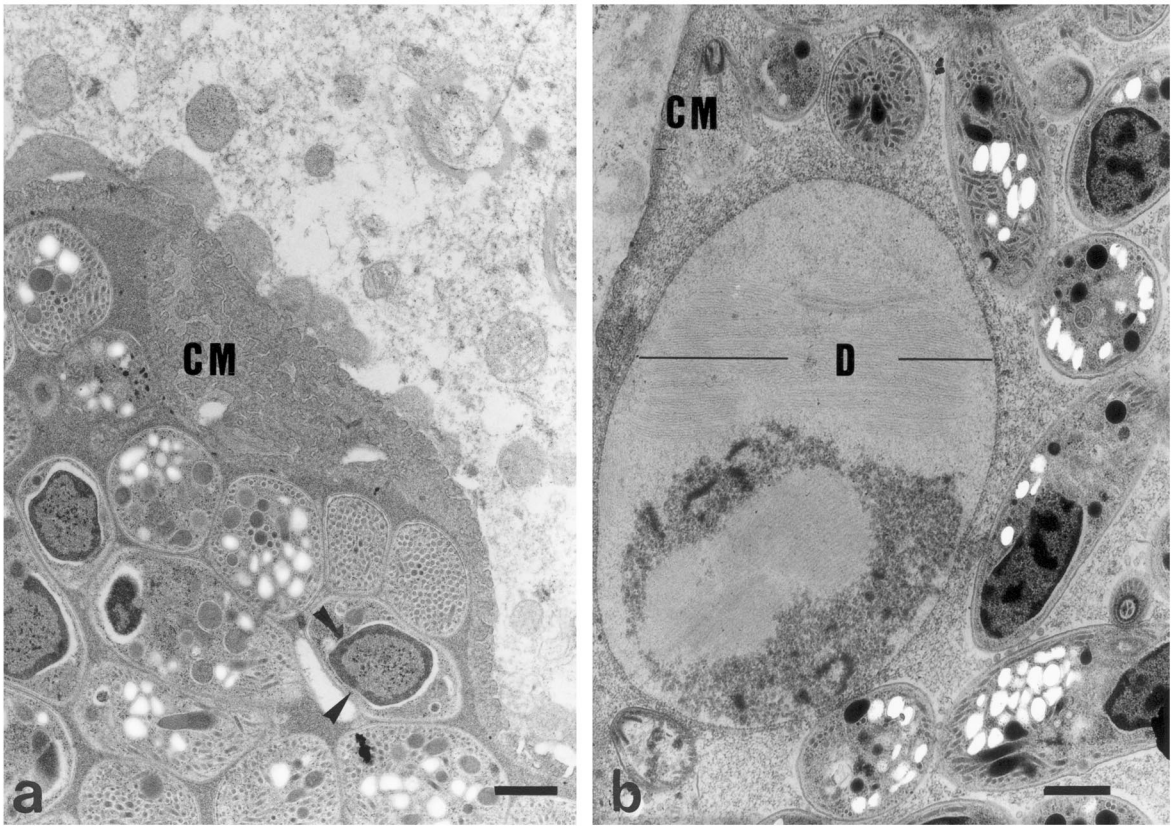

Fig. 10. Electron micrographs of *T. gondii* brain cysts in mice autopsied at different times during or after Fansidar® therapy (bars = 1.1 μm). (a) Chromatin aggregation at the border of the bradyzoite nucleus (►) and multiple, deeply invaginated layers of the cyst membrane (CM). Mouse no. 32, autopsied at day 77 during Fansidar® treatment; (b) large defect (D) within the cyst matrix seen in mouse no. 26, autopsied 13 days after a complete 90-day Fansidar® treatment (CM = cyst membrane).

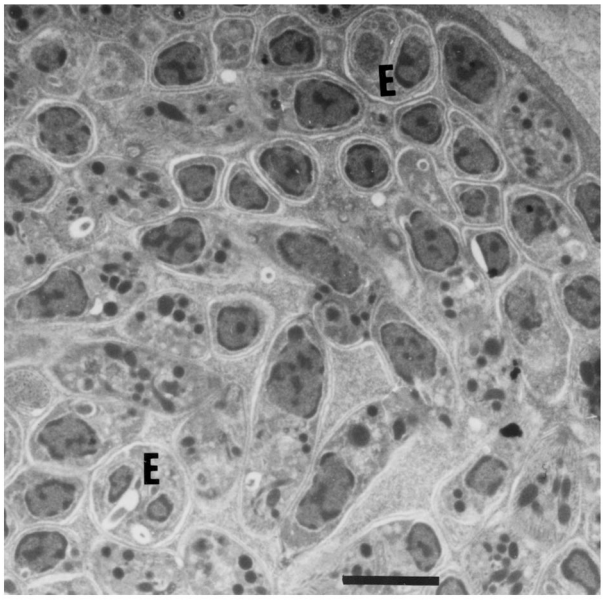

Fig. 11. Multiplying bradyzoites (endodyogeny, E) within a brain cyst of mouse no. 25, autopsied 92 days after a 90-day Fansidar® treatment period. (scale bar, 1.7 μm; CW, cyst wall).

in all treated *Toxoplasma*- infected mice within the first 40 days after Fansidar® application (Fig. 12b). Parasite detection by PCR was negative in all 13 blood samples examined (Table 16).

6.1.4. Discussion

As presumed for the human host, an infection of NMRI mice with the low-virulent 'Gail' strain leads to a non-progressive encephalitis during acute infection and a stable brain cyst number at medium level during chronic infection (personal observations). The results of our study demonstrate a partial efficacy of a low-dose Fansidar® therapy on the cyst stages of *T. gondii* which increases with prolonged therapy. The findings support the recommendations of different treatment trials (McAuley et al., 1994; Roizen et al., 1995) that prenatally infected newborns profit from a prolonged, continuous treatment.

The reduction of the brain cyst load seen in our study is within the reported range of other in vivo models. Using a similar parasite/mouse model but a high-dose, continuous pyrimethamine treatment (3×150 mg/kg/day) Sarciron et al. (1998) observed an even more pronounced reduction of the brain cyst number (77%) within 5 days. Similar to our

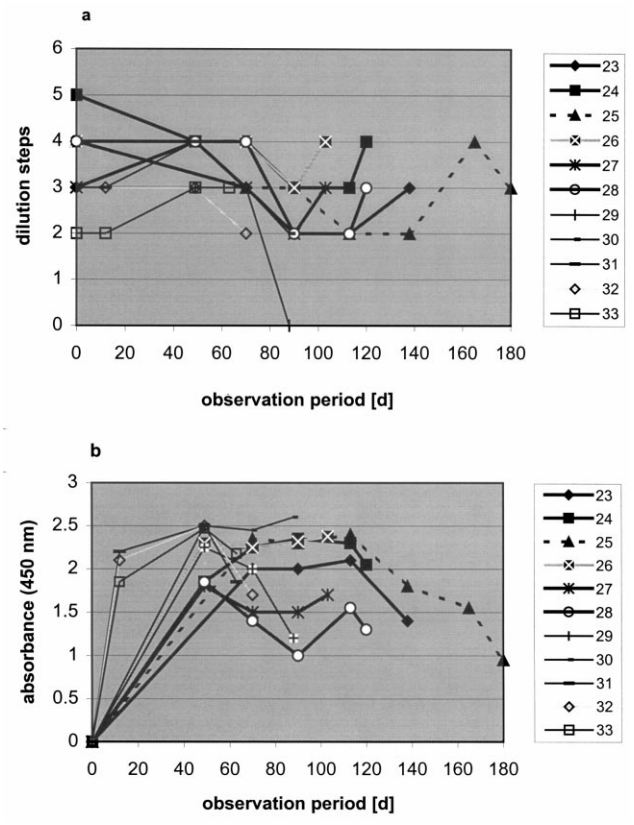

Fig. 12. Antibody development in mice of group C during and after a 90-day Fansidar® treatment period. (a) Individual dye test (DT) titers of mice nos. 23–33. Dilution steps: 4-fold, beginning at 1:64 (0); (b) anti-BSA antibody formation in mice nos. 23–33.

histological findings in short- and long-term treated mice, the cyst reduction was not correlated with an immunological reaction in the brain tissue. An inflammatory response of the cerebral tissue was reported when specific treatment was initiated shortly after *Toxoplasma* infection (Araujo et al., 1993) or before complete maturation of brain cysts (Ferguson et al., 1994) when proliferative stages of the parasite are still present.

Cysts affected by therapy obviously disappear from brain tissue fast and absolutely. A previous report described that there is no difference in the number or the micromorphology of mouse brain cysts whether treatment (50 mg/kg pyrimethamine and 500 mg/kg sulfamethoxypyrazine) was given for 10 days or 25 days (Werner et al., 1979). This confirms our findings that it is difficult to demonstrate the self-destruction of the affected cysts by morphological methods when the brain is examined only 3–4 weeks after the onset of therapy.

A decrease in the average cyst size during in vivo (Ferguson et al., 1994; Sarciron et al., 1998) and in vitro (Ricard et al., 1999) treatment as well as an increase (Werner et al., 1979) was reported. Cyst size, which is not only determined by the time after infection (small = young cyst; large = mature cyst) but also by the *T. gondii* strain used (personal observation), may vary in different mouse/parasite models. Our histological studies did not evidence a significant difference between treated and untreated animals. However, electron microscopy revealed degenerating and lysed bradyzoites which, as suggested previously (Ferguson et al., 1994), will finally be absorbed by the host cell and cause a shrinkage of the cyst. Cyst shrinkage seems a possible explanation for the multimembraneous wall structure seen after treatment.

In our study, the destruction of more than 50% of brain cysts within the first treatment cycle had no effect on the specific antibody response of the animals. This is surprising when we assume that extracerebrally located *T. gondii* cysts will be destroyed to an even higher degree during Fansidar® application. The fast anti-BSA antibody response of the treated mice during the period of cyst destruction on the one hand and the non-responding *Toxoplasma*-specific antibodies together with a weak cellular reaction of the cerebral tissue on the other hand, possibly indicate a specific, immunosuppressive effect of parasitic antigens.

To summarise, a long-term anti-*Toxoplasma* therapy in chronically infected mice is only partially efficacious. We doubt whether a high-dose, more toxic therapy would result in a complete destruction of cysts. As suggested by Werner et al. (1979) it is not the cyst membrane (or the intracerebral location) but the dormant bradyzoite metabolism of selected cysts which limits the efficacy of the drugs applied. However, we could prove that cysts and their bradyzoites return to active metabolism when drug pressure is released. Endodyogeny, which is a rare event in mature cysts was demonstrated in animals autopsied 13 and 92 days after cessation of a 90-day Fansidar® treatment. The activation of bradyzoites, however, is limited to intracystic stages and does not result in a reactivation of the disease as demonstrated by the negative PCR results and the stable low cyst counts. In children, the rising antibody titres (serological rebound) seen after cessation of long-term therapy are also not correlated with clinical symptoms (Djurkovic-Djakovic et al., 2000; Coureur et al., 1991) or the presence of parasites in the peripheral blood (personal observation). Whether the intracystic reactivation of *T. gondii* alone may be responsible for serological rebounds in children could not be demonstrated by the mouse model due to the non significant changes in antibody titres.

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