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HIV infections and AIDS: continuous vigilance needed to contain the epidemic

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The 1 December, known as World AIDS Day since 1988, provides an occasion to raise awareness and take stock of the latest developments in the human immunodeficiency virus (HIV)/acquired immunodeficiency syndrome (AIDS) epidemic. Since it was first recognised in the early 1980s, this epidemic has been associated with high morbidity and mortality. UNAIDS, the Joint United Nations Programme on HIV/AIDS, estimates that in 2007 alone two million people have died of AIDS worldwide, of whom around 270,000 are children under 15 years of age [1]. An additional 33 million people are living with HIV globally; the estimated number of new HIV cases in 2007 was 2.7 million. These figures clearly demonstrate that HIV/AIDS remains a major challenge to public health; therefore measures to contain the epidemic are of paramount importance.

World AIDS Day is an opportunity to shed light on the many activities and initiatives that are being conducted at national and international levels to fight the spread of the disease. From its beginning in late 1995 until now, Eurosurveillance has focussed extensively on HIV/AIDS. The journal has closely monitored the epidemic primarily in Europe but has also reported on worldwide trends. The first publication on HIV/AIDS in March 1996 by F. Cazein et al. entitled Prevalence of HIV-2 infection in Europe [2] was followed by regular annual updates on the situation, and over the years we have covered a wide range of associated aspects of the infection and their impact on public health, such as therapeutic advances including post-exposure prophylaxis, novel testing methods, behavioural factors and prevention measures. In September 2008, a special issue was dedicated to the widespread advances made in Europe in estimating the real number of newly acquired HIV infections based on STARHS (Serological Testing Algorithms for Recent HIV Seroconversion) assays [3]. Next week's issue of the journal will include short communications providing an epidemiological update on the HIV/AIDS situation in Europe while analysing the latest figures on HIV/AIDS surveillance in the WHO European Region, highlighting the situation in intravenous drug users in Europe and reporting on the continuing HIV and other sexually transmitted infection epidemics in the United Kingdom.

The driving forces of the HIV/AIDS epidemic are manifold and transmission patterns vary geographically. In the European Union (EU), the predominant transmission mode remains unsafe sex between men, whereas reported heterosexual transmission is in part attributed to persons from high-prevalence countries outside the EU. In eastern Europe and the Baltic States an important driver of the epidemic is intravenous drug use [4,5]. To stop the spread of the disease it is crucial to have a thorough knowledge of the transmission routes and of other factors contributing to the epidemic as well as to ensure access to testing, treatment and care for all. Furthermore, campaigns are needed to raise awareness of the risk of contracting infection and of the possible preventive measures. If such campaigns are to reach their target audiences, they must be tailored to the existing knowledge, attitudes and behaviour in the general population as well as in specific populations at risk. In this issue of Eurosurveillance an article by S.A. Cowan and J. Haff reports on the results of a survey conducted in Denmark in 2006 on HIV and risk behaviour among men who have sex with men (MSM) [6]. The results show that in this group in Denmark, the numbers of sex partners and unsafe sex practices are increasing compared to those of three earlier surveys conducted since 2000. A total of 33% of the respondents had practised unsafe sex, defined as unprotected anal intercourse with one or more partners of different or unknown HIV status. The number of partners was the strongest predictor of unsafe sex; the probability of having had unsafe sex ranged from 17% in men with one partner to 58% in men with more than 20 partners. HIV status was also a strong predictor; in a bivariate analysis, 49% of HIV-positive men had practised unsafe sex compared to 25% of HIV-negative men. The results of this survey demonstrate a clear need to respond to such ongoing risky behaviour in MSM and should be compared with the findings of similar studies in other countries in Europe and taken into consideration when designing targeted prevention campaigns in the future.

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Phylogenetic analysis of West Nile virus isolated in Italy in 2008

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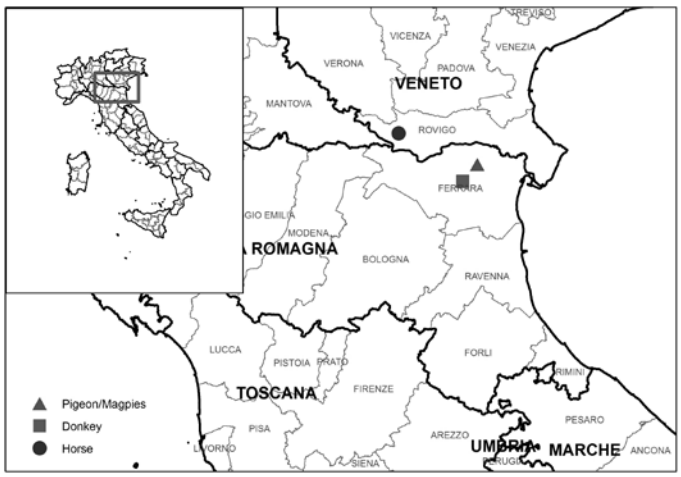
In Italy the first occurrence of West Nile virus (WNV) infection was reported in Tuscany region during the late summer of 1998. In August 2008, the WNV infection re-emerged in Italy, in areas surrounding the Po river delta, and involving three regions Lombardy, Emilia Romagna and Veneto. WNV was isolated from blood and organs samples of one horse, one donkey, one pigeon (*Columba livia*) and three magpies (*Pica pica*). The phylogenetic analysis of the isolates, conducted on 255 bp in the region coding for the E protein, indicates that these isolates belong to the lineage I among the European strains. According to the analysis, both the 1998 and 2008 Italian strains as well as isolates from Romania, Russia, Senegal and Kenya fell in the same sub-cluster.

Introduction

In Italy the first occurrence of West Nile virus (WNV) infection was reported in Tuscany region during the late summer of 1998 [1]. Since 2001, a national surveillance system has been in place in Italy, based on the periodical testing of sentinel-chicken flocks and sentinel horses [2]. Apart from sporadic seroconversions occurring in few horses and in few sentinel chickens, no further WNV outbreaks had been reported before 2008 either in horses or in humans.

Figure 1

Location of equine stables (horse and donkey) and sites (pigeon and magpies) were the animals were found from which West Nile virus (WNV) was isolated; Italy, 2008



In August 2008, the WNV infection re-emerged in Italy, in areas surrounding the Po river delta, involving three regions Lombardy, Emilia Romagna and Veneto. Following the evidence of first outbreaks in equines [3], extensive monitoring was carried out including syndromic surveillance in horses as well as laboratory analysis of samples collected in horse stables and from wild and domestic birds. In the infected stables insect traps were placed, and mosquitoes collected and identified. Mosquitoes, blood, serum and tissue samples from horses and birds within and around the outbreak area were collected and tested both serologically and virologically.

As a result, West Nile virus strains have been isolated from blood samples of one horse of the Rovigo Province and one donkey kept in a stable in the Ferrara Province, and from pools of brain, kidneys, heart and spleen of one pigeon (*Columba livia*) and three magpies (*Pica pica*) caught in the same territory (Figure 1). All these isolates were sequenced and found to be identical considering the 255 bp in the region coding for E protein. Full genome sequencing is ongoing.

Virus isolation and phylogenetic analysis

Virus isolation was performed on Vero E6 cell monolayers, RK13 or C6/36 followed by Vero E6 passages. The growth of WNV was confirmed either by reverse transcription polymerase chain reaction (RT-PCR) or by immunofluorescence (IFA).

Total RNA was extracted from WNV isolates. Viral RNA was reverse transcribed and amplified by using the one step RT-PCR kit (Qiagen, Germany). Available sequences were collected from Genbank and aligned to determining highly conserved genomic sequences flanking the genome region coding for the envelope (E) protein. Based on the alignment, a pair of primers was designed to amplify a 1,058 bp region. PCR products were purified with the Qiaquick PCR Purification kit (Qiagen, Germany) and used for direct sequencing in both directions using the following primers: WN_E_484F: 5'-actcaggcagggagattca -3', WN_E_622R: 5'-ttccgacagtcatcacgtagta -3', WN_E_634F: 5'-ttggtccatcgtgagtggt -3', WN_E_768R: 5'-gcccaatgctatcacagact-3'.

Raw sequence data were assembled using Contig Express (Vector NTI suite 9.1, Invitrogen, USA) and consensus sequence (Genbank FJ471491) aligned with the corresponding sequences deposited in the Genbank database (D00246, AF260967, AY033389, AF317203, AF001570, AF146082, AF130362, AF260969,

AF404757, AF260968, AF001567) with ClustalW [4]. The aligned partial E gene sequences were used to generate a table of pairwise distances to evaluate the variation within the strains and translated into amino acid sequences using Vector NTI suite 9.1 (Invitrogen, USA) and aligned with ClustalW.

The phylogenetic analyses were conducted on 255 bp in the region coding for the E protein by using Phylogeny Interference Program Package (PHYLIP, version 3.6a, [5]).

Results

The 255 bp sequence of the genome region coding for the envelope (E) protein of the WNV isolates showed a 98.8% nucleotide similarity with the strain isolated in Tuscany during the 1998 and a complete similarity (100%) of the deduced amino acid sequence. According to the partial sequences of protein E, the 2008 Italian strain was similar to the Romanian 1996-7, Volvograd 1999, Senegal 1993 and Kenyan 1998 strains. However it showed 3.5% of divergence with the United States and Israeli strains which had almost identical E sequences.

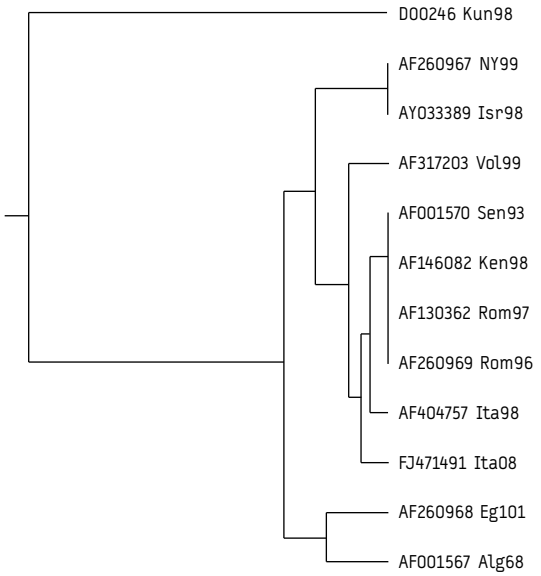
The phylogenetic analysis of the 255 bp of the E gene of the WNV isolates included, with high bootstrap support, the Italian isolate in the lineage I among the European strains. According to the analysis, both the 1998 and 2008 Italian strains as well as isolates from Romania, Russia, Senegal and Kenya fell in the same sub-cluster (Figure 2).

Discussion

Although additional observations on the outbreaks and investigations of the cases are still in progress, the information available allows the assumption that a new epidemic of West Nile disease is occurring in Italy after 10 years of apparent silence. The results of phylogenetic analysis indicate that the current epidemic is caused by a strain of WNV included in the lineage I which showed high nucleotide and amino-acid similarity to the strain responsible for the 1998 outbreak in Tuscany. Up to now, however, there is no evidence of any direct epidemiological link between the two Italian outbreaks. The fact that in both outbreaks the areas where the infection took place were close to wildlife nature reserves, in which a consistent population of wild migratory birds rests, might support the hypothesis of a new introduction of the virus by migratory birds. Additional epidemiological investigations are currently ongoing.

FIGURE 2

Phylogenetic analysis of West Nile virus (WNV) based on 255 bp partial nucleotide sequence of the E gene; isolates from animal samples, Italy, 2008



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A persistent problem with scabies in and outside a nursing home in Amsterdam: indications for resistance to lindane and ivermectin

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An ongoing outbreak of scabies in and outside a nursing home in Amsterdam is described. Despite standard treatment with lindane and ivermectin, many recurrences were observed which suggested resistance to these drugs. After treatment with 5% permethrine, the patients were finally cured.

Introduction

Between September 2007 and March 2008, recurrent scabies occurred among patients and staff members of a nursing home in Amsterdam. All patients could be linked to one "index" patient who died in this nursing home. Outbreaks of scabies in nursing homes are generally caused by patients with a very infectious form of scabies, also called scabies crustosa. In the Dutch guidelines for the treatment of scabies 1% lindane is foreseen as single treatment, in nursing homes combined with ivermectin (200 µg/kg), to be repeated after one week in case of extensive skin infections. It is recommended to treat asymptomatic contacts prophylactically with either lindane or ivermectin.

Outbreak description

In November 2007, the Public Health Service (PHS) in Amsterdam was notified that a nursing home doctor in Amsterdam had been diagnosed with scabies and that another staff member who worked in the same nursing home had been diagnosed with scabies in September 2007. At the same time, the nursing home had received information from a hospital in town that a patient, who had been discharged and transferred to the nursing home in August, probably had had scabies at the time despite previous treatments. This "index" patient had died in September 2007.

Subsequent surveillance in the nursing home showed that two of the 15 patients who stayed on the same floor as the index patient, and eight of the 83 staff members who worked on this floor had symptoms of itching. In five of the 10 affected people scabies was diagnosed by detection of mites. In addition to the standard cleaning procedures regarding clothes, bed linen and floor, all people with symptoms of itching or skin eruptions, including their family contacts, were treated with lindane and ivermectin. Contacts without symptoms received ivermectin only. During the following months, the nursing home was advised to survey patients and staff members actively for itching, and to consult a dermatologist to confirm the diagnosis in case scabies was suspected. Information regarding the scabies outbreak was sent to patients who had been discharged from the nursing home since August 2007.

In December 2007, three patients on another floor of the nursing home developed itching. They were treated with lindane, unfortunately without prior consultation of a dermatologist. In January 2008, scabies was diagnosed, through detection of mites, in two further patients living on yet another floor. They were treated with lindane and ivermectin, while the remaining tenants of that floor as well as staff working on that floor were treated prophylactically with ivermectin. Standard cleaning/washing measures were taken.

Recurrence of scabies in the nursing home

In January 2008 scabies was diagnosed, through detection of mites, for the second time in five staff members. Subsequently they were treated twice with permethrin 5% and ivermectin with one week interval, and afterwards remained free of scabies symptoms for at least three months.

In February and March 2008, the first recurrences of scabies (diagnosed by detection of mites) were reported in two of the patients who had previously been diagnosed with scabies. A room mate of theirs, who had been treated prophylactically with only ivermectin in 2007, was now also diagnosed with scabies by detecting mites. All three patients were subsequently treated twice with permethrin 5% and ivermectin. No further recurrences of scabies were diagnosed for at least three months. Asymptomatic room mates were given ivermectin only, also twice with one week interval.

Also in March, three staff members developed scabies (mites detected) who had received prophylactic ivermectin only. After treatment with the revised regimen, they remained free of symptoms for at least three months.

Scabies outside the nursing home

Before admittance to the nursing home, the index patient had lived in an apartment building with a common laundry. Two co-

tenants had frequently visited her in the nursing home and were diagnosed with scabies by detecting mites and were subsequently treated with lindane and ivermectin in November 2007. The other users of the laundry in this apartment building were at the same time treated prophylactically with lindane. Standard hygiene measures were taken. In January 2008, these two co-tenants developed recurrent scabies (mites detected) and now received ivermectin twice with one week interval. In late February, they had recurrent scabies again (mites detected). Only after treatment with permethrin 5% and ivermectin they remained symptom-free for at least three months.

The daughter in law of the index patient and her husband had suffered from scabies and received repetitive treatments with lindane and ivermectin since September 2007. They contacted the PHS in March 2008 because the treatment had still not been successful. After treatment with 5% permethrin, they remained free of scabies symptoms.

In November 2007 the PHS was notified that an ex-tenant of the nursing home was diagnosed with scabies (mites detected). After treatment with lindane she had recurrent scabies in February 2008. After treatment with permethrin 5% and twice with ivermectin, she remained free of scabies symptoms.

In February 2008, the PHS was notified of yet another recurrent scabies diagnosis (mites detected) in a hospitalised patient who had stayed in the nursing home in September 2007 and developed symptoms of itching after his stay. Having received the information letter from the nursing home, he had been treated for scabies in November 2007 with lindane and ivermectin. When he was hospitalised in December, he was again diagnosed with scabies and treated with lindane and ivermectin for the second time. In February, when he was admitted to hospital again, it appeared that he was still suffering from scabies. He then received the revised treatment, but the results could not be evaluated as he died shortly after.

Also in February 2008, a dermatologist reported an out-patient with scabies crustosa to the PHS. This patient had stayed in the nursing home together with the index patient. She developed symptoms of itching after her stay. Having received the information letter, she had been treated twice with both lindane and ivermectin. As the itching persisted after treatment, she received corticosteroids and subsequently developed scabies crustosa. Three contacts of this patient also to had complaints of itching. This patient, as well as her contacts, later received permethrin 5% and ivermectin and remained free of scabies symptoms.

Discussion

Despite treatment, the correct application of hygienic measures and the prophylactic treatment of contacts, a large number of people developed recurrent scabies in the course of the described outbreak. All of them could be linked to the index patient, who had been treated for various episodes of scabies prior to her stay in the nursing home. Only after treatment with permethrin 5% all involved patients finally got rid of their scabies and remained symptom-free during a follow-up of at least three months. We therefore consider it most likely that resistance to lindane and ivermectin may have played a role in this outbreak [1].

This observational report is the first in the Netherlands which indicates a likely resistance of scabies mites to lindane and ivermectin. Outside the Netherlands, resistance to lindane has been described *in vivo* and *in vitro* [2,3], and for that reason, lindane is not prescribed anymore in the United States; permethrin 5% is now the first choice of treatment [4]. There is only one publication on resistance of scabies mites to ivermectin [3]. But *in vitro* studies have shown that over a period of 10 years, the survival time of the mite has doubled [5]. Resistance to ivermectin should be considered in situations where scabies in not endemic and patients remain having complaints despite treatment [6].

Conclusion

This outbreak of scabies was difficult to control. According to the information obtained from the patients involved in this outbreak, re-infection was not likely. It was more likely that the mites causing this outbreak were not susceptible to lindane and ivermectin.

Note: the issue regarding resistance to lindane is not relevant any more since in the summer of 2008 this drug has been taken off the Dutch market for environmental reasons.

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HIV and risk behaviour among men who have sex with men in Denmark – the 2006 Sex Life Survey

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Behavioural surveys among men who have sex with men (MSM) are important for HIV surveillance. The Danish 2006 Sex Life Survey was carried out as a self administered questionnaire, which was distributed at gay venues and bars and posted on the internet. The questionnaire was completed by 3,141 MSM. We describe the methods, the respondent group and the results of the 2006 Sex Life Survey, and discuss it implications. The main finding of this survey is that 33% of the respondents have practised unsafe sex, defined as unprotected anal intercourse with one or more partners of different or unknown HIV status. In the three previous Sex Life Surveys of 2000, 2001 and 2002, this figure was between 26% and 28%.

Introduction

Following a period of decreasing incidence of newly diagnosed cases of human immunodeficiency virus (HIV) infection from the early 1990s to 2000, the rate of newly detected HIV infections began to rise again in Denmark, as it did in many EU countries [1,2]. The main mode of HIV transmission in Denmark is unprotected sex between men who have sex with men (MSM). An important approach to understand the dynamics of this rising trend and contribute to evidence-based HIV prevention, is to conduct second generation surveillance – that is surveillance which combines monitoring of new HIV cases and indicators of sexual behaviour among persons in the groups at highest risk for infection [3] .

Since 2000, four Sex Life Surveys monitoring sexual behaviour and responses to HIV issues among MSM in Denmark have been carried out in cooperation between STOP AIDS – Gay Men's HIV Organization and Statens Serum Institut, with financial support from the National Board of Health. All four surveys were quantitative analyses with data collection on sexual behaviour and self-reported HIV prevalence among MSM in Denmark [4]. This paper describes the results of the most recent, fourth survey performed in 2006.

Methods

The 2006 survey was carried out between mid-August and mid-October 2006 by handing out questionnaires during the annual Copenhagen Gay Pride event and placing questionnaires in gay bars, clubs and other venues in Copenhagen and in the second largest city in Denmark, Aarhus. Questionnaires were also distributed as inserts in magazines both gay and HIV-related journals. In addition, the questionnaire was posted on several sites on the internet, both gay and HIV-related websites. This sampling method was the same as in the other Sex Life Surveys.

The questionnaire was constructed so that it would be possible to compare the results with those from earlier Danish surveys and with the outcomes of other European surveys among MSM (e.g. Gay Men's Sex Survey by Sigma Research in the United Kingdom and Barometre Gay by INVS in France). Most questions and the recall period of 12 months were identical in all four Sex Life Surveys. The questionnaire was limited to 28 questions in order to be contained within a single paper sheet.

The questions were arranged in four categories: a) demographic data/background data (age, education, residence, homo/bisexual behaviour and HIV status); b) sexual behaviour (frequency of sex, number of partners, unprotected anal sex, etc.); c) knowledge about and attitudes towards HIV and sex-related matters; d) response to various safe sex campaigns.

The internet version of the questionnaire contained exactly the same questions as the paper version, but had a number of additional pop-up double-check questions in case of answers that were inconsistent (e.g. the date of the last positive test being earlier than the year of HIV detection). Both versions were tested in a pilot study of 30 MSM contacted in gay bars in Copenhagen.

Data analysis was performed using Stata version 8. Chi-square test was used for bivariate comparisons, and multivariate logistic regression was applied to assess odds ratios (OR) and significance of independent variables for main sexual behaviour outcomes. A non-parametric test (Kruskal-Wallis) was used to compare number of partners in different groups.

When analysing the data the following definitions were used:

- Unprotected anal sex = penetrative anal sex without a condom, no distinction between insertive and receptive anal sex.
- Unsafe sex = unprotected anal sex when serostatus of the respondent is unknown, or with a partner with unknown HIV serostatus, or with a partner whose HIV serostatus is different from the perceived or known serostatus of the respondent.

In the data analyses different denominators are used, i.e. not the total number of respondents but the number of respondents who provided particular information.

Results

Demographic and background data

A total of 3,141 responses from survey participants were analysed. Of these, 2,026 (64%) responses were obtained from

questionnaires posted on the internet, 468 (15%) from those handed out at the gay pride, 411 (13%) from those disseminated in gay bars or saunas, and 236 (8%) from those distributed via magazines.

The mean age of the respondents was 33 years (range: 15-85 years). Fifty-six percent lived in the Copenhagen Area, 27% in Aarhus, Odense or Aalborg, and 27% outside the large cities.

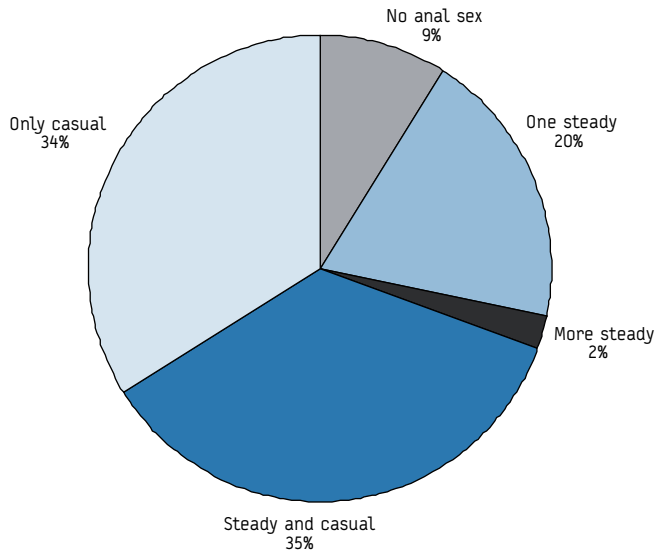
Fifty-five percent had either completed, or were attending post-secondary vocational or post-secondary academic education. This level of education was high compared to the general population, but it did not differ from other surveys among MSM.

During the 12-month study period, 2,755 (88%) of the respondents had sex with men exclusively, whereas 386 (12%) had sex with both men and women (hereafter referred to as homo or bisexual behaviour, respectively). Bisexual behaviour was stated by 343 (17%) of the 2,026 internet respondents, but only by 43 (4%) of those 1,115 who responded to paper questionnaires. This difference can be associated with a geographical pattern, as a higher proportion of paper respondents were Copenhagen residents, and a higher proportion of internet respondents were non-Copenhagen residents. Accordingly, 88 (6%) of respondents in Copenhagen reported bisexual behaviour, compared to 197 (18%) of respondents outside Copenhagen (p<0.001).

Two hundred thirty-one (8%) of the 2,918 respondents who answered this question reported to be HIV-positive, 2,188 (75%) to be HIV-negative, and 499 (17%) stated that they did not know their HIV status. There was a significant difference in geographic distribution, as 10% of the respondents living in the Copenhagen area were HIV-positive whereas among those living in the rest of the country this proportion was only 4% (p<0.001). Among Copenhageners, 17% did not know their HIV-status, and among non-Copenhageners this figure was 19% (non significant).

Figure

Type of anal sex partners reported in 2006 Sex Life Survey, Denmark (n=2,932)



Sexual behaviour

A total of 2,836 (92%) of the 3,095 respondents who answered this question had practised anal sex within the past 12 months. In comparison, 86% of the respondents in the Sex Life Surveys of 2000 and 2002 had practised anal sex, and the percentage was 84% in 2001. A logistic regression analysis controlling for age, residence, HIV status and homo or bisexual behaviour showed the 2006 increase to be significant (OR= 1.9, 95%CI=1.6-2.3, p<0.001), when comparing participants of the present survey to participants of any of the three former surveys.

The average number of anal sex partners reported by respondents in the 2006 survey was 9.4. In the previous three Sex Life Surveys this number was 8. Likewise, the median has increased from two in previous years to three in 2006 (p<0.001).

The proportion of men who had practised anal sex was the same for HIV-positive and HIV-negative men, but HIV-positive respondents had more anal sex partners (mean 17, median 6) than HIV-negative respondents (mean 8, median 3) (p<0.001). Among HIV-negatives, 180 (10%) of the 1,867 respondents who stated the number of anal sex partners had more than 20 anal sex partners, while this was the case for 47 (25%) of 186 HIV-positive respondents who stated number of anal sex partners (p<0.001).

The respondents were asked to state the number of both steady and casual partners during the last 12 months. The terms "steady" and "casual" were not defined in the questionnaire, and it is unknown to what extent partners may have overlapped or succeeded each other.

Thirty-four percent had only casual partners – one or more - during the last 12 months. A similar proportion, 35%, had both steady and casual partners, whereas only 20% had one steady partner. A small group (2%) reported more than one steady partner but no casual partners (Figure). In comparison, in the 2000 Sex Life Survey (the only one of the former surveys that distinguished between steady and casual partners), 26% of the respondents had only one steady partner, 28% had only casual partners and 29% had both steady and casual partners. A similarly small proportion (2%) had more than one steady partner but no casual ones (p<0.001).

The survey did not distinguish between insertive and receptive anal sex. When asked about unprotected anal sex, that is anal sex without condom, it was declared by 58% of the respondents.

Among HIV-positive respondents, 66% had practised unprotected anal sex, while this was the case for 55% of the HIV-negatives and for 60% of respondents who did not know their HIV status (p=0.001).

Among respondents with one steady partner, 73% had practised unprotected anal sex. This proportion was 71% among respondents with both steady and casual partners, 50% among respondents with only casual partners, and 51% among respondents with more than one steady, but no casual partners (p<0.001). Compared to the 2000 Sex Life Survey, the respondents who had only casual partners had a significantly higher proportion of unprotected anal sex in 2006 (p<0.001). For the respondents who had both steady and casual partners, the increase in the proportion of those who had unprotected anal sex was only marginally significant (p=0.06). Among respondents who had only one steady partner, there was no

difference in the proportion of those who had unprotected anal sex between the two Sex Life Surveys.

Among homosexual MSM, 59% had practised unprotected anal sex, compared with 48% of bisexual men (p<0.001).

Among respondents under 30 years of age, 62% had practised unprotected anal sex, compared with 55% of respondents aged 30 years or older (p<0.001). When stratified by number and type of anal sex partners, there was no difference between the age groups, if respondents had only steady partners. Among respondents who had both steady and casual partners, 301 of 404 (75%) respondents less than 30 years old had unprotected anal sex, compared to 341 out of 499 (68%) respondents aged 30 years or more (p=0.04). Among respondents who had only casual partners, 195 of 360 (54%) respondents less than 30 years old had unprotected anal sex compared to 242 of 556 (46%) respondents aged 30 years or more (p=0.02).

Education level or place of residence was not of significant importance with regard to having practiced unprotected anal sex.

Among men who had only had one anal sex partner during the last 12 months (casual or steady), 66% had unprotected anal sex. Among men who had two or more partners, the fraction of those who had unprotected anal sex ranged from 55% (2-5 partners) to 71% (>20 partners).

Unsafe sex

In this survey, unsafe sex is defined as unprotected anal sex when serostatus of the respondent is unknown, or with a partner with unknown HIV serostatus, or with a partner whose HIV serostatus is different from the perceived or known serostatus of the respondent. Of the respondents, 33% stated that they had practised unsafe sex at least once during the last 12 months.

To assess possible predictors of unsafe sex, a multivariate logistic regression analysis was carried out. Six factors turned out to be independently associated with unsafe sex: Number of anal sex partners, HIV status, risk perception, age, education level and frequency of having sex (Table 1). There was no association between unsafe sex and residence, homo- or bisexual behaviour, or whether the questionnaire was submitted online or on paper, neither in bivariate nor multivariate analyses. Having had a new anal sex partner within the last 12 months was a significant predictor for unsafe sex in the bivariate analyses, but not in the multivariate analysis.

The number of partners was the strongest predictor of unsafe sex; the probability of having had unsafe sex ranged from 17% in men with one partner to 58% in men with more than 20 partners (p< 0.001).

HIV status was also a strong predictor. In a bivariate analysis, 49% of HIV-positive men had practised unsafe sex compared to 25% of HIV-negative men (p<0.001). Men who did not know their HIV status were the group among whom unsafe sex was practised by the biggest proportion (60%). This is due to the fact that all unprotected anal sex in this group was considered unsafe sex.

In the three earlier Sex Life Surveys, the proportions of respondents reporting unsafe sex ranged from 26% to 28%. As the populations in the four surveys differed in demographic composition, it is not possible to make a direct comparison. However, a multiple regression analysis shows that the proportion of respondents who practised unsafe sex had increased by 20-30% since the 2000, 2001 and 2002 surveys, when controlling for age, HIV status, education, number of partners, and frequency of sex. Unsafe sex was further stratified by discordant/unknown status. Table 2 presents the different strata of safe/unsafe sexual behaviour.

Risk perception and assessment of the risk of HIV transmission

Unprotected anal sex is known to be the most risky sexual practice for HIV transmission. Respondents were asked to state their perception of the risk of HIV transmission when practising anal sex with and without condom use, and with and without ejaculation, respectively. The majority (88%) stated that the risk of HIV transmission during anal sex without a condom and with ejaculation inside the partner was "risky" or "very risky". As noted above, perceiving the risk as "low" or "not risky" was a predictor of having practised unsafe sex. Whether the respondents perceived the practices to be risky or not, we examined the individual answers according to the level of risk assigned to the different anal sex practices (with and without a condom and with and without ejaculation inside the partner). The way the respondents

Table 1

Frequency and odds ratios (OR) for independent variables which were significant predictors of unsafe sex in a logistic regression analysis; 2006 Sex Life Survey, Denmark

	N (%)*	Multivariate OR
Anal sex partners < 3, >0 Anal sex partners ≥ 3	1,046 (36%) 1,627 (55%)	1 4.4 (3.50-5.62)
HIV-negative** HIV-positive	2,188 (75%) 231 (8%)	1 3.1 (2.20-4.37)
High risk perception*** Low risk perception	2,569 (87%) 377 (13%)	1 2.67 (1.98-3.61)
Age ≥ 30 years Age < 30 years	1,673 (60%) 1,124 (40%)	1 1.7 (1.32-2.06)
Post-secondary vocational or academic education Primary and secondary education	1,526 (55%) 1,266 (45%)	1 1.3 (1.04-1.61)
Frequency of sex: once a month or less often Frequency of sex: several times a month or more often	1,041 (33%) 2,100 (67%)	1 1.3 (1.01-1.61)

OR: odds ratio
* Number and proportion (%) of respondents who answered the question concerned
** Respondents with unknown HIV status were excluded from the analysis
*** High risk perception: attributing great or very great risk of unprotected anal sex; low risk perception: attributing low or no risk of unprotected anal sex

Table 2

Overview of respondents' sexual behaviour within the last 12 months; 2006 Sex Life Survey, Denmark

Behaviour	Number of respondents (%)
No anal sex	231 (8%)
Only protected anal sex	974 (35%)
Unprotected anal sex with concordant partners	663 (24%)
Unprotected anal sex without knowing own and/or partners' HIV status	737 (28%)
Unprotected anal sex with discordant partners	187 (5%)
Total (who stated HIV status and sexual behaviour)	**2,792 (100%)**

ranked the risk levels was used as a marker for knowledge of HIV transmission risk, so that anal sex without a condom with ejaculation inside the partner had to be ranked as more risky than without ejaculation, which in turn had to be ranked more risky than anal sex with a condom. Ninety-seven percent ranked the levels of risk satisfactorily.

HIV testing

Seventy seven percent of the respondents had undergone HIV testing one or more times in their lifetime.

Among respondents who stated the year of the last test, 36% had been tested in 2006. The questionnaire was distributed in the period August-October, so the answers could not reflect test activity in a full year. When including respondents whose last test had taken place in 2006 or 2005 (i.e. max 22 months ago), the figure was 59%. The corresponding figure was 51% in 2001 and 50% in 2002 survey (data were not available in 2000 survey) ($p<0.001$).

There was no difference in whether an individual had been practising unsafe sex during the last 12 months or not, in relation to whether he had ever been tested. However, among men who had practised unsafe sex during the last 12 months and were not HIV-positive, 48% had been tested in 2006 or 2005, while this was the case for only 43% of those who had not practised unsafe sex and were not HIV-positive. Two thirds of the respondents who had unsafe sex also stated how often they had it. Among those, testing frequency did not reflect risk taking; respondents who had unsafe sex once or twice during the last 12 months were more often tested recently than respondents who had unsafe sex 3-10 times, who, in turn, were tested recently more often than respondents who had unsafe sex more than 10 times during the last 12 months. This trend was, however, only marginally significant ($p=0.06$)

Disclosure and condom use with a new partner

In the course of the last 12 months, 66% of respondents had practised anal sex with a new partner with whom they had not previously had anal sex. Of these, 22% did not use a condom during the most recent occasion they had anal sex with a new partner, i.e. they had practiced unprotected anal sex.

Overall, 31% of those who had anal sex with a partner with whom they had not previously had anal sex informed their partner of their HIV status (disclosure) prior to having sex (only 1% disclosed it after sex). The same number of men were informed about their partner's status (received disclosure). There was an almost total overlap in these two groups, indicating that people either practised mutual disclosure or that neither of them disclosed.

TABLE 3

Disclosure of HIV status and condom use; 2006 Sex Life Survey, Denmark

Disclosure/condom	Number of respondents	Proportion of total (%)
No disclosure, no condom	196	11
Condom but no disclosure	975	56
Disclosure but no condom	171	10
Both disclosure and condom	394	23
Total (who provided this information in the questionnaire)	1,736	100

Forty-nine percent of the respondents who had not been using a condom last time they practised anal sex with a new partner disclosed their HIV status, compared with 30% of those who did use a condom. As shown in Table 3, 11% did not use condoms and did not disclose their HIV status the last time they practiced sex with a new partner, matching the study definition of unsafe sex.

Nearly half (48%) of the respondents had met their new partner on the internet. This figure was higher among internet respondents (57%) and lower (33%) among those who submitted paper questionnaires ($p<0.001$). The internet, bars/discotheques and saunas/sex clubs constituted a total of 79% of the answers to the question on where the respondents had met their latest new partners, regardless of the questionnaire source.

Gay magazines, venues and websites

Sixty percent read gay magazines, 82% used websites for homosexuals and 74% frequented gay venues. Fifty nine percent of the respondents used both gay venues and websites, 15% used venues exclusively, 12% only websites, and 14% used none of these.

Discussion

This survey included 3,141 MSM representing 6.4% of the estimated 50,000 MSM in Denmark who in turn constitute 2.5% of the adult male population (aged 15-80 years) [5].

It is not possible to calculate a response rate, nor can it be known if the MSM who were not reached with the questionnaire or who chose not to answer, differ from the respondents in demographical or behavioural parameters. Even though the large number of internet respondents facilitated the inclusion of MSM outside the big cities, it is quite possible that MSM who answered the questionnaire represent a more outgoing and sexually active fragment of the Danish MSM population.

In this survey, only 20% of the respondents appeared to be practising a monogamous sex life with one steady partner, whereas the majority had both steady and casual partners or only casual partners. The extensive change of partners facilitates the spread of sexually transmitted infections, including HIV infection.

The main finding of this survey is that 33% of the respondents have practised unsafe sex, defined as unprotected anal intercourse with one or more partners of different or unknown HIV status. In the three previous Sex Life Surveys of 2000, 2001 and 2002, this figure was between 26% and 28%, indicating an increase of 20-30%, when controlled for population differences. There is no perfect way of dealing with differences when trying to compare different convenience samples, but controlling for factors that were shown to influence the risk of unsafe sex in bivariate analyses of both the present and the former Sex Life Surveys goes some way to overcome this issue. Furthermore, the same logistic regression analysis showed no difference in unsafe sex between the years 2000, 2001 and 2002, when controlling for the same factors. The fact that more respondents were recruited via the internet did not have an independent impact, when different rates of unsafe sex were analysed.

Several studies in other European countries [6-8] have reported increased frequency of unsafe sex among MSM in the early 2000s. However, during the recent years, unsafe sex levels seem to have

stabilised among MSM in some countries [9]. The increase in proportion of MSM practising unsafe sex between the 2002 and the 2006 Sex Life Surveys could have taken place at any time during this four-year period, and only repeated surveys will show if the trend in Denmark is still increasing.

The fact that MSM who are aware of their positive HIV status reported the highest levels of unsafe sex is problematic, but it mirrors recent findings elsewhere [6,10,11].

Among HIV-positive respondents, 66% had unprotected anal sex, but the proportion of those HIV-positive respondents who had unprotected anal sex with partners they did not know to be seroconcordant (of same HIV status) was 49%, suggesting that some amount of serosorting (the practice of having unprotected anal intercourse with a partner believed to be of the same HIV status [12,13]) among HIV-positive MSM takes place. The difference among HIV-negative respondents who have unprotected anal sex (55%) and HIV-negative respondents who have unsafe sex (25%) is even bigger. Whether this is due to HIV-negative MSM practicing active serosorting, or it is merely due to the more easy access that HIV-negative MSM have to seroconcordant partners, is not known. Serosorting among men who perceive themselves to be HIV-negative is only of value if both partners have had no risk of becoming infected since last negative test, and several studies have demonstrated that relying on negative serosorting with casual partners often leads to HIV transmission [10,14,15].

An even stronger predictor of unsafe sex than HIV positivity was the number of anal sex partners. This issue is recurrent in all the previous

Sex Life Surveys as well as in surveys in other countries [16]. In this context it is noteworthy that the average number of anal sex partners has increased since the 2002 Sex Life Survey.

The present survey does not offer an explanation as to why the numbers of partners, unprotected anal intercourse and unsafe sex are increasing. Among the reasons suggested by researchers in the field are treatment optimism, "safe sex fatigue", and the absence of the deterring effect of friends and lovers who are ill [17]. Especially the younger generations have begun their sex life in this day and age when HIV is no longer considered a threat of early death. This may partly explain why in our study younger MSM had unsafe sex more often than the older MSM.

It may be that the findings reflect a general tendency towards a more liberal and uninhibited sex life following a couple of decades of caution. Men's sex life is influenced by other factors than those that have to do with risk and HIV. An additional reason for increase of unprotected anal sex could be a switch from risk avoidance towards risk management strategies, e.g. serosorting.

Although 33% of people practising unsafe sex is a high percentage, there are still many MSM who exclusively had safe sex. The respondents were not asked about the number of partners with whom they had practised safe sex, or how many times.

The survey included assessment of the risk of HIV transmission in the case of unprotected anal intercourse with ejaculation in the partner. However, the participants were not asked to assert whether they practiced insertive or receptive anal sex or both, so some respondents could have interpreted the question in light of their own practices, and not, as intended, as the general possibility of transmitting HIV by ejaculating into the partner, i.e. transmission from the insertive partner to the receptive one.

Men who had a low estimation of the risk were more likely to have practised unsafe sex than men who estimated that the risk was high. However, on the basis of this survey results, it cannot be determined whether individuals choose to practise unsafe sex because they estimate the risk to be low, or they may be rationalising – after having practised unsafe sex – that the risk might not be that high after all.

MSM with an education level corresponding to post-secondary vocational or academic education had a lower risk of having unsafe sex than the less well educated MSM in this survey. The level of knowledge regarding safe sex practice was very high regardless of educational level, so this finding is surprising. Also, education level has not been a significant predictor in the former Sex Life Surveys.

Finally, the frequency of sex was an independent predictor of unsafe sex, but not as strong as the number of anal sex partners.

The overall HIV prevalence in the study was 8%, with a higher HIV prevalence among residents of the capital (10%) than among respondents from the rest of the country (4%). The wide use of the internet questionnaire in the 2006 Sex Life Survey has contributed to a larger proportion of responses from internet respondents living outside of Copenhagen in the 2006 survey than in the previous three Sex Life Surveys. Consequently, the overall HIV prevalence was lower than in the past surveys when it ranged between 10 and 11%. The prevalence estimate obtained by using data from the national surveillance system and the Danish HIV Cohort [18] is 5%. The result of our study is in line with this, taking into account that the survey still contained a disproportionately big fraction of Copenhageners with higher HIV prevalence than in the rest of the country. However, this can not be quantified, since the population distribution of MSM in Denmark is not known. Furthermore, the very high prevalence among the 61 respondents who had received the questionnaire as an insert in a HIV related magazine contributed to increase the overall prevalence.

Practically all respondents ranked different anal sex practice risks in the right order. This indicates a very high level of awareness concerning risky sex behaviours. Also in 2000, respondents demonstrated a good knowledge of risky sex behaviour. The awareness level is thus still high, a fact that may be ascribed to earlier information campaigns.

Future prevention initiatives must not only aim at maintaining this high level but also address the fact that unsafe sex is taking place despite the widespread and thorough knowledge of risks.

More than three-fourths of the respondents had undergone HIV testing one or more times in their lifetime. In other European countries, this figure varies between 50% and 80% [19]. Half of the respondents who had practised unsafe sex (and who had not previously been tested positive) had been tested in 2006 or 2005, implying that half of those respondents who could in principle have been infected within the last 12 months had not been tested within this period [12]. This was the case for a somewhat smaller number of respondents who – according to their questionnaire replies – had run no risk of HIV infection. From a prevention perspective, the point is not to make as many people as possible take the test, but

to make the relevant people take the test – those who have run a risk of being infected.

Two thirds of the respondents had anal sex with a new partner during the last 12 months, confirming the impression of a high partner turnover. Eleven percent did not know the HIV status of their new partner, but they still did not use a condom at the latest intercourse with the new partner. This method of assessing unsafe sex (at the last anal intercourse) adds an additional level to the measure of unsafe sex during the last 12 months.

Nearly half of the respondents had met their new partner via internet. From a prevention perspective, it is relevant that the internet is such a popular contact place. Gay venues such as bars/discos and sauna/sex clubs were used by two thirds of the respondents, and more than half of the respondents used both gay venues and gay websites. The proportion of respondents who used only venues or websites, or neither of these were much smaller (15%, 12% and 14%, respectively. In the light of both internet and gay venues playing a considerable role in the social and sexual life of MSM, preventive efforts focused on both these information media should make it possible to reach a large number of this population.

HIV among MSM is still a serious problem in Denmark and in the rest of Europe, and will continue to be so as a considerable proportion of MSM practise unsafe sex. The present survey demonstrates a high level of knowledge in this target group. However, knowledge is not enough to ensure safe sex practices, and the frequency of unsafe sex among MSM seems to be increasing. This finding has been used in safer sex campaigns conducted by STOP AIDS – Gay Men's HIV Organization, who tailor campaigns to influence attitudes and actions and not just knowledge about HIV transmission [20]. Monitoring developments and trends in the sexual behaviour among MSM is thus important, not only on a national level, but also in a European and a global context. Hopefully, the Danish Sex Life Survey will be continued regularly in the future, and behavioural surveys among MSM on a European scale will be undertaken.

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Parvovirus outbreak in a kindergarten in a municipality in the north of Portugal, April-June 2008

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In response to an alert raised due to epidemic exanthematous rashes in children in a kindergarten, an outbreak investigation was carried out in a municipality in the north of Portugal in late spring 2008. The intention was to establish an aetiological diagnosis and take corrective measures if necessary. The warden at the kindergarten was interviewed, and a self-administered questionnaire was given to parents and staff. Blood samples from seven children with facial erythema were collected for serological investigation. Seventeen cases of erythema infectiosum, due to infection with parvovirus B19, were identified and classified as "confirmed". No cases occurred among the eight adult staff members. An overall attack rate of 38% was observed among the 45 children (born in 2002 and 2003). All cases were mild and without fever. This parvovirus B19 outbreak made it possible to estimate the basic reproduction number (R_0) at between 6 and 8 (or above). Staff members, parents and local clinicians were informed that the infection could pose a risk when caught by people with special clinical conditions. All children had received one dose of measles-mumps-rubella vaccine and 60% had received two doses. The seven children with serologically confirmed parvovirus B19 infection were immune to measles and rubella. All seven were negative for measles- or rubella-specific IgM.

Introduction

The Portuguese vaccination programme includes two routine doses of the combined vaccine against measles, mumps and rubella (MMR), at the recommended ages of 15 months and five or six years [1]. Coverage with the first and second dose of MMR vaccine has reached high and sustained levels in the north of Portugal for years [2]; this also applies to the municipality where this outbreak occurred.

Epidemic exanthematous rashes can have different aetiological causes, and differential diagnosis may be needed in the context of measles elimination programmes in Europe [3]. Previous outbreaks caused by parvovirus B19 have been studied in Portugal [4]. Measles and rubella are statutory reportable diseases in Portugal, and guidelines to study cases of measles were issued in the context of a catch-up vaccination programme in 1998/9 [5]. The Health Ministry has recently issued warnings to all services and health professionals about the possibility of importation of measles due to the international epidemiological situation, and emphasised the need to sustain high vaccination coverage [6]. This is the setting for the alert and response described here.

Alert

In the morning of 16 April 2008, the local health authority (LHA) was contacted by telephone by a nurse working in the school health programme team. She reported that several children in a kindergarten presented spots on the face. The kindergarten warden suspected that the nearby plane trees were causing an allergic reaction to several young children.

Preliminary assessment

In the afternoon of 16 April, two members of the LHA visited the kindergarten premises and spoke with the warden. The team examined six children with the spots. The appearance was strikingly similar to pictures published in the literature describing cases of erythema infectiosum, with the typical "slapped face appearance". All children were in a good physical condition, none had fever or other symptoms, and only one presented a rash in the abdominal region.

It was decided to conduct an outbreak investigation with the main objectives of:

- Testing the hypothesis that it was not a measles or rubella outbreak;
- Establishing an aetiological diagnosis;
- Providing information to the kindergarten community and clinicians on appropriate measures;
- Collecting data on MMR vaccination and taking corrective action if necessary.

Methods

Collection of clinical information

The warden was asked to provide a list with names and birth dates of all members of the kindergarten community (staff and children). Staff members and parents were asked to fill a questionnaire which was collected in the last week of June, a few days before the kindergarten would close for the summer holidays. Just before the holidays, a phone call was made to confirm that no further cases had occurred.

A case was defined as "probable" if erythema on face, extremities or trunk, was observed in members of the kindergarten community between 5 April and 19 June 2008. A case was classified as "confirmed" if in addition to the "probable" case definition it was laboratory-confirmed or had an epidemiological link with a confirmed case.

Written vaccination data from all children and adults were checked by a nurse.

Laboratory study

A nurse visited the kindergarten on 9 May 2008, to collect blood samples from seven of the children who had presented facial erythema and whose disease onset had been 11 to 34 days before. Enzyme immunoassays (EIA) for specific IgG and IgM antibodies levels against measles, parvovirus B19 and rubella were done by two local general practitioners (GPs), who had been treating the children and previously asked informed consent from the parents.

Results

Kindergarten community

The 45 children attending the kindergarten (29 boys and 16 girls) were born between January 2002 and December 2003. There were two groups of children, 20 in class room 1 (13 boys and seven girls) and 25 in class room 2 (16 boys and nine girls). The eight adult staff members were all women, born between September 1954 and August 1972.

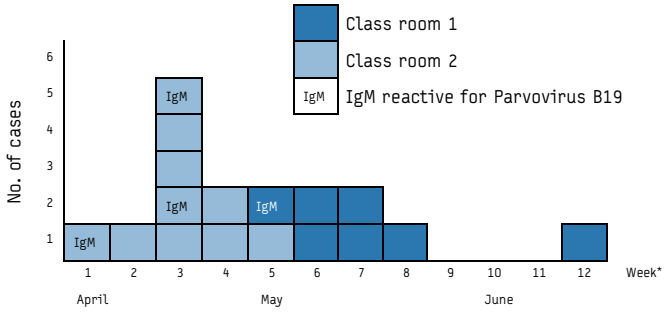
None of the staff members had ever been vaccinated against measles but, following the Portuguese guidelines for their age group, only one was young enough (born in 1972) to have received one dose of that vaccine. All children had received one dose of MMR vaccine at between 15 and 20 complete months of age (mean age at vaccination = 15.9 months). Twenty-seven children (60%) had received a second dose between 60 and 73 complete months of age (mean age at vaccination = 63.9 months). Among the children that had received only one dose, nine had not yet completed six years of age, and the remaining nine had not yet completed the age of seven years.

Epidemiology

In total, 17 cases were observed among the 45 children and none among the eight staff members. The date of onset of the first known case was on 5 April 2008 and the date of onset of the last case on 19 June 2008. The peak of the outbreak was in the third week, when five cases occurred (Figure 1). The attack rate (AR) among the children was 38% (17/45), 35% among the group in class room 1 and 40% among the group in class room 2 (Figure 1; difference not statistically significant: p=0.73). The AR

FIGURE 1

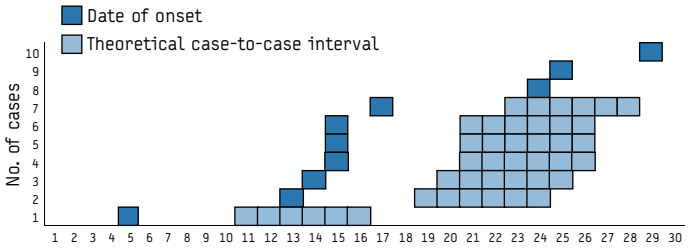
Epidemic curve of the outbreak of erithema infectiosum in a kindergarten; disease onset by week and class room, Portugal, April-June 2008 (n=17)



* First day of first week = 31 March 2008

FIGURE 2

Erithema infectiosum outbreak in class room 2 by date of disease onset, Portugal, April-June 2008 (n=10), with the theoretical case-to-case interval of 6-11 days as proposed by Heegaard and Brown, 2002 [7]



TABLE

Laboratory results of the serological study of kindergarten children with clinical manifestations typical of erythema infectiosum, Portugal, April-June 2008 (n=7)

Case	Ig Class	Measles		Rubella		Parvovirus B19	
Age / Sex MMR doses		Concentration	Interpretation	Concentration	Interpretation	Assay index	Interpretation
4y / F 1	IgG IgM	3,974 mIU/ml -	Immune Negative	160 IU/ml -	Immune Negative	6.22 <1.00	Reactive Not reactive
4y / F 1	IgG IgM	4,012 mIU/ml -	Immune Negative	68 IU/ml -	Immune Negative	5.18 <1.00	Reactive Not reactive
5y / F 2	IgG IgM	4,093 mIU/ml -	Immune Negative	164 IU/ml -	Immune Negative	6.32 1.32	Reactive Reactive
5y / M 2	IgG IgM	5,090 mIU/ml -	Immune Negative	125 IU/ml -	Immune Negative	6.27 <1.00	Reactive Not reactive
6y / M 2	IgG IgM	5,227 mIU/ml -	Immune Negative	75 IU/ml -	Immune Negative	6.67 2.06	Reactive Reactive
6y / F 2	IgG IgM	6,361 mIU/ml -	Immune Negative	340 IU/ml -	Immune Negative	5.66 1.22	Reactive Reactive
6y / F 2	IgG IgM	538 mIU/ml -	Immune Negative	42 IU/ml -	Immune Negative	6.16 1.47	Reactive Reactive

Note: concentration and interpretation of the results as proposed by the assay manufacturer.

was higher among females (41.7%) than among males (24.1%) but the difference was not statistically significant (p=0.29). Five additional cases were reported among the household contacts of the 17 kindergarten cases: four siblings and one parent. None of the staff members became ill.

The days of onset of the 10 cases from class room 2 are graphically represented in Figure 2. If the case-to-case interval is six to 11 days [7], then it is very likely that the first case on 5 April was the primary case, infected outside the kindergarten, while the following six cases were secondary cases, probably infected by the first case. Cases 8 to 10 were a third generation, infected by one or more of the secondary cases (Figure 2). Thus, provided that all children were susceptible before this outbreak and taking into account the definition of the basic reproduction number (R_0) [8], the estimated value of R_0 in this outbreak was 6. However, if 25% or more of infections were asymptomatic [9], the R_0 for this outbreak is likely to have had a value of up to 8 or more.

Laboratory study

Blood samples had been collected from seven of the 17 cases that had occurred before the nurse visited the kindergarten on 9 May. The specific IgM antibody tests for measles and rubella were negative for all seven children tested. Measles IgG concentrations varied from 538 to 6,361 mIU/ml, and all children were classified as "immune". Rubella IgG concentrations varied from 42 to 340 IU/ml and all children were classified as "immune". Regarding parvovirus B19-specific antibodies, all seven children were "reactive" for IgG, but only four were also "reactive" for IgM (Table).

Clinical manifestations

The seventeen erythema episodes were classified as "confirmed cases" of erythema infectiosum. All other members of the kindergarten were classified as "non-cases", while there were no situations compatible with the definition of "probable case".

The 17 cases presented facial erythema, lasting between two and five days (in 16 children) and 10 days in one child. Eight patients had only facial erythema while the remaining nine also had the rash on the trunk and/or extremities. Itching was reported by two children and none of the cases were febrile. All cases were very mild and no clinical complications were observed.

Control and prevention measures

The premises were inspected and the procedures were verified; they complied with the Portuguese legal requirements.

The kindergarten staff was informed about the benign nature of erithema infectiosum and the possible risk for pregnant women and those with anaemia and immunodeficiencies. It was recommended to exclude children form the kindergarten if they developed fever. Strict handwashing procedures after contact with patients were recommended. The same information was issued by letter to all parents.

The medical coordinator of the local National Health Service (NHS) unit was informed about the outbreak, the data to be collected and the measures to be taken. An email explaining the situation and the clinical conditions under which parvovirus B19 infection poses a particular risk was sent to all GPs working at the local NHS unit.

Discussion and conclusion

It was confirmed that the described outbreak was due to infection by parvovirus B19. All seventeen cases unequivocally met the case definition criteria. The three cases that were not reactive for parvovirus-specific IgM (see Table) had very typical clinical symptoms, and the blood samples had been collected 15, 25 and 26 days, respectively, after the onset of symptoms. We are not sure about the reasons for these negative laboratory results, but we think that low sensitivity of the laboratory method cannot be excluded because the levels of parvovirus B19-specific IgM were generally very low, even in the reactive samples. Although it is arguable whether effective preventive measures can be taken [4,8], the usual recommendations were issued.

Several parvovirus outbreaks had been detected and studied in a neighbouring municipality in 2004 [4]. Should there be a connection between these outbreaks and the one described in this paper, it would be consistent with the reported periodicity of between three and seven years for parvovirus B 19 epidemics in a given community [9]. In 2004, the children described here had not been exposed to the infection because they were attending any kindergarten and didn't have much contact with other children. Moreover, seroprevalence data in 2001-02 showed that the infection was rare in young age groups [10]. We therefore believe that our estimated range for R_0 is likely to be valid. Should there be immune children, then the reported R_0 values would be an underestimate.

No cases were observed among staff members, probably because they were all immune. Recent Portuguese seroprevalence data [10] have shown a high proportion of immune individuals in the age groups of the staff members of the described kindergarten. Furthermore, we believe that their professional activity is associated with increased exposure to parvovirus, compared with the general population.

The virus seems to have entered the kindergarten with the first case and spread first into class room 2 and then into class room 1 (Figure 1). For class room 2, we can identify a likely transmission chain (Figure 2). However, this is more difficult for class room 1, where one or more cases seem to be missing in the period from 20 May to 18 June 2008. This may have been the result of a recall bias by parents and staff or of an unidentified transmission chain outside the kindergarten.

We did not recommend vaccination against measles for adult staff members because previous studies have shown that Portuguese women in those age groups are not only immune to measles but have measles-specific IgG levels well above protective levels [11].

We were able to prove that the outbreak was not measles or rubella. Furthermore, all children had received one dose of MMR vaccine and the levels of measles- and rubella-specific IgG among the seven studied children were well above the protection thresholds. Those children who had not received the second MMR dose were still within the age range recommended for that vaccination. Such high coverage values are consistent with what has been observed in the north of Portugal [2] and in the annual internal evaluations in our municipality (unpublished data).

After the described outbreak investigation, a report on imported cases of measles in Portugal was published [12]. Two importation episodes (in 2005 and in 2008) were identified and reported. The measles cases imported in 2005, affecting migrant Romanian communities, were studied by community physicians (see

Acknowledgements) in two neighbouring municipalities, including the one where the present parvovirus outbreak was observed. These experiences have been helpful in the current parvovirus investigations. Once again, our local public health unit was able to quickly respond to an alert due to an eruptive epidemic disease, and would have detected a measles (or rubella) outbreak, if that had been the aetiology of the cases.

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