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Treatment failure of pharyngeal gonorrhoea with internationally recommended first-line ceftriaxone verified in Slovenia, September 2011

M Unemo (magnus.unemo@orebroll.se)[1], D Golparian[1], M Potočnik[2], S Jeverica[3]
1. World Health Organization Collaborating Centre for Gonorrhoea and other Sexually Transmitted Infections, Swedish Reference Laboratory for Pathogenic Neisseria, Department of Laboratory Medicine, Microbiology, Örebro University Hospital, Örebro, Sweden
2. Department of Dermatovenereology, University Medical Centre Ljubljana, Ljubljana, Slovenia
3. Institute of Microbiology and Immunology, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia

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We describe the second case in Europe of verified treatment failure of pharyngeal gonorrhoea, caused by an internationally occurring multidrug-resistant gonococcal clone, with recommended first-line ceftriaxone 250 mg in Slovenia. This is of grave concern since ceftriaxone is last remaining option for empirical treatment. Increased awareness of ceftriaxone failures, more frequent test-of-cure, strict adherence to regularly updated treatment guidelines, and thorough verification/falsification of suspected treatment failures are essential globally. New effective treatment options are imperative.

Background

Neisseria gonorrhoeae has developed resistance to all antimicrobial drugs previously used as first-line treatment for gonorrhoea [1]. Resistance to currently recommended first-line third-generation cephalosporins – cefixime and ceftriaxone – is emerging [1-3], and treatment failures with cefixime have been verified in Japan [4] and several European countries, namely Norway [5], the United Kingdom [6], Austria [7] and France [8]. One failure to treat pharyngeal gonorrhoea with ceftriaxone, the last remaining option for empiric treatment, has also been verified in Europe (Sweden) [9]. It is likely that treatment failures with ceftriaxone will initially accumulate for pharyngeal gonorrhoea because these infections are harder to treat than urogenital infections [1,10,11]. It is of grave concern that during the past year, the first three extensively drug-resistant (XDR) [1] *N. gonorrhoeae* strains that also had high-level ceftriaxone resistance were reported from Japan, France and Spain [8,12,13].

In this emergent situation of fear that gonorrhoea may become untreatable [1,8,12], the European Centre for Disease Prevention and Control (ECDC) has prepared a response plan for the European Union [14]. The World Health Organization (WHO) has published the 'Global Action Plan to Control the Spread and Impact of Antimicrobial Resistance in *Neisseria gonorrhoeae*' [15].

This report describes a ceftriaxone treatment failure of pharyngeal gonorrhoea in Slovenia in 2011, which is the second one strictly verified in Europe (and possibly globally).

Case description

In early September 2011, a Slovenian bisexual woman in her early 30s visited a dermatovenereologist in Ljubljana, Slovenia (Day 1). She had no symptoms of gonorrhoea, however, she was sampled and administered the internationally recommended first-line treatment of 1×250 mg ceftriaxone intramuscularly (Table), based on the fact that she had had unprotected oral and vaginal sex with gonorrhoea-positive casual male partner in late August 2011 in Belgrade, Serbia. The partner could later not be traced in Serbia.

Microscopy of Gram-stained smear of a cervical specimen was negative for *N. gonorrhoeae*. However, two days later (Day 3), a pharyngeal culture was shown to be positive for *N. gonorrhoeae*, while the cervical culture was negative. *Chlamydia trachomatis* DNA was identified in an additional cervical sample, using the COBAS TaqMan CT Test v2.0 (Roche Diagnostics). During a follow-up visit seven days after the initial visit (Day 8), a test-of-cure (TOC) pharyngeal culture was taken and examination showed no signs or symptoms of pharyngeal gonorrhoea, and she was given doxycycline at a dosage of 100 mg twice a day, for seven days, for a concomitant chlamydial infection. However, two days later (Day 10) the TOC culture confirmed gonococci in a pharyngeal sample. About three weeks later (Day 30), the patient returned with symptoms of acute pharyngitis (pain, inflammation and fever) and was given one dose of 250 mg ceftriaxone intramuscularly and

one oral dose of 1 g azithromycin. Finally, a follow-up examination after about four months (Day 173) showed no signs of infection, and a pharyngeal TOC culture was negative for *N. gonorrhoeae* (Table). The patient repeatedly reassured that she had not had any sexual contacts between the ceftriaxone therapy and the TOC.

Characterisation of *N. gonorrhoeae* isolates

The pre- and post-treatment *N. gonorrhoeae* isolates were species-confirmed by sugar utilisation test and Phadebact Monoclonal GC Test (Pharmacia Diagnostics). The isolates were indistinguishable using serovar determination (Bpyut), full-length *porB* gene sequencing, multilocus sequence typing (MLST; ST1901 [12]), and *N. gonorrhoeae* multiantigen sequence typing (NG-MAST; ST1407 [16]). Using Etest (AB bioMérieux), both isolates showed a ceftriaxone minimum inhibitory concentration (MIC) of 0.125 mg/L (Table), and overall indistinguishable antibiograms (cefixime 0.25 mg/L, spectinomycin 16 mg/L, azithromycin 0.5 mg/L, and ciprofloxacin >32 mg/L) and were beta-lactamase-negative. According to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) [17], the MIC of ceftriaxone for these isolates were equal to the resistance breakpoint (>0.125 mg/L). Sequencing of resistance determinants for third-generation cephalosporins [1,8,12,18,19] showed that both isolates contained an identical *penA* mosaic allele XXXIV [12], which has been correlated with decreased susceptibility or resistance to third-generation cephalosporins and treatment failure with cefixime [5,20,21]. In addition, they contained *mtrR* and *penB* alterations that further increase the MICs of third-generation cephalosporins [1,8,12,19].

Discussion

This study describes the second verified case in Europe (possibly globally) of treatment failure of pharyngeal gonorrhoea with the internationally recommended first-line treatment of 250 mg ceftriaxone, the last remaining treatment option. The failure was strictly verified in accordance with WHO recommendations [1,15], i.e. detailed clinical records were obtained, reinfection was excluded as much as possible, pre- and post-treatment isolates were indistinguishable using highly discriminatory typing, ceftriaxone MICs were elevated, and the isolates contained well-known cephalosporin resistance determinants. The reporting of the case was unfortunately delayed because it took several months before the patient returned for follow-up examination and TOC after the third antimicrobial treatment (to prove successful eradication of infections).

This case shows that ceftriaxone at a dosage of 1×250 mg may in rare cases not be enough for treatment of pharyngeal gonorrhoea caused by gonococcal strains with ceftriaxone MICs of 0.125 mg/L. A 250 mg ceftriaxone dose also results in median times of free ceftriaxone above the MIC of only 24.1 h (range: 10.5–52.2 h) for the detected MIC of 0.125 mg/L [22], and rare treatment failures may happen in the lower range. Nevertheless, these cases are likely to be treatable with enhanced ceftriaxone doses or dual antimicrobial treatment that has already been introduced as first-line empiric treatment in the United States [10] and the United Kingdom [23]. It may be crucial to promptly revise also other national and regional treatment guidelines, and a revision of the European guidelines from the International Union against Sexually Transmitted Infections (IUSTI) and WHO [2] are currently in progress.

Table

Details of verified ceftriaxone treatment failure of one case of *Neisseria gonorrhoeae* pharyngeal infection, Slovenia, September 2011

Age (years)/ Sex	Place of exposure	Healthcare clinic (day of presentation)	Symptoms (signs)	Positive diagnostics	Negative diagnostics	MIC (mg/L)[a] Ceftriaxone	MLST (NG-MAST)[a]	Treatment
32/ female	Serbia (Belgrade)	STD (1)	- (-)	GC culture (pharynx) and CT PCR (cervix)	GC culture (cervix) and microscopy (cervix)	0.125	ST1901 (ST1407)	Ceftriaxone 250 mg×1 IM
		STD (8)	- (-)	GC culture (pharynx)	NA	0.125	ST1901 (ST1407)	Doxycycline 100 mg b.i.d., 7 days PO[b]
		STD (30)	Pharyngitis (inflammation in pharynx)	NA	NA	NA	NA	Ceftriaxone 250 mgx1 IM and azithromycin 1 gx1 PO
		STD (173)	- (-)	NA	GC culture (pharynx), CT PCR (cervix)	NA	NA	NA

b.i.d.: twice a day; CT: *Chlamydia trachomatis*; GC: *Neisseria gonorrhoeae*; IM: intramuscular administration; MIC: minimum inhibitory concentration; MLST: multilocus sequence typing; NA: not applicable; NG-MAST: *Neisseria gonorrhoeae* multi-antigen sequence typing; PCR: polymerase chain reaction; PO: per oral administration; STD: sexually transmitted diseases.

[a] MIC (mg/L) as determined by Etest, MLST [12] and NG-MAST [16] of *N. gonorrhoeae* pre- and post-treatment isolates.

[b] Treatment of concomitant *C. trachomatis* infection.

It is worrying that the gonococcus causing this treatment failure was assigned to MLST ST1901 and NG-MAST ST1407, which is a multidrug-resistant gonococcal clone that also shows decreased susceptibility and resistance to cefixime and is spreading worldwide [5,7,8,13,20,21,24-28]. The previously reported treatment failures with cefixime in Norway [5], Austria [7], France [8] and likely in the United Kingdom [6], were caused by this gonococcal clone or its evolving subtypes. This clone has also shown its capacity to develop high-level resistance to ceftriaxone [8,13].

In conclusion, the second case in Europe (possibly worldwide) of clinical failure using standard ceftriaxone treatment for pharyngeal gonorrhoea, caused by an internationally occurring multidrug-resistant gonococcal clone, has been strictly verified in Slovenia. An increased awareness of treatment failures with ceftriaxone, more frequent TOC (all cases of pharyngeal cases may be crucial), strict adherence to appropriate treatment guidelines, which need to be regularly updated based on antimicrobial resistance surveillance data, and thorough verification/falsification of suspected treatment failures (including subsequent tracing of sexual contacts of the index case with the treatment failure) are essential globally. A stronger focus on pharyngeal gonorrhoea, including increased sampling of pharyngeal specimens and promotion of condom use also when practising oral sex, is also crucial because pharyngeal infection is harder to treat than urogenital infection, relatively common, and is frequently an asymptomatic reservoir for infection and emergence of resistances [1,5]. Ultimately, new options for effective treatment of gonorrhoea are imperative.

References

1. Tapsall JW, Ndowa F, Lewis DA, Unemo M. Meeting the public health challenge of multidrug- and extensively drug-resistant Neisseria gonorrhoeae. Expert Rev Anti Infect Ther. 2009;7(7):821-34.
2. Bignell C, IUSTI/WHO. 2009 European (IUSTI/WHO) guideline on the diagnosis and treatment of gonorrhoea in adults. Int J STD AIDS. 2009;20(7):453-7.
3. Cole MJ, Unemo M, Hoffmann S, Chisholm SA, Ison CA, van de Laar MJ. The European gonococcal antimicrobial surveillance programme, 2009. Euro Surveill. 2011;16(42):pii=19995. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19995
4. Yokoi S, Deguchi T, Ozawa T, Yasuda M, Ito S, Kubota Y, et al. Threat to cefixime treatment of gonorrhea. Emerg Infect Dis. 2007;13(8):1275-7.
5. Unemo M, Golparian D, Syversen G, Vestrheim DF, Moi H. Two cases of verified clinical failures using internationally recommended first-line cefixime for gonorrhoea treatment, Norway, 2010. Euro Surveill. 2010;15(47):pii=19721. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19721
6. Ison CA, Hussey J, Sankar KN, Evans J, Alexander S. Gonorrhoea treatment failures to cefixime and azithromycin in England, 2010. Euro Surveill. 2011;16(14):pii:19833. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19833
7. Unemo M, Golparian D, Stary A, Eigentler A. First Neisseria gonorrhoeae strain with resistance to cefixime causing gonorrhoea treatment failure in Austria. Euro Surveill. 2011;16(43):pii=19998. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19998
8. Unemo M, Golparian D, Nicholas R, Ohnishi M, Gallay A, Sednaoui P. High-level cefixime- and ceftriaxone-resistant *N. gonorrhoeae* in France: novel penA mosaic allele in a successful international clone causes treatment failure. Antimicrob Agents Chemother. 2012;56(3):1273-80.
9. Unemo M, Golparian D, Hestner A. Ceftriaxone treatment failure of pharyngeal gonorrhoea verified by international recommendations, Sweden, July 2010. Euro Surveill. 2011;16(6):pii=19792. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19792
10. Workowski KA, Berman S, Centers for Disease Control and Prevention (CDC). Sexually transmitted diseases treatment guidelines, 2010. MMWR Recomm Rep. 2010;59(RR-12):1-110.
11. Moran JS. Treating uncomplicated Neisseria gonorrhoeae infections: is the anatomic site of infection important? Sex Transm Dis. 1995;22(1):39-47.
12. Ohnishi M, Golparian D, Shimuta K, Saika T, Hoshina S, Iwasaku K, et al. Is Neisseria gonorrhoeae initiating a future era of untreatable gonorrhea?: detailed characterization of the first strain with high-level resistance to ceftriaxone. Antimicrob Agents Chemother. 2011;55(7):3538-45.
13. Cámara J, Serra J, Ayats J, Bastida T, Carnicer-Pont D, Andreu A, et al. Molecular characterization of two high-level ceftriaxone-resistant Neisseria gonorrhoeae isolates detected in Catalonia, Spain. J Antimicrob Chemother. 2012 May 7. [Epub ahead of print].
14. European Centre for Disease Prevention and Control (ECDC). Response plan to control and manage the threat of multidrug-resistant gonorrhoea in Europe. Stockholm: ECDC; 2012. p. 1-23. Available from: http://www.ecdc.europa.eu/en/publications/Publications/1206-ECDC-MDR-gonorrhoea-response-plan.pdf
15. World Health Organization (WHO), Department of Reproductive Health and Research. Global Action Plan to Control the Spread and Impact of Antimicrobial Resistance in Neisseria gonorrhoeae. Geneva: WHO; 2012. p. 1-36. Available from: http://www.who.int/reproductivehealth/publications/rtis/9789241503501
16. Unemo M, Sjöstrand A, Akhras M, Gharizadeh B, Lindbäck E, Pourmand N, et al. Molecular characterization of Neisseria gonorrhoeae identifies transmission and resistance of one ciprofloxacin-resistant strain. APMIS. 2007;115(3):231-41.
17. European Committee on Antimicrobial Susceptibility Testing (EUCAST). Breakpoint tables for interpretation of MICs and zone diameters. Version 2.0. Basel: European Society of Clinical Microbiology and Infectious Diseases; 1 Jan 2012. Available from: http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint_tables/Breakpoint_table_v_2.0_120221.pdf
18. Unemo M, Fasth O, Fredlund H, Limnios A, Tapsall J. Phenotypic and genetic characterization of the 2008 WHO Neisseria gonorrhoeae reference strain panel intended for global quality assurance and quality control of gonococcal antimicrobial

resistance surveillance for public health purposes. J Antimicrob Chemother. 2009;63(6):1142-51.

19. Zhao S, Duncan M, Tomberg J, Davies C, Unemo M, Nicholas R. Genetics of chromosomally mediated intermediate resistance to ceftriaxone and cefixime in Neisseria gonorrhoeae. Antimicrob Agents Chemother. 2009;53(9):3744-51.
20. Buono S, Wu A, Hess DC, Carlson JS, Rauch L, Philip SS, et al. Using the Neisseria gonorrhoeae Multiantigen Sequence-Typing Method to Assess Strain Diversity and Antibiotic Resistance in San Francisco, California. Microb Drug Resist. 2012 Jun 11. [Epub ahead of print].
21. Heymans R, Bruisten SM, Golparian D, Unemo M, de Vries HJ, van Dam AP. Clonally related Neisseria gonorrhoeae isolates with decreased susceptibility to the extended-spectrum cephalosporin cefotaxime in Amsterdam, the Netherlands. Antimicrob Agents Chemother. 2012;56(3):1516-22.
22. Chisholm SA, Mouton JW, Lewis DA, Nichols T, Ison CA, Livermore DM. Cephalosporin MIC creep among gonococci: time for a pharmacodynamic rethink? J Antimicrob Chemother. 2010;65(10):2141-8.
23. Bignell C, Fitzgerald M; Guideline Development Group. UK national guideline for the management of gonorrhoea in adults, 2011. Int J STD AIDS. 2011;22(10):541-7.
24. Golparian D, Hellmark B, Fredlund H, Unemo M. Emergence, spread and characteristics of Neisseria gonorrhoeae isolates with in vitro decreased susceptibility and resistance to extended-spectrum cephalosporins in Sweden. Sex Transm Infect. 2010;86(6):454-60.
25. Pandori M, Barry PM, Wu A, Ren A, Whittington WL, Liska S, et al. Mosaic penicillin-binding protein 2 in Neisseria gonorrhoeae isolates collected in 2008 in San Francisco, California. Antimicrob Agents Chemother. 2009;53(9);4032-4.
26. Tapsall JW, Ray S, Limnios A. Characteristics and population dynamics of mosaic penA allele-containing Neisseria gonorrhoeae isolates collected in Sydney, Australia, in 2007-2008. Antimicrob Agents Chemother. 2010;54(1):554-6.
27. Tanaka M, Koga Y, Nakayama H, Kanayama A, Kobayashi I, Saika T, et al. Antibiotic-resistant phenotypes and genotypes of Neisseria gonorrhoeae isolates in Japan: identification of strain clusters with multidrug-resistant phenotypes. Sex Transm Dis. 2011;38(9):871-5.
28. Neisseria gonorrhoeae Multi Antigen Sequence Typing (NG-MAST). Query global sequence and ST database. London: Department of Infectious Disease Epidemiology, Imperial College London and are funded by The Wellcome Trust. Available from: http://www.ng-mast.net/sql/allelicprofile.asp.

Salmonella Paratyphi B var Java infections associated with exposure to turtles in Bizkaia, Spain, September 2010 to October 2011

E Hernández (esther-ha@ej-gv.es)[1], J L Rodriguez[1], S Herrera-León[2], I García[3], V de Castro[1], N Muniozguren[1]
1. Unidad de Epidemiología, Subdirección de Salud Pública de Bizkaia, Bilbao, Spain
2. Unidad de Enterobacterias, Centro Nacional de Microbiología, Instituto de Salud Carlos III, Majadahonda, Spain
3. Laboratorio Normativo de Salud Pública del Gobierno Vasco, Derio, Spain

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Between September 2010 and October 2011, the Unit of Epidemiology in the Department of Public Health in Bizkaia, Spain identified eight cases of *Salmonella* Paratyphi B var Java infection and three cases of infection with its possible monophasic variant 4,5,12:b:- dT+. Six cases reported contact with turtles and *S.* Java was isolated from three of these turtles' habitats. The isolates from the patients and their respective turtles were indistinguishable by pulsed-field gel electrophoresis (PFGE). Although other reptiles can also carry *Salmonella*, turtles pose a special risk, as they are commonly kept as pets for children. This emphasizes the need to give recommendations regarding ownership and handling of aquatic turtles and other reptiles. As parents are often not aware of the risk of infection associated with the presence of turtles in the household, it would be appropriate to inform potential buyers at points of sale about the risk of infection and measures they can take to minimise this risk.

Introduction

Salmonella infections are predominantly acquired through the consumption of contaminated food, but contact with animals may also be an important source of infection [1]. Reptiles are frequent carriers of *Salmonella* in their intestinal tract [2], they usually show no signs of illness and shed the bacteria in their faeces, contaminating the water and any surface in contact with them [3-6].

Several *Salmonella* serotypes have been found in reptile-associated salmonellosis, including *Salmonella* Java, *S.* Poona, *S.* Pomona, *S.* Marina, *S.* Stanley, *S.* Litchfield, *S.* Newport and the most common serotypes, *S.* Typhimurium and *S.* Enteritidis [2-7].

Although other reptiles can also carry *Salmonella*, turtles pose a special risk, as they are commonly kept as pets for children.
S. Paratyphi B infections can cause enteric fever (paratyphoid fever) or gastroenteritis. In some cases, serious complications can occur (septicaemia, meningitis), especially in young children and immunocompromised patients [7].

S. Paratyphi B var Java shares the same somatic and flagellar antigens as *S.* Paratyphi B, but uses d-tartrate as a carbon source. This variant appears to be less virulent, causing infections characterised by watery diarrhoea, abdominal pain and fever, although infection can also be invasive. In sporadic cases and outbreaks, infection with *S.* Java has been associated with consumption of contaminated food, including salads, goat's milk cheese and poultry and with contact with reptiles and tropical fish aquariums [8-11].

The Epidemiology Unit of the Department of Public Health in Bizkaia (a territory of the Basque Country, in the north of Spain, with a population of nearly 2,150,000 inhabitants) identified, between September 2010 and October 2011, 14 cases of *S.* Paratyphi B infection (incidence rate: 0.65/100,000 inhabitants). In Spain, the most common *Salmonella* serotypes are Enteritidis and Typhimurium. *S.* Paratyphi B biovar Java represented 2.1%, 1.4% and 1.7% of the *Salmonella* strains isolated from humans and serotyped at the National Reference Laboratory for *Salmonella* in 2009, 2010 and 2011 respectively. As *S.* Java is an unusual serotype, an investigation was initiated to identify the risk factors.

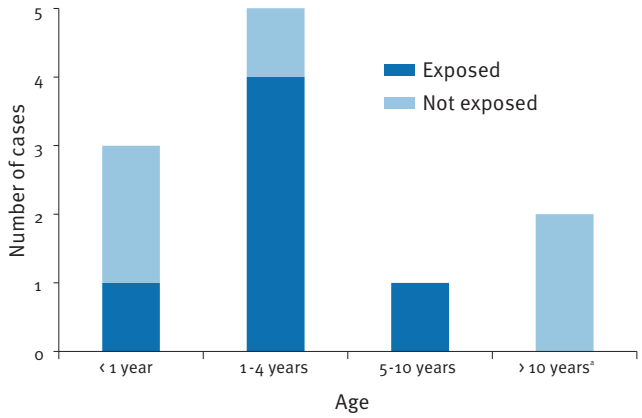
Methods

A case was defined as a patient, resident in Bizkaia, who had an isolate of *S.* Paratyphi B var Java between September 2010 and October 2011.

Adult cases and the parents of the affected children were contacted by telephone and questioned using a standard questionnaire about potential risk factors, such as other cases of gastroenteritis in their environment, travel, consumption of suspected food items and animal exposure. Where contact with turtles was

Figure 1

Cases of *Salmonella* Java and its possible monophasic variant by age group and exposure to turtles, Spain, September 2010–October 2011 (n=11)



[a] Two adults in their mid-20s and early 60s.

reported, a water sample was collected from the turtle's aquarium or terrarium for *Salmonella* testing. Another water sample was taken from the turtle tank at the shop where one of the turtles was bought, for laboratory analysis. The detection of *Salmonella* in the water samples was performed using enzyme-linked fluorescence assay (ELFA) method (bioMérieux´s VIDAS) and by culture (ISO 19250 Water quality-detection of *Salmonella* spp.).

Isolates from patients and environmental samples which were positive for *S.* Paratyphi B were submitted for confirmation to the reference laboratory, National Centre for Microbiology, Carlos III Institute of Health, Madrid, Spain. The strains were typed using phenotypic (lead acetate method) and molecular methods to detect the tartrate reaction [12]. Susceptibility to antimicrobials was tested by the disc diffusion method according to Clinical and Laboratory Standards Institute (CLSI) guidelines [13]. The panel included the following antimicrobials: ampicillin, cefalotin, cefotaxime, amoxicillin/clavulanic acid, tetracycline, streptomycin, kanamycin, gentamicin, nalidixic acid, ciprofloxacin, chloramphenicol, trimethoprim/sulfamethoxazole and a sulphonamide compound (sulfadiazine, sulfathiazole and sulfamerazine sodium). The XbaI-PFGE patterns of strains were compared according to the PulseNet protocol [14].

Three of the 14 cases, identified as *S.* Paratyphi B, were excluded from the investigation because they were not *S.* Java or its variant.

Results

Out of the 14 strains of *S.* Paratyhi B studied, eight were identified as S Paratyphi B variant Java (*S.* Java), three as possible monophasic variants of *S.* Java (S. 4,5,12:b:-), and three as *S.* Paratyphi B sensu stricto. The last three, which came from a family outbreak involving three siblings, produced different clinical manifestations, and were excluded from this description.

The 11 patients from whom *S.* Java or its possible monophasic variant was isolated were not related to each other, and developed a mild disease, with symptoms of gastroenteritis.

Except for two adults in their mid-20s and early 60s, all cases were children aged between three months and

Table

Description of cases and laboratory results, *Salmonella* Paratyphi B var Java infections, Spain, September 2010–October 2011 (n=11)

Case	Age group (years)	Turtle exposure	Pacient		Turtle's water	
			Serotype	PFGE	Result (Serotype)	PFGE
1	5–10	yes	Salmonella Java	Type 1	negative[a]	NA
2	1–4	yes	S. Java	Type 1	negative[a]	NA
3	›10[b]	no	S. Java	Type 1	NA	NA
4	1–4	yes	S. Java	Type 1	S. Java	Type 1
5	‹1	no	4,5,12:b:-	Type 2	NA	NA
6	1–4	no	S. Java	Type 2	NA	NA
7	‹1	yes	S. Java	Type 2	S. Java	Type 2
8	5–10	yes	S. Java	Type 2	S. Java	Type 2
9	‹1	no	4,5,12:b:-	Type 3	NA	NA
10	1–4	yes	4,5,12:b:-	Type 3	negative[a]	NA
11	›10[b]	no	4,5,12:b:-	Type 3	NA	NA

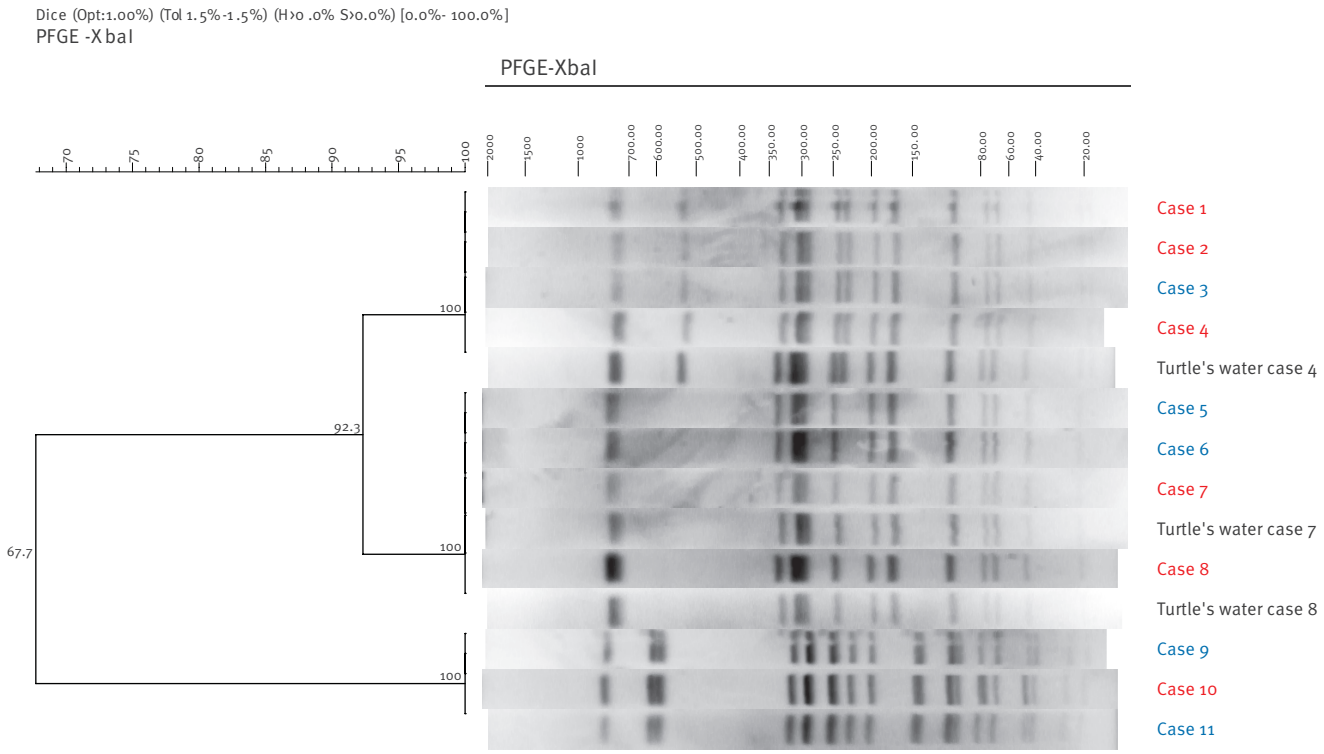
NA: not applicable; PFGE: pulsed-field gel electrophoresis.

[a] These samples were taken with a delay of five to 13 months after the infection.

[b] Adults in their mid-20s and early 60s.

Figure 2

PFGE profiles of cases, *Salmonella* Paratyphi B var Java infections, Spain, September 2010–October 2011



PFGE: pulsed-field gel electrophoresis.
Cases in red: exposed to turtles.
Cases in blue: not exposed turtles.

10 years. Six of the cases among children were male and three were female).

During the interviews, the only common factor found to constitute a risk according to the literature was having been in contact with aquatic turtles during the days before illness onset in six of the nine children, either at home (four cases), or at a relative's house (one case) or at school (one case).

The laboratory results show three different PFGE profiles, which we call type 1, 2 and 3 (Table, Figure 2). All strains were fully susceptible to all antimicrobials tested.

Three of the six samples of turtle's water yielded *Salmonella* Java, with the same PFGE patterns as the bacteria isolated from the children who had contact with them. Two of them were type 2, and the other was type 1. The three negative results came from samples collected more than five months after the infection.

The turtles were purchased at different shops and the supplier or suppliers could not be identified.

The PFGE patterns of isolates of patients with and without turtle exposure were indistinguishable, although the source of infection could not be found. All PFGE profiles were compared with those deposited at the PulseNet network and no match was found.

The water sample taken from the shop where the turtle of case 8 had been bought yielded *Salmonella* serogroup C. This turtle belonged to the subspecies *Trachemys scripta scripta*. The species of the other turtles are not known.

Discussion

Although we lacked a control group, the epidemiological and laboratory findings from our investigation indicate that turtles were the most likely source of infection with *S.* Paratyphi B var Java or its possible monophasic variant in this cluster of cases. Although any *Salmonella* serotype may be carried and transmitted by turtles, *S.* Java has been particularly associated with these reptiles [4].

For the first time, a possible monophasic variant of *S.* Java associated with reptile contact is described.

This is the second time we find an association between contact with turtles and *Salmonella* infection. In 2008, following an increase in *S.* Typhimurium infections in our region, a case-control study was performed, which estimated the odds of infection to be 1.62 times higher if the case had been exposed to turtles (95% confidence interval (CI): 0.68–3.89). In this study, 67/145 (46.2%) of cases were children aged between one and four years and 24/138 (17.4%) of cases reported contact with turtles. The association between reptile exposure and *Salmonella* infection has been described in several countries [2-7,15-18].

Most cases of turtle-associated salmonellosis occur in young children, who are in the most susceptible age spectrum, probably because they usually have a closer contact with these pets, and play with the aquarium water, which is a good medium for the growth of *Salmonella*. Moreover, their hygiene practices tend to be worse than those of adults [2,15]. In addition, parents are often not aware of the risk of infection associated with the presence of turtles in the household.

Not all the cases in this cluster reported exposure to turtles. However, direct contact is not necessary for infection; environmental contamination and symptomatic or asymptomatic patients represent possible sources of infection that may have gone unnoticed. As *Salmonella* bacteria survive in the environment for a long time [2,5], indirect transmission can play an important role.

Three of the six samples of turtle's water tested negative. However, *Salmonella* shedding can be intermittent and increase in response to stress like crowding, living in an environment with inadequate temperature, humidity or cleanliness, transportation, a change of habitat or excessive handling. A negative result doesn't rule out the possibility of intermittent water contamination [2,5]. For this same reason, a mixed infection in the water of the shop where *Salmonella* serogroup C was found is possible.

In the United States of America (USA), the association between contact with small turtles and *Salmonella* infection lead, in 1975, to a ban on the sale and distribution of turtles under 10.2 cm in carapace length, except for scientific or educational purposes. As a consequence, an important reduction in the number of *Salmonella* infections was observed in the following years, especially among children [2-5]. Since then, many sporadic turtle-associated salmonellosis cases have been detected.

In recent years, there has been an increase in the number of reptiles kept as pets, as well as in the number of infections linked to contact with reptiles, including more common serotypes, such as Typhimurium [2-4]. Currently, an estimated 6% of *Salmonella* infections in the USA are caused by direct or indirect contact with reptiles [4]. In February 2012, the Centers for Disease Control and Prevention (CDC) reported 132 cases of *S.* Paratyphi B var. L (+) tartrate + infection between 5 August 2010 and 26 September 2011. The median age of the patients in this outbreak was six years and of the 56 patients interviewed, 36 reported turtle exposure [19].

In Europe, *Salmonella* infection cases attributed to direct or indirect contact with reptiles have also been described, although the number is likely to be underestimated, as in many cases the source of infection is unknown [17]. In Sweden for instance, between 1990 and 2000, 339 reptile-associated *Salmonella* infections were reported, accounting for approximately 5% of all reported cases [5]. In this country, from 1970 to 1994, a certificate was required for the import of reptiles, stating that the animals were free of *Salmonella*, and the commercial distribution of turtles with a carapace length less than 10.2 cm was banned. When import regulations ceased, an increase in the number of cases was observed between 1996 and 1997. After a public education campaign launched in 1997, the number of cases decreased again [20].

Attempts to eliminate *Salmonella* from turtles by antibiotic treatment have not been successful, as the animals readily become reinfected from the environment, food or other turtles and can result in the development of antibiotic resistance. As *Salmonella* shedding may be intermittent and related to stress, it is difficult to determine whether turtles are free of bacteria [2]. For this reason, the way to prevent transmission is to avoid contact of susceptible persons with turtles and to follow strict hygiene practices to minimise the risk of infection.

In the US, apart from the restrictions on the sale of small turtles, there are recommendations published by CDC for preventing reptile-associated salmonellosis, which include washing hands after handling reptiles and keeping reptiles away from food and food preparation areas [21].

Conclusions and recommendations

In conclusion, there is a risk of *Salmonella* infection linked to contact with turtles, which emphasises the need to give recommendations regarding ownership and handling of aquatic turtles and other reptiles kept as pets by young children. These recommendations can also apply to immunocompromised persons. It would also be appropriate to give information to potential buyers at points of sale about the risk of *Salmonella* infection and measures that can be taken to minimise this risk.

A report of this outbreak with the following recommendations was sent to the public health authorities and the Department of Agriculture in Bizkaia so that preventive measures can be taken.

Recommendations given for preventing *Salmonella* infection from turtles included:

- washing hands with water and soap immediately after handling turtles (or other reptiles);
- cleaning and disinfecting surfaces that have been in contact with the animal;
- not using the kitchen to wash the aquarium/terrarium (if the bathroom is used, this should be disinfected after use);
- avoiding contact of the turtle with food (turtles should not live in the kitchen or roam freely in the house);
- avoiding contact of especially susceptible people (children under five years, pregnant women, patients with cancer or undergoing chemotherapy treatment, transplanted patients, persons with diabetes, hepatic conditions or other immunocompromised persons) with turtles and any object that has been in contact with them.

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References

1. Musto J, Kirk M, Lightfoot D, Combs BG, Mwanri L. Multi-drug resistant *Salmonella* Java infections acquired from tropical fish aquariums, Australia, 2003-04. Commun Dis Intell. 2006;30(2):222-7.
2. Centers for Disease Control and Prevention (CDC). Multistate outbreak of human *Salmonella* infections associated with exposure to turtles: United States, 2007-2008. MMWR Morb Mortal Wkly Rep. 2008;57(3):69-72.
3. Mermin J, Hutwagner L, Vugia D, Shallow S, Daily P, Bender J, et al. Reptiles, amphibians, and human *Salmonella* infection: a population-based, case-control study. Clin Infect Dis. 2004;38 Suppl 3: S253-61.
4. Harris JR, Neil KP, Behravesh CB, Sotir MJ, Angulo, FJ. Recent multistate outbreaks of human *Salmonella* infections acquired from turtles: a continuing public health challenge. Clin Infect Dis. 2010;50(4):554-9.
5. Hoelzer K, Moreno Switt AI, Wiedmann M. Hoelzer et al. Animal contact as a source of human non-typhoidal salmonellosis Vet Res. 2011;42(1):34.
6. Wells EV, Boulton M, Hall W, Bidol SA. Reptile-associated salmonellosis in preschool-aged children in Michigan, January 2001-June 2003. Clin Infect Dis. 2004;39(5):687-1.
7. Nagano N, Oana S, Nagano Y, Arakawa Y. A severe *Salmonella* enterica serotype Paratyphi infection in a child related to a pet turtle, Trachemys scripta elegans. Jpn J Infect Dis. 2006;59(2):132-4.
8. Gobin M, Launders N, Lane C, Kafatos G, Adak B. National outbreak of *Salmonella* Java phage type 3b variant 9 infection using parallel case–control and case–case study designs, United Kingdom, July to October 2010. Euro Surveill. 2011;16(47):pii=20023. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=20023
9. Denny J, Threlfall J, Takkinen J, Löfdahl S, Westrell T, Varela C, et al. Multinational *Salmonella* Paratyphi B variant Java (*Salmonella* Java) outbreak, August – December 2007. Euro Surveill. 2007;12(51):pii=3332. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=3332
10. Chart H. The pathogenicity of strains of *Salmonella* Paratyphi b and *Salmonella* Java. J Appl Microbiol 2003;94(2):340-8.
11. Levings RS, Lightfoot D, Hall RM, Djordjevic SP. Aquariums as reservoirs for multidrug-resistant *Salmonella* Paratyphi B. Emerg Infect Dis. 2006;12(3):507-10.
12. Malorny B, Bunge C, Helmuth R. Discrimination of d-tartrate-fermenting and -nonfermenting *Salmonella* enterica subsp. enterica isolates by genotypic and phenotypic methods. J Clin Microbiol. 2003;41(9):4292-7.
13. Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing; twenty-first informational supplement. CLSI document M100-S21. Wayne, PA: CLSI; 2011.
14. Centers for Disease Control and Prevention (CDC). PulseNet Protocols. *Salmonella*. Atlanta: CDC. [Accessed 20 Jun 2012]. Available from: http://www.cdc.gov/pulsenet/protocols.htm
15. Stam F, Römkens TE, Hekker TA, Smulders YM. Turtle-associated human salmonellosis. Clin Infect Dis. 2003;37(11):e167-9.
16. Centers for Disease Control and Prevention (CDC). Turtle-associated salmonellosis in humans – United States, 2006-2007. MMWR Morb Mortal Wkly Rep. 2007;56(2):649-52.
17. Editorial team, Bertrand S, Rimhanen-Finne R, Weill FX, Rabsch W, Thornton L, et al. *Salmonella* infections associated with reptiles: the current situation in Europe. Euro Surveill. 2008;13(24):pii=18902. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=18902
18. Aiken AM, Lane C, Adak GK. Risk of *Salmonella* infection with exposure to reptiles in England, 2004-2007. Euro Surveill. 2010;15(22):pii=19581. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19581
19. Centers for Disease Control and Prevention (CDC). Notes from the field: outbreak of salmonellosis associated with pet turtle exposures-United States, 2011. MMWR Morb Mortal Wkly Rep. 2012;61(4):79
20. De Jong B, Andersson Y, Ekdahl K. Effect of regulation and education on reptile-associated salmonellosis. Emerg Infect Dis. 2005;11(3):398-403.
21. Centers for Disease Control and Prevention (CDC). Healthy Pets Healthy People (HPHP). Is a turtle the right pet for your family?. Atlanta: CDC. [Accessed 20 Jun 2012]. Available from: http://www.cdc.gov/healthypets/spotlight_an_turtles.htm

The case-cohort design in outbreak investigations

O Le Polain de Waroux (olivier.lepolain@hpa.org.uk)[1,2], H Maguire[1,2], A Moren[3]
1. London Region Epidemiology Unit, Health Protection Agency (HPA), London, United Kingdom
2. European Programme for Intervention Epidemiology Training (EPIET), European Centre for Disease Control and Prevention (ECDC), Stockholm, Sweden
3. EpiConcept, Paris, France

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The use of the case–cohort design for outbreak investigations has been limited. Here we discuss its strengths and limitations based on real and fictitious examples. The case–cohort is a case–control study where controls are sampled from the initial population at risk, and may thus include both cases and non-cases. An advantage of the design, compared to traditional case–control studies, is that risk ratios can easily be obtained directly from the cross-product of exposed and unexposed cases and controls (rare disease assumption is not required). We illustrate this in the context of point source gastrointestinal outbreaks and in field studies on vaccine effectiveness. The design is also useful to investigate multiple outcomes with a unique sample of controls or to test hypotheses when different case-definitions (from the most sensitive to the most specific) are used for a particular outcome. Strengths and limitations are presented, and discussed in the context of outbreak investigations.

Introduction

Outbreaks are defined as any excess in the number of cases of disease that would normally be expected in a particular geographic area over a particular period of time [1]. Outbreak investigations differ from standard epidemiological research as they are often conducted under time and resource constraints. Design options mostly depend on the outbreak setting, the size of the outbreak and of the population affected, whether or not the affected population is well defined and its members identifiable, and what measure of association is desired. In addition, the approach may vary depending on the pathogen (or the environmental hazard) and its mode of transmission, as well as time, staff and resource constraints.

The two main study designs generally considered by field epidemiologists in the investigation of outbreaks are the retrospective cohort design and the traditional case–control design. In the retrospective cohort, all members of a defined cohort are included in the study and information on their exposure to different factors is investigated retrospectively [2]. Risk of illness in exposed and unexposed individuals is obtained and the measure of association is the risk ratio (RR). Traditional case–control designs (also called 'cumulative' or 'classic' case–control) offer an efficient alternative when the source population (i.e. the population from which cases arose) is large and/or the outcome rare. Exposures in cases are compared to exposures in a sample of the non-cases (i.e. the controls) drawn from the same at-risk population, and the most common measure of association is the odds ratio (OR).

The case–cohort design is an alternative to the traditional case–control design. In the case-cohort design controls are randomly sampled from the source population, regardless of their disease status.

Although the case–cohort design has gained popularity in large prospective studies [3], its use in outbreak investigations has been limited [4,5]. There is, to the best of our knowledge, no publication that explains, summarises and discusses the use of case–cohort designs in the context of outbreak investigations.

In that context, the aim of this paper is therefore to summarise the theory of the case–cohort design, illustrate its use in four different outbreak scenarios and discuss its strengths and limitations.

Description of the case–cohort design

The foundation of case–cohort design is generally attributed to Prentice who, in 1986, described it as an efficient alternative to a full cohort design in the context of prospective research when the collection and follow-up of covariate information in each cohort member is costly and time-consuming [6]. Similar approaches were suggested by others under the terminology 'hybrid epidemiologic design', 'case–base' design or 'inclusive' case–control design [7-9].

Sampling and sample size

In a case–cohort design, all cases (or a random sample of all cases) and a random sample of the source population (i.e. the controls) are included in the study. The controls may therefore include some of the cases included in the case group [10].

Figure 1 illustrates the sampling of cases and controls in the case–cohort study design, and compares this with the traditional case–control and retrospective cohort designs.

The sampling strategies for controls include the whole range of probabilistic sampling methods used in cross-sectional studies, also including complex sampling designs. There are not many examples, but one is in a case-cohort study during and outbreak in Darfur, Sudan that used complex sampling to recruit controls [4].

Generally a little less statistical power is achieved with a case–cohort study, compared to a traditional case–control study, if both have an equal number of controls, inversely proportional to the primary attack rate (AR). A simple way of estimating the number of controls required for a defined power is to apply sample size calculations used in traditional case–control studies and multiply the number of controls by a weighting factor corresponding to the inverse of the proportion of non-cases in the initial cohort. For example, if the AR is 33% then 50% more controls (as $(1\text{-}0.33)^{-1}=1.5$) will have to be selected than in a traditional case–control study, whereas if the AR is only 5% the number of controls will only need to be increased by 5% (as $(1\text{-}0.05)^{-1}=1.05$). In some situations however, the AR will not be known at the start of the investigation.

Measure of association and analysis

Provided that cases are a random sample of all cases and the controls are sampled randomly from the source population, the cross product of exposed and unexposed cases and controls will yield a true estimate of the crude RR (allowing for sampling error), unlike the traditional case–control study where the OR obtained from the cross product of exposed and unexposed cases and controls will generally overestimate the RR (if true RR>1) or underestimate the true RR (if true RR<1). This inflation – or deflation – of the OR in case–control studies increases as the AR increases – or decreases – and also depends on the magnitude of the true RR (Figure 2).

Standard logistic regression can be used for multivariable analysis, in the same way as in a traditional case–control study, to obtain direct estimates of the adjusted RRs from the model output. This approach, taken in previous case–cohort studies [4,5], is limited however by the lack of precision around the estimates,

Figure 1

Comparing three study designs: case–cohort, case–control and retrospective cohort

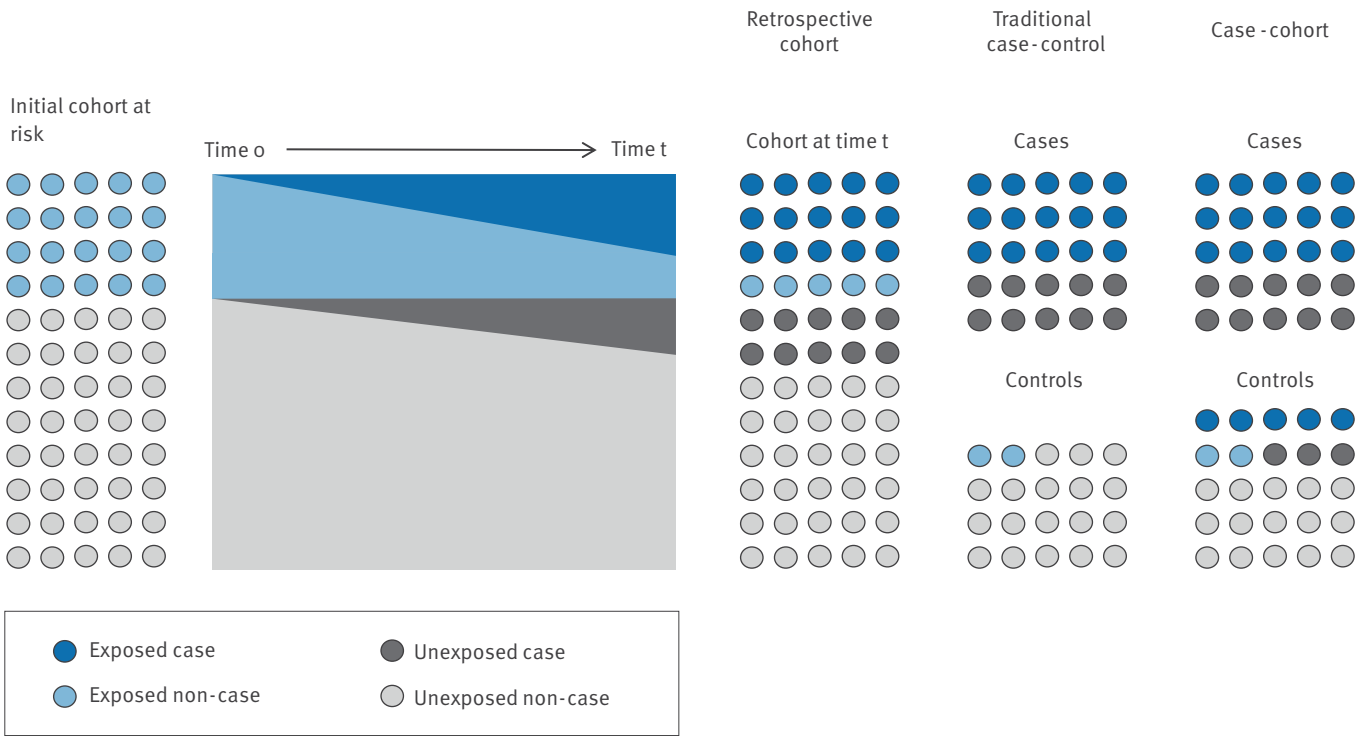
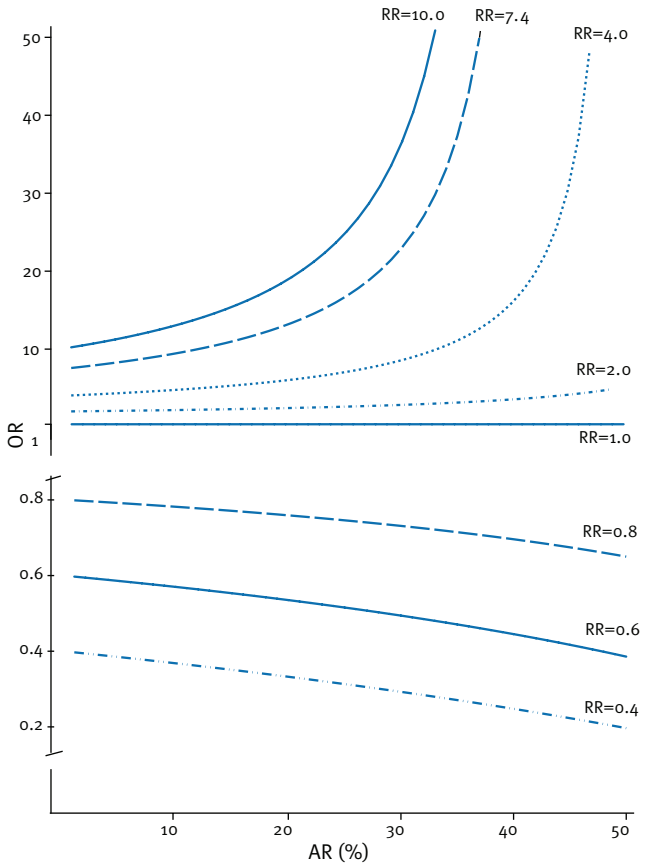


Figure 2

The relationship between odds ratio and risk ratio, for increasing attack rates



AR: attack rate; OR: odds ratio; RR: risk ratio

with standard errors generally being equal or larger than the true standard errors [10]. This may not be a major constraint when a strong association is found for a particular exposure variable; however in situations where weak evidence of an association is found, this should be taken into account in the interpretation of the results. Several solutions have been proposed to deal with this [8,11,12]. Schouten et al. [11] developed pseudo-likelihood risk models using logistic regression with a so called 'sandwich estimator' (or robust variance estimator) derived from the covariate matrix of the model output. Logistic regression is applied in traditional case–control studies, but RRs are obtained directly from the model output. The sandwich estimator adjusts the standard errors of the RR. This approach only requires common statistical software but may be more challenging if software commands are not readily available.

Outbreak scenarios

We will illustrate the case–cohort design through four commonly encountered outbreak scenarios, and discuss its strengths and limitations compared with traditional case–control and retrospective cohort designs. Examples are either based on published outbreak investigations, or, if no such outbreak investigation was published, are fictitious for illustrational purposes. We chose examples which illustrate the design well and cover different types of scenarios where the case–cohort design might be considered.

Scenario 1: A point-source outbreak in a closed setting

Outbreaks occurring in closed settings, such as schools, cruise ships or parties are common. To illustrate design options in this context, let us imagine a Salmonella outbreak following a party attended by 400 people, of whom 100 developed symptoms of diarrhoea within two days following the event and were defined as primary cases (AR: 25%).

If contact details of all participants can be obtained, the first choice would be to conduct a retrospective cohort design. Let us assume that in a retrospective cohort study a particular food item (food x) emerged as the most important risk factor in a univariable analysis, with 80 ill with diarrhoea amongst the 140 exposed (AR: 57.1%) and 20 ill amongst the 260 unexposed (AR: 7.4%), giving a crude risk ratio of 7.4 (95% confidence interval (CI): 4.8–11.4).

Under time and resource constraints of outbreak investigations, there is often a need to collect data on smaller sample sizes, and the use of traditional case–control or case–cohort studies (in this case, nested in the cohort) could be envisaged.

The Table compares the results of the univariable analysis for food x obtained with a retrospective cohort to those obtained in a traditional case–control study and in a case–cohort study, in which the sample size would be half that of the cohort. The true RR is obtained in the case–cohort study, whereas, in the traditional case–control studies, the OR does not approximate the true RR because the overall primary AR is high (25%), as shown in Figure 2.

Table. Comparing measures of association in the retrospective cohort, case–cohort and traditional case–control studies

Attributable risk fractions (the proportion of cases explained by the association=(RR-1)/RR) can also be calculated easily with case–cohort studies.

Arguably, in most outbreaks such as food-borne outbreaks the exact quantification of the risk increase associated with a particular factor may be unimportant

as long as there is evidence that that particular factor is associated with an increased risk, and over- or underestimating the RR may not matter that much.

Scenario 2: A vaccine effectiveness study during an outbreak

Outbreaks provide good opportunities to measure vaccine effectiveness (VE). Traditional case–control studies are often conducted when the source population is too large to conduct a retrospective cohort design [13].

In these studies VE is calculated as 1-OR of being vaccinated, where OR is assumed to approximate the RR. However, in studies of VE it is important to accurately obtain a precise estimate of the true RR. The use of the traditional case–control study in that context should therefore be discouraged given the difficulties in interpreting the OR [14].

The case–cohort design offers a suitable alternative in the context of VE studies during outbreaks, as it allows (i) to obtain true estimates of the RR and (ii) to randomly sample controls from the population without the need to enquire about disease history.

During a mumps outbreak in Switzerland, Richard et al. [15] investigated and compared the VE of two mumps vaccines. Cases were obtained from outbreak and surveillance data and controls were selected from a random systematic sample of GP registers, regardless of children's disease status. Similarly, Carrat et al. [16] used a case–cohort design to investigate influenza VE. Vaccination status in cases of confirmed influenza was compared to the vaccination status in controls randomly selected from GP registers, irrespective of whether or not they suffered from ILI during the influenza epidemic period. The design was particularly useful to obtain true estimates of the RR, and thus the VE, given the high incidence rate of ILI and influenza in the population.

Scenario 3: A food-borne outbreak at a restaurant

Food-borne outbreaks linked to restaurants are common. The use of a retrospective cohort design in restaurant outbreaks is often limited by the lack of identifiable controls, either because the guests' details have not been recorded or because the restaurant management may refuse to release details on their customers [17]. Traditional case–control studies are therefore often seen as the only available option, in which controls are a convenient sample selected from the non-ill meal companions of cases [17-19]. There may be few of these unaffected individuals, or they may not represent the average meal consumption of the customers as they tend to be more similar to the cases with regard to their meal consumption. In a situation where non-ill meal companions were scarce, Giraudon et al. [19] instead used a case–case approach in their investigation of a Salmonella PT1 outbreak linked to a fast-food restaurant in London. They compared consumption in mild cases to that reported by severe cases assuming an exposure dose–response effect.

We suggest that in food-borne outbreaks linked to restaurants, where no customers' list is available, a case–cohort design could be performed, in which meal consumption in the cohort of customers (e.g. based on receipts or any other type of restaurant record) would be compared to meal consumption in cases. Limitations with this approach include the lack of adjustment for the possible confounding effects age and sex, and the assumption that all food and drinks served were consumed. Its advantage is a rapid test of hypotheses, with no need of selection and interviewing controls. This can be particularly useful during ongoing outbreaks where speed is crucial.

Scenario 4: Investigating multiple outcomes

The opportunity to study multiple outcomes is particularly helpful in outbreak situations because, unlike in standard epidemiological research, case definitions are often dynamic. Generally, the case definition is initially broad (sensitive) and is narrowed down (more specific) as more information is gathered (e.g. laboratory confirmation).

With case–cohort studies, hypotheses can be tested with different sets of cases (e.g. from the most sensitive to the most specific case definition) using only one sample of controls.

Moreover, in situations where several outbreaks occur at the same time, especially outbreaks linked to similar

Table

Comparing measures of association in the retrospective cohort, case–cohort and traditional case–control studies

Type of design	Sample size	Number of cases	Type of measure of association	OR or RR (95%CI)
Retrospective cohort	400	100	RR	7.4 (4.8–11.6)
Traditional case–control	200	100	OR	16.0 (8.0–32.0)
Case–cohort	200	100	RR	7.4 (4.2–13.1)[a]

OR: odds ratio; RR: risk ratio.

[a] Variance derived from a first-order Taylor series approximation.

risk factors, the case–cohort design allows for one single control group to be used as reference group to investigate multiple outcomes.

For example, Martin et al. [5] used a case–cohort study to investigate a *Campylobacter* outbreak in the municipality of Söderhamn, Sweden, linked to the consumption of communal water. Although the number of confirmed campylobacteriosis cases was small (n=101) in comparison to the population of Söderhamn (n=27,765), the use of a traditional case–control study was complicated by the fact that another large outbreak of acute gastrointestinal illness (initially thought to affect more than 20% of the residents) occurred simultaneously, possibly including some unconfirmed cases of campylobacteriosis and possibly linked to the same source. A case–cohort study was conducted, and the control group was a simple random sample of the community, thus including some individuals with gastrointestinal illness. The investigation found that consuming communal water increased the risk of both campylobacteriosis and acute gastrointestinal illness, and the risk increased with the amount of water consumed.

Conclusions

We have described the use of the case–cohort design in field epidemiology, and illustrated its strengths and weaknesses through examples.

Among the advantages we identified is that a true estimate of the RR is possible. Although the OR may be good enough in most outbreak situations, there are situations (in particular VE studies) where obtaining a precise estimate of the true RR is important.

Further, the control group represents a random sample of the source population, and detailed disease history is therefore not required. This is particularly advantageous when cases and controls are sampled from different source databases, for instance a surveillance database for cases and a GP practice register for controls.

In addition, the control group can easily be used as a reference group to investigate multiple outcomes.

There are also a few limitations such as reduced statistical power compared with a traditional case–control study and the few analytical challenges, which can be addressed, but need more statistical expertise than a traditional case–control design.

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References

1. Heyman DL, editor. Control of Communicable Disease Manual. 19th ed. Washington, DC: American Public Health Association; 2008.
2. Dwyer DM, Strickler H, Goodman RA, Armenian HK. Use of case-control studies in outbreak investigations. Epidemiol Rev. 1994;16(1):109-23.
3. Kulathinal S, Karvanen J, Saarela O, Kuulasmaa K. Case-cohort design in practice - experiences from the MORGAM Project. Epidemiol Perspect Innov. 2007;4(1):15.
4. Guthmann JP, Klovstad H, Boccia D, Hamid N, Pinoges L, Nizou JY, et al. A large outbreak of hepatitis E among a displaced population in Darfur, Sudan, 2004: the role of water treatment methods. Clin Infect Dis. 2006;42(12):1685-91.
5. Martin S, Penttinen P, Hedin G, Ljungstrom M, Allestam G, Andersson Y, et al. A case-cohort study to investigate concomitant waterborne outbreaks of Campylobacter and gastroenteritis in Soderhamn, Sweden, 2002-3. J Water Health. 2006;4(4):417-24.
6. Prentice RL. A case-cohort design for epidemiologic cohort studies and disease prevention trials. Biometrika. 1986;73:1-11.
7. Kupper LL, McMichael AJ, Spritas R. A hybrid epidemiologic study design useful in estimating relative risk. J Am Stat Assoc. 1975;70(351):524-8.
8. Miettinen O. Design options in epidemiologic research. An update. Scand J Work Environ Health. 1982;8(Suppl 1):7-14.
9. Rodrigues L, Kirkwood BR. Case-control designs in the study of common diseases: updates on the demise of the rare disease assumption and the choice of sampling scheme for controls. Int J Epidemiol. 1990; 19(1):205-13.
10. Rothman KJ, Greenland S, Lash TJ. Case-control Studies. In: Rothman KJ, Greenland S, editors. Modern Epidemiology. Philadelphia: Lippincott Williams and Wilkins; 2008: p. 111-27.
11. Schouten EG, Dekker JM, Kok FJ, Le Cessie S, Van Houwelingen HC, Pool J, et al. Risk ratio and rate ratio estimation in case-cohort designs: hypertension and cardiovascular mortality. Stat Med. 1993;12(18):1733-45.
12. Onland-Moret NC, van der A DL, van der Schouw YT, Buschers W, Elias SG, van Gils CH et al. Analysis of case-cohort data: a comparison of different methods. J Clin Epidemiol. 2007;60(4):350-5.
13. Goodson JL, Perry RT, Mach O, Manyanga D, Luman ET, Kitambi M, et al. Measles outbreak in Tanzania, 2006-2007. Vaccine. 2010;28(37):5979-85.
14. Moulton LH, Wolff MC, Brenneman G, Santosham M. Case-cohort analysis of case-coverage studies of vaccine effectiveness. Am J Epidemiol. 1995;142(9):1000-6.
15. Richard JL, Zwahlen M, Feuz M, Matter HC, Swiss Sentinel Surveillance Network. Comparison of the effectiveness of two mumps vaccines during an outbreak in Switzerland in 1999 and 2000: a case-cohort study. Eur J Epidemiol. 2003;18(6):569-77.
16. Carrat F, Tachet A, Rouzioux C, Housset B, Valleron AJ. Field investigation of influenza vaccine effectiveness on morbidity. Vaccine. 1998;16(9-10):893-8.
17. Baker K, Morris J, McCarthy N, Saldana L, Lowther J, Collinson A, et al. An outbreak of norovirus infection linked to oyster consumption at a UK restaurant, February 2010. J Public Health (Oxf). 2011;33(2):205-11.
18. Barton BC, Mody RK, Jungk J, Gaul L, Redd JT, Chen S, et al. 2008 outbreak of Salmonella Saintpaul infections associated with raw produce. N Engl J Med. 2011;364(10):918-27.
19. Giraudon I, Cathcart S, Blomqvist S, Littleton A, Surman-Lee S, Mifsud A, et al. Large outbreak of salmonella phage type 1 infection with high infection rate and severe illness associated with fast food premises. Public Health. 2009;123(6):444-7.

Epidemiology of *Chlamydia trachomatis* endocervical infection in a previously unscreened population in Rome, Italy, 2000 to 2009

V Marcone (valentinamarcone@libero.it)[1], N Recine[2], C Gallinelli[1], R Nicosia[1], M Lichtner[3], A M Degener[4], F Chiarini[1], E Calzolari[2], V Vullo[1]

1. Department of Public Health and Infectious Diseases, Sapienza University, Rome, Italy
2. Department of Obstetric and Gynaecological Sciences and Urologic Sciences, Sapienza University, Rome, Italy
3. Department of Infectious Diseases, Sapienza University, Polo Pontino, Rome, Italy
4. Department of Molecular Medicine, Sapienza University, Rome, Italy

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As reliable data on *Chlamydia trachomatis* infection in Italy are lacking and as there is no Italian screening policy, epidemiological analyses are needed to optimise effective strategies for surveillance of the infection in the country. We collected data from 6,969 sexually active women aged 15 to 55 years who underwent testing for endocervical *C. trachomatis* infection at the Cervico-Vaginal Pathology Unit in the Department of Gynaecology and Obstetrics of Sapienza University in Rome between 2000 and 2009. The mean prevalence of *C. trachomatis* endocervical infection during this period was 5.2%. Prevalence over time did not show a linear trend. Univariate analysis demonstrated a significant association of infection with multiple lifetime sexual partners, younger age (<40 years), never having been pregnant, smoking, use of oral contraceptives, and human papillomavirus and *Trichomonas vaginalis* infections. Multivariate stepwise logistic regression showed that *T. vaginalis* infection, age under 20 years and more than one lifetime sexual partner remained significantly associated with *C. trachomatis* infection in the final model. Prevalence of *C. trachomatis* in this study was high, even among women aged 25–39 years (5.1%): our data would suggest that a *C. trachomatis* screening policy in Italy is warranted, which could lead to a more extensive testing strategy.

Introduction

Chlamydia trachomatis endocervical/urethral infection, caused by serotypes D to K is the most common bacterial, treatable sexually transmitted infection worldwide [1,2]. As up to 80% of cases are asymptomatic, *C. trachomatis* can be spread unknowingly and remains largely undiagnosed [1,2]. The prevalence of the infection in Europe varies according to the population, setting, country, resource allocation for surveillance and prevention and national reporting system, if there is one. A systematic review of *C. trachomatis* infection among asymptomatic unscreened European women showed that the prevalence ranged from 1.7% (among women aged 15–40 years in the United Kingdom in the mid-1990s) to 17% (among women aged 15–55 years in France in the late 1980s) and was more than 5% in the majority of the countries examined [3,4]. More recently the European Centre for Disease Prevention and Control (ECDC) described surveys from seven countries, estimating a population prevalence of 1.4–3.0% in people aged 18–44 years [5]. They also reported that overall trends over time across Europe appeared to be increasing, from 1990 to 2009, although data were not available from Bulgaria, Czech Republic, France, Germany, Italy, Liechtenstein and Portugal [6]. Moreover, the organisation of the control of *C. trachomatis* infection varied widely, with many countries having no organised activities until 2009 [7].

Pelvic inflammatory disease, tubal sterility or infertility, newborn eye infection or pneumonia and, although controversial, sperm pathology, male sterility and spontaneous abortion or preterm labour, are well-known complications of untreated *C. trachomatis* infection [8-14].

Since treating complications is costly in both psychosocial and financial terms, and is often unsuccessful [15], screening is critical for the early detection and treatment of uncomplicated *C. trachomatis* infection, the control of the overall prevalence of the infection in the population and thus the reduction of transmission and finally for the reduction of treatment costs.

C. trachomatis screening programmes exist in only two European countries (England and the Netherlands) and in the United States: they are opportunistic or pro-active and are mostly directed at young women aged under 25 years [7,16]. Sweden, although lacking

nationally organised screening programmes, is the first country in the world to offer testing for *C. trachomatis* infection, treatment and partner notification – all free of charge – throughout the country. It is also the first to have a national diagnostic and reporting system [5]. In these four countries, after substantial decreases in complication rates of *C. trachomatis* infection at the end of the 1980s and early 1990s, further decreases in pelvic inflammatory disease and ectopic pregnancy rates after 2000 were observed [7,16-20].

Unfortunately, reliable and recent data concerning *C. trachomatis* control in Italy are lacking, except for those in studies such as that of the Italian MEGIC Group (Multicentre Epidemiology Group for Investigation of Chlamydia trachomatis) that reported a prevalence of *C. trachomatis* infection of 3.9% among 1,321 asymptomatic women [21] or that of the STD Surveillance Working Group, which described 809 female incident cases from mainly dermatology and venereology departments and a few gynaecological departments between 1991 and 1996 [22].

There is no screening policy for *C. trachomatis* infection in Italy. A national women's health report released in 2008 suggested for the first time that women should be tested for *C. trachomatis* when they have their first cervical smear test [23]. In order to understand if a screening strategy would be appropriate, the prevalence of the infection needs to be ascertained and there needs to be a preliminary analysis of the epidemiological variables in the population at risk, as well as a surveillance network. No existing epidemiological model can be applied to a different population without analysis and adjustment. New, larger epidemiological analyses are therefore needed in Italy to plan specific and effective strategies for the surveillance and screening of *C. trachomatis* infection in the country.

The purpose of this study was to investigate the prevalence of *C. trachomatis* endocervical infection and its determinants in a large population of sexually active women aged 15–55 years attending an outpatient service of a cervico-vaginal pathology unit in Rome over a 10-year period.

Methods

Patient population

Between January 2000 and December 2009, a total of 7,620 women (aged 13–58 years) attending the outpatient service of the Cervico-Vaginal Pathology Unit in the Department of Gynaecology and Obstetrics of Sapienza University in Rome were examined for genitourinary symptoms or routine gynaecological examination.

A team of gynaecologists collected socio-demographic and behavioural data, as well as clinical data, for each woman during this time, using our model of clinical record taking for sexually transmitted infections – a structured questionnaire. The data were archived as digital files.

The self-administered, structured, paper questionnaire comprised 25 questions on socio-demographic characteristics, sexual behaviour, reproductive history, and tobacco, alcohol and drug use.

Testing for *C. trachomatis* infection, along with testing for human papillomavirus (HPV) and N. gonorrhoeae infection and vaginal wet mount examination, was offered to all sexually active women presenting to the Unit.

Women who refused to be tested for *C. trachomatis* and/or to answer the questionnaire and/or were not sexually active were excluded from the study (n=651).

According to these criteria, a total of 6,969 sexually active women aged 15–55 years who were tested for cervical *C. trachomatis* infection were enrolled. The women were categorised as symptomatic if they presented with either dysuria or pelvic pain or both (symptoms typical of *C. trachomatis* infection). Women not exhibiting either of these symptoms were classified as asymptomatic. They were then further categorised according to whether they were seeking care for family planning, infertility routine gynaecological examination or matters related to pregnancy.

All participating women gave written informed consent. The research was carried out in compliance with the Declaration of Helsinki [24] and was approved by the local ethics committee (reference number 148/11, 2022). Data were stored and managed according to Italian privacy rules [25].

Examinations performed

On a scheduled visit, during the gynaecological examination, an unmoistened sterile speculum was inserted into vagina, so that vaginal walls, fornices and cervix could be evaluated for any erythema and colour and viscosity of any discharge. The pH of the vaginal walls was measured using colorimetric paper. For wet mount examinations, vaginal fluor samples were collected from lateral fornices by a wooden Ayre's spatula, mixed first with saline and then with 10% potassium hydroxide, on two different slides, and immediately observed under a phase contrast microscope [26].

A 'whiff test' using 10% potassium hydroxide was performed for each sample in order to detect abnormal amine production by anaerobes [27].

Wet mount examination allowed the vaginal microflora (predominance of lactobacillary morphotypes) to be assessed and *Trichomonas vaginalis* to be detected (in order to investigate coexisting sexually transmitted infections). In addition, we also looked for bacterial vaginosis-associated clue cells, aerobic

vaginitis-associated pleomorphic bacteria, yeasts and white blood cells.

Samples were taken from the endocervix for detection of *C. trachomatis* and from the ecto-endocervix for detection of HPV DNA, as described below.

Detection of microorganisms

Chlamydia trachomatis

Endocervical swabs were tested for the presence of *C. trachomatis* using the BD ProbeTec ET System (Becton, Dickinson and Company, United States). These assays amplify *C. trachomatis* DNA in separate wells and monitor inhibition of amplification for each specimen using strand displacement amplification and detection by fluorescent energy transfer probes, producing a method-other-than-acceleration (MOTA) score for each specimen The original algorithm involved retesting specimens with MOTA scores between 2000 and 9999. A negative repeat result (MOTA score ‹2000) was considered indeterminate [28].

Human papillomavirus

DNA was extracted from cervical samples using QIAampTissue Kit (Qiagen, Italy) and then genotyped by sequencing a 450-base pair fragment amplified from the L1 region of HPV DNA [29]. Sequence homology was determined using BLAST and ClustalW programs.

Neisseria gonorrhoeae

Identification of *N. gonorrhoeae* was carried out by growth on media selective for pathogenic *Neisseria* species (Oxoid) incubated for up to 48 hours in 5–10% CO_2 at 35–37 °C. Colonies obtained were identified by API NH (bioMérieux) [30].

Statistical analysis

The chi-square test was used to analyse contingency tables; the t-test was used to compare means and odds ratios (ORs), with 95% confidence intervals (CIs), in order to measure the strength of association between *C. trachomatis* infection and behavioural and clinical characteristics and age.

We used the Cochran–Armitage test to assess the possibility of a linear trend in the observed patterns for number of lifetime sexual partners and increasing age.

Statistical tests were considered significant if p was 0.05 or less. A stepwise backward logistic regression analysis, entering the variables significantly associated with *C. trachomatis* infection, was used to assess the effect of more than one variable at a time and to identify possible confounding factors in the range of test values under consideration. Statistical analysis was performed using SPSS version 18.0.

Results

A total of 366 (5.2%) of the 6,969 women sexually active women enrolled in the study tested positive for *C. trachomatis* endocervical infection (Table 1).

Prevalence of *C. trachomatis* infection by year is shown in the Figure: the p value for the chi-square statistic was not statistically significant (p=0.938) (the chi-square test for the resulting 2×10 contingency table tested the null hypothesis of no association against the alternative hypothesis of an association of some sort). Thus prevalence and time appeared not to be associated and were not expected to have a linear correlation over the study period.

A total of 4,620 (66%) of the women were asymptomatic for *C. trachomatis* infection: 256 (5.5%) of them tested positive. This prevalence was slightly higher than that in the 2,349 symptomatic women (4.7%), but the difference was not statistically significant (p=0.1289). Of the 366 women who were positive for *C. trachomatis* infection, 256 (70%) were asymptomatic.

Prevalence was also slightly higher among women without clinical signs of infection (238/4,328; 5.5% compared with those with signs (128/2,641; 4.8%), but this difference was also not statistically significant (p=0.2362).

Univariate analysis of sexual and reproductive history and of age (Tables 1 and 2) highlighted a significant association of *C. trachomatis* infection with age under 40 years, having never been pregnant, smoking, use of oral contraceptives and multiple lifetime sexual partners: women with two to four partners had a slightly higher risk of infection (in comparison with women who had had one partner); women with five to nine partners had double the risk; having had more than nine partners was linked to a threefold higher risk. The p value for the Cochran–Armitage test (p‹0.0001) suggested an underlying positive linear trend between number of lifetime sexual partners and prevalence of infection.

Comparison of the prevalence of *C. trachomatis* infection in stratified age groups with that in women over 49 years of age showed that teenage women aged 15–19 years had the highest increased risk of infection (OR: 4.55 (95% CI: 1.90–10.89); p=0.0002) and that the odds ratios for the remaining strata declined with increasing age. The p value for the Cochran–Armitage test (p‹0.0001) suggested an underlying negative linear trend between age and prevalence of infection.

Further univariate analysis showed that the prevalence of the infection was similar (no statistical significance) whatever the reason for seeking care (Table 2). Condom use was not found to be associated with *C. trachomatis* infection.

The frequency of *C. trachomatis* infection was significantly higher among patients who were also infected

with HPV (OR: 5.50 (95% CI: 4.39–6.89)) and *T. vaginalis* (OR: 4.97 (95% CI: 2.57–9.59)) (Table 3).

Multivariate stepwise logistic regression analysis shows that after backwards elimination, *T. vaginalis* infection (OR: 3.23 (95% CI: 1.61–6.46); p=0.001), age 15–19 years (OR: 2.33 (95% CI: 1.02–5.31); p=0.04) and more than one lifetime sexual partner (OR: 1.50 (95% CI: 1.21–1.87); p=0.000) remained significantly associated with *C. trachomatis* infection in the final model.

We found no cases of gonorrhoea among the first thousand patients referred to the clinic and systematically screened. We then tested *C. trachomatis*-positive cases only, if they showed symptoms or signs of cervicitis: none were positive for *N. gonorrhoeae*.

Discussion

To the best of our knowledge, this is the first study reporting on the epidemiology of *C. trachomatis* infection in Italy in a large sample of a diverse group of women over a long period of time. The mean prevalence of the infection was high (5.2 %) and showed no linear trend over time. The prevalence in asymptomatic women was higher than that observed in 1990 by the MEGIC group (5.5% vs 3.9%, respectively) [21]. In symptomatic women and in those seeking care for infertility the prevalence in our study (4.7% and 4.9% respectively) was similar to that reported by the same group (5.0% and 5.4%, respectively) [21]. These findings may reflect the lack of control and screening activities in Italy.

We also found a high prevalence of *C. trachomatis* infection in pregnant women (5.3%), i.e. those seeking obstetric care (Table 2) which has not been described in Italy and suggests we should consider screening in pregnancy according to CDC guidelines [16]. This strategy could also reduce the rate of obstetric complications due to *C. trachomatis* infection.

Two of the variables independently associated with *C. trachomatis* infection in our study, younger age and multiple lifetime sexual partners (particularly more

Table 1

Univariate analysis of age and sexual and reproductive history of women tested for *Chlamydia trachomatis* infection, Cervico-Vaginal Pathology Unit, Sapienza University, Rome, Italy, 2000–2009 (n=6,969)

Characteristic	Tested for *C. trachomatis* endocervical infection			Odds ratio[a] (95% CI)	P value (t-test statistic)[b]
	Number positive (%)[a]	Number negative[a]	**Total[a]**		
Mean age in years					
15–19	9 (10.8)	74	**83**	4.55 (1.90–10.89)	0.0002
20–24	71 (7.8)	835	**906**	3.18 (1.78–5.70)	0.0000
25–29	86 (5.6)	1,441	**1,527**	2.23 (1.26–3.96)	0.0049
30–34	84 (5.2)	1,519	**1,603**	2.07 (1.17–3.68)	0.0113
35–39	61 (5.1)	1,125	**1,186**	2.03 (1.12–3.66)	0.0166
40–44	29 (4.2)	656	**685**	1.65 (0.87–3.16)	0.1242[c]
45–49	12 (2.7)	429	**441**	1.05 (0.48–2.29)	0.9084[c]
≥50–55	14 (2.6)	524	**538**	1 Reference	–
Mean age per category	32.0 years	34.4 years	**33.2 years**	Difference (those positive vs those negative): −2.4	0.001 (t=−4.610)
Number of lifetime sexual partners					
1	89 (3.4)	2,508	**2,597**	1 Reference	–
2	71 (5.6)	1,191	**1,262**	1.68 (1.22–2.31)	0.0013
3	57 (5.1)	1,063	**1,120**	1.51 (1.08–2.12)	0.0167
4	41 (5.5)	702	**743**	1.65 (1.13–2.40)	0.0094
5–9	54 (7.9)	626	**680**	2.43 (1.71–3.45)	0.0000
≥10	54 (9.5)	513	**567**	2.97 (2.09–4.21)	0.0000
Mean number of lifetime sexual partners per category	2.9	1.7	**2.3**	Difference (those positive vs those negative): 1.2	0.02 (t=2.518)
Ever been pregnant					
Yes	115 (3.8)	2,896	**3,011**	1 Reference	–
No	251 (6.3)	3,707	**3,958**	1.71 (1.36–2.14)	0.0000
Total	**366 (5.2)**	**6,603**	**6,969**	–	–

CI: confidence interval.

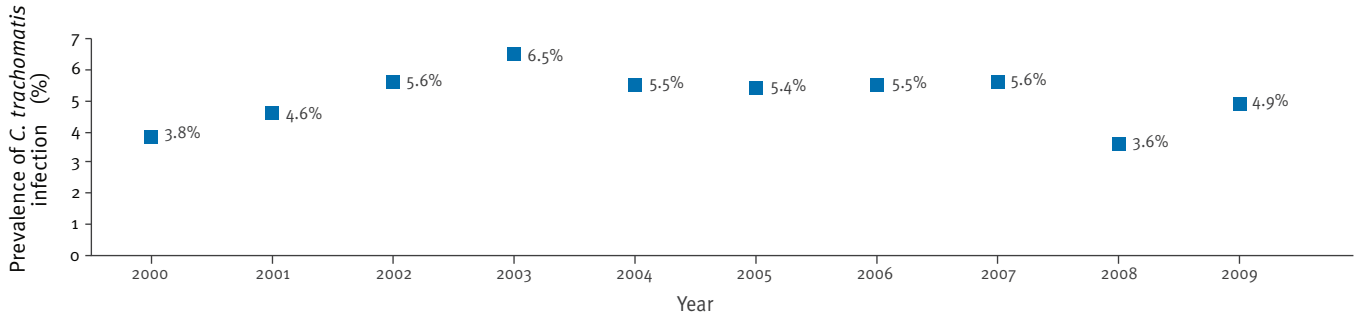
[a] Unless otherwise indicated.

[b] Where relevant. The t-test compares the mean values for women who tested positive for *C. trachomatis* and those who were negative.

[c] Not statistically significant.

Figure

Prevalence of *Chlamydia trachomatis* infection in women tested at the Cervico-Vaginal Pathology Unit, Sapienza University, Rome, Italy, 2000–2009 (n=6,969)



The overall chi-square statistic was 6.255 (the chi-square test for the resulting 2×10 contingency table tested the null hypothesis of no association against the alternative hypothesis of an association of some sort). The p value for the chi-square statistic (p=0.938) was not statistically significant.

Table 2

Univariate analysis of reasons for seeking care, clinical features, contraceptive use and smoker status of 6,969 women attending as outpatients the Cervico-Vaginal Pathology Unit, Sapienza University, Rome, Italy, 2000–2009

Characteristic	Tested for *Chlamydia trachomatis* infection			Odds ratio (95% CI)	P value
	Number positive (%)	Number negative	**Total**		
Reason for seeking care					
Gynaecological	207 (5.3)	3,666	**3,873**	1 Reference	–
Infertility	68 (4.9)	1,331	**1,399**	0.90 (0.68–1.20)	0.4852[a]
Obstetrics	50 (5.3)	889	**939**	1.00 (0.73–1.37)	0.9806[a]
Family planning	41 (5.4)	717	**758**	1.01 (0.72–1.43)	0.9427[a]
Symptoms of *C. trachomatis* infection[c]					
Yes	110 (4.7)	2,239	**2,349**	1.19 (0.95–1.50)	0.1289[a]
No	256 (5.5)	4,364	**4,620**	1 Reference	–
Signs of *C. trachomatis* infection[d]					
Yes	128 (4.8)	2,513	**2,641**	1.14 (0.92–1.42)	0.2362[a]
No	238 (5.5)	4,090	**4,328**	1 Reference	–
Contraceptive use					
None	269 (5.1)	5,025	**5,294**	1 Reference	–
Oral contraceptives	43 (7.3)	546	**589**	1.47 (1.05–2.05)	0.0226
Intrauterine device	20 (5.1)	372	**392**	1.00 (0.63–1,60)	0.9856[a]
Condoms	34 (4.9)	660	**694**	0.96 (0.67–1.39)	0.8370[a]
Smoker					
Yes	120 (6.1)	1,838	**1,958**	1.26 (1.01–1.58)	0.0402
No	246 (4.9)	4,765	**5,011**	1 Reference	–
Total	**366 (5.2)**	**6,603**	**6,969**	–	–

CI: confidence interval.

[a] Not statistically significant.

[b] Dysuria or pelvic pain.

[c] Cervical erythema, inflammation or discharge.

than five), have also been highlighted by research groups worldwide in various populations [7,16,31]. We found that the highest prevalence of infection (10.8%) was associated with a nearly fivefold increased risk of infection (as an independent factor, it showed a two-fold increased risk) in women aged 15–19 years.

Before 2008, *C. trachomatis* control activities in Italy consisted of case management in dermatovenereology clinics with Chlamydia testing for symptomatic people only [7]. C trachomatis testing is currently recommended for women at the time of their first cervical smear test, which takes place when women are 25 years of age in Italy. To the best of our knowledge, no report on the uptake and results of this testing recommendation is yet available. However, our data suggest that women aged under 25 years, and in particular those under 20 years, would be the core population of a good testing policy and a hypothetical *C. trachomatis* screening programme, as in other screening programmes worldwide [7,16]. Thus, the current Italian policy could be ineffective. The high prevalence of infection observed until the age of 40 years – which is a novel aspect of our findings – could also lead to a more extensive testing strategy. Although being aged 25–39 years was not an independent risk factor for infection, our data suggest that older women should also be tested.

Furthermore, as prevalence in women with signs or symptoms of infection did not differ statistically from that in women with no signs or symptoms in this study, case management appears to be an insufficient Chlamydia control activity.

The prevalence of infection among women seeking care for family planning was also high (5.4%): despite the low number of women in our study who sought advice for family planning, given the high number of women who usually attend this type of service and their young age, we suggest that family planning clinics could be sentinel for *Chlamydia* surveillance or an appropriate setting for *Chlamydia* opportunistic screening.

Our data also show that having HPV or *T. vaginalis* infection was associated with a fivefold higher risk of *C. trachomatis* coinfection, as expected in groups at higher risk as a result of age and behaviour [32,33]. In our logistic regression, HPV was not significantly associated with *C. trachomatis* infection, suggesting that age and multiple partners could be possible confounding factors, while *T. vaginalis* infection was an independent risk factor for *C. trachomatis* infection. It is possible that severe inflammation of the cervix due to *T. vaginalis* infection may make the cervix more susceptible to *C. trachomatis* infection. It could therefore be suggested that patients diagnosed with *T. vaginalis* infection should be tested for *C. trachomatis* or even given treatment for *C. trachomatis* infection without being tested, as proposed by Lo et al. [33].

Data on *N. gonorrhoeae* and *C. trachomatis* coinfection in Italy are limited, but our findings on *N. gonorrhoeae* seem to be consistent with those reported in 1998 by a dermatovenereology network, which found that fewer than 1% the infections in 44,438 individuals with sexually transmitted infections were *N. gonorrhoeae* cervical infections [22].

We also found a statistical association of *C. trachomatis* infection with absence of previous pregnancies, use of oral contraceptives and smoking. However, as they were not shown to be statistically associated with infection in the logistic regression final model, age, having multiple lifetime sexual partners and *T. vaginalis* infection are likely to be confounders, in contrast to the findings of others [34-36].

The lack of statistical association between *C. trachomatis* infection and condom use (as a protective factor) is unexpected, given the findings of others [21,37]. This could be considered a result of incorrect condom use and lack of health education. It could also be that some of the women were not entirely truthful when providing details of the type of contraception they used. There are probably some methodological limitations in the epidemiological study of condom effectiveness in

Table 3

Univariate analysis of other sexually transmitted infections in 6,969 women attending as outpatients the Cervico-Vaginal Pathology Unit, Sapienza University, Rome, Italy, 2000–2009

Other sexually transmitted organisms detected	Tested for *Chlamydia trachomatis* infection			Odds ratio (95% CI)	P value
	Number positive (%)	Number negative	**Total**		
Trichomonas vaginalis or HPV[a]	145 (16.9)	714	**859**	5.41 (4.33–6.77)	0.0000
Trichomonas vaginalis	11 (15.7)	59	**70**	4.97 (2.57–9.59)	0.0000
HPV	142 (17.1)	688	**830**	5.50 (4.39–6.89)	0.0000
Neither *Trichomonas vaginalis* nor HPV	221 (3.6)	5,889	**6,110**	1 Reference	–
Total	**366 (5.2)**	**6,603**	**6,969**	–	–

CI: confidence interval; HPV: human papillomavirus.

[a] Women coinfected with *T. vaginalis* and HPV (n=41) are not included.

preventing *C. trachomatis* infection, as has been highlighted by Warner et al. [37].

A new *C. trachomatis* variant was detected in 2006 following an unexpected 25% decrease in the number of infections in a Swedish county [38,39]. As we used the Becton Dickinson ProbeTec – which detects the new variant – the presence or absence of the variant in Italy has no impact on our prevalence data. However, as no data are available on the type and distribution of *C. trachomatis* diagnostic methods used in Italy, nor on whether this variant is present among Italian women, surveillance is also needed to provide such information.

In conclusion, the prevalence and determinants of *C. trachomatis* infection observed in this study seem to highlight the need for a focus on control activities in Italy, with special attention to standardisation of diagnostic tests and women aged under 25 years, who would be the core population of a screening programme.

References

1. Paavonen J. Chlamydia trachomatis infections of the female genital tract: state of the art. Ann Med. 2012;44(1):18-28.
2. World Health Organization (WHO). Prevalence and incidence of selected sexually transmitted infections, Chlamydia trachomatis, Neisseria gonorrhoeae, syphilis and Trichomonas vaginalis: methods and results used by WHO to generate 2005 estimates. Geneva: WHO; 2011. Available from: http://whqlibdoc.who.int/publications/2011/9789241502450_eng.pdf
3. van de Laar MJ, Morré SA. Chlamydia: a major challenge for public health. Euro Surveill. 2007;12(10):pii=735. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=735
4. Wilson JS, Honey E, Templeton A, Paavonen J, Mårdh PA, Stray-Pedersen B, et al. A systematic review of the prevalence of Chlamydia trachomatis among European women. Hum Reprod Update. 2002;8:385-94.
5. European Centre for Disease Prevention and Control (ECDC). Chlamydia control in Europe. ECDC Guidance. Stockholm: ECDC; 2009. Available from: http://ecdc.europa.eu/en/publications/Publications/0906_GUI_Chlamydia_Control_in_Europe.pdf
6. European Centre for Disease Prevention and Control (ECDC). Sexually transmitted infections in Europe, 1990–2009. Stockholm: ECDC; 2011. Available from: http://ecdc.europa.eu/en/publications/Publications/110526_SUR_STI_in_Europe_1990-2009.pdf
7. European Centre for Disease Prevention and Control (ECDC). Review of Chlamydia control activities in EU countries. Stockholm, May 2008. Technical Report. Stockholm: ECDC; 2008. Available from: http://ecdc.europa.eu/en/publications/publications/0805_ter_review_of_chlamydia_control_activities.pdf
8. Haggerty CL, Gottlieb SL, Taylor BD, Low N, Xu F, Ness RB. Risk of sequelae after Chlamydia trachomatis genital infection in women. J Infect Dis. 2010;201 Suppl 2:S134-55.
9. Baud D, Regan L, Greub G. Emerging role of Chlamydia and Chlamydia-like organisms in adverse pregnancy outcomes. Curr Opin Infect Dis. 2008;21(1):70-6.
10. Wagenlehner FM, Naber KG, Weidner W. Chlamydial infections and prostatitis in men. BJU Int. 2006;97(4):687-90.
11. Eley A, Pacey AA, Galdiero M, Galdiero M, Galdiero F. Can Chlamydia trachomatis directly damage your sperm? Lancet Infect Dis. 2005;5(1):53-7.
12. Gonçalves LF, Chaiworapongsa T, Romero R. Intrauterine infection and prematurity. Ment Retard Dev Disabil Res Rev. 2002;8(1):3-13.
13. Diemer T, Ludwig M, Huwe P, Hales DB, Weidner W. Influence of urogenital infection on sperm function. Curr Opin Urol. 2000;10(1):39-44.
14. Paavonen J, Eggert-Kruse W. Chlamydia trachomatis: impact on human reproduction . Hum Reprod Update. 1999;5(5):433-47.
15. Land JA, Van Bergen JE, Morré SA, Postma MJ. Epidemiology of Chlamydia trachomatis infection in women and the cost-effectiveness of screening. Hum Reprod Update. 2010;16 (2):189-204.
16. Workowski KA, Berman S; Centers for Disease Control and Prevention (CDC). Sexually transmitted diseases treatment guidelines, 2010. MMWR Recomm Rep. 2010;59(RR-12):1-110.
17. Sutton MY, Sternberg M, Zaidi A, St Louis ME, Markowitz LE. Trends in pelvic inflammatory disease hospital discharges and ambulatory visits, United States, 1985-2001. Sex Transm Dis. 2005;32(12):778-84.
18. Chandra A, Martinez GM, Mosher WD, Abma JC, Jones J. Fertility, family planning, and reproductive health of U.S. women: data from the 2002 National Survey of Family Growth. Vital Health Stat 23. 2005;(25):1-160.
19. French CE, Hughes G, Nicholson A, Yung M, Ross JD, Williams T, et al. Estimation of the rate of pelvic inflammatory disease diagnoses: trends in England, 2000-2008. Sex Transm Dis. 2011;38(3):158-62.
20. Bender N, Herrmann B, Andersen B, Hocking JS, van Bergen J, Morgan J, et al. Chlamydia infection, pelvic inflammatory disease, ectopic pregnancy and infertility: cross-national study. Sex Transm Infect. 2011;87(7):601-8.
21. Determinants of cervical Chlamydia trachomatis infection in Italy. The Italian MEGIC Group. Genitourin.Med. 1993;69(2):123-5.
22. Giuliani M, Suligoi B, the STD Surveillance Working Group. Sentinel surveillance of sexually transmitted diseases in Italy. Euro Surveill. 1998;3(6):pii=97. Available from: http://www.eurosurveillance.org/viewarticle.aspx?articleid=97
23. Ministry of Health. Lo stato di salute delle donne in Italia. [Women's health status in Italy]. Rome: Ministry of Health; March 2008. Italian. Available from: http://www.ministerosalute.it/imgs/C_17_pubblicazioni_764_allegato.pdf

24. World Medical Association (WMA). Declaration of Helsinki. WMA General Assembly, Seoul, South Korea, October 2008. World Medical Journal. 2008;54(4):122-5. Available from: http://www.wma.net/en/30publications/20journal/pdf/wmj20.pdf
25. Italian Data Protection Authority. Linee guida in tema di fascicolo sanitario elettronico e di dossier sanitario. [Guidelines on the Electronic Health Record and the Health File]. Gazzetta Ufficiale - Serie Generale. 3 Aug 2009;178. Rome: Istituto Poligrafico e Zecca dello Stato. Italian. Summary available from: http://www.guritel.it/free-sum/ARTI/2009/08/03/sommario.html; (English version available from: http://www.garanteprivacy.it/garante/doc.jsp?ID=1672821).
26. Donders GG. Definition and classification of abnormal vaginal flora. Best Pract Res Clin Obstet Gynaecol. 2007;21(3):355-73.
27. Amsel R, Totten PA, Spiegel CA, Chen KC, Eschenbach D, Holmes KK. Nonspecific vaginitis. Diagnostic criteria and microbial and epidemiologic associations. Am J Med.1983;74(1):14-22.
28. Health Protection Agency (HPA). Chlamydia trachomatis infection – testing by nucleic acid amplification tests (NAATs). UK standards for microbiology investigations. Virology. 37(3). London: HPA; 25 May 2012. Available from: http://www.hpa.org.uk/webw/HPAweb&HPAwebStandard/HPAweb_C/1316425069287
29. Manos MM, Ting Y, Wright DK, Lewis AJ, Broker TR, Wolinsky SM. Use of polymerase chain reaction amplification for the detection of genital human papillomaviruses. Cancer Cells 1989;7(17):209-14.
30. Barbé, G, Babolat M, Boeufgras JM, Monget D, Freney J. Evaluation of API NH, a new 2-hour system for identification of Neisseria and Haemophilus species and Moraxella catarrhalis in a routine clinical laboratory. J Clin Microbiol. 1994;32(1):187-9.
31. Franceschi S, Smith JS, van den Brule A, Herrero R, Arslan A, Ahn PT, et al. Cervical infection with Chlamydia trachomatis and Neisseria gonorrhoeae in women from ten areas in four continents. Sex Transm Dis. 2007;34(8):563-9.
32. Griffiths V, Cheung WH, Carlin EM, Ahmed-Jushuf I. Incidence of concurrent sexually transmitted infections in patients with genital warts. Int J STD AIDS. 2006;17(6):413-4.
33. Lo M, Reid M, Brokenshire M. Epidemiological features of women with trichomoniasis in Auckland sexual health clinics: 1998-99. New Zeal Med J. 2002;115(1159):U119.
34. Gall SA. Oral contraceptives and Chlamydia infections. JAMA. 1986;255(1):38-9.
35. Oh MK, Feinstein RA, Soileau EJ, Cloud GA, Pass RF. Chlamydia trachomatis cervical infection and oral contraceptive use among adolescent girls. J Adolesc Health Care. 1989;10(5):376-81.
36. Corbeto EL, Lugo R, Martró E, Falguera G, Ros R, Avecilla A, et al. Epidemiological features and determinants for Chlamydia trachomatis infection among women in Catalonia, Spain. Int J STD AIDS. 2010;21(10):718-22.
37. Warner L, Stone KM, Macaluso M, Buehler JW, Austin HD. Condom use and risk of gonorrhea and Chlamydia: a systematic review of design and measurement factors assessed in epidemiologic studies. Sex Transm Dis. 2006;33(1):36-51.
38. Ripa T, Nilsson PA. A variant of Chlamydia trachomatis with deletion in cryptic plasmid: implications for use of PCR diagnostic tests. Euro Surveill. 2006; Euro Surveill. 11(45):pii=3076. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=3076
39. Herrmann B. A new genetic variant of Chlamydia trachomatis. Sex Transm Infect. 2007; 83(4):253-4.