Rapid Communications

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1. Disease Control and Prevention Center, National Center for Global Health and Medicine, Tokyo, Japan
2. Bacteriology I, National Institute of Infectious Diseases, Tokyo, Japan
3. Clinical Laboratory, National Center for Global Health and Medicine, Tokyo, Japan

Citation style for this article:
Mawatari M, Kato Y, Hayakawa K, Morita M, Yamada K, Mezaki K, Kobayashi T, Fujiya Y, Kutsuna S, Takeshita N, Kanagawa S, Ohnishi M, Izumiya H, Ohmagari N. Salmonella enterica serotype Paratyphi A carrying CTX-M-15 type extended-spectrum beta-lactamase isolated from a Japanese traveller returning from India, Japan, July 2013. Euro Surveill. 2013;18(46):pii=20632. Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=20632

Article submitted on 30 October 2013 / published on 14 November 2013

Emerging drug resistance in Salmonella Typhi and S. Paratyphi is a substantial public health concern. We report what appears to be the first case and isolation of multidrug resistant S. Paratyphi A carrying CTX-M-15-type extended-spectrum beta-lactamase from a Japanese traveller returning from India.

Here, we report the isolation of multidrug resistant S. Paratyphi A producing CTX-M-15-type extended-spectrum beta-lactamase (ESBL) from a traveller returning from India. To our knowledge, this is the first report of S. Paratyphi A with CTX-M-15-type ESBL isolated from a human. Enteric fever, including typhoid fever caused by S. enterica serotype Typhi (S. Typhi) and paratyphoid fever caused by S. enterica serotype Paratyphi A (S. Paratyphi A), is one of the most important febrile illnesses in tropical and subtropical countries, with high rates of morbidity and mortality [1]. In industrialised countries, enteric fever is a common cause of fever in returned travellers [2]. The emergence of drug-resistance in S. Typhi and S. Paratyphi A is an emerging public health problem. Owing to the recent increase of fluoroquinolone resistance, third-generation cephalosporins, such as ceftriaxone or cefotaxime, have become the primary drugs for treatment of enteric fever [3].

No further details on the treatment regimen in India were available. The patient returned to Japan on day 4 of illness owing to sustained fever, diarrhoea, and anorexia. During her travel from India to Japan (days 5−7), the patient took oral combined ofloxacin-cefixime. On day 7, she was admitted to our hospital with diarrhoea and anorexia but without fever. On examination, deep palpation of the lower abdominal area caused mild discomfort, otherwise, physical examination was normal. She did not have any underlying illness, and was not on any regular medication. Eight months prior to the admission at the hospital in India she had been vaccinated against Salmonella Typhi (Vi polysaccharide).

Laboratory analyses
On admission, stool and blood samples were taken for culture. Antimicrobial susceptibility test was performed by broth microdilution in accordance with the Clinical and Laboratory Standards Institutes [4].

The patient’s stool was screened on admission for meticillin-resistant Staphylococcus aureus, vancomycin-resistant Enterococci, and Gram-negative rods resistant to one or multiple agents in the extended-spectrum cephalosporin class and/or that demonstrated elevated minimum inhibitory concentrations (MICs) (>1 mg/L) to imipenem and/or meropenem. Screening culture of stool was positive only for drug-sensitive Escherichia coli and Enterobacteriaceae spp.

Results from blood cultures were positive for S. Paratyphi A two days after admission and S. Paratyphi A was found to be resistant to cefotaxime (MIC = 64 mg/L) and ceftazidime (MIC > 16 mg/L). Addition of clavulanic acid to each cephalosporin lowered the MIC to 0.25 mg/L and 1 mg/L, respectively. This isolate was also resistant to nalidixic acid, and categorised as

Case report
In July 2013, a Japanese woman in her mid-20s was admitted to a hospital in Manali, India for fever over 38.5 °C, diarrhoea, and anorexia, where she was diagnosed with typhoid fever based on clinical symptoms and a non-specified rapid serological test. She had been in India for five weeks prior to the admission. Before her visit to India, she had been on a seven-week trip to China, Myanmar, Thailand, and Nepal. She was treated with parenteral ceftriaxone for three days.

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isolate.

Quinolone resistance genes such as qnrA, qnrB, qnrC, and qnrS were not detected in this isolate as described elsewhere [6-9]. However, plasmid-mediated qnrC, qnrS genes have been previously reported in patients in Middle Eastern countries [11,12].

Discussion and conclusion

Although ESBL-producing organisms are emerging among Enterobacteriaceae, ESBL-producing S. Typhi or S. Paratyphi A have been reported rarely in Nepal and the Middle East [10,11]. CTX-M-15 type ESBL-producing S. Typhi were previously reported in patients in Middle Eastern countries [11,12].

The number of reports on ESBL in S. Typhi and S. Paratyphi A is still limited, and thus, extended-spectrum cephalosporins would still be a reasonable empiric treatment for a suspected case of enteric fever in the current situation. The patient improved on day 8 before antibiotics were changed. The oral treatment by a fluoroquinolone (ofloxacin) prior to the admission might have contributed to her clinical improvement. Other possible explanations include clinical recovery by natural history of S. Paratyphi A, or infection of two different antimicrobial patterns of S. Paratyphi A, i.e., ESBL-S. Paratyphi A and non-ESBL-S. Paratyphi A, with non-ESBL mainly contributing to clinical symptoms.

Potential increase in the plasmid-mediated spread of ESBL in S. Paratyphi A in the future would pose a threat to public health. Judicious use of antibiotics to avoid unnecessary selective pressure on intestinal bacterial flora, and careful microbiological analysis of patients with typhoid fever, especially those returning from the Middle East or south Asia are approaches critical for prevention of the potential spread of multidrug resistant S. Paratyphi A.

Acknowledgements

This work was partly supported by funding from the Research on Emerging and Reemerging Infectious Diseases by the Ministry of Health, Labour, and Welfare, Japan (H24-shinkou-ippan-013).

| Antibiotics                        | MIC (mg/L), interpretation result |
|------------------------------------|-----------------------------------|
| Ampicillin                         | ≥16, R                            |
| Amoxicillin/clavulanic acid        | =16, I                            |
| Cefotaxime                         | =64, R                            |
| Cefotaxime/clavulanic acid         | =0.25, S                          |
| Ceftazidime                        | ≥16, R                            |
| Ceftazidime/clavulanic acid        | =1, S                             |
| Ceftriaxone                        | ≥16, R                            |
| Nalidixic acid                     | ≥16, R                            |
| Ciprofloxacin                      | =0.5, I                           |
| Levofloxacin                       | =1, I                             |
| Azithromycin                       | =16†                              |
| Aztreonam                          | ≥16, R                            |
| Trimethoprim/sulfamethoxazole      | ≥2, R                             |
| Chloramphenicol                    | =4, S                             |
| Tetracycline                       | =2, S                             |

MIC: Minimum inhibitory concentration; R: resistant; S: susceptible; I: intermediate.

* The MIC breakpoint for azithromycin is not provided by the Clinical and Laboratory Standards Institutes. We thus refer to the breakpoints table by the European Committee on Antimicrobial Susceptibility Testing, which mentions that azithromycin has been used in the treatment of infections with Salmonella Typhi (MIC ≤4 mg/L for wild type isolates) [5].

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Table

Minimum inhibitory concentrations of various antibiotics on Salmonella enterica serotype Paratyphi A isolated from a Japanese traveller returning from India, Japan, July 2013

| Antibiotics                        | MIC (mg/L), interpretation result |
|------------------------------------|-----------------------------------|
| Ampicillin                         | ≥16, R                            |
| Amoxicillin/clavulanic acid        | =16, I                            |
| Cefotaxime                         | =64, R                            |
| Cefotaxime/clavulanic acid         | =0.25, S                          |
| Ceftazidime                        | ≥16, R                            |
| Ceftazidime/clavulanic acid        | =1, S                             |
| Ceftriaxone                        | ≥16, R                            |
| Nalidixic acid                     | ≥16, R                            |
| Ciprofloxacin                      | =0.5, I                           |
| Levofloxacin                       | =1, I                             |
| Azithromycin                       | =16†                              |
| Aztreonam                          | ≥16, R                            |
| Trimethoprim/sulfamethoxazole      | ≥2, R                             |
| Chloramphenicol                    | =4, S                             |
| Tetracycline                       | =2, S                             |

MIC: Minimum inhibitory concentration; R: resistant; S: susceptible; I: intermediate.

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Conflict of interest
None declared.

Authors’ contributions
M. Mawatari collected the data and drafted the manuscript; YK and KH participated in the coordination and concept of the manuscript and edited the manuscript and helped with the draft of the manuscript; M. Morita, MO and HI performed and analysed the molecular tests; KY and KM performed and analysed the microbiological tests; TK, YF, S. Kutsuna and NT collected the data and participated in the concept of the manuscript; S. Kanagawa and NO revised the article for intellectual content. All authors read and critically revised the first as well as the subsequent and final drafts of this manuscript.

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We describe the first isolation of an NDM-1-producing Pseudomonas aeruginosa in Italy. In May 2013, a patient with acute lymphoblastic leukaemia and history of prior hospitalisation in Belgrad, Serbia, underwent stem cell transplantation at a tertiary care hospital in Rome, Italy. After transplantation, sepsis by NDM-1-producing P. aeruginosa occurred, leading to septic shock and fatal outcome.

In May 2013, a Pseudomonas aeruginosa strain producing New Delhi metallo-beta-lactamase-1 (NDM-1) was isolated in Italy from a patient previously hospitalised in Serbia and found to be resistant to all antibiotics tested except colistin. Multidrug-resistant and extensively drug-resistant P. aeruginosa represent an increasing therapeutic challenge worldwide. In particular, the ST235 clone is the founder of the successful epidemic clonal complex 235 (CC235), associated with various carbapenemase genes, but very rarely with NDM-1 [1,2].

Case description
In late May 2013, a man in his early 40s with a diagnosis of acute lymphoblastic leukaemia (ALL) in first remission was admitted to the haematology unit of a hospital in Rome, Italy, in order to undergo stem cell transplantation. He had been hospitalised in December 2012 at a general hospital in Belgrad, Serbia. In that hospital, the patient had undergone four courses of chemotherapy (HYPER-C-VAD) from December 2012 to February 2013 with a complete remission of disease.

At hospital admission in Rome, the patient was febrile, in good general condition. According to the haematology ward’s routine procedure adopted for transplantation candidates, nasal, pharyngeal, rectal and groin swabs, as well as blood, urine and stool cultures were screened and found negative for multidrug-resistant bacteria. In brief, all samples were screened for methicillin-resistant Staphylococcus aureus (MRSA), extended-spectrum beta-lactamase (ESBL)-producing Enterobacteriaceae, Acinetobacter baumannii, P. aeruginosa, carbapenem-resistant Enterobacteriaceae, vancomycin-resistant Enterococcus faecium (VRE), using MacConkey and chromogenic agar plates (Becton Dickinson, Heidelberg, Germany). Antimicrobial drug susceptibility testing and minimal inhibitory concentrations (MICs) were obtained by the Phoenix System (Becton Dickinson, Sparks, MD) and interpreted according to the recommendations from the European Committee on Antimicrobial Susceptibility Testing (EUCAST) [3].

Lacking an available HLA-compatible donor, the patient received on 28 May a stem cell transplant from a haploidentical donor, with a high-dose conditioning regimen containing thiopeta, busulfan, and also containing fludarabine-unmanipulated bone marrow as the stem cell source. Prophylaxis of graft-versus-host disease was post-transplant cyclophosphamide, mycophenolate and cyclosporine, as in the Baltimore programme [4]. Two days later he developed severe neutropenia (absolute neutrophil count (ANC) =0.1x10^9/L) and treatment was started with piperacillin/tazobactam, amikacin and vancomycin, with an immediate defervescence. The patient remained neutropenic (ANC <0.1x10^9/L), and fever reappeared some days later. Meropenem was added in substitution of piperacillin/tazobactam.

A pharyngeal swab taken 10 days after transplantation was positive for Klebsiella pneumoniae. Species identification and antimicrobial susceptibilities were determined by the Phoenix System (Becton Dickinson, Sparks, MD) and the tigecycline MIC was obtained by Etest (Becton-Dickinson, United States). The K. pneumoniae isolate was an ESBL producer with resistance to cefotaxime (MIC >4 mg/L) ceftazidime (MIC >8 mg/ml), ertapenem (MIC >1 mg/L) and tigecycline.
On Day 17 after transplantation, the patient rapidly deteriorated and was transferred to the intensive care unit, where he died few hours later from septic shock still in profound aplasia.

The blood cultures of the patient, taken on Day 15, were positive for the ertapenem-resistant *K. pneumoniae*. Another rectal swab was again taken on Day 16 and draining abscess, Rome, May 2013.

| Antibiotics | MIC (µg/mL), interpretation resulta |
|-------------|----------------------------------|
| Amikacin    | ≥16, R                           |
| Aztreonam   | ≥16, R                           |
| Cefepime    | ≥8, R                            |
| Ceftazidime | ≥8, R                            |
| Ciprofloxacin| ≥1, R                           |
| Colistin    | ≤1, S                            |
| Gentamicin  | ≥4, R                            |
| Imipenem    | ≥8, R                            |
| Meropenem   | ≥8, R                            |
| Piperacillin| ≥16, R                           |
| Piperacillin/tazobactam | ≥16/4, R |
| Tobramycin  | ≥4, R                            |

MIC: minimum inhibitory concentration; R: resistant; S: susceptible

a Based on EUCAST interpretive criteria [1].

(MIC 3 mg/L), but remained susceptible to imipenem (MIC ≤1 mg/L), meropenem (MIC ≤1 mg/L) and colistin (MIC ≤1 mg/L), according to interpretive criteria from the EUCAST [3]. The strain was assigned to ST101 by multilocus sequence typing [5] and resulted negative for carbapenemase genes in PCR using previously described primers and protocols [6,7]. Colistin (given every 12 hours for a total daily dose of 9,000,000 IU after a loading dose of 9,000,000 IU) was added to the ongoing antimicrobial treatment.

At Day 15 after transplantation, the patient was still febrile and neutropenic. Another rectal swab was again positive for the ertapenem-resistant *K. pneumoniae*. A perianal abscess appeared on Day 16 and draining yielded *P. aeruginosa* and VRE. This isolate was resistant to all antibiotics tested (Table) except colistin.

The blood cultures of the patient, taken on Day 15, yielded a *P. aeruginosa* with an identical phenotype. Vancomycin treatment was halted, and tigecycline (200 mg/day administered every 12 hours, after a loading dose of 200 mg), rifampicin (600 mg given every 24 hours) and daptomycin (700 mg every 24 hours), were added to the previous treatment.

On Day 17 after transplantation, the patient rapidly deteriorated and was transferred to the intensive care unit, where he died few hours later from septic shock still in profound aplasia.

Molecular characterisation of the *P. aeruginosa* isolate

The *P. aeruginosa* strain isolated from the blood cultures was identified as sequence type (ST) 235 by MLST [8]. The ST235 strain resulted positive for the *bla*NDM-1 gene. Plasmid and total DNA was tested by Southern blot hybridisation with the *bla*NDM-1 gene as probe. Results suggested a chromosomal location of the *bla*NDM-1 gene (data not shown). Total DNA was restricted with HindIII and a genome library was constructed in the HindIII-pZErO-2 kanamycin-resistant vector (Invitrogen, Milan Italy). Transformants were obtained selecting on plates containing 30 mg/L ampicillin and 30 mg/L kanamycin. One transformant carried a HindIII insert of approximately 3.3 KB, which resulted positive in a PCR for the *bla*NDM-1 gene. The insert was fully sequenced using universal primers and the primer walking approach. Comparative DNA sequence analysis showed that the HindIII insert in our construct had 100% of DNA identity to the HindIII-fragment of the ST235 *P. aeruginosa* strain HIABP11 (EMBL ACC. No. KC170992) carrying *bla*NDM-1, that was isolated in France in March 2012 [9]. Based on the DNA sequence of the HIABP11 strain (EMBL ACC No. KC170992), 12,762 kb containing the entire *bla*NDM-1 gene environment and flanking regions were analysed by PCR and sequencing. The presence of ISPa7 was identified upstream of the HindIII insert of approximately 3.3 KB, which resulted positive in a PCR for the *bla*NDM-1 gene. The insert was fully sequenced using universal primers and the primer walking approach. Comparative DNA sequence analysis showed that the HindIII insert in our construct had 100% of DNA identity to the HindIII-fragment of the ST235 *P. aeruginosa* strain HIABP11 (EMBL ACC. No. KC170992) carrying *bla*NDM-1, that was isolated in France in March 2012 [9]. Based on the DNA sequence of the HIABP11 strain (EMBL ACC No. KC170992), 12,762 kb containing the entire *bla*NDM-1 gene environment and flanking regions were analysed by PCR and sequencing. The presence of ISPa7 was identified upstream of the HindIII insert of approximately 3.3 KB, which resulted positive in a PCR for the *bla*NDM-1 gene. The insert was fully sequenced using universal primers and the primer walking approach. Comparative DNA sequence analysis showed that the HindIII insert in our construct had 100% of DNA identity to the HindIII-fragment of the ST235 *P. aeruginosa* strain HIABP11 (EMBL ACC. No. KC170992) carrying *bla*NDM-1, that was isolated in France in March 2012 [9]. Based on the DNA sequence of the HIABP11 strain (EMBL ACC No. KC170992), 12,762 kb containing the entire *bla*NDM-1 gene environment and flanking regions were analysed by PCR and sequencing. The presence of ISPa7 was identified upstream of the HindIII insert of approximately 3.3 KB, which resulted positive in a PCR for the *bla*NDM-1 gene.

Control measures

The ST235 strain could represent a serious risk for potential spreading in the hospital environment.

However, during the hospitalisation and after the identification of the strain, strict contact isolation precautions (CIP), and screening of the personnel were implemented on the haematology ward. CIP included wearing a gown and gloves when entering the patient’s room, promptly removing gloves after care and hand decontamination, as well as using disposable single-use or patient dedicated non-critical equipment such as blood pressure cuff and stethoscope. Moreover, since the hands of healthcare workers are the most common vehicle for the transmission of microorganisms, including *P. aeruginosa* and *K. pneumoniae*, from patient to patient and within the healthcare environment [10], hand hygiene practices were strengthened.

All contacts, room-mates, relatives, and personnel involved in the care of the patient were screened for carriage of this organism. In addition, environmental screening for *Pseudomonas* and *Klebsiella* was performed at several points of the ward, including the rooms where the patient stayed. Neither NDM-1-producing *P. aeruginosa* nor *Klebsiella* were obtained
from these cultures. Moreover, after a one month of strict surveillance, neither any infection nor any colonisation by NDM-1-producing *P. aeruginosa* or Klebsiella were observed on the wards where the patient stayed.

**Discussion**

It has been recently demonstrated that the emergence of multidrug-resistant, metallo-beta lactamase (MBL)-positive *P. aeruginosa* in Russia was largely due to the spread of one dominant clone, CC235 producing VIM-2 [1]. The association of CC235 with MBL genes has been reported outside Russia in several European countries [1]. In particular, VIM-1 was the more common MBL identified in CC235 *P. aeruginosa* from Italy, VIM-4 in Greece, Sweden, Hungary, and Belgium, VIM-13 in Spain, and IMP-29 in France [1]. To the best of our knowledge, only few NDM-1-producing *P. aeruginosa* strains were reported in Europe before this Italian case: two strains were described in Serbia in 2010 and one strain was identified in France in 2012 [2,9]. Both the Italian and French NDM-1 producing *P. aeruginosa* were identified in patients previously hospitalised in Serbian hospitals, where a reservoir of NDM-1-producing CC235 *P. aeruginosa* can be suspected. Moreover, six of 55 travel-associated cases of *Enterobacteriaceae* producing NDM-1 reported in European countries had a link to the Balkan region, presumably to Serbia, Kosovo*, Montenegro, and Bosnia and Herzegovina [11].

* This designation is without prejudice to positions on status, and is in line with United Nations Security Council Resolution 1244/99 and the International Court of Justice Opinion on the Kosovo declaration of independence.

**Acknowledgements**

This work was supported by The Italian FLAGSHIP “InterOmics” project (PB.P05) funded by MIUR and coordinated by the CNR. Renata Galetti’s fellowship was supported by the grant 2012/24864-1, Sao Paulo Research Foundation (FAPESP)

**Conflict of interest**

None declared.

**Authors’ contributions**

All authors of this research paper have directly participated in the planning, execution and analysis of this study

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For the last 60 years, only a few cases of tick-borne encephalitis (TBE) have been detected in Bulgaria. Considering the remarkable increase in TBE morbidity in Europe over the past two decades, we conducted a study of TBE among patients with acute viral meningitis who were hospitalised in Bulgaria during 2009 to 2012. A total of 86 patients with viral meningitis of unknown aetiology during this period were tested. Acute TBE was confirmed in three of these patients. The last TBE case was detected in October 2012; the other two were diagnosed in 2009. To the best of our knowledge, these three patients are the first confirmed TBE cases reported in Bulgaria. The risk of TBE is underestimated in Bulgaria due to the low awareness of medical doctors.

Introduction

Tick-borne encephalitis (TBE) occurs in north and central Europe, Russia, Far East, Asia and Japan [1-3]. The aetiologic agent, tick-borne encephalitis virus (TBEV), is a member of the genus Flavivirus of the family Flaviviridae, which also includes aetiologic agents of yellow fever, dengue, West Nile fever and Japanese encephalitis. Three subtypes of the virus are known: European, Siberian and Far Eastern [4]. The severity of the disease and its outcome depends largely on the causative subtype [4].

TBEV is transmitted to humans through bites of infected *Ixodes ricinus* ticks or by consumption of unpasteurised milk from infected animals [5], usually goats, but also sheep and cows [6]. The incubation period is between 2 and 28 days, most commonly 7–14 days. A shorter incubation period is connected to milk-borne TBE [5].

Between 70% and 98% of TBEV infections are subclinical [5]. In clinically manifested cases, about two thirds of the patients only develop a non-specific febrile syndrome during the first phase of the infection [5]. Neurological disorders, usually meningitis or meningoencephalitis, appear during a second febrile phase. Biphasic febrile illness is typical for infection with the Western subtype of the virus, while patients infected with the Eastern subtype develop only a monophasic course [5].

In Bulgaria, reporting of TBE has been mandatory since 1953, but is rarely reported. Over the past 60 years, only a few cases of TBE have been detected [7,8] which, according to the current European Union case definition criteria [9] would not be considered as confirmed cases. Before our study presented here, the last TBE cases were reported in 2006 [10]: laboratory diagnosis of these and previous cases was based on detection of TBE-specific antibodies by complement fixation assay. Most of these cases were due to consumption of unpasteurised goats’ milk [10]. Surprisingly, the tick vector, *Ixodes ricinus*, is widely distributed in Bulgaria and Lyme borreliosis, caused by borreliae transmitted by the same tick species, is endemic in the country, with about 1,000 cases reported annually [11].

Considering the remarkable increase in TBE morbidity in Europe over the past three decades [12], we conducted a study among patients with acute viral meningitis who were hospitalised in Bulgaria during 2009 to 2012, to determine whether some of the acute viral meningitis cases could be due to infection with TBEV.

Methods

Patients and serum samples

Serum samples from hospitalised patients with acute viral meningitis of unknown aetiology (with or without history of a tick bite, according to the anamnesis) were collected between 2009 and 2012. The samples were drawn by physicians at the infectious diseases units of regional hospitals in the largest districts of Bulgaria (Sofia, Pazardzhik, Plovdiv, Burgas). Serum samples from 86 patients were drawn during the acute phase and 49 sera were collected at the convalescence phase, 7–30 days after the first sample.

Case definition

In this study, we used the 2012 European Union case definition for TBE [9]. A case is classified as probable when a patient met the clinical criteria (symptoms of inflammation of the central nervous system (CNS)) and laboratory criteria (detection of TBE-specific IgM antibodies in a unique serum sample). For a confirmed
TBE case, in addition to meeting the clinical criteria, at least one of the following laboratory criteria was met: (i) TBE-specific IgM and IgG antibodies in blood; (ii) seroconversion or fourfold increase of TBE-specific antibodies in paired serum samples; (iii) detection of TBE viral nucleic acid in blood or CSF.

Enzyme-linked immunosorbent assay
All 135 serum samples from the 86 patients were tested for TBEV-specific IgM antibodies; those that were positive were also tested for IgG antibodies against TBEV using commercially available enzyme-linked immunosorbent assay (ELISA) (Euroimmun, Germany). The test uses highly purified TBEV proteins. Serum samples were diluted 1:101. Peroxidase-labelled anti-human IgM or IgG antibodies and 3,3',5,5' -tetramethylbenzidine (TMB)/peroxide substrate were used to detect specific interactions. Calculated values below 0.8 were interpreted as negative; those between 0.8 and 1.1 were accepted as borderline and those above 1.1 were considered positive.

Polymerase chain reaction
TBEV RNA was detected by reverse transcription (RT) polymerase chain reaction (PCR) based on quantitative real-time technology (TaqMan), as described elsewhere [13]. The system detected a fragment of the 3’ non-coding region of the TBEV genome.

Testing for other pathogens
Serum samples of patients that were found positive for TBEV-specific antibodies were further tested for IgM antibodies against *Borrelia burgdorferi* by ELISA (anti-Borrelia ELISA IgM, Euroimmun, Germany), West Nile virus by ELISA (West Nile virus IgM capture – DxSelect, Focus Diagnostics, United States) and immunofluorescence assay (IFA) (Euroimmun, Germany) and against yellow fever virus by IFA (Euroimmun, Germany).

Results
Samples from 86 patients with viral meningitis of unknown aetiology during 2009 to 2012 were tested to detect acute TBE: three confirmed TBE cases were
found. The last TBE case was detected in October 2012; the other two were diagnosed in 2009. The place of residence of the three confirmed cases, as well as the previous not-confirmed cases reported since 1953, is shown (Figure).

Case 1
A teenager residing in Velingrad (Pazardzhik district, south Bulgaria) was admitted to the regional hospital in early 2009 with high fever (40 °C) and malaise. The patient’s temperature returned to normal (four days after admission) and then about a week later, the patient’s condition again deteriorated, with fever, headache, stiff neck, sore throat, nausea, vomiting and a depressed mood. The patient had a history of possible tick exposure in the forest surrounding the village. Cerebrospinal fluid (CSF) collected four days after admission showed a high number of leucocytes (160/µL; norm: 0–5/µL) with 75% granulocytes (norm: 60–70% lymphocytes, up to 30% monocytes), high protein content (125 mg/dL; norm: 15–45 mg/dL) and normal glucose level (0.31 mmol/L; norm: 0.22–0.44 mmol/L). TBEV was detected by real-time RT-PCR in a serum sample taken the same day. The patient was transferred to a hospital in Sofia and a second CSF sample was obtained on 22 April 2009. CSF pressure was increased, the leucocytes count was increased (400/µL) with 65% lymphocytes, the protein content was decreased slightly but was still high (100 mg/dL) and the glucose level was still normal (0.30 mmol/L). *Mycobacterium tuberculosis* was isolated from this CSF sample. A serum sample drawn the same day showed high titres of TBEV-specific IgM antibodies by ELISA. IgG antibodies against TBEV were not found.

Case 2
In early September 2009, a person aged in their early 20s was admitted to the regional Plovdiv hospital (south Bulgaria) with fever (38.5 °C), headache, fatigue, nausea and vomiting. Physical examination revealed stiff neck, muscle soreness, conjunctivitis, stupor and abnormal reflexes with pain in joints. The symptoms started 5–6 days before hospital admission. The patient lived in a village with a high risk for exposure to tick bites (many village residents had had tick bites). CSF analysis showed an increased count of leucocytes (301/µL, with 82% lymphocytes, slightly elevated protein (56 mg/dL), and normal glucose level (0.38 mmol/L). After initial improvement within a week, the patient’s condition worsened again with fever, severe headache and prominent dizziness. CSF analysis of the sample collected at that time reflected the worsened condition of the patient: the leucocytes count reached 447/µL, with 90% lymphocytes and the protein level was remarkably elevated (134 mg/dL); the glucose level was normal (0.28 mmol/L). The patient gradually recovered within a month after symptom onset. Paired serum samples from this patient – one upon admission and a second during the convalescence – were tested by ELISA: the first sample was positive and the second borderline for TBEV-specific IgM antibodies. IgG antibodies were detected by ELISA in the second serum sample, but not in the first.

Case 3
A resident of the Burgas district (east Bulgaria) in their late 20s was admitted to the regional hospital in late September 2012 with fever (37.5–38 °C), considerable numbness in the muscles and weakness. Physical examination revealed mild neck stiffness, mild left hemiplegia and hypeaesthesia of the limbs. The patient’s symptoms started two days before admission. Upon admission, a tick was found on their body and was removed. Four days later, the patient’s condition improved, after a further four days, the fever, weakness and numbness in muscles was exacerbated. CSF analysis showed slightly elevated leucocyte count (60/µL) and protein level (74 mg/dL); the glucose level was normal (0.38 mmol/L). A serum sample taken at that time and a later sample taken 9 days later, were tested by ELISA: in both samples, IgM and IgG antibodies specific to TBEV were detected. The patient was discharged in an improved condition three weeks after the admission.

The serum samples of the three patients tested negative by ELISA and IFA for West Nile virus and IFA for yellow fever virus, and were also negative for IgM antibodies to *Borrelia burgdorferi* by ELISA. Bacterial culture from the CSF samples of the three patients was negative.

Discussion
Earlier investigations, carried out between 1974 and 2002, confirmed that TBEV was present in ticks in Bulgaria: 6,849 ticks were investigated and eight TBEV strains were isolated [14]. TBE cases in humans have been occasionally reported in Bulgaria (Figure); however, the fact that cases do occur – even though only diagnosed and reported sporadically – and are associated with tick bites or consumption of unpasteurised milk, shows that TBEV circulates in the country. Given that patients who develop neurological symptoms represent a small proportion of those infected, it can be predicted that the number of TBEV-infected people in Bulgaria is many times higher.

There has been significant increase in the number of registered cases of TBE in Europe, Russia and the Far East since 1990 [12]. Since then, about 10,000 to 12,000 clinical cases are reported per year in 30 TBE-endemic countries in Europe and Russia [12]. The epidemiology of TBE after 1990 is characterised not only by a global increase in the number of cases but also by an expansion of risk areas. For example, a significant increase in TBE was recorded in Sweden in the last decade (2000–2011), especially in 2011, when there was a record annual number of TBE cases in Sweden [15]. New endemic areas in Switzerland were identified by detection of TBEV RNA in field-collected ticks in 2007–2010 [16]. Since September 2012, given the importance and
spread of TBE in the European Union, the European Commission included TBE in the list of communicable diseases covered by epidemiological surveillance in the Member States [9].

In all three patients described here, the typical biphasic course of the disease was noted. These patients are, to the best of our knowledge, the first confirmed cases in Bulgaria, having been laboratory confirmed by PCR and IgG ELISA.

Usually, IgM and IgG antibodies to TBEV are present by the time CNS involvement manifests itself in the second phase of TBE [12]. However, TBEV RNA is very rarely detected by PCR during the vireamc second phase of the disease [13]. Surprisingly, we detected TBEV infection by RT-PCR in the first case. This patient proved to have a mixed infection with M. tuberculosis, which could have promoted the primary progressive course of the meningo-encephalitis, as previously reported [17].

The detection of three cases among the 86 patients tested shows that TBE is probably not uncommon in Bulgaria. The risk of TBE is underestimated in Bulgaria because of the low awareness of medical doctors. TBE should be considered for patients with various manifestations of CNS infection in Bulgaria.

Acknowledgements

This work was supported by the Global Emerging Infections Surveillance Response System (GIES) funds DOD # NAMRU3.2008.0003.

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This study examines the *Salmonella* status in reptiles kept in households with children suffering from gastroenteritis due to an exotic *Salmonella* serovar, to obtain information on possible transmission paths. A number of affected households (n=79) were contacted, and almost half (34/79) comprised at least one reptile in the home. Of the households, 19 were further studied, whereby a total of 36 reptiles were investigated. Samples were taken from the reptiles including the oral cavity, the cloaca, the skin and, in the case of lizards, the stomach, and isolation of *Salmonella* strains was performed using repeated enrichment and typing. Where the *Salmonella* serovars of the infected child and the reptile were identical, typing was followed by pulsed-field gel electrophoresis (PFGE). Bearded dragons (*Pogona vitticeps*) constituted 19 of 36 examined reptiles. Altogether 319 *Salmonella* isolates were investigated and 24 different serovars identified in the reptiles. In 15 of 19 households, an identical serovar to the human case was confirmed in at least one reptile (including 16 of all 19 bearded dragons examined). The results demonstrate that reptiles and especially bearded dragons shed various *Salmonella* serovars including those isolated from infected children in the respective households. Hygiene protocols and parents’ education are therefore highly necessary to reduce the risk of transmission. From a terminological point of view, we propose to call such infections ‘Reptile-Exotic-Pet-Associated-Salmonellosis’ (REPAS).

**Introduction**

According to Thomas et al. [1] the potential of captive and wild animals to transmit salmonellae to humans should not be underestimated, and epidemiological studies on sources of human salmonellosis should simultaneously investigate both the human cases and the wild and domestic animals in contact with them. A study in 1997 from Canada also estimated that three to five per cent of human *Salmonella* cases were associated with exposure to exotic pets (including reptiles, sugar gliders, and hedgehogs) and involved a great variety of *Salmonella* serovars with for example *S. Stanley*, *S. Poona*, *S. Jangwani*, *S. Pomona*, *S. subspp. IV 48:g,251:-* (former *S. Marina*) [2].

Numerous reports exist on the prevalence of *Salmonella enterica* in captive reptiles, with recent publications demonstrating a higher prevalence in lizards (up to 76%) compared to tortoises and turtles [3,4]. Geue and Löschner [5] showed that reptile collections with purchased animals had a significantly higher prevalence of *Salmonella* than collections from pure breeders. Furthermore, animals from pet shops were more frequently affected (89%) than wild caught animals (59%).

Beside the reptiles per se, the reptile feed, in the form of rodents, can also cause infections in humans. In England, a *S. Typhimurium* definite phage-type was identified as a source of salmonellosis in humans with a strong association to those keeping reptiles, and was also confirmed in frozen feeding-mice originating from a specific rodent breeding facility [6]. In a further outbreak of *S. Typhimurium* related to snakes in the United States (US), pulsed-field gel electrophoresis (PFGE)-patterns identical to the human isolates were confirmed for isolates from mice used to feed the snakes as well as the snakes and the environment [7].

Among reptiles, turtles have been reported to be the most common source of *Salmonella* in the 1970s [8]. However, later studies and surveys indicate that other reptile species, especially lizards, may play a more important role [9-13]. Measures targeted at the prevention of turtle-associated salmonellosis in the US and Canada in the 1970s temporary helped to reduce its occurrence, however reptile-associated salmonellosis is suspected to be a resurgent problem and estimated to cause three to 11% of all human salmonellosis cases in these countries [2,14,15].
Most reports of reptile-associated salmonellosis concern babies (less than one year of age) and young children (up to six years old). However, adults can be affected, especially immunocompromised hosts, and patients with impaired gastric acid production [16]. Fatal outcomes following reptile-associated salmonellosis (RAS) in babies have been observed [17,18]. In Germany, a recent study [19] reported an increasing number of salmonellosis related to reptiles, with most patients being less than one year old. The aim of this study was therefore to obtain data on possible links between captive reptiles and salmonellosis in children by examining both the children with salmonellosis and all reptiles kept in the respective households.

Methods
The study was conducted from July 2010 to October 2011. Within this period, the National Reference Centre (NRC) for Salmonella and other Bacterial Enterics at the Robert Koch Institute (Wernigerode, Germany) examined samples from 206 households with salmonellosis in children not older than three years. Of the Salmonella isolates, 65% (134/206) did not belong to S. Typhimurium and S. Enteritidis and were therefore of interest for this study. The responsible federal health institutes were asked to contact these households. A total of 79 parents, corresponding to 79 households, were successfully contacted by mail and asked about the presence of reptiles in the respective households. Thirty-five of 79 parents confirmed having reptiles and were contacted by phone to attend this study. As inclusion criteria for participation, parents had to agree that all reptiles in the respective household could be sampled and their health status assessed, and the time period between detection of clinical salmonellosis in the child and the sampling of the reptiles was not more than three weeks.

Species and health status of the reptiles were assessed and sampling was conducted following established guidelines using sterile cotton swabs (Heinz Herenz, Hamburg, Germany). Swabs were taken from the oral cavity, the cloaca, and the skin on the ventral region of the reptile. For lizards, an additional swab sample was taken from the stomach. Bacterial isolation and identification were conducted with repeated enrichment and examination of several colonies in each sample, in order to find as many different Salmonella serovars as possible: All samples were immediately plated onto sheep blood agar as well as XLT-4 (RV) medium (Oxoid, Wesel, Germany). This medium was cultured aerobically for 24h at 39°C. A sample was then plated onto sheep blood agar as well as XLT-4-Agar (Oxoid, Wesel, Germany) and cultured aerobically for further 24h at 39°C. From the RV bouillon culture, 1 ml was transferred to a new tube containing RV, and enrichment as well as culture was repeated. This procedure was repeated again, so that in total isolation and enrichment cycles. From Salmonella-suspicous colonies, at least five different colonies were subcultured on Brilliant Green agar (Sifin, Berlin, Germany) for confirmatory testing with biochemical methods. Confirmed colonies were agglutinated against Salmonella surface antigens (sera from Sifin, Berlin, Germany) and all strains were typed at the NRC according to the White-Kauffmann-Le Minor scheme [20].

The isolates of identical serovars confirmed in the infected child and the reptile were compared using PFGE. PFGE was carried out according to the standardised protocol for subtyping Salmonella [21]. 40 strains (human and animal) were investigated by PFGE. In case of minor pattern differences between the reptile and the human isolate of the respective household, the PFGE was repeated. Interpretation of the PFGE results followed recent recommendations from the literature [22-24]: Identical PFGE patterns were considered to represent the same epidemiological type. Depending on the time the outbreak has been going on and if person to person spread is the prominent feature, two to three [23] or up to four differences [22] in PFGE restriction fragment pattern are considered to be the result of a single genetic event, and isolates can be designated as ‘subpatterns’ or related patterns. Given the maximum time period (several weeks between initial infection of the child and final sampling of the reptile) for this study, more than two differences were considered to represent an epidemiologically-significant difference.

Results
Nineteen households met the inclusion criteria for participation in the study and reptiles were thus investigated. Altogether, 36 reptiles were kept in the households and included in this study (per household between 1 and 7, on average 1.9). Details are listed in Table 1. In these 19 households, Salmonella serovars isolated from the children belonged mainly to subspecies I (12 households), but also subspecies II (4 households), subspecies IIIa (1 household), subspecies IIIb (2 households) and subspecies IV (3 households) (Table 1). One S. Newport strain (6,8:e,h:11,2:267, d-tartrate- negative and malonate positive) was investigated in Paris (Institut Pasteur) because of its unusual biochemical properties. The strain exhibited two atypical characteristics: D-tartrate-negative and malonate positive. The ‘e,h’ and the ‘1,2’ flagellar phases have been confirmed by fliC and fliB sequencing. The multilocus sequence typing (MLST) profile (ST118) was identical to those of serovar Newport lineage II [25].

Investigated households were spread well over Germany, including nine (of 16 possible) federal states (Bavaria, Baden-Württemberg, Berlin, North Rhine-Westphalia, Rhineland-Palatinate, Saxony, Saxony-Anhalt, Schleswig-Holstein, Thuringia). From the investigated households, all children except one were less than 15 months of age, most (11/19) were less than six months. All children showed clinical symptoms of gastroenteritis including fever, with some being critically ill. Except for three serovars (S. Eastbourne 9,12:e,h:1,5; S. subspec. IV 44:z4,223--; S. Monschau
## Table 1a
Comparison of *Salmonella* serovars in reptiles and salmonellosis-affected children living in the same households, with results of pulsed-field gel electrophoresis when the serovars were identical, Germany, July 2010–October 2011

| House- | Child | Reptile species (type of reptile) | Serovars identified in the reptile | Localisation on reptile (if serovar identical to child) | PGFE result (number of pattern differences) |
|--------|-------|----------------------------------|-----------------------------------|--------------------------------------------------------|---------------------------------------------|
| hold   |       |                                  | N                                | Non-identical isolates to the child (localisation on the reptile) |                                             |
| 1      | 11    | S. Eastbourne 9,12:e,h:1,5       | 3                                | S. subspec. I serol. rough (sc, ch); S. subspec. IIb 50:r:z (cl) | Ch, cl, sk                                  |
|        |       | *Pogona vitticeps* (l)           | 1                                | –                                                      | Cl                                          |
| 2      | 3     | S. Ealing 35:g,m,s:-             | 1                                | S. Eastbourne 9,12:e,h:1,5 (cl)                        | –                                           |
| 3      | 8     | S. Cotham 28:i,1,5               | 3                                | S. Tennessee 6,7:229:- (cl); S. subspec. I serol. rough (cl) | Sk s (2)                                    |
| 4      | 2     | S. subspec. IV 44:24,223:-       | 3                                | S. Eastbourne 9,12:e,h:1,5 (cl); S. Tennessee 6,7:229:- (cl) | St i                                        |
| 5      | 6     | S. Tennessee 6,7:229:-           | 3                                | S. Enteritidis 9,12:g,m:, phage type 8/7 (cl); S. Kisarawe 11:k:e,n,x (cl) | Sk i                                        |
| 6      | 35    | S. Monschau 35:m,t:-            | 1                                | –                                                      | Cl                                          |
|        |       | *Pogona vitticeps* (l)           | 2                                | S. Pomona 28:y:1,7 (cl)                                | Cl                                          |
| 7      | 2     | S. subspec. IIIa 41:24,223:-     | 4                                | S. subspec. IIb 42:1:235: (cl); S. Paratyphi B Var.dT: Java 4,5,12:b:1,2; phage type Worksop (cl); S. Florida 1,6,11,25:d:1,7 (cl) | Cl i                                        |
|        |       | *Pantherophis guttatus* (a)      | 1                                | S. subspec. IIb 42:k:235 (cl)                          | –                                           |
|        |       | *Chameleo calyptratus* (l)       | 1                                | S. Pomona 28:y:1,7 (cl)                                | –                                           |
| 8      | 3     | S. subspec. IIlb 14,24:210:z     | 1                                | S. subspec. IIlb 42:k:235 (cl)                        | –                                           |
|        |       | *Pantherophis guttatus* (a)      | 2                                | S. subspec. IIlb 14,24:210:z (cl)                     | Cl                                          |
|        |       | *Pantherophis guttatus* (a)      | 1                                | –                                                      | Cl                                          |
| 9      | 7     | S. Newport 6,8:e,h:1,2:767      | 2                                | S. subspec. IIlb 14,24:210:z (cl)                     | Cl                                          |
|        |       | (d-tartrate-,malonate+)          |                                  | –                                                      | –                                           |
| 10     | 13    | S. Kandla 17:229:-              | 2                                | S. Kisarawe 11:k:e,n,x (ch, cl)                        | Cl                                          |
|        |       | *Pogona vitticeps* (l)           | 2                                | S. Kisarawe 11:k:e,n,x (cl)                            | Cl                                          |
| 11     | 5     | S. Monschau 35:m,t:-            | 2                                | S. Tennessee 6,7:229:- (sk); S. Carmel 17:l,v:e,n,x (ch, sk, st) | –                                           |
|        |       | *Pogona vitticeps* (l)           | 2                                | S. Tennessee 6,7:229:- (cl); S. Carmel 17:l,v:e,n,x (sk) | –                                           |
|        |       | *Python sp. (s)                  | 0                                | –                                                      | –                                           |
|        |       | *Python sp. (s)                  | 1                                | S. subspec. IIlb serol. rough (ch)                     | –                                           |
|        |       | *Python sp. (s)                  | 2                                | S. subspec. IIlb serol. rough (cl); S. subspec. IIlb 50:1 (cl) | –                                           |
|        |       | *Python sp. (s)                  | 1                                | S. subspec. IIlb serol. rough (cl)                     | –                                           |
|        |       | *Python sp. (s)                  | 1                                | S. subspec. IIlb serol. rough (ch)                     | –                                           |

PFGE: pulsed-field gel electrophoresis; serol.: serological specificity; sp.: species; subspec.: subspecies; l: lizard; s: snake; t: tortoise/turtle; ch: choana; cl: cloaca; sk: skin; st: stomach.

Where the serovars from the child and the reptile were identical, PFGE was performed; i: identical pattern; s: similar pattern.
**Table 1B**  
Comparison of *Salmonella* serovars in reptiles and salmonellosis-affected children living in the same households, with results of pulsed-field gel electrophoresis when the serovars were identical, Germany, July 2010–October 2011

| Household | Age (months) | *Salmonella* serovar | Reptile species (type of reptile)<sup>a</sup> | Serovars identified in the reptile<sup>b</sup> | Non-identical isolates to the child (localisation on the reptile)<sup>b</sup> | Localisation on reptile<sup>b</sup> of serovars identical to child | PGFE result<sup>c</sup> (number of pattern differences) |
|-----------|--------------|-----------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|-------------------------------|
| 13        | 3            | S. subspec. II 42:r:-  | *Pogona vitticeps* (l)          | 2                               | S. Kisarawe 11:k:e,n,x (ch, cl) | Ch, st, cl, sk                  | i                             |
| 14        | 11           | S. Potsdam 6,7;l,v:e,n,z | *Testudo hermanni* (l)          | 1                               | –                              | –                              | s (1)                         |
| 15        | 7            | S. Jangwani 17:8:1,5   | *Pogona vitticeps* (l)          | 2                               | S. Kumasi 30:z10:e,n,z15 (ch, cl) | Cl                              | i                             |
| 16        | 5            | S. Kumasi 30:z10:e,n,z15 | *Physignathus cocincinus* (l)   | 1                               | –                              | –                              | i                             |
| 17        | 5            | S. subspec. IV 48:z,251: | *Pogona vitticeps* (l)          | 2                               | S. Eastbourne 9,12:e,h,1,5 (cl, sk) | Ch                              | s (2)                         |
| 18        | 3            | S. subspec. IV 44:z,23: | *Pogona vitticeps* (l)          | 2                               | S.Kisarawe 11:k:e,n,x (ch, cl, st) | Sk                              | i                             |
| 19        | 3            | S. Eastbourne 9,12:e,h,1,5 | *Pogona vitticeps* (l)          | 1                               | –                              | –                              | i                             |

PFGE: pulsed-field gel electrophoresis; serol.: serological specificity; sp.: species; subspec.: subspecies;

<sup>a</sup> l: lizard; s: snake; t: tortoise/turtle.

<sup>b</sup> ch: choana; cl: cloaca; sk: skin; st: stomach.

<sup>c</sup> Where the serovars from the child and the reptile were identical, PFGE was performed; i: identical pattern; s: similar pattern.
35:m,t:-) found to have caused infections of two children each, the serovars isolated from the children differed from each other.

A total of 319 Salmonella isolates were investigated and 24 different serovars were identified in the reptiles. The number of serovars within individual reptiles varied between one and four. Only from one snake was no Salmonella isolated. In ten reptiles with Salmonella serovar identical to the salmonellosis-affected child, no other Salmonella serovar but that identical to the child was isolated (Table 1).

In 15 of the 19 examined households, an identical serovar to that of the salmonellosis-affected child was found in at least one reptile. In six reptiles, the respective identical Salmonella serovar was isolated from the oral cavity and in two lizards from the stomach. In 17 reptiles with the same serovar as the child, Salmonella was detected in the cloaca, and in seven the skin swab sample was positive. The identical serovars found in the infected children as well as in the reptiles in the respective households belonged to the following subspecies: I (10), II (1), IIIa (1), IV (3) (details for individual reptiles and also non-identical serovars are presented in Table 1).

Bearded dragons (Pogona vitticeps) were the most common species kept in households with salmonellosis in children (13 of 19 households). Furthermore 16 of 19 of the examined bearded dragons carried an identical Salmonella serovar to the human isolate in the respective household. In corn snakes (Pantherophis guttatus), an identical serovar in the snake and the salmonellosis-affected child was found in three of five examined animals from two different households. Only one tortoise and one turtle were kept in households investigated in this study. These animals, with each an identical serovar to the salmonellosis-affected child, were respectively located in two households. Details are listed in Table 2.

In nine households, the isolates from the infected child and from the reptile had an identical PFGE profile (PFGE data not shown). In six households the human and reptile isolates presented related PFGE patterns (repeated PFGE, Figure 1). Details are also given in Table 1.

**Discussion**

During the fifteen months period of this study, 65% (134/206) of all Salmonella infections in children detected at the NRC were not due to the serovars S. Typhimurium and S. Enteritidis, which are normally transmitted via food ingestion. It is therefore possible that other sources of infection than food, i.e. reptiles in households, could be of relevance. Nearly half (34/79) of the parents who were contacted and questioned, answered that reptiles were present in the respective households. Even though only limited information exists on the presence of reptiles in Germany in general, this percentage by far exceeds recent estimates that reptiles were only kept in 1.2% of all German households in 2010 [26]. This highlights that the presence of reptiles in households appears to coincide with the occurrence of salmonellosis due to exotic serovars in children. This assumption is also supported by a study in the US demonstrating that children with

### Table 2

| Reptile species                  | Number of animals | Number of households with this species | Identical serovars found in the salmonellosis-affected child and the reptile |
|----------------------------------|-------------------|----------------------------------------|--------------------------------------------------------------------------|
| Bearded dragon (Pogona vitticeps)| 19                | 13                                     | 11                                                                     |
| Corn snake (Pantherophis guttatus)| 5                 | 3                                      | 2                                                                      |
| Other snakes                     | 5                 | 1                                      | 0                                                                      |
| Chameleons spp.                  | 3                 | 2                                      | 0                                                                      |
| Chinese water dragon (Physignathus cocincinus) | 2     | 1                                      | 1                                                                      |
| Chinese pond turtle (Mauremys reevesii) | 1     | 1                                      | 1                                                                      |
| Hermann’s tortoise (Testudo hermanni) | 1     | 1                                      | 1                                                                      |
| Total                            | 36                | 19†                                    | 15†                                                                    |

Spp.: species.

† The total does not equate the sum of the numerical values in the remaining rows of the column because a given household could comprise more than one reptile species.
confirmed *Salmonella* infections had more contact to reptiles and cats in comparison to a control group [27].

In most published case reports on RAS, only reptile faecal samples or faecal swabs were used for *Salmonella* detection. In contrast, this study was designed to obtain as much information as possible on *Salmonella* serovars shed by the reptiles. Sampling of the oral cavity (and stomach) as well as the cloaca should provide information on shedding via both orifices and if *Salmonella* are present within the whole digestive system, whereas sampling from the skin should demonstrate whether *Salmonella* may also be transmitted via direct contact with the animal. Repeated enrichment and culture was necessary to provide reliable information on the *Salmonella* status in the reptiles examined. Combining the molecular typing using PFGE with other available data, such as serological typing as in this study, is highly recommended for accurate analysis and comparison of samples [24].

The extensive sampling and testing protocol used in this study is a possible explanation for the high prevalence of *Salmonella* (in 35 of 36 reptiles) found among the reptiles investigated. Intermittent shedding of *Salmonella* in reptiles and the wide array of collection and sampling techniques have been proposed to be the main reason for the variability in detection rates [28]. None of the reptiles examined showed clinical signs indicative for salmonellosis. In consequence, a high prevalence of *Salmonella* in reptiles should generally be assumed, and reptiles should be considered positive for *Salmonella* until the contrary has been proven, as reported previously [29].

Up to four different serovars were found within one reptile. Reptiles were frequently colonised with the same serovars within a given household. This indicates that *Salmonella* as a part of the normal flora can spread amongst individuals within captive reptile collections and therefore probably shed over long periods of time. These results are in accordance with observations that if one reptile carries *Salmonella*, nearly all other reptiles of the respective owner are also affected [5].

Most *Salmonella* isolates were found in cloacal swabs. However, since in some reptiles the identical serovar to the salmonellosis-affected child was only found on...
the skin or the stomach, it can be assumed that shedding via the cloaca is intermittent. A negative cloacal or faecal sample will therefore not prove that the animal does not harbour *Salmonella*. Given the anatomy and behaviour of most reptiles, the presence of *Salmonella* on skin samples was not surprising but underlines the general transmission risk if handling the reptiles is not followed by appropriate hand washing. In conclusion, considering the various shedding sites, several sampling points should be used to increase the detection rate of *Salmonella* in reptiles.

In the majority (15/19) of the examined households, an identical serovar was found in the infected child and at least one reptile from the respective household. The epidemiological association between these isolates was first confirmed using biochemical typing. PFGE confirmed all reptiles, with either completely identical patterns (nine households) or only minor differences in up to two fragments indicating single genetic variations (six households, Figure 1), as described in disease outbreaks [24]. Most serovars found belonged to subspecies I and some have already been described as part of the reptile flora or even as potential human pathogens [2,8,28]. One serovar, *S. Tennessee* was isolated from four reptiles in three households. However, so far there seems to be no specific serovar that is of special zoonotic importance. In consequence, at the moment all *Salmonella* strains found in reptiles need to be considered to be potentially infectious for children.

An important finding of this study was that the reptile species involved could play an important role in transmission of *Salmonella* to children. Little data exists on the proportion of reptile species kept as pets in Germany. In a post-mortem survey, about 17% were water turtles, 34% tortoises, 22% snakes and 27% lizards (including 1.8% bearded dragons) [30]. In one of the authors' clinic specialising in reptile medicine, about 12% of more than 1,000 reptiles presented in the period of the running study were bearded dragons. However, in this study, the majority (13/19) of all households studied kept bearded dragons (*Pogona vitticeps*), and identical *Salmonella* strains to the salmonellosis-affected child were confirmed in 16 of 19 of these reptiles. In contrast, only one turtle and one tortoise were kept in the affected households studied. This result is in strong contrast to early reports of possible RAS in humans, where mainly turtles were suspected to be a source of infection. Interestingly, more recent case reports also indicate that bearded dragons present a risk for transmission of salmonellosis to young children [31,32].

Weiss et al. [12] reported a marked increase in infections in infants caused by *S. Tennessee* in Germany in 2008 and indicated the possibility of bearded dragons being a natural reservoir for this serovar, as this species was kept in seven of 16 households affected and *S. Tennessee* was confirmed in three households. Furthermore, probable transmission from bearded dragons has also recently been described in adult humans [33]. One explanation for the increasing number of reports on bearded dragons as a source of *Salmonella* infection in humans in recent years might be that this species is becoming more popular as a pet, and that bearded dragons are more often handled and petted due to their peaceful nature compared to several other reptile species. Since nearly all children included in this study were too young to handle reptiles themselves, it is likely that vectors, such as the parents or the environment may have played a role. Furthermore it is conceivable that bearded dragons naturally harbour a variety of potentially zoonotic *Salmonella* serovars. The fact that more than one serovar of *Salmonella* was isolated in 13 of a total of 19 bearded dragons supports this assumption.

Husbandry conditions and the households' hygiene status were not assessed according to a protocol, as this was not agreed upon with the owners. However, the overall impression was that most of the households visited demonstrated at least some deficits that needed corrections and advice, e.g. the reptile terrarium placed in the kitchen or in the same room as the child's bed. Further investigations in the environment of the reptile and possible interactions between the reptile and the persons in the households could give important information on possible transmission paths. None of the owners was aware of any risk of infection due to reptiles before, and all were therefore either willing to improve the situation or decided not to keep reptiles any longer.

All publications cited above indicate that although infections attributed to exposure to reptiles and other exotic pets represent only a small proportion of all human salmonellosis cases, it is likely an underestimated and growing problem in Europe [34] and in the US [35] that deserves closer attention. In 2007 more than 500,000 reptiles were imported to Germany only via the Frankfurt/Main airport [36]. According to the European Surveillance System (TESSy) database, the number of infections affecting children younger than three years with serovars associated to reptiles also increased in the last years (Figure 2). Beside the focus on exotic serovars as in this study, it should be kept in mind that there is also a potential risk for the transmission of *S. Typhimurium* and *S. Enteriditis* due to reptiles or as already described, via the reptile feed [6,7]. Also these common serotypes can be shedded by reptiles and therefore have a potential to cause salmonellosis in humans. For example, in this study *S. Paratyphi* B variant Java was isolated from a corn snake. Although the identical serovar has not been found in the child from the respective household, this isolate belongs to phage type Worksp and is therefore of relevance for human infections.

From a terminological point of view, in addition to an earlier suggestion (RAS [37]), we propose that *Salmonella* infections related to reptiles as observed...
in this study be called ‘Reptile-Exotic-Pet-Associated-Salmonellosis’ (REPAS) to give an indication of a possible source of exposure. The main argument for this proposal is that over the last years, the way of trading reptiles has changed considerably and this will probably continue in the future. The main risk of Salmonella transmission from reptiles to humans is not due to European wild species, but to ‘exotic’ (non-native) reptile species, as the results of this study also demonstrate. Furthermore, following recent examinations, Salmonella shedding is higher in reptiles kept in captivity in comparison to wild reptiles [5,28] and ‘pet’ reptiles are obviously in closer contact to humans. These arguments justify the inclusion of ‘exotic pet’ into the term describing the problem. The risk to human health connected to the reptile pet market has been highlighted recently [38] and the accurate description of the problem using REPAS might be important to convey the problem in education and assist risk managers in giving recommendations to harmonise animal welfare and public health.

Figure 2
Occurrence of ‘Reptile-Exotic-Pet-Associated-Salmonellosis’ (REPAS) serovars in children less than three years-old in the European Union, 2007–2010

Salmonella Arizona includes subspecies IIIa and subspecies IIIb. Arrows indicate where REPAS cases have increased in 2010 compared to previous years.
Data from the European Surveillance System (TESSy) (29 May 2012).

Concluding, emphasis on educational measures will be key to reduce the risk of Salmonella transmission from reptiles to children. Professionals dealing with reptiles (pet shop owners, veterinarians, breeders) should be aware of such a risk and, together with those dealing with human health, should be responsible to inform more about this problem. Given the fact that most owners in this study were unaware of the risk of Salmonella transmission from reptiles, we recommend that, in pet shops, any new exotic pet owner be provided with appropriate information sheets on Salmonella prevention. In the authors’ opinion the risk of REPAS can easily be minimised, using a reasonable management protocol without the need to remove reptiles from households with young children.

Acknowledgements
The authors thank François-Xavier Wei1, Centre for Reference and Research on Salmonella, Enteric Bacterial Pathogens Unit, Institut Pasteur, Paris, for the confirmation of the unusual S. Newport. We thank the Member States in the FWD network for submitting detailed serovar data, and Johanna Takkine1, European Center for Disease Prevention and Control (ECDC) for the European data. Furthermore, we thank Marita Wahnfried, Susanne Kulbe, Dagmar Busse for skillful technical assistance, and Rachel Marschang, University of Hohenheim, for linguistic support.
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Infectious diseases are (re-) emerging in Europe. Tropical infectious diseases such as chikungunya and dengue fever have recently been transmitted in Europe in part due to global air traffic and climate change [1,2]. This calls for a proactive public health response to (re-) emerging infectious disease threats both in Europe and globally as well as careful monitoring of infectious disease drivers. Such drivers are globalisation and environmental change, social and demographic change and public health system factors [3]. In order to address this need, the European Centre for Disease Prevention and Control (ECDC) has developed the European Environment and Epidemiology (E3) Network [4].

The long term goal of the E3 Network is to gain a better understanding of drivers of disease and the environmental and socio-economic determinants of public health threats. Such an understanding will strengthen the capacity for formulating public health intervention strategies, and increase the effectiveness of prevention and control measures and general policies within the European Union [5].

The E3 Network provides access to climatic/environmental geospatial data for epidemiologic analysis that are currently collected and analysed by a variety of European agencies, public health institutes, as well as research organisations. The E3 Network will serve as a platform for collaborative dataset compilation, advanced analyses, data processing, and reporting and monitoring of data for risk assessments. It can also be used to enhance the rapid detection of emerging public health threats driven by environmental factors through prediction tools and forecasting models. To facilitate this objective, it will promote the exchange and sharing of the outputs of this collaborative effort through the E3 Geoportal service (https://e3geoportal.ecdc.europa.eu/).

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