Impact factor 6.15

Eurosurveillance

Europe's journal on infectious disease epidemiology, prevention and control

Vol. 18 | Weekly issue 13 | 28 March 2013

Research articles

www.eurosurveillance.org

Anthrax among heroin users in Europe possibly caused by same *Bacillus anthracis* strain since 2000

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Citation style for this article:
Grunow R, Klee SR, Beyer W, George M, Grunow D, Barduhn A, Klar S, Jacob D, Elschner M, Sandven P, Kjerulf A, Jensen JS, Cai W, Zimmermann R, Schaade L. Anthrax among heroin users in Europe possibly caused by same Bacillus anthracis strain since 2000. Euro Surveill. 2013;18(13):pii=20437. Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=20437

Article submitted on 18 October 2012 / published on 28 March 2013

Injection anthrax was described first in 2000 in a heroin-injecting drug user in Norway. New anthrax cases among heroin consumers were detected in the United Kingdom (52 cases) and Germany (3 cases) in 2009-10. In June 2012, a fatal case occurred in Regensburg, Bavaria. As of December 2012, 13 cases had been reported in this new outbreak from Germany, Denmark, France and the United Kingdom. We analysed isolates from 2009-10 and 2012 as well as from the first injection anthrax case in Norway in 2000 by comparative molecular typing using a high resolution 31 marker multilocus variable-number tandem repeat analysis (MLVA) and a broad single nucleotide polymorphism (SNP) analysis. Our results show that all cases may be traced back to the same outbreak strain. They also indicate the probability of a single source contaminating heroin and that the outbreak could have lasted for at least a decade. However, an additional serological pilot study in two German regions conducted in 2011 failed to discover additional anthrax cases among 288 heroin users.

Introduction

Anthrax infections occur world-wide, but are more frequent in countries with subtropical climate for example in southern Europe, the Balkans, south-east Asia, South America and Sub-Saharan Africa. Primary infections predominantly occur in ungulates, whereas human infections are rather rare and usually associated with contact to infected animals or contaminated animal products, such as meat, fur, bone meal, wool or hair [1,2]. Human to human transmission is rare.

Figure 1

Published laboratory-confirmed anthrax cases in heroin users 2000 to 2012 as of 31 December 2012 (n=69)

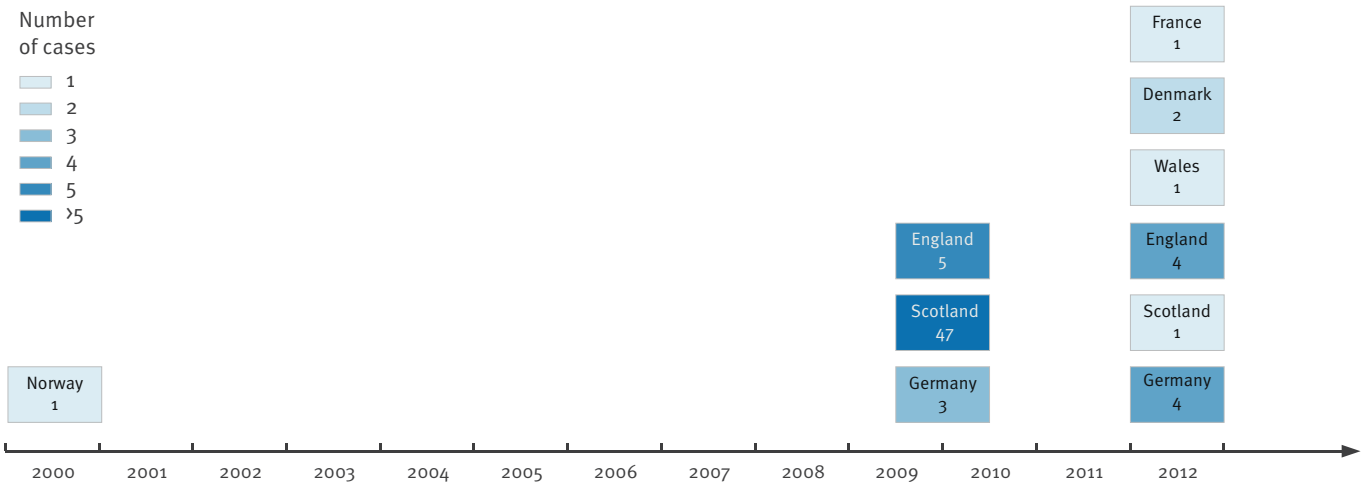


Table 1

Isolates from anthrax cases in injecting drug users between 2000 and 2012 used for molecular typing

Designation	Year of isolation	Source
A112a, A112b[a]	2009	Friedrich-Loeffler-Institute, Jena, Germany
A138	2010	Friedrich-Loeffler-Institute, Jena, Germany
A294	2012	Statens Serum Institute, Copenhagen, Denmark
A306	2012	Statens Serum Institute, Copenhagen, Denmark
A303[b]	2012	Charité Berlin, Robert Koch Institute, Berlin, Germany
A315/1, A315/2[c]	2000	Norwegian Institute of Public Health, Oslo, Norway

[a] A112a and A112b refer to different colony morphologies of the same isolate.

[b] Only DNA from clinical sample, skin swab, available.

[c] Isolates from the same case, below summarised as A315_2000.

In humans, depending on the route of infection, four types of anthrax infections have been defined [3]. Cutaneous anthrax (*Pustula maligna*) is the most frequent (approx. 95%) and least severe form. It is characterised by infection of the skin, most probably via micro lesions or injured skin. Other forms are intestinal and respiratory anthrax. Injection anthrax is a recently defined type of anthrax which is transmitted by subcutaneous, intramuscular or intravenous injection of contaminated drugs. Patients typically present a massive oedema around the injection site often leading to compartment syndrome or necrotising fasciitis [4-6]. Since the clinical picture strongly differs from the classical picture of cutaneous anthrax [7], injection anthrax is difficult to diagnose and treat adequately. Classical signs i.e. papules, vesicles or eschar are often missing, whereas complications such as septic and cardiovascular shock, meningitis and death despite antibiotic therapy, occur more often than in cases of classical cutaneous anthrax [8,9]. Furthermore, the differential diagnosis is hampered by unspecific skin and soft tissue infections that injecting drug users (IDUs) often develop around the site of injection. Despite medical treatment, we calculated a case fatality rate of over 30%, based on the currently available published information and which depends on time and considered patients [10].

Bacillus anthracis is a gram-positive, spore-forming, aerobic rod-like bacterium which grows in chains and is surrounded by a capsule under microaerophilic conditions (cultivation at 5% CO_2 percentage of air) [2]. The bacterium is classified as biological Hazard Group 3 agent [11]. In Germany and at European level, a suspected or laboratory-confirmed anthrax infection according to the case definition is notifiable and has to be reported to the German health authorities who forward the information to the European surveillance system (TESSy) operated by the European Centre for Disease Prevention and Control (ECDC) in Stockholm, Sweden. Particular characteristics of anthrax spores are their extreme resistance against unfavourable environmental influences over a long period of time, enabling them to survive for decades. Spore decontamination requires autoclaving or appropriate chemical disinfectants such as peracetic acid or formaldehyde [12].

The diagnosis of anthrax is mainly based on polymerase chain reaction (PCR) directed against the virulence plasmid markers and isolation of the pathogen from clinical samples [2]. Specific antibody detection can support the diagnoses or discover anthrax cases in epidemiological investigations [13,14].

Early antibiotic therapy [3] is required and schemes for chemoprophylaxis as well as passive and active vaccination are available although vaccines are only approved in some European countries [4,15].

Three outbreaks of injection anthrax in heroin users have been detected so far in Europe (Figure1). The first case was reported from Norway in 2000 [16] and in 2009-10, a total of 119 similar cases occurred in Scotland of which 47 were laboratory-confirmed anthrax cases. Further five cases were reported from England and three from Germany with one case retrospectively detected by serology in a heroin user recovered from a severe disease compatible with injection anthrax [4,17,18]. The most recent outbreak started in 2012 and as of December 2012 at least 13 cases occurred in Germany, France, Denmark and United Kingdom [10,19-24], five of whom died. Since all cases of injection anthrax identified involved exclusively active heroin users, contaminated heroin appears to be the most likely source of infection [4].

In our study we were interested to investigate by molecular typing of the isolates whether the first observed case of injection anthrax in 2000 was related to the more recent cases. Moreover, it is not known if the detected cases of injection anthrax over time reflect the real number of infections or if they are the tip of an iceberg. To investigate this question, a retrospective pilot study among German IDUs, including a questionnaire and serology, was initiated and this study is presented here in brief.

Table 2

Code values for the 31 markers used in multilocus variable-number tandem repeat analysis (MLVA)

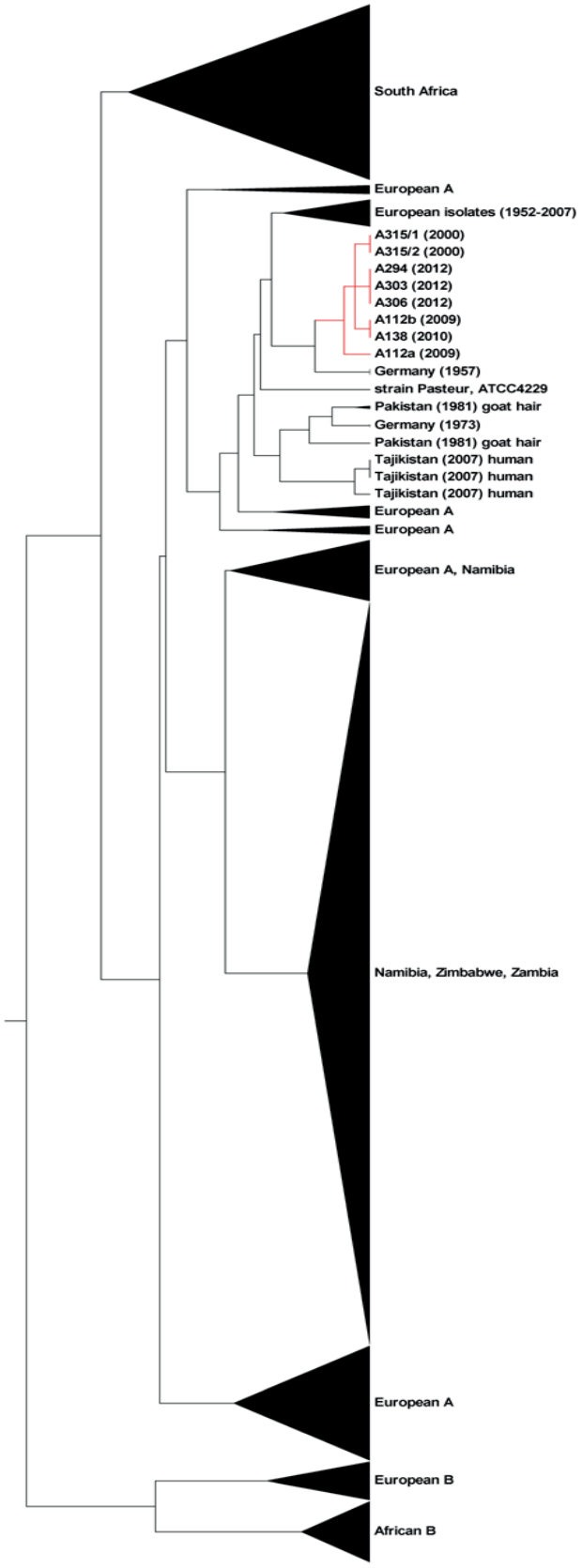
MLVA-Marker	Expected Fragment Lengths (bp)					Observed Fragment Lengths (bp)	Code-No. [24]	reference Ames ancestor
	A112a (2009)	A112b (2009)	A138 (2010)	A294, A303, A306 (2012)	A315/1, A315/2 (2000)			
Bams 1	422	422	422	422	422	425-429	13	2
Bams 3	609	609	609	609	609	616	30	8
Bams 5	307	307	307	307	307	304-305	5	26
Bams 13	454	454	454	454	454	456	30	13
Bams 15	616	616	616	616	616	616-619	46	5
Bams 21	676	676	676	676	676	673-676	9	25
Bams 22	735	735	735	735	735	727-731	13	16
Bams 23	651	651	651	651	651	644-648	10	53
Bams 24	595	595	595	595	595	604-609	9	70
Bams 25	376	376	376	376	376	379-380	3	17
Bams 28	493	493	493	493	493	500-501	14	14
Bams 30	862	889	889	889	889	876/902-906	72 / 75	15
Bams 31	772	772	772	772	772	776	64	8
Bams 34	425	425	425	425	425	431-433	7	64
Bams 44	417	417	417	417	417	422-425	8	4
Bams 51	493	493	493	493	493	501	9	4
Bams 53	236	236	236	236	236	234-235	8	9
CG3	158	158	158	158	158	157	2	9
pXO1	135	135	135	135	135	133	9	9
pXO2	137	137	137	139	141	136/138/140	7 / 8 / 9	6
vrrA	314	314	314	314	314	311-312	4	9
vrrB1	229	229	229	229	229	226	17	9
vrrB2	162	162	162	162	162	159-160	14	13
vrrC1	616	616	616	616	616	621-630	57	10
vrrC2	604	604	604	604	604	603-605	19	57
VNTR 12	115	115	115	115	115	113-114	6	4
VNTR 16	273	273	273	273	273	266-270	8	4
VNTR 17	386	386	386	386	386	386-387	4	6
VNTR 19-2	99	99	99	99	99	96-97	5	4
VNTR 23	197	197	197	197	197	195-196	4	8
VNTR 35	109	109	109	109	109	109-110	3	4

A112a and b, A138 Germany (2 different cases)
A294, A306 Denmark (2 different cases)
A303 Germany
A315/1, A315/2 Norway (2 isolates from same case)

Code numbers are identical with copy numbers of the repeat sequences, where possible. For the purpose of normalisation the appropriate copy code numbers are added for the Ames Ancestor strain as deduced from the sequence available at GenBank, accession no.: AE017334.2 GI:50082967. Expected fragment lengths are taken from the values of alleles provided by Lista et al. in 2006 [27] and deduced from sequences of *Bacillus anthracis* available at Genbank. Values of alleles not published were artificially added by interpolation using the appropriate repeat lengths.

Figure 2

Cluster analysis of *Bacillus anthracis* outbreak isolates from Europe, Asia and southern Africa (n=904)



Dendrogram based on multilocus variable-number tandem repeat analysis (MLVA-31) typing of isolates. Diamond shapes indicate the 'heroin anthrax' isolates. For the cluster analysis by unweighted pair group method with arithmetic mean (UPGMA) a categorical coefficient was used.

Methods

Molecular typing of *Bacillus anthracis* outbreak strains

Bacterial isolates found in drug-related clinical anthrax cases are shown in Table 1. Template DNA for PCR was either isolated using the DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany) or by boiling of colony material in PBS in a thermoblock at 110 °C for 20 min.

Multilocus variable-number tandem repeat analysis (MLVA) for analysis of 31 markers was performed with multiplex PCR and capillary electrophoresis as described [25,26]. Code numbers in Table 2 reflect the copy numbers of the repeat sequences, where possible. For the purpose of normalisation, the appropriate copy code numbers are added for the Ames Ancestor strain as deduced from the sequence available at GenBank, accession No.: AE017334.2 GI:50082967. Expected fragment lengths are taken from the values of alleles provided by [27] and deduced from sequences of *B. anthracis* available in Genbank. Values of alleles not published were artificially added by interpolation using the appropriate repeat lengths. Data were processed using Bionumerics software package version 5.10 (Applied Maths). For cluster analysis by unweighted pair group method with Arithmetic Mean (UPGMA) a categorical coefficient was used.

Single nucleotide polymorphism (SNP) analyses for the 13 canonical SNPs (canSNPs) described by Van Ert et al. in 2007 [28] as well as for the two SNPs, SNP1173928 and SNP1053700, which were identified to be specific for the outbreak strains from 2009-10 [29] were performed using conventional PCR and sequencing of the appropriate regions with standard methods. Further SNPs (A.Br.011, SNP5013862, SNP1967560, SNP1118831, SNP1530761, SNP3287006 and SNP3836105) with increasing specificity for the 'heroin isolates' [29] were additionally included.

The methods and results for the pilot study on retrospective case finding are presented under a separate heading.

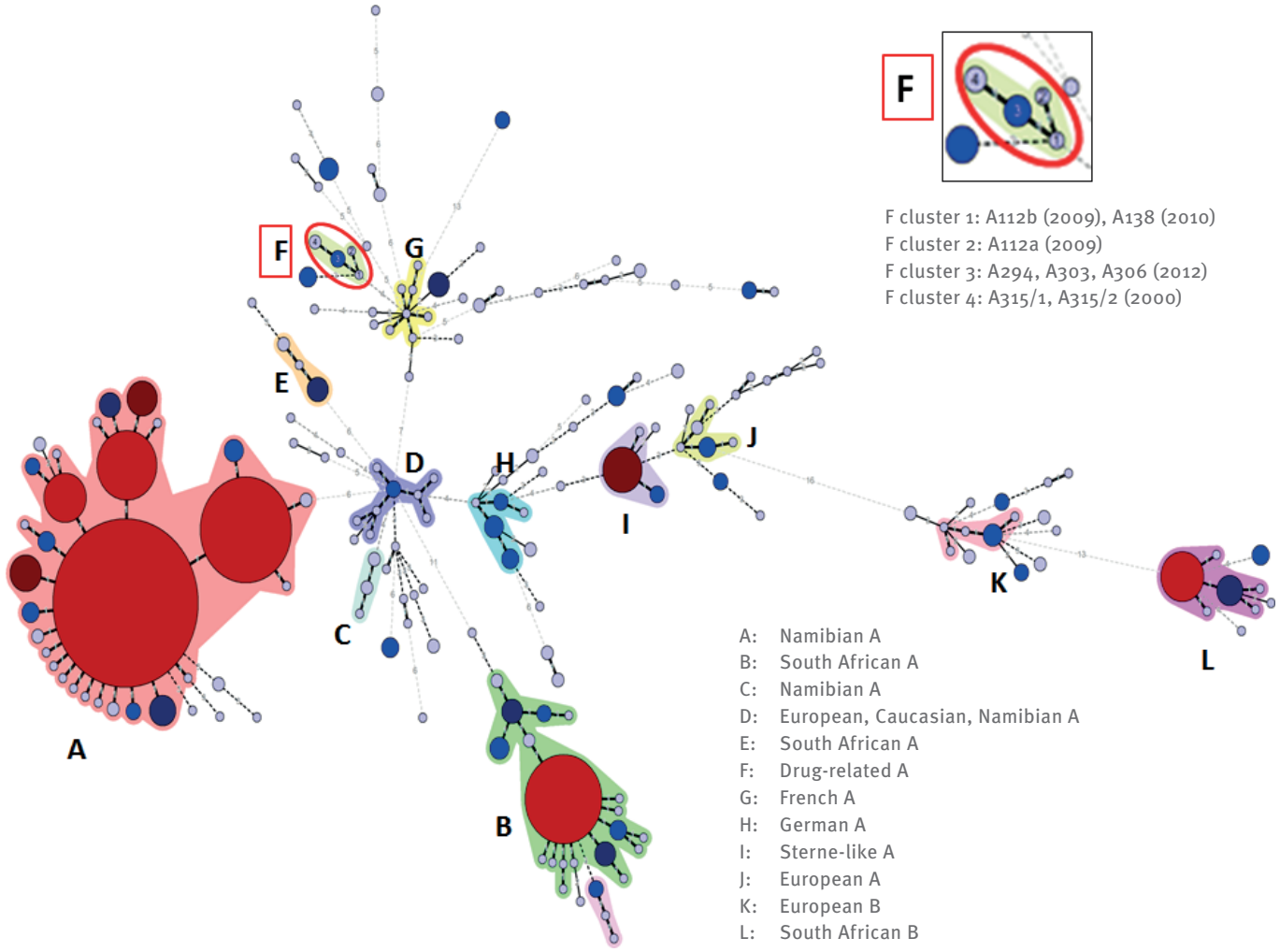
Results

Multilocus variable-number tandem repeat analysis (MLVA)

The results from the MLVA showed that 'heroin anthrax' isolates including strain A315_2000 belong to the European A cluster. Interestingly, two slightly different colony morphologies were observed for isolate A112, and further subcultivation resulted in subclones A112a and A112b. These subclones differed by three repeat units in marker Bams30. All other MLVA markers were identical in all isolates tested, except for the marker for the virulence plasmid pXO2 (Table 3). The oldest isolates from the year 2000 (A315/1 and A315/2) from the same patient in Norway, held nine AT repeats;

Figure 3

Minimum spanning tree isolates of *Bacillus anthracis* typed by 31-marker multilocus variable-number tandem repeat analysis (MLVA) (n=904)



Clustering of multilocus variable-number tandem repeat analysis (MLVA) profiles was done using a categorical coefficient. MLVA-Genotypes (GT) are displayed as circles. The size of each circle symbolises the number of isolates of this particular GT. GTs differing in only one marker are combined in a complex seen as a coloured halo if at least three GTs fulfil this criterion. Distances between circles do not reflect the correct phylogenetic distances. The letters A and B in the legend reflect the main MLVA clusters as defined by Keim et al in 2000 [26]. The F-complex comprises the eight drug-related isolates.

the outbreak isolates from 2009-10 had seven repeats and the latest isolates from 2012 carried eight repeats. A comparison of the heroin-related strains with a collection of 904 isolates from Europe, Asia and southern Africa is shown in Figure 2.

The minimum spanning tree of the investigated isolates is shown in figure 3. The F-complex comprises the eight drug-related isolates arranged according to the number of different markers (one each between the clusters). The sequence of clusters is not expected to reflect the true ancestry of the isolates because of the high mutability and possible homoplasy in both markers Bams30 and pXO2. Interestingly, both the F- and the G-complex are clearly differentiated from the rest of the European and worldwide isolates by seven variable-number tandem repeat (VNTR)-markers, though still differing from each other by another four VNTR-markers. Whether or not this indicates an ancient common group of ancestors could only be answered by including the VNTR-data of strains from the east Turkish region, which are considered near relatives of the heroin-based isolates by the SNP-analysis.

Table 3

Single nucleotide polymorphism (SNP) for clustering and strain identification in isolates from anthrax cases in injecting drug users between 2000 and 2012 (n=8)

Method	Strains 2009-2012 in this study	Strain A315_2000 in this study	Other outbreak strains with reference to Price et al.[28] [30]	Peculiarities
13 SNPs PCR [28]	Present indicating as a member of A.Br.008/009 group	Present indicating as a member of A.Br.008/009 group	A.Br.008/009 group; 36 isolates including outbreak type strain Ba4599 were typed to this group	384/1,033 worldwide isolates were typed to A.Br.008/009 group representing a subset of Trans-Eurasian group [28]
A.Br.011 PCR [29]	Present indicating as a member of the A.Br.008/011 lineage	Present indicating as a member of the A.Br.008/011 lineage	A.Br.008/011 outbreak type strain Ba4599	120 other isolates from different countries, including six Turkish isolates [29]
3 additional SNPs [29] - 5013862 - 1967560 - 1118831	Present	Present	Present outbreak type strain Ba4599	Present in Turkish isolates A0149 and A0264 but not in four other Turkish strains [29]
3 additional SNPs [29] - 1530761 - 3287006 - 3836105	Present	Present	Present outbreak type strain Ba4599	Present in Turkish isolates A0149 and A0264 but not in four other Turkish strains [29]
2 additional SNPs [29] - 10553700 - 1173928	Present	Present	Present in 36 isolates including outbreak type strain Ba4599	Not in A0149 and A0264 and four other Turkish strains [29]

Single nucleotide polymorphism (SNP) analyses

As already shown for the isolates from the outbreak of 2009-10, canSNP genotyping (13 SNPs) of all isolates tested in this study revealed that they belong to the A.Br.008/009 group, the so-called Trans-Eurasian group [28]. The presence of seven further SNPs (A.Br.011, SNP5013862, SNP1967560, SNP1118831, SNP1530761, SNP3287006 and SNP3836105) with increasing specificity, that were used to identify the closest relatives of the outbreak isolates, were also confirmed (Table 3). In addition, all isolates shared the two SNPs (SNP1173928 and SNP1053700) that were shown to be distinctive for the anthrax cases in heroin users. The presence of these two SNPs was confirmed in 36 isolates from the outbreak in 2009-10 [29]. Most markedly, these two SNPs were also identified in the Norwegian isolates from the year 2000, in addition to all other tested SNPs characteristic for the more recent outbreak isolates. In summary, all strains isolated between the years 2000 and 2012 which were analysed in our and in previous studies shared the same 22 SNPs including the two highly distinctive 'heroin-specific' SNPs.

Pilot study on retrospective case finding in Germany

Retrospective case finding was performed by two approaches and is here presented in brief. First, medical personnel from 10 academic medical centres from different parts of Germany responded to a questionnaire to discover retrospectively anamnestic indications for possible clinical signs and symptoms of injection anthrax for the period between January 2009 and spring 2011. For this clinical search the following case definition of injection anthrax was used: 'All cases with history of current heroin use and with clinical picture of massive oedema, compartment syndrome and/or a necrotising fasciitis at the injection site are considered possible injection anthrax.'

Second, a serological investigation was conducted for which 288 serum samples were available. In spring 2011, 44 anonymised sera were obtained in cooperation with the Ministry of Justice of Baden-Wuerttemberg from 20 correctional facilities located in this federal state. Two hundred forty-four capillary blood samples collected as dried blood spots were collected from May until July 2011 in framework the DRUCK-Study, a sero- and behavioural survey directed on the risk of viral infections among injecting drug users in Berlin. This study was piloted by the HIV/AIDS, sexually transmitted and blood-borne infections unit, department for Infectious Disease Epidemiology, Robert Koch Institute, Berlin, in close collaboration with low threshold drop-in facilities in Berlin. Questions on previous clinical signs and symptoms compatible with drug-related anthrax were added to the questionnaire. Informed consent was provided by all study participants and the study was approved by the Federal Commissioner for Data Protection and Freedom of Information as well as the respective responsible Ethics Review Committee.

The sera were tested for specific antibodies against the anthrax toxin component protective antigen (PA) using an accredited in-house ELISA (specificity 100%; sensitivity 1:16,000 for sera, 1:24,000 for plasma) and Western blot analysis (specificity 100%; sensitivity 1:200,000 for sera, 1:100,000 for plasma). In the case of positive results it was intended to test against further anthrax antigens (LF, EF).

The clinical study identified one male heroin consumer from the north of Germany, with acute soft tissue inflammation, abscess and sepsis by anamnestic investigation, but serology did not confirm anthrax. Questionnaires accompanying the serological study revealed anamnestic clinical signs that could correspond with injection anthrax in 34 of 288 individuals (compartment syndrome n=24, 8.3%; fasciitis n=10, 3.5%). All 288 sera were negative for antibodies against PA in ELISA and also Western blot where tested.

Discussion

Through molecular analyses we obtained evidence that a similar source of contamination of heroin, causing injection anthrax in IDUs, could have been active at least since the year 2000. The typing approaches showed that the *B. anthracis* isolates from the first injection anthrax patient in Norway in 2000 was almost identical in the studied key elements for molecular typing, with the strain causing an outbreak among IDUs and represented in the study by six isolates obtained from five clinical cases between 2009 and 2012. Earlier SNP analyses of a large strain collection showed the outbreak strain is closest related to strains from a certain region in Turkey, while it is not related to strains from Afghanistan or Pakistan, the countries assumed to be the primary source of the heroin consumed in Europe [4,29]. However, higher numbers of typed strains from these regions might be required to localise where the putative contamination of the heroin occurred.

In our study, the MLVA-profile from seven isolates and one blood sample revealed the presence of four highly related genotypes, differing only in two highly mutable markers, Bams30 and pXO2. While the former difference was found in the same sample from a patient in the 2009 outbreak (A112a and b), the plasmid marker differed between the isolates from the cases in 2000, 2009-10 and 2012, respectively. All other markers tested were identical, and therefore the four different MLVA genotypes can be considered as one strain causing the outbreak.

Previous studies confirmed that MLVA markers are stable during routine bacteriological diagnosis and passage in mice and rabbits [30,31], but the analysis of animal outbreaks in Namibia showed that especially the markers Bams30 and pXO2 are frequently mutating. Deviations in only one highly mutable marker, particularly the plasmid marker pXO2, in temporally distant isolates from a defined endemic region are well known [25]. The putative evolutionary analysis generating minimum spanning trees clusters such isolates into single complexes. Combined with epidemiological data gained in the investigation of outbreak scenarios, such complexes of highly related genotypes can be considered the same outbreak strain.

MLVA data are placing the group of anthrax isolates related to drug abuse in a distinct but closely related cluster on the background of about 900 isolates from Europe, Asia, and southern Africa. Without the possibility to compare the clinical isolates with any isolates from the source of infection it remains speculative whether the differences found are the result of newly arising mutations during the course of infection or whether the drugs injected by the IDUs were already contaminated with different genotypes. In the latter case, these markers could be useful in tracing back the source of contamination to its origin.

In view of the current understanding of the evolutionary development of *B. anthracis* [28], measurable variations in repetitive sequences would be expected to occur only during replication within an infected host. Earlier investigations on *B. anthracis* strains after in vivo passage in artificially infected laboratory animals failed to reveal newly arisen mutations in any marker [30,31]. In nature rather slowly proceeding or even chronic courses of disease, occurring in host species with low susceptibility for anthrax may be the cause for the emergence of new genotypes. Whether this is the case also in human hosts can only be unravelled once the source of infection is available for comparison.

The results taken together show that all investigated isolates are closely related and it can be concluded that they belong to the same *B. anthracis* strain despite small observed variations.

For SNP analyses we followed the schemes described by van Ert et al in 2007 and Price et al in 2012 [28,29]. The whole cascade of investigation including two very specific SNPs indicates that the isolate A315 obtained from a heroin user with anthrax in Norway in 2000 was identical in all markers with the here investigated isolates from 2009 to 2012. Furthermore the results showed that our strains were completely identical with the previously analysed strains from the 2009-10 and 2012 outbreaks [29,32]. These analyses provide further evidence that all isolates from anthrax patients probably infected by contaminated heroin samples belong to one similar outbreak strain.

This may imply that the contamination of heroin is an on-going process and could occur during the processing of heroin samples in facilities exposed to the identified strain of *B. anthracis*. It cannot be excluded that more infections not recognised by clinical examination or laboratory investigation have occurred, especially if the sudden death of an IDU was ascribed to heroin intoxication. The outbreaks identified may represent

the tip of an iceberg, recognised because high numbers of individuals were affected and the elevated awareness of clinicians as the result of the outbreak in 2009-10. Thus, it is highly important to maintain this awareness by clinicians, microbiological laboratory staff and public health authorities as well as street workers and heroin users themselves. Early detection of new cases will improve the chances of successful treatment of an otherwise often fatal injection anthrax infection and allow the immediate implementation of preventive measures. Besides injection anthrax, further forms of application of heroin causing diverse manifestations of anthrax e.g. respiratory anthrax after inhalation, should be taken into account in this context.

It is possible in general to infer a previous exposure to *B. anthracis* from the detection of anthrax-specific antibodies in sera [14,33,34]. Serology could be applied to support diagnoses in late stages of acute anthrax and in retrospective epidemiological investigations to discover patients who had been exposed to *B. anthracis* or have recovered from a clinical disease compatible with anthrax [6]. However, a serological pilot study including 288 heroin consumers performed in two regions of Germany, Berlin and south-west Germany during 2009 to 2011, did not reveal possible additional anthrax cases. The limitations of this study were firstly that samples were only available from two regions in Germany and it can thus not be excluded that positive cases in other regions were missed. Secondly, the number of investigated IDUs was low due to the limited access to individuals in framework of this study. However, to our knowledge, there have been no further publications so far on serological studies focusing on anthrax in IDUs.

In conclusion, high resolution MLVA31 and SNP13 plus a cascade of SNP analyses and two very specific SNPs identified all studied isolates from the anthrax outbreak in IDUs in 2009-10 and 2012 as the same strain. Most interestingly, the Norwegian isolate from the year 2000 was also identified by all applied methods as the same outbreak strain. It can be concluded that most likely the outbreak has been going on since at least the year 2000 with a highly probable similar source of contamination which might be still active. It seems probable that cases have remained undetected since this time and a joint international epidemiological investigation could contribute to clarify this issue. Identification and abolishment of the contamination source would be the most effective preventive measure. However, this is a challenge that could be only achieved by close cooperation of scientists, public health authorities and law enforcement agencies. Awareness by physicians and patients is most important for an early and effective treatment.

Acknowledgements

We would like to thank all participants in the serological study for providing the samples. Expert technical assistance by Tatjana Franz is highly appreciated. We also thank Anna Rohleder for editorial support in preparation of the manuscript and Ursula Erikli for copy-editing. The work was partially supported by the EU Joint Action QUANDHIP, EAHC contract number 2010 21 02/ A-100 905.

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Barebacking among men who have sex with men recruited through a Swedish website: associations with sexual activities at last sexual encounter

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Citation style for this article:
Berg RC, Tikkanen R, Ross MW. Barebacking among men who have sex with men recruited through a Swedish website: associations with sexual activities at last sexual encounter. Euro Surveill. 2013;18(13):pii=20438. Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=20438

Article submitted on 17 April 2012 / published on 28 March 2013

The research topic of barebacking emerged in the mid-1990s. Since then, a multitude of studies, largely from the United States, have produced invaluable knowledge of factors that help explain the behaviour among men who have sex with men (MSM), and that may contribute to HIV risk reduction programming and advice to counsellors working with barebackers. Given the scant empirical research about barebacking among European MSM, we conducted a survey among 3,634 MSM recruited through a web community in Nordic countries. The objectives of the study were twofold: to describe the sexual activities associated with barebacking behaviour at last sexual encounter, and to evaluate the relationship of barebacking with relevant variables. Men who reported barebacking (n=356) and men who did not (n=3,278) were compared. On the basis of the results of the analyses, the sociosexual profile of barebackers drawn was one that is at increased risk of acquiring human immunodeficiency virus (HIV) and other sexually transmitted infections due to their sexual practices, particularly unprotected anal intercourse, but also group sex and rimming. In a multivariate logistic regression analysis, the likelihood of engaging in barebacking was higher for MSM who reported more frequent HIV testing (odds ratio (OR)=5.16), a higher number of female sex partners (OR=16.80), using gay cruising places (OR=1.51) and gay chat rooms (OR=2.11).

Introduction

After nearly two decades of research about barebacking, the term, which first emerged in the gay press in the mid-1990s [1], remains inconsistently operationalised. While some researchers specify it as intentional condomless anal intercourse among men who have sex with men (MSM) in human immunodeficiency virus (HIV) risk contexts [2-5], others define it as intentional unprotected anal intercourse (UAI) with a non-primary male partner [6,7], or as intentional UAI with a casual or primary partner of any HIV status [8]. A 2009 review [9] concluded that the term had evolved semantically and appeared to hold different meanings across serostatus and cultural groups. Based on interviews with 120 MSM regarding the term barebacking, Carballo-Diéguez and colleagues summarised that MSM by and large understood it as 'condomless anal sex', but that much variation existed. The researchers concluded by suggesting a more HIV prevention-focused distinction between sexual behaviours that were 'intentional and may result in HIV-primary transmission from those that are not' [2].

Although the term barebacking remains elusive and in some communities may have passed into more general usage as a neologism for condomless anal intercourse between men [10-12], research on the issue has produced invaluable knowledge of factors that help explain this behaviour among MSM, and that may contribute to HIV risk reduction programming and advice to counsellors working with barebackers. Existing literature on barebacking was recently summarised in a comprehensive review [9]. With a view to understand reasons for bareback sex, the author positioned empirically identified factors associated with the behaviour in a conceptual framework. It showed that bareback sex was associated with lower age, lower educational attainment, being HIV-positive, recreational drug use, gay community involvement and type of sociocultural environment. Barebacking was also closely associated with both engaging in unprotected sex and having casual partners, which raise added concerns with regard to transmission of HIV and other sexually transmitted infections (STIs) [9].

Men who have sex with men and human immunodeficiency virus

Despite the small size of this community, MSM are the population most severely affected by HIV in European Union and European Economic Area (EU/EEA) countries, accounting for 38% of all new HIV diagnoses in 2010 [13]. Worrying trends show that from 2004 to 2010, the number of HIV diagnoses in this group increased

by 42%, from 7,621 to 10,854 [13]. Surveys mapping behavioural surveillance in Europe [14,15] reveal that just over half (14 of 27) of EU/EEA countries report an established behavioural surveillance system, complicating the evaluation of development in risk behaviour. Trends recorded through behavioural surveillance can offer important insights into corresponding trends in disease incidence over time [14]. Trend data on sexual behaviours among MSM are scarce, and none include information on intention to practice UAI [14,16]. However, according to several reports increases in HIV diagnoses among MSM are linked to an increase in high-risk sexual behaviour (e.g. [17,18]). In England, Dodd et al. [18] identified a significant increase in reporting unprotected anal sex, including UAI with partners of an unknown or discordant serostatus, in recent years. In many countries, the resurgence in HIV diagnoses is linked to STIs [19-21], including syphilis incidence, which in Sweden between 2000 and 2007 was up to 28 times higher among MSM than in the general male population [22].

Surprisingly, little research exists on the range of barebackers' sexual behaviours, beyond their engaging in unprotected sex and having casual partners, that can serve to inform HIV prevention initiatives. As an exception, Léobon and Frigault [23], who have completed one of the few studies that exist about barebacking among MSM in Europe, found that MSM who reported engaging in bareback sex were also more likely to report rimming and group sex. The study results suggested that one in four men engaged in bareback sex in the past year and that compared to respondents from the other three sites, respondents from the bareback website reported having significantly more bareback sex with casual partners [23].

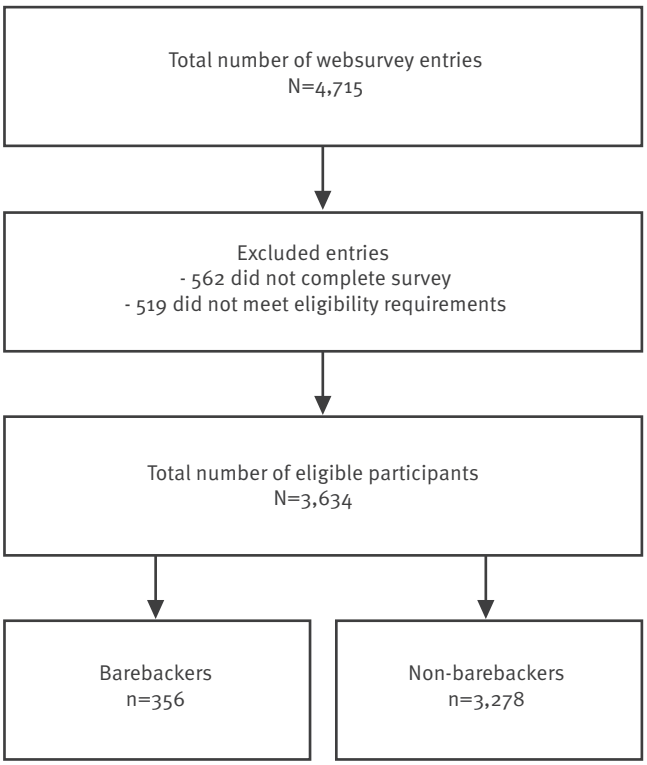
In general, despite a growing body of literature on barebacking, with the exception of Elford and colleagues [24], Léobon and Frigault [23], and Léobon et al. [8], few studies have examined barebacking among European MSM; still fewer have considered the characteristics of last sexual encounter related to bareback sex. Single-event recall like last sexual encounter helps minimise recall bias and is a valid representation of sexual behaviours over longer periods of time [25]. Our objectives were to (i) estimate the occurrence of barebacking and describe sexual activities associated with bareback sex at last sexual encounter among MSM and (ii) to evaluate the relationship of barebacking with HIV testing, having sex with women, and the use of gay cruising places and chat rooms.

Methods

A full description of the study methods has been previously reported [26]. In brief, study eligibility requirements included being MSM, above the age of 15, and having Swedish as the preset language in the Qruiser community, the largest online community in Nordic countries for lesbian, gay, bisexual and transgender persons. Through Qruiser, eligible MSM were invited

Figure

Flowchart of survey participation, March-April 2008



to complete a socio-behavioural survey. The website's banner and pop-up advertisements which invited study participation stated that study completers were eligible to enter into a drawing of travel vouchers. The survey was available for two weeks in March-April 2008, during which time about 50% of the Qruiser community's 51,814 member accounts that met the criteria for being included in the study, logged into the site at least once.

The survey was divided into four sub-sections, including one covering socio-demographic information. The second and third subsections focused on health and sexual behaviours, such as the last sexual encounter with a man. In the final section, two sets of questions were asked about the respondents' thoughts about HIV and their need for information and services concerning HIV, STIs, and safer sex. For the present analyses, descriptive statistics and bivariate analyses were used to evaluate sexual activities at last sexual encounter related to barebacking. In addition, for objective two, four predictor variables implicated in HIV/STI transmission were assessed in univariate logistic regression models: HIV testing, having sex with women, the use of gay cruising places, and the use of chat rooms. Variables with a p value ‹0.001 were included in a multivariate logistic regression model and the odds ratio (OR) with their respective 95% confidence intervals

Table 1

Group differences for men who have sex with men who engaged in barebacking (n=356) and those who did not engage in barebacking (n=3,278), recruited through a web community in Nordic countries, 2008

Variables (last sexual encounter)	Engaged in barebacking (%)[a]	Did not engage in barebacking (%)[a]	Test for difference p value
Found sex partner through internet	142 (57.9)	840 (47.0)	0.002
Had sex at cruising location	49 (13.8)	314 (9.6)	0.015
Had sex with ≥2 people	59 (16.6)	274 (8.4)	<0.001
Had sex with unknown casual partner	141 (39.6)	948 (28.9)	<0.001
Engaged in UAI	166 (46.6)	792 (24.2)	<0.001
Engaged in rimming	138 (38.8)	823 (25.1)	<0.001
Engaged in fisting	21 (5.9)	52 (1.6)	<0.001
Engaged in oral sex	301 (84.5)	2,682 (81.9)	0.243
Received semen in mouth	100 (28.1)	604 (18.4)	<0.001
Ejaculated in partner's mouth	72 (20.2)	592 (18.1)	0.348
Engaged in mutual masturbation	212 (59.5)	2,079 (63.4)	0.148
Communicated about HIV-status	98 (58.3)	732 (43.0)	<0.001

HIV: human immunodeficiency virus; UAI: unprotected anal intercourse.

[a] Percentages were calculated on the basis of the number of cases for which information was available.

(CI) were calculated using SPSS 18.0. In these analyses, the question that served as the outcome variable (barebacking) was worded 'Have you during the past year had unprotected anal intercourse with a casual partner with whom you beforehand decided not to use a condom?' in line with similar studies [6,7]. The protocol for the study was approved by the institutional review board at the University of Gothenburg.

Results

There were 4,715 websurvey entries. Of these, 3,634 were eligible entries from MSM who had been sexually active in the past year (1,081 men either did not complete the survey or failed to meet the eligibility requirements). The sample was predominantly gay (74%), HIV-negative (85%) and almost 96% lived in Sweden. Nearly ten percent of the respondents (n=356) said they had engaged in barebacking in the past year (this was a binary 'yes'/'no' question and we did not ask frequency of barebacking, which likely varied). Additional details regarding the response rate and sample have previously been reported elsewhere [26].

Sexual activities at last sexual contact associated with barebacking

In terms of objective one, situations regarding last sexual encounter, barebacking was associated with finding the sexual partner through the internet, having sex at a cruising location, having sex with two or more people, having sex with an unknown casual partner, and engaging in unprotected anal intercourse. Additionally, sexual activities associated with barebacking at last sexual contact included rimming, fisting, and oral sex with sperm taken in the mouth. At last sexual contact, barebackers were also more likely to have communicated about HIV-status with their partner. For all bivariate analyses, $p < 0.01$, except for having had sex at a cruising location where $p=0.015$. The association between barebacking and oral sex, ejaculating in the partner's mouth, and mutual masturbation was not statistically significant ($p>0.05$) (see Table 1).

Specifically, the analyses showed that MSM who reported bareback sex were more likely to have found their last sex partner through the internet (142/245) than non-barebackers (840/1,786) and had last sex at a gay sauna, sex cinema or other cruising area (49/356 barebackers and 314/3,278 non-barebackers). For 59/356 of barebackers and 274/3,278 of non-barebackers, the last sexual encounter involved two or more partners, and barebackers were also more likely to have engaged in sex with an unknown casual partner (141/356 vs 948/3,278). Compared to men not reporting bareback sex, barebackers were more likely to have communicated about HIV-status at last sexual contact (see Table 1).

Predictors of barebacking

Results for the second objective showed that all four predictor variables (HIV testing, having sex with women, using gay cruising places, using chat rooms) were significantly associated with barebacking in the

Table 2

Multivariate logistic regression analysis summary for predicting barebacking

Variables	Beta weight	Wald chi-squared	Odds ratio (95% CI)	p value
HIV tests	1.64	167.99	5.16 (4.03–6.61)	<0.001
Female sex partners in past year	2.82	490.83	16.80 (13.09–21.56)	<0.001
Used cruising location in past year	0.41	10.83	1.51 (1.18–1.94)	<0.001
Used gay chat weekly	0.75	35.52	2.11 (1.65–2.70)	<0.001

CI: confidence interval; HIV: human immunodeficiency virus

univariate logistic regression analyses (all p<0.001). Men who reported barebacking within the past year reported a mean of 6.5 (standard deviation (SD)=9.7) HIV tests compared to 4.2 (SD=5.3) tests among men who did not report bareback sex. The mean number of female sex partners in the past year was less than one for both barebackers and those not reporting bareback sex (0.9 (SD=2.6) vs 0.50 (SD=2.0)). In total 41.3% (1,256/3,044) of non-barebackers reported using a cruising location in the past year, while the corresponding number for barebackers reporting this behaviour was 63.9% (209/327). Similarly, 43.0% (153/356) of barebackers said they used gay chat rooms weekly, while 29.7% (975/3,278) of their non-barebacking counterparts reported this activity.

In the multivariate logistic regression analysis, the likelihood of engaging in barebacking was higher for those MSM who reported more frequent HIV testing in the past year (OR=5.16), a higher number of female sex partners in the past year (OR=16.80), using gay cruising places in the past year (OR=1.51), and using gay chat rooms at least weekly (OR=2.11). Confidence intervals and final beta weights are shown in Table 2. The Cox and Snell's pseudo R-square was 0.479 and the Hosmer-Lemeshow goodness of fit chi-square test was 243.6, degrees of freedom (*df*)=3, p<0.001. Keeping in mind that a multivariate logistic regression is considered to represent a good fit with the data when the chi-square is statistically non-significant, the four-variable predictor model did not have a significant fit. The model's predictive power was 85.0% and it had a correct classification of barebacking of 11.6%.

Discussion

In this study of MSM recruited from a general gay-interest website, the sample of MSM was diverse with respect to their enacted sexual behaviours. Barebackers were more likely than men not reporting bareback sex to engage in a variety of sexual behaviours associated with higher risk for HIV/STI transmission.

Overall, although 'only' 10% of men indicated they engaged in bareback sex (discussed in [26]), the barebackers in this sample had a higher sexual risk profile, above and beyond barebacking, than other MSM as has been reported elsewhere [6,7,24]. This included nearly half (47%) of barebackers reporting that last sexual encounter involved UAI, 40% reporting that the last sexual encounter was with an unknown casual partner and many stating that it involved rimming, group sex, and taking semen in their mouth. Additionally, barebackers were more likely to report that the last sexual encounter was at a cruising location and multivariate analyses showed that those who reported using gay cruising places were almost twice as likely to engage in bareback sex compared to those who did not frequent such arenas. These findings may suggest, as Léobon and colleagues [8] found in their study of MSM in France, that barebackers have an adventure-oriented sexuality, characterised by sexual risk behaviours across multiple dimensions (acts, frequency, partners, setting) that place them at risk of infection. Indeed, qualitative research among MSM in New York City [27] found no distinctive patterns of factors motivating bareback sex, excepting libidinal and erotic desires that men could not or chose not to control. Other studies report similar findings [3,6].

Consistent with previous empirical research [7,24,28], findings in the present study also extend sexual health professionals' knowledge about the impact of the internet by demonstrating a relationship between barebacking and using the internet for sexual networking. The multivariate analysis showed that weekly use of gay chat rooms was associated with a two-fold increase in the odds of engaging in barebacking and barebackers were more likely than non-barebackers to report having found their last sexual partner through the internet. Also studies carried out in the United States (US) have affirmed the role of the internet in meeting bareback partners [28,29]. Among self-identified

barebackers in New York City, 56% met their last barebacking partner in an online chatroom or website. This venue also yielded the most number of partners [28]. As suggested by our own and other studies [7,29], the internet has become a social community in which many MSM are exposed to and comfortable in the context of sexual risk taking. Léobon and Frigault [23] found that among barebackers in France, the internet was the main environment used for seeking sexual encounters and they suggested that this behaviour was facilitated by specialised sex-oriented venues on the internet. In fact, while recognising that the serves as an important tool in the development of men's social and sexual identity, it has been suggested that the internet plays a growing role in facilitating sexual networking among MSM [26,30]. This may especially be the case for seropositive MSM. According to Elford and colleagues' [24] findings, HIV-positive MSM in London were more likely to find their HIV-positive bareback partners online, presumably because serostatus disclosure was easier online than offline.

Results from our present study show that a segment of MSM engaged in sex with women (almost one in five MSM), supporting earlier findings [7], and that barebackers relative to MSM not reporting this behaviour were significantly more likely to have sex with women. These men may represent an important epidemiological link between the broader MSM and heterosexual communities. Our previous research has suggested that many barebackers also do not identify as gay [26], mirroring results in the review by Millet et al [31], which concluded that a sizeable proportion of non-gay identified men of all ethnicities engage in homosexual sex and often do not disclose such behaviour to their female sex partners. That MSM in our study, especially barebackers, have sexual contact with both men and women increases the likelihood that these men may serve as bridge contacts, responsible for transmission of HIV and other STIs between sexual networks.

To this point, we have highlighted distinctions between barebackers and non-barebackers. On the other hand, it was encouraging that 44% of the sample had communicated about HIV with their last sexual partner. This fact, along with the previous points made, have bearing on future research and intervention initiatives, a point we address below. First, it must be highlighted that in multivariate analysis HIV testing was a significant predictor of barebacking. Barebackers not only reported more frequent HIV testing, but they were also more likely to have communicated about HIV-status with their most recent sexual partner, suggesting that these men negotiate safety around unprotected sex. Our quantitative results fit with a recent qualitative, US-based study, which also discovered that barebackers used strategies to "lessen risk of HIV transmission", such as strategic positioning and relying on knowledge of the reduced infectiousness of partners on successful antiretroviral treatments [5].

As described in this analysis of mostly Swedish MSM, profound differences between barebackers and MSM not reporting bareback sex were identified that may inform future data collection and prevention approaches. A first step is to document and understand the interplay between demographic, psychological, and sociocultural characteristics of barebackers in various areas of Europe, and other regions, using different recruitment strategies. This requires greater resolution about the definition of barebacking, perhaps best accessible through phenomenological research about the behaviour. Subsequent research should include more comprehensive qualitative and quantitative data collection to identify and understand risk trends among barebackers, as well as ways to reach these men, including those that may be harder to reach for outreach and intervention (e.g. those not identifying as gay), and to identify potential leverage points (i.e. changeable key mediators and moderators) to reduce the risk of HIV/STI exposure, infection, and reinfection. A consideration of barebacking as an indicator of behavioural intention to engage in UAI could be valuable, given that behavioural intention is a critical determinant of a person's behaviour [32]. Presently, while surveys have documented a general consensus concerning the main behavioural indicators for MSM, there is considerable diversity between EU/EEA countries [14] and intention is not incorporated. Additional work on relevant indicators in today's behavioural surveillance systems on MSM in Europe [14] as well as Global AIDS response progress reporting [16] seems valuable.

The lack of behavioural surveillance of sexual risks related to HIV and STIs among MSM [14], including barebacking, complicates both the estimation of developments across time and space, as well as the planning and evaluation of prevention programmes. Specifically, barebacking as a behaviour among European MSM has only recently, and limitedly, been explored in academic literature. The current analysis not only explored patterns of sexual behaviour and risk of barebackers and non-barebackers but also potential harm reduction strategies of barebackers, such as communicating about HIV status with sex partners. Future programmes must be appropriately tailored to meet the needs of barebackers, and, as other HIV prevention professionals [4,5,27,33], we suggest that prevention campaigns focusing on barebackers should reinforce harm reduction, given that they seem to already incorporate it in their sexual liaisons. For example, barebackers reported communicating about HIV-status with their sex partners and frequently testing for HIV. Harm reduction strategies include not only open discussions about infection risks and sexuality with potential sex partners and frequent HIV/STI testing and monitoring of sexual health, but also limiting numbers of partners, serosorting, withdrawal before ejaculation, and strategic positioning. In studies of gay and bisexual men in the US, researchers [5,29,34] identified serosorting and strategic positioning as

frequently used harm reduction techniques among men who bareback. Carballo-Diéguez and colleagues' [27] point is well taken in that public health interventions directed at men who bareback must acknowledge the power of libidinal desires while seeking to encourage safer avenues for sexual satisfaction, including pre- or post-exposure prophylaxis. Another consideration for European HIV prevention responses is researchers' [5] warning that continued reliance on HIV prevention messages involving reiteration of risk could intensify barebackers' attachment to unsafe sexual behaviours. The researchers propose that health promoters instead work with the inner contradictions that barebackers express and facilitate spaces for men who bareback to discuss their behaviour and its justification [5].

Continuing from above, MSM who are unaware of their HIV-positive status contribute disproportionately to the transmission of HIV [17], thus testing and counselling may help prevent secondary transmissions. There is also a need for the development, implementation and evaluation of creative and scientifically sound offline, but perhaps particularly online, interventions to affect the diversity of MSM, to prevent a variety of risk behaviours and promote health among MSM. It is important to note here that it can be a challenge to reach high-risk MSM such as barebackers through intervention campaigns. Especially MSM who also have sexual relationships with women may not recognise themselves in HIV prevention programmes primarily targeting gay-identified men. Thus, it will be crucial to develop messages that can be accessed without publicly acknowledging homosexual behaviour, such as internet campaigns and posters placed in public places where men have sex, community-based health and social centres and popular gathering places within the broader community. In this sense, the joint work of the range of education and prevention professionals involved in the setting of MSM (e.g. non-governmental organisations, sexual health clinics, public health offices), not to mention barebackers and other MSM groups, represent a key way forward in the control of HIV/STIs among MSM. Such prevention initiatives have the potential to reduce the rate of new HIV infections among MSM and their partners, members of communities that currently carry a disproportionate burden of the HIV epidemic.

This study is not without limitations. First, the observed associations are based on cross-sectional data. Future studies using a prospective cohort design will be necessary to evaluate the significance and stability of sexual behaviours among barebackers over time. Although the internet provides a data collection mode that may minimise response bias among other limitations, these results remain based on self-reported data and their potential limitations. Another important caveat is that our non-random sample with participant self selection through the Qruiser website limits the ability to generalise the results to other MSM populations. Samples recruited through the internet have been found to be more urban, younger, single, and have higher education [35,36]. It is likely that men who are more closeted about their same-sex preference are less likely to visit gay websites and volunteer for research about MSM. In northern Europe, MSM seem to display sexual behaviours that differ from their counterparts in other countries, such as in the US (see e.g. [26]). These limitations notwithstanding, using 'last sexual encounter' as recall period likely minimised recall bias because it is a valid representation of sexual behaviours over longer periods of time [25]. Additionally, our data provide important insights into patterns of risk behaviour among not only an understudied group of MSM at elevated risk for HIV, but also among a northern European population disproportionately affected by HIV infection rates.

Acknowledgements:

The Swedish National Board of Health and Welfare and The Swedish Institute for Communicable Disease Control.

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