

Table. Incidence of resistance/decreased susceptibility to key antimicrobial agents in isolates of *Salmonella enterica* serovars Typhi and Paratyphi A, United Kingdom, 2001–2006*

Year	No. studied	% <i>S. Typhi</i> resistant to					No. studied	% <i>S. Paratyphi A</i> resistant to				
		C	A	Tm	Cp _L	Cp _H		C	A	Tm	Cp _L	Cp _H
2001	170	24	23	23	35	0	232	28	27	27	23	2
2002	150	18	17	17	35	1	149	10	9	10	39	3
2003	218	20	20	21	43	1	177	17	18	17	65	12
2004	215	23	23	24	47	2	221	5	5	5	70	14
2005	222	29	29	29	62	2	217	7	7	7	60	12
2006	240	23	24	24	68	2	278	2	3	2	64	9

*C, chloramphenicol; A, ampicillin, Tm, trimethoprim, Cp_L, ciprofloxacin MIC 0.25–1.0 mg/L; Cp_H, ciprofloxacin MIC >1.0 mg/L. No isolates exhibited resistance to ceftriaxone or cefotaxime; of 50 *S. Typhi* and 40 *S. Paratyphi A* isolated in 2005 and 2006, the MIC to azithromycin by E test (AB Biodisk, Solna, Sweden) was not greater than 8 mg/L for *S. Typhi* and 12 mg/L for *S. Paratyphi A*, which corresponds to those of drug-sensitive controls of the respective serotypes.

enteric fever in the United Kingdom has been seriously jeopardized. In cases of treatment failures, commonly used alternative antimicrobial agents have included third-generation cephalosporins such as ceftriaxone. The macrolide antimicrobial azithromycin is also being increasingly used, particularly for patients with hypersensitivity to penicillins (5). With this in mind, 50 *S. Typhi* and 40 *S. Paratyphi A* strains isolated from January 2005 through December 2006, which exhibited resistance to ciprofloxacin at 0.125 mg/L, were tested for resistance to azithromycin by Etest. Results indicated that none of the isolates of *S. Typhi* exhibited MICs >8 mg/L, which corresponded to the MIC to azithromycin of a drug-sensitive control strain of *S. Typhi* (range 4–8 mg/L, MIC₉₀ 6 mg/L). For *S. Paratyphi A*, none of the isolates exhibited MICs >12 mg/L, corresponding to that of a drug-sensitive control strain of this serovar (range 6–12 mg/L, MIC₉₀ 10 mg/L). Although there are no definitive data on resistance levels for azithromycin in relation to treatment of typhoid and paratyphoid, these findings suggest that resistance to this antimicrobial agent in terms of treatment efficacy has not yet been jeopardized.

These results indicate that the availability of effective antimicrobial agents for the treatment of typhoid and paratyphoid infection is becoming increasingly limited for patients in the United Kingdom. Nevertheless, despite the dramatic upsurge in the oc-

currence of strains with decreased susceptibility, ciprofloxacin still remains the drug of choice for many physicians. It is reassuring that in cases of treatment failure, third-generation cephalosporins such as ceftriaxone and macrolide antimicrobial agents such as azithromycin appear to be viable alternatives.

**E. John Threlfall,*
Elizabeth de Pinna,*
Martin Day,* Joanne Lawrence,*
and Jane Jones***

*Health Protection Agency, London, UK

References

1. Threlfall EJ, Ward LR. Decreased susceptibility to ciprofloxacin in *Salmonella enterica* serotype Typhi, United Kingdom. *Emerg Infect Dis.* 2001;7:448–50.
2. Rowe B, Ward LR, Threlfall EJ. Ciprofloxacin and typhoid fever. *Lancet.* 1992;339:740.
3. Aarestrup FM, Wiuff C, Mølbak K, Threlfall EJ. Is it time to change the break points for fluoroquinolones for *Salmonella* spp.? *Antimicrob Agents Chemother.* 2003;47:827–9.
4. Health Protection Agency. Foreign travel-associated illness in England, Wales and Northern Ireland—2007 report. London: Health Protection Agency; 2007.
5. Threlfall EJ, Day M, De Pinna E, Lewis H, Lawrence J. Drug-resistant enteric fever in the UK. *Lancet.* 2006;367:1576.

Address for correspondence: E. John Threlfall, Health Protection Agency Laboratory of Enteric Pathogens, Centre for Infections, 61 Colindale Ave, London NW9 5EQ, UK; email: john.threlfall@hpa.org.uk

Usutu Virus Sequences in *Culex pipiens* (Diptera: Culicidae), Spain

To the Editor: *Usutu virus* (USUV) is an arbovirus and a member of the mosquito-borne cluster within the *Flavivirus* genus. USUV belongs to the *Japanese encephalitis virus* antigenic group, which is closely related to pathogens such as *West Nile virus* (WNV) (1).

USUV has been isolated from a human in the Central African Republic and from several mosquito species from tropical and subtropical Africa (2). In late summer 2001, USUV emerged in central Europe and caused deaths in several species of resident birds in Austria (3). However, monitoring of USUV in dead birds from 2003 through 2005 showed that the absolute numbers of USUV-associated bird deaths declined, although USUV detection persisted in bird tissues (4). This decrease in USUV-associated bird deaths was attributed to herd immunity in the bird population (5). In the summer of 2005, USUV was detected in a blackbird in Hungary. The complete genomic sequence of the Hungarian USUV strain shared 99.9% identity with the strain circulating in Austria since 2001 (6). On the other hand, neutralizing antibodies against USUV have been detected in sera of resident and migrant birds

in the United Kingdom without causing an obvious reduction in the bird population (7).

From May through October 2006, monitoring of flaviviruses in mosquitoes was performed in the northeast region of Spain (Catalonia). This monitoring was implemented in the 3 main wetlands of the region: Aiguamolls de la Empordà (Girona Province) near France, where WNV was detected in dead horses in 2000 (8); Delta del Llobregat (Barcelona Province); and Delta de l'Ebre (Tarragona Province). Mosquitoes were collected by mosquito control services in these areas. Female mosquitoes were classified and grouped in pools according to date, species, and localization. During this period, 436 pools belonging to 9 mosquito species were collected. The most abundant species was *Culex pipiens* (n = 168).

Viral RNA was recovered from mosquito pools by homogenization and viral RNA extraction with QIAamp Viral RNA Mini Kit (QIAGEN, Valencia, CA, USA), and then generic reverse transcription (RT)-nested PCR was used to identify flaviviruses (9). This procedure was used to amplify a specific fragment of the NS5 gene within the flavivirus genome. The 143-bp amplification product was detected by electrophoresis and purified by using QIAquick PCR Purification Kit (QIAGEN). Sequencing reactions were performed with ABI Prism BigDye Terminator Cycle Sequencing v.3.1 Ready Reaction (Applied Biosystems, Foster City, CA, USA), and analyzed by using an ABI PRISM model 3730 automated sequencer (Applied Biosystems). Assembly of the consensus sequences and translation into amino acid sequences was performed with Larsergene DNASTAR group of programs (DNASTAR Inc., Madison, WI, USA). Comparisons with published sequences of known flaviviruses were performed by searches with FASTA program in EMBL database (available from www.ebi.ac.uk/embl/) to iden-

tify the detected agent and to study the level of homology.

One pool of *Cx. pipiens* captured in the middle of August 2006 from Delta del Llobregat, in a typical Mediterranean climate that contained 3 female mosquitoes, was positive for flaviviruses. That positive pool was obtained from the center of the village of Viladecans, where different common migratory and sedentary birds such as *Passer domesticus*, *Hirundo rustica*, or *Delichon urbica* feed and nest. The Spanish USUV sequence showed 97.97% homology to USUV strain SAAR-1776 from South Africa; it showed 94.94% similarity with USUV strain Vienna 2001 from Austria and USUV strain Budapest from Hungary, with 2-nt and 5-nt differences, respectively (Figure). All of these were synonymous mutations and thus did not result in amino acids replacements. The homology data showed that the Spanish strain belongs to USUV species and is more related to the African USUV isolates than to central European isolates.

To date, no bird deaths observed in Barcelona Province have been associated with viral encephalitis. However, this region is where the USUV-specific RT-PCR-positive samples were obtained from *Cx. pipiens* mosquitoes. One possible explanation for these findings is that Spanish USUV could be naturally avirulent for birds because the African strains of USUV appear to be in Africa. Alternatively, USUV and other related viruses such as WNV may have been circulating

in Spain for many years, as a result of regular reintroduction by birds migrating from Africa. Under such circumstances, natural genetic resistance, herd immunity, and cross-protective immunity caused by related viruses likely provided at least some protection against symptomatic infections. The discovery of USUV-specific RNA, most related to the African strains of USUV, in *Cx. pipiens* in Spain extends previous evidence (7,10) that USUV and related flaviviruses such as WNV are being introduced into western Europe from Africa, presumably by migratory birds.

Acknowledgments

We thank Erika Serrano for technical help and Francesc Xavier Abad for NBS3 laboratories management.

**Núria Busquets,* Anna Alba,*
Alberto Allepuz,*
Carles Aranda,†
and José Ignacio Núñez***

*Centre de Recerca en Sanitat Animal, Barcelona, Spain; and †Universitat Autònoma de Barcelona, Barcelona, Spain

References

1. Kuno G, Chang GJ, Tsuchiya KR, Karabatsos N, Cropp CB. Phylogeny of the genus *Flavivirus*. *J Virol*. 1998;72:73-83.
2. Adam F, Diguette J-P. Virus d'Afrique (base de données). Centre collaborateur OMS de référence et de recherche pour les arbovirus et les virus de fièvres hémorragiques (CRORA); Institut Pasteur de Dakar [cited 2008 Mar 1]. Available from <http://www.pasteur.fr/recherche/banques/CRORA>

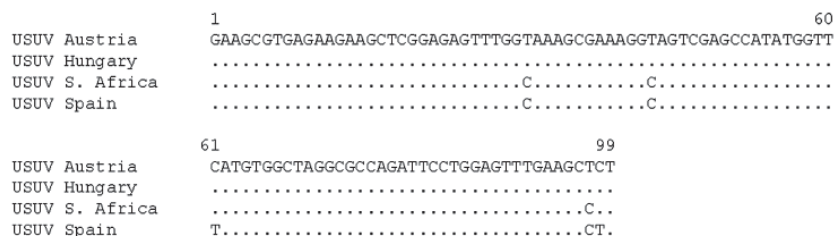


Figure. Comparison at nucleotide level of sequenced fragment among related Usutu virus (USUV). Dot indicates coincident nucleotide. The partial nucleotide sequence of detected Spanish USUV has been deposited in the GenBank database under accession no. AM909649. S. Africa, South Africa.

3. Weissenböck H, Kolodziejek J, Url A, Lussy H, Rebel-Bauder B, Nowotny N. Emergence of Usutu virus, an African mosquito-borne *Flavivirus* of the Japanese encephalitis virus group central Europe. *Emerg Infect Dis.* 2002;8:652–6.
4. Chvala S, Bakonyi T, Bukovsky C, Meister T, Brugger K, Rubel F, et al. Monitoring of *Usutu virus* activity and spread by using dead bird surveillance in Austria, 2003–2005. *Vet Microbiol.* 2007;122:237–45.
5. Meister T, Lussy H, Bakonyi T, Šikutová S, Rudolf I, Vogl W, et al. Serological evidence of continuing high *Usutu virus* (*Flaviviridae*) activity and establishment of herd immunity in wild birds in Austria. *Vet Microbiol.* 2008;127:237–48.
6. Bakonyi T, Erdélyi K, Ursu K, Ferenczi E, Cörgő T, Lussy H, et al. Emergence of *Usutu virus* in Hungary. *J Clin Microbiol.* 2007;45:3870–4.
7. Buckley A, Dawson A, Moss SR, Hinsley SA, Bellamy PE, Gould EA. Serological evidence of *West Nile virus*, *Usutu virus* and *Sindbis virus* infection of birds in the UK. *J Gen Virol.* 2003;84:2807–17.
8. Murgue B, Murri S, Zientara S, Durand B, Durand JP, Zeller H. West Nile outbreak in horses in Southern France, 2000: the return after 35 years. *Emerg Infect Dis.* 2001;7:692–6.
9. Sánchez-Seco MP, Rosario D, Domingo C, Hernández L, Valdés K, Guzmán MG, et al. Generic RT-nested-PCR for detection of flaviviruses using degenerated primers and internal control followed by sequencing for specific identification. *J Virol Methods.* 2005;126:101–9.
10. Figuerola J, Jiménez-Clavero MA, Rojo G, Gómez-Tejedor C, Soriguer R. Prevalence of West Nile virus neutralizing antibodies in colonial aquatic birds in southern Spain. *Avian Pathol.* 2007;36:209–12.

Address for correspondence: Núria Busquets, Unitat de Malalties Víriques, Edifici CRESA, Universitat Autònoma de Barcelona, Bellaterra 08193, Spain; email: nuria.busquets@cresa.uab.es

Letters

Letters commenting on recent articles as well as letters reporting cases, outbreaks, or original research are welcome. Letters commenting on articles should contain no more than 300 words and 5 references; they are more likely to be published if submitted within 4 weeks of the original article's publication. Letters reporting cases, outbreaks, or original research should contain no more than 800 words and 10 references. They may have one Figure or Table and should not be divided into sections. All letters should contain material not previously published and include a word count.

Rotavirus P[4]G2 in a Vaccinated Population, Brazil

To the Editor: Gurgel et al. provide an early examination of postmarketing surveillance data from Brazil, one of the first countries to implement routine childhood immunization with Rotarix vaccine (1). In a community with reported vaccination coverage of 50%, the P[4]G2 strain was detected in all 21 rotavirus-positive stool samples identified during November 2006–February 2007. Although monitoring effectiveness of Rotarix against P[4]G2 strains is of interest (2), the small sample size, short duration of surveillance, and lack of a comparison group preclude firm assessment of an association between P[4]G2 predominance and vaccination.

Because Rotarix was introduced in Brazil in March 2006, most children >12 months old (66 [51%] of 129) in the study were ineligible for vaccination. Genotype P[4]G2 was the only strain identified even in older children, which suggests either a change in disease ecology from vaccination or the random circulation of P[4]G2 strains in the community. Ongoing hospital-based surveillance during 2006 in 3 regional countries that had not introduced rotavirus vaccine (El Salvador, Guatemala, and Honduras) showed that P[4]G2 was the predominant circulating strain (prevalence 68%–81%). Thus, as previously documented (3,4), the predominance of P[4]G2 strains after Rotarix introduction in Brazil could represent a natural shift unrelated to vaccination.

Evaluation of vaccine effectiveness against specific strains will allow full assessment of the public health impact of vaccination. Although the data are sparse in the study from Gurgel et al., a comparison of the odds of vaccination among rotavirus-positive (cases) versus rotavirus-negative (controls) children shows 80% vac-

cine effectiveness against P[4]G2 strains among infants <1 year of age, in accordance with recently published data from a controlled trial (5). To further elucidate vaccine impact, we are providing support for vaccine effectiveness studies in Nicaragua and El Salvador and conducting strain monitoring before and after licensure throughout Latin America.

**Manish M. Patel,*
Lucia Helena de Oliveira,†
Ana Maria Bispo,†
Jon Gentsch,*
and Umesh D. Parashar***

*Centers for Disease Control and Prevention, Atlanta, Georgia, USA; and †Pan American Health Organization, Washington, DC, USA

References

1. Gurgel RQ, Cuevas LE, Vieira SCF, Barros VCF, Fontes PB, Salustino ET, et al. Predominance of rotavirus P[4]G2 in a vaccinated population, Brazil. *Emerg Infect Dis.* 2007;13:1571–3.
2. Glass RI, Parashar UD, Bresee JS, Turcios R, Fischer TK, Widdowson MA, et al. Rotavirus vaccines: current prospects and future challenges. *Lancet.* 2006;368:323–32.
3. Desselberger U, Iturriza-Gomara M, Gray JJ. Rotavirus epidemiology and surveillance. *Novartis Found Symp.* 2001;238:125–47.
4. Santos N, Hoshino Y. Global distribution of rotavirus serotypes/genotypes and its implication for the development and implementation of an effective rotavirus vaccine. *Rev Med Virol.* 2005;15:29–56.
5. Vesikari T, Karvonen A, Prymula R, Schuster V, Tejedor JC, Cohen R, et al. Efficacy of human rotavirus vaccine against rotavirus gastroenteritis during the first 2 years of life in European infants: randomised, double-blind controlled study. *Lancet.* 2007;370:1757–63.

Address for correspondence: Manish M. Patel, Viral Gastroenteritis Section, Centers for Disease Control and Prevention, 1600 Clifton Rd NE, Mailstop A47, Atlanta, GA 30333, USA; email: aul3@cdc.gov