the ecologic system could contribute to the emergence of human leishmaniasis in urban areas.

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References

1. Urbanization; an increasing risk factor for leishmaniasis. Wkly Epidemiol Rec. 2002;44:365–72.
2. Abranches P, Lopes FJC, Conceição-Silva FM, Ribeiro MMS, Pires CA. Kala-azar in Portugal. III. Results of a survey on canine leishmaniasis performed in the Lisbon region. Comparison of urban and rural zones [in French]. Ann Parasitol Hum Comp. 1983;58:307–15.
3. Miró G, Montoya A, Mateo M, Alonso A, Garcia S, Garcia A, et al. A leishmaniasis surveillance system among stray dogs in the region of Madrid: ten years of serodiagnosis (1996–2006). Parasitol Res. 2007;101:253–7.
4. Tselentis Y, Gikas A, Chaniotis B. Canine reservoirs and leishmanioses in Portugal. PhD Thesis. 2002. p. 45–57.
5. Ferrel JP, editor. World class parasites, an urban area of Brazil. Vet Parasitol. 2006;140:54–60.
6. Alves-Pires C, Os flebótomos (Diptera, Psychodidae) dos focos zoonóticos de leishmaniasis em Portugal. PhD Thesis. Universidade Nova de Lisboa, Portugal; 2000.
7. Alves-Pires C, Os flebótomos (Diptera, Psychodidae) dos focos zoonóticos de leishmaniasis em Portugal. PhD Thesis. Universidade Nova de Lisboa, Portugal; 2000.
8. Alves-Pires C, Afonso MO, Santos-Gomes G, Dedet JP, Pratlong F. The phlebotomines of Portugal. X–Natural infestation of Phlebotomus perniciosus by Leishmania infantum MON-1 in Algarve [in French]. Parasite. 2001;8:374–5.

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TaqMan Assay for Swedish Chlamydia trachomatis Variant

To the Editor: Chlamydia trachomatis (CT) is the most prevalent bacterial sexually transmitted infection worldwide. Recently, a new variant of CT (swCT) has been reported in Halland County, Sweden. A total of 12 swCT specimens were sequenced and found to have the same deletion, a 377-bp deletion in the cryptic plasmid (1). Because the deletion was found in the target area of 2 commercial CT nucleic acid amplification tests (Roche, Basel, Switzerland, and Abbott Laboratories, Abbott Park, IL, USA), screening tests have produced false-negative results for patients infected with this new Swedish variant (I). In specific regions of Sweden, the proportion of all detected CT cases attributable to swCT ranges from 13% to 39%; a considerable number of chlamydia infections have escaped detection by commonly used test systems (I).

Although the first 2 studies to monitor potential spread of the swCT variant outside Sweden (Ireland and the Netherlands) did not detect swCT, a third study (Norway) did identify this variant (2–4). Subsequently, the European Surveillance of Sexually Transmitted Infections network and the European Center for Disease Prevention and Control launched an initiative, consisting of a short questionnaire, to learn more about this swCT variant problem outside Sweden (5).

However, quick monitoring of the spread of the swCT variant has been hampered by lack of a direct test to detect this swCT variant and by lack of a readily available positive control. We therefore constructed a positive control by using a clinical specimen of the swCT variant in which the deletion was present (forward swCT 5′-TCC GGA TAG TGA ATT ATA GAG ACT ATT TAA TC-3′ reverse swCT 5′GTT GTG TGT ACT AGA GGA CTT ACC TCT TC-3′) (2). The specimen was obtained in Sweden (by B.H.) and confirmed as swCT by the method described by Ripa and Nilsson (6). The obtained 98-bp amplicon was subsequently cloned in a pGEM-T Easy Vector (Promega Benelux b.v., Leiden, the Netherlands) and transformed in Escherichia coli DH5α. After extraction the plasmid was verified for the correct insert by sequencing and quantified as described (7). This positive control is available for researchers and clinicians free of charge.

Subsequently, we developed a real-time PCR (TaqMan assay) that specifically detects the swCT variant by using a probe that spans the 377-bp left and right gap border sequences: probe- swCT 5′[MGB] GGA TCC GTT AGG ATG-3′. One copy of cloned positive swCT control could be detected in our swCT assay. We selected 10 copies per PCR as positive swCT control for each run. A total of 239 recent samples known to be CT positive and identified with techniques detecting the swCT variant were retrospectively analyzed with our new swCT real-time PCR for 3 cohorts: 1) 30 real-time PCR CT-positive clinical samples (CT prevalence in the population, 1.8%) from the Department of Medical Microbiology and Infection Prevention, VU University Medical Center, Amsterdam, the Netherlands; 2) 57 Becton Dickinson (Franklin Lakes, NJ, USA) CT-positive samples (CT prevalence in the sexually transmitted disease population, 7.3%) from the Department of Infectious Diseases, South Limburg Public Health Service, Heerlen, the Netherlands; and 3) 152
CT-positive culture samples (CT prevalence in the population, average 15% [8]) from the Faculty of Medicine, St. Petersburg State University, St. Petersburg, Russia, and from the Laboratory of Microbiology, D.O. Ott Research Institute of Obstetrics and Gynaecology, St. Petersburg, Russia.

Cohort 1 consisted of cervical swabs in 2-sucrose-phosphate (2SP) transport medium, stored at −80°C. Cohort 2 consisted of frozen dry swabs that had been shaken for 10 s in 1 mL 2SP transport medium before sample preparation. Cohort 3 consisted of positive cultured samples. DNA extraction used 200 μL 2SP and was performed with the NucliSens easyMAG (bioMérieux, Boxtel, the Netherlands); the DNA was eluted in 110 μL 2SP [7]. Presence of CT DNA was reconfirmed for all samples with our in-house PCR. Sensitivity of this assay was determined by using a previously described serial dilution of lymphogranuloma venereum (LGV) strain L2 and was assessed at 0.01 inclusion-forming units [9]. Amplification and detection were performed with an ABI Prism 7000 sequence detection system (Applied Biosystems, Foster City, CA, USA) by standard PCR conditions of the manufacturer, with 45 cycles. The Swedish variant was found in none of the 3 cohorts tested. Sensitivity and specificity were confirmed by using 12 swCT variant samples from Sweden, which were all positive according to our swCT TaqMan assay.

Our new swCT TaqMan assay, combined with the positive control (which can be obtained by contacting S.M.), will be a helpful tool for determining whether this Swedish CT variant is present outside Sweden, other than in the 2 case-patients identified in Norway. We did not find any evidence of the swCT variant in the Netherlands or St. Petersburg, Russia, each of which is near Scandinavia (Table). Recently, the C. trachomatis LGV strain was discovered in the Netherlands in a population of men who have sex with
men. In this instance, the real-time TaqMan assay also proved helpful in determining spread (10).

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References

1. Soderblom T, Blaxhult A, Fredlund H, Herrmann B. Impact of a genetic variant of Chlamydia trachomatis on national detection rates in Sweden. Euro Surveill. 2006;11:E61207.1.
2. de Vries HJC, Catsburg A, van der Helm JJ, Beukelaar EC, Morré SA, Fennema JSA, et al. No indication of Swedish Chlamydia trachomatis variant among STI clinic visitors in Amsterdam. Euro Surveill. 2007;12:E070208.3.
3. Lynagh Y, Crowley B, Walsh A. Investigation to determine if newly-discovered variant of Chlamydia trachomatis is present in Ireland. Euro Surveill. 2007;12:E070201.2.
4. Moghaddam A, Reinton N. Identification of the Swedish Chlamydia trachomatis variant among patients attending a STI clinic in Oslo, Norway. Euro Surveill. 2007;12:E070301.3.
5. de Laar V, Ison C. Europe-wide investigation to assess the presence of new variant of Chlamydia trachomatis in Europe. Euro Surveill. 2007;12:E070208.4.
6. Ripa T, Nilsson PA. A Chlamydia trachomatis strain with a 377-bp deletion in the plasmid causing false-negative nucleic acid amplification tests. Sex Transm Dis. 2007;34:255–6.
7. Catsburg A, van der Zet W, Morré SA, Ouburg S, Vandenbroucke-Grauls CM, Savelkoul PH. Analysis of multiple single nucleotide polymorphisms (SNP) on DNA traces from plasma and dried blood samples. J Immunol Methods. 2007;321:135–41.
8. Savitcheva A, Smirnova T, Pavlova N, Bashmakova M, Shishkina O, Novikov B, et al. Diagnostic and treatment of genital Chlamydia trachomatis infection in St. Petersburg and Leningradskaya Oblast. In: Domeika M., Hallen A., editors. Chlamydia trachomatis infection in Eastern Europe. Uppsala (Sweden): Uppsala University; 2000.
9. Morré SA, Sillekens P, Jacobs MV, van Arle P, de Blok S, van Gemmen B, et al. RNA amplification by nucleic acid sequence-based amplification with an internal standard enables reliable detection of Chlamydia trachomatis in cervical scrapings and urine samples. J Clin Microbiol. 1996;34:3108–14.
10. Morré SA, Spaargaren J, Fennema JSA, de Vries HJC, Peña AS. Real-time PCR for the rapid one-step diagnosis of Chlamydia trachomatis. Letters to the Editor. 2005;11:1311–2

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Table. Published studies and the current study on screening for the swCT variant*

| Location                        | Ct+, no. detected | swCT variant, no. detected | Reference |
|---------------------------------|-------------------|----------------------------|-----------|
| Amsterdam, the Netherlands      | 75                | ND                         | (3)       |
| Dublin, Ireland                 | 750               | ND                         | (4)       |
| Oslo, Norway                   | 47                | 2                          | (5)       |
| St. Petersburg, Russia          | 152               | ND                         | This study|
| Heerlen, the Netherlands        | 57                | ND                         | This study|
| Amsterdam, the Netherlands      | 30                | ND                         | This study|

*swCT, Swedish Chlamydia trachomatis variant identified in Halland County, Sweden; Ct+, C. trachomatis DNA; ND, not detected.
†2 female patients: 1 originally from Sweden, 1 from Norway.

Highly Pathogenic Porcine Reproductive and Respiratory Syndrome, China

To the Editor: Since April 2006, a highly pathogenic disease caused by unknown agents and characterized by high fever and a high proportion of deaths in pigs of all ages, emerged in some swine farms in Jiangxi Province, People’s Republic of China. The morbidity rate was 50%–100% and mortality rate was 20%–100%. In the next several months, the disease spread rapidly to most provinces of China. In almost all affected swine herds, the following clinical signs were observed: high and continuous fever, anorexia, red discolorations in the body, and blue ears; in the late phase of the disease, diarrhea and other clinical signs might be seen due to the secondary infections. Clinical samples (from lungs, kidneys, liver, and lymph nodes) were collected from animals in different provinces and sent for laboratory diagnosis. DNA and RNA were extracted from the tissue homogenate and PCR or reverse transcription–PCR (RT-PCR) was conducted to detect porcine reproductive and respiratory syndrome virus (PRRSV), classic swine fever virus, porcine circovirus, and pseudorabies virus, respectively (1). In clinical samples, only PRRSV was found to be the dominant virus (48 of 50 samples were PRRSV posi-