to low concentrations of rifampin. To confirm that this effect was a result of persistence rather than generation of resistant mutants, we transferred the colonies growing after transient rifampin exposure of Beijing strain 1585 to a medium containing 8 mg/L rifampin. Their growth was completely inhibited, and molecular analysis did not detect any of the most prevalent rifampin resistance–associated mutants (data not shown).

We believe that these results provide a possible explanation for the otherwise unrealistically high (apparent) mutation frequency reported by de Steenwinkel et al. (*2*). If these strains are capable of persisting at low concentrations of rifampin, this extended period would provide a window for the generation of mutants during or after exposure. Stress may also play a role; *rpoB* gene mutants have shown to exhibit a stringent-like response (*7*), and defective *rpoB* activity as a result of low-level rifampin exposure could induce a similar response. If rifampin induces a stress response, the situation may be analogous to the high mutation rates seen after quinolone exposure (*8*).

In summary, our data show that the high apparent *M. tuberculosis* strain mutation frequency reported by de Steenwinkel et al. (*2*) may be a result of the higher tolerance to rifampin of some Beijing strains. This tolerance likely results in a specific window of rifampin concentrations that, possibly combined with subsequent error-prone replication/outgrowth, enables the generation and selection of new mutants, rather than the selection of preexisting mutants. When interpreted in the light of our observations, the unexpected results of de Steenwinkel et al. could help explain the association of Beijing genotype strains with drug resistance and relapse (*9*,*10*). Drug levels achieved during treatment may be much more critical in preventing the accumulation of rifampin-resistant mutants for these strains than for other genotypes.

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References

1. Werngren J. *Mycobacterium tuberculosis* Beijing type mutation frequency. Emerg Infect Dis. 2013;19:522–3. http://dx.doi.org/10.3201/eid1903.121001
2. de Steenwinkel JEM, ten Kate MT, de Knegt GJ, Kremer K, Aarnoutse RE, Boeree MJ, et al. Drug susceptibility of *Mycobacterium tuberculosis* Beijing genotype and association with MDR TB. Emerg Infect Dis. 2012;18:660–3. http://dx.doi.org/10.3201/eid1804.110912
3. Bergval I, Kwok B, Schuitema K, Kremer K, van Soolingen D, Klatser P. Pre-existing isoniazid resistance, but not the genotype of *Mycobacterium tuberculosis* drives rifampin resistance codon preference in vitro. PLoS ONE. 2012;7:e29108. http://dx.doi.org/10.1371/journal.pone.0029108
4. Werngren J, Hoffner SE. Drug-susceptible *Mycobacterium tuberculosis* Beijing genotype does not develop mutation-conferred resistance to rifampin at an elevated rate. J Clin Microbiol. 2003;41:1520–4. http://dx.doi.org/10.1128/JCM.41.4.1520-1524.2003
5. Ford CB, Shah RR, Maeda MK, Gagneux S, Murray MB, Cohen T, et al. *Mycobacterium tuberculosis* mutation rate estimates from different lineages predict substantial differences in the emergence of drug-resistant tuberculosis. Nat Genet. 2013;45:784–90. http://dx.doi.org/10.1038/ng.2656
6. den Hertog AL, Visser DW, Ingham CJ, Fey FH, Klatser PR, Anthony RM. Simplified automated image analysis for detection and phenotyping of *Mycobacterium tuberculosis* on porous supports by monitoring growing microcolonies. PLoS ONE. 2010;5:e11008. http://dx.doi.org/10.1371/journal.pone.0011008
7. Koch A, Mizrahi V, Warner DF. The impact of drug resistance on *Mycobacterium tuberculosis* physiology: what can we learn from rifampin? Emerging Microbes & Infections. 2014;3:e17. http://dx.doi.org/10.1038/emi.2014.17
8. Gillespie SH, Basu S, Dickens AL, O'Sullivan DM, McHugh TD. Effect of subinhibitory concentrations of ciprofloxacin on *Mycobacterium fortuitum* mutation rates. J Antimicrob Chemother. 2005;56:344–8. http://dx.doi.org/10.1093/jac/dki191
9. Devaux I, Kremer K, Heersma H, van Soolingen D. Clusters of multidrug-resistant *Mycobacterium tuberculosis* cases, Europe. Emerg Infect Dis. 2009;15:1052–60. http://dx.doi.org/10.3201/eid1507.080994
10. Huyen MN, Buu TN, Tiemersma E, Lan NT, Dung NH, Kremer K, et al. Tuberculosis relapse in Vietnam is significantly associated with *Mycobacterium tuberculosis* Beijing genotype infections. J Infect Dis. 2013; 207:1516–24. http://dx.doi.org/10.1093/infdis/jit048

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Bacteria in Dairy Products in Baggage of Incoming Travelers, Brazil

To the Editor: International air travel can lead to the rapid global dissemination of infectious agents. Unlike products and byproducts of animal origin imported between countries under agreements that legally establish sanitary standards, products introduced into a country illegally or irregularly do not follow specific standards and can come from any source, thereby posing a risk to the health

status of a country. Animal products transported clandestinely in baggage can contain infectious agents harmful to animal and human health (*1*–*4*). We investigated *Brucella* spp., *Mycobacterium bovis*, and *Mycobacterium avium* subsp. *paratuberculosis* (MAP) in dairy products seized from baggage of passengers on flights at the 2 main international airports (Guarulhos Airport, São Paulo, and Galeão Airport, Rio de Janeiro) in Brazil.

During 2010–2011, 12 missions were instigated by the International Agriculture Surveillance (VIGIAGRO/MAPA) in airports to detect and seize unauthorized dairy products carried by passengers; 195 products were collected from multiple flights from different destinations. Baggage was scanned by using an x-ray machine and, on detection of a product, was opened by the owner in the presence of a federal agriculture inspector. To avoid contamination, the products were not opened and were sent to the designated Ministry of Agriculture, Livestock and Food Supply Laboratory in their original packaging. All seized products were packed according to the International Air Transport Association standards (*5*) and transported by commercial aviation with official monitoring to the laboratory.

After completing real-time quantitative PCR (Promega, Madison, WI, USA) using TaqMan technology (Life Technologies, Darmstadt, Germany), we extracted DNA directly from the sample (*6*,*7*). The technique for the detection of MAP and eryD *Brucella* (except strain 19 *Brucella abortus*) and also using the region RD4 to detect *M. bovis* were proposed by Irange et al. (*8*). To detect *M. bovis*, we used the primers *M. bovis*-88-F (5′-CGC.CTT.CCT.AAC.CAG.AAT.TG-3′), *M. bovis*-88-R (5′-GGA.GAG.CGC.CGT.TGT.AGG-3′) and to detect *Brucella,* we used Bru-Eri-Taq-92-F (5′-GCC.ACA.CTT.TCT.GCA.ATC.TG-3′) and Bru-Eri-Taq-92-F (5′-GCG.GTG.GAT.AAT.GAA.ATC.TGC-3′).

We analyzed 35 containers of dulce de leche, a caramelized milk paste confection, from Argentina (n = 30), Angola (n = 1), and Uruguay (n = 4). We tested all specimens for *Brucella* spp. and MAP, and 32 for *M. bovis*. We detected MAP in 1 specimen from Argentina and 1 from Uruguay, *Brucella* spp. in 3 specimens from Argentina and 1 from Uruguay, and *M. bovis* in 1 specimen from Argentina.

Three containers of liquid milk from the United States were collected and analyzed for the presence of MAP; 2 were analyzed for *M. bovis* and *Brucella. Brucella* was detected in 1 specimen. Five containers of powdered milk were seized: 2 from Chile, 2 from Angola, and 1 from Portugal. *Brucella* was detected in 1 container from Chile; *Brucella* and *M. bovis* were found in 1 container from Angola. Four containers of yogurt were seized, 1 each from the United States, China, Angola, and South Africa. MAP was detected in those from Angola and South Africa, and the yogurt from South Africa also showed *Brucella*.

We analyzed samples from 147 cheeses that were confiscated from baggage owned by travelers from 21 countries, mainly from Italy (24.5%), Portugal (22.4%), and France (14.3%). *M. bovis* was identified in 18 (17.5%) cheeses collected from Italy, Portugal, Spain, the United States, the Netherlands, Lebanon, Morocco, and Norway. MAP was amplified in specimens from 13 cheeses from Spain, United States, Iraq, Israel, Norway, Peru, France, and Portugal, the last 2 countries showed the highest occurrence. *Brucella* was detected in 62 of the cheeses analyzed, which originated in Bolivia, Chile, Iraq, Lebanon, and Morocco (n = 1 in each country), Netherlands, Israel, and Norway (n = 2 in each country), Turkey and Spain (n = 3 in each country), United States, France and England (n = 4 in each country), Portugal (n=10), and Italy (n = 23).

Both *M. bovis* and *Brucella* were detected in 13 (8.8%) cheeses (1 each from Spain, Netherlands, Morocco, and Norway; 4 from Portugal, and 5 from Italy); *Brucella* and MAP were detected in 4 (2.7%) cheeses (Spain, France, Portugal, and Iraq). Co-amplification of the 3 genes (*Brucella* + MAP + *M. bovis*) occurred in 3 (2%) cheeses (United States, Norway, and Portugal). Among the cheeses analyzed, 84 (57.1%) contained isolates that amplified >1 of the genes for the 3 bacteria examined.

Of the 166 dairy products analyzed, *Brucella* was detected in 70 (42.1%). Cheeses were the most seized products (n = 121) and had the highest number of *Brucella*-positive results (62/121[51.2%]). *Brucella* was detected in dairy products that originated in Argentina, Spain, France, Iraq, Israel, Italy, Lebanon, Portugal, and Turkey; it was detected in 4 (21%) of the 19 cheeses from France and in 3 of the 4 (75%) cheeses that originated in Spain. *M. bovis* was detected in dulce de leche from Argentina, powdered milk from Chile, and in cheeses from Spain, Netherlands, Italy, Lebanon, Morocco, Norway, and Portugal.

Bacteria can be introduced into a country through contaminated animal products that are brought across borders illegally. The risk may be even greater when these products are carried in passengers' baggage on international flights because of the growing number of international travelers and the wide range of origins of these passengers. Greater attention should be given to agricultural surveillance at airports to mitigate the risk for introduction of these products.

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References

1. Schneider H. Good governance of national Veterinary Services. Rev. Sci. Tech. 2011;30:325–38 [cited 2011 Apr].
2. Hartnett E, Adkin A, Seaman M, Cooper J, Watson E, Coburn H, et al. A quantitative assessment of the risks from illegally imported meat contaminated with foot and mouth disease virus to Great Britain. Risk Anal. 2007;27:187–202. http://dx.doi.org/10.1111/j.1539-6924.2006.00869.x
3. Brückner GK. Ensuring safe international trade: how are the roles and responsibilities evolving and what will the situation be in ten years' time? Rev Sci Tech. 2011;30:317–24.
4. de Melo CB, de Sa MEP, Alves FF, McManus C, Aragão LF, Belo BB, et al. Profile of international air passengers intercepted with illegal animal products in baggage at Guarulhos and Galeão airports in Brazil. SpringerPlus. 2014;3:69. http://dx.doi.org/10.1186/2193-1801-3-69
5. International Air Transport Association. 3.6.2 Division 6.2—infectious substances. 2011 Jan 1 [cited 2011 Aug 10]. http://www.iata.org/whatwedo/cargo/dgr/Documents/DGR52_InfectiousSubstances(DGR362).pdf
6. Millar BC, Jiru X, Moore JE, Earle JAP. A simple and sensitive method to extract bacterial, yeast and fungal DNA from blood culture material. J Microbiol Methods. 2000;42:139–47. http://dx.doi.org/10.1016/S0167-7012(00)00174-3.
7. Dias NL. *Staphylococcus aureus* identification, evaluation of the enterotoxigenic potential and methicillin resistance by the PCR technique in dulce de leche samples in the Sete-Lagoas microregion, in the State of Minas Gerais; Brazil (dissertation) [in Portuguese]. Belo Horizonte, Minas Gerais, Brazil: Federal University of Minas Gerais, 2010.
8. Irenge LM, Walravens K, Govaerts M, Godfroid J, Rosseels V, Huygen K, et al. Development and validation of a triplex real-time PCR for rapid detection and specific identification of *M. avium* sub sp. *paratuberculosis* in faecal samples. Vet Microbiol. 2009;136:166–72. http://dx.doi.org/10.1016/j.vetmic.2008.09.087.

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Evidence of Evolving Extraintestinal Enteroaggregative *Escherichia coli* ST38 Clone

To the Editor: Several clones of extended-spectrum β-lactamase (ESBL)–producing extraintestinal pathogenic *Escherichia coli* (ExPEC) have globally expanded their distribution, including multilocus sequence types (MLSTs) ST38, ST131, ST405, and ST648 (*1*). ExPEC infections often originate from the patient's own intestinal flora, although the degree of overlap between diarrheagenic *E. coli* and ExPEC pathotypes is unclear. Relatively little is known about antimicrobial drug resistance in the most common diarrheagenic *E. coli* groups, including enteroaggregative *E. coli* (EAEC), and bacterial gastroenteritis is generally managed without use of antimicrobial drugs.

The ability of diarrheagenic *E. coli* to cause extraintestinal infections has been shown in previous studies: a study among children in Nigeria linked EAEC to uropathogenic clonal group A (*2*), and a study in Brazil showed that EAEC markers were present in 7.1% of the *E. coli* isolates from urinary tract infections (*3*). Neither of these studies identified clonal lineages of EAEC specifically associated with extraintestinal infections.

We conducted this study to establish the presence and characteristics of ESBL-producing EAEC in a well-defined collection of ESBL-producing isolates (*4*). The isolates were from human and animal sources in Germany, the Netherlands, and the United Kingdom. The study was conducted at Public Health England during January–April 2013.

DNA from 359 ESBL isolates (*4*) was screened for the presence of the EAEC transport regulator gene (*aggR*), located on the EAEC plasmid, by using a real-time PCR assay and the following primers and probe: AggR_F 5′-CCATTTATCGCAATCAGATTAA-3′ AggR_R 5′-CAAGCATCTACTTTTGATATTCC-3′, AggR_P Cy5-CAGCGATACATTAAGACGCCTAAAGGA-BHQ. The amplification parameters were 50°C for 2 min, 95°C for 2 min, and 40 cycles at 95°C for 10 s and at 60°C for 20 s. Isolates positive for *aggR* were confirmed to be *E. coli* by using the Omnilog GenIII MicroPlate (Biolog, Hayward, CA, USA). Serotyping was done by using standard methods (*5*).

The phylogroup was determined for each isolate, and isolates were then assigned to 1 of the 4 major *E. coli* groups: A, B1, B2, and D (*6*). A microarray was used to detect ESBL genes, such as $bla_{\text{CTX-M}}$, at the group level, as previously described (*4*). The antimicrobial drug susceptibilities of EAEC isolates were determined by using the agar incorporation method, as described in the British Society for Antimicrobial Chemotherapy guidelines (*7*).

Virulence factors associated with intestinal and extraintestinal infection